

**XXVI INTERNATIONAL
ECO-CONFERENCE® 2022
21–23th SEPTEMBER**

XII SAFE FOOD



PROCEEDINGS

NOVI SAD, SERBIA

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SAFE FOOD

PROCEEDINGS

2022

Organizer:



Ecological Movement of Novi Sad

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Official host of the XXVI International Eco-conference 2022 – Institute for Nature Conservation of Vojvodina Province in Novi Sad

THE ECOLOGICAL MOVEMENT OF THE CITY OF NOVI SAD AN IMPORTANT DECISION OF ITS PROGRAMME COUNCIL

Since 1995, the Ecological Movement of the City of Novi Sad organizes "Eco-Conference® on Environmental Protection of Urban and Suburban Areas", with international participation. Seven biennial conferences have been held so far (in 1995, 1997, 1999, 2001, 2003, 2005, 2007, 2009, 2011, 2013 and 2015). Their programs included the following environmental topics:

- Session 1: Environmental spheres: a) air, b) water, c) soil, d) biosphere
- Session 2: Technical and technological aspects of environmental protection
- Session 3: Sociological, health, cultural, educational and recreational aspects of environmental protection
- Session 4: Economic aspects of environmental protection
- Session 5: Legal aspects of environmental protection
- Session 6: Ecological system projecting (informatics and computer applications in the field of integrated protection)
- Session 7: Sustainable development of urban and suburban settlements-ecological aspects.

Conference participants have commended the scientific and organizational levels of the conferences. Conference evaluations have indicated that some aspects are missing in the conference program. In addition, since a team of conference organizers was completed, each even year between the conferences started to be viewed as an unnecessary lag in activity.

Eco-Conference® on Safe Food

With the above deliberations in mind, a decision was made that the Ecological Movement of the City of Novi Sad should embark on another project – the organization of Eco-Conferences® on Safe Food. These Conferences were planned to take place in each even year. Preparations for the first Eco-Conferences® on safe food started after the successful completion of the Eco-Conference® '99.

So far four Eco-Conferences® have been held (in 2000, 2002, 2004, 2006, 2008, 2010, 2012 and 2014.) focusing this general theme.

Theme of the Eco-Conference®

By organizing the Eco-Conference® on Safe Food, the organizer wishes to cover all factors that affect the quality of human living. Exchange of opinions and practical experiences should help in identifying and resolving the various problems associated with the production of safe food.

Since 2007 Eco-Conference gained patronship from UNESCO and became purely scientific Conference.

Objectives of the Eco-Conference®

- To acquaint participants with current problems in the production of safe food.
- To make realistic assessments of the causes of ecological imbalance in the conventional agricultural production and the impact of various pollution sources on the current agricultural production.
- Based on an exchange of opinions and available research data, to make long term strategic programs of developing an industrialized, controlled, integral, alternative and sustainable agriculture capable of supplying sufficient quantities of quality food, free of negative side effects on human health and the environment.

Basic Topics of the Eco-Conference®

Basic topics should cover all relevant aspects of the production of safe food.

When defining the basic topics, the intention was itemize the segments of the production of safe food as well as the related factors that may affect or that already have already been identified as detrimental for food safety and quality. The topics include ecological factors of safe food production, correct choice of seed (genetic) material, status and preparation of soil as the basic substrate for the production of food and feed, use of fertilizers and pesticides in integrated plant protection, use of biologicals, food processing technology, economic aspects, marketing and packaging of safe food.

To paraphrase, the envisaged topics cover the production of safe food on the whole, individual aspects of the production and their mutual relations, and impact on food quality and safety.

Sessions of the Eco-Conference®

1. Climate and production of safe food.
2. Soil and water as the basis of agricultural production.
3. Genetics, genetic resources, breeding and genetic engineering in the function of producing safe food.
4. Fertilizers and fertilization practice in the function of producing safe food.
5. Integrated pest management and use of biologicals.

6. Agricultural production in view of sustainable development
7. Production of field and vegetable crops.
8. Production of fruits and grapes.
9. Livestock husbandry from the aspect of safe food production.
10. Processing of agricultural products in the framework of safe food production.
11. Economic aspects and marketing as segments of the production of safe food.
12. Food storage, transportation and packaging.
13. Nutritional food value and quality nutrition.
14. Legal aspects of protecting brand names of safe food.
15. Ecological models and software in production of safe food.

Attempts will be made to make the above conference program permanent. In this way will the conference become recognizable in form, topics and quality, which should help it find its place among similar conferences organized elsewhere in the world.

By alternately organizing conferences on environmental protection of urban and suburban areas in odd years and conferences on safe food in even years, the Ecological

Movement of the City of Novi Sad is completing its contribution to a higher quality of living of the population. Already in the 19th century, Novi Sad was a regional center of social progress and broad-mindedness. Today, owing first of all to its being a university center, Novi Sad is in the vanguard of ecological thought in this part of Europe.

It is our duty to work on the furtherance of the ecological programs of action and, by doing so, to make our contribution to the protection of the natural environment and spiritual heritage with the ultimate goal of helping the population attain a higher level of consciousness and a higher quality of living.

Director of the Ecological
Movement of Novi Sad
Nikola Aleksic

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FOREWORD

Ecological Movement of Novi Sad continuously for twenty-six times is successfully organizing international scientific Eco-conference. Every odd year a conference devoted to the ecological problems of cities and suburbs is organized and even years a conference devoted to safe food is organized. This year the program of the conference represent various aspects of safe food production. Population nutrition and in this regard, correctness and biological value of food represents not only local but also a global problem, which is why this problem attaches great importance worldwide. Population nutrition is a multilayered problem of population and includes quantitative and qualitative indicators which have a great impact on the health and prosperity of the human population, but also represent the social, political, economic, and environmental problem.

It is estimated that in the world, every ninth inhabitant is starving or about 800 million, of which 98% in developing countries, 500 million in Asia and the Pacific region and 23 million in Africa. The reasons for insufficient amounts of food on a global scale are numerous: local and regional wars for which it is not possible to cultivate the land, the production of fuels from vegetable products, increasing production and thus consumption of meat, which requires a greater quantity of plant products, food price growth and reproduction materials for which farmers are not able to establish manufacturing.

In addition, there is lack of agro-technical knowledge, corruption, inefficient use of natural resources, environmental pollution, global climate change and others. It is believed to be due to climate change by 2030.

Yields of cultivated species reduced by approximately 30%, while, at the same time, there will be a reduction in arable land per capita, which in 1970, amounted to 3205 in 1990, to 2372, and in 2050, it will be reduced to 1500 m². At the same time it is estimated that the Earth's globe in 2050 to live more than ten billion people. An interesting fact is claiming that 30,000 edible plant species in the world, only four species: rice, wheat, maize and potatoes provide 60% of energy needs in nutrition.

Using large edible plant species on a large scale would contribute to partially solving the problem of hunger, and probably better quality food. There is also an

opinion that in the world, enough food is produced and that will be in the future be able to provide increased needs. The problem is the global distribution of agricultural products, their price and the huge losses that arise in the course of their processing, storage and transport. This among other things is confirmed by the fact that especially in developed countries, but not only there, the problem of obesity seems a growing health problem.

It stems not only from the relatively low prices of food and in this regard the entry of excessive amounts of calories, but also from an inadequate diet, intake of foods rich in carbohydrates and fats.

Based on the above, one can reasonably ask how it fits the concept of safe food and organic agricultural production to overcome its lack of quantity, especially in economically underdeveloped countries, but also in other parts of the world. It would be wrong to reduce the problem of hunger only in economically undeveloped countries, it exists, and in countries in transition and prosperity, it is true to a lesser extent. Due to the lack of application of chemicals (fertilizers, pesticides, etc.) still yields compared to conventional production to organic production is the lower about 20%, a price on that technology manufactured goods is also increased by about the same percentage.

Organic production is encouraged from the state budget funds, which means that the poorer classes of the population is co-funding the consumption of organic food to the upper classes of citizenship that such foods can buy. Additionally, organic food production deserves attention for several reasons. Consumption of organic food contributes to the health of the population, increasing environmental awareness not only among food producers but also among consumers, it provides effective management in smaller areas, as well as the export of such manufactured food for which there is a significant demand in the world market.

Problem of safe food is very complicated, so the program and this year's conference is designed so that it covers all aspects of this issue: climate, soil, genetics, fertilizers, integrated crop protection, sustainable development of agriculture, food safety, production of fruit and grapes, livestock production, food processing, storage, transport and packaging, nutritional value, legal aspects and environmental models and software in the production of safe food.

Ecological Movement of Novi Sad and the co-organizers hope that the conference will contribute to better understanding and addressing specific environmental issues regarding the production of healthy full-value food and thanks to everyone who contributed to the organization and holding together with the message what attitude we should have toward nature, since natural resources are borrowed from future generations.

Prof. Dr. Miroslav Malešević

INTRODUCTORY PRESENTATION



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Review paper

CHALLENGES AND SOLUTIONS FOR AGRICULTURAL PRODUCTION IN THE UPCOMING ERA

Abstract

The aim of this work was to provide an overview of the current and upcoming challenges of the overall agricultural and especially animal production from a global perspective and in the Balkan region with an analysis of possible solutions.

Accordingly, to the forecasts, the agricultural sector, and especially the sector of animal production will be challenged with many issues in the upcoming period (climate change, increase in human population, a deficit of natural resources and energy, higher environmental protection demands, increased consumer standards). Furthermore, in the Balkan region, besides this, there is a number of more elementary problems (bad politics, low transfer of knowledge in practice, underutilization of available resources).

However, there is a solution for every problem and it is up to the scientific sector to ensure the application of new technologies and knowledge in order to enable the sustainability of the food supply of the human population in the Balkan region.

Key words: *agriculture, animal production, climate change and resources,
precision technologies, insect farming, artificial mea*

INTRODUCTION

According to US Census Bureau (2016; figure 1), the global human population, by the year 2050, will increase to over 9 billion people. FAO (2011) emphasised that the population growth will result in an increase in food consumption by 50–60% compared to the present consumption. Also, it is expected that the global population will become wealthier indicating that will prefer to select animal products (meat, milk and eggs) resulting in a significant increase in the number of domestic land animals (Picture 1).

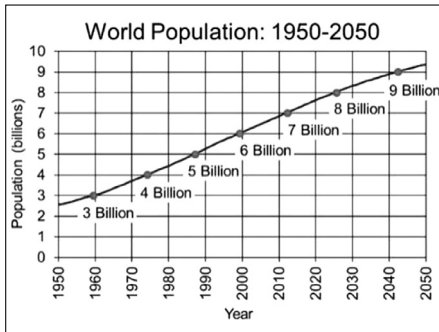
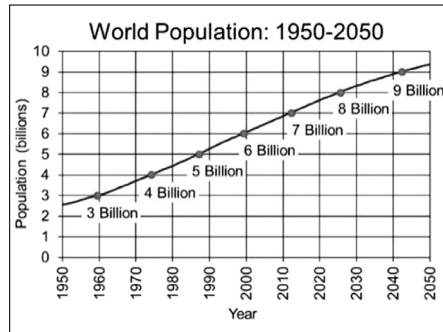


Figure 1. Expected population growth (US Census Bureau, 2016)



Picture 1. Expected increase of land animals (FAO, 2011)

According to the UN (2022), the world population could peak at nearly 11 billion (or higher (Consensus for Action, 2013) around the year 2100. Expected growth has been the consequence of the increase in surviving rate to reproductive age, significant changes in fertility rates, growing urbanization and migrations worldwide.

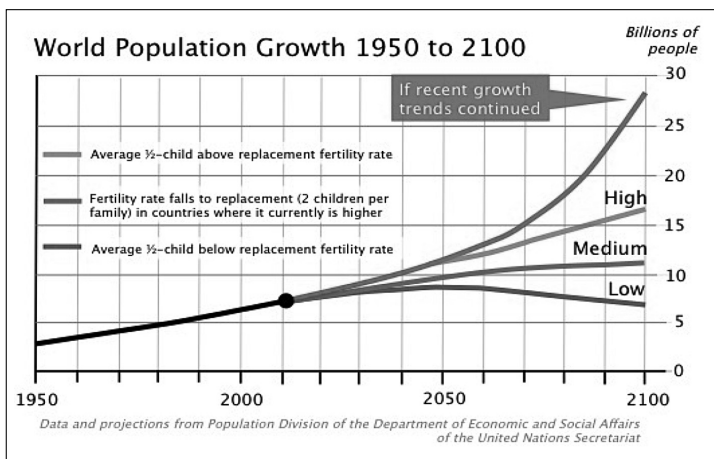


Figure 2. World population trends till year 2010

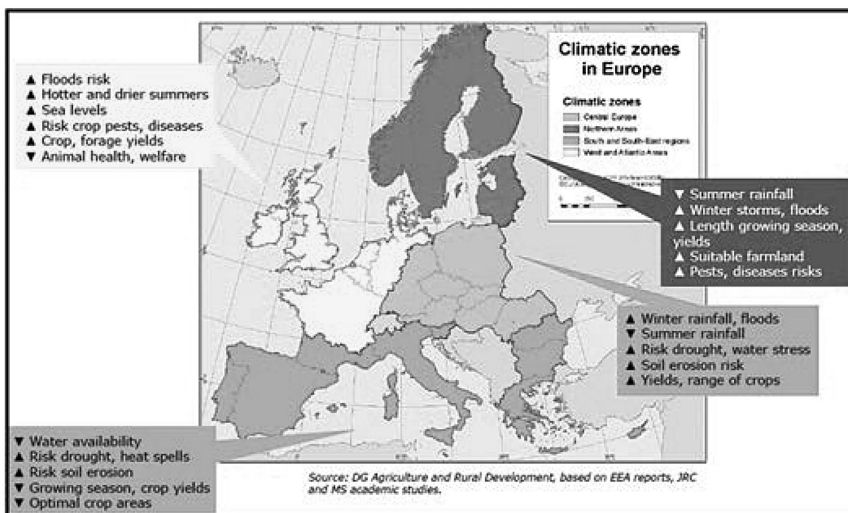
On the other hand, considering the constant trends in the reduction of the birth rate in developed countries and the significant decline in number of births in China in 2021 (only 10.6 million children were born, which is 7.52 births per 1000 inhabitants, which is the lowest number of births since the year 1949 (Chinese National Bureau of Statistics), it is realistic to expect different scenarios from the extreme increase in the number of inhabitants in the world.

Regardless of the scenario of the world's human population, the basic need of all inhabitants is food, therefore the agricultural sector as a producer remains one of the primary and most important activities in ensuring the survival of the human population.

THE CHALLENGES OF AGRICULTURAL PRODUCTION AND RESOURCES

Agricultural production worldwide in the future period will have to manage a number of significant challenges: fast population growth, growing demand for energy, lack of resources, accelerated urbanisation, changes in diet, ageing inhabitants in rural areas in developed countries, increased competition in the global markets, deficiency of access to funds in developing countries, and changes in climate (EPRS, 2016).

How will agricultural production in the European Union be impacted by changes in climate conditions? Accordingly, to European Commission, DG AGRI (2016) the change in rainfall will represent a severe problem in many European regions. Furthermore, increasing temperatures, variability and seasonality as well as increasing frequency of extreme events, heatwaves, droughts, storms and floods across the European Union are some of the anticipated changes (Picture 2 and Picture 4).



Picture 2. Predicted impact of changes in climate on European Union (EC, DG AGRI, 2016)

Furthermore, IPCC (2007) stated that changes in climate will affect agricultural production and especially animal production worldwide. In support of the stated, Battisti and Naylor (2009) predicted that by the year 2050, most of the world will be exposed to median temperatures during the summer period that will be higher than the highest registered temperatures. While, Reiczigel et al. (2009) in Hungary, as well as Dunn et al. (2014) in United Kingdom indicated an increase in heat stress days per year resulting in serious problems in the animal production sector. Furthermore, Popović et al. (2013, 2022) pointed out that the production of oil flax and buckwheat will also face difficulties in terms of forecasted changes in climate and temperature rising.

Also, the natural resources required to enable global food and agricultural production are limited and will not grow. Therefore, the required increase in agricultural production has to be managed in the terms of the growing deficiency of natural resources: agricultural land, water, nutrients, and people knowing what to do and how to produce efficiently and environmentally sustainable (Picture 3).



Picture 3. Availability of natural resources in terms of changing climate conditions (CPA Canada, 2022)

Furthermore, to minimize the consequence of global food and agricultural production on the environment, waste and greenhouse gasses (GHG) emissions must be reduced. The latest results (IPCC, 2018) highlighted that a number of climate change effects could be avoided in the case of limiting global warming to 1.5°C compared to 2°C, for example, lower global sea level rise. The limitation of global warming could give people and ecosystems more time to adjust and remain below appropriate risk thresholds. Finally, limiting global warming to 1.5°C demands immediate, far-reaching and outstanding changes in all aspects of civilisation.

What about Balkan region? The perspectives? The analysis of the general situation in the Balkan region, indicates accelerated inflation of all valuables, energy, commodities and services, as well as, what is extremely dangerous for society, inflation of

general social values. Those trends are partially caused by the COVID-19 pandemic and associated speculation on world markets and inflation as well as the transition from a communist regime to some sort of so-called capitalism. Results of that changes are losing confidence in state institutions, pronounced pessimism in the population and a remarkably prominent trend of depopulation of the working-age population. Such a deeply corrupt society and an environment of insecurity and loss of confidence in knowledge and skills have resulted in an apparent economic decline of the region, which is particularly pronounced in the field of agricultural production resulting in an extreme decrease in production which *currently amounts to about 30% self-sufficiency*. Furthermore, a common characteristic of the Balkan region is the loss of interest in higher education related to agricultural sciences and the rapid decline in the number of students at related faculties, as well as the loss of interest in agricultural production and the progress and modernization of the food production sector.



Picture 4. Drought in Europe: Shipping threatened in Germany as Rhine water levels continue to drop (Euronews, 11/08/2022)

Also, the agricultural sector, is burdened with a number of soluble problems, like inadequate policies, irrational utilization of resources, lack of application of knowledge in the field, insufficient transfer of knowledge from higher education institutions to practice, low level of education of producers, and generally neglected branch with extremely high potential for progress. *So, Balkan does have resources, land, water, and people, but...*

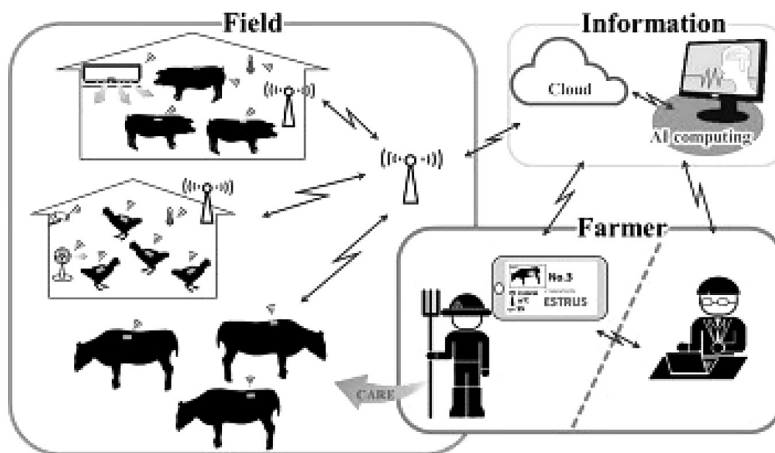
WHAT ARE THE SOLUTIONS?

In the scenario of significant growth of the human population, in order to prevent global food insecurity, the sustainable intensification of the total agricultural and animal production sector needs to be enabled in the following conditions: increased demand for animal products decreased necessary resources (agricultural land, water),

and various tools and production methods that could be applied. In these conditions, intensification of animal production implies an increase in animal density and a decrease in the stockperson per animal ratio. In the scenario of increasing herd size and decreasing availability of employees on the farm, precision animal production technologies impose as the optimal solution.

Precision animal farming technologies imply the usage of diverse sensors/detectors and managing of big data in order to deliver simple information regarding animal health status, production on a daily, monthly or some other basis, and welfare. The information concerning the production, reproduction and welfare could be available at the individual and herd levels.

That information enables a farmer to optimize the farm management and accordingly realize an efficient and sustainable production.



Picture 5. IoT sensors for smart livestock management
(Iwasaki et al., 2019)

Precision animal farming is not a new science (for instance, test-day records as a managing tool were used for a few decades) but the applicability and availability of precision animal farming techniques have been significantly improved due to the accelerated development of computer science and the availability of inexpensive sensors developed for the video gaming industry.

How does it work? Remote sensors such as cameras, microphones, thermometers and accelerometers monitor or capture information such as images, sound, heat or motion from groups or individual animals. The data from the sensors could be stored in external drives or sent directly to a processing node and then processed by algorithms. An algorithm represents a formula or step-by-step set of operations that are used to solve a specific problem or some class of problems. For instance, the feature variable percentage of time pigs are lying down is used to determine the biological outcome of “lameness”, or the number of coughs is used to detect the biological outcome of “respiratory disease”.

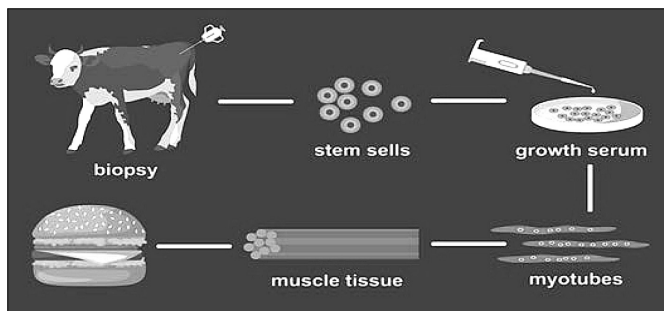
By applying precision agriculture technologies, we are able to facilitate our business and increase the profitability of our farm business. The choice and applicability of precision technology systems largely depend on the characteristics of each farm and the management method of each farmer. Therefore, the decision to apply is the individual decision of each farmer. New technologies are available and new systems are emerging on the market every day, it is up to us to select, apply and put them into operation in order to optimize our business.

Research, development and innovation have made the agricultural sector in Europe competitive and effective in producing enough quantity of quality food for the citizens of Europe. Application of new technologies and new knowledge, and by improvement of our production system on daily basis, we can be economically and environmentally sustainable.

But, there are some rumours that animal production pollutes! According to the Greenpeace analysis (2020), farm animals and the fed production in Europe are producing more greenhouse gases every year than all of the bloc's cars and vans jointly. Nevertheless, the information and relevance of the information depend on the source of the information and the intention to place the same information. How much animal production really contributes to the total anthropogenic emission of greenhouse gases needs to be adequately scientifically assessed (which has not yet been carried out). However, the fact is that different lobbies (vegans, artificial/cultured meat, insects...) promote their ideas very strongly in terms of marketing.

Cultured Meat – Meat without Harming a Single Animal

'Cultured meat, produced in bioreactors without the slaughter of an animal, has been approved for sale by a regulatory authority for the first time. The development has been hailed as a landmark moment across the meat industry' (Guardian, 2020).



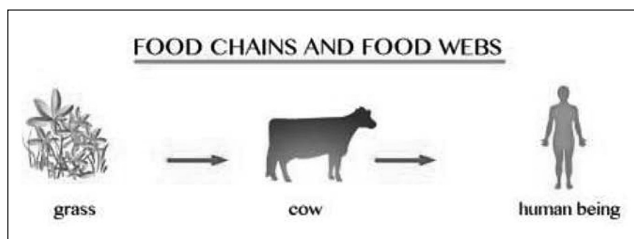
*Picture 6. The production of cultured meat
(Cultured meat, 2022)*

Cultured meat could be represented as an early-stage technology with some possible benefits and challenges, so... There are lots of unanswered questions regarding the cultivated meat, the question of impact on human health, impact on the environment,

the emission of greenhouse gasses, water consumption, footprint ... The information placed in the media that artificial/cultivated meat will save the planet from GHG emissions is not scientifically proven, and there are no scientific estimates of the extent to which meat production will contribute to the reduction of GHG, and how it will affect the human health and welfare? The only estimates currently available are regarding the financial turnover in the artificial meat market in the amount of 140 billion euros in the next few years. So, potentially good business for investors and not so good for consumers.

Vegan diet

A vegan diet implies a plant-based diet and avoiding all foods that come from animals. There are some opinions that *'if farm animals stopped landing on everyone's plates 14.5% of all human-related emissions would be offset'* ECOPEANUT (2021). However, there are potentially several obstacles to the transition of the human population to a vegan diet, for example, the lack of resources for the production of sufficient food of plant origin for the entire human population and the impossibility of using certain plant species that ruminants process very efficiently into high-value products for human nutrition (Picture 7). Furthermore, there are conflicting opinions and evidence regarding the impact of a vegan diet on human health. Finally, the extinction of domestic animals will result in catastrophic long-term consequences on the pedological quality and structure of agricultural land that would directly endanger existing plant production.

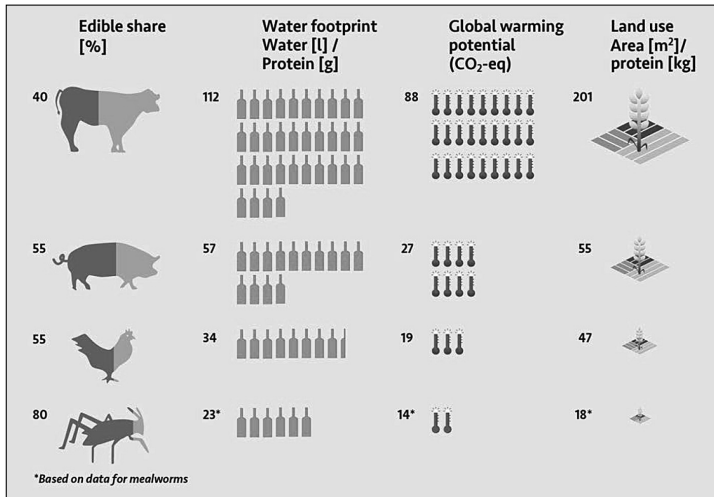


Picture 7. The basics – food chain (Gloster EDU, 2019)

Insect farming

Insect farming represents a rapidly growing industry, with a large number of farms worldwide rearing insects. Farmed insects, or *"mini-livestock,"* imply the rearing of insects such as crickets and mealworms as food or feed for animals. Reverberi (2020) stated that eating collected insects was a common practice in many countries for hundreds of years (edible insects represent traditional foods in over 100 countries of Asia, Africa, and South America) while farming and processing insects into a food ingredient represent a new developing industry. Small companies in North America

and Europe are processing cricket (and mealworm) powder into packaged food (energy bars, pasta, and chips). There are some opinions that insect agriculture implies lower greenhouse emissions, and therefore lower environmental impact compared to animal farming. Furthermore, farming insects that is the production of insects' protein requires less resources than the production of animal protein (XiaoZhi Lim (2022), Picture 8).



Picture 8. Comparison of various food sources (XiaoZhi Lim, 2022)

CONCLUSION

Forecasts indicate that the agricultural sector, and especially the sector of animal production will be faced up with a number of obstacles in the future period. One of the most pronounced challenges is unquestionable changes in climate in the direction of worsening conditions for agricultural production. Then there is a need for a significant increase in the amount of produced food and feed caused by the expected rapid incline in the human population that is supposed to be realised in terms of natural resources and energy decline. Furthermore, agricultural production must ensure a high level of environmental protection and meet increasingly high consumer standards. *So how do you bring seemingly contradictory settings together?* There are different solutions, from alternative ways of producing the necessary proteins for the nutrition of the human population to smart solutions (precision agriculture) that will enable the satisfaction of all modern challenges and sustainable food production. However, the problems of agricultural production on a global level and on a local level, for example in the Balkan region, are somewhat different. Apart from the already mentioned challenges, the Balkan region is also burdened by more elementary problems such as bad policies, unorganized production systems, negligible transfer of knowledge into production, and general despondency of the population and loss of faith in the possibility of improvement. However, it is up to the scientific sector to reverse the

trends and establish modern efficient and sustainable agricultural production and ensure the application of new technologies and knowledge in order to enable the sustainability of the food supply of the human population in the Balkan region.

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Прегледни рад

ИЗАЗОВИ И РЕШЕЊА ЗА ПОЉОПРИВРЕДНУ ПРОИЗВОДЊУ У НАДОЛАЗЕЋОЈ ЕРИ

Извод

Циљ овог рада је био да се из глобалне перспективе и у региону Балкана сагледају актуелни и предстојећи изазови укупне пољопривредне, а посебно сточарске производње, уз анализу могућих решења.

Сходно томе, према прогнозама, пољопривредни сектор, а посебно сектор сточарске производње биће суочени са многим питањима у наредном периоду (климатске промене, повећање људске популације, дефицит природних ресурса и енергије, већи захтеви за заштиту животне средине, повећани потрошачки стандарди). Штавише, у региону Балкана, поред овога, постоји и низ елементарнијих проблема (лоша политика, слаб трансфер знања у пракси, недовољно коришћење расположивих ресурса).

Међутим, за сваки проблем постоји решење и на научном сектору је да обезбеди примену нових технологија и знања како би се омогућила одрживост снабдевања храном људске популације у региону Балкана.

Кључне речи: *пољопривреда, сточарска производња, климатске промене и ресурси, прецизне технологије, узгој инсеката, вештачко месо*



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Review paper

FOOD CONTROL SYSTEMS AND PROTECTION OF HUMAN HEALTH

Abstract

Providing a sufficient amount of safe food with defined desirable quality parameters is an imperative for every producer, but also an unmatched demand of a modern consumer. Globalization of the food market, development and introduction of novel foods and new production processes, ever more present centralized method of preparing and distributing food, growing demand for minimally processed food with increasing use of ready to eat food, as well as the creation of ever longer and more complex food chains, have contributed to a significant increase of risk level in the area of its safety. The issue of food spoilage and its safety is an indispensable part of all national structures' activities involved in public health care, which, in recent decades has gained a new integrated approach, known as „One Health”.

Key words: *food spoilage, foodborne diseases, food control, „One Health”*

INTRODUCTION

Given that modern food trade and transport have an international character, food safety is a common issue and subject of attention of both developed and developing countries (Vesković Moračanin et al., 2015). In order to respond to the challenge, the governments of many countries have established new institutions, standards and methods for regulating food safety and increased investments in systems for controlling potential hazards. The very fact that withdrawal of a product from the market can lead to serious financial losses, damage to manufacturer's reputation, as well as to the occurrence of foodborne illness, and in the worst case, even fatal outcomes, contributed to the issue of food safety being at the forefront of national policies today.

The principles „from field to table” (Italy), „from farm to fork” (England) or „from producer to consumer” (Germany) are synonyms on which the new integrated food

safety system is based in the European Union, as well as in our country (Vesković Moračanin et al., 2015; Vesković and Đukić, 2018). The principle suggests that food safety begins, before all, at the level of primary production, i.e. production on the agricultural land and in the barn, and ends with the consumption of food by the final consumer. This system establishes the responsibility of all participants in the integrated food chain, starting with production, processing, and ending with food transport and distribution (Vesković-Moračanin et al., 2014). In addition to the defined and shared responsibility, other actual challenges are directed towards the need to: *i*) reduce economic losses caused by food spoilage, *ii*) lower the cost of the food production process, *iii*) reduce the possibility of transmitting pathogenic microorganisms, as well as *iv*) meet the growing needs of consumers for *ready-to-use* food, which has a fresh taste, high nutritional and vitamin value, and which, in addition, is minimally processed and treated with preservers (Vesković-Moračanin et al., 2015; Vesković and Đukić, 2018).

Apart from the above requirements, according to FAO projections, in decades to come, agricultural and food production will have to increase by at least 70% in order to be able to keep up with the expected growth of the world's population. At the same time, it is completely realistic to expect that the aforementioned necessary increase in production will open up new challenges in the area of food quality and safety, as well as human health (WHO, 2022). The issue of food spoilage and its safety is an indispensable part of all national structures' activities involved in public health care, which, in recent decades has gained a new integrated approach, known as „One Health”.

FOOD SPOILAGE

In the 21st century, food spoilage with its consequent loss is of great importance. In addition to direct material damage, a significant loss is also reflected in lost work, used water, energy, land, as well as other resources that enter the food production chain (Lipinski et al., 2013).

Food spoilage is a natural, metabolic process that leads to sensory changes in the texture, smell, taste, or appearance of food that becomes undesirable or unacceptable for human consumption. (Doyle, 2007; Nychas and Panagou, 2011). Irrespective of its origin (vegetable or animal), due to its composition (moisture, proteins, lipids, carbohydrates and other organic and mineral substances) food represents an ideal environment for the development of unwanted microbiological, chemical and physical processes that lead to the emergence of unpleasant sensor changes, i.e. spoilage. The nutritional value of the food does not change during the mentioned process, while the sensory properties (color, smell, texture and edibility) become changed, and the food itself is unusable for human consumption (Rahman, 2007). Although microorganisms are the most common carriers of the food spoilage process, this process does not always lead to illness in consumers, given that pathogenic microorganisms or their toxins do not have to be present in spoiled food. However, the resulting changes in sensory properties mean that this type of food cannot be used further in human diet.

Explaining the processes of food spoilage, certain ecologists believe that the created harmful metabolic products, primarily, characteristic unpleasant smells, represent a

protective mechanism of microorganisms – the cause of spoilage, which in this way keep the necessary nutrients for themselves, while other potential users of the same food, e.g. large animals, including humans, are repulsed (Burkepile et al., 2006).

Food spoilage is a major concern of the entire world population, with developing parts of the world particularly affected. At the same time, the research results indicate that there is a global lack of information about this problem, especially in the part of quantifying food loss in relation to the etiology of the cause, as well as the extent of economic damage that accompanies such loss (FAO, 2011). In parallel with these unfamiliarities, there are no adequate assumptions about the potential costs that could be spent on activities aimed at reducing or preventing food loss due to food spoilage. On the other hand, expert predictions suggest that food production should be significantly increased in order to meet global demand in the future (Fonnesbech et al., 2005). Due to all of the above, the issue of food spoilage is of great international importance, both from the aspect of efforts to suppress and reduce hunger in the world, improve its safety during the intended period of use, and from the economic aspect, due to the damage caused by food losses.

It sounds paradoxical but the data of the United Nations Department of Economic and Social Affairs (DESA) indicate that the food sector at the beginning of the XXI century has produced enough food for the needs of the entire human population. On the other hand, it is estimated that more than 690 million people in the world are still hungry. In addition to the different economic development of the countries, the aforementioned situation is conditioned by the fact that a third of the produced food is unused due to its spoilage or some other reason (Gustavsson et al., 2011). Research has shown that in developing countries, due to the lack of modern processing and preservation technologies, as well as adequate storage methods, much more food is lost during the production/processing phase and immediately afterwards. In contrast, in industrialized countries one-third of food spoilage and write-offs occur at the retail or consumer level (FAO, 2011). The highest percentage of food loss was found in root vegetables (40–50%), fruits and vegetables (35%), fish and seafood (30%), cereals (20%), meat, oilseeds and dairy products (20%) (FAO, 2019).

The worrying data is that in the countries with medium and high incomes, food is to a large extent wasted, even if it is still suitable for human consumption, but also due to its increased production in relation to the actual needs of end users.

Regardless of the level of economic development and maturity of the system in the country, it is necessary to strive to reduce food losses to a minimum. Considering the importance of this problem, many countries in the EU have, as part of their national policies in the area of food production, and an important part of their strategies, raised the issue of reducing food loss and wastage, asking that legally binding activities that will contribute to this reduction be established within the legislation (UNEP, 2021). However, in most countries, legislation is designed in such a way that food safety is treated separately from the issue of food spoilage. Despite such a prevailing approach, from the microbiological-economic point of view, the mentioned areas cannot be separated or considered individually. The occurrence of diseases with accompanying health problems, as well as economic losses due to foodborne diseases, are directly related and complementary to economic losses due to food spoilage (Di Renzo et al., 2015).

THE MOST COMMON FOOD BORNE DISEASES

Food safety issue is receiving ever-increasing attention worldwide since high correlation has been found between consumer diet and health. As much as the essential need for food is, so is safe food consumption a basic human right. Food safety is a global issue with significant implications on human health. World Health Organization (WHO) has been warning that at least two billions of people worldwide get sick from eating unsafe food. Unsafe food contains dangerous agents or contaminants, which can lead to the appearance of diseases in people, or to the creation of an increased risk of developing chronic diseases. Such contaminants can reach food in many different ways during processing, and they can also be found in it due to poor or inadequate production and hygiene conditions. The most common danger for the creation of unsafe food is the presence of various pathogenic microorganisms, parasites, mycotoxins, residues of veterinary drugs and pesticides (Vesković et al., 2011).

Experience so far has shown that food safety is a common problem and subject of attention, both in developed and developing countries. Research indicates that the most common risks to food safety, and thus directly to human health risks, are greater in developing countries than in developed countries (Gizaw, 2019). However, highly developed countries, such as Japan, the US, etc., have also experienced incidents where hundreds and thousands got sick or died (Fung et al., 2018). Based on a comprehensive overview, it can be said that the level of risk varies depending on the sanitary condition of the soil (Đukić et al., 2008, 2011), climatic conditions (Vesković and Đukić, 2018), the way people eat, the level of their social status, that is, the height of the state public revenue and established infrastructure.

The constant need for a larger amount of food due to the increase in the population, the increase in the volume of its production, the establishment of complex transport and supply chain with trade globalization, as well as the existence of an inevitable demand for a lower price and increased competitiveness, contributed to an increased number of incidents in the area of disease occurrence that are directly related to food. Also, the uncontrolled and frequent application of agrochemicals in primary agricultural production, along with the appearance of an increasing number of new pathogens with changes in their virulence, further increases the risk of disease. The emergence of antibiotic resistance of microorganisms, which is consequently followed by an intense increase in the number of multiresistant species, is also very worrying. At the root of this problem is, of course, the uncontrolled and inadequate use of antimicrobial agents in animals, with the consequent incalculable harmful effect on this issue.

Foodborne diseases today are public as well as the main problem in the whole world. The WHO defines them as "diseases of an infectious or toxic nature that are or suspected to be food and waterborne". There are various agents like bacteria, viruses, and parasites causing foodborne diseases, among which, the bacterial agents are more prevalent. According to estimations of the Centers for Disease Control and Prevention (CDC, 2021), of more than 250 different food-borne diseases registered in total, over 48 million people get sick from a foodborne illness every year, 128.000 are hospitalized, and 3.000 die. The most severe cases of the disease occur in infants, small children, the elderly, immune-compromised (such as those with HIV/AIDS, cancer,

diabetes, kidney disease, and transplant patients), but also in healthy people who are exposed to high doses of the causative germs. The largest number of causative agents of these diseases are bacteria (about 66%), while other causative agents are viruses, parasites, natural and synthesized chemical substances, as well as microorganism toxins, among which, in addition to bacterial toxins, mold toxins are particularly important (Škrinjar et al., 2013).

The gate of infection for foodborne pathogens and their toxins is gastrointestinal tract where preliminary symptoms are most commonly registered. Nausea, vomiting, abdomen cramps and diarrhoea are frequent symptoms of the diseases.

Foodborne diseases significantly burden the world economy on an annual basis. The estimated costs of registered incidents in the US economy ten years ago, and which refer only for the purposes of notifying consumers, withdrawing food from market circulation and compensating damaged users in lawsuits, were approximately 7 billion dollars per year (Hussain and Dawson, 2013). According to the reports of U.S. Department of Agriculture Economic Research Service (USDA ERS, 2018) the total economic burden was about \$17.6 billion, an increase of about \$2 billion, or 13 percent, over the 2013 ERS estimate of \$15.5 billion.

Table 1. The most common animal products and foodborne bacteria causing disease in humans

Type of product	Foodborne bacteria	Disease symptoms
Fresh and thermally underprocessed meat and meat products	<i>Campylobacter jejuni</i> , <i>Escherichia coli</i> , <i>Salmonella</i> spp. <i>Listeria monocytogenes</i>	Abdominal pain, nausea, vomiting, diarrhoea
Fresh milk and dairy products	<i>L. monocytogenes</i> , <i>Salmonella</i> , <i>Shigella</i> , <i>Staphylococcus aureus</i> , <i>Campylobacter jejuni</i>	Abdominal pain, nausea, vomiting, diarrhoea, increased temperature
Fresh eggs and thermally underprocessed egg products	<i>Salmonella</i> Enteritidis	Abdominal pain, nausea, vomiting, diarrhoea, increased temperature

The 2018 World Bank report indicates that the total productivity loss associated with foodborne diseases in low- and middle-income countries is estimated at \$95.2 billion per year, while the annual cost of treating illness caused by contaminated food is estimated at \$15.6 billion dollars (World Bank, 2019). Therefore it can be said for sure that foodborne diseases represent a significant problem for individuals, communities and the entire food industry (Fung et al., 2018). Epidemiologic data worldwide have shown that food of animal origin is in 50 –90% cases involved in etiology of foodborne disease. According to the European Food Safety Authority (EFSA, 2014) data, eggs and egg products are considered one of the most important vectors of

foodborne outbreak (22.8%), while meat and meat products account for 20.2%, seafood 13.8%, and cheese for 5.4%. Today, several thousands of different products of animal origin can be found on the global food market. Analyses have shown that fresh, thermally unprocessed food is a very suitable environment for growth and propagation of foodborne pathogens, which classifies it in the high-risk foodborne disease (Table 1).

It is considered that *Salmonella* spp., excluding *S. typhi*, and *Campylobacter* spp. are major causes of foodborne diseases in the US, England and Australia (Scallan et al., 2011). Today, the most common is norovirus with an estimated 15 million cases, followed by *Campylobacter* spp., which is responsible for almost 5 million cases. When it comes to meat chain, classical zoonoses, such as tuberculosis, cysticercosis, trichinellosis or anthrax infection became much less important (Bunčić et al., 2019), while causative agents like *Campylobacter*, *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes* and *Yersinia enterocolitica*, mostly excreted by asymptomatic animals or coming from the surrounding environment, became predominant (Nastasijević et al., 2020).

In 2018, 26 EU member states reported a total of 5.146 outbreaks caused by foodborne pathogens and 48 outbreaks caused by waterborne pathogens. 4.588 ill was hospitalized, while the number of deaths was 40. Campylobacteriosis was the most commonly reported zoonosis. Apart from *Campylobacter*, causative agents of these outbreaks were *Salmonella*, norovirus, bacterial toxins other than *Clostridium botulinum* and *Campylobacter* (EFSA, 2019). WHO (2019) reports that 70%, of the total of approximately 1.5 billion registered diarrhoeas was caused by biologically contaminated food, whereby these poisoning, primarily in undeveloped countries, were responsible for deaths of approximately 3 million children. Morbidity caused by food poisoning in Europe is second right behind respiratory diseases, with estimated 50.000–300.000 cases of acute gastroenteritis annually per million inhabitants (Luchansky, 1999).

FOOD SAFETY

Food control – historical aspects

The issue of food safety is probably as old as the human race itself. The first elements are contained in the intuitive recognition and subsequent avoidance of foods that are naturally toxic and harmful to consume. As human patterns of living, eating, habits and food characteristics changed, the issue of food safety took the form of formalization, and later the framework of legislation. In the beginning, there was, as in laws of ancient Israel, some advice on foods to avoid, ways of preparing them, as well as the importance of food hygiene during consumption. In the third book of Moses (the book of Leviticus), from 2000 BC, it is said that Moses introduced laws to protect his people from food-related diseases, which included mandatory washing of clothes and bathing after animal sacrifices. It is also stated that the Chinese, Greeks and Romans had similar thoughts (Mossel et al., 1995), and that the Egyptians stored and

marked their food thousands of years ago. In 375 BC, the Indian teacher, philosopher and economist, Chanakya, mentioned the issue of food adulteration in his book „Arthashastra”.

Observed throughout history, the concern for food quality and safety increased and over time took its regulatory form. The early period of legislation was, first of all, based on the need to prevent the possibility of food adulteration, as well as the need for food to be accurately described. The first English law on food from 1202 (*the Assize of Bread*, proclaimed by King John of England) referred to the ban on the use of ground peas or beans during the production of bread, which is equivalent to nowadays bans on food adulteration. Also, in 1266, the UK parliament prohibited unsafe food. The term „unwholesomeness” was introduced as official terminology in public life and legislation. Four centuries later, in 1646, the American colonists passed a similar regulation on the origin of bread, and a century and a half later (in 1785) they passed *the Massachusetts Act against Selling Unwholesome Provisions*, which can be considered America's first food safety law. The aforementioned law aimed to prevent the sale of adulterated food in Canada and the USA, primarily tea, coffee, milk, etc., given that the turnover of these goods at that time was extremely high (up to 50% of the total amount) (Mahmoud, 2020).

Since then, and up to the present day, many key milestones have occurred in the field of food safety. The most important can be considered: the formation of the US Department of Agriculture (USDA) in 1862; the establishment of the Food and Drug Administration (FDA) in 1906, which in the same year adopted the first American laws dealing with food safety in public supply procedures – *the Pure Food and Drug Act* and *the Federal Meat Inspection Act*. The first law prescribed norms that prevented the production, transportation and sale of adulterated food and drugs, while the second (the Federal Meat Inspection Act) regulated the trade of meat and meat products, providing that animals must be slaughtered, as well as that meat processing must take place in sanitary conditions (Johnson, 2016). The 19th and 20th centuries are marked as a period with a high prevalence of food-related diseases (e.g. botulism, tuberculosis, typhoid fever, etc.). At the same time, the mentioned diseases had the highest incidence and prevalence, as well as the mortality rate in the whole world. That is why laws on food safety, as well as accompanying sanitary conditions during its production, have increasingly been the subject of national legislation. This trend was particularly pronounced after World War II, when many countries of the world developed food laws that remained, with certain changes, in effect until two decades ago (Johnson, 2016; Mahmoud, 2020).

Food control – today

In order to be able to respond to the complex challenges that appeared at the end of the 20th and the beginning of the 21st century, modern food safety control systems were directed to follow the latest scientific and professional achievements, to work on the basis of the concept of risk analysis and to be in line with international standards and the practices recommended by *Codex Alimentarius* (CXC 1-1969).

Namely, after the outbreak of an epidemic caused by *Escherichia coli* O157:H7 (1993) in the USA, it was necessary to thoroughly and critically review the existing food safety system, as well as to find a faster, more efficient and safer system than the previous one. Parallel to this incident in Europe, in the United Kingdom, an outbreak of bovine spongiform encephalopathy (BSE) was recorded, which had great economic consequences in the world, due to the ban on the import and export of beef. At the same time, this epidemic has significantly undermined public confidence in food safety systems in Western Europe. In such an environment, there was an increased interest in the implementation of the system of the *Hazard analysis critical control points* (HACCP) worldwide. Although the basic principles of this system were developed several decades earlier, intensive work has been done on its implementation in the field of food production since the nineties of the last century. The aforementioned concept, based on seven principles, in this area was harmonized with the recommendations of *Codex Alimentarius*.

The most significant downside of the former traditional control method, which anticipated testing of a representative final product at the end of the production process, was the fact that the results obtained could not provide a high level of warranty regarding the safety, stability and microbiological quality of the product. There was a high risk of the unwanted presence of pathogenic microorganisms, because it was impossible to claim with certainty that the untested part of the finished product does not contain pathogenic microorganisms, that is, that the entire production line of the given product is safe for consumption

Many parts of the world, including our country, have HACCP implemented in their food safety systems today, as a mandatory regulatory requirement. HACCP is regarded as a systemic approach to ensure safety and as a better method than end-product testing. This preventive system, which is managed by the food industry today, implies an objective assessment of all potential hazards (biological, chemical, physical), as well as the establishment of appropriate control along the food production chain, which aims to eliminate or reduce the risk of an unsafe product. In other words, at the base of the system lies a preventive approach, not inspection one (Herrera, 2004). In addition to risk assessment and adequate risk management, the system involves continuous record keeping, which proves that the established system is alive and that the stipulated requirements have been met. In modern conditions of food production, the importance of applying HACCP is immeasurable, given that unsafe food can lead to serious financial losses due to the withdrawal of products from the market, damage to the producer's reputation, but also to the occurrence of foodborne illness, which in some cases can have a fatal outcome.

To date, the EU has, in its 28 member countries, proactively adopted food laws that imply the mandatory application of HACCP. The same conditions apply to other countries (i.e. third countries) intra-trading with EU member states. Besides, EFSA has been established in Europe since 2002, which is responsible for risk assessment (EFSA, 2018).

Today's food safety policy of our country is fully based on the EU food safety policy, and aims to protect the domestic consumer, while, at the same time, creating conditions for the smooth functioning of the single food market. By implementing the

national Food Safety Act, as well as other accompanying legal acts, along with the implementation of the HACCP system, the entire food chain "from farm to table/fork" is covered in an integrated manner. The implementation of the aforementioned legal norms enables the achievement of prescribed food hygiene in all stages of production, guarantees the creation of a safe finished product, takes care of the health and well-being of animals, plant health and affects the prevention of environmental contamination. Also, established legislation obliges food producers to be ready at all times to provide adequate evidence of the proper and safe functioning of the system. The requirements of the standard are identical, both for food produced for the domestic market (e.g. meat) and for food intended for export from our country.

FOOD SAFETY: A HOLISTIC APPROACH

Regardless of the fact that foodborne diseases are different, that some of them are zoonotic and some are not, the most effective form of their prevention is the constant application of the integrated, preventive holistic approach of „*One Health*” (formerly One Medicine). This approach implies the implementation of systematic surveillance throughout food production chain, with the mandatory connection of human health with animal health and environmental health. Mandatory integration and exchange of information on animal and human health is the key to the effectiveness of any national, as well as global, health system (FAO–OIE–WHO, 2010). One Health's integrated approach arose from the need to manage food safety with a high level of certainty, as well as to properly understand all aspects relevant to the occurrence of risks and hazards to human, animal and environmental health. By collecting and analyzing all significant data on human diseases that can be food-related, the health consequences of the occurrence of potential foodborne diseases, as well as the accompanying economic losses in the economy, are reduced at the same time. The application of One Health in veterinary and public health represents a significant potential for preventing the occurrence of health incidents, but also an incentive for the development of "healthy" national economies (Boqvist et al., 2018).

Knowledge of potential sources of disease, ways of transmission of pathogens, degree of exposure to environmental influences, level of humans-to-animals contact, as well as the level of direct human-to-human contact, are important factors for defining disease control strategies. In addition, it is necessary to look at consumer behavior, the existence of trends in diet and the type of food consumed, the nature of current economic incentives, as well as trade and politics trends, etc (Zinsstag et al., 2011).

A deep understanding of all socio-economic factors in the food chain, starting with food producers, regardless of whether they are small farmers or large entities in the food business, to the consumers themselves, contributes to the adequate selection of appropriate health protection methods. Also, many experiences from the past, both positive and those that led to mistakes, should be thoroughly analyzed in order to be able to serve for the enhancement of future food safety systems. It is obvious that this approach requires a strong and integrated cross-sectoral cooperation of national economic and health institutions. In other words, only a coordinated, collaborative,

multidisciplinary and cross-sectoral approach to solving existing and unknown risks within the interaction of people - animals - ecosystem, may have sufficient strength and ability to respond to new and unknown challenges and threats to human health (Mackenzie et al., 2019).

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Прегледни рад

СИСТЕМИ КОНТРОЛЕ ХРАНЕ И ЗАШТИТА ЗДРАВЉА ЉУДИ

Сажетак

Обезбедити довољну количину безбедне хране са дефинисаним пожељним параметрима квалитета је императив сваког произвођача, али и неприкосновен захтев савременог потрошача. Глобализација тржишта хране, развој и увођење „нове хране” и нових производних процеса, све заступљенији централизовани начин припреме и дистрибуције хране, растућа потражња за минимално прерађеном храном, уз све већу употребу *ready to eat* хране (хране спремне за конзумирање), као и стварање све дужих и сложених ланаца хране, допринели су значајном повећању нивоа ризика на пољу њене безбедности. Питање кварења хране и њене безбедности неизоставан је део активности свих националних структура укључених у јавно здравство, које је последњих деценија добило нови интегрисани приступ, познат као „једно здравље”.

Кључне речи: *кварење хране, болести које се преносе храном, контрола хране, „Једно здравље“*

**SOIL AND WATER AS THE BASIS
FOR AGRICULTURAL PRODUCTION
OF HEALTHY SAFE FOOD**



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Original Scientific paper

**ANALYSIS OF THE PRESENCE OF HEAVY METALS
IN THE SOILS OF THE HILLY-MOUNTAINOUS AREAS
OF BALKAN PENINSULA WITH THE ASSESSMENT
OF ITS POTENTIAL FOR THE FRUIT GROWING:
CASE STUDY OF THE LJUBOVIDJA RIVER BASIN,
POLIMLJE, MONTENEGRO**

Abstract

The paper presents results of the analysis of the presence of heavy metals with an assessment of its potential for the further development of fruit growing in North Montenegro, and in this specific case at the territory of the River Basin Ljubovidja, which is the hilly-mountainous area

of the Balkan Peninsula. Sampling and further analysis showed that the dominant type of soil in this area is District Cambisol. In the second phase, we conducted further research on the presence of heavy metals in the area of Pavino Polje, in the municipality of Bijelo Polje, Montenegro. The results of the analysis finally showed that there is no presence of heavy metals in the samples we collected during the field visit. The studied area is recommended for the establishment of organic production. It is particularly suitable for the production of fruit growing, vegetables and fodder is also recommended.

Keywords: *Heavy metals, mountainous areas, fruit growing, organic production, Polimlje, Montenegro, Balkan Peninsula*

INTRODUCTION

At the end of the 20th century, significant changes occurred in the world: social, economic, and political (Zejak et al., 2022). Population explosion, industrialization, urbanization and exploitation of natural resources are creating problems globally (Nagpal et al., 2020). The problem of land degradation is more and more one of the most important issues that should be treated very seriously. Large quantities of different kinds of elements are discharged into the environment as contaminants each year by human activities. Heavy metal toxicity and its lack of biodegradability and long-lasting duration in the environment while entering the food chain are considered to be one the most serious potential pollutants of the ecosystem (Popović, 2002; Pakzad et al., 2016; Kastratovic et al., 2016). No or low degraded soil and well-protected lands are today a special asset of every society. Proper management of this resource is an obligation and is part of all wise decisions of those who deal with agrarian policies and sustainable management.

According to the official statistic (MONSTAT), Montenegro is with 0.84 ha per inhabitant of the total agricultural land. In relation to this criterion, the country is in the second position in the European continent after Ireland. On the other hand, this country is poor in fertile land, because many meadows have been turned into pastures, and pastures into forest land; and finally barren land counts for 23% of the territory. That is why Montenegrin agriculture is characterizing the so-called rural agriculture, i.e. production took place just for satisfying farmers' own needs (Fustic and Spalević, 2003; Fustic et al., 2005). Land degradation in Montenegro is caused by numerous natural and anthropogenic factors. The distinctly hilly and mountainous area of Montenegro (94% with a slope of over 5%), a very dynamic and scattered relief, a developed and dense network of watercourses, an abundance of precipitation (1000-5000 mm per year) and other climatic characteristics are factors of crucial importance for provoking of water and wind erosion. This is contributed by the high share of erodible and non-resistant soils substrates, the devastation of plant cover and bare land, which make up 23% of the total territory, the damages are enormous, multiple and incalculable. Another type of land degradation is conditioned by excess floods that threaten 26,000 ha in flat areas with varying intensity covering only 5% of Montenegro. Applying soil conservation measures, these potentially fertile lands should be brought to intensive agriculture production – culture for more intensive use in agriculture. Technological development

caused an increase in the use of natural resources by exploitation of mineral raw materials, industrialization and urbanisation – construction of infrastructure facilities and settlements, which "consumed" a considerable area, mostly the most fertile land. With this particular form of degradation through conversion – land use change, Montenegro has lost 800-1000 ha per year in the last 40-50 years (Fustic, 2005).

In Montenegro, the transition from conventional agriculture to organic production is increasing. This method of production is well accepted by those who are engaged in soil conservation. Unlike conventional agriculture, which, due to the application of pesticides and fertilizers, led to the loss of individual plant and animal species, organic farming is based on the principles of protection and preservation of plant and animal species and the environment. Organic production is based on the biological balance of the system land – plant – animal – human. According to the Codex Alimentarius definition, it is a "holistic" production system that promotes and strengthens the agro-ecosystem and health, including biodiversity, biological cycle, and soil. Organic production is one of the world's leading trends in agriculture that is continuing to grow. Organic production of agricultural and other products is based on the application of organic production methods at all stages of production, aims to reduce the use of chemicals, and excludes the use of GMOs and products consisting of or derived from GMOs, as well as the use of ionizing radiation. Organic farming aims to produce safe food, of high quality, in an environmentally sustainable way, maintaining the genetic diversity of agro and ecosystem, preserving the environment, maintaining and improving soil fertility, reducing all forms of pollution, and producing food of high nutritional value, improving health and making a profit (Popović et al., 2014; 2019; Zejak et al., 2022; Ikanović and Popović, 2020). The land we want for the future must be carefully treated and well protected today.

MATERIALS AND METHODS

Study area. Montenegro is a country with great variability, ranging from sand and rock coasts (some corresponding to a ria coast), karst plateaus, large (intra-montane) fields, high mountains holding a (peri) glacial imprint, canyons, and more; all of it comprised in only 13 812 km² and within an elevation range of 2535 m. It is characterized by a Mediterranean climate, with warm and dry summers and autumns, and relatively cold winters with heavy snowfall on the continent (Zejak et al., 2022). The average altitude is 1,086 m; the lowest point: is the Adriatic Sea 0 m; the highest point: is Prokletije (Zla Kolata, 2534 m a.s.l., Dobra Kolata, 2528 m); Durmitor Kuk (Bobotov Kuk, 2522 m). According to data provided in the papers of various authors (Zejak et al, 2022; Spalevic et al., 2019; 2020), the structure of land use in Montenegro is as follows: agricultural land: 38.2% (estimated in 2018); arable land: 12.9% (estimated in 2018); crops: 1.2% (estimated in 2018); pasture: 24.1% (estimated in 2018); forest: 40.4% (estimated in 2018); other: 21.4% (estimated 2018). Irrigated land 24 km² (2012). The Map of Montenegro with the studied region of River Basin Ljubovidja is presented in Figure 1. North Montenegro, where the studied region is located is mainly mountainous. In this neighbouring region, the highest peaks of Montenegro

are found, including Komovi (2487 m a.s.l.) and Zla Kolata (2535 m a.s.l.) in the Prokletije Mountains of the same Polimlje Region. Nevertheless, this area of Polimlje is quite densely populated and includes the towns of Plav, Andrijevica, Berane, and Bijelo Polje. The rivers in this region drain to the Black Sea, and some of them form deep canyons crossing limestone formations. Further downstream, they form broader valleys flowing through softer Palaeozoic material (Spalevic, 2011).

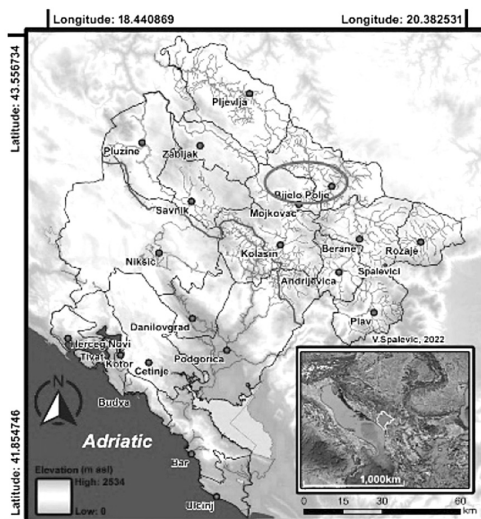


Figure 1. Map of MNE, Ljubovidja basin and Pavino Polje, (Zejak et al., 2022)

Table 1. Maximum allowed concentrations of dangerous and harmful substances in soils based on standards of Ex-Yugoslavia (mg/kg of air-dry soil)

Element	Official Gazette of the Republic of Serbia 23/1944	Rulebook Of Council Of Europe 2092/91	Rulebook on methods organic herbal of production, Official Gazette of FRY 51/2002
Cadmium	3	2.0	0.8
Lead	100	100.0	50.0
Mercury	2	1.0	0.8
Arsenic	25	-	10.0
Chrom	50	50.0	30.0
Nickel	300	-	-
Copper	100	50.0	50.0
Zinc	300	150.0	150.0
Boron	50	-	-
Molybdenum	-	-	10.0
Cobalt	-	-	30.0

Source: Kastori et.al., 2003.

Climate. Based data of more than 70 years (1948–2021) for temperature and precipitation, the study area is characterized by a mountain Mediterranean climate (Rajović, 2009) with rainy autumns and springs, cold winters, and a deficit of precipitation in summer months (Table 2.)

Table 2. Precipitation and temperature for the period 1948–2021 recorded at Bijelo Polje

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>Max daily precipitation in mm</i>												
Max	68.6	92.8	73	93.3	42.6	58.5	97.8	55.8	95.6	157.6	101.6	79.4
Aver.	23.4	22.8	21.5	24	21.9	20.6	21.7	21.1	25.3	29	29	23.5
St.D.	15.6	18.6	13	15	9.9	12.4	15.2	11.9	17.2	24.6	16.2	14.7
<i>Mean monthly temperatures in °C</i>												
Max	2.9	5.8	7.8	12.6	15.8	18.2	20.8	20.9	17.7	12.6	8.6	4.4
Min	-5.6	-5.2	-0.7	6.1	9.8	14.1	16.2	14.3	11.3	6.2	-1.6	-4.7
Aver.	-1.6	0.8	4.6	8.9	13.3	16.3	18.1	17.7	14.3	9.4	4.5	0.1
St.D.	2.2	2.7	2.1	1.3	1.3	1	1.1	1.4	1.5	1.4	2.1	2.2
<i>Max. daily temperatures in °C</i>												
Max	15.4	20.9	25.6	28.1	32.4	35.5	36.8	39.2	36	29.5	23	19.2
Aver.	11.7	14.5	20.1	23.6	27.6	30.4	32.8	32.8	29.4	24.8	18.6	13.6
St.D.	2.8	3	3.1	2.3	2.2	2.5	2	2.5	2.6	2.6	2.8	3.3
<i>Min. daily temperatures in °C</i>												
Min	-27.6	-24.5	-16.5	-7.5	-4	0	1.2	2.6	-4	-7.2	-15.4	-21.7
Aver.	-15.1	-13	-8.4	-2.8	0.9	4.8	6.5	6.1	2.3	-2.5	-7.3	-12.6
St.D.	5.3	4.7	4.1	1.8	2	1.8	2.1	1.5	2.5	2.3	3.7	4.6

Source: Data from the Hydrometeorological Institute of Montenegro

The absolute maximum air temperature ever recorded was 39.2 °C. Winters are severe, with negative temperatures as low as -27.6 °C. The average annual air temperature, t_0 , was 8.9 °C. The average annual precipitation, H year, was 873 mm. The temperature coefficient for the region, T , was calculated at 0.99. The torrential rain, bb , was calculated at 84.7 mm.

The geological structure and soils of the area. The study area at large consists of various types of sediment, magmatic and metamorphic rocks generated in the long, Palaeozoic to Quaternary, interval. Most of the terrain is underlain by Mesozoic formations of carbonate composition, while magmatic and silico-clastic rocks are substantially less present. Paleozoic geological formations consist of sedimentary and metamorphic, silico-clastic rocks found mostly in the north-eastern parts of Montenegro, while Cainozoic rocks of carbonate and clastic composition occur almost in all

regions of Montenegro. The research part related to geology and soil is based on previous geological and pedological studies (Fustic and Djuretic, 2000), who analyzed all geological formations and soils of Montenegro. Furthermore, we collected some soil samples for chemical and physical analysis. The grain size composition of the soil was determined by the pipette method. The soil samples were air-dried at 105°C sifted through 2 mm sieve and dispersed using sodium pyrophosphate. Total carbonates were determined by the volumetric Scheibler method; the soil reaction (pH in H₂O and nKCl) was determined with a potentiometer; the content of the total organic matter was determined by the Kozman method; easily accessible phosphorous and potassium were determined by the Al-method and the adsorptive complex (y₁, S, T, V) was determined by the Kappen method (Spalevic et al., 2011). Certain soil analyzes in Montenegro for the presence of heavy metals were carried out as part of the "program for testing harmful substances in the soil for the year 2007". The analyzes were done by the ministry of tourism and environmental protection. The program was implemented by the public institution "Center for ecotoxicological testing of Montenegro" in accordance with the rulebook on permitted amounts of hazardous and harmful substances in soil and methods for their testing (official gazette of the Republic of Montenegro 18/97). The investigation includes analyzing land in 15 settlements that are most exposed to pollution. The total content of heavy metals in the soil was determined after preparing the samples in a closed microwave system under high pressure Milestone Ethos 1 according to US EPA method 3051A (2007).

RESULTS AND DISCUSSION

Soil is the basic substrate for plant production, and that is why it is necessary to determine whether it is suitable for this purpose, from the point of view of the content of harmful substances and heavy metals, in order to produce health-safe food (Vukeljić et al., 2002). This is possible if the determined content of heavy metals is compared with the limit value for the respective element. The limit values for the maximum allowed concentration of heavy metals in the soil (Table 1) represent significant support in the assessment of soil pollution with these predominantly toxic elements (Kastori et al., 2003; Đukić, 2003). With the sudden scientific and technological development in all branches of the economy, the emission of heavy metals increased, which thus became significant pollutants (Vukeljić et al., 2002). Plants grown on contaminated soils and useful in nutrition endanger the life and health of people and animals (Triphati et al., 2001). The concentrations of Pb, Cd, Cu and Mn in sixteen imported NPK fertilizers, which are most often used in Serbia, were determined by the method of flame atomic absorption spectrometry. The obtained results show that the content of heavy metals in samples of different fertilizers varies significantly, which depends on the ratio of N:P:K in the examined samples of fertilizers, as well as on the origin of the fertilizers themselves. The highest content of Cu was found in coloured, mixed NPK fertilizers originating from Hungary, Greece and the Netherlands, and exceeds the value of the maximum permissible concentration of Cu that can be found in the soil. The Mn content in the Hungarian NPK fertilizer (10:10:20) is ten times higher than the

average Mn content in the soil. These data indicate that it is necessary to permanently control the content of heavy metals in imported fertilizers, in order to reduce soil, underground and surface water pollution (Milinović et al., 2008). The maximum permitted amount of hazardous and harmful substances in soil (Tab. 3), in Montenegro, can lead to its pollution, and which is caused by the improper use of mineral fertilizers and plant protection products by legal and natural persons, as well as by the discharge of waste matter from various sources, are specified in the Rulebook on permitted amounts of hazardous and harmful substances in soil and methods for their testing ("Official Gazette of Montenegro", 18/97).

Table 3. Maximum allowed quantities (MDK) of dangerous and harmful substances, according to the current Ordinance

No.	Element	Chemical label	MDK in the soil in MNE, mg/kg	MDK in the soil in Serbia, mg/kg dry soil	Average value in the soil, mg/kg
1.	Cadmium	Cd	2.0	3.0	< 0.50
2.	Lead	Pb	50.0	100.0	17.37
3.	Mercury	Hg	1.5	2.0	0.0527
4.	Arsenic	As	20.0	25.0	< 5.0
5.	Nickel	Ni	50.0	50.0	17.75
6.	Fluorine	F	300.0	300.0	144.0
7.	Copper	Cu	100.0	100.0	22.5
8.	Zinc	Zn	300.0	300.0	95.0
9.	Boron	B	5.0	50	3.0
10.	Cobalt	Co	50.0	30.0	14.25
11.	Molybdenum	Mo	10.0	10.0	< 5.0
12.	Potassium	K	–	–	207.65
13.	Phosphorus	P	–	–	492.5
14.	Nitrogen	N	–	–	0.15

The research also covers the area of Bijelo Polje. The content of hazardous and harmful substances in the soil in the Municipality of Bijelo Polje was analyzed at four locations. The results of the analysis of the samples indicate the presence of lead (Pb) in concentrations above the MDK at the location of City Landfill 1. At the aforementioned location of City Landfill 1, an increased concentration of cadmium (Cd), chromium (Cr), copper (Cu), and zinc was also determined (Zn). The concentration of fluorine (F) at the Zaton 1 location is above the MDK. At the locations of City Landfill 1 and Zaton 1, there is an evident increase in lead concentration compared to the previous year, while at the other two locations, a decrease in lead concentration can be observed compared to the previous year. The content of polyaromatic hydrocarbons

exceeds MDK at the location of City Landfill 1. Except for the location Zaton 1, at all other locations, there is an increase in the concentration of polyaromatic hydrocarbons compared to the previous year.

In the final report of the Ministry, it was concluded that the condition of the soil in relation to the content of dangerous and harmful substances can be characterized as satisfactory. On the basis of the monitoring carried out, at the targeted locations, it was found that in nine municipalities, it was polluted as a result of inadequate disposal of municipal waste. In the municipality of Nikšić, inadequate disposal of industrial waste causes an increased content of dangerous and harmful substances. In most municipalities, there is a negative impact of traffic, i.e. exhaust emissions, which is a significant source of pollution of the surrounding land, at the same time endangering air quality. In relation to the study area of in the Polimlje, Montenegro region, a soil samples were analyzed, from a plot of pasture/meadow culture (Figure 1) in the Pavino Polje, municipality of Bijelo Polje, at a location of the altitude of 800 m.a.s.l., with a sampling depth of 0.3 and 0.5 m of the Ah portion of the profile. Then an analysis was carried out for the presence of heavy metals by the national and authorized laboratory at the Public institution Center for Ecotoxicological Testing – CETI in Podgorica (Montenegro). This analysis by using the method AOAC (Official, method 990.08 – Metals in solid wastes, AOAC Official methods of analysis (1995), Chapter 9, p. 31). The K content was determined on an AA-6701F Atomic Absorption Spectrophotometer, and the P content was determined on the Sequential Plasma Spectrometer ICPS-7500. Both instruments are products of Shimadzu. The results of the tests in CETI-Podgorica are given in Tab.3. The majority of microelements belong to the group of heavy metals, which are characteristically toxic to plants, animals and humans in larger quantities quote from Brankov et al. (2006).

Maximum permitted amounts (MDK) of dangerous and harmful substances, according to the current Ordinance (Official Gazette of the Montenegro, No. 18/97), it was concluded that the concentration of cadmium (Cd) is below the permitted concentration, more precisely, that it is only 0.05 mg/kg, while 2mg/kg is otherwise permitted. The concentration of 2mg/kg in the sample indicates that the presence of lead (Pb) in the controlled soil is about 30% lower than the permitted level, mercury (Hg) is only 0.005, which is a trace presence in the sample compared to the permitted 1.5mg/kg. Arsenic (As) is recorded with around 25%, and Chromium (Cr) the same with around 25%. Nickel (Ni) 17.75 mg/kg of the permitted 50 mg/kg. Fluorine (F) is recorded with about 50% of the permitted concentration, Copper (Cu) is present with 22.5mg/kg, while its presence is permitted in the amount of 100 mg/kg. Zinc (Zn) was found in the sample in a concentration of about 30% of the allowed: Boron (Bo) was recorded with 3 mg/kg, which is 2 mg/kg less than the allowed 5 mg/kg in the soil sample. Cobalt (Co) was found in the amount of 14.25 mg/kg, and the permitted concentration is 50 mg/kg. Molybdenum (Mo) is above 5mg/kg, while the permissible concentration of molybdenum is 10mg/kg of the sampled soil. The moisture content of the sampled soil during testing was 9.35%. The nitrogen content is 0.15%, phosphorus 492.5 mg/kg, and potassium 207.65 mg/kg. Based on the results (Tab. 3.) of laboratory tests in the report of Public institution CETI – Podgorica, it was determined that the tested soil samples

corresponds to the conditions of the Rulebook on permitted concentrations of hazardous and harmful substances in soil and methods for their testing (Official Gazette of Montenegro, No 18/97).

CONCLUSION

- At the studied area of Pavino Polje, analyzes of soil samples did not determine the presence of heavy metals. As a consequence it is possible to organize organic production in this region, but it is necessary to take care of the locality itself in such a way that possible existing or potential sources of its pollution would be avoided.
- The relief, climate and soils of the North Montenegro, their existing condition and soil use would be arranged for organic agriculture, so that this area can be used for the possible production of: (1) planting of continental fruits such as raspberries, blackberries, blueberries, apples, pears and plums; (2) field crops (buckwheat, millet, potato, barley, oats, rye), and the production of (3) certain types of bulk fodder (hay, silage) for domestic animals raised in the organic farming system.
- The soil, climate and relief, with their current state and existing basic features, favour the further development of organic agricultural production, especially in the studied area and region of northern Montenegro.

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Оригинални научни рад

**АНАЛИЗА ПРИСУСТВА ТЕШКИХ МЕТАЛА У ЗЕМЉИШТИМА
БРДСКО-ПЛАНИНСКОГ ПОДРУЧЈА БАЛКАНСКОГ ПОЛУОСТРВА
СА ПРОЦЈЕНОМ ЊЕГОВОГ ПОТЕНЦИЈАЛА ЗА УЗГОЈ ВОЋАРСКИХ
КУЛТУРА: СТУДИЈА СЛУЧАЈА СЛИВА РИЈЕКЕ ЉУБОВИЋЕ,
ПОЛИМЉЕ, ЦРНА ГОРА**

Извод

У раду је приказана анализа присуства тешких метала са процјеном његовог потенцијала за узгој воћарских култура у земљиштима сјевера Црне Горе, слива ријеке Љубовиће, која се налази у брдско-планинском подручју Балканског полуострва. Узимање узорака је показало да је доминантни тип земљишта овог подручја дистрични камбисол, што се подударило са претходним истраживањима Фустиха и Ђуретића (претходног вијека), Спалевића и Зејака (овога вијека). У другој фази, свели смо даље истраживања на присуство тешких метала на подручје Павиног Поља, које је по својим физичко-географским карактеристикама репрезент овог подручја из општине Бијело Поље. Полазна хипотеза је била да је ово подручје потенцијално погодно за даљи развој воћарства, а ова истраживања и предлози су ишли ка томе да се овдје даље иде ка успостављању органске производње. Резултати анализа су на крају показали да у узорцима отворених профила, а

који су прикупљени током рада на терену, не постоји присуство тешких метала. Предметни локалитет је препоручљив од стране струке и науке за даљи рад на заснивању органске производње. Посебно је погодан за производњу воћа, као примарне гране, а препоручљиво је и гајење поврћа и крмног биља.

Кључне речи: *Тешки метали, планинска подручја, воћарство, органска производња, Полимље, Црна Гора, Балканско полуострво.*



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Original Scientific paper

AUTOMATED ECOMONITORING OF WATER IN THE FUNCTION OF SOIL AND WATER PROTECTION

Abstract

With the construction of large industrial and similar facilities, the threat of water and soil pollution is increasing. It is often considered that improperly disposed waste or discharged dirty water will not significantly harm nature. Incidents rarely occur in large plants, but they do happen from time to time. All of this points to the need for permanent monitoring of the influence of people, but also of natural phenomena, on the quality of soil and water. Monitoring of water quality also detects contamination of the soil through which the water passes. Since no one can predict if and when some emergency situations will occur, such monitoring must be carried out continuously, and the measured data must be sent to the cloud in real time, so that they are available to the persons in charge. Healthy-and safe water is a guarantee of environmental protection and the production of healthy and safe food.

Key words: *water pollution, soil pollution, remote measurement*

INTRODUCTION

Human activity implies large industrial plants, a large amount of people in one place, which results in large amounts of solid and liquid waste. Modern societies have organized collection and disposal of solid waste, and controlled collection and treatment of wastewater. Despite this, we are witnessing the uncontrolled occurrence of various types of water and soil pollution at certain time intervals. Most often, such things happen by accident, carelessness, negligence or caused by a technical malfunction, but unfortunately, there are also cases when it is done knowingly.

The goal of this study was to create monitoring of water and soil and assess the current situation, based on which timely and proper reactions would be made in the necessary situations in order to ensure health-safe water and create conditions for the production of healthy and safe food.

WHY HEALTHY SAFE WATER IS ESSENTIAL FOR THE PRODUCTION OF HEALTHY FOOD

Water is directly and indirectly connected to the soil where food is grown. Plants draw water directly for their growth and development, and incorporate this water directly into the fruits.

In nature, underground water is found under the surface layer of the soil, at a greater or lesser depth. Groundwater is usually "connected" with surface water, and surface water has a great influence on the level and quality of ground water.

Also, in agriculture, irrigation is carried out by pumping underground water from wells or by pumping water from rivers, lakes, ponds or similar water sources. In this case, we serve water directly to the plants on the leaves and roots, without being sure of the quality of the water.

THE MOST IMPORTANT PARAMETERS FOR GROUNDWATER MEASUREMENT

By measuring the quality of groundwater, we indirectly measure the quality of the soil. Groundwater washes certain substances from the ground, and by measuring the water, we also measure the quality of the soil.

The most interesting parameters to monitor in groundwater are water level, water temperature, pH value, electrical conductivity, presence of carbohydrates and others.

The level of underground water is a parameter that largely gives us information about soil moisture, the need for irrigation and drainage, or systems for regulating underground water. Also, if we have water intake for irrigation from a well, it tells us about the current capacity of the well.

The temperature of the underground water must be stable because of the maintenance of microorganisms in the soil. It is a common phenomenon that cooling and heating systems, like heat pumps use underground water in the technological process. Unaware of the fact that such processes negatively affect the temperature of the underground water, the devices heat up the underground water in the summer, while cooling it in the winter. Such procedures affect the temperature of the soil even more.

The acidity and alkalinity of groundwater is a good indicator of the quality of the soil, its changes during the year or over a period of several years. The agronomic profession also takes care of the pH value of the soil. Soil quality testing is done periodically and automated water monitoring provides this information instantly in real time. It is visible how the rainy season affects the pH value and how the application of certain agrotechnical means affects the pH value. With automated monitoring, it is possible to accurately monitor the influence of external factors on the quality of the soil.

Electrical conductivity is data that gives us a picture of the general composition of water. This data indirectly shows the proportion of dissolved minerals, salts and heavy metals in the water. Electrical conductivity is expressed in μS .

Sensors for the detection of hydrocarbons in underground water can timely detect oil derivatives in small quantities. Oil is naturally found in the environment, but it

often happens that due to constructions in nature, oil is uncontrolled in underground water and soil. Uncontrolled occurrence of oil in water and soil occurs most often due to spillage from various tanks (hand tanks, transport and work machine tanks...), but also from oil pipelines or oil plants.

In addition to measuring underground water, it is also necessary to measure the quality of surface water, both stagnant and flowing..

In flowing water, the most common problems that occur are the lack of dissolved oxygen, E.coli and salmonella bacteria, the presence of hydrocarbons in the water, the presence of heavy metals and more.

The probe for measuring the amount of dissolved oxygen directly measures the oxygen concentration expressed in mg/l. The lack of oxygen in the water causes the death of plant and animal life in the water. The most common cause of lack of oxygen in water is the direct discharge of fecal water or slurry into streams, canals and rivers. The reduced concentration of oxygen in the water is in direct correlation with the temperature of the water.

The measurement of the redox potential of water, whether ORP indicates the oxidation potential of water or the reduction potential of water. This indicator can indicate the appearance of E.coli and salmonella bacteria. It is expressed in mV. This parameter is also correlated with water temperature.

Hydrocarbons and the appearance of minerals and salts in flowing water is a problem similar to problems in other waters, underground and stagnant waters..

In addition to the chemical analysis of water, it is important to measure and monitor water level and water temperature movements. It has already been stated before that the amount of dissolved oxygen and the redox potential have a great dependence on the water temperature. Temperature is often elevated at low water levels, i.e. at low flows.



Picture 1. Stagnant water (<https://www.v-elin.hr/>)

Places where negative processes in terms of biological and chemical changes in water most often occur are stagnant waters, lakes, ponds, retention ponds and the like. In stagnant waters, negative processes in terms of water quality are often the product of natural processes, plant and animal life. These waters are referred to as "stagnant

waters", waters with a reduced amount of oxygen, with an excessive amount of vegetation and algae, with a large amount of sludge that is the product of rotting plant species and animal excrement. Such "stale waters" sometimes turn into "pickled waters". These are waters with completely negative parameters in terms of the amount of dissolved oxygen, redox potential and pH value. Precisely because of these negative natural phenomena, but also because of waters where people and human activities have a negative impact. It is necessary to regularly monitor the quality of such water. These waters, although stagnant, affect the quality of the surrounding soil, and slowly affect the quality of underground water as well. Special attention should be paid to the quality of standing water if such water is used in irrigation systems.

It is known that large installations, traffic, thermal energy plants, landfills, composting plants, water purifiers, biogas installations and other plants emit certain substances into the air. All this has an impact on people's quality of life (Vrbanec, 2021)

AUTOMATED DATA COLLECTION

To measure water parameters, a probe, data collection equipment and equipment for powering the measuring and communication equipment are required.

Stations for the automatic measurement of individual measuring quantities are equipped with channels for connecting sensors and communication equipment.

A significant part of this measurement system is the VEMOGS remote stations. These stations are designed specifically for collecting measurement data and sending all data in real time to the Internet and the cloud. Data is transmitted via GSM/GPRS public communication channels, which is a fairly cheap way of communication. Also, VEMOGS remote stations are adapted for power supply from batteries supported by photovoltaic modules. Such a construction of remote stations ensures long-term functioning of the system without the need to replace batteries, while functioning in all seasons.

Data on the Internet, in the cloud, are adapted for easy access and review. All authorized persons receive their own access code and can freely access the data. The data can be viewed in real time, the archive can be viewed, and if necessary, the measurement data can be exported in excel format.

CONCLUSION

Monitoring and protection of water is an important link in preserving the soil and the environment.

The measurement system enables timely, real-time information on changes in water parameters to be presented and documented. Such information makes it possible to prevent further pollution of water and soil, but also opens up the possibility that, often with very simple activities, water treatment improves water quality. Some of the simple methods for increasing the amount of dissolved oxygen are water aeration. In the event of bad pH indicators, it is possible to intervene for a short time with the addition of

various neutralizers. In general, with elevated "bad parameters" of water, if possible, by increasing the water flow, or by adding certain amounts of clean, fresh water, we dilute the polluted water. Of course, there are other, longer-term and somewhat more expensive methods of treating water and the area around water in order to protect water.

So, with small operations and human activity, it is possible to preserve water that is healthy and clean. Safe water is a guarantee of soil preservation, and such soil is necessary for growing healthy, safe and tasty food.

Caring for water begins with monitoring water quality and the changes that occur in the water. Safe water is a guarantee of environmental protection and the production of healthy and safe food.

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Оригинални научни рад

АУТОМАТИЗИРАНИ ЕКОМОНИТОРИНГ ВОДЕ У ФУНКЦИЈИ ЗАШТИТЕ ЗЕМЉИШТА И ВОДА

Сажетак

Изградњом великих индустријских и сличних објеката пријетња за загађење вода и земљишта све је већа. Често се сматра да непрописно одложени отпад или испуштена прљава вода неће битно наштетити природи. У великим погонима ријетко долази до инцидентних догађаја, али они се с времена на вријеме ипак догађају. Све ово упућује на потребу трајног мониторинга утјецаја људи, али и природних појава на квалитету земље и воде. Мониторингом квалитете воде у правилу се детектира и загађеност тла кроз које вода пролази. Како нитко не може предвидјети хоће ли и када доћи до појаве неких ванредних ситуација, такав мониторинг потребно је проводити континуирано, а измјерене податке у реалном времену слати у „облак“, како би били доступни особама задуженим за праћење. Здравствено безбедна вода гаранција је заштите животне средине и производње здравствено безбедне хране.

Кључне речи: *загађење вода, загађење земље, даљинско мерење*



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Original Scientific paper

THE STRUCTURE OF VERTISOL SOIL IN THE AREA OF THE MUNICIPALITY OF SMEDEREVO

Abstract

The objective of this study was to investigate the structure of Vertisol, the aggregate distribution, and the stability of structural aggregates. The investigation was conducted in the municipality of Smederevo in eastern Serbia. At ten locations, disturbed samples, as well as soil samples for structure analysis were taken. The distribution of aggregates shows the high amount of macro aggregates, low content of aggregates 2-3 mm size, and according to the structural coefficient satisfactory structure. The stability of macroaggregates to dispersion by water in the arable layer of Vertisol is excellent. The results of this research could be used in cultivation practices planning since there is a possibility of improving the structure of Vertisol.

Key words: *Vertisol, soil structure, dry and wet sieving*

INTRODUCTION

The mechanical elements of the soil are connected into structural aggregates of different sizes, shapes, stability, and porosity. Soil structure is the result of the complex interaction between soil's physical, chemical, and biological properties. Of all types of structure, the most favorable is a granular structure with structural aggregates, larger and smaller, that do not have a certain geometric shape, but their rough surface prevents the aggregates from sticking together in a compact mass and such a structure maintains the loose state of the soil. If the topsoil is granular, the water enters easily and seed germination is better (Vučić, 1987). In a prismatic structure, the movement of water in the soil is predominantly vertical and water circulates with difficulty (Brouwer et al., 1985). Soil structure is not permanent and can be improved utilizing cultivation practices.

Vertisol belongs to a group of heavy-textured soils with a high clay content of the montmorillonite type. Vertisol is characterized by phenomena associated with swelling and shrinkage; storage of moisture causes swelling and loss of water causes shrinking. Water-air properties are poor due to the high proportion of capillary and subcapillary pores. The major property contributing to the importance of Vertisols is their high water-holding capacity, but because of the high wilting point, the available water is limited. Because of their low permeability, inadequate irrigation of these soils may result in waterlogging and a buildup of salinity unless adequate artificial drainage is provided. Moderate wetting of these soils provides the best conditions for utilizing the great production potential of Vertisol.

The structure of Vertisol is related to the water regime because of the pressure which results from the swelling and shrinkage processes. Prismatic structural aggregates are a typical feature of the Vertisol structure. Due to the phenomena of self-mulching, a thin loose layer with a granular structure can be created on the surface (Ćirić, 1984)

Therefore, the objective of this study was to investigate the structure of Vertisol, the aggregate distribution, and stability of structural aggregates, and based on the obtained results, to give an agronomic assessment of this soil.

MATERIAL AND METHOD

The surveyed area covers the undulating lowland area of the southern end of the Pannonian basin. It extends near the Danube River. The relief of this area is meso-relief. The altitude of the investigated area ranged from 130 to 259 meters above sea level. The climate belongs to the moderate continental type influenced by the proximity of the Danube River. In the investigated area, the largest part of the land is used for agricultural purposes, and these are mainly orchards of smaller areas.

To determine external and internal soil morphology, at ten locations pedological profiles, pits were opened. From each location, from the arable layer, disturbed samples were taken to analyze the mechanical composition. Also, soil samples were taken to analyze the soil structure according to Bošnjak et al. (2012).

Laboratory analysis

Soil laboratory tests were performed in the Laboratory for Pedology and Soil Water Regime in the Faculty of Agriculture in Novi Sad. The physical and water-physical properties of the soil were analyzed on the samples taken.

The following physical properties of the soil were examined:

- Mechanical composition - determined by the pipette method, and preparation of samples for analysis with Na-pyrophosphate according to Thun,
- Textural class - determined based on Tommerup's classification.

The structure of the soil was examined, that is, the analysis of the aggregate distribution (dry sieving) and the stability of structural aggregates in water (wet sieving).

Dry sieving was determined by the standard procedure according to the Savinov method (Bošnjak et al., 2012). Wet sieving of the soil was performed to determine the stability of the structural aggregates according to the adapted method of Elliott (Elliott, 1986).

The Structure coefficient and Mean weight diameter are used to evaluate the soil structure. The structural coefficient (K_s) is calculated based on the formula (Шейн & Гончаров, 2006):

$$K_s = \frac{a}{b}$$

where a is the content of macro aggregates from 0.25 mm to 10 mm, and b is the content of aggregates smaller than 0.25 and larger than 10 mm.

Mean weight diameter (MWD) was calculated based on the formula (Hillel, 2003):

$$MWD = \sum_{i=1}^n x_i w_i$$

where x_i is the mean diameter of the class of stable aggregates (μm), and w_i is the weight percentage of the class of stable aggregates to the mass of the total sample.

Statistical analysis

The research results were processed statistically by the analysis of variance (ANOVA) method using the TIBCO Statistica 14.0.0.15 software program (TIBCO, 2020). "One-way ANOVA" was used to compare the results of the structural analysis between aggregate fractions. The significance of differences between treatment means was determined by Duncan's test for a significance threshold of 5%.

RESULTS AND DISCUSSION

Mechanical composition

The classification of the International Society of Soil Science is based on the principles of particle division according to Atterberg and is accepted as the basic classification of the mechanical composition of the soil (Belić et al., 2014).

At all locations, soils are classified as loamy clay (data not shown). According to FAO-WRB classification (IUSS Working Group WRB, 2015), the soil belongs to Haplic Vertisol. The surface layer of Vertisol contains from 27 to 44 % of clay. Vertisols may have 30 to 80 percent clay content. The lower limit of swell/shrink activity is approximately less than 35 %. Increasing the content of sand reduces the swelling capacity of the soil (McGarry, 1996).

Soil structural analysis

The soil structure is an indicator of soil fertility. Soils with good soil structure have favorable aeration, water mobility and capacity, heat regime, and root development potential. Poor soil structure increases vulnerability to drought and erosion and can limit the availability of some nutrients (Shepherd, 2000).

Dry sieving

The distribution of soil aggregates, as they exist in the field, is similar to the distribution of fragments only when the energy input is low enough to avoid significant changes in the size of the aggregates (Díaz-Zorita et al., 2002).

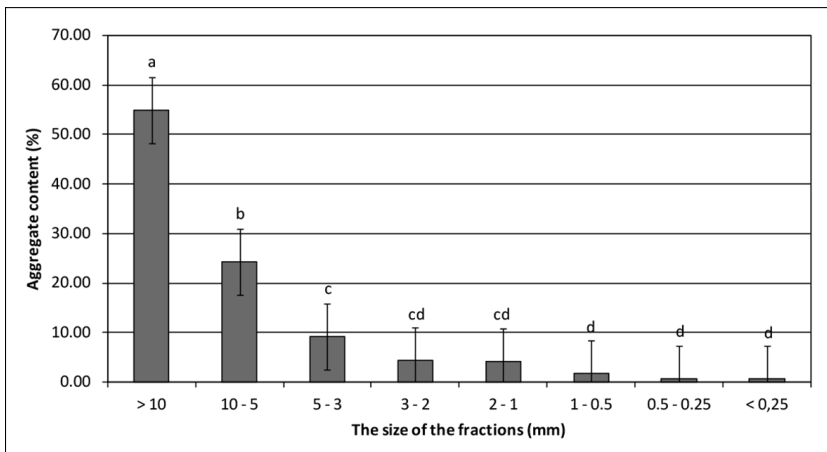


Figure 1. Distribution of structural aggregates of the arable layer of the Vertisol. Vertical lines indicate a standard error and different letters indicate significant differences between aggregate fractions.

The results of dry sieving (Figure 1) show that the arable layer of Vertisol has the highest content of macro aggregates >10 mm in size (54.96 %), and the lowest amount of aggregates smaller than 0.25 mm (4.85%). The total content of aggregates between 0.25 and 10 mm is 44.44 %. Even though from an agronomic point of view the structure cannot be evaluated only by considering the ratio of individual fractions of structural aggregates, the fact remains that aggregates of 0.25-10 mm are the basis for the formation of favorable water-air properties of the soil (Vučić, 1964).

Statistically, significantly higher content of the largest fraction, >10 mm, as well as the aggregate fraction 10-5 mm was determined in relation to the content of the other fractions. Differences in aggregate content of fractions in the range of 1 to 5 mm

were not determined, as well as statistically significant differences between fractions smaller than 1 mm. The higher content of smaller aggregates especially aggregates <0.25 mm, indicates a greater dispersion and a greater susceptibility to wind and water erosion.

The investigated Vertisol is characterized by low content of aggregates 2-3 mm in size, about 4,3 % on average, and a high amount of macro aggregates. Vučić (1987) points out that the smallest evaporation is from the soil that has structural aggregates of 2-3 mm size and the highest when the aggregates are 10-15 mm. Also, the rate of water permeability decreases with an increase in the content of dispersed aggregates.

Wet sieving

The results of wet sieving of the soil samples (Figure 2) show that the lowest content of aggregates was in the < 53 μm fraction (12.59 %), and the highest amount of aggregates after wet sieving of the 0-30 cm layer was in the 2000-250 μm fraction (46.41 %). Based on Kačinski's classification, the stability of macroaggregates to dispersion by water in the arable layer of Vertisol is excellent.

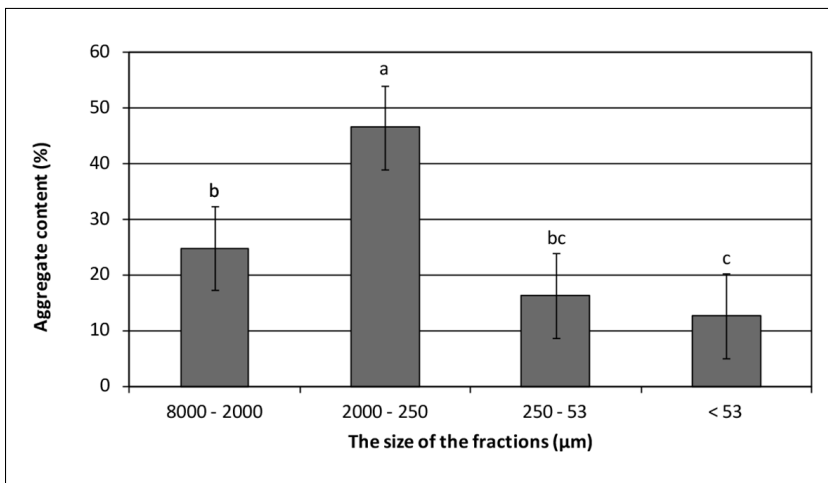


Figure 2. Stability of structural aggregates of the arable layer of the Vertisol. Vertical lines indicate a standard error and different letters indicate significant differences between aggregate fractions.

Statistical analysis revealed significant differences in the content of aggregates between fractions. The analysis indicates significantly higher content of aggregates 2000-250 μm , stable fractions, and significantly lower content of smaller, more unstable fractions of aggregates.

The soil infiltration and filtration, irrigation erosion, the degree of soil compaction, soil crusting, and porosity, that is, the overall fertility of the soil depends on its structure. Soil, especially in irrigation conditions, is exposed to a greater degree of water impact, which can cause unwanted consequences to a certain degree (Bošnjak, 1999). In the conditions of irrigation, land degradation is a consequence of improper exploitation of the irrigation system and the application of poor-quality water.

Structural coefficient and mean weight diameter

If the structure coefficient is greater than 1.5, the soil has a good structure, if it is between 1.5 and 0.67, it is satisfactory, and if it is less than 0.67, the soil has an unsatisfactory structure (Gajic et al., 2014).

The value of the structural coefficient of Vertisol is about 1.00 (Figure 3), that is the obtained results indicate a satisfactory structure of the investigated soils.

Mean weight diameter (MWD) indicates the size distribution of the aggregates and is a measure of the stability of the macroaggregates to water disturbance since the aggregates remaining on each sieve must be stable to the wetting process. The value of the mean weight diameter of the structural aggregates is on average 1.8 for investigated Vertisol soils (Figure 3).

Statistically, the use of any single parameter such as the MWD to characterize a distribution of aggregates is incomplete. Also, the soil with more clay particles gives more undecomposed aggregates, which is not always a sign of a favorable agronomic evaluation of the structure, i.e. a higher coefficient does not necessarily mean a better structure. The importance of assessing the stability of structural aggregates is in monitoring the dynamics and trends in structural changes that are a consequence of land use (Díaz-Zorita et al., 2002; Vučić, 1987).

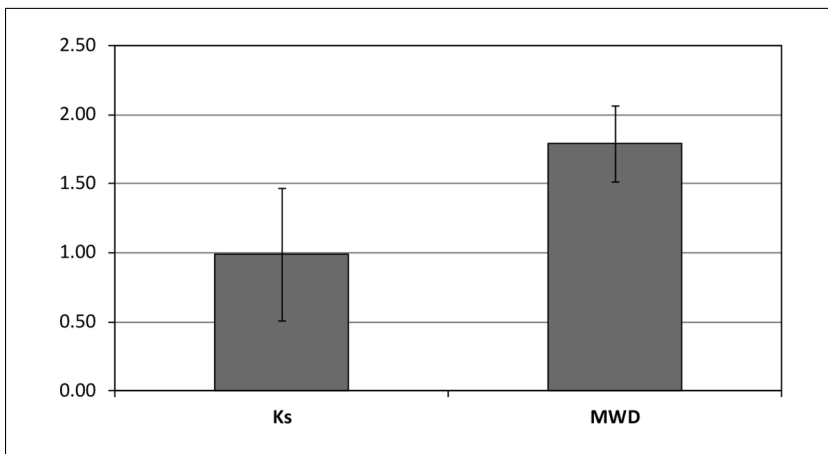


Figure 3. Structure coefficient (K_s) and mean weight diameter (MWD) values. Vertical lines indicate a standard error.

CONCLUSION

The following conclusions can be drawn based on laboratory research of Vertisol soils:

Based on the presence of fractions of mechanical particles, it can be concluded that the examined surface layer of the soil belongs to loamy clay. The surface layer of Vertisol contains from 27 to 44 % of clay.

Based on the results of dry sieving, it can be concluded that the surface layer has the highest content of macro aggregates >10 mm, and the smallest content of aggregates <0.25 mm, responsible for greater dispersion and greater susceptibility to wind and water erosion.

The distribution of aggregates obtained by wet sieving indicates excellent stability of macroaggregates to dispersion by water in the arable layer of Vertisol. Analysis of wet sieving revealed the highest content of aggregates 2000-250 μm , stable fractions, and significantly lower content of unstable fractions of aggregates.

The structural coefficient indicates a satisfactory structure of the tested soils.

The obtained results indicate the possibility of improving the structure of Vertisol and can be used in planning agricultural production.

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Оригинални научни рад

СТРУКТУРА ЗЕМЉИШТА ТИПА СМОНИЦЕ НА ПОДРУЧЈУ ОПШТИНЕ СМЕДЕРЕВО

Резиме

Циљ рада је био да се испита структура смонице, дистрибуција агрегата и стабилност структурних агрегата према расплињавању у води. Истраживање је обављено у општини Смедерево у источној Србији. На десет локација узети су узорци у поремећеном стању и узорци земљишта за анализу структуре. Дистрибуција агрегата показује већу заступљеност макроагрегата, низак садржај агрегата величине 2-3 мм, а према коефицијенту структурности задовољавајућу структуру. Стабилност макроагрегата у обрадивом слоју смонице је одлична. Резултати овог истраживања могу да се користе у планирању пољопривредне производње јер постоји могућност побољшања структуре смонице.

Кључне речи: *смоница, структура земљишта, суво и мокро просејавање*

**GENETICS, GENETIC RESOURCES,
BREEDING AND GENETIC ENGINEERING
IN THE FUNCTION OF HEALTHY
SAFE FOOD PRODUCTION**



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Original Scientific paper

GREEK STRAWBERRY TREE (*Arbutus andrachne* L.) GENOTYPES PROPAGATION

Abstract

In the period 2014-2015 possibility of generative and vegetative propagation of the rare fruit tree Greek Strawberry tree (*Arbutus andrachne* L.) genotypes, located in natural stands, on the middle course, from the left side of Konjska River, in South part of North Macedonia are investigated. The Type 2 is characterized with the largest seeds (in average, seed area 4.31 mm², perimeter 8.72 mm and 1.81 mm width). The results show that the seed from the Greek Strawberry tree genotypes (*A. andrachne* L.) has low germination percent and very low survival rate of the seedlings. The exception from this rule is the Type 2 with about 78 % survived exemplars after four months observations. The highest germination percent has the Type 4 (25 %). Rooting of cuttings gives the higher percent of live plants. Rooting in sand in average gives better results than the rooting in vermiculite. The Type 2 is characterized with the highest ability of rooting cuttings in sand (49.3 %).

Key words: *Genotype, seed, germination, cutting, seedling*

INTRODUCTION

Member of *Ericaceae* family, Greek Strawberry tree (*Arbutus andrachne* L.) is an evergreen, bushy shrub or a small tree up to 12 m, which grows along the Mediterranean coast spontaneously, separately or in association with the related species *Arbutus unedo* L. (Strawberry tree). It is widely distributed from the East Mediterranean to Northern Black Sea area (Bertsouklis and Papafotiou, 2009). The phylogeographic investigations show that during the Last glacial maximum (21.000 years ago) *A. unedo* was extinct from the East Mediterranean coast because the minimum

monthly temperatures were below 4 °C limit for its survival, and after that again colonized from the North African glacial refugium (Santiso et al., 2016). The *A. andrachne* seems that through Last glacial period was decimated but not extinct. Greek Strawberry tree (*A. andrachne* L.) is present mostly along the coast of East Mediterranean, and as more tolerant to low winter temperatures than *A. unedo*, it penetrates through river valley and gorges deeper into mainland. It is founded in similar geographical configuration, in river gorges of the two west tributaries of the river Vardar on the territory of North Macedonia in purified composition through the harsh winter weather conditions, with absence of related and tenderer *A. unedo*. One location is extent the lower course of Crna River, and the other is the middle course of Konjska River (Em et al., 1974).

Strawberry tree (*A. unedo* L.) fruits, which are similar to Greek Strawberry tree (*A. andrachne* L.) fruits, have traditionally been used for human consumption in the Iberian Peninsula and other Mediterranean regions (Molina et al., 2011). In Spain and Portugal, it is one of the most important wild fruit species and its raw fruits are usually consumed in the field and sometimes used for home made dessert (Tardío et al., 2006). New studies suggest potential uses of Strawberry tree fruits (*Arbutus unedo* L.) in the food industry and raising commercial orchards for fruit production (Alarcão-E-Silva et al., 2001). According to some authors (Celikel et al., 2008), both Strawberry and Greek Strawberry trees belong to the group of new, underutilized fruit tree species. Very important advantage of Greek Strawberry tree (*Arbutus andrachne* L.) is that in late autumn has uniformly matured fruits which are ripen simultaneously, considering the short blooming period in spring. In autumn at the Strawberry tree (*Arbutus unedo* L.) can be found flowers, immature, underripe and ripe fruits, as a result of discontinuous blooming period in warmer subtropical conditions. The ripening of Greek Strawberry tree fruits lasts about 7 months, while at the Strawberry tree lasts over 12 months (Santiso et al., 2016). More prominent winter dormancy and short simultaneously ripening of the Greek Strawberry tree with, represent its adaptation to the more severe climatic conditions of the East Mediterranean, which mean a useful genetic predisposition, and an opportunity to introduce this fruit species in commercial fruit production. Generally, *A. unedo* is characterized as a difficult-to-root (Şeker et al., 2010). Generative or vegetative propagation of selected types of Greek Strawberry genotypes is necessary to provide material for commercial use and orchard design and to reduce the natural habitat degradations (Sulusoglu, 2012). The aim of this paper is to be done a first step to investigate possibility to start a positive selection by the propagation abilities of Greek Strawberry tree (*Arbutus andrachne* L.) genotypes, founded in North Macedonian dendroflora and to choose those which will be most suitable for creation of rootstocks and varieties for cultivation of this interesting fruit tree in intensive orchards.

MATERIALS AND METHODS

It is performed investigation of the propagation characteristics of eight Greek Strawberry tree (*Arbutus andrachne* L.) genotypes from locality Ampiratorov Hill from the left side of Konjska River, municipality of Gevgelija, North Macedonia, 75 km

closest air line distance from the Aegean Sea, and 250 km from Adriatic Sea. The location is on 41°10'07" N latitude, 22°23'06" E longitude, altitude of about 350-400 meters on poor skeletal soil over gabbro or limestone, where dominate the special variant of submediterranean pseudomaquis (*Carpinetum orientalis phillyretosum*), exactly because the presence of *A. andrachne* in it (Em et al., 1974).

The examination of the seed characteristics e.g. seeds cross section area (SA), seed length (SL), seed width (SW), seed perimeter (SP) is made by using scanning device for obtaining high resolution images which are processed with the digital image processing method that performs precise analysis of the object dimensions (Markovski and Velkoska-Markovska, 2015) through computer software "ImageJ" (IJ). Except the measures of the seed (area, perimeter, height and width) it is used also shape descriptors for determination of Greek Strawberry genotypes seed form with help of some IJ software equations, such as: Circularity – $4\pi \cdot \text{area} / \text{perimeter}^2$, Aspect ratio (AR) – major axis/minor axis, Roundness – $4 \cdot \text{area} / (\pi \cdot \text{major_axis}^2)$, Solidity – area/convex area. It is investigated by the 50 seeds in three repetitions from eight Greek Strawberry tree genotypes, with surface sowing in humus soil substrate in germination chamber at the temperature of 25 °C and 85 % humidity. The germination control is performed periodically every seven or more days from the setting. In those conditions it is monitored the growth of the seedlings and the survival rate (SR). The seed vigor is analyzed through six periods, wherein the germination percent for the period is divided with the number of past days in the period and the sum of the quotients of the six periods gives the seed vigor (Kolekevski et al., 2004). About 50 cuttings with 3 - 4 buds, or at approximate length of 20 cm, with two to three one third reduced leaves are left on the every cutting. The cuttings were investigated in three repetitions from every genotype and were stuck on 3 - 4 cm depth into sand (SR) or in vermiculite (VR) substrate.

The data are statistically analyzed by ANOVA and Fisher's multiple comparisons testing at a level of 0.05. Pearson correlation matrix and Principal component analysis and ANOVA are performed using the Minitab and Xlstat softwares.

RESULTS AND DISCUSSION

The mother plants, which the plant material is collected from, are in excellent condition, without any kind of important diseases and pests presence. Seed morphology is an evolutionary trait contributing to genetic diversity (Aniszewski et al., 2001). For the determination of germination ability of Greek Strawberry tree genotypes, the quality and dimensions of the seed are very important. Seed morphology has influence on many later seed characteristics (water relation and seed dispersal, emergence, survival and seedling establishment), so the large and heavy seeds germinate rapidly with high survival and fast growth as compared to small seeds (Koechl et al., 2014). The germination of the smaller seeds is generally faster and that providing greater competitive advantage in early developing stages, but the larger seeds germinating slowly and have higher germination (Souza and Fagundes, 2014). The measurements show statistically important differences at the dimensions and shape of the seed. Type

2 is characterized with the largest seed. The highest statistic difference is found in relation to cross the section area of the seed. Type 2 has statically significant larger cross section area and thicker seed compared to six of the investigated genotypes (Tab. 1). The vigor of the seedlings depends on the seed size and large seeds tend to produce more vigorous seedlings compared (Yanlong et al., 2007). Regarding to the perimeter of the seed, the Type 2 varies statistically from only 3 genotypes. The most elongated seed has the Type 6. It is interesting to note that the differences in seed length are not so expressed such as the seed width. It is recorded statistically significant differences in seed shape. The Types 8, 9 and 2 have the most circular form. The Type 4 has the most ellipsoidal seed considering for the smallest seed width (Tab. 1). The same Type is characterized with statistically significant deviations of the values for AR and roundness. As a result of the convex parts domination at the area of seed, the Type 4 is characterized with the lowest value for solidity (Tab. 1).

Table 1. Seed dimension of *A. andrachne* genotypes

Genotype	Area mm ²	Perimeter mm	Length mm	Width mm	Circularity	AR	Round	Solidity
Type1	3.71 ^c	8.68 ^a	3.05 ^a	1.53 ^d	0.63 ^c	1.98 ^b	0.50 ^{de}	0.97 ^{ab}
Type2	4.31 ^a	8.72 ^a	3.04 ^a	1.81 ^a	0.71 ^{ab}	1.71 ^{cd}	0.60 ^{ab}	0.97 ^{ab}
Type4	3.33 ^{de}	8.16 ^b	3.01 ^a	1.40 ^e	0.62 ^c	2.17 ^a	0.47 ^e	0.96 ^b
Type5	2.32 ^f	6.43 ^d	2.16 ^c	1.36 ^e	0.70 ^b	1.60 ^d	0.63 ^a	0.97 ^{ab}
Type6	4.01 ^b	8.46 ^{ab}	3.09 ^a	1.66 ^c	0.70 ^b	1.87 ^b	0.54 ^{cd}	0.97 ^{ab}
Type7	3.22 ^e	7.70 ^c	2.73 ^b	1.52 ^d	0.69 ^b	1.86 ^{bc}	0.56 ^{bc}	0.97 ^{ab}
Type8	3.51 ^{cd}	7.65 ^c	2.66 ^b	1.68 ^{bc}	0.75 ^a	1.61 ^d	0.63 ^a	0.98 ^a
Type9	4.09 ^{ab}	8.40 ^{ab}	2.94 ^a	1.76 ^{ab}	0.72 ^{ab}	1.67 ^d	0.61 ^a	0.98 ^a
Average	3.56	8.02	2.83	1.59	0.69	1.81	0.56	0.97

* Means that do not share a letter are significantly different

The evaluations show that the seeds from the Greek Strawberry tree have very low germination in the average (16 %). According to some investigations the seed germination is influenced by some external factors such as air temperature and shows to an optimal temperature of 20 °C a rate of 19.2 % (Hammami et al., 2005). Some authors suggest that the germination of the *Arbutus* genus is originally very nit and erratic (Şeker et al., 2010). The influence of different temperatures over Greek Strawberry tree seed germination is investigated and it is concluded that the lower temperatures (10 °C and 15 °C) of incubation in agar medium (Murashige/Skoog) affect to high percents of seed germination (84 % to 90 %), while at the higher temperatures (20 °C and 25 °C), practically there is no germination or it is very low (1 %) (Bertsouklis and Papafotiou, 2013). During the field investigation, around the mother plants it is noted high number of young seedlings, which is sign for high germination potential of the

seed. Generally, large seeds are expected to have higher seed germination ability than the small seeds (Koechl et al., 2014). Beginning of the germination dynamics shows that after 20 days from the sowing only two of genotypes have bigger percent of germination over 5 % (Fig. 1). Only the Type 7 has statistically significant deviation to the starting germination from half of the investigated genotypes. Until the 40th day the three genotypes stand out by the increased seed germination (Type 7, Type 8, and Type 1). Again, only the Type 7 has statistically significant difference in relation to five of the investigated genotypes. In the fifth period is observed a rapid germination of the other genotypes (Type 2 and Type 4). So, the statistical advantage of the genotypes with faster germination is significantly decreasing. In the last period it is noted accelerated germination of some of the genotypes that have been long time inactive, such as the Types 4 and 8, which have twice as bigger germination compared with germination in the fifth period. In the last period the Type 4 reaches the highest germination of 25 % among the all investigated genotypes.

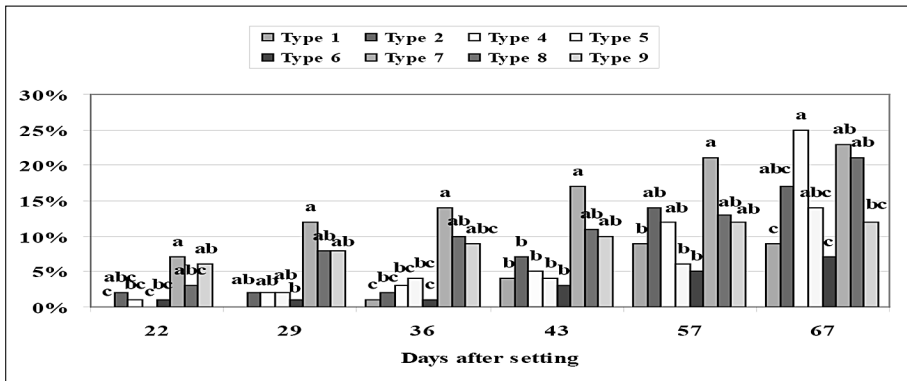


Figure 1. Seed germination of *A. andrachne* genotypes

Determination of the seed vigor best explains the germination dynamic periods (Tab. 2). Most uniform germination through all six periods has the Type 7. With uniform germination are characterized also the Types 8 and 9. These three Greek Strawberry tree genotypes have the highest seed vigor among the investigated genotypes. Also, it is noted that the mentioned three genotypes have increased seed vigor up to the third period, and then mildly decreasing of the seed vigor. Another group of genotypes (Type 1, 2, 4 and 6) are characterized with slight increasing to the third period and with a rapid increasing of the seed vigor at the end of the observation. The Type 5 is characterized with different dynamics than the other genotypes, or this Type has increasing of the seed vigor up to third period, stagnation to the fifth period and sudden increasing in sixth period. The observation of further growth of the seedlings shows that from the total number of emerged seedlings with appearance of cotyledon leaves, after thirty days, begins the decline of the surviving plant number. Entirely are survived only the seedlings from genotypes 2, 5 and 7 (Fig. 2).

Table 2. Seed vigor of Greek Strawberry tree genotypes

Geno-type	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Seed vigor (Total)
Type 1	0.00	0.00	0.03	0.09	0.16	0.13	0.41
Type 2	0.09	0.07	0.06	0.16	0.25	0.25	0.88
Type 4	0.05	0.07	0.08	0.12	0.21	0.37	0.90
Type 5	0.00	0.07	0.11	0.09	0.11	0.21	0.59
Type 6	0.05	0.03	0.03	0.07	0.09	0.10	0.37
Type 7	0.32	0.41	0.39	0.40	0.37	0.34	2.23
Type 8	0.14	0.28	0.28	0.26	0.23	0.31	1.49
Type 9	0.27	0.28	0.25	0.23	0.21	0.18	1.42

Already it is noted statistic significant difference among the Types 2 and 7 with some of other Types. After 90 days it comes to sudden decreasing or halving the number of surviving seedlings at the most of genotypes, and also occurs the statistical significant difference between the Type 2 with all other genotypes. Except the Type 2, significant statistical difference is noted at Type 5 relating to the number of seedlings with one of the investigated genotypes. At the end of observation, the number of surviving seedlings of the Type 2 is decreased for only 22 %, while among the other genotypes decreasing is from 91 % to 73 % (Fig. 2).

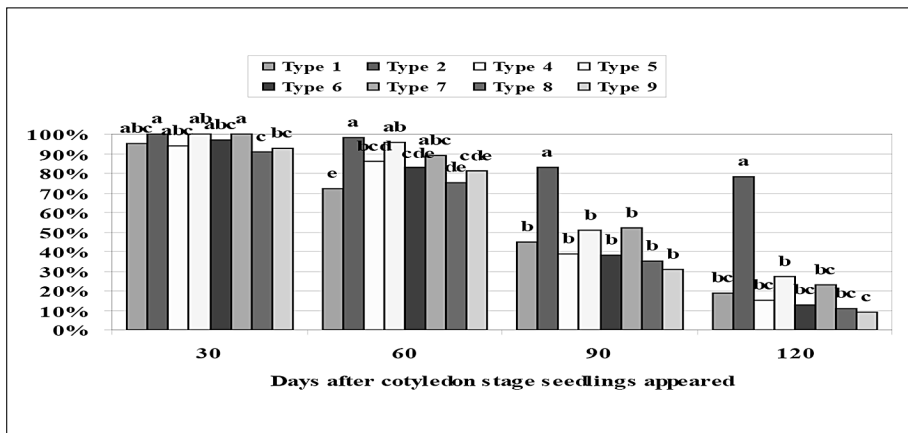


Figure 2. Survival rate of *A. andrachne* genotypes seedlings

The reason for drastic decreasing of the seedling surviving number and reason for prematurely dying of the seedlings from the genotypes of Greek Strawberry tree is

unknown. The fact that most of the genotypes seedlings are affected with this phenomenon shows that it is the factor which is referred to whole Greek Strawberry tree species. That fact additionally increases the value of the Type 2 with the high number of surviving seedlings in further investigations. It is known that the species from genus *Arbutus* live in symbiosis with arbutoid mycorrhizae. Sometimes the presence of wrong incompatible microbiota in the ectomycorrhizae can cause dying of the seedlings of *Arbutus* species (Becerra et al., 2007).

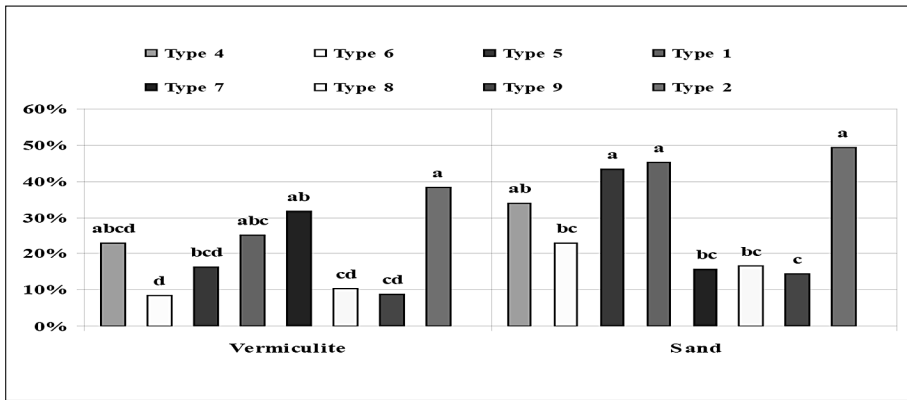


Figure 3. Rooting of softwood cuttings from *A. andrachne* genotypes

The rooting of cuttings from the genotypes amounts 25 %, in average. Using high concentration of biostimulator (IBA) at the related species *Arbutus unedo* is achieved the very high percent of rooting (up to 87 %) of the cuttings of some local Turkish genotypes (Sulusoglu, 2012). Statistical significant difference between the rooting in sand (SR) and rooting in vermiculite (VR) does not exist. Generally, the cuttings from the genotypes are rooting better in sand (30.1 %). The investigation shows that the influence of substrate sand over rooting of cuttings from the Greek Strawberry tree genotypes is higher than the influence of the genotype. Analyzed individually, only the genotype 7 has bigger, but statistically insignificant difference to the rooting in vermiculite (31.6 %) than in sand substrate (15.7 %). Type 2 is characterized with the highest rooting percent in vermiculite substrate (38.2 %). The Types 2 and 6 have statistically significant greater ability for rooting in vermiculite in relation to many of the other genotypes (Type 5, 8, 9 and 6) (Fig. 3). Only two (Type 1 and Type 5) from the investigated genotypes have statistically significant higher percent of rooting in sand, than in vermiculite. The statistical difference is higher in sand substrate rooting of cuttings. The Types 2, 1 and 5 have statistically greater difference in relation to other types, with exception of Type 4. The greater ability for rooting in sand substrate has again the Type 2 (49.3 %). It can be said that the rooting of cuttings as a vegetative way of propagation of Greek Strawberry tree genotypes, is more efficient than the germination of the seeds.

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РАЗМНОЖАВАЊЕ ГЕНОТИПА ДРВА ГРЧКЕ МАГИЊЕ (*Arbutus andrachne* L.)

Резиме

У периоду 2014-2015. године истраживана је могућност генеративног и вегетативног размножавања генотипова ретког воћног дрвета Грчке магиње (синоними: Грчка планика, Голо дрво, Го човек) (*Arbutus andrachne* L.), смештених у природним састојинама, на средњем току, са леве стране Коњске реке, на јужном делу Северне Македоније. Тип 2 карактеришу највећа семена (просечна површина семена 4,31 мм², обим 8,72 мм и ширина 1,81 мм). Резултати показују да семе генотипова дрвећа грчке магиње има низак проценат клијавости и ниску стопу преживљавања садница. Тип 2 се одликује највећом способношћу укорјењавања резница у песку (49,3 %). Изузетак је Тип 2 са око 78% преживелих примерака након четири месеца посматрања. Највећи проценат клијавости има тип 4 (25 %). Укорјењавање резница даје највећи проценат живих биљака. Укорјењавање у песку у просеку даје боље резултате од укорјењавања у вермикулиту.

Кључне речи: *Генотип, семе, клијање, резница, расад*



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Original Scientific paper

PRODUCTIVITY OF GLUTEN-FREE PSEUDOCEREAL *Fagopyrum esculentum* Moench – NOVOSADSKA BUCKWHEAT ON THE CHERNOZEM

Abstract

Buckwheat is successfully cultivated in the mountainous area. Buckwheat seeds are used in food, while leaves and flowers are used in pharmacy, they have a preventive effect on human health: hypertension, arteriosclerosis, constipation, etc. Buckwheat is also of great economic importance in beekeeping, because of nutritionally valuable honey. In this study, the productivity of “Novosadska buckwheat” grown in experimental plots of the Institute of Agriculture and Vegetables, on chernozem in 2022 was analyzed. “Novosadska buckwheat” showed that it has excellent genetic potential and achieved excellent performances in an unfavorable year for production. The average yield of buckwheat was 1.11 ± 0.20 t ha⁻¹, plant height 152.00 ± 0.32 cm, plant height to 1st inflorescence 27.00 ± 7.83 cm, which shows that buckwheat can be successfully planted in plain areas.

Key words: *Fagopyrum esculentum* Novosadska buckwheat, gluten-free grain, productivity, chernozem.

INTRODUCTION

Buckwheat – *Fagopyrum esculentum* Moench. is a pseudocereal; it belongs to *Polygonaceae* family. It is a fast-growing, broad leaf annual crop of warm-season with small white or pink flowers (Sattel et al., 1998). The flower clusters develop into brown triangular seeds that consist of a groat surrounded by a thick hull (Myers & Meinke, 1994). There are other commercially available species of buckwheat, such as *F. tartaricum*, *F. sagittatum*, *F. emarginatum* but the most common species is *F. esculentum* (Treadwell & Huang, 2008). Buckwheat got its name from the fact that seeds are similar to the beech tree's seeds that are called boekweit in Dutch (Myers & Meinke, 1994). The origin of buckwheat is believed to be mountainous part of south western China, it was cultivated in China around 5000 years ago (Boland, 2013; Chen, 2001). It spread through Asia to Europe and the New World experienced the introduction of the crop in the 17th century with the movement of European colonists (Treadwell & Huang, 2008). Buckwheat grain contains: carbohydrates (85%), proteins (12%), fats (2%), minerals (potassium, 218 mg; phosphorus, 254 mg; calcium, 21 mg; magnesium, 85 mg; iron, 3.2 mg; sodium, selenium, fluorine, as well as trace elements: iodine, zinc, bromine, etc.), then vitamins (B₁, B₂, P, E), dietary fiber, phytosterols, contains bioflavonoid rutin (which has a positive effect on the blood system) and lysine (an essential amino acid important for bone growth and blood regeneration) as well as agrimin (reduces the risk of sterility). Buckwheat flower can be used as a cough medicine and to ease breathing, while the fresh leaves have a pronounced bactericidal effect, they can be applied directly to open wounds or cuts. In this study, the possibility of using buckwheat harvest residues was analyzed. Harvest residues and residues after grain processing can be used in different ways, such as: fodder, mulch, for making compost, in further industrial processing, for the production of biofuels and for ploughing under. Buckwheat stem has pharmaceutical value, because it contains the substance rutin, which increases capillary resistance and is used as a means to stop bleeding (Popović et al., 2017; 2021). Buckwheat biomass in the ripening period can be used to obtain biogas or to produce pellets. If above-ground biomass is used for the preparation of solid fuels, briquettes or pellets, after combustion in boiler plants, ash rich in potassium and calcium remains. Biomass can also be used as green fodder (Popović et al., 2021). Plowing buckwheat biomass, if the goal of cultivation is sideration, is carried out after the flowering of the plants in order to use it for the production of honey. The best quality siderate for plowing is obtained from biomass after the flowering of plants. Vegetative biomass plowed in the flowering phase is quickly mineralized in the soil so that the next crop has mineral salts available at the time when it needs them the most. Buckwheat also has great agrotechnical importance because, as a crop of dense sowing, it covers the soil well and suppresses weeds. In addition, it can absorb phosphorus from less accessible forms and prevent its leaching into deeper layers and underground watercourses. Buckwheat plants have a short vegetation period, so we can grow them as a follow-up or side crop in very different climatic and soil conditions. (Glamočlija et al., 2015; Popović et al., 2017; 2021).

The very favorable agro-ecological conditions of this region provide exceptional opportunities for this type of production. Given that buckwheat achieves the best yields

in mountainous terrain, the aim of this study was to examine the productivity of buckwheat in the plain area on chernozem.

MATERIAL AND METHODS

The production trials were carried out on the trial plots of the Institute of Field Crop and Vegetable, in Bački Petrovac. This study shows the production of buckwheat, a variety of “Novosadska” grown on chernozem. Sowing was done in three repetitions, on an area of 10m² (5×2=10m²), at the optimal time, in mid-April. Cultivation technology applied in the crop was varietal. The crop was irrigated five times during the growing season with 30 mm water. The harvest was done at technological maturity. Before harvesting, 10 buckwheat plants were taken from each plot for morphological analysis. The yield was taken after harvest from each plot and converted to 13% moisture. The obtained results were processed using descriptive statistics and presented tabularly and graphically.

For sowing buckwheat, it is best to use seeds from the previous year, with a seed germination rate of at least 95%. In lowland areas, buckwheat is sown in the second half of April, and at higher altitudes (in our country up to 1,100 m) in May. As a follow-up crop, buckwheat is sown until the end of June, and for the production of fodder successively, approximately in five periods in order to extend the mowing period as much as possible. Common buckwheat is sown both in narrow and wide rows. Buckwheat is sown in close succession at an inter-row distance of 12 cm with grain drills, with a consumption of 60-80 kg ha⁻¹ of seed. Wide-row sowing of buckwheat is carried out at an inter-row distance of 25-50 cm and a spacing of 1.5 cm between plants in the row and is carried out with seed drills for wide-row crops with the consumption of 40-50 kg ha⁻¹ of seeds. This method of sowing buckwheat is common in arid areas and on weedy lands. Buckwheat sowing depth is 4-6 cm. After sowing, the surface can be rolled if necessary. Supplementary pollination of buckwheat is carried out by placing beehives with bees around the crop at the beginning of flowering. In densely sown crops, weeds can be controlled before sowing with the herbicide preparations Devrinol 45-F, Acetochlor-90 or during the growing season with the preparation Focus ultra. If buckwheat is grown for the needs of the pharmaceutical industry, the plants are cut about 30 days after germination, because that's when they have the highest content of rutin. As ripe buckwheat fruits are easily shed, harvesting should be done as soon as possible or the crop should be sprayed beforehand with defoliant due to accelerated leaf shedding (eg Reglone forte) or preparations that prevent grain shedding (Spodnam DP or NU film), (Popović i sar., 2013; 2017; 2019).

Soil properties. The soil in its natural state rarely provides the most favorable conditions for the growth of crops, that is why man, by applying various agrotechnical measures, changes its physical, chemical and biological properties and adapts them to his needs. The physical composition of the soil is an important factor for water, air, heat and nutrition regime, thus fertility, and to a significant extent determines the suitability of the soil for agricultural production (Popović, 2010; Glamočlija et al., 2015). Productive agricultural soils have the ability to promote root growth, preserve moisture and

supply the plant with water, to retain, increase the availability and enable the circulation of mineral substances, ensure optimal gas exchange and stimulate the activity of the biological phase. All these properties are partly a function of physical properties and processes in the soil. The chemical reaction of the soil in "Bački Petrovac" is weakly alkaline with 4.7% CaCO₃. The soil is well supplied with total nitrogen (0.2%), readily available phosphorus and potassium (37 and 36 mg/100g).

Meteorological conditions. The successful production of cultivated plants largely depends on meteorological conditions, and above all, on the amount of precipitation and air temperature. These two factors determine the climate of an area and significantly influence the growth and development of crops and applying of all agrotechnical measures. In the examined year of 2022, the average air temperature was 20 °C and the total amount of precipitation was 162 mm. The production year was very unfavorable for crop production.

RESULTS AND DISCUSSION

The yield formation of buckwheat plants depends on the characteristics of the genotype, agroecological factors and the applied cultivation technology. Manifestation of certain traits and their dominant influence on the production of yield can be different in different conditions of the external environment. This study included the monitoring of morphological traits important for yield, such as stem height, plant height up to 1 inflorescence, stem thickness, leaf length and width, spikelet length and weight of 1000 grains. The results of the research for productive parameters of "Novosadska buckwheat" are shown in table 1. "Novosadska buckwheat" showed that it has excellent genetic potential and achieved excellent performances in an unfavorable year for production. The average yield of buckwheat was 1.11±0.20 t ha⁻¹, plant height 152.00±0.32 cm, plant height to the 1st inflorescence 27.00±7.83 cm (graph. 1-2), stem thickness 1.00 ±0.18 cm, leaf length 8.04±0.74 cm, leaf width 9.10±0.57 cm and leaf mass 1.02 ±0.15g which shows that buckwheat can be successfully grown in plain areas.

Table 1. Productivity of Novi Sad buckwheat for chenosema

Parameter	Average value	Min	Max	Std. Dev.	IV*
Grain yield, t/ha	1.11	1.02	1.22	0.20	0.22
Plant height, cm	152.00	148.80	153.40	3.25	4.60
Plant height until 1 st inflorescence, cm	27.00	22.92	34.00	7.83	11.08
Stem thickness, cm	1.00	0.85	1.10	0.18	0.25
Leaf length, cm	8.04	7.50	8.55	0.74	1.05
Leaf width, cm	9.10	8.60	9.40	0.57	0.80
Leaf mass, g	1.02	0.89	1.10	0.15	0.21

* IV – variation interval; Autor's calculation

In the world, there is an increasing interest in buckwheat, which is why the areas are increasing every year. In 2020, the area under buckwheat amounted to 1,856,913.0 ha, the grain yield was 975.0 kg/ha, and the production was 1810816.0 million tons. (Popović et al., 2022).

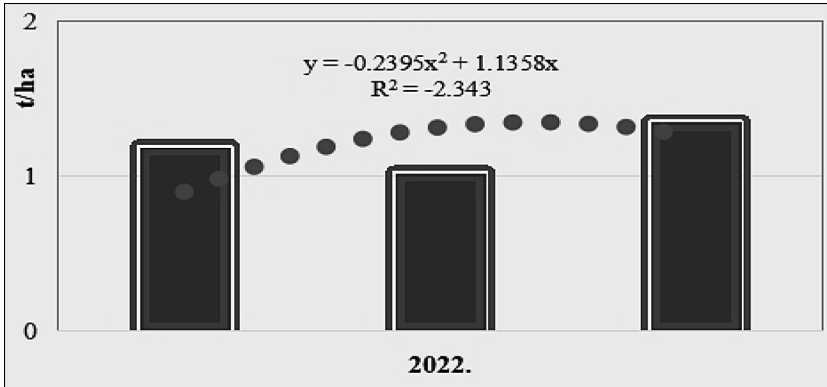


Figure 1. Buckwheat grain yield in arid year, Bački Petrovac, Serbia

The height of the plant is an important feature that significantly affects the harvest index and grain yield. The height of the tree is significant in the competition for life factors. Buckwheat has tall plants, so it smothers and shades weeds with its habit and achieves good yields. The conditions of the external environment have a very significant influence on the morphological and productive properties of buckwheat. By examining the height of the tree, it is evident that the examined values are the result of the influence of meteorological factors during the vegetation period. In addition to the external environment, the genetic potential of the examined variety also has a significant influence on tree height (Popović et al., 2017).

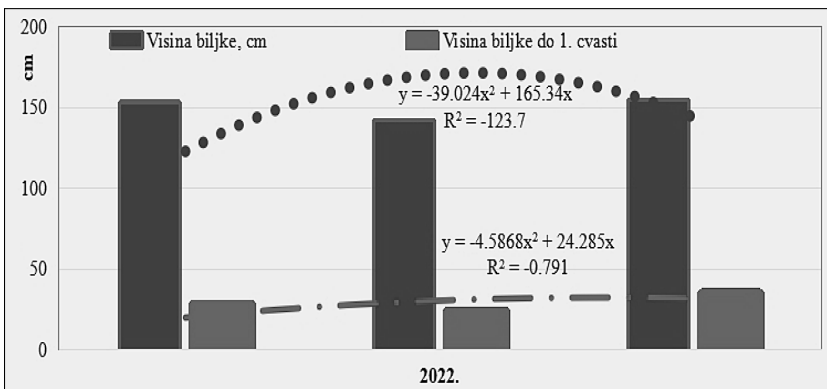


Figure 2. Buckwheat plant height and height to the 1st inflorescence, cm

Taylor et al. (2001) emphasizes the importance of aboveground biomass development in suppressing weeds and preventing their growth. Grain yield is the result of many developmental and physiological changes during the life cycle of a plant. In the formation of yield, the importance of several components is highlighted: the number of inflorescences per unit area, the number of fruiting trees per plant, the number of grains per plant, the weight of 1000 seeds and the harvest index (Glamočlija et al., 2015). The productivity of these components depends on the weather conditions in the growing season, the assortment and the applied cultivation technology (Popović et al., 2017). Stability in achieving the expected yield is one of the most desirable features for the genotype to be recommended for widespread cultivation. The expression of quantitative traits in plants is conditioned by the influence of the genotype, the environment and their interaction, which occurs as a result of the genotype's response to changes in the external environment (Baker, 1990; Glamočlija et al., 2015; Popović et al., 2017; 2019; 2022). The formation of grains and the creation of yield takes place mainly at the expense of the breakdown of reserve substances and their transfer from older and photosynthetically inactive parts of the plant, such as the stem and older leaves, to the ear. According to estimates, carbohydrate reserves in the stem contribute to the total yield of buckwheat with about 10-12% in optimal agroecological conditions and with more than 40% in conditions of drought and heat stress (Austin et al., 1977). All over the world doctors as well as nutritionists are mentioning health problems linked to the consumption of cereals containing gluten as sources of allergies and intolerances. Rice, quinoa, amaranth and buckwheat are proposed as alternatives. Buckwheat doesn't contain any gluten so it can fit perfectly in a diet low or free from gluten. Quality of buckwheat grains is varies from: protein content 12-18.6%, fat content 3-4%, starch 63-66%, Oleic acid 42%, Linolic acid 32%, Palmitic acids 16% (Tomotake et al. 2006). Buckwheat is known in France by "galettes de blé noir" traditional crepes made from buckwheat flour, especially in Brittany; in Eastern Europe, the toasted buckwheat groats "Kasha" are used to preparing traditional dishes (in soups etc.), in Russia buckwheat flour is also prepared as blinis; in Asia, is known buckwheat pasta "Soba noodles"; herbal tea called "Sobacha" and buckwheat is delicious in any salad.

CONCLUSION

The Novosadska variety grown on chernozem in a dry year achieved excellent morpho-productive properties. The yields it achieved were significantly higher than the average world yields, on the basis of which we can conclude that buckwheat can be successfully grown in our country and in the plains.

Buckwheat is becoming more and more important in the world and in our country in the economic, ecological and social sense. A growing trend for gluten-free buckwheat based products highlights the need for development of new products in functional food.

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Оригинални научни рад

ПРОДУКТИВНОСТ БЕЗГЛУТЕНСКОГ ПСЕУДОЖИТА *Fagopyrum esculentum* Moench. – НОВОСАДСКЕ ХЕЉДЕ НА ЧЕРНОЗЕМУ

Сажетак

Хељда се успешно узгаја у планинском подручју. Хељдино семе се користи у исхрани, док се листови и цветови користе у фармацији јер превентивно делују на здравље људи, код хипертензије, артериосклерозе, затвора итд. Хељда има и велики привредни значај у пчеларству, због нутритивно вредног меда. У овој студији анализирана је продуктивност новосадске хељде гајене на огледним парцелама Института за ратарство и повртарство, на чернозему у 2022. години. Новосадска хељда је показала да има одличан генетски потенцијал и постигла одличне перформансе у неповољној години за производњу. Просечан принос хељде износио је $1,11 \pm 0,20 \text{ t ha}^{-1}$, висина биљке $152,00 \pm 0,32 \text{ cm}$, висина биљке до прве цвасти $27,00 \pm 7,83 \text{ cm}$, што показује да хељда може успешно да се гаји и у равничарским пределима.

Кључне речи: *Fagopyrum esculentum*, новосадска, без глутена, продуктивност, чернозем.



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Original Scientific paper

BANKUT WHEAT

Abstract

"Bankut" varieties of wheat had great economic importance for wheat production in Vojvodina and other areas of our country. Among the varieties of this wheat, the most significant were the "Bankut 1201" and "Bankut 1205" varieties. These varieties were created by Laslo Baroš on a farm in Bankut, Hungary. He wanted to create a winter variety of wheat suitable for the conditions of Potisje, better than Banatka. Populations of domestic Banat and world-famous Manitoba wheat served as parents for hybridization. He crossed the "Bankuti" variety no. 5 separated from "Banatka" with "Manitoba". As a result of this hybridization, several varieties were created, among which variety no. 1201. The same combination gave, just a little later, variety no. 1205 (Bekrić, 1989).

Key words: *wheat, commercial quality, technological quality*

INTRODUCTION

"Bankut 1205" is a winter wheat. Variety that came to Vojvodina during the Second World War. Since liberation, the Institute for Agricultural Research in Novi Sad has worked on its improvement (Mišić, 1995). Similar to the variety Bankut 1201, it was characterized by high biological plasticity, so it could be successfully grown outside the Vojvodina area. After the Second World War, it became the leading variety in production, so that since the fifties, together with Bankut 1201, it has occupied more than 80% of the wheat areas of Vojvodina (Mišić, 1995). Bankut 1205 retained its dominant position in the assortment of Vojvodina until the first years of the seventh decade when varieties from the Mediterranean area took over the primacy in production.

History of growing bankut wheat

In the ten-year period from 1930 to 1939, on an average area of 2.114.100 ha, a very low grain yield was achieved, only 1.14 t/ha. The leading variety was Bankut 1201, and the supplementary variety was Bankut 1205. The ten-year period from 1951 to 1960 was also included in the studies. On an average of 1.902.000 ha, the yield was 1.32 t/ha, which is 15.8% more than it was in the period from 1930 to 1939. The leading varieties in this period were Bankut 1205 and Bankut 1201. From 1961 to 1965, on an average of 2.009.200 ha, a yield of 1.78 t/ha was achieved, which is 56.1% more compared to the period from 1930 to 1939. In addition to Bankut 1205, Bankut 1201 wheat was also represented.

The trend of increasing the average yield of wheat continued in the five-year period from 1966 to 1970. Then it was grown as a supplementary variety to Bankut 1205. On the average area of 1.467 ha, the yield was 2.00 t/ha, 75.4% higher than in the period from 1930 to 1939. Compared to the period from 1961 to 1965, a 27.4% higher average yield was achieved. The average yield of the individual sector in the period from 1961 to 1965, when Bankut 1205 was the most widespread variety in production and the supplementary variety was Bankut 1201, was 52.9% of the yield of the social sector (Bekrić, 1989). From 1966 to 1970, with the supplementary variety Bankut 1205, the average yield of the individual sector was 57.6% of the average yield of the social sector.

Variability of agronomic characteristics of wheat

In addition to the variety and applied agrotechnics, varying meteorological conditions between years is one of the main causes of varying grain yield and technological quality of wheat (Hristov and Mladenov, 2007). In the growing season of 2008/2009. year, an experiment was carried out in order to examine the variability of grain yield, the number of grains per m² and the weight of 1000 grains. The variety Bankut 1205 had the lowest number of grains per m² (Jocković et al., 2010). The mode of inheritance and components of phenotypic variability for the number of grains per ear of wheat were studied when five different genotypes of wheat were crossed (NS Rana 2, Bankut 1205, NS 732, Atlas 66, and Siete Cerros 66). In the F1 offspring of all crossings, dominance was manifested, and in one part of the offspring, superdominance. The calculated value of the average degree of dominance indicates that in the inheritance of the number of grains per ear, in the crosses of the mentioned genotypes, it is about superdominance (Petrović et al. 1994). Heredity, phenotypic variability, and components of variance for spike length and number of spikelets per spike were studied in 50 cultivars including Bankut 1205 from different selection centers. The coefficient of variation for the number of spikelets per spike ranged from 4.7% for Bankut 1205 to 12.4% for the Norin 10 variety. The phenotypic analysis of variance showed that the genetic variance accounted for a larger part of the total phenotypic variability for spike length (81.082%) and the number of spikelets per spike (57.36%) than the influence of environmental factors on the expression of the analyzed yield components. (Zečević

et al., 2004). The main goal of wheat breeding is to obtain varieties with high genetic potential for yield, which would be best suited for certain growing regions, heterogeneous in terms of climate and soil. As yield is a complex property, the result of a series of quantitative properties, for a successful breeding effect it is necessary to know the characteristics of genotypes, but also the interaction between genotypes and environmental conditions, to make the best possible use of the genetic yield potential and create more adaptable genotypes (Borojević, 1982). Stability tests of the class index were performed in a sample of 22 wheat genotypes, among which the Bankut 1205 variety was tested. According to the regression coefficient, among the most stable genotypes are Ana, Kratka, Talent, Florida, Sremica, Concordia, Aibian, and Italy. The variety Bankut 1205, with long and heavy spikes, showed the highest mean values of the spike index. Genetic analysis of divergent wheat genotypes grown in our agro-ecological conditions contributes to their better understanding and facilitates the selection of parents for crosses in future wheat breeding programs (Petrović et al., 2002).

The inheritance of the number of primary roots in F1 and F2 generations was investigated by diallel crossing of 4 varieties of winter wheat ("Sava", "Libellula", "Bankut -1205" and "Kruševačka 9083"). The number of primary roots is inherited partially dominantly and intermediately. The parental varieties differed in the number of primary roots. The number of primary roots is controlled by additive and non-additive gene effects. "Libellula" and "Sava" had more recessive genes than "Bankut-1205", which had more dominant genes in F1. In F2, "Sava", "Bankut-1205" and "Kruševačka 9083" had similar recessive-dominant proportions of genes, but "Libellula" had more recessive genes. Heritability in the broad sense was very high.

Regression analysis of covariance and variance in diallel crosses was conducted to determine the effects of genes on the number of grains per ear of five wheat genotypes: NS Rana 2, Bankut 1205, NS 732, Atlas bb. Graphical regression analysis showed that the number of grains per ear is determined mainly by the effects of the dominant gene, as well as by the unequal distribution of dominant and recessive genes between the five genotypes of parental wheat (Dimitrijević et al., 1994). In one study, attention was paid to the problems of open pollination of wheat. Six different genotypes were selected for this purpose: San Pastore, Bankut-1205, Novi Sadska Rana-2, NS-732, Nizija, and Siete Cerros. The frequency of open-pollinated plants in relation to the total number of plants was 3.8 in the pure variety NS-732, which indicates that approximately 26 open-pollinated plants per m², i.e. 260.000 phenotypically different plants per m²/ha, can be expected in the crop of the tested variety. 39 different genotypes of wheat were selected in order to examine the variability of yield, the number of grains per m², and the weight of 1000 grains. A field trial was set up at Rimski šančevi in the 2008/2009 season. Based on the obtained results, we determine significant differences in the yield, the number of grains per m² and the weight of 1000 grains among the tested genotypes. The Dragana variety had the highest average grain yield (9.25 t/ha), and the Banatka variety had the lowest (3.08 t/ha). The highest number of grains per m² (51532) was the variety Sana, and the lowest number of grains per m² (22360) was the variety Bankut 1205. The highest weight of 1000 grains (57.1 g) was the variety KG 56, and the lowest weight of 1000 grains (33.2 d) has variety NS 3-5299/2 (Jocković et al., 2010).

Technological characteristics

The variety Bankut has a white, spindly, and thistle spike with a red grain. According to the ripening time, it ripens three days before Banatka. It had very good resistance to low temperatures, heat and air drought, and high temperatures during the grain filling phase. As with Bankut 1201, its stem was over 140 cm high, with insufficient resistance to lodging. Despite that, it suppressed increasingly low-yielding varieties such as "Banatka", "Dakota" and others. It was equal to Bankut 1201 in resistance to grain shedding. Like Bankut 1201, it did not have satisfactory resistance to powdery mildew (*Ustilago tritici*), leaf and stem rust (*Puccinia recondita tritici* and *Puccinia graminis tritici*). The quality of her flour and bread was very good and slightly better compared to Bankut 1201. It had a medium-large vitreous grain, with a weight of 1000 grains up to 40 g and volumetric grain weight of up to 84 kg. It achieved the best results on more fertile soils and in conditions of improved technology. It was registered by the Commission for the recognition of Varieties as a variety of AP Vojvodina, central Serbia, Kosovo, Kumanovo, and Tetovo regions.

In support of this, the quality of Bankut wheat of the agricultural farm from Dobrica was tested. The technological quality of wheat is attached:

Table 1. Commercial quality of Bankut wheat

Bankut 1205		
Test parameters		Rating value
Water content (%)		12.8
Volumetric mass (kg/Hl)		81.30
Broken and coarse grains (%)		3.6
Corroded grains (%)		1.4
Spoiled grains (%)	Fusarium	0.4
	The rest	2
Content of total impurities (%)		6.9

Table 2. Technological quality of Bankut wheat

Bankut 1205		
Test parameters		Rating value
Water content (%)		12.8
Volumetric mass (kg/Hl)		81.30
Grinding (%)		63.0
Wet gluten content (%)		34
Water absorption power (%)		59.3
Test development (min)		3.2

Test stability (min)	1.3
Degree of softening (FJ)	76
Area (cm ²)	61.7
Quality number	8.8
Quality class	B1
Energy (cm ²)	41.0
Tensile strength (EJ)	162
Stretchability (mm)	156
O/R ratio	1.0
Maximum resistance (EJ)	180
Maximum Viscosity (AJ)	1265
Content of separable impurities (%)	3.6

SUMMARY

Bankut 1205 maintained its dominant position in the assortment of Vojvodina until the first years of the seventh decade when varieties from the Mediterranean area took over the primacy in production. The tested sample of the variety Bankut 1201 belongs to technology group B1, the relationship between resistance and stretchability is not ideal. The stretch it self is extremely good. The hectoliter mass is also high.

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Оригинални научни рад

БАНКУТ ПШЕНИЦА

Сажетак

„Банкути“ пшенице су имале велики привредни значај за производњу пшенице у Војводини и другим подручјима наше земље. Између ових пшеница најзначајније су биле сорте „Банкут 1201“ и „Банкут 1205“. Ове сорте створио је Ласло Барош на газдинству у месту Банкут у Мађарској. Он је желео да створи озиму пшеницу за услове Потисја, бољу од Банатке. Као родитељи за хибридизацију послужиле су популације домаће Банатске и чувене светске пшенице „Манитобе“. Он је укрстио линију „Банкути“ бр. 5 издвојену из „Банатке“ са „Манитобом“. У резултату ове хибридизације створено је више линија између којих се истичала линија бр. 1201. Иста комбинација дала је, само нешто касније, линију бр. 1205 (Бекрић, 1989).

Кључне речи: *пшеница, комерцијални квалитет, технолошки квалитет*



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Original Scientific paper

THE INFLUENCE OF DIFFERENT SUBSTRATE MIXTURES ON SEEDLINGS QUALITY AND ECONOMICALLY HIGH YIELD OF TOMATO, PEPPER AND BASIL

Abstract

In this paper, the influence of different substrate mixtures on seedlings quality and yield of tomato, pepper and basil were investigated. The experiments were carried out in the plastic greenhouse and experimental field of the Institute for Medicinal Plant Research "Dr. Josif Pančić" in Pančevo, during year 2016. Four treatments (different substrate mixtures) and a control treatment (soil from the investigated location) were tested. The best characteristics of morphological traits of transplants were achieved using the commercial Klasmann Potgrond H substrate. On the other hand, the plants that were grown on the compost produced during the production and processing of medicinal plants (IMPR) had the highest yield of fruits (tomatoes and peppers) and yield of fresh aboveground mass (basil).

Key words: *substrate mixtures, tomato, pepper, basil, yield*

INTRODUCTION

There is almost no part of the world where tomatoes, peppers and basil are not used. They are used as a fresh vegetable or as a raw material for processing and various product preparation. In order to enable transport over long distances and a long period of manipulation and storage, new hybrids with "long shelf life" properties have taken

over 90% of the domestic market. Such hybrids are more suitable for producers. But, without pleasant taste, aroma (without any, in some cases) and texture, they are not appreciated among consumers and processing technologists. The major disadvantage of old varieties is its softness and inability to survive on store shelves for more than a day or two. However, in recent times, old varieties that have a recognizable taste and aroma are increasingly "requested", such as tomato varieties: *Novosadski jabučar*, *Volovsko srce*, *Roma* and others, and pepper varieties *Kurtovska kapija*, *Dukat*, *Župska rana*, *Šorokšari*, *Somborka*, *Strižanka* or basil variety *Sitnolisni aromatični* and similar. One of the above-mentioned varieties is the *Volovsko srce* variety. This variety has taken a special place in our region primarily because of its specific taste and smell, but also because of the tradition in its consumption. It is a variety characterized by large fruits and specific color, and distinctive heart-like shape, which is how this variety got its name. One of the most important shortcomings in the production of this variety is the impossibility of long-term storage, due to the fact that its fruits ripen quickly and cannot withstand long-term transport. The second variety is the *Župska rana* pepper variety. *Župska rana* is a variety of early pepper. It is highly valued and cultivated in these areas. It is one of the tastiest pepper varieties. It often has good growth and stem height and is very yielding. The fruits are very long and fleshy and it is suitable for both greenhouse and field production. When the fruits are fully ripe, they are distinctly red in color. The third variety is the small-leaved aromatic basil variety. This variety of basil is the most cultivated in our country. It tolerates our agroecological conditions well, achieving a satisfactory yield and quality every year (Filipović et al., 2016). It is mostly used as a spice, but also in religious ceremonies and as an ornamental plant. In addition, it also contains significant amounts of essential oil of good quality. Since all three plant species, i.e. the mentioned varieties, are grown from seedlings for that purpose, it is necessary to provide appropriate growing media that can be used for the production of quality transplants (Filipović and Ugrenović, 2013). The market offers a large number of commercial substrate mixtures, which often have high prices and sometimes unsatisfactory quality. Choosing the substrate which can improve the plants' traits during the cultivation of selected tomato, pepper and basil varieties is another important task. Therefore, the aim of this research was to determine the possible influence of different autochthonous substrate mixtures on seedling quality and productivity of selected tomato, pepper and basil varieties.

MATERIAL AND METHODS

The following plant material was used in the research: tomato (*Lycopersicon lycopersicum* L.) variety *Volovsko srce* produced by "Superior" d.o.o. from Velika Plana, pepper (*Capsicum annum* L.) variety *Župska rana* produced by the Institute for Vegetable Crops from Smederevska Palanka and basil (*Ocimum basilicum* L.) variety *Sitnolisni aromatični* produced by the Institute for Medicinal Plant Research "Dr Josif Pančić" from Belgrade. Research was conducted during 2016 in the greenhouse and experimental field of the Institute for Medicinal Plant Research "Dr Josif Pančić" located in Pančevo (44°52'20"N; 20°42'06"E; 74 m.a.s.l.).

During mid February 2016, seeds for the production of seedlings were sown in the greenhouse without additional heating. The seedlings were transplanted into the four examined substrates and one commercial substrate after the appearance of the first regular leaf (end of March 2016). Seedlings were produced in plastic round pots with a diameter of 10.5 cm, a depth of 9.0 cm and a substrate volume of 525 cm³. After three months, more precisely in the middle of May 2016, developed seedlings were transplanted in the field, when the seedlings reached the most optimal stage for reception. Planting was done manually, for tomato/pepper at a distance between rows of 80 cm, and in a row 40 cm plant from plant, while for basil the distance between rows was 50 cm, and in a row 30 cm plant from plant. In the experimental field, oil flax was grown (*Linum usitatissimum* L.) as a pre-crop to all examined species. The trial was based on a completely randomized block design with a base plot size of 24.5 m² (10.0 m x 2.45 m) in four replications. The impact of different types of substrate mixtures was investigated and compared with the control variant: as a control variant, marshy black soil type (collected at the experimental location) was used. This soil has the following agrochemical characteristics: pH value = 5.4, humus content = 2.3%, P₂O₅ content 3.6 mg/100 g of soil and K₂O 36.2 mg/100 g of soil. The second variant was a mixture (25% marshy black soil type : 25% sand : 50% compost created from the waste obtained from green areas (parks and gardens) with the following agrochemical properties: pH in KCl = 6.98, humus content = 3.86%, CaCO₃ content = 3.0%, P₂O₅ 6.8 mg/100 g soil and K₂O 10.3 mg/100 g soil. The fourth variant was represented by the compost created during the production and processing of medicinal plants (IMPR – product of the Institute for Medicinal Plant Research "Dr. Josif Pančić" from Belgrade). The properties of this compost are: pH in KCl = 6.02, humus content = 3.86%, nitrogen = 2.2%, 0.46% P₂O₅ and 0.48% K₂O, 2%, Fe 0.6%, Zn 0.08% and 26% organic matter. The last, fifth variant was represented by the commercial substrate Klasmann Potgrond H, which is a mixture of black sphagnum peat and very fine white sphagnum peat to which soluble fertilizer and microelements have been added, pH values 5.5-6.5, 1, 5 kg/m³ has an average of 14% N, 16% P₂O₅ and 18% K₂O (Glamočlija et al., 2015). Meteorological data during the experimental vegetation period were obtained from the meteorological station of the Institute "Tamiš" Pančevo (Table 1).

Table 1. Meteorological data for vegetation period in 2016

Parameters	May	June	July	August	Sum / Average
Precipitation	26.8	160.6	103.2	14.2	304.8
Temperatures	18.2	23.3	23.0	21.1	21.1

During the growing season, classic measures of care of seedlings and old plants from pests, diseases and weeds were applied. The first watering was done immediately after planting, and subsequents were done as needed.

Ten plants were taken from each repetition (some in the seedling stage, some for the purposes of measuring examined traits in adult plants). In our research, the follo-

wing were measured: seedling height (cm), seedling leaf number, seedling stem thickness at the base (mm), seedling root mass (g/plant), fruit width (cm), fruit length (cm) and fruit/fresh herb yield (g/plant). In the case of tomatoes and peppers, only three harvests were carried out, while the other harvests were not recorded, which, depending on the year, range from 10 to 15. Tomato and pepper fruits were harvested at the technological stage of ripening, and the basil plants were harvested by hand with a sickle at the cut height of 8 cm above the ground. The first mowing of the above ground mass of basil was done in full bloom at the end of July. The second harvest was done at the beginning of October (Filipović et al., 2016). The obtained data were processed with indicators of descriptive statistics: mean value, standard deviation (StdDev) and coefficient of variation (Cv). Statistical analysis was performed with the statistical software Statistica for Windows 10 (STATISTICA, 2010).

RESULTS AND DISCUSSION

The influence of different substrate mixtures on various traits of seedlings and fruits of tomato *Volovsko srce* variety is shown in table 2.

Table 2. Different mixtures of substrates and parameters nursery-plants and tomato fruit

Tretmans	Nursery-plants – Protected area				Open field					
	Height of seedlings (cm)	Leaf number per plant	The thickness of the stem in the grounds (mm)	Mass of seedling root (g/plant)	Width fruit (cm)	Fruit length (cm)	First harvest - fruit yield (g/plant)	Second harvest - fruit yield (g/plant)	Third harvest - fruit yield (g/plant)	Fruit yield per plant (g/plant)
Control	16.0	6.0	6.8	1.1	6.1	6.2	218.6	330.8	259.5	808.9
Mixture (25:25:50)	19.7	7.3	7.6	1.6	8.9	8.0	355.4	345.3	236.4	937.1
Compost – plant and wood waste	22.2	7.8	8.2	2.0	7.2	6.7	210.4	308.2	339.9	858.5
Compost – IMPR	22.7	8.2	8.4	2.0	11.3	9.0	491.8	518.9	436.0	1446.7
Potgrond H	23.2	8.7	9.4	2.1	5.3	8.4	213.3	312.0	288.2	813.5
Average	20.8	7.6	8.1	1.8	7.7	7.7	297.9	363.0	311.9	972.9
StdDev	2.789	0.974	0.901	0.434	2.59	1.51	140.5	109.34	100.60	307.80
Cv (%)	13.42	12.82	11.17	24.61	33.50	19.74	47.18	30.12	32.25	31.64

The highest tomato seedling height was achieved by the plants grown on the Potgrond H substrate (23.2 cm). Seedlings produced on compost IMPR (22.7 cm) and compost from plant and wood waste (22.2 cm) had almost identical seedling heights. As expected, the lowest tomato seedling height was recorded in the control treatment (16.0 cm). The highest number of leaves per plant was recorded for the Potgrond H substrate (8.7), and the lowest number of leaves per plant was recorded in the control variant (6.0). A similar trend was observed for the stem thickness at the base and the root mass of the tomato seedlings of the *Volovsko srce* variety. In the open field, the widest and longest tomato fruits were achieved by plants produced by transplants grown on the compost IMPR, which were 11.3 cm wide on average and 9.0 cm long, respectively 8.9 cm wide and 8.0 cm long. Tomato plants produced from transplants grown on Potgrond H substrate had the smallest average fruit width (5.3 cm), while the smallest fruit length was recorded in the control treatment (6.2 cm). Regarding the most productive harvest, the best results were achieved during the second harvest, with an average of 363.0 g of fruit weight per plant. Overall, the highest individual yield of tomato fruits after three harvests was achieved by plants whose seedlings were produced on compost IMPR (1446.7 g/plant), while the lowest fruit yield per plant was recorded on plants from the control treatment (808.9 g/plant). As can be seen, in the examined year, the agroecological conditions were favorable for the cultivation of this tomato variety. *Volovsko srce* is an early tomato variety, and it ripens in 80 days from planting. Some amounts of cow manure have an extremely good effect on the yield of tomatoes (Castro Pacheco, 2021). Growing tomato seedlings on different substrate mixtures, with a special emphasis on mixtures with vermicompost, gives satisfactory results, and the best results are obtained with the combination of vermicompost and zeoplant (Đorđević et al., 2004). In Malek's research (2012), the *Volovsko srce* variety achieved a yield of 38 t/ha when treated with 40 g/plant of cow manure, which is an economically justified yield when growing tomatoes. Fruit yield of *Volovsko srce* cultivar at Bangladesh Agricultural University farm after induced mutation treatment had yield per plant ranging from 2.2 to 3.9 kg (Nahyan et al., 2014).

The influence of different substrate mixtures on various traits of seedlings and fruits of the *Župska rana* pepper variety is shown in table 3.

When it comes to the production of pepper seedlings of the *Župska rana* variety, the best results (seedling height, number of seedling leaves, seedling stem thickness at the base and seedling root mass) were achieved by plants that were grown on the substrate Potgrond H. Slightly lower values of the mentioned indicators were recorded for seedlings that were grown on IMPR compost and compost from plant and wood waste. The lowest values of examined traits of pepper seedlings were achieved by plants produced on a mixture-based substrate and on the control treatment. As for the cultivation of pepper variety *Župska rana* in the open field, the largest fruits (the highest values of width and length) were recorded in plants with seedlings produced on the IMPR compost based mixture and seedlings produced on the substrate Potgrond H. The weight of the fruit, as well as the number of fruits per plant, play a decisive role in the formation of the yield of peppers. Overall, for all three harvests, the highest yield of pepper fruits was achieved using transplants grown on substrate based on IMPR compost (283.9 g/plant). Next in rank was the yield achieved by plants whose seedlings

Table 3. Different mixtures of substrates and parameters, nursery-plants and pepper fruit

Tretmans	Nursery-plants – Protected area				Open field					
	Height of seedlings (cm)	Leaf number per plant	The thickness of the stem in the grounds (mm)	Mass of seedling root (g/plant)	Width fruit (cm)	Fruit length (cm)	First harvest - fruit yield (g/plant)	Second harvest - fruit yield (g/plant)	Third harvest - fruit yield (g/plant)	Fruit yield per plant (g/plant)
Control	15.8	5.9	6.3	0.16	4.1	7.5	30.7	37.7	32.7	101.1
Mixture (25:25:50)	18.5	6.9	7.0	0.17	5.7	11.0	63.4	76.3	58.6	198.3
Compost – plant and wood waste	20.7	8.1	7.5	0.19	4.8	7.9	35.1	57.1	48.4	140.6
Compost – IMPR	20.1	8.7	8.1	0.20	5.7	11.2	91.9	112.6	79.5	283.9
Potgrond H	20.8	9.4	8.4	0.20	5.3	11.0	71.6	84.0	52.0	207.5
Average	19.2	7,80	7.44	0.18	5.12	9.7	58.5	73.5	54.2	186.3
StdDev	1.971	1.334	0.806	0.019	0.853	2.110	28.575	34.582	21.544	73.481
Cv (%)	10.28	17.12	10.84	10.66	16.69	21.69	48.82	47.04	39.74	39.45

were produced on the Potgrond H substrate (207.5 g/plant), which was about 27% less, while the lowest fruit yield per pepper plant was recorded on plants from the control treatment (101,1 g/plant). In research by Đukić et al. (2003) the average length of the fruit of the variety Župska rana was 16.7 cm, and the width was 4.95 cm, while the weight of the fruit was 94.65 g. This pepper variety can be successfully grown in conventional as well as organic growing conditions in which it achieves more than satisfactory results (Bicikliski et al., 2018).

The influence of different substrate mixtures on examined traits of seedlings and yield of the fresh above-ground part of basil plant of the *Sitnolisni aromatični* variety is shown in table 4.

As with the previous two plant species, the best results of the examined morphological traits (height of seedlings, number of leaves, thickness of the stem and mass of seedling roots) were achieved by the plants grown on the Potgrond H substrate, which are closely followed by the values of seedlings grown on the IMPR based compost. The control treatment had the lowest values for examined traits, but also for both individual and total yield of fresh above-ground biomass. The highest values (563.4 g/plant) of the total yield of the fresh above-ground biomass were obtained from plants whose seedlings were grown on IMPR compost, while the lowest values were achieved by plants whose seedlings were grown on compost from vegetable and wood. waste (308.4 g/plant), in the control (327.0 g/plant) and on the substrate Potgrond H (331.7 g/plant).

Table 4 Different mixtures of substrates and parameters nursery-plants and basil herba

Tretmants	Nursery-plants – Protected area				Open field		
	Height of seedlings (cm)	Leaf number per plant	The thickness of the stem in the grounds (mm)	Mass of seedling root (g/plant)	Fresh aboveground yield – First harvest (g/plant)	Fresh aboveground yield – Second harvest (g/plant)	Total fresh aboveground yield (g/plant)
Control	9.2	6.3	3.4	0.07	180.8	146.2	327.0
Mixture (25:25:50)	9.5	6.9	3.9	0.11	229.9	203.5	433.3
Compost – plant and wood waste	10.0	7.5	4.2	0.13	152.8	155.6	308.4
Compost – IMPR	10.6	7.6	4.3	0.13	265.7	297.7	563.4
Potgrond H	11.4	8.0	4.3	0.13	179.8	151.9	331.7
Average	10.1	7.3	4.0	0.11	201.8	191.0	392.8
StdDev	0.846	0.616	0.361	0.028	52.100	67.863	110.105
Cv (%)	8.36	8.49	9.02	25.17	25.82	35.54	28.03

The results obtained in the research of Čolik (2015) showed that there is a significant difference between basil seedlings grown on soil and on substrate. In this case too, the quality of the substrate affected the yield of basil. Thus, the mass of the fresh basil leaf grown on the substrate was 24.57 g, which is 9.38 g more than the one grown on the soil. Beatović et al. (2009) conducted a study on the effect of different substrates on basil yield, in which the most suitable substrate for basil production was a combination of Gaj peat and manure in a ratio of 70%:30%. Hewidy et al. (2014) conducted a study in which loamy soil was enriched with peat, compost and vermicompost. The best results when growing basil seedlings were obtained in a combination of soil and compost 70%:30%. The achieved values were in accordance with the values obtained during fertilizing with vermi-compost and biological fertilizers in the research of Rezaee and Nabavi (2012).

CONCLUSION

Based on the conducted research and analysis of the data obtained during the application of different substrate mixtures on seedling quality and productivity of selected tomato, pepper and basil varieties, it was concluded that the best results of the examined traits (seedling height, number of leaves, thickness of the stem and mass

of seedling roots) were achieved in plants that were grown on the substrate Potgrond H. Satisfactory results were also achieved using seedlings grown on the IMPR based compost mixture. As for the productive traits of the tomato crop, the highest yield of fruits was achieved using seedlings grown on the IMPR compost based mixture, while the lowest yield of fruit per plant was recorded for plants from the control treatment. The same situation was recorded when measuring the yield of pepper fruits and the yield of the fresh aboveground biomass of basil, where the highest values of this trait were achieved in plants produced on IMPR compost based mixture.

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Оригинални научни рад

УТИЦАЈ РАЗЛИЧИТИХ СУПСТРАТНИХ СМЕША НА КВАЛИТЕТ РАСАДА И ЕКОНОМИЧНО ВИСОК ПРИНОС ПАРАДАЈЗА, ПАПРИКЕ И БОСИЉКА

Резиме

У раду је истраживан утицај различитих супстратних смеша на квалитет расада и продуктивност усева парадајза, паприке и босиљка. Истраживања су обављена на локацији колекције Института за проучавање лековитог биља „Др Јосиф Панчић“ у Панчеву, у току 2016. године. Испитивано је четири варијанте супстрата и контролна варијанта (земљиште без супстрата). Најбоље карактеристике морфолошких показатеља оствариле су биљчице произведене на комерцијалном супстрату за производњу расада Класманн Поттронд Х. С друге стране, биљке које су произведене на компосту насталом у производњи и преради лековитог биља, код свих испитиваних врста имале су највеће плодове (парадајз и паприка) и принос свежег надземног дела (босиљак).

Кључне речи: *супстратне смеше, парадајз, паприка, босиљак, принос*

**INTEGRATED PLANTS PROTECTION,
PESTICIDES APPLICATION AND EFFECTS
OF RESIDUES**



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Original Scientific paper

APPLICATION OF NORMAL-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF SOME PESTICIDE RESIDUES IN APPLE JUICE

Abstract

This paper describes a new, precise and accurate normal-phase high-performance liquid chromatography (NP-HPLC) method with ultraviolet-diode array detector (UV-DAD) for determination of some organophosphorus insecticide residues in different apple juice samples. The analyses were performed using a LiChrosorb CN analytical column (250 x 4 mm; 5 µm), a mobile phase composed of *n*-hexane and dichloromethane with a volume ratio (80/20, V/V), a flow rate of 1 mL/min, a constant column temperature of 25 °C and UV detection at 220 and 270 nm. The obtained results showed that analysed apple juice samples did not contain detectable residues of investigated pesticides.

Key words: NP-HPLC method, organophosphorus insecticide residues, apple juice samples

INTRODUCTION

Organophosphorus insecticides are widely used because of their relatively low toxicity, but their improper use can cause their presence in agricultural products, serious environmental pollution, as well as human health impairment. Through primary agricultural products, such as fruits and vegetables, they can also be found in processed products for human consumption, such as fruit juices. Apple juice is one of the most used juices in the world with many positive effects on human health. Maximum Residue Levels (MRLs) for pesticides in food are legally regulated in most countries

to guarantee consumer safety. In the European Union, the MRLs are determined by Regulation No. 396/2005 (2005), and those for apple and apple products amount to: 0.05 mg/kg for parathion, 0.01 mg/kg for fenitrothion, and 0.02 mg/kg for malathion.

Analytical methods for the determination of pesticides at very low concentrations are particularly needed for monitoring pesticide residues in food and environmental samples. The most applied analytical methods for the determination of pesticide residues are GC (Gas Chromatography) and LC (Liquid Chromatography) equipped with various detectors (Alder, et al., 2006). However, it is usually necessary to sample preparation, such as extraction or concentration of the sample before their chromatographic determination.

In previous research, we developed and validated several analytical methods for the determination of some pesticide residues in water and apple juice using reversed-phase high-performance liquid chromatography (RP-HPLC) (Velkoska-Markovska et al., 2018a, 2018b, 2020), but because the solubility of parathion, fenitrothion and malathion in non-polar solvents, such as *n*-hexane is very good, the aim of this paper was to investigate the possibility of their determination in apple juice by means of normal-phase high-performance liquid chromatography (NP-HPLC).

MATERIAL AND METHODS

Reagents and Chemicals. The Pestanal analytical standards of parathion (98.8 % purity), fenitrothion (95.2 % purity) and malathion (97.2 % purity) were manufactured by Sigma-Aldrich (Germany), and HPLC-grade *n*-hexane and dichloromethane were purchased by Merck (Germany). For analysis, 100 % apple juice samples, produced by three different producers (A, B, C), were purchased from local supermarkets.

Equipment. The HPLC analyses were carried out using an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography system equipped with: vacuum degasser (G1322A), binary pump (G1312B), autosampler (G1329B), a thermostatted column compartment (G1316A), UV-VIS diode array detector (G1316B) and ChemStation software. An analytical column of the type LiChrosorb CN (250 x 4 mm, 5 mm) produced by Merck (Germany) was used for the chromatographic separation and determination of pesticide residues in apple juice samples. An ultrasonic bath “Elma” was used for the better dissolving of the stock and sample solutions, and for the solid-phase extraction (SPE) was used a vacuum manifold Visiprep (Supelco, Sigma-Aldrich).

Preparation of Stock Solutions. The stock solutions of parathion, fenitrothion and malathion were prepared by separately dissolving of 0.0113, 0.0173 and 0.0223 g, respectively, of the pure analytical standards in *n*-hexane in 25 mL volumetric flasks. The solutions were degassed for 15 min in an ultrasonic bath and kept in a refrigerator at 4 °C. These stock solutions were used to prepare a series of working standard solutions and for fortification of apple juice samples in order to test method validation.

Sample preparation. Before the analysis, the apple juice samples were filtered through a 0.45 µm nitrocellulose membrane filter (Millipore, Ireland). For the method validation, specificity, selectivity, linearity, precision expressed as repeatability of the

retention time, peak area and peak height, and accuracy were tested. For that purpose, 1 kg of apple juice samples were prepared, by spiking with three sets of concentrations for each analysed pesticide, namely: 0.035; 0.05 and 0.06 mg/kg (for parathion); 0.007; 0.01 and 0.012 mg/kg (for fenitrothion); and 0.014, 0.02 and 0.024 mg/kg (for malathion). Samples to which no pesticides have been added were used as blanks. For each concentration level, 5 samples were prepared ($n = 5$). In order to quantitatively determine the presence of analysed pesticide residues, the analytes were concentrated by solid-phase extraction using Supelclean ENVI-18 columns (0.5 g, 6 mL, Supelco), and then NP-HPLC analysis was performed. Volume of 20 μ L was injected from each sample. The solid-phase extraction procedure consists of the following steps: conditioning the columns (by passing 5 mL of acetonitrile and then 5 mL of water at a flow rate of 2 mL/min); passing the sample (1 kg of apple juice sample was passed through the conditioned column at a flow rate of 8-10 mL/min); washing the column (by passing 5 mL of water); drying the column (under vacuum for 20 min); and elution of the components of interest (with two portions of 2 mL each of acetonitrile). The eluates were evaporated to dryness using nitrogen at a temperature of 40 °C, and then the dry residue was dissolved with 1 mL of *n*-hexane using a Vortex for 1 min. Before performing the HPLC analysis, the final extract was filtered through a 0.45 μ m Iso-Disc PTFE syringe filter (Supelco) and transferred into appropriate vials for analysis. The volume of 20 μ L was injected from each sample.

RESULTS AND DISCUSSION

As a result of the fact that the solubility of parathion, fenitrothion and malathion in *n*-hexane is very good, the possibility of developing NP-HPLC method for the qualitative and quantitative determination of parathion, fenitrothion and malathion in apple juice samples was considered. An intermediate LiChrosorb CN column (250 x 4 mm, 5 μ m) was used for the development of the NP-HPLC method. LiChrosorb CN represents an intermediate stationary phase that can be used with typical normal-phase and reversed-phase eluents. When using the reverse-phase mode of chromatography, the properties of this stationary phase are similar to those of silica gel, but the presence of nitrile groups changes the selectivity of silica gel. In relation to certain groups of compounds, cyanopropyl-silica gels possess a satisfactory retention ability both in normal-phase and in reverse-phase chromatography mode (Lough and Wainer, 1996).

Non-polar solvents, such as *n*-hexane and dichloromethane, in different volume ratios were used for the preparation of the mobile phase. Moreover, in order to develop a simple NP-HPLC method, the chromatographic process was conducted by applying isocratic elution, at a constant column temperature of 25 °C.

The choice of the wavelength at which the chromatographic analyses were performed was based on the UV spectra of the components of interest recorded in a solution of *n*-hexane and dichloromethane, with a volume ratio of 80/20 (Figure 1).

As can be seen from the recorded UV spectra, the three components: parathion, fenitrothion and malathion show a band with a maximum at a wavelength around 220 nm (Figure 1). For the components parathion and fenitrothion, one more band was

observed, the maximum of which lies at a wavelength around 280 nm for parathion and 270 nm for fenitrothion. For these reasons, the HPLC analysis for the simultaneous determination of parathion, fenitrothion and malathion in apple juice samples with a normal-phase elution mode was carried out at a wavelength of 220 nm.

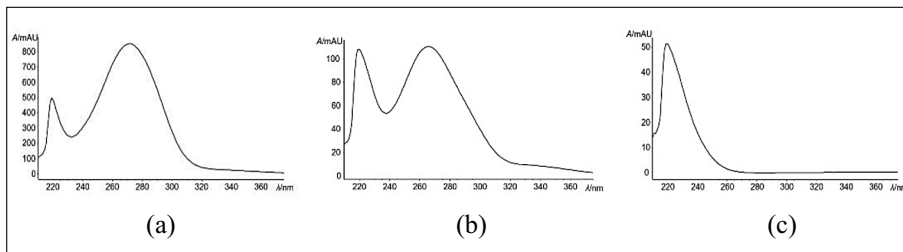


Figure 1. UV spectra of parathion (a), fenitrothion (b) and malathion (c) recorded in a solution of *n*-hexane and dichloromethane, with a volume ratio of 80/20

Additionally, the chromatographic process was also monitored at 270 nm because fenitrothion and parathion show maximum absorption and greater intensity of their chromatographic peaks at this wavelength. A series of preliminary tests have been performed in order to obtain optimal conditions for the separation of analytes with a symmetrical shape of the chromatographic peaks and satisfactory values for the purity index. For this purpose, the volume ratio of *n*-hexane and dichloromethane in the mobile phase was changed. The optimal conditions for the separation of parathion, fenitrothion and malathion by means of normal-phase liquid chromatography under isocratic elution on an analytical column LiChrosorb CN (250 mm x 4 mm; 5 μm) were obtained using a mobile phase composed of *n*-hexane and dichloromethane with volume ratio (80/20, *V/V*), flow rate of 1 mL/min, constant column temperature of 25 °C and UV detection at 220 nm and 270 nm (Figure 2). Under these experimental conditions, the baseline was calm, without pronounced noises, and the chromatographic

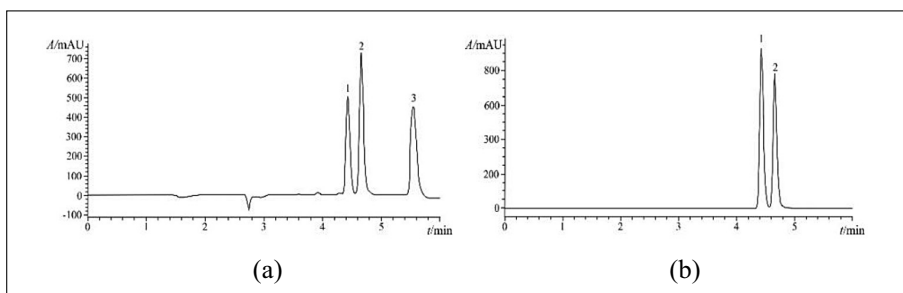


Figure 2. Chromatograms obtained from a standard mixture of 0.89 μg parathion (1); 1.31 μg fenitrothion (2) and 3.46 μg malathion (3) at 220 nm (a) and 270 nm (b) on a LiChrosorb CN column (250 mm x 4 mm; 5 μm)

peaks were sharp, symmetrical and completely separated (Figure 3). In support of this finding are the calculated values for the separation factor (α) of two adjacent chromatographic peaks ($\alpha > 1$) and the resolution of adjacent peaks ($R_s > 2.90$) (Table 1). The values for the dead time of the column (t_0), the retention time (t_R) of the analytes, their retention factor (k'), the separation factor (α) and the resolution (R_s) of the adjacent peaks are shown in Table 1. The time required for this analysis was about 6 min.

Method validation was performed according to EU regulations and EU documents (European Commission, 2010; Document, 2011), and for that purpose, the specificity, selectivity, linearity, precision, accuracy and limit of quantification were tested. The validation of developed method was carried out at 220 nm for the malathion, and at 270 nm for the parathion and fenitrothion.

Table 1. Data on retention time (t_R), retention factor (k'), separation factor (α) and resolution (R_s) of the examined components obtained by the developed method

Compound	t_R /min	k'	α	R_s
parathion	4.40	0.64	1.12	2.90
fenitrothion	4.62	0.72	1.46	9.49
malathion	5.49	1.05	–	–
<i>n</i> -hexan/dichloromethane (80/20, V/V)	2.68	–	–	–

Specificity and selectivity. To confirm the specificity of the developed method for determination of analysed residues in apple juice samples, UV-diode array detection was used to check the peak purity and analyte peak identity. The purity index for all analytes was greater than 999, which means that the chromatographic peak was not affected by any other compound. Identification of the components of interest was performed by comparing the retention times of the analytical standards with those of the same components in the apple juice sample and by monitoring the values for the match factor obtained by overlapping the UV spectra of the pure analytical standard and the absorption spectrum of the same analyte present in apple juice samples. The identity of the analytes was also confirmed by the obtained values for the matching factors (> 998). According to EU criteria ((European Commission, 2010), to further prove the selectivity of the method, in Figure 3 presented chromatograms of a standard mixture of the components of interest with a concentration corresponding to the MRL (a), a blank sample (sample of apple juice in which no pesticides were added) (b) and an apple juice sample spiked with pesticides at a concentration equal to the MRL (c).

Linearity. The linearity of the developed method was determined by construction of calibration curves with triplicate injections (20 μ L) of the spiked apple juice samples in the range from 30 % less than MRL to 20 % above (Table 2). The calculated results for multiple correlation coefficients ($R^2 \geq 0.99$) indicated that the proposed method

has a good linearity. However, the values obtained for the multiple correlation coefficients (R^2) when the peak area was taken as dependent variable were greater than that obtained when the peak height was taken as dependent variable. Hence, the further calculations were performed according to the peak area.

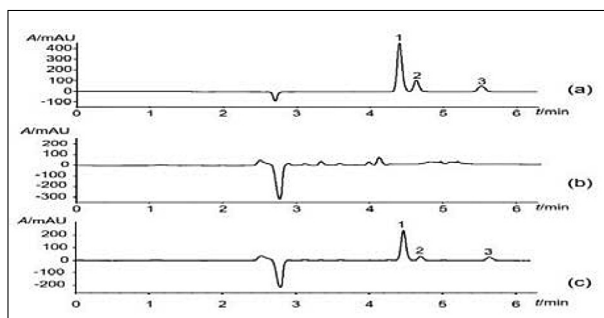


Figure 3. Chromatograms obtained from a standard mixture of parathion (1), fenitrothion (2) and malathion (3) with a concentration equal to the MRL (a), a blank sample (b) and a sample of apple juice enriched with pesticides with a concentration equal to the MRL (c) at 220 nm

Table 2. Statistical data for linearity of the method

Compound	Linearity range (mg/kg)	Regression equation	R^2
Parathion	0.035 – 0.05	$^1y = 83864x - 2866.4$	0.9982
		$^2y = 19210x - 665.38$	0.9980
Fenitrothion	0.007 – 0.01	$^1y = 33818x - 199.31$	0.9988
		$^2y = 14678x - 101.99$	0.9969
Malathion	0.014 – 0.02	$^1y = 19783x - 207.4$	0.9984
		$^2y = 5322.7x - 74.432$	0.9936

1y = peak area, 2y = peak height

Limit of quantification. The limit of quantification (LOQ) was determined by spiking an apple juice sample with concentration correspond to 30 % less of MRL for each analyte. The signal-to-noise ratio (S/N) at this concentration level was found to be > 10 and therefore, the LOQ was estimated to be 0.035, 0.007 and 0.014 mg/kg of parathion, fenitrothion and malathion, respectively, in apple juice sample, which is acceptable for determining the pesticide residues according to the EU rules ((European Commission, 2010). *Precision.* The precision was expressed as repeatability of obtained results from five successive injections (20 μ L) of the spiked apple juice samples at MRL of each analyte (Table 3). The values of RSD for retention time, peak area and peak height indicated a good precision of the method.

Tab. 3. Statistical data for Intra-day precision retention time, peak area and height, n=5

Compound		\bar{x}	SD	RSD (%)
Parathion	retention time (min)	4.40	0.002	0.05
	peak area	1372.26	29.98	2.18
	peak height	284.26	6.81	2.39
Fenitrothion	retention time (min)	4.62	0.006	0.13
	peak area	136.23	10.83	7.95
	peak height	42.76	3.22	7.53
Malathion	retention time (min)	5.49	0.016	0.29
	peak area	179.21	19.70	10.99
	peak height	28.67	2.76	9.64

Table 4. Results from recovery experiments (n = 5)

Compound	Fortification level ($\mu\text{g}/\text{kg}$)	Total analyte found ($\mu\text{g}/\text{kg} \pm \text{SD}$)	Recovery (%)	RSD (%)
Parathion	0.035	0.0347 ± 0.000006	99.19	0.19
	0.05	0.0505 ± 0.00036	101.08	0.71
	0.06	0.0598 ± 0.00057	99.74	0.95
Fenitrothion	0.007	0.0070 ± 0.00016	99.69	2.25
	0.01	0.0099 ± 0.00032	99.22	3.23
	0.012	0.0119 ± 0.00015	99.65	1.27
Malathion	0.014	0.0138 ± 0.00033	98.78	2.39
	0.02	0.0195 ± 0.00099	97.71	5.10
	0.024	0.0232 ± 0.0012	96.59	5.33

Accuracy. The method accuracy was determined by recovery studies in apple juice samples spiked with the analysed pesticides at three concentration levels (Table 4). Obtained values for recovery and for relative standard deviation were within following ranges 96.59–101.08% and 0.19–5.33%, respectively, and they are in accordance with the EU criteria ((European Commission, 2010). Consequently, it can be concluded that the proposed method was accurate and suitable for the determination of the target pesticide residues in apple juice samples. The method has been successfully applied to the analysis of residues of the investigated pesticides in different apple juice samples, produced by different manufacturers. No residues of parathion, fenitrothion and malathion were found in the analysed samples in a concentration corresponding to the MRL or higher.

CONCLUSIONS

A new possibility of successful determination of parathion, fenitrothion and malathion residues in apple juice samples using NP-HPLC method and UV-DAD has been presented. The proposed method carried out on a LiChrosorb CN analytical column (250 x 4 mm; 5 µm) using a mobile phase composed of *n*-hexane and dichloromethane with a volume ratio (80/20, *V/V*), a flow rate of 1 mL/min, a constant column temperature of 25 °C and UV detection at 220 nm and 270 nm can be used to qualitatively and quantitatively determine the tested pesticides in apple juice samples in concentrations corresponding to the MRL, after solid-phase extraction. Obtained results showed that analysed samples did not contain detectable residues of investigated pesticides.

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Оригинални научни рад

ПРИМЕНА ТЕЧНЕ ХРОМАТОГРАФИЈЕ ВИСОКИХ ПЕРФОРМАНСА НОРМАЛНЕ ФАЗЕ НА АНАЛИЗУ НЕКИХ ОСТАТАКА ПЕСТИЦИДА У СОКУ ОД ЈАБУКЕ

Извод

Овај рад описује нову, прецизну и тачну течну хроматографију високих перформанси нормалне фазе (NP-HPLC) са ултраљубичастим диодним детектором (UV-DAD) за одређивање неких остатака органофосфорних инсектицида у различитим узорцима сока од јабуке. Анализе су обављене коришћењем Lichrosorb CN аналитичке колоне (250 x 4 mm; 5 µm), мобилне фазе састављене од *n*-хексана и дихлорометана са запреминским односом (80/20, V/V), протоком од 1 mL/min, константна температура колоне од 25 °C и UV детекција на 220 и 270 nm. Добијени резултати су показали да анализирани узорци сока од јабуке не садрже детектабилне остатке испитиваних пестицида.

Кључне речи: *NP-HPLC метода, остаци органофосфорних инсектицида, узорци сока од јабуке*



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Original Scientific paper

SPINOSAD APPLICATION IN PROCESS OF INTEGRATED PEST MANAGEMENT AGAINST *RHYZOPERTHA DOMINICA* F. IN STORED SMALL GRAINS

Abstract

This study aimed to evaluate insecticidal activity of Spinosad applied at doses of 0.25, 0.5, and 1.0 mg kg⁻¹ (ppm) on *Rhyzopertha dominica* in wheat, barley, rye, oats and triticale grains. Mortality was assessed after 7 and 14 days, while impact on progeny production was assessed after 8 weeks. In the lowest dosage after 7 days of exposure, Spinosad application affected mortality ranged from 94.5% (wheat) to 100% (barley). After 14 days, all doses of Spinosad achieved mortality of 100%. All three applied dosages of Spinosad prevented progeny emergence. Damaged grain and dust in Spinosad treated samples were not recorded which represent the most ideal small grain protection. Spinosad has been identified as a natural and promising alternative to stored-grain protectants.

Key words: *R. dominica*, small grains, Spinosad, insecticidal activity

INTRODUCTION

Pest infestation has the great influence on stored grain goods. The lesser grain borer *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) is the major cause of insect damaged kernels (IDK) in wheat. *R. dominica* spends most of its life inside kernels, feeding on its endosperm, which causes damage and changes in grain physicochemical properties. Adult feeding activities produce large amounts of dockage, most of which consists of ovoid granules of undigested endosperm mixed with a finer floury part.

Application of chemical insecticides has a crucial role in the quick and efficient control of this pest, which is followed by the occurrence of undesirable residues in grains and resistance of particular populations of stored-product pests. The aforementioned problems demand the necessity of finding alternative, environmentally friendly solutions, and omit or significantly reduce negative effects of the application of synthetic insecticides. Natural originating insecticides, such as Spinosad, will contribute to solving these problems in the near perspective. Spinosad is a natural product, obtained by fermentation of soil actinomycete, *Saccharopolyspora spinosa* Mertz & Yao. Resistance of *R. dominica* to Spinosad has not yet been found (Andrić et al., 2019).

This is a primary pest which can destroy stored wheat completely. Perišić et al. (2017) were concluded that small grains – wheat, barley, rye, and triticale can be considered as "nutritionally suitable" for *R. dominica* development, but triticale is the most susceptible to attack and suitable for development of *R. dominica*. Based on the aforementioned, it is important to determine is the protection of all stored small grains can be uniform or it must take care of grain species, especially during application of natural originated insecticides and because of covered way of life as specificity of *R. dominica* (life cycle mainly spends in the grain feeding by grain endosperm). Special attention must be direct to the "grain effect", i.e. variation in the pest sensitivity caused by different grain species.

MATERIALS AND METHODS

The experiment was conducted in the Center for Small Grains in Kragujevac. The tested adult population of *R. dominica* was reared on the whole wheat kernels under laboratory conditions (temperature $26\pm 1^\circ\text{C}$ and relative humidity – r.h. $60\pm 5\%$). In the experiment 2-4 weeks old adults were used. Five species of small grains were tested: wheat (Vizija variety), barley (Rekord variety), oats (Vranac variety), rye (Raša variety) and triticale (Favorit variety). All varieties originate from the Center of Small Grains Kragujevac, Serbia. Grain samples with the moisture content ranging from 11-12 % were included in the experiment.

For examination of insecticidal efficacy pesticide Laser 240 SC (active substance Spinosad-240g/L;Dow AgroSciences, Austria) was used. Efficacy of applied insecticide on small grains was determined according to methods for evaluation of the biological efficacy of insecticides in storage pests suppression (OEPP/EPPO, 2004a,b). The 0.5 kg lots of each grain samples were weighed on an analytical balance (Mettler 609-B6, Zurich, Switzerland), and placed into jars (1000 ml in volume). Afterward, grain samples were treated with 5 ml of aqueous solutions of Spinosad in amounts of 0.25; 0.5 and 1.0 mg kg⁻¹ (ppm) of grain. Grain labeled as a control A was treated with 5 ml of distilled water, while grain used as a control B stayed untreated. Firstly, treated grain was manually shaking for 30 seconds and afterward were mixed on a rotary mixer for 10 min.

Plastic vessels (200 ml in volume) were filled with 50 g of treated, as well as untreated grain, and placed in a thermostat chamber with controlled conditions, 26

$\pm 1^{\circ}\text{C}$ temperature and $60 \pm 5\%$ r. h. After 24 h, 25 adults of *R. dominica* were released into each vessel (except in control B) and the vessel was topped with a cotton cloth and fixed with a rubber band. Four plastic vessels was set up for every variant, i.e. four replications. Insect mortality was determined after 7 and 14 days of contact with treated or untreated grain types. After the last mortality count, both dead and living adults were removed and all vessels were returned to the incubator for 8 additional weeks under the same (described) conditions.

After 8 weeks, progeny emergence/suppression was determined by counting insects in treated and control grain samples. During F_1 generation counting, whole grain, damaged grain, and dockage from each vessel were separated and weighed on an analytical scale (Mettler 609-B6, Zurich, Switzerland). The whole procedure was repeated twice. Recorded data of insecticide efficacy were expressed as percentage mortality with calculated standard error ($\% \pm \text{SE}$). Before analysis, the percentage of mortality was transformed using *arcsine*, while data for the amount of insect-damaged grains and dockage were transformed by a square root. Differences in means for all data were statistically analyzed with one-way ANOVA and Tukey-Kramer (HSD) post hoc test (at $P = 0.05$).

RESULTS

In the lowest dosage, after 7 days of exposure, Spinosad application affected mortality from 94.5% (wheat) to 100% (barley). The high mortality of *R. dominica* ($\geq 98\%$) with dosages application of 0.5 and 1 mg kg^{-1} in all tested grain species was achieved. Spinosad application in the dosage of 1 mg kg^{-1} caused 100% of the *R. dominica* mortality, except in triticale where mortality was slightly lower (99.5%). In the present research, Spinosad application caused 100% of *R. dominica* mortality after 14 days of exposure, for every applied rate, in all examined grain species (Table 1).

Table 1. Mortality of *R. dominica* adults ($\% \pm \text{SE}$) after 7 and 14 days of exposure in different grain types treated with Spinosad

Insecticide	Dosage (mgkg^{-1})	Mortality ($\% \pm \text{SE}$) after exposure in small grains				
		Wheat	Barley	Rye	Oat	Triticale
		After 7 th day of exposure				
Spinosad	0.25	94.5 \pm 0.2 ^{b*}	100.0 \pm 0.0 ^a	97.5 \pm 0.2 ^b	96.5 \pm 0.3 ^b	95.5 \pm 0.2 ^b
	0.5	99.0 \pm 0.2 ^a	100.0 \pm 0.0 ^a	99.5 \pm 0.0 ^{ab}	99.5 \pm 0.0 ^{ab}	98.0 \pm 0.0 ^{ab}
	1.0	100.0 \pm 0.0 ^a	100.0 \pm 0.0 ^a	100.0 \pm 0.0 ^a	100.0 \pm 0.0 ^a	99.5 \pm 0.0 ^a
Control A	0	2.0 \pm 0.1 ^c	1.0 \pm 0.1 ^b	2.0 \pm 0.1 ^c	1.0 \pm 0.1 ^c	1.0 \pm 0.1 ^c
<i>F</i>		719.30	91823	457.78	341.83	266.37
<i>P</i>		< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

		After 14 th day of exposure				
Spinosad	0.25	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a
	0.5	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a
	1.0	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a	100.0±0.0 ^a
Control A	0	2.0±0.1 ^b	1.0±0.1 ^b	2.0±0.1 ^b	1.0±0.1 ^b	1.0±0.1 ^b
<i>F</i>		686458	918203	686458	918203	91203
<i>P</i>		<0.05	<0.05	<0.05	<0.05	<0.05

* Within every period of exposure, values across columns marked with same letters do not show a statistical difference; Tukey-Kramer (HSD) test for $P>0.05$; $df=7.56$. Control A – untreated, infested grain with *R. dominica*

Ten weeks after the treatment, all three applied dosages of the examined insecticide prevented progeny emergence. The suitability of grain species for *R. dominica* development was varying. The greatest progeny number in control samples was determined in triticale (432.5), followed by rye (177.1) and wheat (128.5), while oats had the lowest suitability (36.4) (Table 2).

Table 2. Average number of the *R. dominica* progeny in grains (\pm SE) ten weeks after insecticide Spinosad treatment

Insecticide	Dosage (mg kg ⁻¹)	Progeny number average (\pm SE) in small grains				
		Wheat	Barley	Rye	Oat	Triticale
Spinosad	0.25	0	0	0	0	0
	0.5	0	0	0	0	0
	1.0	0	0	0	0	0
Control A		128.5±1.0	82.1±0.8	177.1±0.8	36.4±0.3	432.5±0.8
<i>F</i>		31.81	25.12	41.81	20.21	50.89
<i>P</i>		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

– Control A – untreated, infested grain with *R. dominica*

The smallest mass of damaged grain was found in infested, untreated oats (0.44 g), while the greatest mass of damaged grains was weighed in triticale (10.26 g). The presence of dockage in all treated samples with Spinosad was not recorded. The highest mass of the dockage was measured in a control sample of triticale infested with *R. dominica* (5.2 g), followed by rye (3.8 g), significantly lower in wheat (2.04 g) and barley (1.05 g), and the smallest in oats (0.27 g) (Table 3.).

Table 3. Mass of damaged grains and the dockage ($g \pm SE$) after sieving of the *R. dominica* progeny from Spinosad treated grains

Insecticide	Dosage, $mg\ kg^{-1}$	Mass of damaged grains and the dockage ($g \pm SE$) in grain types				
		Wheat	Barley	Rye	Oat	Triticale
		Damaged grains				
Spinosad	0.25	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a
	0.5	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a
	1.0	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a	0 \pm 0.0 ^a
Control A	0	6.83 \pm 0.3 ^b	3.04 \pm 0.2 ^b	6.85 \pm 0.2 ^b	0.44 \pm 0.1 ^b	10.26 \pm 0.2 ^b
<i>F</i>		33.77	14.08	18.04	3.53	23.29
<i>P</i>		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
		Dockage				
Spinosad	0.25	0.00 ^a	0.01 \pm 0.0 ^a	0.05 ^a	0.00 ^a	0.01 ^a
	0.5	0.00 ^a	0.01 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	1.0	0.00 ^a	0.00 ^a	0.01 ^a	0.00 ^a	0.00 ^a
Control A	0	2.04 \pm 0.1 ^b	1.05 \pm 0.1 ^b	3.80 \pm 0.2 ^b	0.27 \pm 0.0 ^b	5.20 \pm 0.2 ^b
<i>F</i>		29.75	24.35	47.78	10.47	11.04
<i>P</i>		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

* Within every period of exposure, values across columns marked with same letters do not show a statistical difference; Tukey-Kramer (HSD) test for $P > 0.05$; $df = 7.56$;

– Control A – untreated, infested grain with *R. dominica*

DISCUSSION

In the present research, Spinosad caused 100% mortality of *R. dominica* after 14 days of exposure, for every applied rate, in all examined grain species. Numerous studies determined complete control of *R. dominica* in wheat with Spinosad, after 14 days of exposure (Fang et al., 2002; Vayias et al., 2009; Vayias et al., 2010; Athanassiou et al., 2011; Subramanyam et al., 2012; Nayak and Daghli, 2017).

Athanassiou et al. (2008) recorded that the mortality of *R. dominica* increased with dosage, exposure interval of Spinosad, and temperature. In present study the highest dose was 1 ppm. This dose was recommend for used to discriminate between susceptible and resistant populations for future resistance monitoring programs (Nayak and Daghli, 2017).

Our study combined research on the efficiency of Spinosad on all small grains (wheat, barley, rye, oats, and triticale). By examining the application of Spinosad on different food commodity, Fang et al. (2002) were among the first who found a difference in the efficiency of Spinosad in the control of *R. dominica* on four classes of wheat (hard red winter, hard red spring, soft red winter and durum wheat). During the examination of grain species (wheat, barley, rice, and maize), depending on the efficacy of Spinosad, Athanassiou et al. (2008) and Vajas et al. (2009) revealed that *R. dominica*

mortality was the lowest in maize, after 7 days of exposure. After 7 days of exposure, Chintzoglou et al. (2008) recorder the same lower mortality on maize (80%) treated with 0.025 ppm of a.i. and no differences of Spinosad efficacy in wheat and barley. Listed authors determined no significant differences in mortality of *R. dominica* after 14 days of exposure in comparison with the commodities. Athanassiou and Kavallieratos (2014) investigated the Spinosad efficacy on *R. dominica* in the applied rate of 1 ppm on wheat, barley, rye, and maize and reported that overall mortality ranged from 92.8 to 100 % after 7 days of the exposure. After 14 days of exposure, all adults were dead in all treatments, which is in agreement with our results.

The influence of commodity (wheat, barley, rice, rye, and maize) on the efficiency of spinosad was determined in controlling of *Sitophilus oryzae* L., *Prostephanus truncatus* (Horn), *Tribolium confusum* (Jacquelin du Val), *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* (Stephens), *Plodia interpunctella* (Hubner). Although tested small grain species differed in properties, Spinosad effectiveness in exterminated small grains was not different. According to Athanassiou et al. (2008) the "grain effect" is manifesting at the most in pest species less susceptible to Spinosad, while in *R. dominica*, as very susceptible, differences between different grain species could be masked.

Present study revealed that the examined insecticide prevented progeny emergence. Similar results were reported by Vayas et al. (2009), Athanassiou et al. (2011) and Nayak and Daghli (2017), who draw the same conclusions for Spinosad application in dosages of 0.1, 0.5 and 1 mg kg⁻¹, after 10 weeks of exposure. Athanassiou and Kavallieratos (2014) revealed that offspring emergence was ≤0.4 adults/vial in all tested commodities (wheat, barley, rye, and maize), with total suppressed only in wheat.

Present results show that *R. dominica* progeny production and feeding damage varied considerably depending on the small grain species, so the influence of commodity on the development of this pest was significant. In the present study, the highest progeny was found in untreated triticale, as well as the mass of damaged grains and dockage. The amounts of damaged kernels and dockage were in correlation with the emergence of progeny in untreated, infested samples. Damaged grain and dust in Spinosad treated samples were not recorded which represent the most ideal small grain protection and especially in the world where permitted limit for wheat is 2.5 damaged grain/100 g of total grain.

CONCLUSIONS

In this laboratory bioassays, Spinosad application was efficient in the suppression of *R. dominica* adults in small grains. All three dosages of examined insecticide were prevented progeny emergence and that is very important parameter for making decision about long protecting potential in stored grains. Untreated grain samples, infested with *R. dominica*, in relation to the other tested samples, showed the biggest change in mass of damage grain and dockage which clearly show a positive aspect of the use of Spinosad.

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Оригинални научни рад

ПРИМЕНА *SPINOSAD-a* У ИНТЕГРАЛНОЈ ЗАШТИТИ СТРНИХ ЖИТА ОД *RHYZOPERTHA DOMINICA* F.

Сажетак

Истраживање је спроведено у циљу оцене инсектицидне активности Спиносада, примењеног у дозама од 0,25, 0,5, и 1,0 мг кг⁻¹ (ппм) на *Рхузопертха доминица* у зрну пшенице, јечма, ражи, овса и тритикалеа. Смртност је процењена након 7 и 14 дана, док је утицај на стварање потомства оцењен након 8 недеља. При најнижој дози након 7 дана излагања, примена Спиносада је изазвала смртност од 94.5% (пшеница) до 100% (јечам). Након 14 дана, све дозе Спиносада оствариле су смртност од 100%. Све три примењене дозе Спиносада спречиле су појаву потомства. У узорцима третираним Спиносадом није забележена појава оштећених зрна и прашине што представља савршену заштиту зрна стрних жита. За Спиносад је утврђено да представља природну и одговарајућу алтернативу синтетичким инсектицидима стрних жита.

Кључне речи: *Rhyzopertha dominica*, *стрна жита*, *Спиносад*, *инсектицидна активност*



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Original Scientific paper

THE INFLUENCE OF PESTICIDES ON PLANTS, SOIL MICROORGANISMS AND FOOD SAFETY IN PLANT PRODUCTION

Abstract

Pesticides, is chemical agents for plant protection, and are mostly used in agriculture and forestry (90%), i.e. in plant production. In addition to accumulating in the environment, pesticides act on plants, microorganisms and other members of the biocenosis, and through the food chain, they reach the human body, where they exhibit active biological effects. In this study, the impact of pesticides on plants, soil microorganisms and food safety in crop production is considered.

Key words: *pesticides, plants, microorganisms, biological effect, food safety*

INTRODUCTION

The use of pesticides, i.e. chemical agents for plant protection, is the most radical measure of yield protection against pests, diseases and weeds. Pesticides include more than ten thousand preparations, based on 600 chemical compounds. Their intensive and uncontrolled application causes them to accumulate in the soil, especially in the fertile surface layer, where they affect beneficial microorganisms and other soil

organisms, carbon and nitrogen cycling and other biogenic elements, as well as plant nutrition. From the soil, pesticides get into groundwater, rivers, drinking water, and through food chains into the plant, animal and human body (Đukić et al., 2007; Lakić et al., 2018; Ikanović and Popović, 2020; Ikanović et al., 2020; Božović et al., 2020; Rajčić et al., 2020). In addition to dichloro-diphenyl-trichloroethane (DDT), the use of which was prohibited in most countries since the 1970s, a large number of other pesticides, which are used to increase yields in modern crop production, have negative effects on the environment and human health (Bagi and Bodnar, 2012; Anđelković, 2018). It has been proven that pesticides have an active biological effect, toxic, mutagenic and carcinogenic, on living organisms. Numerous studies have shown high correlations between exposure to pesticides and the occurrence of various types of cancer (Beard, 2006; Bassil et al., 2007). For the above reasons, the reduction of pesticide use, along with the intensification of preventive and biological protection measures, are the basic goals of sustainable agriculture and sustainable development. In this paper, the impact of pesticides on plants, soil microorganisms and food safety in crop production is discussed.

INFLUENCE OF PESTICIDES ON PLANTS, SOIL MICROORGANISMS AND FOOD SAFETY

Although pesticides are also used in veterinary medicine, the greatest application, as chemical agents for plant protection, is still found in agriculture and forestry (90%), that is, in plant production. They include more than ten thousand preparations, based on 600 chemical compounds. Pesticides were created precisely as a result of man's desire to produce a larger amount of healthy food, that is, to achieve higher yields in agriculture, but also the need to eradicate diseases, in which pests play the role of transmitters. However, although the application of pesticides represents the most radical measure of yield protection against pests, diseases and weeds, the impact that pesticides have on other members of the biocenosis, and through food chains on the health safety of food, is very complex and diverse (Đukić et al., 2007; Stajkovic et al., 2009).

Different types of pesticides exhibit numerous physiological activities in plant organisms. The impact of pesticides on plants begins from the moment of contact and penetration through the root, stem and leaf. Most herbicides and insecticides with systemic action, acaricides and some fungicides quickly penetrate and move around the plant and have a general effect on the entire plant organism. When developing plants are treated, pesticides penetrate the plant organism to the greatest extent through the leaves (cuticles and pores), in the form of liquid and vapor. In the absence of moisture in the plant, pesticides penetrate through the fatty components of the cell wall via the lipid pathway. Pesticides that are not able to move, are localized at the places of initial penetration into the plant and they have a local effect. The resistance of plants to the action of pesticides is determined by the anatomical and morphological characteristics of the plant, growth stages, applied agrotechnical measures in plant production, chemical composition, doses and forms of preparations. The basis of the resistance of

different plant species to pesticides is their biochemical differences in the exchange of matter during the physiological reaction to these compounds. Depending on the applied dose and type of preparation, pesticides introduced into the soil can change the composition of the soil microflora. There is no doubt that the intensive and uncontrolled application of pesticides causes their accumulation in the soil, especially in the fertile surface layer, where they affect beneficial microorganisms and other soil organisms, the cycle of carbon and nitrogen and other biogenic elements, as well as the nutrition of plants (Đukić et al., 2007, Stajkovic et al., 2009). In addition to plants, soil microorganisms are a key factor that enables the cycling of matter in all ecosystems on the planet and the maintenance of life in the biosphere (Đukić and Đorđević, 2004; Šarčević-Todosijević et al., 2020). A general indicator of the effect of pesticides on the microflora is the biological activity of the soil or the intensity of soil respiration (sorption of O₂, release of CO₂). Soil fungicides and fumigants, as a rule, have a negative effect on soil microflora. Fumigants are strong inhibitors of soil microorganisms and are therefore often called soil sterilizers. They prevent the development of heterotrophic microorganisms and stop soil respiration. Fungicides that do not pass into the gaseous phase, that is, do not have a fumigation effect, show a less pronounced sterilization effect, which is caused by their uneven distribution in the soil and heterogeneous soil structure. Đukić et al. (2007) point out that different types of soil fungi are differently sensitive to fungicides. Among the saprophytes, representatives of the genera *Trichoderma*, *Fusarium* and *Penicillium* are the most resistant to fungicides, so in soils treated with fungicides, the mentioned genera dominate in the population of soil fungi. In the doses recommended against soil pests, organochlorine insecticides generally do not have a negative effect on the abundance of soil microorganisms. They have a stimulating effect on the development of certain groups of microorganisms, while at higher doses they initially suppress and then stimulate the soil microflora. Herbicides break down relatively quickly in the soil and their application in the recommended doses does not have a negative impact on the soil microflora. Đukić et al. (2007) state that production doses of herbicides: products of phenyl carboxylic acid, chlorine-substituted aliphatic acids, sym-triazine do not have a clearly expressed effect on the qualitative and quantitative composition of soil saprophytic fungi, bacteria and actinomycetes. However, when herbicides are introduced into the soil in increased doses, there is a temporary regrouping of the composition of the microflora (Đukić and Đorđević, 2004; Đukić et al., 2007; Stajkovic et al., 2009). When considering the impact of pesticides on soil microorganisms, it is important to mention the microbiological transformation of pesticides in the soil. Đukić et al. (2007) point out that in this process in the soil, as well as in the culture of microorganisms, stable products of pesticide transformation can be formed, whose presence and accumulation in the environment and plants is harmful. Favorable conditions for the microbiological synthesis of such compounds, in combination with pesticides, are also created by high doses of applied nitrogen fertilizers. Therefore, during the application of chemical plant protection agents, it is necessary to take into account their interaction in the system pesticides – mineral fertilizers - microorganisms (Đukić et al., 2007).

From the soil, pesticides get into groundwater, rivers, drinking water, and through food chains into plant, animal and human bodies. Although the use of the oldest pe-

sticide dichloro-diphenyl-trichloroethane (DDT) has been banned in many countries since the 1970s, it has not yet been completely eliminated from the environment, and exposure to the pesticide is still widespread. In developed countries, the slow elimination from the body means that a large part of the population still has detectable levels of DDT, or its metabolite DDE, in their serum or adipose tissue. In developing countries, the pesticide is still used for vector control and a significant proportion of infants have daily intakes above recommended levels. However, recent breast cancer research, conducted using cohort studies, has allowed for a more rigorous assessment of the role of DDT in the etiology of the disease. However, recent breast cancer research, conducted using cohort studies, has allowed for a more rigorous assessment of the role of DDT in the etiology of the disease (Beard, 2006).

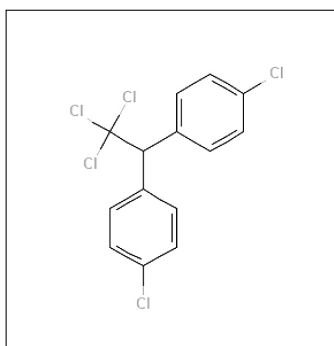


Figure 1. Chemical structure of dichloro-diphenyl-trichloroethane (DDT) (source: National Library of Medicine/National Center for Biotechnology Information <https://pubchem.ncbi.nlm.nih.gov/compound/Clofenotane#section=2D-Structure>)

A large number of other pesticides, which are used to increase yields in modern crop production, have negative effects on the environment and human health (Bagi and Bodnar, 2012; Anđelković, 2018). It has been proven that pesticides have an active biological effect, toxic, mutagenic and carcinogenic, on living organisms (Đorđević et al., 2020). Toxins are substances that are dangerous for living organisms due to the harmful effects they cause in cells, tissues, organs and biochemical processes. The fate of toxins in the body depends on the way they are ingested; toxic substances that are present in food and water enter the body through the digestive tract (Anđelković, 2018). Margni et al. (2002) state that the intake of pesticide residues through food results in the highest exposure of the human body to their toxic effect, as much as 103 to 105 times greater than the intake of pesticides into the body through drinking water or inhalation. Based on this, they emphasize that monitoring pesticide residues in food should be a priority in the production of health-safe food (Margni et al., 2002). However, the organophosphate pesticide parathion is easily adsorbed through the skin, lungs or digestive tract and is equally toxic regardless of how it enters the human body (Anđelković, 2018). The common biochemical mechanism of action of organophos-

phates in the composition of this group of pesticides is based on inhibition of the activity of the enzyme AChE (acetylcholinesterase) of blood, brain and other tissues. Inhibition of AChE causes the accumulation of ACh (acetylcholine) at the central and neuroeffector synapses of the cholinergic nervous system, which leads to impaired neurotransmission. Irreversible AChE inhibitors can thus lead to muscle paralysis, convulsions and bronchoconstriction, and death due to asphyxiation. In addition to the above, organophosphates also have a non-specific effect on other enzyme systems, as well as a general toxic effect, especially on the liver and blood components (Massoulié et al., 2008; Pohanka, 2011).

Mutagens are environmental agents that change the genetic information of organisms. A significant number of mutations introduce the cells of living organisms into malignant processes, so mutagens are carcinogens at the same time. Cancer is one of the leading causes of death in the world. Experiments performed on cell transformation in *in vitro* conditions, using DNA isolated from cancer cells, indicated the genetic mechanisms of carcinogenesis. Based on empirical data confirmed in numerous experimental models, it was concluded that mutation or activation of one gene is not enough, but can be a trigger for a cascade process, that is, mutation or activation of a number of other genes. There are three types of evidence for this claim: evidence based on DNA analysis from different stages of carcinogenesis; evidence based on the increased expression in cell cultures and experimental animals of oncogenes, that is, genes that encode proteins, which are capable of inducing carcinogenesis in a living organism; as well as evidence from epidemiology. Namely, all cancer cells have a clonal origin, and the incidence of cancer increases with age (Zimonjić i sar., 1990; Marinković and Marinković, 2012, Đorđević et al., 2020). It has been proven that certain types of pesticides can play a significant role in the described genesis of malignant processes in the body. Numerous studies have shown high correlations of exposure to pesticides with the occurrence of lymphoma, leukemia and solid tumors (especially brain and prostate). Illnesses generally occur as a result of long-term exposure to high doses of pesticides, and in some cases, specific pesticides have been identified that caused the illnesses (Bassil et al., 2007).

Back in 1983, Fujii and Inoue established specificity in the action of pesticides on organisms. They tested on soybeans the mutagenic activities of several types of pesticides used in agriculture. For several types of pesticides (lebaycid, diazinon, EPN, karpfos), no mutagenic activity was determined. An increase in the mutation rate was found for ecatin. Then, tests were carried out for ecatin using *in vitro* or *in vivo* methods on microorganisms. However, systematic tests of bacteria gave negative results in all cases, so the authors conclude that the manifestation of mutagenic activity of ecatin is specific for plants (Fujii and Inoue, 1983).

Brkić et al. (2006), using the Ames test, examined the genotoxic properties of the herbicide GAL-57, containing two active ingredients; bentazon and dicamba. Taxons *Salmonella typhimurium* and *Escherichia coli* were tested. The authors state that the results showed that there was neither a biological nor a statistically significant increase in the mutation factor, on the basis of which they concluded that the herbicide GAL-57, has no genotoxic properties (with or without metabolic activation) under experimental conditions in the Ames test (Brkić et al., 2006).

Results like those obtained by Brkić et al. (2006) are encouraging. However, sustainable, rational and safe use of pesticides in crop production is necessary. The absence of a legal framework for the application of pesticides, significantly increased uncontrolled use of low quality pesticides in many parts of the world. With the aim of protecting the environment and human health, the maximum allowable concentrations (MACs) of pesticides and their decomposition products, which can be present in food, have been determined. These concentrations do not lead to health damage or any physiological effect in the most sensitive individuals (Bagi and Bodnar, 2012).

Reducing the use of pesticides, along with the intensification of preventive and biological protection measures, are the basic goals of sustainable agriculture and sustainable development. Biopesticides represent one of the most significant discoveries of biotechnology. Biopesticides reduce environmental pollution and enable preservation of food safety (Đukić et al., 2007; Šarčević-Todosijević et al., 2019; Popović et al., 2019; Stevanović et al., 2019). It is important to mention the use of microbial antagonists in the control of plant pathogens. Antagonistic microorganisms have shown the potential to inhibit the growth and proliferation of various phytopathogens, with little or no negative side effects on the environment.

CONCLUSION

Intensive and uncontrolled application of pesticides causes their accumulation in the soil, in which they affect beneficial microorganisms and other soil organisms, the cycling of biogenic elements, as well as the nutrition of plants. From the soil, pesticides get into groundwater, rivers, drinking water, and through food chains into the plant, animal and human body, in which they exhibit an active toxic, mutagenic and carcinogenic effect. Therefore, in the protection of the environment and the production of safe food, it is necessary to reduce the use of pesticides, while intensifying preventive and biological protection measures.

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Originalni naučni rad

UTICAJ PESTICIDA NA BILJKE, MIKROORGANIZME ZEMLJIŠTA I ZDRAVSTVENU BEZBEDNOST HRANE U BILJNOJ PROIZVODNJI

Rezime

Pesticidi, su hemijska sredstva za zaštitu bilja, i najviše se upotrebljavaju u poljoprivredi i šumarstvu (90%), odnosno u biljnoj proizvodnji. Pored akumuliranja u životnoj sredini, pesticidi djeluju na biljke, mikroorganizme i ostale članove biocenoza, a preko lanaca ishrane, dospavaju i u organizam čoveka, u kojem ispoljavaju aktivna biološka dejstva. U ovoj studiji razmatra se uticaj pesticida na biljke, mikroorganizme zemljišta i zdravstvenu bezbednost hrane u biljnoj proizvodnji.

Ključne reči: *pesticidi, biljke, mikroorganizmi, biološko dejstvo, zdravstvena bezbednost hrane*



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Original Scientific paper

ESSENTIAL OILS FROM BULGARIAN *ROSA ALBA* L. AND *ROSA DAMASCENA* MILL. AMPLIFY THE ANTIFUNGAL EFFECT OF NYSTATIN

Abstract

Rosa alba L. and *Rosa damascena* Mill are the most popular Bulgarian essential oil-bearing plants. The purpose of this work is to investigate the antifungal activity of both essential oils individually and in combination with nystatin against human and plant pathogens, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*. Fungal cultures were grown in a beer agar medium. The agar dilution method was used to analyze the antifungal activity. Antifungal effects of both oils (in concentrations of 10, 25, and 50 mg/L and in combination with 50 mg/L nystatin as well) were determined by measuring the inhibition zones diameter 48 and 120 hours post inoculation and were presented as an antifungal index (AI). The results showed a decrease in the radial growth of *Aspergillus flavus* culture, depending on the oil concentration. The most sensitive inhibition was observed of both of oils at a concentration of 50 mg/l, which was more pronounced at 48 hours. Combination of *R. alba* L. oil with nystatin exhibited over additive antifungal effect that was more efficient than the respective combination of *R. damascena* Mill. with nystatin against *A. niger*. The over-additive effect for both combinations was the most pronounced at 50 mg/l oil and 48h post-inoculation. These data indicate that rose oil could be utilized as a topical disinfectant in clinical practices.

Key words: *Rosa alba* L., *Rosa damascena* Mill. oil, *Aspergillus fumigatus*,
Aspergillus niger, *Aspergillus flavus*, antifungal activity

INTRODUCTION

Aspergilli species are ubiquitous fungi found in air, soil, plants, and decaying organic materials. Aspergillosis is an opportunistic infection that can attack the lungs, ears, eyes, digestive system, kidneys, and brain. The growth of Aspergilli in foodstuffs is toxicologically significant since some species are known to produce mycotoxins like aflatoxins-B1, B2, G1, and G2, aspergillic acid, hydroxyaspergillic acid, and oxalic acid. The consumption of moldy products can cause mycotoxicoses, and more importantly, some mycotoxins are potent carcinogens (Bennett & Klich, 2003).

Nystatin is one of the commonly used antifungal drugs administered orally for the treatment of infections caused by fungi. Nowadays, the problem with fungal resistance is very serious and many researchers are focused on developing new antifungal agents from medical and aromatic plants and their products.

Essential oils have been long recognized as antibacterial, antifungal, antiviral, insecticidal, and antioxidant agents (Bassole & Juliani, 2012). According to Aoudou et al., (2010) antifungal activity against the strain *Aspergillus flavus* is due to some major compounds as geraniol, nerol, and geranial (Aoudou et al., 2010). Nedkov et al. (2009) have found about 214 components in rose oils through HPLC, GC, and GC-MS. The composition of *R. alba* L. oil is similar to *R. damascena* Mill. (Nedkov et al., 2009), with the same major compounds, but with different proportions of the major compounds. Many scientists reported about antimicrobial properties of *R. damascena* Mill. essential oil (Shohayeb et al., 2014). A study by Mileva et al. (2014) indicates a weak activities against the fungal strains *A. niger* and *A. flavus* of some plant essential oils belonging to the Rosaceae family. Due to the develop resistance to nystatin, it is interesting to explore its antifungal effect in the combinations with rose oils. The aim of the present work is to explore antifungal activity of nystatin in combination with essential oils of *Rosa alba* L. and *Rosa damascena* Mill. against *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*.

MATERIAL AND METHODS

Plant material

Essential oil from *Rosa alba* L. and *Rosa damascena* Mill. F. Trigintipetala Dieck were provided by Institute for Roses and Aromatic Plants (IRAP) in Kazanlak.

Fungal strains

A. fumigatus, *A. niger*, and *A. flavus* – from the Mycological Collection at the Institute of Microbiology, Sofia, were used throughout and maintained at 4 °C on beer agar, pH 6.3.

Mycelial growth inhibition

Oils and their combinations with nystatin were screened for their antifungal activity against *A. fumigatus*, *A. flavus*, and *A. niger* by employing the standard discs diffusion technique. The fungal cultures were grown on beer agar and 7-day-old culture was used for antifungal assay (v/v) tests. Inoculum (100 µl) were applied on the surface of the beer agar plate and spread by using sterile glass spreader. Stock solutions of rose oils in ethanol were used for preparing filter paper discs for assay. The sterile discs (5 mm diameter Whatmans filter paper No 42) were soaked through adding 10µl adequate concentration to prepare following discs: rose oil 25 µg/disc, nystatin 50 µg/disc and combination of (rose oil 500 µg and nystatin 50 µg)/disc, after drying. The incubation was carried for 48 h at 28 °C. The sensitivity to nystatin and the different oils individually and their combination with nystatin was determined by the diameter of the inhibition zone.

Antifungal activity assay by agar dilution method

Potato dextrose agar (PDA) medium with different concentrations of essential oils (10, 25, 50 µg/mL), nystatin (50 µg/mL) and their combination were prepared by adding the appropriate quantity of essential oil/and, or nystatin to the melted medium, followed by manual rotation of the Erlenmeyer flask to disperse in the medium. Plates were incubated at 28°C. The antifungal index (AI) was calculated according to Wang et al., (2005): $AI = (1 - Go/Gc) \times 100$; Go = diameter of growth zone in the test plate, Gc = diameter of growth zone in the control plate. Each experiment was performed three times, and the data were averaged (Wang et al., 2005).

RESULTS AND DISCUSSION

The total number of compounds identified in *R. alba* L. and *R. damascena* Mill. oils were determined by Mileva et al., (2014). The essential oils have following major components: geraniol (*R. alba* – 18.28% and *R. damascena* – 14.13%), β – citronellol (18.00%; 13.56%), nerol (7.74%; 8.34%), eugenol (0.38%; 0.64%), methyleugenol (0.1%; 2.05%) and β – caryophyllene (3.7%; 4.08%).

The oils contain the same substances, but differ by their ratios. *R. alba* oil contents more geraniol and citronellol, and *R. damascena* oil has more nerol, eugenol, methyleugenol and β – caryophyllene.

The essential oils concentrations were chosen based on published data by Mileva et al. (2014). The inhibition zones of combination of rose oil and nystatin are larger than individually treatment. Diameter of fungal radial growth is presented in Table 1. Antifungal activity has been calculated as antifungal index and illustrated in Figures 1, 2 and 3.

The strongest activity of essential oils against *A. fumigatus* was found at 48 h exposition (Figure 1) at concentration 10 µg/ml, AI for *R. alba* and *R. damascena* were

56.66% and 53.33% respectively. The combinations showed trend to weak intensification of the effect in comparison with individually supplementation. The antifungal effects were lower at 120 h post inoculation in comparison to that at 48 h.

The effect of tested oils and their combinations with nystatin against *A. flavus* is expressed as AI (Figure 2). The cultivation in the presence of the rose oils also result to inhibition of *A. flavus* radial growth (Table 1), more clearly expressed on 120 h. The effect was higher at the variant of nystatin plus *R. damascena*, AI – 66.67%, than combination of nystatin and *R. alba*, AI – 55.56%.

Table 1. Diameter of fungal radial growth and influence of essential oils and their combination with nystatin

Mean diameter of radial growth (sm)						
µL/mL rose oil, individually or in combination with 50 µg/ml Nystatin	<i>A. flavus</i>		<i>A. niger</i>		<i>A. fumigatus</i>	
	48h	120h	48h	120h	48h	120h
Control	3	4,5	4,4	8	3	7
Nystatin	2,3	4	2,4	6	2,7	5,7
<i>R. alba</i> L. 10	2,3	4	2,5	6,5	1,3	6,5
<i>R. alba</i> L. 25	2,3	3,5	2,5	7,5	2,7	6,7
<i>R. alba</i> L. 50	2,1	2	4,4	8	3	6,5
<i>R. alba</i> L. 10 + Nystatin	2	4,5	1,5	5	1,2	6
<i>R. alba</i> L. 25 + Nystatin	1,9	4	1,5	7,4	1,7	4,5
<i>R. alba</i> L. 50 + Nystatin	1,6	2	0,9	4	2	5,7
<i>R. damascena</i> Mill. 10	2	4	2,5	8	1,4	6,5
<i>R. damascena</i> Mill. 25	2,5	4	4,4	8	2,8	6,5
<i>R. damascena</i> Mill. 50	2	2	4,4	8	2,5	7
<i>R. damascena</i> Mill. 10 + Nystatin	2	4	1,6	6,5	1,2	6
<i>R. damascena</i> Mill. 25 + Nystatin	1,5	3,3	1,7	6,5	2	5,5
<i>R. damascena</i> Mill. 50 + Nystatin	1,5	1,5	2,4	7,5	1,72	5,17

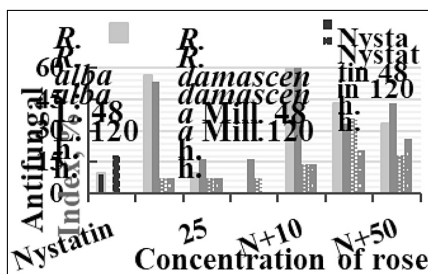


Figure 1. Antifungal activities of *R. alba* and *R. damascena* essential oil, and their combination with nystatin against *A. fumigatus*.

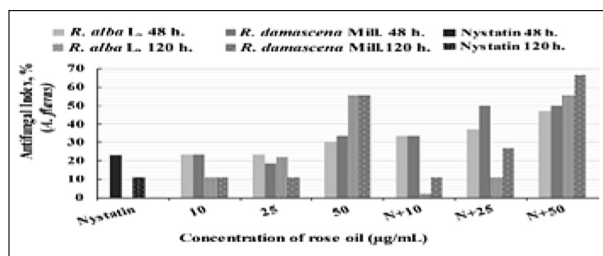


Figure 2. Antifungal activities of *R. alba* and *R. damascena* essential oil, and their combination with nystatin against *A. flavus*.

The activities of the two oils and nystatin, individually and in combination expressed as AI against *A. niger*, are shown in (Figure 3). The inhibition of radial growth of *A. niger* by tested substances, was more considerable after 48 h cultivation, than that of 120 h. After application of nystatin, AI was 45.45% at 48 h, and 25% at 120 h post inoculation. The rose oil concentration dependence of AI was observed after 48 hours and 120 hours exposition when applying the combination of nystatin with *R. alba* oil, with the highest value of AI at 50 µg/ml – 79.54%. and at 120 h the effect was much lower – 50.14%. The effect of combination nystatin/ *R. damascena* oil was the highest at 25 µg/ml *R. damascena* oil, AI – 61.36%.

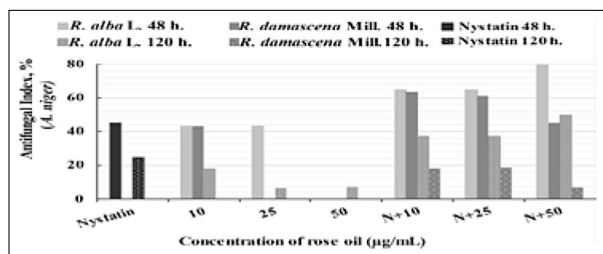


Figure 3. Antifungal activities of *R. alba* and *R. damascena* essential oil and their combination with nystatin against *A. niger*.

The activities of the two oils and nystatin, individually and in combination expressed as AI against *A. niger*, are shown in (Figure 3). The inhibition of radial growth of *A. niger* by tested substances, was more considerable after 48 h cultivation, than that of 120 h. After application of nystatin, AI was 45.45% at 48 h, and 25% at 120 h post inoculation. The rose oil concentration dependence of AI was observed after 48 hours and 120 hours exposition when applying the combination of nystatin with *R. alba* oil, with the highest value of AI at 50 µg/ml – 79.54%. and at 120 h the effect was much lower – 50.14%. The effect of combination nystatin/ *R. damascena* oil was the highest at 25 µg/ml *R. damascena* oil, AI – 61.36%.

CONCLUSION

The combinations of Bulgarian *R. damascena* Mill. and *R. alba* oils with nystatin are promising effective antifungal formulations against *A. flavus* and *A. niger*, which pharmacological treatment is challenging.

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Originalni naučni rad

ETERIČNA ULJA IZ BUGARSKE ROSA ALBA L. I ROSA DAMASCENA MLIN. POJAČIVAČI ANTIFUGALNOG EFEKTA NISTATINA

Sažetak

Rosa alba L. i *Rosa damascena* Mill su najpopularnije bugarske biljke koje sadrže eterično ulje. Svrha ovog rada je da se ispita antifungalna aktivnost oba eterična ulja pojedinačno i u kombinaciji sa nistatinom protiv ljudskih i biljnih patogena, *Aspergillus fumigatus*, *Aspergillus niger* i *Aspergillus flavus*. Kulture gljivica su uzgajane u podlozi sa pivskim agarom. Za analizu antifungalne aktivnosti korišćena je metoda razblaživanja agara. Antifungalni efekti oba ulja (u koncentracijama od 10, 25 i 50 mg/L i u kombinaciji sa 50 mg/L nistatina) su utvrđeni merenjem prečnika zona inhibicije 48 i 120 sati nakon inokulacije i predstavljeni su kao antifungalni indeks (AI). Rezultati su pokazali smanjenje radijalnog rasta kulture *Aspergillus flavus* u zavisnosti od koncentracije ulja. Najosetljivija inhibicija je primećena kod oba ulja u koncentraciji od 50 mg/l, koja je bila izraženija nakon 48 sati. Kombinacija ulja *R. alba* L. sa nistatinom pokazala je aditivni antifungalni efekat koji je bio efikasniji od odgovarajuće kombinacije *R. damascena* Mill. sa nistatinom protiv *A. niger*. Efekat prekomernog dodavanja za obe kombinacije bio je najizraženiji pri 50 mg/l ulja i 48 sati nakon inokulacije. Ovi podaci ukazuju da bi ružino ulje moglo da se koristi kao lokalno dezinfekciono sredstvo u kliničkoj praksi.

Ključne reči: *Rosa alba* L., *Rosa damascena* Mill. ulje, *Aspergillus fumigatus*,
Aspergillus niger, *Aspergillus flavus*, antifungalna aktivnost

**AGRICULTURAL PRODUCTION
FROM SUSTAINABLE DEVELOPMENT
ASPECT**



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Original Scientific paper

IMPACT OF FOOD PRODUCTION AND CONSUMPTION ON THE ENVIRONMENT, GEOGRAPHICAL APPROACH

Abstract

Food travels long distances to reach our plates. A concept related to carbon footprint is measuring "food miles" which shows how environmentally desirable that food is. This study, – which follows the „from farm to plate” approach – analyses examples of different stages when measuring it, and the possibilities and difficulties of its reduction. The use of local products, the short food supply chain concept, good cooperation, and communication play an important role. For better understanding, a questionnaire survey was conducted with consumers, to find out what they know about this concept, and about the transport, production, and packaging of the purchased food.

Key words: *food miles, local product, short food supply chain, awareness, consumption*

INTRODUCTION

Food mileage is an unusual topic of everyday conversation because, yet few people are aware that it is currently a problem that needs to be solved (Leavens, 2017). During our shopping, we ourselves are at a current point in this process. Nowadays, environmental awareness has become part of everyday life. We can meet a lot of movements that try to present humanity with a more sustainable world, thus ensuring the future of the next generations and our Earth. The concept of food kilometers expresses the distance traveled by food. Besides introducing some international examples, this study focuses on Hungarian consumers, to find out what they know about this concept and what their attitude toward environmental protection is, related to it.

THE CONCEPT OF FOOD MILEAGE

The first time we encountered the questions coinciding with the concept of "food miles" was in the 1994 report of Tim Lang (2006) who presented his report to the Sustainable Agriculture Food and Environment (SAFE) association. The professor revealed in detail how production volumes have increased over the past 50 years, and how large-scale changes food distribution has undergone. This was based on the food kilometer report written that year, which reported on the dangers of the distance traveled by food. He also touched on how consumer habits were adjusted accordingly. Consumers have gradually become accustomed to convenience over the years. Today, it is natural for them to find all kinds of products on the shelves of stores, and most of them have a very wide range to suit their needs. Thanks to globalization and large-scale urbanization, the food intended for human consumption can have an increasingly long journey behind it before reaching its final destination.

LEGAL REGULATIONS REGARDING PLACE OF ORIGIN OF FOOD PRODUCTS

The local farmers' market is a market in which the farmer sells his products in the county of his commercial region or within a distance determined for his own goods circulation. "[...] supplies a retail or catering establishment or public catering establishment within the region or within a maximum distance of 40 km as the crow flies from the location of the farm in Hungary..."(FVM decree 52/2010).

Based on the provisions of the American legislation, a distance of 400 miles (644 km) is specified, in contrast to the environment with a radius of 40 km in Hungary. In addition to distance – which is measured as the crow flies, – this definition also includes the movement of goods within the current state. These measures are mainly due to the huge distances resulting from geographical conditions.

SHORT FOOD SUPPLY CHAINS

In 2012, thanks to the legislative changes, the process of developing farmers' markets was greatly simplified. Since then, the opening of these markets has been non-stop, even at an increasingly rapid pace. This also shows the growing consumer demand for the products available there. A short food supply chain (SFSC), – as defined for the first time in the EU's rural development policy for 2014-2020 – by the EU (Regulation, 2013), is a supply chain involving a limited number of economic operators committed to cooperation, local economic development, and maintaining close geographical and social relations between food producers, processors, and consumers. As a result of this regulation, urban and rural farmers who sell their products directly to consumers, with minimum intermediaries, have increased in number in recent years. The growth in SFSCs can be linked to a shift in consumer behavior, which has shown an increased appreciation of local food and related attributes such as the place of origin,

traditional production methods, and processing techniques. It could be even more efficient with good communication strategies to share information about products between producers. The situation is very similar in North America, as well, but not recognized by everyone everywhere the same way.

In Hungary, with the support of domestic producers, the argument in favour of SFSC is that the chains are also more reliable from an environmental point of view. They may seem like a more reasonable choice compared to traditional retail channels, as they are more sustainable. In the next part of the study, different aspects, and examples of thinking about environmental protection are shown.

Transportation

The largest part of greenhouse gas emissions is generated directly in relation to food kilometers, i.e. during transportation. According to Tim Lang (2006), the emission of carbon dioxide gas is distributed among the ways of transport in the following ratio: 60% is generated by road, 20% by air, and 10-10% by rail and sea. This distribution is still recognized in research. However, the level of emission increases proportionally with the distance. Raw materials transported by train can travel up to ten times the distance compared to trucks, while the level of emissions remains the same. Compared to this, in the case of huge distances, the most suitable tool for reducing greenhouse gases is the cargo ship. This is followed by the railway, the car, and finally the airplane. As an example, the harmful effect of transporting one ton of food by plane can be almost seventy times greater than that transported by cargo ship. Vehicles used for different types of transport can produce widely different emissions. Flying the distance from Boston to Chicago (about 850 miles) is far more polluting than shipping from China to California by freighter, which is nearly 11,000 miles.

The distances covered by individual foods can now be calculated with various internet calculators. With these, we mainly get only an approximate value. Based on LCA (life cycle assessment) calculations for the United States by L. Weber and H. Scott Matthews, 83% of the harmful environmental impact from the production to the consumption of food occurs during the production processes. Shipping accounts for only 11% of the total, and within that only 4% is the final transportation stage, during which it reaches the place of processing to the retailer (Weber & Matthews, 2008).

An example of sugarcane cultivation in the Hawaiian Islands

The location of the processing and related side processes may differ from the original location of the cultivation process. Based on the example of the following sugar farm, we highlight how big distances can exist in reality, in cases where the place of cultivation and processing are separated from each other. Consumers would not even think that semi-processed foods can and do travel such huge distances between individual sub-processes.

One of the Hawaiian Islands, Maui is famous for its sugar cane farming. More precisely, it was until the owner company, the Hawaiian Commercial & Sugar Com-

pany (HC&S), closed it in 2016 after about 145 years of active operation. Using the example of former sugar cultivation, we highlight how important it is during the production of a product to carry out the necessary activities, if possible, if not directly, but in its close surroundings. The plant grown here was only processed locally to the state of raw cane sugar. From there it was transported to California, where it was further refined into white sugar near San Francisco. However, this is by no means the end of the process. After that, the refined sugar was sent all the way to New York, where the packaged end products were made one by one. With this, we achieve the result that the sugar has covered a distance of up to 15,000 kilometers before the coffee is consumed in a cafe located just a few kilometers from the sugar cane cultivation.

The role of production conditions

There are countless circumstances that play a role in the individual steps of the processes. We examine the factors from the infrastructure built around the production process. Stationary energy is the one that significantly influences the harmful emissions of a given production unit. For better understanding, we present an examination of different Australian tomato plantations (Roggeveen, 2014). The main aspects were the types of fuel supplying stationary energy and the plant's technological equipment. The answer is clear, coal proved to be the most harmful to crops, followed by diesel, and finally electric energy. According to technological development, high and medium categories were investigated. It goes without saying that a higher standard entails a higher cost. This way, however, it is possible to ensure a constant, ideal temperature, ventilation, and continuous water supply with the help of the established automatic irrigation system. After the static energy, the other huge problem is caused by the by-products and wastes formed and preserved during the processing. This part accounts for almost a quarter of the total value of harmful emissions.

The tomato seedlings were mainly transported to the farms by truck. The year-round supply achieved by this route is also only 1% contaminated at these plantations. Emissions during transport are practically negligible. This is especially important when foods needlessly travel long distances between stages of processing. When traveling, it is suggested to avoid empty flights. It is not uncommon for a van or small truck to not be fully loaded. By paying attention to this, half-full flights could be avoided. It is interesting that calculated on an annual time interval, the amount emitted by trucks is still four times bigger than the environmental load accumulated during the transport of nutrients and other growth-stimulating substances arriving from Europe by cargo ship.

The case of the cultivation conditions of different tomato producers shows that the distance is not always the most relevant since colonies close to each other were observed.

The role of the relationship between partners

Before the vegetables and fruits reach the store shelves, they go through the hands of many people. Their individual attitude related to environmental protection can also

greatly contribute to the successful direction of the reduction of environmental pollutants formed during production. It is important how they manage their own business, and what kind of relationship they have with their partners and other members of the supply chain.

Unsurprisingly, the factors that help reduce emissions depend primarily on the financial situation. First of all, the applicable system that helps to reduce the level of emissions must be assessed and its application must be sought. However, this method must be affordable and not have a negative impact on the success of the business economy. It also requires that alternative solutions are available, which people can access in different ways. People in question must also want the change and strive to use such a system, the design of which is far from easy.

Weather conditions may seem like a small factor at first, but they play a huge role in well-functioning agricultural farms. By definition, it is advisable to start cultivation and production where the given conditions are ideal.

Processing of meat-based foods

An exceptionally large proportion of greenhouse gases originate primarily in the form of methane from the excreta associated with livestock and the gases emitted by them. The majority of people are aware of the fact that transportation is harmful to the environment in large quantities, but few people know that agriculture is a much more important factor. Livestock farms account for 18% and other agricultural industries for 11% of all greenhouse gas emissions. Significantly more water is used in the production of meat and dairy products than in the case of plant-based foods.

Consumers are also not aware of how much damage meat processing causes to the environment. Among the other factors, we can also mention soil degradation, the use of petroleum, and deforestation. Solutions to the problem should be widely sought within the meat processing sector. This would probably take less time than for consumers to reduce their consumption of animal foods. Meat processing companies could reduce emissions by investing more energy in proper manure management and researching other feed alternatives to avoid large amounts of methane gas. They should also have a new perspective on the way they handle the remaining unprocessed, inedible by-products, waste, and various packaging.

Considerations of packaging

The packaging accompanies the products all the way from the processing phase to the end of use by consumers (Marsh and Bugusu, 2007). Its task is to protect the given product from harmful environmental effects: chemical, biological and physical. In addition, the traceability and nutrition information on the displayed label are very important and useful information for consumers. The design and execution of the packaging greatly contribute to the life of the final product during its time on the shelves. The range of materials traditionally used for packaging includes glass, plastic, metal, paper, and various types of combinations. Researchers are constantly trying to

find solutions that can reduce the environmental impact of packaging materials. During processing, they are increasingly trying to use smaller amounts of packaging material. Fortunately, most of the materials used can be recycled.

During the preparation of a final product, packaging plays a role at several points. There are strict legal requirements for the packaging process and the use of materials. By complying with these conditions and by carefully selecting and using materials, it is possible to stimulate the extent of pollution reduction. To survive long distances, the food needs a greater degree of safety. The label informs the distributor, the location of the production process, and the place of origin, but it is not possible to obtain precise data from this about the level of environmental impact for the consumer.

FINDINGS OF THE SURVEY OF CONSUMERS

The key question for reducing the food mileage is whether consumers know this concept and how much they care about it, since their habits and attitude are decisive, and their willingness is necessary for the situation to improve. Primary research based on the answers of a questionnaire survey was applied to find out how much attention the consumers pay to the environmental impact of their food. Would they possibly change their consumption habits if they learn about new aspects? The evaluation of their answers shows how much they care about the place of origin and other environmentally important information about individual foods during their shopping preferences. A total of 146 people filled out the questionnaire which was evaluated with statistical methods.

*Table 1: What do “food miles” mean for the respondents of the survey?
(source: own compilation)*

Distance, the food traveled	47,26%
Other (related to the environment, value, time, quantity, export-import)	nutritional 10,96%
I don't know	41,78%

One of the first questions was about the meaning of food miles. More than 40% did not have any idea about it. The answers are summarised in Table 1. From the answers to the open question, we see that people have some information, but most of them do not know and understand the reasons; and that the price is important for them. With correlation analyses, it can be established that the importance of quality, price, and brand name is independent of gender. Regardless of gender, age, or place of residence, the quality of a given product is more important (90,41% say so) than its place of origin. There is no significant difference between different age groups whether they read the labels or not. Reading the label surprisingly does not mean that the consumer is reading every detail on it (Figures 1.)

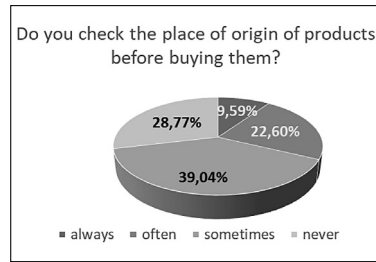
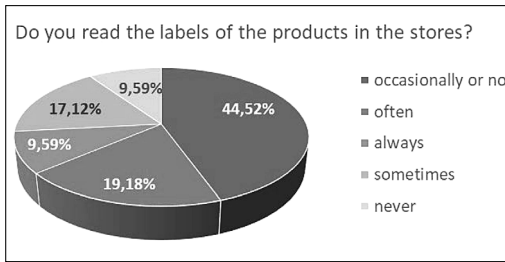


Figure 1: a) How often do the respondents read the labels of products they purchase, b) including the place of origin? (source: own compilation)

When shopping, the choice of local products is listed as the most important aspect to a small extent. Figure 2 shows that women prioritize the choice of local products on the scale of importance. This may indicate that women shop more consciously and that they trust local products more than imported goods.

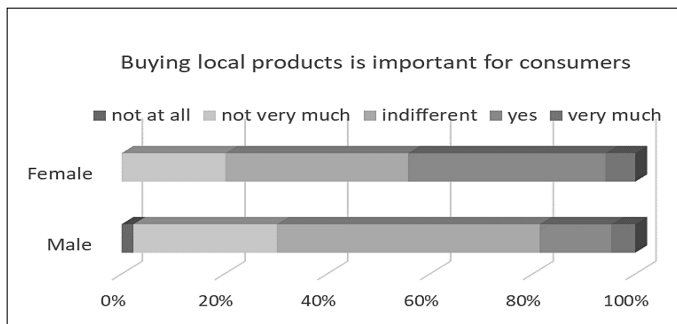


Figure 2: Importance of buying local food (source: own compilation)

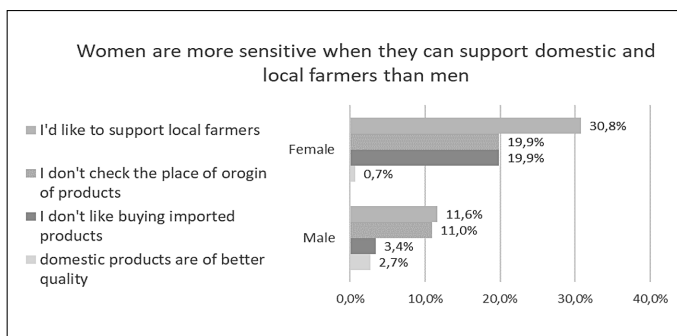


Figure 3: Reasons for the importance of the place of origin (source: own compilation)

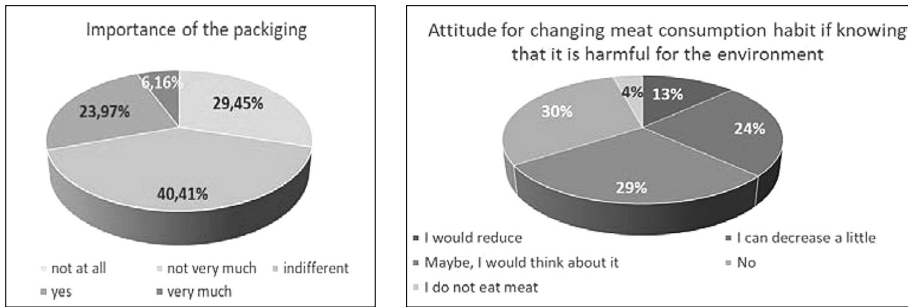


Figure 4: Importance of packaging (left) and meat consumption (right) (source: own compilation)

In addition, women – thanks to their more empathic nature - pay more attention to supporting the prosperity of domestic producers and economies through conscious shopping. The distribution of various reasons can be seen in Figure 3.

It can be seen from Figure 4 that respondent consumers have an indifferent feeling about packaging and that people who consume meat do not want to change their habits.

SUMMARY

The quantity of agricultural products must increase to meet the rapidly growing global population. If the conditions do not change, by the 2050s the current level of greenhouse effect emissions may result in an increase of nearly 80%. Since production conditions affect harmful emissions to such a large extent, it is certain that small producers and local products have an advantage compared to goods coming from far away. The selection of the way of transport is always primarily based on the quantity to be transported, and the best when trying to make full use of the capacity of a freight car. The management of raw materials would require more attention, especially in relation to water and energy consumption. To minimize the use of water, they should optimize their system in such a way that they can reuse the wastewater.

From the point of view of both retailers and nature conservation, it is important to get rid of packaging materials as simply and cheaply as possible, so that they are not wasted and have no further negative effects. They try to encourage consumers to buy individual products in bulk. To obtain better values, continuous monitoring would be necessary, the implementation of which would require a lot of human resources. In keeping with this trend, the European Commission is supporting multi-actor projects in which researchers and agri-food practitioners cooperate. Unfortunately, the information on the label on the packaging is not sufficient to reflect the level of environmental impact for consumers. Information sharing with them needs more improvement.

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Оригинални научни рад

УТИЦАЈ ПРОИЗВОДЊЕ И ПОТРОШЊЕ ХРАНЕ НА ЖИВОТНУ СРЕДИНУ, ГЕОГРАФСКИ ПРИСТУП

Извод

Храна путује велике удаљености да би стигла до наших тањира. Концепт који се односи на угљенични отисак је мерење „миља хране“ што показује колико је та храна еколошки пожељна. У овој студији, која прати приступ „од фарме до тањира“, анализирају се примери различитих фаза мерења, могућности и потешкоће његовог смањења. Употреба локалних производа, концепт кратког ланца снабдевања храном, добра сарадња и комуникација игра важну улогу. Ради бољег разумевања, спроведена је анкета са потрошачима, како би се сазнало шта знају о овом концепту, као и о транспорту, производњи и паковању кушњене хране.

Кључне речи: *прехрамбени миље, локални производ, кратак ланац снабдевања храном, свест, потрошња*



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Original Scientific paper

THE ROLE OF FOOD AND DRINK HUNGARIKUMS IN TOURISM AND HOSPITALITY

Abstract

In this paper, we first present some of the so-called Hungarikums, foods and drinks that belong to the most well-known Hungarian cuisine. It is a tradition in Hungary that events and tourist events including those organized for residential communities, villages, smaller towns, and even national and international visitors are based on local specialties, including these traditional products. We focus on two well-known Hungarikum main dishes and their consumption, presenting case studies for thematic events, and festivals that are introducing them. This study analyses the important role these dishes play in the Hungarian tourism and hospitality.

Key words: *Hungarikum, fish soup, mutton stew, festival, program tourism*

INTRODUCTION

Every nation is proud of its outstanding accomplishment, special economic achievements, natural and cultural values, its unique quality products, cuisine, food, and drink specialties. What Germanicum is for Germans, Polonicum for Poland, Italicum for Italy, Serbicum for Serbians, etc., Hungarikum is for Hungarians.

Hungarikums are products that are unique to Hungary, they are special, of unique quality, and can be found in Hungary or produced in Hungary using a special method. This method has to be applied everywhere the same way, always under the same conditions, having a precise description or recipe. Therefore, they have unique natural, economic, or cultural values that can only be found in Hungary (Tózsza, 2016) and they are protected by law regarding national values and Hungarian heritage adopted in 2012 (Farkas, et al., 2022).

HUNGARIKUM FOODS AND DRINKS

Famous traditional and unique products of Hungary can be suggested for becoming a precious value of Hungary, to belong to the Hungarikum list. The decision is made by the Hungarikum Committee. There are different types of these products, this study focuses on foods and drinks. The following thematic Hungarikum list is not complete due to space limitations, it includes the internationally most well-known and famous Hungarikums. Firstly, raw materials and herbs are mentioned. These include ground paprika from Kalocsa, Hungarian acacia, acacia honey, paprika from Szentes, Piros Arany, Erős Pista, onion from Makó, ground paprika from Szeged.



*Figure 1: From the left: Piros Arany, Erős Pista (Hungarikum Bizottság, 2013)
(Hungarikum Committee, 2013)*

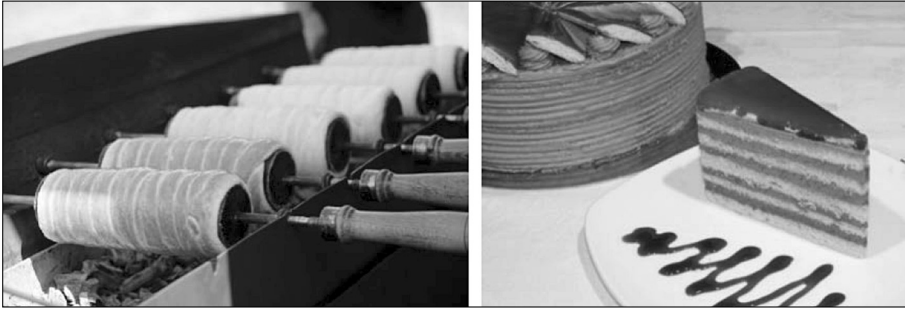
*From the right: Ladies making Szeged pepper garlands
(Agrárminisztérium – Hungarikum Főosztály, 2022)*

Hungarikums also include **preserved meats**, such as the Csabai sausage, Csabai thick sausage, Gyulai sausage, Gyulai paired sausage, Debreceni paired sausage, Herz Classic Téliszalámi salami, and products made from a fattened goose.



*Figure 2: Csabai sausage and Csabai thick sausage
(Hungarikum Bizottság, 2013)*

Sweets include the chimney cake (kürtós kálács, or called Dorongfánk in Transdanubia), dumplings, the so-called friend's ear (barátfüle), Rigójancsi, Dobostorta, Rákóczy curd cake.



*Figure 3: On the left: Chimney cake, Kürtőskalács (Hungarikum Bizottság, 2013)
On the right: Dobostorta (Egal-Team Kft., 2022.)*

The Hungarian well-known **drinks** are pálinka, marc pálinka, Törley sparkling wine (champagne), and UNICUM bitter liqueur. Hungarikums are Egri bikavér red wine with a special historical past, Tokaj aszú, produced in the wine region of the Tokaj Mountains (and cannot be made anywhere else), as well as in the summer heat cooling and refreshing spritzer (called fröccs), for which soda water is used, and it is also among Hungarikums.



*Figure 4: Spritzer made from white wine with soda water
(Hungarikum Bizottság, 2013)*

Among the main dishes, Hungarikums are the fish soups (Bajai, Tiszai), the Karcagi mutton stew, and the Hungarian goulash soup. We have to mention that the gastronomic and catering heritage of Károly Gundel and the Gundel Restaurant are also considered Hungarikums.

EVENTS BUILT ON HUNGARIKUMS

Hungarikum foodstuffs belong to the national heritage of Hungary. They are made in a strict traditional way; they have a high added value and also comply with the quality policy of the European Union. Their production is not characterized by large-scale production, but rather by family businesses, primary producers, and small and medium-sized enterprises.

More and more programs related to hospitality and tourism are being built around these Hungarikum dishes in the country. We consider it important to mention that this is characteristic not only in Budapest – the capital city – but also in the countryside. Besides, it has economic significance, it is justified that one of the elements of rural development could be supporting these events. There are one-day and longer event series, which also include programs related to the preservation of tradition, culture, and community building. Festivals and cultural events attract visitors, often from the surrounding settlements, but often from distant parts of the country and abroad, thus spreading the settlement's good reputation (Véha, et al., 2012).

Events connected to Hungarian cuisine are: the Fish Cooking Festival in Baja; Csaba Sausage Festival; Zamat Festival in Debrecen; Hungarikum Festival, PORVA; Hungarikum Festival Szeged; Local Product Festival – Hungarikum Festival – Zither Festival (Kecskemét); Hungarikum Piknik, Eger; Mutton Cooking Festival in Karcag; Kürtőskalács Festival; Mohács bus tour; Szolnok Goulash Festival; etc.

In the following, we present the two case studies of events related to Hungarikum dishes chosen based on focus group interviews with gastronomy festival visitors: the Fish Cooking Festival in Baja and the Mutton Cooking Festival in Karcag. Observations were made on these festivals in 2022, and the findings are given along with photo illustrations.

FISH COOKING FESTIVAL IN BAJA

Baja is a fishing town. The flavor of the fish soup and the way it is served are unique. It is served with so-called "match pasta" and the juice of the fish soup jus and the cooked fish slices are served separately.



Figure 5: The way of serving the Baja fish soup (Anon., 2016)

In Bács-Kiskun county, they are not fascinating and wearying the visitors with historical data. Instead of that, tourists are enticed by various entertaining programs, presentations of the county's cultural and historical landmarks, ethnographic curiosities, and gastronomy. the Fish Cooking Festival in Baja is one of these events, organised every year at the same time, on the second Saturday of July, where tens of thousands of people come.

The first occasion was in 1996, the 300th anniversary of the founding of the city. Tens of thousands have visited it every year since then, as light music concerts and art programs are also part of the festival, in addition to cooking fish soup (hvg.hu, 2016). For years, Baja fish soup – which is considered a Hungarikum – is cooked in almost 2,000 cauldrons, in Szentárvány square. This Festival is also mentioned in the Guinness Book of Records as "the occasion for preparing and eating the largest amount of food prepared according to the same recipe in one place" (Info, 2020.). Ten-person cooking teams are formed from residents, acquaintances, and friends of the city and surrounding settlements. When the fish soup is ready, they eat it, and since it goes best with it, they drink local red wine (Barna, 2017).

In 2022, the Fish Cooking Festival in Baja was held on July 8-9. Around 20,000 servings of fish soup were sold during the event. Besides the delicious fish soup, the visitors could enjoy various programs prepared for them by the organisers. In addition to the commemorative band and acoustic music concerts that ensured the festival atmosphere, there was a painting exhibition, presentation of a fine art collection from Marosvásárhely, as a twin-city of Baja, cruises on the Danube, boating, folk music and folk-dance programs, craft fair, dog school presentation, and many other smaller programs. In the evenings, well-known DJs provided a good atmosphere.



*Figure 6: Pictures from the Fish Cooking Festival in Baja
(Bácskai Napló – Baja Híroldala, 2022.)*

MUTTON COOKING FESTIVAL IN KARCAG

In the Nagyunság region, the most characteristic animal husbandry was in the Great Plain, where there was a tradition of consuming mutton meat. Karcag, the capital of Nagyunság, the mutton stew was prepared in a unique way compared to the recipes of other settlements in Unság. So, the Karcag Mutton Stew was included in the

UNESCO National Register of Intangible Cultural Heritage in 2009 as "the Karcag tradition of Kunság Mutton Stew". And in 2013, as a so-called "Hungarikum by law", it was included in the Collection of Hungarikums under the name "Karcagi Birkapörkölt" (Hungarikum Bizottság, Hungarikumok Gyűjteménye – Magyar Értéktár, 2016).

The head and claws of the sheep are burnt, then the meat is almost fried without adding water, so it is roasted like that, and then all is cooked together with the tripe and offal. For seasoning, only onion, ground and piped peppers, and salt are used. The cooking takes place in huge cauldrons, large enough to fit the whole mutton sheep in it (Hungarikum Committee, Collection of Hungarikumok – Magyar Értéktár, 2013). The meat of different mutton-sheep animals is never cooked together. As the saying goes: "one sheep – one leg". From a sheep with a live weight of 50-55 kg, remains approx. 26-28 kg after the cleaning preparation. It needs a 30-liter big cast iron pot to just fit inside (Figure 7) and makes about 56-60 portions of stew (Retromuzsika.hu, 2022).



Figure 7: Karcag mutton stew (Karcagi Birkafőző Fesztivál, 2022)

Since 1999, the mutton cooking competition has been held in Karcag every year on the last Saturday of June (Karcagi birkafőző fesztivál 2022, 2022.) In 2022, this event took place on June 24-25 (Karcag, 2022).

This year, mutton stews was made in more than 200 cauldrons. Chefs of various ages came from all over the country to find out who cooks it better (Daróczy, 2022). In addition to delicious food and drinks, visitors were also traditionally treated to cultural events. Guests could take part in a folklore festival, various concerts, craft fairs, children's programs, and there was a street party, too (Open Gates Hungary Kft., 2022.).

On the first day of the festival, Friday, there were operetta hits, old hits, children's games, crafts, Wine Yard, and Rock Yard. The cooking competition was on Saturday.



*Figure 8: Pictures from the Karcag Mutton Cooking Festival
(Karcagi Birkafőző Fesztivál, 2022)*

SUMMARY

The dishes that have received the honorary title of "Hungarikum" are made with reliable ingredients and traditional recipes, so consumers and guests are assured that the food they have chosen is special and of high quality. They also know what they can expect because it is always made the same way. Programs, village days, and festivals organized around Hungarian food increase the number of guests visiting the settlement, the number of tourists is increased and due to international visitors, it ensures a good reputation abroad. These are also important sustainable tourism attractions since local products are used for cooking as raw materials. The events organized in addition to the usual cooking competitions meet the interests of several age groups and play an important role in presenting and preserving traditions. In addition, music concerts and other cultural programs are touching the crowd unnoticed. These festivals are also good locations for the advertising activities of various companies, which can appeal to a new audience with their presentations. We can therefore say that the usually multi-day programs organized around Hungarikum food have economic and cultural significance not only from the point of view of the local residents.

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Оригинални научни рад

УЛОГА ХРАНА И ПИЋА ХУНГАРИКУМ У ТУРИЗМУ И УГОСТИТЕЉСТВУ

Извод

У овом раду најпре представљамо неке од такозваних Хунгарикума, јела и пића која спадају у најпознатију мађарску кухињу. У Мађарској је традиција да се манифестације и туристичке манифестације, укључујући и оне које се организују за стамбене заједнице, села, мање градове, па чак и домаће и међународне посетиоце, заснивају на локалним специјалитетима, укључујући ове традиционалне производе. Фокусирамо се на два позната главна јела Хунгарикума и њихову конзумацију, представљајући студије случаја за тематске догађаје и фестивале који их представљају. Ова студија анализира значајну улогу ових јела у мађарском туризму и угоститељству.

Кључне речи: *Хунгарикум, рибља чорба, овчија чорба, фестивал,
програмски туризам*

**PRODUCTION OF FIELD AND VEGETABLE
CROPS FROM THE POINT OF HEALTHY
SAFE FOOD**



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Original Scientific paper

THE SIGNIFICANCE OF SAFE FOOD IN TRAVEL AND TOURISM DEVELOPMENT IN THE POST-COVID-19 ERA

Abstract

The purpose of this paper is to analyze the current situation of multidimensional food safety assurance as well as the significance of food safety in travel and tourism development in the post-Covid era. The paper also investigates the place and role of food safety in the Travel & Tourism Development Index (TTDI index). The method of observation, analysis, synthesis, comparative and inductive-deductive methods were used in the paper. The research shows that food safety is neglected and that the TTDI index did not include food safety as a significant indicator in sub-index changes, indicating that umbrella tourism organizations did not recognize the importance of food safety as an indicator of the development of a tourist destination.

Key words: *Food, safe, health, Covid-19, TTDI index*

INTRODUCTION

Tourism is a rapidly growing industry, which has to face many challenges during its various processes. Besides the most common topics of sustainability, climate change and conditions of a pandemic caused by the virus Covid-19, another important aspect of the industry is food safety.

We are entering a new, unprecedented global economic and social era following the Covid-19 pandemic, in which there will be opportunities and threats for the goods and services provided. It seems that food safety is neglected, even though sustainable food supply and risk-free food safety are closely related to each other and are very important for safety in tourism destinations. Although the food industry is one of the most important global industries with significant implications for the world economy, very little research has been done to cover this topic and address its specificity, issues, or performance assessment.

In recent years, the impact of COVID-19 has further demonstrated the need for the T&T sector to build resilience and has highlighted the role that socioeconomic conditions, healthcare, food safety, social protection, working conditions and ICT investment play in sector development and longevity.

Safety in tourism has gained much more importance in the period during and after the Covid-19 crisis. However, the sector and its customers have probably changed permanently.

The paper aims to investigate the role of safe food in the development of tourism as well as potential innovations within the era of the COVID-19 crisis after framing them within the four issues of the food sector: food safety, bioactive food compounds, food security, and food sustainability that are directly affected by the pandemic.

This paper discusses an innovative approach for measuring tourism competitiveness using a new Travel & Tourism Development Index (TTDI index) and investigates the place and role of food safety in the Travel & Tourism Development Index (TTDI index).

LITERATURE REVIEW

Understanding food safety hazards is essential to avoid potential negative health impacts in the food supply chain in a post-Covid-19 pandemic era. Developing strategies for virus direction in foods plays a vital role in food safety and verification (Zhang, et al. 2021). Three main transmission means of SARS-CoV-2 have been proposed and discussed, namely, human-to-human contact transmission (He et al. 2020; Zhang et al. 2020), aerosol transmission (Li et al. 2020; Anderson, 2020), and droplet transmission (Galbadage et al. 2020). Moreover, it has been suggested that the virus can transmit via the digestive tract, but its role and significance need further observation and research (Wong et al. 2020). Limited attention has been focused on the transmission of SARS-CoV-2 in food and the food supply chain. During the COVID-19 pandemic, all categories of the food supply chain, including fresh vegetables, fruit, bakery items, perishable goods, and food grains, have been highly compromised (Ivanov, Dolgui, 2020). Food safety is one of the four pillars of the food system that has been badly affected by the COVID-19 pandemic (Galakanakis, 2020).

When the virus contaminates the food, the risk of infection may increase during transportation due to the lack of strict control of the transport pathway. (Zhang et al. 2021). The food system is comprehensive, multifaceted, highly interconnected, and has the potential to address food security, safety, nutrition/quality, and manufacturing allocation (Abbaspourrad et al. 2017). Studies have found that SARS-CoV-2 can be transported via the surface of contaminated droplets in the form of an aerosol, which appears in the air at the department store's entrance. Thus, at the end of the food chain, people still suffer from the risk of infection in food consumption (Liu et al. 2020).

Surveys conducted in 2020 investigated the food consumption behavior during the pandemic period, attempting to predict the post-Covid-19 era as well (Skalkos et al. 2021). A US study in major metropolitan areas showed that patterns for major food groups seem to stay the same. Still, a large share indicated that they had been snacking

more because of the beginning of the pandemic, which was offset by a sharp decline in fast food consumption (Chenarides, et al. 2021). A Swiss study revealed that consumers considered having more time to prepare meals themselves as being particularly important to achieving healthier food consumption (Hansmann et al. 2020). A unique panel survey of representative households in Addis Ababa implied, at least indirectly, that the aggregate food value chains have been resilient to the shock associated with the pandemic (Hirvonen et al. 2021). The Covid-19 pandemic led to millions of infections and deaths worldwide, changing dramatically what we perceived as norms and impacting society, health systems, governmental policies, and businesses. The food sector is no exception, as the consequences of this “*Black Swan*” socio-economic event has changed the way we think, buy and consume food by accelerating pre-existing innovation trends (Askew, 2020) marking a “before” and “after” period. The role of mapping trends and predicting consumer behavior toward new technologies, services, and products for transitioning beyond Covid-19 will be highly beneficial as per approaches described by Busse and Siebert (Busse, Siebert, 2018) and Suanda et al. (Suanda et al., 2018). According to Galanakis, 2020 on a long-term basis, the pandemic affects the whole food sector in four main domains: food safety, bioactive food ingredients, food security, and sustainability. In the post-pandemic era, the research in the food sector was abruptly redirected to four main domains:

1. Food safety became a critical issue to limit the spreading of the virus in the food chain among producers, farmers, retailers, and consumers
2. The demand for foods containing bioactive compounds increased rapidly, as consumers were looking to adopt healthier diets and boost their immune systems

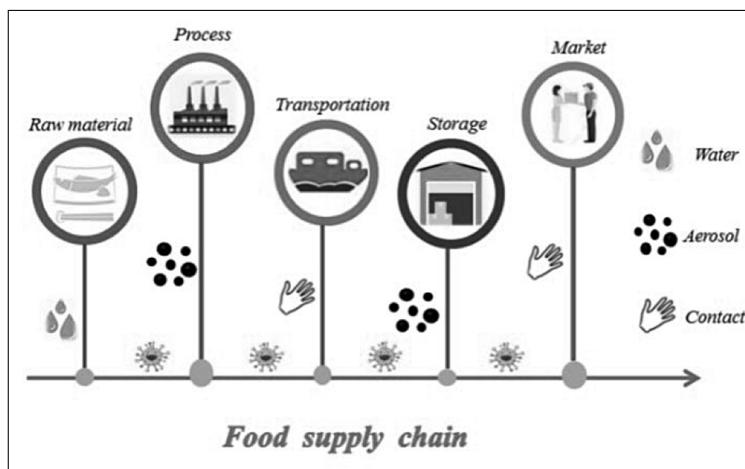


Figure 1. The possible transmission of SARS-CoV-2 in a food supply chain via three main means: water; aerosol, and contact

Source: Zhang, W., He, H., Zhu, L., Liu, G., & Wu, L. (2021). Food safety in Post-COVID-19 pandemic: challenges and countermeasures. *Biosensors*, 11(3), 71.

3. The lockdown of billions of people worldwide has led to food shortages and food security problems related to price spikes and panic buying
4. The sustainability and resilience of the food sector is another issue to be addressed to restrict relevant crises in the future (Boyaci-Gunduz, et al. 2021; Galanakis, 2020; Rizou et al.2020).

Food systems affect human health directly and indirectly, and today it is more urgent than ever that they should become sustainable. In 2015, the United Nations (UN) 2030 Agenda for Sustainable Development declared the 17 Sustainable Development Goals (SDGs), which comprise an urgent call for action by developed and developing countries in a global partnership (<https://sustainabledevelopment.un.org/>).

The current food systems are highly dependent on animal-based protein sources that are not sustainable from an environmental point of view but also from a health and food security perspective (Galakanis, 2020).

Food insecurity is growing due to the economic crisis caused by the Covid-19 pandemic (World Food Program, 2020). Both developing and developed countries are facing the same situation due to increased food insecurity during the Covid-19 pandemic, while vulnerable and low-income population groups are more severely affected.

THE TRAVEL AND TOURISM DEVELOPMENT INDEX

The competitiveness of tourist destinations is important, especially as countries strive for a bigger market share. However, competitiveness is a complex concept, encompassing various aspects that are very difficult to measure. When the World Economic Forum published its 2019 edition of the Travel & Tourism Competitiveness Report (TTCR), growth in the Travel & Tourism (T&T) sector was achieving new records. Two years later, the T&T industry looks very different. Demand in this sector was one of the hardest hit by the COVID-19 pandemic, leaving companies and tourism-driven national economies severely affected by shutdowns, travel restrictions, and the disappearance of international travel. Fortunately, there are positive signals, and analysis shows that recovery has started, albeit not at the same pace across the globe or in the same market segments.

Since 2007, World Economic Forum (WEF) has developed the first report on the World Travel & Tourism Competitiveness Index (TTCI). For the past 15 years, this report has become the leading benchmark and measure in the world tourism industry (Travel & Tourism Development Index 2021: Rebuilding for a Sustainable and Resilient Future).

However, in the past two years, the severe damage to the Travel & Tourism sector (T&T) due to the impact of the Covid-19 pandemic has completely changed the approach of WEF. WEF has made important changes between the TTCI and the TTDI, which is presented through the theme of the 2021 report: “Rebuilding for a Sustainable and Resilient Future.”

The Travel & Tourism Development Index (TTDI) is a direct evolution of the Travel & Tourism Competitiveness Index (TTCI). The TTDI benchmarks and measures “the set of factors and policies that enable the sustainable and resilient

development of the Travel and Tourism (T&T) sector.” Some of the most notable framework and methodology differences between the TTCI and TTDI include the additions of new pillars, including Non-Leisure Resources, Socioeconomic Resilience and Conditions, and T&T Demand Pressure and Impact.

The new TTDI framework is designed to support this pivot in strategy and practice. Sustainable management of tourism demand that maximizes benefits for local communities while mitigating negative side effects such as overcrowding will also become a vital component of T&T development as the sector recovers.

The number of subindexes has been increased from four to five with the addition of a T&T Sustainability subindex. The Natural and Cultural Resources subindex has been renamed T&T Demand Drivers. The Environmental Sustainability pillar and the new Socioeconomic Resilience and Conditions and T&T Demand Pressure and Impact pillars have been grouped together under a new T&T Sustainability subindex to emphasize how vital sustainability is for long-term T&T sector development (Travel & Tourism Development Index 2021: Rebuilding for a Sustainable and Resilient Future, 2022).

FOOD SAFETY AND TTDI – DOES THE FOOD SAFETY HAVE A PLACE IN TTDI?

Due to the magnitude of the pandemic, one of the aspects that might be worth studying is the behavioral change linked to the perception of the risk posed by Covid-19. According to the protection motivation theory, the evaluation of the severity of a threat is one of the cognitive processes behind the decision to engage in protective behavior (Villacé-Molinero et al., 2021).

Under these circumstances, the World Economic Forum (WEF, 2020) demands that all stakeholders urgently come together to minimize the impact on public health and limit its potential for further disruption to lives and economies worldwide.

Looking at the pillars of TTDI, it can be concluded that safe food is dealt with by two pillars: Pillar 2 – Safety and Security and Pillar 3- Health and Hygiene. According to TTDI indicators make up each pillar. Table 1. shows indicators of pillar Safety and Security (6 indicators). Safety and security are critical factors in determining the success of a country’s T&T sector.

This pillar measures the extent to which a country exposes locals, tourists and businesses to security risks. In addition to creating barriers to T&T investment, countries with a high incidence of crime or violence are likely to deter visitors, making it less attractive to develop the T&T sector in those places. The costliness and occurrence of common crime and violence, police reliability, and terrorism and armed conflict are considered.

It is unusual that there is no mention of food safety anywhere. We are of the opinion that safe food is a very important indicator of the safety of a tourist destination, especially in post- Covid-19 era. For the above reason, we looked at which indicators make up the pillar Health and Hygiene. Table 2. shows indicators of *pillar Health and Hygiene*.

Table 1. Indicators of pillar Safety and Security

Pillar 2: Safety and Security
Business costs of crime and violence
Reliability of police services
Safety walking alone at night
Homicide rate
Global Terrorism Index
Organized violence, deaths

Source: Travel & Tourism Development Index 2021: Rebuilding for a Sustainable and Resilient Future, 2022

Table 2. Indicators of pillar Health and Hygiene

Pillar 3: Health and Hygiene
Physician density
Use of basic sanitation
Use of basic drinking water
Hospital beds density
Accessibility of healthcare services
Communicable disease incidence

Source: Travel & Tourism Development Index 2021: Rebuilding for a Sustainable and Resilient Future, 2022

This pillar measures healthcare infrastructure, accessibility and health security. COVID-19 has highlighted the potential impact of communicable diseases on the T&T sector. In particular, the pandemic has demonstrated how important a country's healthcare system is when it comes to mitigating the impact of pandemics and ensuring safe travel conditions, and workforce availability and resilience. In general, if tourists or sector employees do become ill, the country's health sector must be able to ensure that they are properly cared for, as measured by the availability of and access to physicians, hospital beds and general healthcare services. Moreover, access to safe drinking water and sanitation is important for the comfort and health of travelers and locals alike. Food safety and security don't exist as an indicator of this pillar. The modern food industry is among the key partners of today's global tourism. As part of the tourism processes, tourists buy and consume local food in the local catering facilities. Furthermore, tourists are usually willing to try out gastronomy specialties during their travels. Food safety is important for tourists although it is not always part of their conscious behavior at the destination. Because of that, we think safety food should be included as an indicator of this pillar.

CONCLUSION

The Covid-19 pandemic crisis has created a new era. Food sector experts will have to face many significant challenges: ensuring food safety and food security, introducing Industry 4.0 tools to reduce losses and waste of food, as well as identifying alternative and safe protein sources that meet the nutritional expectations of consumers. At the same time, they should introduce innovations fast enough with the imminent economic crisis in the era of the COVID-19 pandemic as well as offer acceptable and economically competitive products and develop functional foods fortified with bioactive compounds and antioxidants that promote health and support consumers' immune system. Post-COVID tourism must focus on the development of new tourist products in the area of selective forms of tourism, emphasizing the well-being of tourists, health, safety, and security issues, and sustainability.

In the context of indicator analysis of TTDI it was concluded that food safety does not exist as an indicator in each pillar, which is completely unclear and unjustified.

There is an urgent need to identify and develop cross-sectoral policies which promote and support a healthy, safe and sustainable food system in a post-covid-19 era.

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Оригинални научни рад

ЗНАЧАЈ ЗДРАВСТВЕНО БЕЗБЕДНЕ ХРАНЕ У ТУРИЗМУ У ПОСТ КОВИД ПЕРИОДУ

Abstract

Циљ рада је да укаже на мултидимензионалност осигурања безбедности хране, као и на значај здравствено-безбедне хране у сектору туризма и путовања у пост-Ковид периоду. У раду се истражује место и улога здравствено-безбедне хране у Индексу развоја туризма и путовања (TTDI index). Методологија рада укључила је коришћење метода опсервације, анализе, синтезе, компаративну и индуктивно-дедуктивну методу. Истраживање показује да је безбедност хране занемарена и закључено је да ТТДИ индекс није укључио безбедност хране као значајан индикатор у променама подиндекса што имплицира да крвене туристичке организације нису препознале значај безбедности хране као индикатор развоја туристичке дестинације.

Кључне речи: *храна, безбедност, здравље, Ковид-19, ТТДИ индекс*



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Original Scientific paper

STUDENTS 'ATTITUDES TOWARD THE USE OF FOOD ADDITIVES

Abstract

Nowadays it is hard to imagine the food industry without the use of food additives. Additives make up about ten percent of the food which the average adult consumes. Additives added to food products are usually labeled with E – numbers, which are unique and specific for each compound. These labels refer to artificial and natural additives whose use is approved in the countries of the European Union. The results of the research showed a weak awareness of students about the use of additives in food products.

Keywords: *additives, food products, student attitudes, food, E- numbers*

INTRODUCTION

Food additives are both natural and synthetic substances that are added to food products in order to preserve sensorial properties (taste, smell, color, appearance), to improve texture or have some other technological function (Kaptan and Kayısoglu, 2015). Today, there is about 25,000 additives in use worldwide.

In the 1980s, the global food production system was motivated to increase the consumption of certain food products. In order to meet the multiple demands of consumers, the food industry found a solution in the use of food additives. During the production process of various food products, additives are necessary because their use ensures safety, product quality and extension of shelf life (Ilback and Busk, 2000). In addition, additives improve some attributes of the food itself, such as quality and sensory properties and thus make the product attractive to consumers (Sohár, 2005).

Food safety is recognized as a global problem. Consumers are increasingly concerned about food safety. Many studies have confirmed that consuming excessive amounts of synthetic food additives can cause respiratory, dermatological, neurolo-

gical, and gastroenterological problems (Randhava and Bahna, 2009). Henson and Traill (1993) point out that food safety is in inverse proportion to the risk that consuming certain foodstuff carries. Based on that, it is believed that the risk can be expressed as the probability that the consumer is not in danger of consuming certain food. Experts believe that the potential risk to human health from regular consumption of food containing additives is negligible compared to the benefits which the use of additives has in food production (Emerton and Choi, 2008).

However, although the consumers are somewhat aware of the benefits of food additives, they still tend to look at their presence in food with a certain amount of caution and try to avoid buying such products (Wandel, 1997). Emerton and Choi explain this mistrust with the fact that during the development of additives and their application in food processing, additives are associated with the use of bad raw materials, poor technological processing, as well as health problems. In addition to the facts stated by Emerton and Choi, there is another reason for mistrust, which is the name of food additives.

The names given to additives are mainly unknown to consumers. According to the Rulebook on food additives, the labeling of additives sold individually or in a mixture with other additives and/or other food ingredients, which are intended for sale to the final consumer, must provide information about the name and E number of each additive or the name under which it is sold (Rulebook, 2018). Further confusion and the reason for consumers' suspicion comes from E numbers. This fact can be attributed to the insufficient knowledge of consumers. As a result of a lack of information and commitment to consumers, when general information and knowledge about additives are concerned, during the 90s there was a growing interest in the consumption of organic food (Brokman and Biren, 2011).

This topic is still popular and arouses interest among scientists. As food safety has become a global problem, more and more scientific papers investigate consumers' perception of the use of additives in food industry. The works also emphasize communication between state institutions, the government, and the media, which are somewhat obliged to inform consumers about reports related to the testing of food additives and their use in the food industry. Food safety research has shown that consumers tend to make rational food choices if they have some knowledge about the detrimental health effects associated with a particular additive present in a specific food (Redmond and Griffith, 2003a).

Several studies dealt with consumers' attitudes, knowledge, and perceptions about food additives. Altug and Elmaci (1995) conducted research among students, doctors, housewives, nurses, high school students, kindergarten teachers and employees of the Department of Food Engineering at Ege University. The study showed that regardless of the group respondents belong to, most consumers had little knowledge about food additives and believe that additives are harmful to health. In a similar study, Wandel (1997) points out the negative attitude of consumers towards the numbering system, ie. naming the additives on food labels with E numbers. In 2007, Kim, Na and Kim conducted a survey involving high school students. The obtained results indicate that most of the respondents are not familiar with the additives used in food production, and equally have difficulties in identifying them on the product label.

A similar study was conducted at the University of Mauritius by Shaheen Koyratty and Aumjoud (2012). The obtained results showed that 65% of the respondents do not read or check the labels on food products and have scarce knowledge about food additives.

The aim of this paper was to examine the attitudes of students at the University of Novi Sad toward the use of additives in food products.

MATERIAL AND METHODS

The research was conducted in the period from 01.04.2022. to 01.05.2022. based on research conducted by Shaheen (2012) at the University of Mauritius with certain modifications. The research involved a total of 151 students at the University of Novi Sad, the second largest university center in the Republic of Serbia.

The research was conducted using a survey method. After data collection, the results were processed by descriptive analysis.

The questionnaire consisted of three parts. The first part was related to the socio-demographic characteristics of respondents. The second part consisted of multiple-choice questions, with the aim to assess the knowledge of the students about additives and their presence in food products. The third part involved the questions related to the students' perception of food additives and their presence in food products. The respondents were provided responses based on the standard 1-5 Likert scale (1 – not agree at all, up to 5 – completely agree).

RESULTS AND DISCUSSION

Analysis of socio-demographic characteristics of respondents

In Table 1 the results of socio-demographic characteristics of the respondents are presented.

Table 1. Socio-demographic characteristics of the respondents

Gender (Participation %)	Men	Women
	52,3	47,7
Age	Age (man and women)	Participation (%)
	19-23	49,7
	24-26	34,4
	27-30	11,3
	more than 30 years	4,6

Source: Author, 2022.

The sample of 151 respondents included students of different levels of study, aged from 19 to 30 years. Among the surveyed students, there were slightly more men (52.3%) than women (47.7%). The largest number of students was on basic academic studies (36.4%), while the smallest number of respondents were doctoral students (9.3%).

RESULTS OF THE STUDENT SURVEY ON KNOWLEDGE OF ADDITIVES AND THEIR APPLICATION IN FOOD PRODUCTS

Table 2. Example of multiple choice questions for assessing knowledge of additives

Questions	Options and correct answer
In your opinion, what is the role of additives in food products?	a) Treatment and/or prevention of certain diseases (e.g. cancer) b) <i>Extending the shelf life of food products and improving their taste and color.</i> c) Increasing the mass of food products.
What is the health effect of food coloring such as tartrazine (E102).	a) <i>May cause hyperactivity in children.</i> b) It can improve the eyesight of the elderly. c) It can cause carcinogenic diseases in younger people.
Why does the presence of aspartame in the product require the additional claim "contains phenylalanine" on food label?	a) <i>Warns people who are sensitive to phenylalanine to take precautions.</i> b) Informs diabetics that snacking is e.g. tortilla chips safe to consume. c) To emphasize the benefits of aspartame in a product such as e.g. tortilla chips.
The statement "Diet" on the bottle of a soft drink indicates the following:	a) Non-alcoholic drinks are without additives. b) <i>Non-alcoholic drinks do not provide energy.</i> c) Non-alcoholic drinks can replace lunch.

Source: Author, 2022.

The total of ten questions referred to the knowledge about food additives and their application in food products. According to the results, the majority of the respondents (over 70%) knew the exact definition of food additives, their role and the difference between natural and artificial additives. This result is quite encouraging, considering that research conducted in previous years (Kim Na and Kim, 2007) showed that regardless of the group of respondents, knowledge about food additives was limited.

When the function of sodium sulfate and tartrazine in food is concerned, more than 50% of respondents showed that they are well informed. Tartrazine is an orange food color. It is mostly used in the food industry, but also it has application in the pharmaceutical, textile, and cosmetic industries. Tartrazine can cause side effects on human health, such as hyperactivity of children, allergies, asthma (Rovina et al., 2017). So-

dium sulfate can also cause allergic reactions of asthmatics. On the other hand, when it comes to the meaning of the number E330 (citric acid), the opinions of respondents are divided. Only 36% of respondents gave the correct answer, while 33.3% of them believe that this number represent the amount of citric acid that is allowed in food. The issues related to aspartame and diet products are often linked, since aspartame is an artificial sweetener that is most often found in diet products. When it comes to aspartame, more than half of the respondents (55.3%) gave the correct answer. What is particularly interesting is that 45.3% of respondents believe that products with the statement "diet" on the label do not contain any additives.

Preservatives are known as antimicrobial agents, and they belong to the group of food additives. Their function is to protect food from spoilage caused by microorganisms. According to the Rulebook (2018) there are 45 substances that can be used as preservatives. Although these substances are harmless if used in allowed quantities, some of them may have adverse effects on human health. Some of the side effects are rash, itchy skin, shortness of breath, sneezing (Silva and Lidon, 2016). In this study, 27% of respondents explain that the statement "No preservatives" on label means that the food product does not contain additives.

The last part of the survey referred to the students' perception of food additives and their presence in food products. Students expressed their attitudes using a Likert scale numbered from one to five. Number one indicates that the student does not agree at all with the given statement, and number five indicates that the student completely agrees with the given statement.

Out of the total number of respondents, only 2.67% of them regularly read the label on food product and thus get information about the ingredients and the presence of additives. In a study conducted by Koyratty et al. (2014) the obtained percentage is much higher and amounts to 35%. Mentioned research involved a larger number of respondents (180). Similar research conducted in Norway (Wandel, 1997) and Australia (Williams et al., 2004) showed that information about food additives is the most desirable and the most often read detail on the products label. It should be noted that these studies were also conducted on a larger sample.

A worrying fact is that 62.66% of students do not read the products label at all, while 43.33% of respondents believe that food that contains additives have a negative impact on human health. Although the respondents in the previous part of the survey showed a solid knowledge about food additives, based on these data, the question is how they choose their products. Most of them, 56.66%, are willing to pay more for a product which does not contain any additives.

On the other hand, 49.33% of respondents do not agree with the statement that the use of additives has some benefits, and they believe that food containing additives is of poorer quality. The use of additives in the food industry is regulated by the Rulebook on food additives. Food producers must comply with this policy. However, although this fact is true, distrust towards producers exists. Almost half of the respondents, 48%, believe that manufacturers do not operate in compliance with this rulebook.

On the other hand, most of them believe that products that contain the statement "without additives" on label, do not contain them indeed.

CONCLUSIONS

Based on the results of the conducted survey about students' knowledge and application of additives in food products, it can be concluded that a small number of students read the labels on food products and thus are not adequately informed about the product and its composition. It indicates the lack of their knowledge about the presence of additives in food and their effects on the health. It can also be concluded that students have different opinions when it comes to the presence of certain additives and their use in the food industry as well as their adverse effects to consumer health. This indicates the need to discuss this topic with young people in order to raise the level of their knowledge about food additives.

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Оригинални научни рад

СТАВОВИ СТУДЕНАТА О УПОТРЕБИ АДТИВА У ПРЕХРАМБЕНИМ ПРОИЗВОДИМА

Сажетак

Прехрамбена индустрија данас се тешко може замислити без адитива. Адитиви чине око десет одсто хране коју просечан одрасли човек конзумира. Адитиви који се додају прехранбеним производима обично се означавају као Е – бројеви и ти бројеви су јединствени и утврђени за свако једињење. Ове ознаке се односе на вештачке и природне адитиве чија је употреба одобрена у земљама Европске Уније. Резултати истраживања су показали слабу упућеност студената о употреби адитива у прехранбеним производима.

Кључне речи: *адитиви, прехранбени производи, ставови студената, храна, Е-бројеви*

**FRUIT AND GRAPES PRODUCTION
FROM THE STANDPOINT OF HEALTHY
SAFE FOOD**



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Original Scientific paper

GENOTYPE EXPRESSION OF TRADITIONAL PEAR VARIETY SINEC DEPENDING ON ECOLOGICAL FACTORS

Abstract

This paper presents the results of an examination of the phenological characteristics, pomological properties and chemical composition of the fruits of the traditional pear landrace *Sinec* in three regions of the Republic of North Macedonia (Kumanovo, Debar and Kriva Palanka). The pear *Sinec* blooms in mid – April. The fruits ripen from the second decade of August (in Kumanovo and Kriva Palanka) and to the end of August (the Debar region). The landrace pear *Sinec* forms intermediate small fruits, while larger ones are formed in the region of Kumanovo and Debar. The fruits contain on average 13.9 % soluble dry matters, 13.04 % total sugars and 0.33 % total acids. For all investigated fruit properties are found statistically significant differences except for the content of total acids in the fruit.

Key words: *traditional landrace, flowering, ripening, pomology, chemical traits*

INTRODUCTION

In the past there was an extensive sorting of traditional varieties of pears, such as Karamanka, Letna Kajkushka, Zimska Kajkushka, Tiranka, Zimorka, Erebasma, Trupnjak, Mustabej, *Sinec*, Tatlikuti, Shalganka etc. (Avramovski et al., 2005). Their common characteristics were vitality and longevity with an average age of 100-150 years old. It should be noted that they were well suited to local conditions and had a natural resistance to some diseases making the use of chemical plant protection insignificant and unnecessary (Stancevic, 1983).

Striving towards maximum benefits, the application of new industrial varieties, replacement of local varieties and landraces by foreigners, significantly reduced the pear genetic diversity. The intensification of agricultural production and introduction into production of new, relatively speaking better varieties of pears, introduced diseases that destroyed the entire sorting both domestic and foreign (Selamovska and Nikolic, 2012).

Today traditional landraces are true rarity to be encountered and mainly appear sporadically in yards. The individual trees can be found in the displaced areas and hilly and mountainous areas, where extensive agriculture is still being practiced (Selamovska, 2013a). This unenviable situation imposed the necessity to preserve traditional domestic pear sorting. Through trials efforts are being made to revitalize the pear in Macedonia.

MATERIAL AND METHODS

In this paper the traditional pear landrace *sinca* is studied. When choosing varieties for specific soil and climatic conditions it is necessary to know the specific genetic, morphological and pomological properties of landraces, their behavior toward various soil and climatic conditions, start of giving fruit and yield, sensitivity or resistance to various biotic and abiotic factors, convenience for storage, transport and other.

Hence, arose the plan to work in this paper by examining certain important traits, such as flowering and ripening time; fruit features like fruit mass (g), fruit dimensions-length and width (mm), fruit firmness (g/cm²), fruit taste, fruit texture, length of stalk (cm) and seed number per fruit. Also, the chemical fruit traits such as total sugar (%), total acid (%) and soluble dry matters (%) are examined. The morphological characteristics were determined of the *Pyrus* descriptor as developed by the International Board of Plant Genetic Resources (IBPGR). The classification of fruits was done according to the Rubcov classification (Mratinic, 2000). The fruit mass was weighed on the Mettler analytical laboratory scale and fruit firmness was measured with a FTOS penetrometer. The extraction of seeds was done manually and then the number of filled (healthy) seeds per fruit was counted. The total sugar and the soluble dry matter were measured with a Carl Zeiss Jena – DDR/713457 refractometer. The total acids were determined by a standard method of titration (NaOH).

The examinations were performed on three localities and their vicinity in Macedonia: Kumanovo region (v. Stracin), Kriva Palanka region (v. Konopnica) and Debar region (v. Mogorche).

All outreach analyses were performed on cooperation with professionals from the Agency for Individual Agriculture Development. This research was a part of the scientific-research projects which were funded by the Ministry of Agriculture, Forestry and Water Economy of Macedonia.

The region of Kumanovo belongs to the continental – mediterranean – climate vegetation – soil area, which combines the impact of east-continental and continental climate. The average annual air temperature is between 11.8°C to 13.6°C (in average 12.7°C). The average annual amount of precipitation in the region of Kriva Palanka

amounts 460 -583mm. According to the thermal designation by Gračanin, Kumanovo region has moderately warm climate (Filipovski et al., 1996).

The regions of Kriva Palanka and Debar fall within the warm - continental climate vegetation – soil area. This area represents the transition between Mediterranean and continental climate. The mean annual temperature is 9.6°C to 11.8°C (on average 10.9°C). The valley where Kriva Palanka is situated is characterized by lower daily high temperature and dry climate (9.6-10.1°C) compared to Debar that has humid climate (11.6-11.8°C) with an annual amount of rainfall 600-700mm in Kriva Palanka and 800-900mm in Debar. The soil temperature at a depth of 20cm ranges from 11.1°C to 12.8°C and depth of 50cm from 12.3°C to 13.1°C. Although in this area cinnamon forest soils can be found, it is dominated by the lessiv variety. In the past, the forests in the region of Kriva Palanka were used for extensive grazing as pasture (Filipovski et al., 1996).

The obtained results were statistically processed by analysis of variance. The significance of differences between the average values were tested using LSD test (0.01%, 0.05%). The correlation between the morphological and chemical-physiological characteristics of the fruit is calculated according to the Röemer – Orhal categorization.

RESULTS AND DISCUSSION

In Macedonia, pear Sinec is encountered in the regions of Kriva Palanka, Kumanovo, Debar and Skopje. It is found under several synonyms: Sinec (Kriva Palanka, Skopje), Sinac, Sinjak (Kumanovo), Sinci, Sinici, Since (Debar). It grows in hilly to mountainous conditions, at an altitude of 300-500-1000m. Soliter trees are found on natural pastures, fields, yards, etc. According to the morphological features, the pear Sinec is high, branched tree with upright branches and represents vital and long-living fruit which is abundantly fruitful. Almost all fruits are collected by locals, mostly for their own use, and only a small portion is sold to local markets (Selamovska and Nikolic, 2012; Selamovska et al., 2013b, 2014, 2019). Sinec is a traditional variety resistant to *Psylla pyri*, one of the most pear phytophagic sap-sucking insect (Dimetrovski, 1974).

Depending on the climatic conditions, the variety has different flowering times (Milutinovic et al., 1998). It starts to flower in the middle of April, with the difference that in the surroundings of Debar it flourishes a few days later compared to Kumanovo and Kriva Palanka. And in the region of Skopje, the variety forms flowers earlier (Selamovska et al., 2013b). According to Gjurgjevic and Shoshkic (1968), the traditional pear variety Sinec belongs to the group of early flowering varieties. According to the maturation time, Sinec is a summer traditional variety and the fruits ripen in the second third of August. In the region of Kumanovo and Kriva Palanka, they ripen earlier in comparison with Debar, where they are mostly found. The reason for the later flowering and ripening of the variety in the Debar region is the cold climate, the higher altitude and the higher amount of precipitation during the year compared to the other regions.

Table 1. Morphological pear fruit properties grown on three localities

Locality	v. Stracin (Kumanovo)	v. Konopnica (Kriva Palanka)	v. Mogorche (Debar)	Average
FWg (g)	61.20 ± 15.58	39.20 ± 7.49	60.80 ± 11.85	53.73 ± 12.59
FL (cm)	51.10 ± 4.51	51.90 ± 4.61	55.80 ± 4.10	52.93 ± 2.51
FWd (cm)	46.40 ± 4.90	40.00 ± 3.30	46.00 ± 3.86	44.13 ± 3.59
Ratio FL:FWd	1.11 ± 0.09	1.29 ± 0.12	1.22 ± 0.09	1.21 ± 0.09
FSL (cm)	41.00 ± 3.59	51.00 ± 6.26	39.70 ± 4.60	43.90 ± 6.18
NoSs	0.80 ± 0.79	1.10 ± 1.29	1.20 ± 1.40	1.03 ± 0.21
FF (g/cm ²)	1301.00 ± 292.10	755.00 ± 40.89	1470.00 ± 581.28	1175.33 ± 373.70

* Data are average of n=10 ± SD, Abbreviations: FWg – Fruit weight, FL – Fruit length, FWd – Fruit width, Ratio FL : FWd – Ratio of fruit length : Fruit width, FSL – Fruit stalk length, NoSs – Number of seeds per fruit, FF – Fruit firmness

The results obtained during the analysis of the properties of the fruits are given in Table 1. The fruits of the *Sinec* variety have a cushioned pear-shaped and asymmetric shape (form 1.3-1.4). The fruit is of an irregular shape and in relation to the cup is curved and spreads, and the stalk narrows down. The fruit's exoderm is rough and with basic green colour. The average weight of the fruit is 53.73 g, i.e. the *Sinec* variety forms medium to small fruits (according to Rubcov's classification). In terms of length, it has been concluded that the fruits are with an average length of 52.93 mm and an average width of 44.13 mm. Large fruits are formed in the region of Kumanovo and Debar with an average weight over 60.00 g for which statistically significant differences have been determined. Regarding the mass, the length and width of the fruits, there were ascertained statistically significant differences in the region of Kumanovo and Debar. According to the climate conditions it is quite expected that the fruits in the Debar region will be enlarged because the climate and orographic conditions are very favourable for growing pears. This region is characterized by sufficient amount of precipitation throughout the year and high relative humidity. The pear is a fruit plant which in dry conditions produces smaller and less quality fruits with a higher quantity of stone cells (Dimitrovski, 1974; Milutinovic et al., 2005). The stalk of the fruit is medium thick and long with an average height of 43.90 mm which make suitable for growing in windy and hilly mountainous conditions (Niketic, 1951; Mratinic, 2000). The fruits of the region of Kriva Palanka are characterized by a longer stalk, for which statistically significant differences are determined.

The fruits formally produce 1.0 healthy seeds, and according to the classification of Nyeki et al. (1998), the *sinec* variety forms a very small amount of seeds in the fruit, indicating triploidity of the variety, as well as the tendency to partenocarpic fruits. The largest number of seeds form the fruits of pears grown in the region of Kriva Palanka and Debar, for which statistically significant differences have been determined. The fruit mesocarp is juicy with a medium-sized taste (aroma, sweetness), a rough texture and a large amount of stone cells. When the fruit is ripened mesocarp rots.

Otherwise, the fruits are stored for two to three weeks. The fruit firmness is one of the more important and commonly utilized measurements of a fruit's quality and it is very significant characteristic when it comes to transport and storage of fruits. Namely, firmer fruits are more easily transported and stored. The average fruit firmness is 1175.3 g / cm². In Debar region Sinec variety forms the firmest fruits.

Table 2. Chemical pear fruit properties grown on three localities

Locality	v. Stracin (Kumanovo)	v. Konopnica (Kriva Palnaka)	v. Mogorche (Debar)	Average
SDM (%)	12.00 ± 1.17	14.80 ± 0.71	14.90 ± 0.45	13.90 ± 1.65
TSC (%)	11.01 ± 0.59	14.30 ± 0.63	13.80 ± 0.74	13.04 ± 1.77
TAC (%)	0.51 ± 0.04	0.13 ± 0.01	0.35 ± 0.02	0.33 ± 0.19
Ratio TSC:TAC	21.51 ± 1.27	107.80 ± 4.90	39.43 ± 2.45	56.24 ± 45.52

* Data are average of n=10±SD, Abbreviations: SDM – Soluble dry matters, TSC – Total sugar content, TAC – Total acid content, Ratio TSC:TAC – Ratio of total sugar content : total acid content

The average content of soluble dry matter in fruits is 13.9%, total sugars 13.04% and total acids 0.33% (Table 2), depending on the areas where pears are grown. High content of soluble dry matter has fruit in the region of Kriva Palanka and Debar The sweetest fruits of pears with the highest content of total sugars are those grown in the region of Kriva Palanka, and hence, because of this feature, they are used for making homemade brandy of high quality. The lowest content of total sugars has the fruits grown in the region of Kumanovo. Except for the content of total acids in the fruit for other chemical properties statistically significant differences were found. In all the parameters tested, statistically significant differences were found due to the very different climatic conditions of cultivation, except for the total acids. This is the result of the large amount of stone cells that degrade during the maturation process. Namely, the polysaccharide – cellulose in the cell wall decomposes, thus the amount of soluble sugars increases rapidly. Then the mesocarp rots and is converted into acids, which is a variety characteristic.

Table 3. Correlation between the researched pear fruit parameters grown on three localities

Locality	FWg:FL	FWg:FWd	FF:FWg	SDM:TSC
v. Stracin (Kumanovo)	0.707	0.985	0.235	0.148
v. Konopnica (Kriva Palanka)	0.626	0.972	-0.045	-0.230
v. Mogorche (Debar)	0.760	0.940	0.493	0.242

The weight of the fruits is strongly correlated with the length of the fruits at two sites, Stracin and Konopnica, and very strong at the site Mogorče, while the width of the fruits at all three sites has a complete correlation. In the other parameters the interdependence ranges from very low to medium (Table 3, Table 4).

Table 4. Correlation between the researched pear fruit parameters grown on three localities (continue from Table 3)

Locality	SDM:TAC	TSC:TAC	FF:TSC	FF:TAC
v. Stracin (Kumanovo)	0.271	0.447	-0.838	-0.464
v. Konopnica (Kriva Palanka)	0.173	0.434	-0.769	-0.253
v. Mogorče (Debar)	-0.310	0.230	-0.615	-0.363

CONCLUSION

Based on the research carried out about the pear variety Sinec grown at three different sites, the following conclusions can be drawn:

Depending on the climatic conditions Sinec starts to flower in the middle of April. Sinec is a summer pear variety whose fruits ripen in the second decade of August. The fruits in the region of Kumanovo and Kriva Palanka mature early.

Sinec forms medium-sized fruit with a long stalk and a very small number of seeds in the fruit. The fruit mesocarpe is juicy, medium-flavored with respect to flavor and sweetness, rough texture and a large amount of stone cells. When the fruit is ripened mesocarp rots.

Fruit pears on average contain 13.9% soluble dry matter, 13.04% total sugars and 0.33% total acids depending on the breeding area. The parameters examined have statistically significant differences due to the very different climatic conditions of cultivation.

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Оригинални научни рад

ИЗРАЖАВАЊЕ ГЕНОТИПА ТРАДИЦИОНАЛНЕ СОРТЕ КРУШКА СИНЕЦ ЗАВИСНО ОД ЕКОЛОШКИХ ФАКТОРА

Извод

У раду су приказани резултати испитивања фенолошких карактеристика, помолошких својстава и хемијског састава плодова традиционалне сорте крушке Синец у три региона Републике Северне Македоније (Куманово, Дебар и Крива Паланка). Крушка Синец цвета средином априла. Плодови сазревају од друге декаде августа (у Куманову и Кривој Паланци) до краја августа (Дебарски крај). Крушка сорте Синец формира средње ситне плодове, док се крупнији формирају у региону Куманова и Дебра. Плодови садрже у просеку 13,9 % растворљивих сувих материја, 13,04 % укупних шећера и 0,33 % укупних киселина. За сва испитивана својства воћа утврђене су статистички значајне разлике осим за садржај укупних киселина у плоду.

Кључне речи: *традиционална сорта, цветање, сазревање, помологија, хемијска својства*



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Original Scientific paper

CONTENT OF SOME ANTIOXIDANTS IN THE FRUITS OF AUTOCHTHONOUS APPLE VARIETIES

Abstract

The content of vitamin C, total phenols, anthocyanins, flavan-3-ols and the antioxidant fruit activity of 7 apple varieties (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka) were examined. Most of the autochthonous varieties had better properties and higher antioxidant activity compared to the standard variety Idared. The autochthonous variety Bela tetovka had the highest content of total phenols (7544.59 mg/kg FW), flavan-3-ols (1019.957 mg/kg FW) and antioxidant fruit activity (87.2% inhibition). A positive correlation was found between total phenols and flavan-3-ols with the antioxidant activity, as well as between flavan-3-ols and phenols. Moderate negative correlation was detected between the content of vitamin C and flavan-3-ols.

Keywords: *vitamin C, total phenols, anthocyanins, flavan-3-ols, antioxidant activity*

INTRODUCTION

Fruits are a rich source of antioxidants that have a preventive, curative and therapeutic effect on human health, destroying free radicals that cause cancer and degenerative diseases. The content of antioxidants depends on the genotype, environmental conditions, cultivation way, method of fruit storage and processing (Bassi et al., 2017).

Vitamins are necessary for maintaining human life and health, ensuring growth and development of the body. Insufficient intake of certain vitamins in the body leads to various diseases called hypovitaminosis and avitaminosis. One of the strongest

antioxidants is vitamin C. It is contained in smaller (4-20 mg/100g pear, apple, plum, walnut, apricot, cherry, peach) or bigger quantities (50-1008.3 mg/100g citrus, strawberry, blueberry, black currant, pomegranate, hawthorn, rose hips and actinidia) (Latocha et al., 2010; Selamovska and Miskoska-Milevska, 2021; Rahman et al., 2008).

Polyphenols are very important compounds found in fruits, that have antioxidant, anti-inflammatory, anticancer and antimutagenic action, antiallergenic and antimicrobial action (Hertog et al., 1993; Goldner et al., 2015). They provide protection against infections, reduce the risk of chronic diseases, cardiovascular and neurodegenerative diseases (Vauzour et al., 2010). **Flavonoids** are a class of plant secondary metabolites, polyphenolic antioxidants, which belong to the group of soluble coloured pigments. They are classified into 12 subclasses in terms of chemical structure, including flavonols, flavans, catechins, isoflavonoids etc. (Panche et al., 2016). They are most common in fruits (especially berry fruits) and grapes. Flavonoids are necessary for the production of vitamin C (Cook and Sammon, 1996). **Flavonols** belong to flavonoids and represent building blocks of proanthocyanidins. They are found in apples, strawberry fruits and grapes. Campferol, quercetin, myricetin, rutin and ficetin are especially present. **Flavanols** or catechins are the 3 hydroxy-derivatives of flavanones. They include flavan-3-ols, mainly responsible for the astringency, bitterness, and nutrient structure (Ivanova and Dimovska, 2010). Flavon-3-ols include catechins and their derivatives (catechin, epicatechin, epigallocatechin, galliccatechin, epicatechin-3-O-gallate). They are mostly present in apple, blueberry, strawberry and grape. **Anthocyanins** are a group of over 500 different compounds that contribute to the red, purple, and blue colour of many plants. They belong to the group of phenols. The most important representatives of the group of anthocyanidins found in fruits and grapes are: pelargonidine, cyanidin, peonidine, delphinidine, petunidine and malvidin. They are most common in berry fruit species (black currant, blueberry, strawberry, raspberry), grapes and some tropical species. (Panche et al., 2016; Khoo et al., 2017).

Macedonia is plentiful in autochthonous fruit varieties which represent a rich starting material in the further selection process. The aim of this study is to detect and determine the content of specific biologically active substances (antioxidants) in the fruits of each autochthonous apple variety.

MATERIAL AND METHODS

The study was part of the scientific project *Antioxidant activity of fruits of autochthonous varieties and populations of fruits, vegetables and grapes*. The laboratory tests were performed in the oenological laboratory at the Institute of Agriculture in Skopje.

Seven autochthonous varieties of apple (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka) were taken as material for analysis. Comparative studies were performed in relation to the standard variety Idared.

The content of vitamin C (mg%) was examined by the volumetric method, according to the method of Murray. The content of total phenols, anthocyanins and flavan-3-ols was determined by the spectrophotometric method, expressed in mg/kg FW.

Their content was determined with Agilent 8453 UV-VIS spectrophotometer. Determining of the antioxidant activity was performed as an anti-radical activity against the stable product DPPH (2,2-diphenyl-1-picrylhydrazil).

According to the XLStat test 2014 5.03, a correlation analysis was made between the examined parameters. The coefficient of determination R^2 and certain general standard parameters, such as maximum and minimum values, average value and standard deviation for each of the parameters were calculated.

RESULTS AND DISCUSSION

In Table 1, results from the chemical analysis performed on the fruits of some autochthonous apple varieties were given.

Table 1. Results from the chemical analysis performed on fruits of autochthonous apple varieties

<i>Variety</i>	<i>Vitamin C (mg %)</i>	<i>Total phenols mg/kgFW</i>	<i>Anthocyanins (mg/kgFW)</i>	<i>Flavan 3 ols mg/kgFW</i>	<i>Antioxidant activity (% inhibition)</i>
Ubavo cvetka	12.0	3668.59	19.63	291.420	53.1
Shareno blago	7.0	3475.31	8.22	366.399	52.6
Prespanka	11.0	5012.37	13.61	579.940	69.9
Tetovka	7.0	3518.51	2.90	450.609	50.9
Karapasha	7.0	4387.83	3.70	898.645	61.6
Kozharka	9.0	4975.00	6.15	393.035	63.2
Bela tetovka	9.0	7544.59	1.30	1019.957	87.2
Idared	13.0	2482.30	1.37	143.884	44.5
Average	9.37	4383.06	7.11	517.980	60.37

The content of vitamin C in the fruits of the apple varieties had average value of 9.37 mg%. The obtained results for the content of vitamin C were about 15.52% of the total antioxidant activity. Compared to the standard variety, all autochthonous varieties had smaller content of vitamin C, except Ubavo cvetka (12.0 mg%) and Prespanka (11.0 mg%). The standard variety Idared had the biggest content of vitamin C of the total antioxidant activity (29.2%), while Bela tetovka the smallest (10.3%). The data on the content of vitamin C in apple corresponded to the available literature data (Boyer and Liu, 2004) according to which, apple fruits contained an average of about 5.7 mg/100g of vitamin C, i.e. vitamin C participated with less than 0.4% of the total antioxidant activity. Vitamin C was a powerful antioxidant, but according to the authors, the antioxidant activity of apples was caused more by other antioxidant components.

The average content of total phenols in apple varieties was 4383.06 mg/kg FW. All of the autochthonous apple varieties had a higher content of total phenols compared to the standard variety Idared. The highest content of total phenols was found in Prespanka (5012.37 mg/kg FW) and Bela tetovka (7544.59 mg/kg FW). A positive correlation was found between the content of total phenols and the antioxidant activity of the fruits. Our results corresponded with Murillo et al. (2012), who also, found a positive correlation between polyphenol content and the antioxidant activity. Kaur et Kapoor (2005) found a positive correlation between the content of phenols and anthocyanins in some fruit species.

The average content of anthocyanins in the fruits of the autochthonous varieties was 7.11 mg/kg FW. Almost all of the apple autochthonous varieties, with the exception of Bela tetovka, had higher content of anthocyanins compared to the standard variety. The highest content of anthocyanins was detected in Ubavo cvetka (19.63 mg/kg FW) and Prespanka (13.61 mg/kg FW).

The accumulation of anthocyanins is primarily influenced by genetic and external factors (light, temperature, etc.). According to Honda et al. (2002), during the synthesis of anthocyanins in apple fruits, five genes were expressed, of which the level of expression correlated with the concentration of anthocyanins. Anthocyanins in apples were found in the cellular vacuoles of the epidermis and subepidermis (Dayton citation according to Tešović et al., 1999). The main anthocyanin component in the epidermis of apple fruits according to Sun and Francis (1967) was cyanidin-3-galactoside, followed by cyanidin-3-arabinoside and cyanidin-7-arabinoside. In the fruits of wild and cultivated apple varieties, Tešović et al. (2012) determined 3 anthocyanin components.

The average content of flavan-3-ols in the fruits of the autochthonous apple varieties was 517.98 mg/kg FW. All of the apple varieties had higher content of flavan-3-ols compared to the standard variety. The highest content of flavan-3-ols was observed in Bela tetovka (1019.957 mg/kg FW). There was a strong and statistically significant positive correlation between flavan-3-ols and phenols. Moderate negative correlation between vitamin C and flavan-3-ols was detected. The other compared parameters had no significant correlation. The variety Bela tetovka had low content of vitamin C, but high content of flavan-3-ols. These results were similar to the results of Boyer and Liu (2004). According to them, most of the content of antioxidants in apples was made of flavonoids.

The antioxidant activity of apple fruits had an average of 60.37% inhibition. All autochthonous apple varieties had higher antioxidative activity in fruits compared to the standard variety. Bela tetovka had the highest fruit antioxidant activity (87.2% inhibition). A strong and statistically significant correlation between the total phenols and flavan-3-ols and the antioxidant activity was determined. The variety Bela tetovka had the highest content of total phenols and flavan-3-ols and also had the biggest antioxidant activity in fruits.

The antioxidant fruit activity depended on the biotype, type and age of the plant material. The greatest antioxidant activity was found in strawberries, blackberries and red raspberries. Fruits and leaves of fruit species had great antioxidant activity. With aging, the content of total phenols in leaves reduced and their antioxidant ability

decreased (Wang and Lin, 2000). In some apple and strawberry varieties the antioxidant activity was 12- 64 mM FRAP (Kaur and Kapoor, 2005).

In Tables 2, 3, 4 and 5, the statistical data and the correlation values of the examined parameters in the autochthonous varieties of apples were given.

Table 2. Maximum and minimum values, average value and standard deviation for each of the parameters

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. Deviat.
Vitamin C	8	0	8	7,0000	13,0000	9,3750	2,3867
Phenols	8	0	8	2482,3000	7544,5900	4383,0625	1532,3433
Anthocyanins	8	0	8	1,3000	19,6300	7,1100	6,5160
Flavan-3-ols	8	0	8	143,8840	1019,9570	517,9861	301,2189
Antiox. activity	8	0	8	44,5000	87,2000	60,3750	13,4958

In the apple varieties, strong and statistically significant correlation of total phenols and flavan-3-ols and the antioxidant activity was found. This indicated presence of other chemical compounds that have inhibitory or intensifying effect on the tested compounds (phenols and flavan-3-ols). That meant, in case of increasing of the value of total phenols, as well as of flavan-3-ols, the antioxidant activity in the apple fruits would drastically increase. There was a strong and statistically significant correlation between the flavan-3-ols and phenols. This meant that the values of phenols, flavan-3-ols and the antioxidant activity in apple fruits were directly related. Apart from the moderate negative correlation between vitamin C and flavan-3-ols, the other compared parameters had no significant correlation (Table 3).

Table 3. Correlations between tested parameters

Variables	Vitamin C	Phenols	Anthocyanins	Flavan 3 ols	Antiox. activity
Vitamin C	1	-0,1935	0,3731	-0,4721	-0,1518
Phenols	-0,1935	1	-0,1469	0,8117	0,9885
Anthocyanins	0,3731	-0,1469	1	-0,3105	-0,1130
Flavan-3-ols	-0,4721	0,8117	-0,3105	1	0,8284
Antiox. Activity	-0,1518	0,9885	-0,1130	0,8284	1

Values in bold are different from 0 with a significance level $\alpha=0,05$

The value of p (0.0001 – 0.0111) was significantly below the given alpha = 0.05, which showed high statistically significant correlation of the values of total phenols and flavan-3-ols on the antioxidant activity, as well as high correlation between phenols and flavan-3-ols. (0.0144). There was a value overlap of the mentioned parameters which was almost absolute (Table 4).

Table 4. p values between tested parameters

Variables	Vitamin C	Phenols	Anthocyanans	Flavan three ols	Antiox. activity
Vitamin C	0	0,6462	0,3626	0,2376	0,7197
Phenols	0,6462	0	0,7286	0,0144	< 0,0001
Anthocyanans	0,3626	0,7286	0	0,4542	0,7900
Flavan-3-ols	0,2376	0,0144	0,4542	0	0,0111
Antiox. Activity	0,7197	<0,0001	0,7900	0,0111	0

Values in bold are different from 0 with a significance level alpha=0,05

The highest coefficient of determination was between the phenols and the antioxidant activity, i.e. about 97.7% of the variations in the value of the antioxidant activity were explained by the variations in the value of phenols. Also, the high coefficient of determination between the flavan-3-ols and the antioxidant activity of 68.6%, as well as the coefficient of determination of 65.9% between the flavan-3-ols and phenols should be mentioned (Table 5).

Table 5. Coefficient of determination R²

Variables	Vitamin C	Phenols	Anthocyanans	Flavan 3 ols	Antiox. activity
Vitamin C	1	0,0374	0,1392	0,2228	0,0230
Phenols	0,0374	1	0,0216	0,6589	0,9772
Anthocyanans	0,1392	0,0216	1	0,0964	0,0128
Flavan-3-ols	0,2228	0,6589	0,0964	1	0,6862
Antiox. activity	0,0230	0,9772	0,0128	0,6862	1

CONCLUSION

According to the values obtained for the examined parameters, the autochthonous apple varieties compared to the proposed standard variety Idared showed higher values. This indicated that these varieties had high quality properties, were characterized by

high antioxidant activity for the species and had a strong positive effect on human health. The autochthonous variety Bela tetovka had highest content of total phenol, flavan-3-ols and antioxidant fruit activity. The standard variety Idared had the lowest content of total phenols, anthocyanins, flavan-3-ols and antioxidant fruit activity, but the highest content of vitamin C.

A strong positive correlation of total phenols and flavan-3-ols and the antioxidant activity was determined. There was a strong positive correlation between flavan-3-ols and phenols. Moderate negative correlation between vitamin C and flavan-3-ols was observed. The other compared parameters had no significant correlation.

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САДРЖАЈ НЕКИХ АНТИОКСИДАНТА У ПЛОДОВИМА АУТОХТОНИХ СОРТИ ЈАБУКА

Сажетак

Испитивани су садржај витамина С, укупни феноли, антоцијани, флаван-3-оли и антиоксидативна активност плодова у седам аутохтоних сорти јабука (Лепоцветка, Шарено благо, Преспанка, Тетовка, Карапаша, Кожара, Бела Тетовка). Већи део аутохтоних сорти имају боље карактеристике и више антиоксидативних активност у односу на стандардну сорту Ајдаред. Аутохтона сорта Бела Тетовка има највиши садржај укупних фенола (7544,59 mg/kg FW), флаван-3-ола (1019,957 mg/kg FW) и антиоксидативну активност плодова (87,2% инхибиције). Утврђена је већа позитивна корелација између укупних фенола и флаван-3-ола са антиоксидативном активношћу, као и између флаван-3-ола и фенола. Детерминисана је умерена негативна корелација између садржаја витамина С и флаван-3-ола.

Кључне речи: *витамин С, укупни феноли, антоцијани, флаван-3-оли,
антиоксидативна активност*



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Original Scientific paper

COMPARISON OF QUALITY PROPERTIES OF FRESH AND SOLAR DRIED *GOJI BERRIES* (*LYCIUM BARBARUM*)

Abstract

In this research the quality properties of fresh and solar dried goji berries (*Lycium barbarum*) were investigated and compared. For that purpose, freshly harvested goji berries, the JB2 variety were used. Before the drying, the berries were treated in several ways, immersion of berries in boiling water and in 1 % ascorbic acid solution; immersion of frozen berries in 1 % ascorbic acid solution; berries without treatment (control). The following parameters were analyzed in fresh and dried goji berries: the total dry matter, moisture, total acids, mineral matters (ash), glucose, fructose, vitamin C, protein, fat and total polyphenols. For that purpose, the standard laboratory methods were used. Sensory properties were estimated by scoring method (max. 20).

Key words: *goji berries, solar dryer, quality and sensory properties*

INTRODUCTION

Goji berry (*Lycium barbarum*) is deciduous shrub tree of *Solanaceae* family, that grows in China, Tibet and other parts of Asia (Adiletta et al., 2015). It is also known

as Chinese wolfberry, possibly because Chinese farmers observed wolves eating these berries (Kulczyński, 2016). Due to its short vegetation period, goji berry is successfully cultivated in Europe (Wojdyłoa et al., 2018). In the last few years, the cultivation of goji berries has become increasingly popular, both in the Balkans and in Northern Macedonia (Karakashova et al., 2020).

Goji berries have been an important part of traditional Chinese and Asia medicine for more than 2000 years due to its nutritional value, some pharmacological activities, and functional properties (Kulczyński, 2016; Ağagündüz et al., 2021). Goji fruits are orange-red, approximately 2 cm long ellipsoids berries, with sweet-and-tangy flavor. They are an excellent source of carotenoids (0.03–0.5% d.w.), mainly of zeaxanthin, which are natural pigments responsible for yellow, orange, and red color (Amagase et al., 2009). Goji berries are also rich in ascorbic acid (approx. 42 mg/100 g) and vitamins E, B₁, B₂, and B₆ (Llorent-Martínez et al., 2013). Some other compounds identified in the berries include polyphenols – with quercetin and kaempferol derivatives of rutinoides as dominant fractions – chlorogenic and caffeic acids, and small amounts of caffeoylquinic and p-coumaric acids (Rocchetti et al., 2018). Further, goji berries contain carbohydrates (arabinose, rhamnose, xylose, galactose, mannose and glucose) (Montesano et al., 2016), organic acids (malic acid, citric acid, shikimic acid and fumaric acid) (Mikulic-Petkovsek et al., 2012), and many minerals (potassium, sodium, phosphorus, magnesium, iron, calcium, zinc, and selenium) (Llorent-Martínez et al., 2013). Goji berries comprise also fatty acids (hexadecanoic acid, linoleic acid and myristic acid) (Blasi et al., 2017) and amino acids (proline, betaine and taurine) (Potterat, 2010). Due to their chemical composition, goji berries are used in the pharmaceutical, food and cosmetics industry (Karakashova et al., 2020). Goji berry is a fruit with a wide range of biological effects, proven in various human, clinical and in-vitro studies. Goji berry is a food that has a positive effect on human health, which is mainly attributed to the presence of important chemical compounds (Mikulic-Petkovsek et al. 2012). The recommended daily intake for fresh goji berries is 25 to 120 g (Amagase and Farnsworth, 2011).

Drying is the oldest way of preserving food. During the drying process, a large amount of water is released, the mass and volume of fruits are reduced, and there is an increase in the concentration of dry matter, with all the components from its composition (Vereš, 2004). Also, the drying process is intended to remove water from foodstuff in order to prevent microbial spoilage and chemical alterations thus prolonging shelf-life (Cuccurullo et al., 2012; Cinquanta et al., 2010). During the drying of the raw material, not all of the free-water content must be removed, as this could cause a negative impact on the elasticity and ability to rehydration (Vereš, 2004).

Sun drying is one of the oldest and the most economical way of drying. Drying of fruits can be performed in natural atmospheric conditions on the sun or in solar dryers. In North Macedonia, there are favorable conditions for using solar drying, because there are about 250 sunny days during the year (Vasilevska-Popovska, 2009). During the sun drying there is a risk of damage due to dust and insect infection. Generally, the dehydration causes damages in texture, color, taste and nutritional value of food due to the high temperatures and required long time of drying (Adiletta et al., 2015).

Dried fruit should have an appropriate aroma and color to the variety and a good

ability to rehydrate (increase in volume) when immersed in hot water, for 10 minutes, after which it should have a taste and smell corresponding to the fruit from which it is obtained. Also, dried fruit should not have: any physiological damage to fruits due to over-drying, etc.; no foreign odors and tastes and to not contain mechanical and biological impurities (Vračar, 2001). The purpose of the present research is to study the impact of pre-treatment and the process of solar drying on chemical and sensory properties of dried goji berries, the variety JB2.

MATERIALS AND METHODS

Sample preparation. Goji berries trees, variety JB2 (Figure 2-a), are grown in the region of Skopje, North Macedonia. Goji berries, varieties JB2 reaches the minimum yield up to 5 kg per tree. This variety have small berries, with orange-red in color. The berries ripen successively and therefore the harvest takes place several times, starting in June until October. After the harvest, the fresh goji berries (JB2) were inspected and selected only berries in proper technological maturity, without mechanical and physiological changes or damage.

Drying experiments. In order to obtain quality dried products, it is necessary to prepare goji berries, through several technological operations: selection, washing, pre-treatment of berries, drying, packaging and storage. After washing, goji berries should be drained and then exposed to appropriate pre-treatments, in order to prevent discoloration, as well as changes in chemical composition. In order to find out appropriate pre-treatment of goji berries, in this research were applied different method before the drying process. There were used: JB2-I- control variant – (without treatment); JB2-II-variant – immersion of berries in boiling water, cooling and immersion in 1 % ascorbic acid solution, 5 minutes; JB2-III-variant – immersion of frozen fruits in 1 % ascorbic acid solution, 5 minutes. The treated goji berries were placed on wooden drying shelves, with plasticized grids and an area of 1 m². During the drying process the wooden drying shelves were changed, in order the goji berries to be dried evenly and faster.

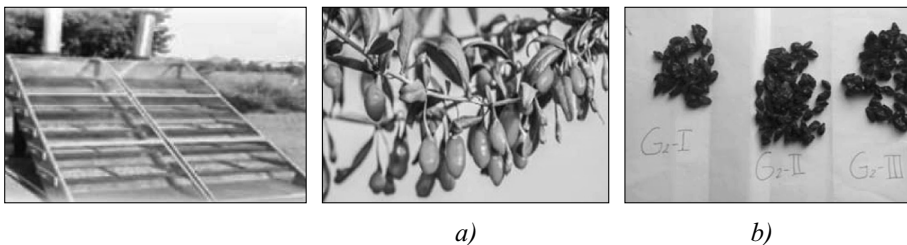


Figure 1. Solar dryer Figure 2. Fresh (a) and solar dried (b) goji berries JB2

The process of drying was performed at solar dryer, that is made of metal lined with galvanized sheet metal and glass, set at an angle, through which the sun's rays penetrate (Figure 1). The natural solar energy heated the air in the solar dryer, causing

it the process of drying berries. There are intake openings for fresh air, and in the upper part there are exhaust openings (ventilators) for the wet and hot air, which maintains the natural air circulation. The temperature was reaching from 55 to 65 °C, and at night from 30 to 45 °C, which had a favorable effect on the drying of goji berries. The temperature, humidity and airflow were the factors that significantly affect the evaporation of the water from the fruit (Karakashova, 2003). The drying time of goji berries ranged from 36 to 48 hours, what directly depends on the availability of the sun, the flow and humidity of the air, and the properties of the raw material that is being dried.

The dried goji berries (Figure 2-b), in order to be successfully preserved for a longer period of time, need to be packed in paper bags to stabilize, and afterwards to be packaged in appropriate packaging with suitable air porosity. The dried berries were stored in suitable storage conditions, protected from influence of the air moisture and direct sunlight.

Quality properties. To determine the quality properties of fresh and dried goji berries, standard laboratory methods were used and the following chemical parameters were determined: total dry matter, in the dryer at 105 °C up to constant mass (Vračar 2001); soluble dry matter by refractometric method; total acids, with 0.1 M NaOH solution and 1 % phenolphthalein solution as an indicator; the mineral matters (ash) in a Muffle oven, at a temperature of 525 ± 25 °C (Vračar 2001); glucose, fructose and sucrose by applying HPLC-method with RI-detector; vitamin C, with 0.1 N-solution J_2 and 1 % starch solution; the proteins were determined with the Kjeldahl method; the fats by applying Soxhlet method (Vračar 2001); total polyphenols with Folin–Ciocalteu method, at the absorbance 765 nm wavelength (Lamuela-Raventós, 2018).

To determine the quality properties, it is also especially important to evaluate the sensory properties, both for fresh and dried goji berries. Sensory evaluation has been performed by point system method, with a maximum of 20 points in relation to all properties (color-max. 7; taste-max. 4; odor-max. 4 and fruit condition and appearance-max. 5 points) (Karakashova and Babanovska-Milenkovska, 2012).

The results obtained from the quality properties analysis were processed using standard statistical methods, Microsoft Excel 2010 as well as the statistical software package R 3.3.3.

RESULTS AND DISCUSSION

In this research were made comparison of quality properties of fresh and dried goji berries. The quality properties were estimated according to the analysis of some chemical parameters and sensory properties.

Comparison of chemical properties of fresh and dried goji berries. Chemical composition means the content of all compounds in the product, including water. The specificity of the chemical composition depends on the variety, climatic conditions, agro-technical measures and the degree of maturity (Obradović, 2011). From a technological point of view, all the components present in the fruit, except water, make up the total dry matters, which are composed of soluble components and insoluble matters. Quality varieties of fruit have a higher content of total dry matter and thus higher nutritional value (Katalinić, 2006).

Total dry matters content. The total dry matter of the fresh goji berries, JB2 variety was estimated as 22.22 %. In the dried goji berry of the JB2 variety, the total dry matter in the control variant (JB2-I) was 86.91 %, 85.54 % in the JB2-II variant, and 86.39 % in the variant JB2-III. From the obtained results it can be concluded that the average value of the total dry matters in the dried goji berries for the variety JB2 was 86.28 %. From the applied variants, a statistically significant difference was determined between the control (JB-I) and variants JB2-II and JB2-III for the total dry matters content, at $p = 0.05$ and $p = 0.01$.

Soluble dry matters content. According to Donno et al., (2015), the content of soluble dry matters in goji berry was 11.63 %, and from the study of Zhang et al. (2016), the content of soluble dry matters in eight goji berry genotypes grown in Ningxia – China, was with an average value of 19.3 %. According to the data obtained from our research, the content of soluble dry matters was 16 % in goji berries of the JB2 variety.

Sugar content. According to the data of study (Adiletta et al., 2015), glucose and fructose are the most common sugar in goji berries. In the fresh goji berries, JB2 variety was found 7.74 % of glucose content, fructose content was lower with a value of 5.24 % and the value of sucrose was 0.57 %.

The highest average value in terms of glucose content was determined in dried goji berries, variant JB2-III (12.03 %), and the lowest was determined in a control variant (JB2-I) with 9.46 %. From the applied variants, a statistically significant difference has been found between the control variant (JB2-I) and the variant JB2-III, at $p = 0.05$ and $p = 0.01$ in terms of glucose content. In sun-dried goji berries of the JB2 variety, the highest value for the fructose content was determined in the variant JB2-III (12.82 %), and the lowest value of 9.64 % was found in the control variant (JB2-I). The fructose content of goji berries of the JB2-II variant was 11.56 %. Between the control variant (JB-I) and variant JB2-III, has been found a statistically significant difference at $p = 0.05$, in terms of fructose content.

Total acids content. In the last stage of maturation there is a decrease in the content of acids, and an increase in the content of sugars. According to Obradović (2011), the average acids content in the fruit ranges from 0.2 to 1.2 %, and the pH is around 3.5. The content of total acids, expressed as malic acid, in fresh goji berries variety JB2 had a value of 0.53 %. The average value for the content of total acids, expressed as malic acid, in the sun-dried berries of the variety JB2 had a value of 2.47 %. Dried goji berries, variant JB2-III, was characterized by the highest value (2.99 %) of total acids, expressed as malic acid, and the lowest value was determined in the variant JB2-I (1.95 %). With the statistical processing of the obtained results for the content of total acids of all variants of dried goji berries (JB2), was determined that their average values of all variants had not shown statistically significantly difference from each other, at $p = 0.05$ and $p = 0.01$.

Vitamin C content. In our study, the amount of vitamin C in fresh goji berries, the variety JB2 was estimate on 85.68 mg/100 g. According to Vulić et al. (2016), the fresh goji berries grown in the Republic of Serbia has the amount of 59.47 mg/100 g vitamin C.

In the dried berries JB2 variety, the highest content of vitamin C of 35.65 mg/100 g had the control variant JB2-I, while the content of vitamin C was 18.17 mg/100 g in the dried berries JB2-II, and 24.56 mg/100 g in the variant JB2-III. The average value of the dried goji berries JB2 variety, from the applied variants, was 26.1 mg/100 g in relation to the content of vitamin C. From the results obtained from the statistical analysis, regarding the content of vitamin C, it was concluded that there are statistically significant differences between the three applied variants ($p = 0.05$). There were no statistically significant differences found between the variant JB2-II and JB2-III ($p = 0.01$).

Total mineral matters (ash) content. Fresh fruits usually contain mineral matters in the range of 0.3 to 0.8 % (Obradović 2011). From the research was found that the fresh goji berries of the JB2 variety were characterized by the amounts of 1.71 % total mineral matters (ash). According to Donno et al. (2015), the content of total mineral matters (ash) in fresh goji berries was determined on 0.95 %. In the dried goji berries of the JB2 variety, the mineral matters were 4.16 % in the variant JB2-III (highly present), 4 % in the variant JB2-II, and 3.86 % in the control variant JB2-I. With the statistical processing of the obtained results for the content of mineral matters of all variants for the analyzed variety JB2, it was determined that their average values had not statistically significantly difference from each other at $p = 0.05$ and $p = 0.01$.

Protein content. According to the results obtained from our analysis, the fresh goji berries, variety JB2 had a protein content of 7.37 %. Donno et al. (2015) determined that the protein in fresh goji berries grown in northern Italy is 12.20 %. The highest value for the protein content of 15.53 % was found in the dried goji berries of the JB2-II variant, in the variant JB2-III was determined protein content of 15.19 % and the lowest protein content was found in the control variant JB-I (13.72 %).

Fat content. According to the results, a fat content of 2.57 % was determined in the examined fresh variety JB2. According to Endes et al. (2015), goji berry has content of 4.11 % fats. The highest presence of fats was determined in dried goji berries of the JB2-III variant (11.04 %), while in the control variant JB2-I and in the variant JB2-II, 8.19 % and 7.82 %, respectively, fats were determined.

Total dietary fiber content. According to the data obtained from this examination, the content of total dietary fibers in the fresh goji berries JB2 variety was 7.74 %. Endes et al. (2015) found that dietary fibers in goji berries in Turkey are present in quantities of 7.3 %.

Total polyphenol content. According to the research data, the variety JB2 of goji berries contains 121 mg/100 g total polyphenols. Donno et al. (2015) determined the content of total polyphenols in the analyzed goji berry samples with a value of 268.35 mg/100 g.

Among the three variants of sun-dried goji berries, the JB2 variety, the total polyphenols were mostly present in the variant JB2-III (1425.4 mg/100 g), while the value of total polyphenols was 703.9 mg/100 g in the control variant JB2-I, and 581.5 mg/100 g in the JB2-II variant. For the applied variants, it was determined a statistically significant differences in relation to the total polyphenols between the control JB2-I, JB2-II and JB2-III variants, at $p = 0.05$ and $p = 0.01$.

Sensory properties of the fresh and dried goji berries. The appearance, color, taste and texture of the goji berries can determine by the sensory analysis. From the

obtained results, the fresh goji berries of the JB2 variety were characterized with a total of 15 points (out of 20 max.). For the dried goji berries, in terms of color (max. 6), the highest value had JB2-I variant (4.5 points). The JB2-II variant had the lowest value for smell (3 points) and for texture (3.5 points), (out of 4 and 5 max., respectively). In terms of taste (max. 5), the highest value had JB2-III variant (3 points). The total points (max. 20) from the sensory analysis had the highest value for JB2-III variant (15 points) and the lowest value had the JB2-II variant (10.5 points).

CONCLUSION

According to the obtained results of chemical composition and sensory properties of goji berries, variety JB2, it can be concluded that it has very important nutrients and good qualitative properties. In relation to pre-drying treatments, in variant JB2-II, was notice a loss of color and partially of some components of the chemical composition that are easily soluble in water. Dried goji berries JB2-III proved to be a good variant of pre-treatment, when frozen goji berries were immersed in a 1 % solution of ascorbic acid. There were obtained the highest values for the content of glucose, fructose, total acids, ash, fats content, content of total polyphenols. This variant of dried goji berries had also good sensory evaluation, what includes appropriate texture, slight color change and had the highest total points 15 (out of max. 20).

The interest, production and promotion of goji berry is constantly growing in the Republic of North Macedonia, due their good chemical properties. In dried goji berries are retain most of the nutrients from the fresh berries. We recommend solar drying to the producers and emphasize to increase the production of dried fruits.

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Originalni naučni rad

POREĐENJE KVALITETNIH SVOJSTVA SVEŽIH I SUŠIH GODŽI BOBICA (*Licium barbarum*)

Apstrakt

U ovom istraživanju ispitana su i upoređena svojstva kvaliteta svežih i solarno sušenih godži bobica (*Licium barbarum*). U tu svrhu korišćene su sveže ubrane godži bobice, sorte JB2. Pre sušenja bobice su tretirane na više načina, potapanjem bobica u ključalu vodu i 1% rastvor askorbinske kiseline; potapanje smrznutih bobica u 1% rastvor askorbinske kiseline; bobice bez tretmana (kontrola). U svežim i sušenim goji bobicama analizirani su sledeći parametri: ukupna suva materija, vlaga, ukupne kiseline, mineralne materije (pepeo), glukoza, fruktoza, vitamin C, proteini, masti i ukupni polifenoli. U tu svrhu korišćene su standardne laboratorijske metode. Senzorna svojstva su procenjena metodom bodovanja (maks. 20).

Ključne reči: *Godži bobice, solarna sušara, kvalitet i senzorna svojstva*

**LIVESTOCK PRODUCTION
FROM THE STAND POINT OF HEALTHY
SAFE FOOD**



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Original Scientific paper

THE VARIABILITY OF BIOCHEMICAL PARAMETERS IN BLOOD SERUM AND MILK OF HOLSTEIN COWS REGARDING THE AGE AND HEALTH STATUS

Abstract

In order to determine the variability of biochemical parameters, blood and milk were sampled from 25 Holstein cows for a period of three months. The differences in the analyzed parameters due to the animal's health status (risk of acidosis or ketosis or normal health status; defined according to the fat/protein ratio) and animal's age (parity classes) were determined. The results of this research indicate the variability in biochemical parameters in both blood serum and milk as a result of variance in animals' health status and due to various age of animal implying that animals' response depends on animal age. Obtained results should be considered in the modelling for the prediction of metabolic disorders occurrence.

Key words: *Holstein cows, biochemical parameters, milk, blood, metabolic disorders*

INTRODUCTION

One of the most challenging livestock productions is dairy cattle farming because the farmer must examine a big quantity of data every day and understand how to react in time to prevent potential problems in production (Gantner, 2020; Gantner et al., 2021). Furthermore, to optimize the management and realization of the genetic potential of dairy cows, the farmer must be conscious of the genetic and environmental aspects of different characteristics of interest for milk production. The term effective

dairy cattle farming means gravidity and calving on an annual basis, with the transitional period and the beginning of lactation being the most stressful period in the production cycle of dairy cows (Gantner, 2020). Various factors, such as changes of ration, negative energy balance, reduced food intake, weight loss and hypocalcemia can cause sets of different disorders in this period (Ametaj, 2017). After giving birth and during the first month of lactation, the most common metabolic disorders that appear in dairy cows are sub-acute and acute ruminal acidosis, laminitis, ketosis, fatty liver, displaced abomasum, milk fever, downer cow, retained placenta, liver abscesses, metritis, mastitis and bloat (Ametaj, 2017). Additionally, it is significant to point out that the occurrence of one metabolic disorder is highly associated with another one (Suthar et al., 2013; Ametaj, 2017). Accordingly, cows impacted by milk fever are more prone to mastitis, retained placenta, metritis, ketosis; at the same time cows impacted by acidosis are more tending to laminitis, milk fever, mastitis, and fatty liver (Suthar et al., 2013; Ametaj, 2017). Ruminal acidosis in subacute and acute forms frequently occurs in high-producing dairy herds in early lactation and cows with a high intake of dry matter (Ametaj, 2017), while ketosis usually occurs as a result of negative energy balance (usually due to increased daily milk production). Since subclinical disorders produce high economic losses to dairy farmers through therapy costs, decreased production, decreased reproduction characteristics and increased involuntary culling (Suthar et al., 2013), it is very important to detect and prevent disorders in the sub-clinical phase.

The purpose of this study was to determine the effect of cows age (parity) on the variability of biochemical parameters in blood serum and milk of Holsteins regarding the cow's health status (based on F/P ratio classes).

MATERIAL AND METHODS

The study was conducted in the east of Croatia on an indoor dairy cattle farm. The cows included in the research were healthy and did not have any disorders. The cows were fed with a total mixed ration with the addition of mixture accordingly to the daily milk production. During the three months of research, blood and milk was sampled, on monthly basis, from 25 Holstein cows (mean milk production near 40 kg/day, Table 1). Blood samples were taken from the coccygeal vein into tubes with lithium heparin anticoagulant (Becton Dickinson, Plymouth, England, UK) and centrifuged (1.500 g/10 min at 4°C) in order to separate the plasma. Milk samples were taken into clean tubes and centrifuged (12.000 g/30 min at 4°C) for plasma separation. The determination of the biochemical parameters in blood and milk plasma was performed operating an automatic clinical chemistry analyzer Beckman Coulter AU400 (Beckman Coulter, Germany). The concentration of β -hydroxybutyrate (BHB) was determined utilizing commercial kits (Randox Laboratories Ltd, Crumlin, UK) following the enzymatic colorimetric method. The test-day records of animals included in the research, obtained during the regular milk recording, were taken from the central database of the Croatian Agency for Agriculture and Food. Test-day records were corrected accordingly to the ICAR guidelines (2017). With reference to the F/P value, records

were divided into three classes: F/P ratio < 1.1 (acidosis risk); F/P ratio in [1.1, 1.5] (normal status); F/P ratio > 1.5 (ketosis risk). Table 1 presents basic statistical parameters of daily production (daily milk yield, daily fat and protein content along with F/P ratio) accordingly to the parity classes (cows in II. parity and cows in III. and higher parities).

Table 1. Basic statistical parameters of daily production traits of Holstein cows

Trait	N	Mean	SD	Minimum	Maximum
II. parity					
DMY, kg	28	38.79	8.33	18.60	55.70
DFC, %	27	3.50	0.94	2.12	6.94
DPC, %	27	3.34	0.25	2.70	3.81
F/P ratio	27	1.05	0.28	0.65	1.94
III.+ parity					
DMY, kg	47	39.61	9.48	19.90	59.80
DFC, %	47	4.02	1.26	1.99	8.61
DPC, %	47	3.41	0.36	2.66	4.26
F/P ratio	47	1.19	0.43	0.56	3.24

* DMY – daily milk yield; DFC – daily fat content; DPC – daily protein content; F/P – fat/protein

The variability of biochemical parameters due to F/P ratio classes separately for each parity class (II., and III. +) was tested using the least square means in the GLM procedure in SAS (SAS Institute Inc., 2019). The following statistical model was used:

$$y_{ijkl} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3 \ln(305/d_i) + b_4 \ln^2(305/d_i) + M_j + D_k + e_{ijkl}$$

Where:

y_{ijkl} = estimated biochemical parameters;

μ = intercept;

b_1, b_2, b_3, b_4 = regression coefficients (lactation curve by Ali and Schaeffer, 1987);

d_i = days in milk ($i = 11$ to 345 day);

M_j = fixed effect of experiment month k ($k =$ May, June, July),

D_k = fixed effect of F/P ratio classes (acidosis risk, normal status, ketosis risk),

e_{ijkl} = residual.

Scheffe's multiple comparisons in PROC GLM (SAS) were used to test the significance

($p < 0.05$) of the differences in biochemical parameters due to F/P ratio classes.

RESULTS

The values of the biochemical parameters in the blood serum due to F/P ratio classes separately for each parity class are presented in Table 2. The highest values of γ -glutamyl transferase (GGT), urea (UREA), β -hydroxybutyrate, Fe and Ca were determined in cows in the second lactation with an F/P ratio lower than 1.1 that is in cows at risk of acidosis. The lowest values of protein (PRO), albumin (ALB) and Fe were observed in cows in second parity in ketosis risk (F/P > 1.5). The concentration of triglyceride in blood plasma did not show variability due to the F/P ratio regardless of the parity class. The concentration of all biochemical in blood serum, with exception of glucose, was higher in older cows at risk of acidosis prevalence. Furthermore, almost all biochemical parameters (except urea, β -hydroxybutyrate, and Ca) were lower in older cows at risk of ketosis occurrence.

Table 2. LSmeans of the biochemical parameters in blood serum in regard to F/P ratio classes separately for each parity class

Trait	F/P ratio < 1.1		F/P ratio in [1.1, 1.5]		F/P ratio > 1.5	
	Acidosis risk		Normal status		Ketosis risk	
Parity	II.	III.+	II.	III.+	II.	III.+
Aspartate amino transferase (U/L, AST)	143.51	152.16 ^a	176.36	122.61 ^a	148.31	63.20 ^b
γ -glutamyl transferase (U/L, GGT)	33.18	39.59	31.62	31.05	32.42	25.96
Glucose (mmol/L, GUK)	3.00	2.96	3.10	3.16	3.21	2.85
Urea (mmol/L, UREA)	4.41	4.58	3.88	4.79	3.07	4.30
Protein (g/L, PRO)	84.59	84.67	84.82	83.95	82.35	87.78
Albumin (g/L, ALB)	31.70	32.23	32.13	32.61	30.88	29.41
Triglyceride (mmol/L, TGC)	0.12	0.11	0.11	0.12	0.11	0.13
β -hydroxybutyrate (mmol/L, BHB)	0.47	0.47	0.31	0.59	0.36	0.41
Fe (μ mol/L)	24.48	25.04	25.08	23.53	20.33	12.90
Ca (mmol/L)	2.13	2.20	2.11	2.17	2.10	2.15

* Values within the same row and parity class marked with different letter differ statistically significant ($P < 0.05$)

The variability of the biochemical parameters in milk regarding F/P ratio classes separately for each parity class is presented in Table 3. The highest value of GGT and the lowest values of glucose, urea, albumin, Fe and Ca in milk were determined in cows in the second lactation that was at risk of ketosis prevalence. Also, the highest

values of aspartate aminotransferase (AST), glucose and Ca in milk were observed in younger cows in normal status. Furthermore, older cows with acidosis risk had a higher concentration of GGT, glucose, urea, protein, albumin, and Fe than the younger ones. On the other hand, older cows at ketosis risk had a lower concentration of GGT, glucose, protein, and Fe than the younger animals implying that animal's age, besides the individual health status, affects the concentration of biochemical parameters both in blood serum and milk.

Table 3. LSmeans of the biochemical parameters in milk in regard to F/P ratio classes separately for each parity class

Trait	F/P ratio < 1.1		F/P ratio in [1.1, 1.5]		F/P ratio > 1.5	
	Acidosis risk		Normal status		Ketosis risk	
Parity	II.	III.+	II.	III.+	II.	III.+
Aspartate amino transferase (U/L, AST)	13.99	13.12 ^a	17.66	16.56 ^a	16.39	33.17 ^b
γ -glutamyl transferase (U/L, GGT)	312.46	322.87	374.49	377.08	419.42	354.25
Glucose (mmol/L, GUK)	0.57	0.69 ^a	0.61	0.43 ^a	0.53	0.27 ^b
Urea (mmol/L, UREA)	5.33	5.58	4.84	5.61	3.74	4.85
Protein (g/L, PRO)	35.48	36.22	34.81	37.07	34.86	34.48
Albumin (g/L, ALB)	22.28	22.68	21.85	23.21	21.04	21.17
Fe (μ mol/L)	22.80	27.50	21.24	26.43	17.30	14.53
Ca (mmol/L)	3.16	3.10	3.37	3.39	3.15	3.41

* Values within the same row marked with different letter differ statistically significant (P<0.05)

DISCUSSION

The inadequate feeding management and imbalanced ration (regarding the forage to concentrate ratio) accompanying the reduced absorption capacity of short-chain fatty acids through the rumen due to underdeveloped rumen papillae after parturition significantly increases the probability of metabolic disorders prevalence (Dieho et al., 2016). The occurrence of some metabolic disorders frequently results in variations in the concentration of biochemical parameters both in the blood and milk of dairy cows. Understanding the essentials underlying the development of metabolic disorders by using biochemical parameters of blood and milk to assess animal health could improve disease prevention.

The aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT) as important catabolic enzymes play a significant role in proper animal liver function. Similarly like in this research, Liu et al. (2012) determined that GGT enzyme concentrations in milk were higher than in blood plasma, while in the case of AST

concentrations were the opposite (higher in blood plasma). The highest concentration of urea in the blood serum and milk of animals at risk of acidosis could indicate inefficient utilization of nitrogen from food. Another useful parameter is milk urea nitrogen (MUN) because it shows nitrogen metabolism during the whole 24 hours. Stefanska et al. (2020) pointed out that in cows with low rumen pH, MUN rises significantly.

The results of this research indicate the differences in biochemical parameters in both blood serum and milk due to animals' health status and due to animal age implying that animals' response depends on animal age. Obtained should be considered in the modelling for the occurrence prediction of metabolic diseases.

CONCLUSIONS

The obtained results indicate the variability in biochemical parameters in both blood serum and milk due to animals' health status (classes of F/P ratio that is acidosis or ketosis risk) and due to animal age (parity classes) implying that animals' metabolism and response to feeding management and production needs depends on animal age. Obtained should be considered in the modelling for the occurrence prediction of metabolic diseases.

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Оригинални научни рад

ВАРИЈАБИЛНОСТ БИОХЕМИЈСКИХ ПАРАМЕТАРА У КРВНОМ СЕРУМУ И МЛЕКУ ХОЛСТАЈН КРАВА У ОДНОСУ НА СТАРОСТ И ЗДРАВСТВЕНО СТАЊЕ

Сажетак

У циљу утврђивања варијабилности биохемијских параметара узорковани су крв и млеко од 25 холштајн крава у трајању од три месеца. Утврђене су разлике у анализираним параметрима у зависности од здравственог статуса животиње (ризик од ацидозе или кетозе или нормалног здравственог стања; дефинисано према односу масти/протеина) и старости животиње (паритетне класе). Резултати овог истраживања указују на варијабилност биохемијских параметара у крвном серуму и млеку као резултат варијансе здравственог стања животиња и због различите старости животиња, што имплицира да одговор животиња зависи од старости животиње. Добијене резултате треба узети у обзир при моделирању за предвиђање појаве метаболичких поремећаја.

Кључне речи: *Холштајн крава, биохемијски параметри, млеко, крв, метаболички поремећаји*



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Original Scientific paper

LOAD OF SOIL, FOOD, ANIMAL FEED AND BIOLOGICAL MATERIAL WITH RADIOACTIVE RESIDUES

Abstract

The uses of nuclear energy for peaceful purposes (nuclear weapon testing, nuclear reactor accidents, industrial and medical use of radioactive compounds) and application of phosphate mineral fertilizers in agricultural production lead to a substantial environmental contamination. Land contaminated with radionuclides represents the first link in the food chain and results in radioactive contamination of crop and livestock production. The activity concentrations of radionuclides gamma emitters were determined using low-level gamma spectrometry on high resolution HPGe detector system produced by ORTEC. The concentrations of thorium and uranium in all the samples were analysed by a technique of inductively coupled plasma using mass spectrometry (ICP-MS 7700, Agilent). The samples of the soil, foodstuff (meat and meat products, milk and dairy products, fish, honey), animal feed (complete mix, concentrated pre-mix feed, phosphate mineral feed, fish meal) and biomaterials (internal organs, urine) were examined. The results revealed the presence of the following natural radionuclides: K-40, Ra-226, Th-232, U-235 and U-238. The presence of Cs-137, that is, an anthropogenic (artificially produced) radionuclide has also been confirmed. Based on the results obtained, it can be concluded that the highest concentration of activity originates from natural radionuclide of potassium-40 in all tested samples. Also, in this study, the examined content of the tested radionuclides in different environmental samples provides interesting additional information for approximate calculation of the average input of radioactive residues.

Key words: *natural radionuclides, artificial radionuclides, biosphera*

INTRODUCTION

The use of nuclear energy in science, agriculture, industry, transport and energy, and even in regular operating conditions can cause environmental contamination. Accidents at nuclear power plants (Chernobyl, 1986; Fukushima-Daiichi, 2011) are

also major sources of environmental contamination with radioactive substances. Numerous studies on the degree of contamination of the cycle of agricultural production with artificial radionuclides examine the origin, transfer and migration routes, as well as their content in the final agricultural products – food and animal feed (Mitrović al. 2008).

Radionuclides of natural origin contribute equally to the radiation load of the biosphere, and the organisms that live in it, as those created artificially. Natural radioactivity consists of radioactive elements that originate from the time of the formation and evolution of the Earth and the first beginnings of the development of the entire living world. Tests show that there are very significant differences in the content of natural radionuclides between individual locations on the Earth's surface, and their amount varies from place to place. In nature, the largest contribution to the activity comes from the natural radionuclide ^{40}K . Natural radionuclides that have always been present on Earth include ^{235}U , ^{238}U , ^{232}Th , ^{226}Ra and ^{222}Rn (Levant, 1996). All living beings have been exposed to the low-intensity radiation of these elements from nature since their creation on the Earth. This is background radiation. The living world is evolutionarily adapted to the natural level of radiation and reacts to the increased presence of radioactive substances in its environment with changes in basic life processes. This results in pathological changes in cells, tissues and organs, and in the cases of drastic increase of radiation levels, these changes can have a fatal outcome. Radioactive elements emit ionizing radiation that, passing through the tissue, causes a series of biological consequences (Veriš et al., 2009), such as: inhibition of cell division, mutation (transformation) of cells, as well as a series of genetic effects (chromosome damage).

MATERIALS AND METHODS

Sampling: soil samples were collected from several locations in Vojvodina and Central Serbia. Sludge samples were collected from the bottom of the Danube in the region of Novi Sad. The samples were dried at 105 °C to constant mass (IAEA, 1989). All mechanical contaminants, mainly small stone pieces and plant material were removed. The samples were then sieved and homogenized as fine powder. Food samples of fish, dairy products (powdered milk, powdered whey, powdered eggs), honey and meat were collected from different sales points, and samples of animal feed (fish meal, premixes, mineral nutrients) from regular production. The samples weighing about 300 g were prepared and packed in cylindrical geometry-Marinelli beakers ($V=250\text{ml}$) and measured on the cap of detectors.

Analysis: Activity concentrations of radionuclides gamma emitters were determined by the method of low-level gamma spectrometry on high resolution HPGe coaxial detector system produced by ORTEC with nominal efficiency of 28%, resolution (FWHM) at 1.33 MeV ^{60}Co of 1.67 keV and and Peak-to-Compton ratio 67:1. The detector was shielded with the cylindrical lead shield of 10 cm wall thickness. The gamma

spectra were acquired and analyzed using the GammaVision® software. The precision and accuracy of the method was tested using a certified reference material LR 320 (Deutscher kalibrierdienst, Germany). Typical measurement time was 100 ks. Activity concentrations of thorium and uranium in all the samples were analyzed by a technique of inductively coupled plasma with mass spectrometry, on the Agilent ICP-MS 7700 (Carol S., 2007). The samples were prepared by wet digestion using Ethos, Labstation Microwave, Milestone (Pappas et al., 2002).

RESULTS AND DISCUSSION

In all tested soil and river sludge samples (Table 1), the long-lived artificial radionuclide ^{137}Cs was found. Its average activity in soil samples was ~ 6 Bq/kg, while in river sludge samples it was slightly higher than 15 Bq/kg. The measured activity of ^{40}K , ^{232}Th and ^{226}Ra in all tested samples has the values typical for Vojvodina soil (Bikit et al., 2010). The mean values of natural ^{238}U activity concentration range from 33 to 41 Bq/kg, and the mean values for natural ^{235}U activity in all tested samples are ~ 2 Bq/kg (Mihaljev et al., 2015).

Table 1. Activity concentration of cesium-137, potassium-40, thorium-232, radium-226, uranium-235 and uranium-238 in soil and sludge samples

Locality	Activity					
	^{137}Cs [Bq/kg]	^{40}K [Bq/kg]	^{232}Th [Bq/kg]	^{226}Ra [Bq/kg]	^{238}U [Bq/kg]	^{235}U [Bq/kg]
Vojvodina (n=11)	$\bar{A}=5.41$ VI=4.10-9.10	$\bar{A}=531$ VI=344-741	$\bar{A}=47.9$ VI=43-54	$\bar{A}=42.3$ VI=41-51	$\bar{A}=36.4$ VI=29.2-52.5	$\bar{A}=1.87$ VI=1.51-2.71
Central Serbia (n=18)	$\bar{A}=5.94$ VI=0.91-16.7	$\bar{A}=353$ VI=70-629	$\bar{A}=38.3$ VI=32.2-49.6	$\bar{A}=39.8$ VI=32.2-49.6	$\bar{A}=41.0$ VI=31.0-66.8	$\bar{A}=2.10$ VI=1.59-3.43
Novi Sad Danube- sludge (n=5)	$\bar{A}=15.4$ VI=13.8-17.0	$\bar{A}=548$ VI=494-640	$\bar{A}=34.8$ VI=19.6-43.9	$\bar{A}=5.46$ VI=4.15-8.12	$\bar{A}=33.4$ VI=11.6-51.8	$\bar{A}=1.72$ VI=0.60-2.66

\bar{A} – Average value of activity concentration;

VI – variation interval (span) = min. value – max. value

Based on the results shown in Table 2, it can be concluded that the presence of ^{137}Cs , as a biologically significant radionuclide, was determined in the samples of small blue fish (sprat, mackerel, sardines) with an average value of 3.05 Bq/kg and in samples of some dairy products (milk powder, whey powder) with an average value of 1.99 Bq/kg. The measured ^{40}K activity ranged from 32 Bq/kg (honey) to 894 Bq/kg (milk powder).

Table 2. Activity concentration of cesium-137, potassium-40, thorium-232, radium-226, uranium-235 and uranium-238 in food samples

Vrsta uzorka	Aktivnost					
	¹³⁷ Cs [Bq/kg]	⁴⁰ K [Bq/kg]	²³² Th [Bq/kg]	²²⁶ Ra [Bq/kg]	²³⁸ U [Bq/kg]	²³⁵ U [Bq/kg]
Sitna plava riba (n=15)	$\bar{A}=3.05$ VI=2.55-4.13	$\bar{A}=122$ VI=101-162	$\bar{A}=1.94$ VI=1.03-3.33	$\bar{A}=2.91$ VI=1.23-4.88	$\bar{A}=8.78$ VI=3.0-17.0	$\bar{A}=0.45$ VI=0.15-0.87
Mlečni proiz- vodi (n=10)	$\bar{A}=1.99$ VI=0.80-4.12	$\bar{A}=535$ VI=113-894	$\bar{A}=2.08$ VI=1.22-3.12	$\bar{A}=4.52$ VI=1.37-15.8	$\bar{A}=17.23$ VI=9.0-31.6	$\bar{A}=0.88$ VI=0.46-1.62
Med (n=11)	< 0.5	$\bar{A}=53$ VI=32-84	$\bar{A}=1.33$ VI=0.5-2.0	$\bar{A}=4.86$ VI=1.9-6.0	$\bar{A}=13.8$ VI=10-21	$\bar{A}=0.71$ VI=0.5-1.0
Meso (n=12)	< 0.5	$\bar{A}=123$ VI=87-132	$\bar{A}=0.98$ VI=0.53-1.94	$\bar{A}=22.6$ VI=6.96-68.3	$\bar{A}=5.51$ VI=1.3-8.2	$\bar{A}=0.28$ VI=0.07-0.42

\bar{A} – Average value of activity concentration;

VI – variation interval (span) = min. value – max. value

The activity of other natural radionuclides (²³²Th, ²²⁶Ra, ²³⁸U, ²³⁵U) had the usual values, typical for certain types of tested samples.

Table 3. Activity concentration of cesium-137, potassium-40, thorium-232, radium-226, uranium-235 and uranium-238 in animal feed samples

Sample type	Activity					
	¹³⁷ Cs [Bq/kg]	⁴⁰ K [Bq/kg]	²³² Th [Bq/kg]	²²⁶ Ra [Bq/kg]	²³⁸ U [Bq/kg]	²³⁵ U [Bq/kg]
Fish meal (n=16)	$\bar{A}=4.08$ VI=0.6-10.4	$\bar{A}=448$ VI=145-583	$\bar{A}=1.76$ VI=0.56-2.61	$\bar{A}=4.13$ VI=1.02-11.0	$\bar{A}=28.4$ VI=22.3-37.7	$\bar{A}=1.46$ VI=1.14-1.93
Premixes (n=14)	$\bar{A}=1.03$ VI=0.37-2.8	$\bar{A}=321$ VI=99-604	$\bar{A}=2.62$ VI=1.58-3.84	$\bar{A}=3.29$ VI=1.09-9.69	$\bar{A}=14.5$ VI=5.0-26.0	$\bar{A}=0.74$ VI=0.26-1.34
Mineral feed (n=5)	< 0.5	$\bar{A}=52$ VI=20.2-80.0	$\bar{A}=14.0$ VI=2.43-46.6	$\bar{A}=6.57$ VI=1.36-9.59	$\bar{A}=486$ VI=32-1474	$\bar{A}=24.9$ VI=1.65-75.7

\bar{A} – Average value of activity concentration;

VI – variation interval (span) = min. value – max. value

Fish meal contains dried and ground whole fish or fish parts and represents an important source of nutrients in the diet of domestic animals. Fish are significant indicators of the aquatic environment they inhabit, and the determination of activity level of natural radionuclides in protein feed of animal origin is highly important (Jovanović et al., 2001).

In some fishmeal samples, the presence of artificial radionuclide ¹³⁷Cs was determined with a maximum value of 10.4 Bq/kg, and in premixes the maximum measured

value of ^{137}Cs was 2.8 Bq/kg. The obtained results (Table 3) show the presence of natural radionuclide K-40 in all analyzed samples, with an activity level of 145-583 Bq/kg in fish meal and 99-604 Bq/kg in premixes. In some samples of fishmeal (table 3), moderately elevated concentrations of radium-226 (11 Bq/kg) and uranium-238 (37.7 Bq/kg) were found, and they were 9.69 Bq/kg (^{226}Ra) and 26 Bq/kg (^{238}U) in the premixes. Other examined natural radionuclides (^{232}Th , ^{235}U) in fish meal and premixes had the activity of less than 4 Bq/kg. The feed for domestic animals contains mineral ingredients, yet in inadequate amounts and undesirable ratio. Mineral feedstuffs such as monocalcium phosphate (MCP) are inorganic compounds intended for satisfying animals' needs for calcium and phosphorus, which are essential for bone formation and play an important physiological role in all cells of the body. Monocalcium phosphate $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ is produced from natural phosphates (apatite, phosphorite). The principle includes their decomposition by action of mineral acids to obtain soluble hydrogen-phosphates. Dissolution of natural phosphates results in the deposition of uranium from phosphate ore in final products, that is, mineral feedstuffs (MCP), and consequently reaches the body of humans and animals (Vranješ et al., 2017).

The results shown in Table 3 reveal that the average activity of thorium-232 in mineral feed samples was 14 Bq/kg, and reached as high as 46.6 Bq/kg in one sample of MCP. The highest measured value for ^{235}U was 75.7 Bq/kg. It should be emphasized that activity concentration of even 1474 Bq/kg for ^{238}U was determined in one sample. This is particularly important because the radioactive decay of radium nucleus produces radon (^{222}Rn), a radionuclide that is the most responsible for atmospheric contamination (especially in enclosed areas). Radium is an analogue of calcium in the body, and is incorporated in the bones of animals and humans (~80%).

Game animals populating certain geographic locations can be considered excellent bio indicators for pollution with radioactive residues, having in mind their consumption of non-processed food of plant origin and their movement across a specific habitat. Still, one should bear in mind that radionuclide accumulation is affected by various features such as endogenous (age, sex, health condition of the animal) and exogenous (geographic position, hydrographical conditions, soil, climate, flora) factors (Mihaljev et al., 2019).

Table 4. Activity concentration of cesium-137, potassium-40, thorium-232, radium-226, uranium-235 and uranium-238 in samples of biological material

Sample type	Activity					
	^{137}Cs [Bq/kg,L]	^{40}K [Bq/kg,L]	^{232}Th [Bq/kg,L]	^{226}Ra [Bq/kg,L]	^{238}U [mBq/kg,L]	^{235}U [mBq/kg,L]
Wild animal kidneys (n=10)	< 0.5	\bar{A} =175 VI=108-341	\bar{A} =1.70 VI=0.58-4.93	\bar{A} =8.63 VI=3.56-16.6	\bar{A} =120.3 VI=38.6-463	\bar{A} =6.18 VI=1.98-24
Game animals' urine (n=21)	< 0.5	\bar{A} =253 VI=128-381	\bar{A} =17.9 VI=0.77-106	\bar{A} =22.2 VI=4.18-29.3	\bar{A} =42.3 VI=17.4-232	\bar{A} =2.17 VI=0.89-11.9

\bar{A} – Average value of activity concentration;
VI – variation interval (span) = min. value – max. value

In all tested game samples (table 4), potassium-40 is the dominant natural radionuclide with an average activity concentration of $^{40}\text{K}_{\text{av}}=175$ Bq/kg (in kidneys) and $^{40}\text{K}_{\text{av}}=253$ Bq/L (in urine). In the body, potassium is under homeostatic control and is little influenced by environmental variations (Mihaljev et al., 2018). Thorium-232 is a metallic naturally occurring element that is radioactive. Due to the insolubility and very low specific activity, thorium can be found in biosphere in very small amounts. This was also confirmed in our results: in the majority of analyzed samples of kidneys and urine of wild animals the activity of thorium-232 was below 5 Bq/kg. However, thorium-232 activity in some urine samples of wild boars (*Sus Scrofa*) reached a value of 106 Bq/L. Considering the nutritive habits (omnivores) and free movement of in nature, this game species can be useful in the process of environmental control as the bio indicators of radioactive contamination. Also, increased activity of uranium isotopes ($^{238}\text{U}_{\text{av}}=120.3$ Bq/kg) and $^{235}\text{U}_{\text{av}}=6.18$ Bq/kg) was apparent in kidney samples, which is in line with the fact that kidney is a critical target organ for natural uranium (Đurić et al., 1996).

CONCLUSION

The presence of radionuclide ^{137}Cs in soil, foodstuffs and animal feed is mostly the result of the accident at the "Lenin" nuclear power plant in Chernobyl in 1986.

The main contribution to radioactive contamination comes from gamma-emitting radionuclides from the ^{232}Th and ^{238}U series as well as from ^{40}K . The radioactivity of the soil in all the tested samples is in approximately the same ranges, while the deviations are primarily influenced by the type of soil. It can also be concluded that soil samples from all analyzed locations do not indicate an increase in radioactivity that could threaten food production. It is to be emphasized that activity of radionuclides in the examined samples was significantly lower than maximum permissible values set by the relevant legal provisions (Official Gazette of RS, 36/2018).

Control of the content of radioactive substances in the biosphere is a basic parameter that ensures maximum radiation safety of the population. From the point of view of protecting nature from the harmful effects of ionizing radiation, the knowledge about the routes and methods of transmission of radionuclides through the environment is of fundamental importance.

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**ОПТЕРЕЋЕНОСТ ЗЕМЉИШТА, НАМИРНИЦА,
ХРАНЕ ЗА ЖИВОТИЊЕ И БИОЛОШКОГ МАТЕРИЈАЛА
РАДИОАКТИВНИМ РЕЗИДУАМА**

Сажетак

Мирољубиво коришћење нуклеарне енергије (испитивање нуклеарног оружја, несреће нуклеарних реактора, индустријска и медицинска употреба радиоактивних једињења) и примена фосфатних минералних ђубрива у пољопривредној производњи доводе до значајног загађења животне средине. Земљиште контаминирано радионуклидима представља прву карику у ланцу исхране, а самим тим и радиоактивну контаминацију биљне и сточарске производње. Концентрације активности радионуклида, гама емитера, одређене су методом гама спектрометрије ниског нивоа на ХПГе детекторском систему високе резолуције произвођача ОРТЕЦ. Концентрације торијума и уранијума у свим узорцима анализиране су техником индуктивно спрегнуте плазме са масеном спектрометријом (ИЦП-МС 7700, Агилент). Испитани су узорци земљишта, различитих намирница (меса и производа од меса, млека и млечних производа, рибе, меда), хране за животиње (потпуне смеше, концентроване предсмеше-премихи, фосфатна минерална хранива, рибље брашно) и биолошког материјала (унутрашњи органи, урин). Резултати показују да је у испитаним узорцима утврђено присуство природних радионуклида: К-40, Ра-226, Тх-232, У-235 и У-238. Од вештачких (произведених) радионуклида детектован је антропогени радионуклид Цс-137. Може се закључити да је калијум-40, у свим испитиваним узорцима пре-доминантни природни радионуклид у односу на друге радионуклиде.

Кључне речи: *природни радионуклиди, вештачки радионуклиди, биосфера*



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Original Scientific paper

THE CORRELATION BETWEEN SOME DAILY MILK TRAITS AND BIOCHEMICAL PARAMETERS IN PLASMA AND MILK OF DAIRY COWS

Abstract

Aiming the determination of the relationship between some daily milk traits (daily lactose content and somatic cell count) and biochemical parameters samples of blood and milk were taken during three months of research from 25 Holstein cows reared on an indoor dairy cattle farm. The relationship between the analyzed traits was determined using the Pearson Correlation Coefficients. The conducted statistical analysis indicates high variability of relationship strength, from negligible to strong relationship. A weak or negligible relationship was determined between the daily lactose content in milk and all analyzed biochemical parameters in plasma, while with the biochemical parameters in milk relationship was stronger. The correlation coefficients between the somatic cell count (normal and transformed) and biochemical parameters in plasma were higher than with the daily lactose content. Determined correlations should be considered when designing systems for the early prediction of mastitis occurrence on dairy cattle farms.

Key words: *daily lactose content, somatic cell count, biochemical parameters, dairy cattle*

INTRODUCTION

Modern, market-oriented dairy cattle farming, on the one hand, is constantly under the influence of demands to raise the level of environmental protection, while at the same time the race for profitability does not stop. In the search for new solutions, a

significant place is occupied by some already existing ones, but in a new and innovative way. Mastitis, inflammation of the udder parenchyma of dairy cows, is one of the most common diseases of dairy cattle, present in clinical and subclinical form and affecting the general health of the herd, and is associated with increased use of antimicrobial drugs and associated resistance to the causative agent of the disease (Ebrahimi et al., 2019). Since the dairy industry requires high-quality milk (with low somatic cell count and high fat and protein content) for the production of dairy products, economic losses due to mastitis result from quality deterioration and reduced milk production (Halasa et al., 2007). Preventing and/or controlling subclinical mastitis consequently reduces the greenhouse gas emissions per unit of product on a farm resulting in improved profits for the farmers through reductions in milk losses, optimum culling rate and reduced feed and other variable costs (Özkan Gülzari et al., 2018). Therefore, the development of useful new models for predicting disease incidence and monitoring the prevalence of mastitis is a priority. Although the number of somatic cells in milk is a generally accepted method for monitoring the occurrence of mastitis, some authors state that lactose is the most promising parameter (Pyorala, 2003). The low lactose content is correlated with increased somatic cell count in milk, while lactose content < 4.5% is an indicator of mastitis (Babnik et al., 2004). Inflammation in the mammary gland leads to cell damage and reduced lactose synthesis (Kitchen, 1981) (Silanikove et al., 2009). It also causes a disruption of the blood-milk barrier with reduced production and secretion from the epithelial cells of the udder, which causes a change in the composition of the milk, as well as the biochemistry of the blood due to the release of various harmful toxins in the mastic udder. Therefore, it is necessary to identify accurate milk and blood biomarkers of inflammation in mammary tissue in order to use them in designing new alternative diagnostic approaches for rapid detection and confirmation of mastitis in dairy animals (Benić et al., 2018).

The objective of this research was to determine the relationship between some daily milk traits (daily lactose content and somatic cell count) and biochemical parameters in plasma and milk samples.

MATERIAL AND METHODS

The research was performed in the east of Croatia on an indoor dairy cattle farm. During the three months of research, milk was sampled, on monthly basis, from 25 Holstein cows with an average daily milk yield of around 40 kg/day. The cows included in the research were healthy and did not have any disorders. The cows were fed with a total mixed ration (TMR) with the addition of mixture accordingly to the daily milk production. Blood samples were taken from the coccygeal vein into tubes with lithium heparin anticoagulant (Becton Dickinson, Plymouth, England, UK) and centrifuged (1.500 g/10 min at 4°C) in order to separate the plasma. Milk samples were taken into clean tubes and centrifuged (12.000 g/30 min at 4°C) for plasma separation. The determination of the biochemical parameters in blood and milk plasma was performed operating an automatic clinical chemistry analyzer Beckman Coulter AU400 (Beckman Coulter, Germany). The concentration of β -hydroxybutyrate (BHB) was determined

utilizing commercial kits (Randox Laboratories Ltd, Crumlin, UK) following the enzymatic colorimetric method. The test-day records of animals included in the research, obtained during the regular milk recording, were taken from the central database of the Croatian Agency for Agriculture and Food. Test-day records were corrected accordingly to the ICAR guidelines (2017), and the somatic cell count was transformed using the log function. Table 1 presents basic statistical parameters of daily milk traits (lactose content and somatic cell count) of the animals included in the research.

Tab.1. Basic statistical parameters of daily milk traits (lactose content and somatic cell count)

Trait	N	Mean	SD	Minimum	Maximum
DLC, %	74	4.464	0.221	3.570	4.850
SCC	72	1420674	2205157	31818	10844297
ISCC	72	29.169	1.950	24.923	33.336

* DLC – daily lactose content in milk sample,%; SCC – somatic cell count;
ISCC – log value of somatic cell count

The relationship between some daily milk traits (daily lactose content and somatic cell count) and biochemical parameters in plasma and milk samples were determined using the Pearson Correlation Coefficients.

RESULTS AND DISCUSSION

The correlation coefficients between the daily milk traits (daily lactose content and somatic cell count) and biochemical parameters in plasma presented in Table 2. ranged between 0.001 (negligible relationship) to -0.605 (strong relationship). A weak or negligible relationship was determined between the daily lactose content in milk and all analyzed biochemical parameters in plasma. A positive relationship existed between the daily lactose content and aspartate aminotransferase, γ -glutamyl transferase, urea, albumin and Fe concentration. Furthermore, between the daily lactose content and glucose, protein, triglyceride, β -hydroxybutyrate and Ca concentration negative relationships were determined. The correlation coefficients between the somatic cell count (normal and transformed) and biochemical parameters in plasma were higher than with the daily lactose content indicating a stronger relationship between the somatic cell count than with the lactose content. The highest determined correlation coefficient of -0.605 between the somatic cell count and concentration of Fe in plasma indicates a decrease in plasma Fe in case of mastitis occurrence. A moderate negative correlation was also determined between the SCC and AST (-0.339), and GGT (-0.247) indicating a decrease of aspartate aminotransferase and γ -glutamyl transferase in a case of SCC increase or mastitis occurrence. On the other hand, a moderate positive corre-

lation between the SCC and glucose (0.329), and triglyceride (0.225) indicate an increase in glucose and triglyceride concentration in plasma if SCC increases.

Correlation coefficients between the daily milk traits (lactose content and somatic cell count) and biochemical parameters in milk are presented in Table 3. The highest and positive correlation coefficient was determined between the daily lactose content and glucose (0.307) and Fe concentration in milk (0.269) indicating simultaneous fluctuation of the defined parameters. The relationship between the daily lactose content and other biochemical parameters in milk was negligible or weak.

Table 2. Correlation coefficients between daily milk traits (lactose content and somatic cell count) and biochemical parameters in plasma

Trait	DLC, %	SCC	ISCC
Aspartate amino transferase (U/L, AST)	0.157 0.186	-0.339 0.004	-0.494 <0.001
γ -glutamyl transferase (U/L, GGT)	0.067 0.573	-0.247 0.038	-0.356 0.002
Glucose (mmol/l, GUK)	-0.075 0.531	0.329 0.005	0.315 0.008
Urea (mmol/L, UREA)	0.056 0.637	0.101 0.401	-0.111 0.356
Protein (mmol/L, PRO)	-0.185 0.117	0.077 0.522	0.161 0.180
Albumin (g/L, ALB)	0.001 0.995	-0.038 0.751	-0.090 0.457
Triglyceride (mmol/L, TGC)	-0.111 0.352	0.225 0.059	0.259 0.029
β -hydroxybutyrate (mmol/L, BHB)	-0.007 0.952	-0.094 0.441	0.020 0.868
Fe (μ mol/L)	0.142 0.231	-0.605 <0.001	-0.523 <0.001
Ca (mmol/L)	-0.091 0.445	-0.075 0.537	-0.128 0.286

* DLC – daily lactose content (%); SCC – somatic cell count;
ISCC – log value of somatic cell count

Somatic cell count (normal and log value) was positively correlated with the AST, GGT, and Ca concentration in milk, while a negative correlation was determined with the glucose, urea, protein, albumin and Fe concentration in milk. Conversely to the correlation with the AST and GGT in plasma, the correlation between the SCC and

transferases was a positive indicating increase of catabolic enzymes in the milk of animals with increased somatic cell count. Understanding conditions underlying the development of mastitis using blood and milk biochemical parameters to assess animal health will improve disease prevention. Different deviations were observed in the results of blood and milk yields, and finding appropriate relationships between these parameters individually in blood and milk is crucial for understanding the health and production status of animals.

Table 3. Correlation coefficients between daily milk traits (lactose content and somatic cell count) and biochemical parameters in milk

Trait	DLC, %	SCC	ISCC
Aspartate amino transferase (U/L, AST)	-0.100 0.402	0.398 0.001	0.379 0.001
γ -glutamyl transferase (U/L, GGT)	-0.005 0.966	0.336 0.004	0.225 0.056
Glucose (mmol/l, GUK)	0.307 0.009	-0.399 0.001	-0.510 <0.001
Urea (mmol/L, UREA)	0.064 0.593	-0.054 0.652	-0.134 0.262
Protein (g/L, PRO)	0.091 0.450	-0.071 0.553	0.011 0.929
Albumin (g/L, ALB)	0.164 0.170	-0.235 0.047	-0.212 0.074
Fe (μ mol/L)	0.269 0.022	-0.277 0.019	-0.265 0.024
Ca (mmol/L)	0.056 0.640	0.172 0.148	0.143 0.232

* DLC – daily lactose content, %; SCC – somatic cell count;
ISCC – log value of somatic cell count

Aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT) are important catabolic enzymes that play an important role in animal liver function. Liver dysfunction is often associated with mastitis, and some studies (Liu et al., 2012) have shown that GGT enzyme activities in milk are higher than in blood plasma. Though some studies have found a significant increase in serum AST activities in mastitic cows compared to apparently healthy cows, these elevated levels of aminotransferases (AST) may be indicative of cellular damage, particularly in the liver and myocardium, and subsequent leakage of cellular enzymes into the serum (Amany & Dina, 2008) (Al-ataish et al., 2018). Lactation has a great influence on the biochemical parameters in

the blood of cows, reflecting the metabolic needs, and the activity of AST in the blood is very important. AST acts as a catalyst in linking the metabolism of amino acids and carbohydrates. Accordingly, changes in their activity in the blood may be a consequence of their increased activity in cells (primarily the liver), but also a reflection of damage to the cellular structure. In some studies (Djokovic et al., 2017), a statistically significant higher ($P < 0.01$) AST activity in blood serum was found in groups of cows in early lactation compared to groups of cows in mid-lactation, while no significant difference ($P > 0.05$) in AST value in milk serum. It has been observed that the levels of AST activity in milk are higher than those found in blood serum (Batavani et al., 2003) (Ping Liu et al., 2012) and it is suggested that the main source of AST in normal and mastitic milk is a secretory cell of the mammary gland, which is consistent with our results. On the other hand, some authors (Mohamed, 2014) report that a significant difference was found in AST activity in the blood serum of cows at the beginning compared to the middle of lactation, without a significant difference in enzyme activity in milk, where AST activity was significantly lower in milk than in blood in both stages of lactation.

A negative correlation between glucose in milk was found in relation to SCC and LSCC, and a possible explanation is that glucose is transferred from milk to the extracellular pathway to maintain the osmotic balance between the extracellular environment and milk with an increase in Na^+ and Cl^- when the body suffers from mastitis (Hu et al., 2021). In addition, hypoglycemia may result from the accumulation of immune cells at the site of infection, which reduces the availability of glucose in the local area. During inflammation, the glucose demands of the immune system may increase to amounts similar to those required for high milk yields (Habel & Sundrum, 2020). Inflammation of the mammary gland caused a significant increase in the concentration of glucose in the blood plasma despite a rather significant decrease in the extraction of glucose by the mammary gland, and it can be considered a protective mechanism due to the supported increased energy demand of the immune system (Silanikove et al., 2014). The higher correlation coefficient found between albumin in milk compared to albumin in plasma is consistent with the claims that although the major site of albumin synthesis is in the liver and that it enters milk by leakage through the epithelial tight junction from the bloodstream, is the main source of the increase in albumin content in milk in inflammatory conditions the gland itself (Batavani et al., 2007). A significantly ($P < 0.05$) lower level of albumin was reported in subclinical (2.44 g/dl) and clinically (2.31 g/dl) infected animals than in animals of the control group (2.94 g/dl) (Singh et al., 2014). α -lactalbumin is a part of lactose synthetase, and a reduction of it, especially with higher leukocyte counts, agrees with reduced lactose synthesis during mastitic conditions (Haenlein et al., 1973). Serum levels of some minerals in subclinical mastitic animals reveal that subclinical mastitis involves a decrease in the iron concentration of 9.9 ± 0.8 compared to the control of 18.6 ± 1.4 (Al-autaish et al., 2018). Restriction of availability of Fe (hypoferremia) represents one of the anti-microbial defense mechanisms. Ganz (2018) reported that a common mechanism of hypoferremia during inflammation is a cytokine-driven increase in hepcidin, which decreases the concentration of ferroportin and thereby reduces iron fluctuation into the extracellular fluid. Although some authors report a drop in the level

of calcium in the blood and milk of infected cows (Yildiz & Kaygusuzoğlu, 2005; Zaki et al., 2010; Hassanin, 2019) in our research, negative correlation coefficients were determined for calcium from plasma, and positive correlation coefficients for calcium in milk. A significantly higher level of calcium was recorded in the plasma of sub-clinical and clinically infected animals compared to the control group of animals (Singh et al., 2014), and it could be a consequence of a decrease in the milk yield of infected animals, therefore less total calcium is excreted in milk.

CONCLUSIONS

The analysis of the relationship between some daily milk traits (daily lactose content and somatic cell count) and biochemical parameters in plasma and milk indicates high variability of relationship strength (from negligible to strong relationship). A weak or negligible relationship was determined between the daily lactose content in milk and all analyzed biochemical parameters in plasma, while with the biochemical parameters in milk relationship was stronger. The highest and positive correlation coefficient was determined between the daily lactose content and glucose and Fe concentration in milk.

The correlation coefficients between the somatic cell count (normal and transformed) and biochemical parameters in plasma were higher than with the daily lactose content indicating a stronger relationship between the somatic cell count than with the lactose content. The highest correlation coefficient was determined between the somatic cell count (SCC) and concentration of Fe in plasma indicating a decrease in plasma Fe in case of mastitis occurrence. A moderate negative correlation was determined between the SCC and aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT). Conversely to the correlation with the AST and GGT in plasma, the correlation between the SCC and transferases in milk was positive indicating an increase of catabolic enzymes in the milk of animals with increased somatic cell count.

Determined correlations should be taken into account when designing systems for the early prediction of mastitis occurrence on dairy cattle farms.

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Оригинални научни рад

КОРЕЛАЦИЈА ИЗМЕЂУ НЕКИХ ДНЕВНИХ ОСОБИНА МЛЕКА И БИОХЕМИЈСКИХ ПАРАМЕТАРА У ПЛАЗМИ И МЛЕКУ МЛЕЧНИХ КРАВА

Сажетак

У циљу утврђивања везе између неких дневних особина млека (дневног садржаја лактозе и броја соматских ћелија) и биохемијских параметара, током тромесечног истраживања узети су узорци крви и млека од 25 крава Холштајн расе узгојених на млечној фарми затвореног типа држања. Однос између анализираних особина одређен је коришћењем Пирсонових коефицијената корелације. Спроведена статистичка анализа указује на велику варијабилност јачине односа, од занемарљиве до јаке везе. Утврђен је слаб или занемарљив однос између дневног садржаја лактозе у млеку и свих анализираних биохемијских параметара у плазми, док је код биохемијских параметара у млеку однос био јачи. Утврђене корелације треба узети у обзир приликом пројектовања система за рано предвиђање појаве маститиса на фармама млечних говеда.

Кључне речи: *дневни садржај лактозе, број соматских ћелија, биохемијски параметри, млечна говеда*



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Original Scientific paper

THE USE OF BIOWASTE AS A SILAGE BY-PRODUCT FOR SUSTAINABLE FOOD AND FEED PRODUCTION

Abstract

Worldwide, food production is on the rise, as well as the amount of waste. In recent years, rising disposal costs, environmental regulations, and awareness have created the need to find sustainable alternatives to waste management. There are various options to turn food waste into value-added products such as compost, fertilizer, soil improvement supplements, biogas, medical products, livestock feed, etc. The work showed the application of food waste in the form of raw materials for silage and the preparation of high-quality animal feed. In this way, waste was converted into a new by-product of the food industry for the preparation of high-quality silages

Keywords: *by-product, bio waste animal feed, silage*

INTRODUCTION

Food processing operators are characterized by industrial processes that produce significant amounts of byproducts that, under the new EU waste regulation, can be reused or recycled with favorable environmental impacts and an important impact on the economy. Each sub-sector produces different types of byproducts that can be treated or reused in different processes or industries. Each waste producer carries with it responsibility in waste management, which includes certain obligations. The waste producer is obliged to draw up a waste management plan and organize its implementation if it produces a certain amount of non-hazardous waste or hazardous waste annually. It is also obliged to obtain a report on waste testing and renew it in case of a change in technology, changes in the origin of raw materials, or other activities that would affect the change in the character of waste and keep the report for at least five

years, or to obtain a corresponding exemption certificate in accordance with this law. The waste producer is obliged to ensure the application of the principles of the waste management hierarchy, collect waste separately in accordance with the need for future treatment, and store waste in a way that minimally affects human health and the environment. Currently, the food industry seeks to reduce the amount of waste material produced during the production cycle. There is growing interest in the commercial use of different generated side products during different production processes. Replacement of edible feed crops with human-inedible biomass in animal diets is a potential strategy that could reduce food-feed competition and mitigate the environmental impacts of livestock, (Salami *et al.*, 2019).

The cost of many traditional feeds restricts their use in many countries, and producers are turning to alternative feed sources because they may supply the energy and protein requirements for reproduction but can contain plant secondary compounds that affect various components of the reproductive cycle, (Blache *et al.*, 2008). Feed technology involves the processing of ingredients and the manufacture of animal feeds and is an integral part of animal production systems to provide high-quality and nutritious food, with the objective is to transform low-quality ingredients into higher-value feed components and improving nutrient utilization of compound feeds, (Abdollahi *et al.*, 2020). Bio-waste is created depending on the type of culture in part before the raw material of vegetables enters the food factory, during purification, then in the processing depending on culture and after processing, where pieces are extracted, and when the cut product does not correspond in shape, size, and color, while a smaller part of the waste is created during the packaging process due to waste.

MATERIALS AND METHODS

In order to obtain initial results on the possibility of bio-waste ensiling, different mixtures of vegetables were ensiled in the Laboratory for Nutrition of Domestic Animals, at the Faculty of Agriculture – University of Belgrade. Laboratory mini silos were used for rape, with a hermetic opening, a volume of 1.5 liters, and one-way emissions. Each silo is filled with 1100-1,200 grams of compressed bio-waste. Experimental silos are stored at room temperature. For ensiling in experimental laboratory silos, it was used: peas from production, broccoli cubes, broccoli bites, vegetable waste, vegetable waste mixed vegetables, tomatoes, parsnip, corn sugar grain, and corn leaves. In order to eliminate the undesirable moisture content, it was used: wheat grain, a covered whole grain of wheat, and a corn crop. Experimental mixtures with different combinations of bio-waste vegetables and foods in dry form, as well as in different relationships were ensiled.

Analytical DM content of oven-dried samples and concentrate diet ingredients were determined by drying at 105 °C for 5 h. Samples were ground through a 1-mm diameter screen. Ash was determined by combustion at 550 °C for 6 h. The CP content was determined by the micro-Kjeldahl method (method 988.05; AOAC, 2002) using K₂SO₄/Se catalyst-Kjeltabs S 3.5, on the Kjeltac Auto 1030 Analyzer-Tecator System. Ether extract content was determined by extraction using diethyl ether in the Soxhlet apparatus (method 920.39; AOAC, 2002). Neutral detergent fiber (NDF) content was

determined using heat-stable α -amylase (A3306 Sigma Chemical Co., St Louis, MO, USA) and sodium-sulfite (Official Method 2002:04; AOAC, 2002). The acid detergent fiber (ADF) and acid detergent lignin (ADL) content were determined according to the Official Method: 973.18 (AOAC, 2002).

RESULTS AND DISCUSSION

Ensiling is a technological process of conservation of high-moist plants (the most common moisture content is 60-80%), intended primarily for the nutrition of domestic animals. Feed conservation is done using acids that are obtained by the activity of bacteria in lactic-acid fermentation. As a result of the whole process, obtained is product silage, which is used in the diet of animals on farms throughout the year.

It was ensiled three different mixtures of vegetable waste from food processing, which are present in table 1. Results of standard chemical analysis and quality of silage fermentation are shown in tables 2-7.

Table 1. Composition of experimental silages, %

Silage 1		Silage 2		Silage 3	
Corn leaves and knobs	23,04	Tomato	10,29	Corn leaves and knobs	26,32
Vegetable mixture waste	9,82	Broccoli petty	14,83	Green beans	22,30
Vegetable leaves	11,00	Broccoli cubes	13,37	Vegetable mixture waste	12,18
Parsnip	8,30	Green beans	20,69	Parsnip	9,66
Broccoli cubes	9,94	Corn leaves	13,83	Sugar corn grain	7,01
Green beans	14,15	Tomato	4,89	Wheat grain	22,53
Corn cover	23,75	Corn flour	22,10	/	/

Table 2. The results of the standard chemical analysis of silage 1

Parameter	As Feed, %	In DM, %
Moisture	70,38	–
Dry Matter	29,62	–
Crude protein	3,19	10,77
Ether extract	0,69	2,34
Ash	0,71	2,40
Neutral detergent fiber-NDF	12,95	43,72
Acid detergent fiber-ADF	3,18	10,73
Acid detergent lignin-ADL	0,05	0,17

Table 3. Total organic acid content and silage quality rating per DLG system for silage 1

	Acid	Participation %	Content, %	Points	Rating
1.	Lactic acid	5,33	0,008	0	Quality Class V Bad quality
2.	Acetic acid	94,67	0,144	0	
3.	Butyric acid	/	/	10	
4.	pH	3,58		9	
	Σ				

Table 4. Total organic acid content and silage quality rating per DLG system for silage 2

	Acid	Participation %	Content, %	Points	Rating
1.	Lactic acid	73,58	0,39	20	Quality Class I Very good quality
2.	Acetic acid	26,42	0,14	10	
3.	Butyric acid	/	/	10	
4.	pH	3,80		50	
	Σ				

Table 5. The results of the standard chemical analysis of silage 2

Parameter	As Feed, %	In DM, %
Moisture	70,65	–
Dry Matter	29,35	–
Crude protein	3,50	11,93
Ether extract	0,75	2,57
Ash	0,62	2,10
Neutral detergent fiber-NDF	12,52	42,65
Acid detergent fiber – ADF	3,32	11,30
Acid detergent lignin – ADL	0,08	0,28

The flow of conservation and intensity of change will depend on a number of factors but mostly those that condition the successful development of lactic-acid fermentation such as food moisture content, environmental anaerobicity, carbohydrate content, and temperature, (Ivetić A., 2017). These factors enable conditions in which desirable microorganisms will dominate during plant fermentation and enable you to obtain high-nutritional quality silage. Silage 1 consists of a parsnip, broccoli, green beans, corn leaves, waste vegetable, and their leaves from food production. Silage 2

consists of broccoli, green beans, tomatoes, and peas. Silage 3 consists of tomatoes, green beans, sugar corn grains, and leaves. Experimental silages 2 and 3 were characters in high-quality quality fermentation, as opposed to experimental silage 1. Acetic acid was presented in experimental silage 1 with 94.67% DM. On the contrary, in high-quality silages 2 and 3, the presence of lactic acid was 66.67 – 73.58 % DM. It is necessary to highlight the benefit of such ensiling with vegetable waste which is that no experimental silages have had the participation of butyric acid.

Table 6. The results of the standard chemical analysis of silage 3

Parameter	As Feed, %	In DM, %
Moisture	69,39	–
Dry Matter	30,61	–
Crude protein	4,12	13,50
Ether extract	0,98	3,20
Ash	1,05	3,44
Neutral detergent fiber-NDF	18,74	61,21
Acid detergent fiber-ADF	8,98	29,33
Acid detergent lignin-ADL	0,13	0,41

Table 7. Total organic acid content and silage quality rating per DLG system for silage 3

	Acid	Participation %	Content, %	Points	Rating
1.	Lactic acid	66,67	0,40	20	Quality Class I Very good quality
2.	Acetic acid	33,33	0,20	9	
3.	Butyric acid	/	/	10	
4.	pH	4,04		9	
	Σ			48	

The main goal of plant ensiling is to preserve the nutritional value of the green mass of plants during conservation so that the nutrient value is as close as possible to the nutritional value they had before conservation, (Ivetić A., 2018). The content of crude protein CP was in the range of 10,77 – 13,50 % DM, which is satisfactory in relation to some of the poorer ones in CP such as corn silages. Because, of low fermentation quality and reduced nutritional value animals are consumed in small quantities due to distaste and subsequently less utilization of SP, (Stojanović *et al.*, 2020).

Lower SM content in silages and a slower decline in pH value may facilitate the development of undesirable bacteria. Arriola *et al.*, (2021) suggest that this may explain more modest improvements in aerobic stability than inoculation in low SM content

compared to those with medium or high DM content. DM content was smaller in experimental silages and was 29-30%.

Also, it could be expected that silage nutrient concentrations and silage fermentation products would affect methane yield and that these factors could be used to predict the methanogenic potential of the silages, (Weiby *et al.*, 2022). Understanding the variability of quality silage, milk production of lactating dairy cows fed alfalfa silage-based diets and the nexus between quality silage and milk yield is vital for silage quality improvement while contributing to profitable milk production, (Tharangani *et al.*, 2022). The indigestible part of the animal feed is the content of the lignin. A very positive effect of these silages on lignin content was observed, which ranged from 0.17-0.41% DM, which is tenfold and more, less than the content of lignin in the silages that are made from traditional crops. Nowadays, plant-based human food products are considered to be more sustainable than animal-derived products, (Wehrmaker *et al.*, 2022).

CONCLUSION

The sustainability of food and feed production means ensuring that agriculture remains sustainable and successful in the long term. The concept and goal of sustainability can be achieved by creating a common value in which food and feed production and environmental values are joined together. This means providing agriculture production benefits to suppliers and customers, while at the same time following with a positive impact on the planet, humans, and animals. All food and beverage production processes lead to by-products from waste materials that remain after the completion of production activities. A certain amount of bio-waste is generated during the food production process. Depending on the type of product, as well as the production cycle, bio-waste occurs during receiving vegetables raw materials, washing, processing raw vegetable materials, packaging, and manipulating in the warehouse of finished products, as well as in retail. There are various options to turn food waste into value-added products such as compost, fertilizer, soil improvement supplements, biogas, medical products, livestock feed, etc. This work it is presented the application of food vegetable waste in the form of raw materials for silage and the preparation of high-quality animal feed. In this way, waste was converted into a new by-product of the food industry for the preparation of high-quality silages.

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Оригинални научни рад

UPOTREBA BIO OTPADA U VIDU SPOREDNOG PROIZVODA ZA SILIRANJE U CILJU ODRŽIVE PREHRAMBENE PROIZVODNJE I PROIZVODNJE HRANE ZA ŽIVOTINJE

Rezime

Širom sveta proizvodnja prehrambene industrije je u daljem porastu; kao i količina otpada. Poslednjih godina, rastući troškovi odlaganja, ekološki propisi i svest stvorili su potrebu da se pronađu održive alternative za upravljanje otpadom. Postoje različite opcije da se bio otpad prehrambene industrije pretvori u proizvode sa dodatom vrednošću kao što su kompost, đubrivo, suplementi za poboljšanje zemljišta, biogas, medicinski proizvodi, stočna hrana i dr. U radu je prikazana primena bio otpada prehrambene industrije u vidu sirovina za siliranje i pripremu konzervisane hrane za ishranu životinja. Na ovaj način je bio otpad konvertovan u novi sporedni proizvod prehrambene industrije za pripremu visoko kvalitetne silaže .

Ključne reči: *bio otpad, sporedni proizvod, ishrana životinja, silaža*

**APPLICATION OF FOOD TECHNOLOGY
IN THE PRODUCTION OF HEALTHY
SAFE FOOD**



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Original Scientific paper

NEW TYPE OF COOKIE PRODUCT WITH DEHYDRATED PEACH

Abstract

Osmodehydrated and lyophilized peach is characterized by upgraded dehydration effectiveness and enriched chemical and mineral matter content, and as such, is an interesting material to be added to the cookies' formulation. Produced cookie samples with different levels of peach dehydrated by two different methods, were subjected to the nutritive and technology quality parameters testing. Obtained results showed that the addition of higher levels of dehydrated peach enhanced all nutritive, while simultaneously decreased most of the technological quality parameters. The optimal addition of osmodehydrated and lyophilized peach to the cookie formulation was determined to be 15%. Testing consumers' attitude showed high appreciation of cookies with dehydrated peach sensory characteristics and high positive attitude towards presented new product thus pointing at its' high potential for market acceptability.

Key words: *osmotic dehydration, lyophilization, sugar beet molasses, peach, cookies*

INTRODUCTION

Combinations of different material dehydration methods have provided numerous beneficial results. Combination methods of osmotic dehydration with lyophilization, for example, has several advantages. This combined method has the possibility to lower the application volume of a high energy-demanding process that gives excellent product quality (lyophilization) via application of a low energy requirements pre-treatment of osmotic dehydration (Ciurzynska et al., 2016; Igual et al., 2019; Filipović et al., 2022a).

Sugar beet molasses is characterized by high dry matter content and rich nutritive composition, and due to high driving force for the water removal during osmotic dehydration has the potential to be a good osmotic solution. Its specific chemical composition, sensory properties, low cost and reuse of by-products of different industry, presents molasses as a better choice than some other, usually used osmotic mediums (Lončar et al., 2021).

Cookies are very popular wheat-based product consumed in the whole world, due to their different flavours, affordable price, long shelf life and readiness to eat. In effort of health improvement and increase of conscience regarding diet and health interaction, cookies' formulation adjustment is needed (Blanco Canalis et al., 2020).

Cookies are a complex system where every ingredient has an essential purpose, and every change in standard formulation usually leads to cookies' dough changes that affect final product quality, hence every addition of new raw material to the cookies' formulation, requires precise testing and quality optimization (Blanco Canalis et al., 2017; Filipović et al. 2022b).

In this research, most of the results produced in scientific project titled: "Production and implementation of innovative product from domestic peach with improved sensory and nutritive properties" are systemized and elaborated in effort of presenting new type of cookie product supplemented with combinedly dehydrated peach.

PEACH COMBINED OSMOTIC DEHYDRATION AND LYOPHILIZATION PROCESS

In published papers: Filipović et al., 2022a; Filipović et al., in press, osmotic dehydration process performed under atmospheric pressure, at constant temperature of 20, 35 or 50 °C, during 1, 3 or 5 hours in molasses of 60, 70 or 80% concentration and successive lyophilization process of following parameters: pressure of the 1.6 Pa, condenser temperature of -57 °C and duration of the lyophilization of 2, 4, 5 or 6 hours, were described.

The obtained results from the research showed that all three osmotic dehydration parameters (process temperature and duration and molasses concentration) statistically significantly affected dry matter content and a_w values of combinedly dehydrated peach. Osmotic dehydration process, in combination with lyophilization, contributed to enhancing overall dehydration effectiveness, by increasing obtained dry matter content values of successive dehydration process, reducing time and energy consumption of high energy demanding single-stage lyophilization process. Exceptional peaches samples' a_w values reduction in lyophilization stage contributed to the synergetic dehydration method with samples' microbiological stability, obtaining dehydrated product of only 0.433 of a_w value. Dehydrated peach chemical content was preserved, while mineral matter content was highly enriched, due to molasses, as an osmotic solution, application. As the optimal parameters of combined osmotic dehydration and lyophilization process were determined: osmodehydration process temperature of 20 °C, during 5 hours in 80 % molasses concentration and 5-hour successive lyophilization process (Filipović et al., 2022a; Filipović et al., in press).

COOKIES WITH DEHYDRATED PEACH PRODUCTION

Cookies samples were produced with and without the addition of lyophilized and osmodehydrated and lyophilized peach at different levels of addition. In the effort of obtaining the same moisture content and comparability of different cookie dough while applying material of different moisture content, the quantity of distilled water addition was adjusted to every cookie sample with dehydrated peach formulation, providing the same level of dough moisture content through the investigated cookie samples.

On produced cookie samples chemical, mineral, total carotenoid and polyphenol content, antioxidative activity, technological quality, instrumental texture and colour and descriptive sensory analysis were performed (Filipović et al., 2022b).

QUALITY OF COOKIES WITH DEHYDRATED PEACH

The results of cookies with dehydrated peach addition quality testing and optimization showed that different dehydration methods had statistically significantly different impacts on the nutritive characteristics of cookies, where molasses application in the osmotic treatment stage of the combined dehydration method had a positive effect on cookies with dehydrated peaches' overall nutritive composition. Cookies with 10% of combinedly dehydrated peach addition nutritive quality indicates a significant increase in chemical, mineral, and phenol content, and antioxidative activity. Peaches' combined dehydration method positively influenced certain cookies' technological quality parameters in comparison to cookies with the same amount of lyophilization peaches addition. The results of the descriptive sensory analysis showed that with the addition of up to 10% of dehydrated peaches to the cookie formulation, there was a positive effect on all sensory responses, providing a favorable peach note to overall taste and flavor (Filipović et al., 2022b).

In table 1 results of the Z-score analysis of cookies, with and without the addition of lyophilized and osmodehydrated and lyophilized peach, are shown. Presented results show segment Z-score S1 - S6, which corresponds to Z-score results of chemical, mineral matter and phenolic compounds content, technological quality parameters, instrumental colour and descriptive sensory analysis responses, respectively. The addition of peach dehydrated by combined method led to the increase of segment Z-score values for all nutritive cookie characteristics, while segment Z-score values for technological cookie characteristics declined with the addition of dehydrated peach to the cookies' formulation, especially with the addition of peach dehydrated by the combined method. Total Z-score values, mathematically combine all segment Z-scores and point at the optimal combination of all tested cookies' nutritive and technological responses. The addition of dehydrated peach by combined method to the cookies formulation, produced an optimal combination of tested quality characteristics. The optimal addition of osmodehydrated and lyophilized peach to the cookies formulation was determined to be in the quantity of 15% (Filipović et al., 2022b).

Table 1. Z-score analysis of cookies with and without the addition of lyophilized and osmodehydrated and lyophilized peach (Filipović et al., 2022b)

Sample description and (number)	Segment Z-score						Total Z-score
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S
Control (1)	0.00	0.09	0.00	0.98	0.75	0.81	0.54
2.5% of lyophilized peach addition (2)	0.16	0.03	0.05	0.43	0.64	0.79	0.38
5% of lyophilized peach addition (3)	0.27	0.00	0.10	0.18	0.58	0.78	0.31
2.5% of lyophilized and osmo-dehydrated peach addition (4)	0.16	0.16	0.08	0.75	0.57	0.77	0.48
5% of lyophilized and osmo-dehydrated peach addition (5)	0.28	0.28	0.14	0.60	0.53	0.60	0.45
10% of lyophilized and osmo-dehydrated peach addition (6)	0.49	0.49	0.22	0.52	0.47	0.49	0.47
15% of lyophilized and osmo-dehydrated peach addition (7)	0.71	0.65	0.45	0.44	0.39	0.31	0.49
20% of lyophilized and osmo-dehydrated peach addition (8)	0.84	0.83	0.63	0.27	0.34	0.13	0.46
25% of lyophilized and osmo-dehydrated peach addition (9)	1.00	1.00	1.00	0.04	0.25	0.00	0.45

CONSUMERS' TEST OF NEW COOKIE PRODUCT WITH DEHYDRATED PEACH

Cookie product with lyophilized and osmodehydrated peach, of experimentally determinated optimal formulation (quantity of dehydrated peach addition of 15%), was produced in higher quantities, in effort of conducting consumers' test and evaluating acceptability of new type of cookie product on large scale of unspecified consumers (576 randomly selected consumers of different sociodemographic characteristics).

Table 2 shows 3 general and 5 specific questions (Filipović et al., 2016), that consumers were asked on the conducted survey.

Table 2. Consumers' survey questions

Question type	Question no.	Question
General questions	Q1	Do you read declarations on cookies' packaging? (Answer: YES/NO)
	Q2	Does your health condition require special diet? (Answer: YES/NO)
	Q3	Based on your opinion, does the food affect health condition? (Answer: YES/NO)
Specific questions	Q4	Is the cookies' with dehydrated peach colour acceptable? (Score from 1 to 9, where 9 is the highest score)
	Q5	Is the cookies' with dehydrated peach taste acceptable? (Score from 1 to 9, where 9 is the highest score)
	Q6	Is the cookies' with dehydrated peach smell acceptable? (Score from 1 to 9, where 9 is the highest score)
	Q7	Is the price of cookies' with dehydrated peach significant issue in buying decision? (Score from 1 to 9, where 9 is the highest score)
	Q8	Would you buy cookie with dehydrated peach and implement it in your diet? (Score from 1 to 9, where 9 is the highest score)

Table 3 shows results of the consumers' answers to the conducted survey, from which it can be seen that with the age, level of education and income increase, percent of the positive answers to the reading of the declarations, increased also. Younger consumers were not exposed to the health issues that would require special diet, which changed with the age increase. In general population, there is a low percent of consumers whose health condition requires special diet – 18.90%. Large share of consumers in all groups positively answered the question regarding effect of the food on health condition.

Answers to the specific questions regarding acceptability of colour, taste and smell of the cookies with dehydrated peach were very high (close to maximal value of 9). Cookies colour was highly evaluated by all consumers, while some lower scores were marked at middleaged consumers, with high level of education and with the highest income level. Taste and smell were better scored at older and higher educated and population with the highest income. Consumers' answers to the questions from Q4 to Q6 indicate to highly appreciated sensory characteristics of cookies with dehydrated peach.

Cookie price was marked with significantly high scores and evaluated as important buying parameter, especially at younger population.

Answers to the last question, would consumers buy cookies with dehydrated peach and implement it in their diet, showed high level of positive attitude towards presented new product and indicated on high potential for marked acceptability of newly developed cookie type with dehydrated peach.

Table 3. Consumers' answers to the survey questions

		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
		Positive answers percent (%)				Consumers average score			
Consumers age	<18	15.34	0.00	84.61	8.89	7.72	8.86	6.43	3.86
	18-30	63.76	21.80	100.00	8.91	7.70	8.74	4.63	5.40
	31-50	74.70	17.96	100.00	8.20	8.50	8.52	4.82	6.91
	>50	82.32	16.07	96.82	8.74	8.49	7.50	5.75	5.50
Education level	Elementary	41.99	15.00	100.00	8.83	7.71	8.91	6.42	5.14
	Medium	65.11	18.05	100.00	8.97	7.85	8.31	5.31	5.77
	Higher	87.17	20.09	85.57	8.91	8.39	7.20	6.60	4.80
	High	74.43	18.91	100.00	8.38	8.63	8.51	4.30	6.66
Income level	Low	48.00	10.10	100.00	8.79	7.49	8.39	5.10	4.50
	Medium	79.53	29.49	97.02	8.65	8.29	8.12	5.83	6.88
	Higher	80.10	10.50	88.51	8.55	8.77	7.62	4.50	6.30
	High	77.20	14.39	100.00	8.31	8.97	8.37	4.16	6.23
Sex	Male	68.14	17.08	96.71	8.37	8.37	8.30	4.60	5.86
	Female	62.55	19.60	99.01	8.80	8.30	8.29	5.24	6.23
Total in all groups		64.53	18.90	98.15	8.61	8.33	8.32	5.04	6.04

CONCLUSION

From presented results it can be concluded that:

- Osmotic dehydration process, as pretreatment to lyophilization, increased total dehydration efficiency, reducing duration and energy consumption in high energy demanding lyophilization process.
- Due to molasses, as an osmotic solution application, antioxidative activity, chemical and mineral matter content of combinedly dehydrated peach was increased.
- Cookies with 10% addition of dehydrated peach were nutritively enriched, with significant increase chemical, mineral and polyphenol content and antioxidative activity.
- Some technological quality parameters were enhanced by using combinedly dehydrated peach in comparison to lyophilized peach.
- The optimal addition of osmodehydrated and lyophilized peach to the cookies formulation was determined to be in the quantity of 15%.
- Results of consumers' test showed high appreciation of cookies with dehydrated peach sensory characteristics by large group (576) of randomly selected consumers.
- Consumers showed high positive attitude towards presented new product and indicated on its' high potential for market acceptability.

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НОВА ВРСТА КЕКС ПРОИЗВОДА СА ДЕХИДИРАНОМ БРЕСКВОМ

Резиме

Осмотски дехидрирану и лиофилизовану брескву карактерише унапређена ефикасност дехидратације и обогаћен хемијски и минерални садржај, и као таква, интересантан је додатак у сировинском саставу кекса. Испитани су нутритивни и технолошки параметри квалитета произведених узорака кекса са различитим нивоима брескве дехидриране помоћу два метода. Добијени резултати су показали да је додатак виших нивоа дехидриране брескве унапредио све нутритивне, док је истовремено смањио већину технолошких параметара. За оптималну количину додатка осмотски дехидриране и лиофилизоване брескве у сировински састав кекса одређена је количина од 15%. Испитивање ставова потрошача је показало високу прихватљивост сензорних карактеристика кекса са дехидрираном бресквом и високу прихватљивост нове врсте кекс производа и указало је на његов висок тржишни потенцијал.

Кључне речи: *осмотска дехидратација, лиофилизација, меласа шећерне репе, бресква, кекс*



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Original Scientific paper

HOME BREWING OF CRAFT BEER

Abstract

Craft beer has been considered an alternative to industrial beers for years. One of the main reasons lies in the relatively simple and fast production process as well as the desire for unique tastes and all-around personalization of the beer consumption experience. One of the reasons is the relatively cheap production of craft beer in limited quantities. Water, barley, hops and yeast are used as basic ingredients for making craft beer. Increased infrastructure, already familiar technology, marketing and availability of the same have led to the fact that craft beer is regularly consumed and the price of the packages - brewing kits themselves for production has become cheaper. This paper tried to investigate the possibility of producing craft beer at home.

Key words: *craft beer, wheat beer, hops, yeast, fermentation*

INTRODUCTION

Beer is a refreshing drink made by alcoholic fermentation from an aqueous extract of sweetened barley with hops. Water, barley, hops and yeast are used to make craft beer. The quality of all raw materials has a decisive influence, often even crucial, on the quality of the finished product i.e. beer (Grujić et al., 2000). The nutritional value of beer is great. It is rich in nutrients, carbohydrates, amino acids, minerals, vitamins and phenolic compounds (Gerhäuser, 2005). The healing properties of beer have been confirmed in many works. Beer has a beneficial effect on the psyche, thus encouraging good mood, relaxing and reducing stress (The Brewers of Europe, 2008). Craft beer has become very popular in Serbia a few years ago, and its appeal continues to grow despite expectations. Namely, the demand for craft beers, which are specific in that they are

made in small, independent breweries where the production is individualized and specialized has increased in the world. Craft beers offer a different experience and atmosphere than traditional industrial beers because of the way they are made. However, this also brings higher production costs per unit, as well as the price that people are increasingly willing to pay in order to enjoy the original taste and unique experience. However, the whole story of beer began in America a long time ago, where individual producers began to slowly break into the market and where today they have a significant share.

HISTORY OF CRAFT BEER PRODUCTION

Craft beer was slowly being introduced as an alternative to traditional, industrial beer in America, so in the last decade of the 20th century, it began to gain popularity by taking market share from large American and international breweries. Until 1971 in the United States, the market was held by a few large companies which did not make high-quality beer due to a huge volume of production. In the same year, small craft brewing which would produce quality beer as in the past gained attraction (Glover, 1999). According to Murray and O'Neill, "the number of small specialty breweries in the United States has increased dramatically since 1980, and this change is related to the signing of President Jimmy Carter's 1976 law that allows domestic beer production nationally." Since 1997 American breweries surpassed for the first time the number of German ones, a nation that still enjoys the strongest brewing tradition and the highest per capita beer consumption in the world (Carroll and Swaminathan, 1992). Adhering to established recipes, creativity and inserting a dose of innovation, they laid the foundations of craft beer production and continued to step forward. Although the COVID-19 pandemic affected the sale and consumption of beer in general and craft beer in particular, its use still occupies a decent part of the market. The primary reason for the larger decline in sales was the transition from the sale of beer in bars and restaurants to the sale of packaging in stores, and this advantaged large producers who were already in this market. Today, it is almost impossible to find a city in America that does not serve "domestic" craft beer, while places that offer only their own beers can easily fall victim to the same market trap of limited variety of offers that has upset large breweries. One example of this is a recent Budweiser ad that reminded the public that the company's roots were originally from a microbrewery and it provides strong anecdotal evidence that even the largest producers are feeling the effects of growing market share in the craft beer industry. Although it has suffered a significant decline in the past two years, this industry has seen undisputed growth in the last 10 years or so. The constantly growing percentage of market share and sales gives strong support to the idea that microbreweries start the market, and artisan beer lovers can really be seen as important and attractive consumers in the market. When we think of innovation, authenticity, phenomenal aroma, and unforgettable taste - that's exactly what craft beer is about. Today, Serbia can boast of many producers who open the first independent small breweries where magic is created, contributing daily to the production of craft beer, as well as its promotion.

GERMAN WHEAT BEER (HEFEWEIZEN)

When it comes to the consumption of craft beer in the world, most consumers prefer IPA (India pale ale), while lagers are represented in a smaller percentage. German wheat beer is no exception, so it is generally more attractive to people who come from Europe or those who like a stronger taste of beer. American beers generally have a milder taste than European ones. Most people seem to either like or dislike German wheat beers, and their opinions are usually based on previous experiences with them. Those who like German wheat beers including Hefeweizen probably had the opportunity to try a sample with the appropriate level of esters and phenols obtained by fermentation. Those who do not like wheat beers have probably tried some of the poor quality, where the character of the yeast was domineering or completely out of balance. In the end, it all comes down to individual preferences. Jaime Hurado says that wheat beers have spread in the American world of specialized brewing. According to him, every brewery and practically every microbrewery has wheat beer of some style, whether it is fermented with domestic yeast or a special Bavarian or Flemish yeast used for wheat beer. According to Picarieli et al., wheat beer is a traditional light top-fermented beer brewed with at least 50% malt (e.g. German wheat beer) or no malt (e.g. Belgian Witbier) wheat (*Triticum aestivum*) as an additive to barley malt (*Hordeum vulgare*). Hefeweizen is a traditional Bavarian wheat beer brewed with at least 50% wheat malt and yeast that give the product a distinctive taste of cloves and bananas. The very low content of hops allows the fundamental taste of wheat to dominate the product. Hefeweizen is a light, pleasant beer with a hazy look and soft texture. It has a high level of protein and yeast in the solution that makes Hefeweizen a cloudy or at least foggy beer. Although most breweries are familiar with the murky German Hefeweizen, there is also a filtered, clear version called Kristallweizen. Hefeweizen can be a variety of colors from pale yellow to dark gold and has a large, thick and creamy white foam. The balance between bitterness and sweetness in these beers is usually balanced, where none dominates although some beers may have increased sweetness. E. Schulz states that there is a difference between two types of wheat beer: Hefeweizen and Kristallweizen. This second type no longer contains yeast and by filtering the beer it gets a nice shine. Hefeweizen differs in the yeast cells left in the beer after bottling and has approximately 1 million cells/ml while wheat cloudy beer has up to 10 million cells/ml.

TECHNOLOGICAL PROCESS OF CRAFT BEER PRODUCTION

Bavarian Hefeweizen TrueBrew™ Ingredient Kit, a Bavarian wheat beer kit from the American producer TruBru was used for this test. The description of this kit states that this is a "real Bavarian treat". The website <https://bsghandcraft.com/bavarian-hefeweizen-truebrew-ingredient-kit> also states that this beer should be served in a tall glass with a slice of orange. Also, to feel the traditional blurry look of beer, it should be served *mit Hefe* ("with yeast") by swirling the bottle with a little remaining beer in it to make sure you get all the remaining yeast. The production of this beer fully

followed the procedure described on the manufacturer's website <https://bsghandcraft.com/bavarian-hefeweizen-truebrew-ingredient-kit>, and the ingredients used were:

Malt: 2 packs of 3.3 pounds (1.49 kg) of liquid wheat malt extract

Yeast: 11.5 g of wheat yeast Fermentis SafAle WB-06

Hops and aromas: 1 oz (29.57 ml) of German Hallertau Mittelfrüh hops

150 g of fermenting sugar was used as an additional ingredient



Picture 1: TruBru™ kit of Bavarian wheat beer

Preparation

Before starting the process of making beer, it was important to check that everything from the equipment was ready. The equipment used for this experiment Bavarian Hefeweizen TrueBrew™ Ingredient Kit contained almost all of the necessary ingredients. Additional equipment included an industrial thermometer, disinfectant Io Star Sanitizer and an aichose optical refractometer model COMINHKPR124469. A set of 5-gallon home brewing equipment (18.92 liters) including disinfection, fermentation and bottling equipment as well as a cooking pot with a capacity of at least 3.5 gallons (13.24 liters) for malting and hops with water were prepared and set in place. Proper access to running water and the burner or stove was checked before starting the process, as well as finding the cold and dark place for storing the fermenter. Approximately two packages of 24 empty beer bottles were prepared in advance. Unlike in Europe, where the most common beer bottle size is that of a 500 ml, people in America use 12 ounce (354.88 ml) bottles as in this case. 16 (473.17 ml) ounce bottles are used less

often, and they are also equated to one pint (A pint is a unit of measures which is used primarily in the United States, Great Britain, and Ireland).

Brewing

The process of brewing beer is the first and extremely important step in the process of obtaining craft beer because as in other similar processes, too much temperature or lack of mixing can later greatly affect the quality of beer or lead to spoilage. The cooking pot was filled with approximately 2.5 gallons (9.46 liters) of water and heated.

Two packs of liquid wheat malt extract were added to water heated to 160°F (71.11°C). According to the manufacturer's recommendation, special care was taken to add a small amount of malt to the hot water in the cooking pot and stir at the same time until the malt dissolved. The mixture of malt and water was gently heated to boiling point and left to boil for 15 minutes. When 15 minutes had elapsed, 1 ounce of German Hallertau Mittelfrüh hops was added to the cooking pot and cooking continued for the next 45 minutes at a moderate temperature with regular stirring. After 45 minutes, the fire was turned off and the boiling was over.

Cooling

A mixture of malt, hops and water called wort can be cooled with a wort cooler or by placing a covered cooking pot in an ice bath. In this case, an ice bath was used until the pot was no longer warm to the touch. The recommended maximum temperature at which yeast can operate is 80 °F (26.67 °C). In this case, the temperature was measured with an industrial temperature gauge and when it reached that value, the cooled wort was poured into a disinfected fermenter, and water was added to a final volume of 5 gallons. Then we started sampling and measuring the initial density of beer, which was 1.054, which confirmed that the brewing process was successfully completed because the value is within the normal range for this beer, which is 1.047 – 1.056 g / cm³ (11.7 - 13.8 ° P).



Picture 2: Beer brewing and cooling

Fermentation

When the mixture has cooled down to the needed temperature, the yeast seeding process started. Thus, the cooling process ended and the most important part of the production of craft beer, fermentation, began. The packaging of Fermentis SafAle WB-06 wheat yeast was carefully opened and the contents were sprinkled on the surface of the wort. The fermenter was then hermetically sealed and moved to a cool, dark place at approximately 70°F. Due to the high temperatures in Las Vegas, the fermenter was placed near an air conditioning source.

The yeast began to ferment the wort, converting malt sugars into alcohol and CO₂. This process usually begins within 24-48 hours of brewing and ends in about 3-7 days. Fermentation was measured until the 10th day when it was already certain that it was nearing the end.

The result – Craft beer

When the primary fermentation was completed after 14 days, the final phase of making craft beer began. Bottling of beer resulted in secondary fermentation in which carbonation was obtained and the final taste formed. Bottling equipment consisted of household utensils which were disinfected using the same iodine solution used in the disinfection process before primary fermentation. Two packs of 48 12-ounce bottles were prepared for bottling, as well as enough caps for closing.

The prepared carbonation solution was obtained by mixing 150 g of carbonation sugar with 1 liter of boiling water. This filling solution was then mixed with fermented beer. In this part of the craft beer production process, it was very important to pay attention to the cleanliness of all devices. The bottles were filled with beer and sealed. They were then transferred back to a cool, dark place at 70°F where they remained for the next two weeks to carbonize. The recommended time frame for carbonation of this



Picture 3: Bottling of beer

type of beer is from 10 to 14 days. After 28 days or 4 weeks from the primary fermentation, tasting and measurement of the final density of beer were conducted. One of the cooled bottles was opened and the final density was measured using the same apparatus used to measure the initial density. The final density was 1.015, which is in the range for this type of beer 1.008 – 1.016 g / cm³ (2.1 - 4.1°P).

The emergence of kraft beer coincides with the strengthening of consumer awareness of the importance of quality and pure original beer ingredients. Apart from quality and aroma, craft brewing differs from conventional beer-making in terms of visual appearance on the market. Interesting forms of packaging, illustrations, or some striking detail on the packaging can pique the interest of the customer and attract their attention (Meler, 2005). Special attention is paid to the design of the label, so consumers have the opportunity to enjoy a modern and unique design, and regarding bottles, in addition to the standard bottle sizes, 25.36 ounces (0.75 liters) bottles are also used (<https://www.ugostiteljstvo.com/zanimljivosti/craft-pivarstvo>). Although it is considered to be the least favorite color in the world, brown is still often used in marketing and is believed to help create a healthy feeling, connection with the ground, as well as a sense of order and conventionality. It is often used in food packaging because it can indicate that the food is natural (<http://www.artitudesdesign.com>). Analyzing (bottle design), a large number of respondents (48.9%) found that the aesthetic design of products has a great influence on the purchase of craft beer (Kelin, 2018).

CONCLUSION

Craft beers are becoming universally recognizable and more people appreciate them. One of the most important differences between craft and industrial beer is in the way it is prepared. The word "craft" itself is close to the word "skill", so we can freely say that craft brewing is a skill of making beer. Unlike industrial, craft beer is made in a way that the yeast stimulates fermentation at the top, the so-called top fermentation, at higher temperatures. This kind of fermentation does not end with the complete conversion of sugar into alcohol, so such beers are sweeter, thicker, fuller in taste and generally of better quality. During the production of this type of beer, brewers are also allowed to add ingredients of their choice when making beer, which will achieve a unique color and taste and will make the beer a unique product. The main task for the largest beer producers on the world market in the future will be to increase competitiveness due to the existence and expansion of numerous small (micro) breweries covering local markets around the world, focused on quality, specialties and individuality, such as craft breweries. Brewing beer seems like a complicated process, but it is very simple as long as the rules are followed. The advice when buying the kit was to pay special attention to the cleanliness of the dishes, which, as with most similar products, is the most important part of the process. Inadequate and unclean equipment can very easily lead to spoilage or altered taste of beer, which is why the manufacturers of these kits and sellers insist on buying quality iodine-based sanitizers. The specific climatic conditions in Las Vegas have been taken into account in the preparation of this

beer, and special attention has been paid to keeping the beer at the required temperature, light, and humidity during the fermentation and storage process. The bottling job was facilitated by great equipment and good organization. The beer itself was rated by the examiners as being of good quality. Special praise was given to the foam that lasted longer than prescribed and the taste that was described as stronger than that of commercial beers of the same or similar type. Taking into account this data, we believe that the preparation of this beer was successful and that there is potential for further individual and commercial production.

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Originalni naučni rad

PROIZVODNJA ZANATSKOG PIVA U KUĆNIM USLOVIMA

Abstrakt

Zanatsko ili kraft pivo se već godinama spominje kao alternativa industrijskim pivima. Jedan od glavnih razloga leži u relativno prostom i brzom procesu proizvodnje kao i izraženom željom za jedinstvenim ukusima i svejevrnom personalizacijom iskustva konzumiranja piva. Kao jedan od razloga se spominje i relativno jeftina proizvodnja zanatskog piva u ograničenim količinama. Za pravljenje zanatskog piva kao osnovni sastojci koriste se voda, ječam, hmelj i kvasac. Povećana infrastruktura, već poznata tehnologija, marketing i dostupnost istih su doveli do toga da se zanatsko pivo redovno konzumira a i cena samih paketa-kitova za proizvodnju je pojeftinila. Ovaj rad je pokušao da istraži mogućnost proizvodnje zanatskog piva u kućnim uslovima.

Ključne reči: *zanatsko pivo, pšenično pivo, hmelj, kvasac, fermentacija*

**ECONOMIC STANDPOINT OF HEALTHY
SAFE FOOD PRODUCTION
AND MARKETING**



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Original Scientific paper

COOPERATION OF FORENSIC ACCOUNTING AND FORENSIC AUDIT IN FOOD PRODUCTION COMPANIES

Abstract

Independent forensic accounting in food manufacturing companies essentially collaborates with independent forensic auditing. This is done continuously and should be seen as an essential link from which real improvement of all work processes can result. It is of great importance for manufacturing companies. Positive effects in companies that have introduced a realistic connection between forensic accounting and forensic auditing are essentially realized in the short term after implementation. Forensic accounting and forensic auditing introduced by the top management essentially contribute to the security of top management's financial reporting, because such reports are based on research in the company itself, on the basis of documents that exist in the company and are found in the company's accounting and finances. In this way, the business of the entire company improves overall, there is greater security in business. As the last effect of those activities, it can be pointed out that the company's operations become more economical. Essentially, after such an implementation, making management decisions is safer and more realistic.

Keywords: *forensic accounting and auditing, management, top management reporting*

INTRODUCTION

Forensic accounting in the company has continuous cooperation with other auditors and subjects:

- Internal auditors,
- External auditors,
- Forensic auditors,
- State authorities (tax),
- To banks,
- Appraisal houses and others.

Forensic accounting and forensic auditing are hired by the company's top management to perform certain tasks in the company or by state authorities, banks, etc. and all with the aim of detecting fraud and prosecuting it [1-5].

The forensic audit introduced by a company, and especially by a food production company, represents a special audit service, and the subject of interest of forensic auditors is financial statements, which are based on the application of accounting and auditing skills in the form of targeted, focused and detailed audit procedures, in order to available documentation revealed fraud in the company itself [6-10].

Therefore, a forensic audit should reveal illegalities, primarily in financial statements. The presentation of the functional cooperation of forensic accounting and forensic auditing should be observed especially in companies engaged in food production, because there is objectively a great responsibility in them [11-12].

COOPERATION OF FORENSIC ACCOUNTING AND FORENSIC AUDITING IN FOOD PRODUCTION COMPANIES

Presentation of the functional cooperation of forensic accounting and forensic auditing it is given in the form of a display in Figure1.

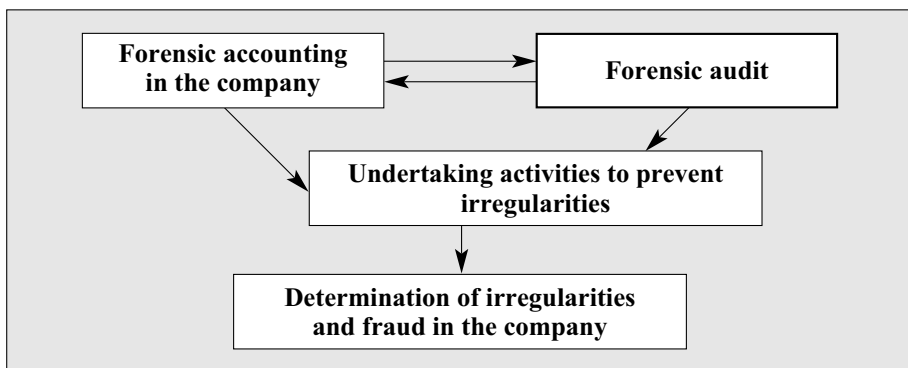


Figure 1. Presentation of the functional cooperation of forensic accounting and forensic auditing Source: Authors (2022).

MODELS RELATED TO FINANCIAL REPORTING OF TOP MANAGEMENT IN COMPANIES ENGAGED IN FOOD PRODUCTION

The authors presented the possible financial reporting primarily intended for the top management of the company, and the presentation itself is given in the form of the presentation of Figure 2, where they presented the most important driving incentives for compiling realistic financial reports in the company's operations, which can be used by the top management.

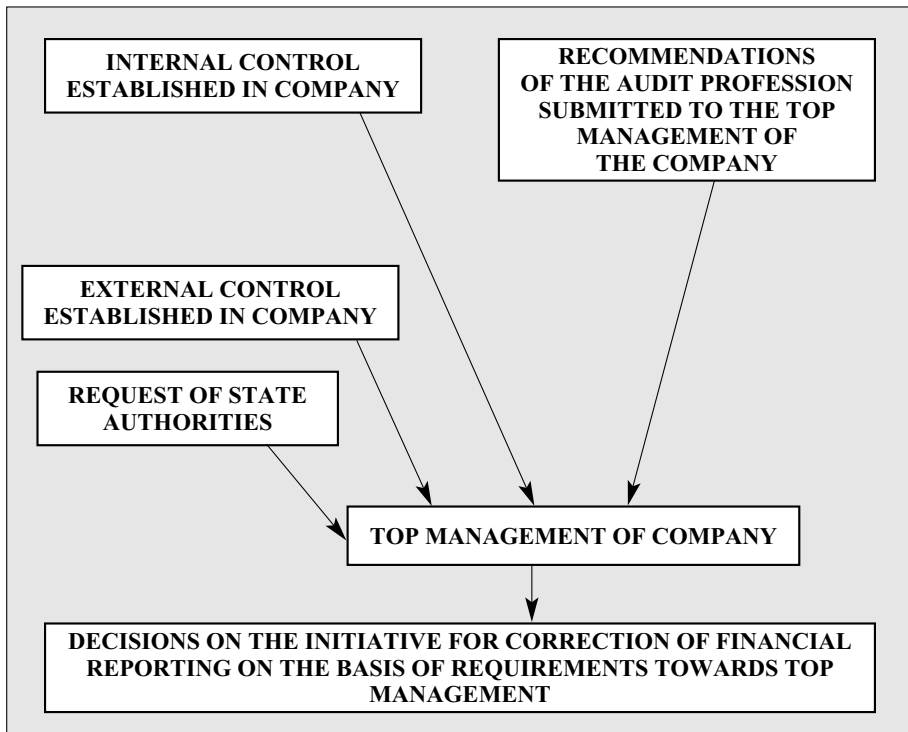


Figure 2: A model based on the initiative of entities to improve financial reporting in the company Source: Authors (2022).

AN INITIATIVE TO CHANGE FINANCIAL REPORTING IN COMPANIES ENGAGED IN FOOD PRODUCTION

The initiative to change financial reporting in companies engaged in food production can come not only from internal audit but also from other bodies in companies. Forensics can also be a driver, and an overview of possible activities is given in Table 1.

Table 1: Application of presentation and standard presentation in the process of reporting to the top management of the company

PRESENTATION METHOD	ADVANTAGES	DEFICIENCY	NOTES ON THE APPLICATION OF REPORTING TO THE TOP MANAGEMENT ARE GIVEN IN WORDS IN ORDER OF IMPORTANCE
<i>PRESENTATION</i>	Interactive Flexible Easier acceptance Helps to consider difficult questions and solutions May increase the chance of enforcement Contributes to focusing on priority issues	Not all evidence can be presented It can lead to difficulties in presenting complex data which can lead to misunderstandings Presentation skills and active involvement of two people are required Good preparation is necessary Possibility of dominance of one person or a particular problem The user can still request a report	GREAT INFLUENCE ON THE BUSINESS OF THE COMPANY
<i>STANDARD REPORT</i>	Good for detailed reporting and complex data It can provide general information and context The evidence is immediately available to the reader	They require more preparation time Long reports can remain unread until the end Sometimes hard to accept They may be untimely presented due to delays in preparation	MEDIUM IMPACT ON THE BUSINESS OF THE COMPANY

CONCLUSIONS

Forensic accounting and forensic audit in a company engaged in food production, which has introduced forensics into regular operations, realizes a whole series of activities and continuous cooperation with other entities that are of vital importance for the functioning of the entire company. It should be pointed out that forensics cooperates with auditors (internal, external, forensic experts engaged on a specific issue by companies or state authorities, banks, etc.). All activities of forensic accounting and forensic auditing are carried out with all parts or sectors in the company. It is an activity that

requires the coordination of all parts of the company, but above all the activities that are carried out within the framework of obtaining documentation in the financial and accounting sector.

The goal of introducing forensics is the systematic detection of fraud in the company, which is of great importance for the survival of a company engaged in food production. Frauds are most often committed by company employees. Detection of fraud is done after receiving the documentation, but also by processing it.

Forensic audit is a special audit service, which is related to forensic accounting, as pointed out by the authors of the paper. This cooperation is based on the financial reports obtained from the company, which are based on the application of accounting and auditing knowledge of people who perform forensic work.

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Оригинални научни рад

САРАДЊА ФОРЕНЗИЧКОГ РАЧУНОВОДСТВА И ФОРЕНЗИЧКЕ РЕВИЗИЈЕ У КОМПАНИЈАМА ЗА ПРОИЗВОДЊУ ХРАНЕ

Сажетак

Независно форензичко рачуноводство у компанијама које се баве производњом хране суштински сарађује са независним форензичком ревизијом. То се врши континуирано и треба га посматрати као суштинску везу из које може произаћи реално побољшање свих процеса рада. То је од велике важности за производне компаније. Позитивни ефекти у компанијама које су увеле реално успостављање везе између форензичког рачуноводства и форензичке ревизије суштински се остварују у кратком року након имплементације. Форензичко рачуноводство и форензичка ревизија које су увели у пословање врх топ менаџмента суштински доприноси сигурност финансијског извјештавања топ менаџмента, јер су такви извјештаји засновани на истраживањима у самој компанији, на основу докумената који постоје у компанији и који се налазе у рачуноводству и финансијама компаније. На тај начин пословање целе компаније се укупно побољшава, већа је сигурност у пословању. Као последњи ефекат тих активности може се истаћи да пословање компаније бива и економичније. Суштински након такве имплементације доношења управљачких одлука, је сигурније и реалније.

Кључне речи: *форензичко рачуноводство и ревизија, управљање, извештавање топ менаџмента*



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Original Scientific paper

MACRO-ECONOMIC RESPECT FOR HETEROGENEOUS ENVIRONMENTAL FACTORS IN COMPANIES ENGAGED IN THE PRODUCTION OF HEALTHY SAFE FOOD

Abstract

Macroeconomic consideration of numerous heterogeneous environmental factors that affect the work of companies that are mainly engaged in the production of safe food requires the top management in these companies to introduce in its analysis a whole range of new ways of observing these factors. This is important for the real business of the mentioned companies. Respect for the economic environment of companies is of great importance for the work and functioning of companies engaged in responsible social production. As a rule, the results of their activities can affect a large number of people, especially from the health safety aspect, which can have implications for the whole society in one economy. One of the modern approaches to doing business in such companies is the analysis of the economic environment of companies engaged in the production of safe food within their overall business. The goal of conducting such activities is to raise the level of realistic perception of the impact of the environment on the possible business of companies, especially within the business of companies that are predominantly engaged in the production of safe food.

Keywords: *health safe food production, company environment*

INTRODUCTION

Top management in companies that produce healthy food has a constant need to find new ways to improve the overall management of the companies in which it performs management functions.

One of the ways in which the level of improvement of the overall business in the work of the mentioned companies could be raised is the management that will clearly adopt the observation of the economic environment of the company.

This is of special importance for companies engaged mainly in the production of safe food, where it is necessary to achieve a high level of safety in food production (Chen, 2019; Popović, 2014; Popović et al., 2017; Novaković et al., 2018).

In companies that produce health-safe food, safety can be achieved based on the application of realistic reports within the companies themselves (Popović et al., 2015; Popović et al., 2018, Popović et al., 2020, Radović et al., 2019; Vitomir et al., 2019).

However, it should be noted that security in the operation of the company is possible both by applying and respecting the real economic environment of the company (Tomas-Miskin, S., 2021; Tomas-Miskin, S., 2021; Tomas-Miskin, S., 2021).

Practical improvement of the overall business in these companies can be defined as a significant contribution of authors who expressed their views in emphasizing the importance of respecting the economic respect of the environment of companies in companies engaged mainly in food production. food, especially when it comes to healthy food.

MACROECONOMIC IMPACTS ON THE BUSINESS OF HEALTH FOOD COMPANIES

It is reflected in the existence of a strong macroeconomic influence of state bodies on economic trends, which may have implications for the work of many companies engaged in food production.

The representations are given in the form of the representations in Figure 1-2.

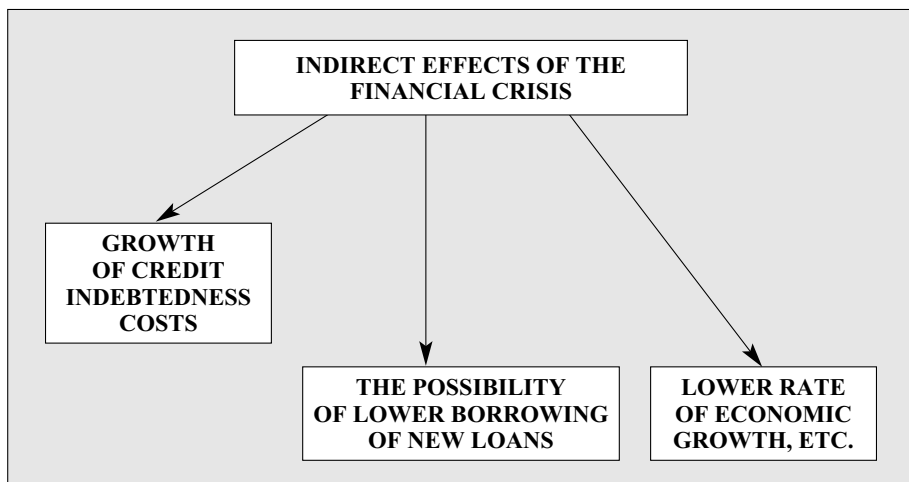


Figure 1: Overview of indirect effects of the financial crisis in small countries

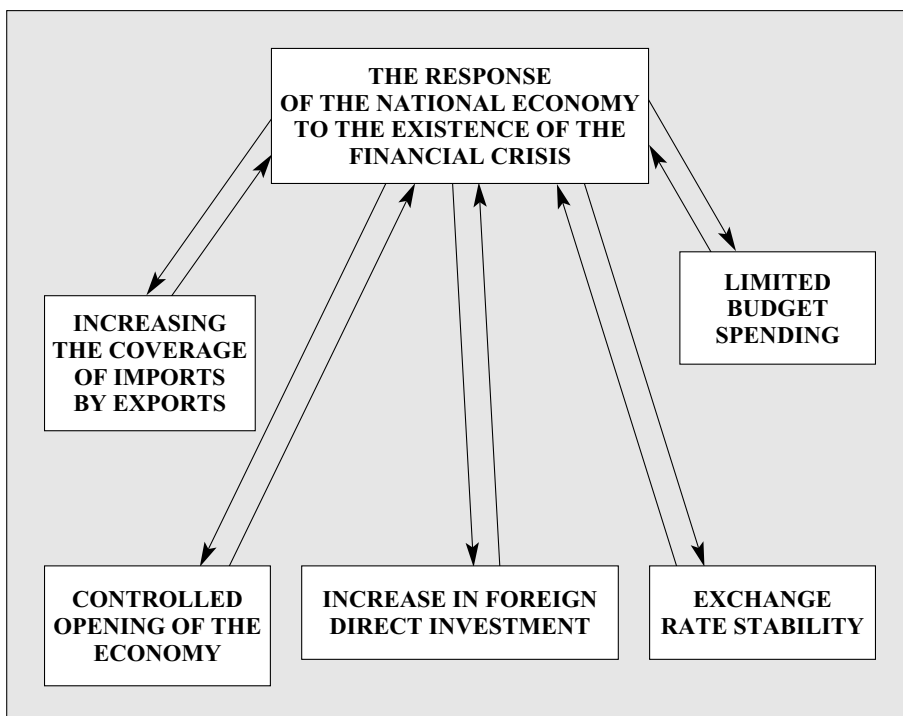


Figure 2: Overview of indirect effects of the financial crisis in small countries

The existence of the economic environment in the Republic of Serbia is of great importance for the real business of companies, i.e. companies should respect the existence of an environment that affects the possible business of companies.

The research was conducted in the Republic of Serbia, which essentially seeks to apply positive experiences, and this paper focuses on the existence of an economic environment towards companies, emphasizing that these companies should be viewed through the prism of food companies, especially in safe food.

In this practical presentation of the importance of the analysis of the economic environment from the point of view of macroeconomic observation of the impact on the company's business.

GENERAL MODEL OF THE IMPACT OF THE ECONOMIC ENVIRONMENT ON THE WORK OF COMPANIES OPERATING IN THE FIELD OF HEALTHY FOOD SAFETY

The general model of the impact of the economic environment on the work of companies operating in the field of healthy safe food can be shown in Figure 3, which shows the impact of the macro-economic environment on the business of companies.

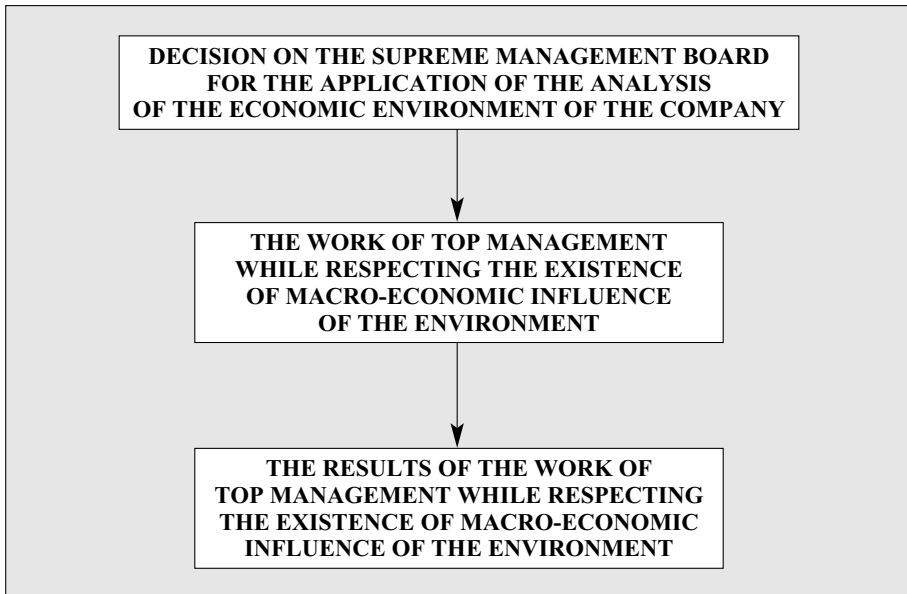


Figure 3. General model of the impact of the economic environment on the work of companies operating in the field of healthy food safety

Based on the presentation of the general model, which shows the real respect for the analysis of the economic environment at work and business of companies that are mainly engaged in the production of safe food, it can be seen that there are the following important characteristics:

1. The connection of numerous environmental factors to the work of companies,
2. The connection of economic factors of the environment to the work of companies,
3. Relationship between the influence of state bodies from the economic environment as a factor influencing the business of companies that produce safe food,
4. The decision and the will to implement the decision of the top management on the application of continuous analysis of the economic environment that may have an impact on the regular operations of the company, in order to achieve significantly better overall business results.

CONCLUSIONS

The processes of actually perceiving the impact of the economic environment on the business of a large number of companies cannot bypass companies that produce health-safe food. The application of the analysis of the macroeconomic environment to the work of companies engaged in production and organization of production is of great importance for the company's operations, especially companies that produce safe food.

The application of business principles that will take into account the analysis of the environment is just one of the ways in which it is possible to improve production in companies that produce healthy food.

One of the examples of positive application in business is the application of environmental analysis, namely the analysis of the macro-economic environment to the real business of many companies that produce food, especially healthy food.

The authors came to these observations by observing the work and business of companies in the field of health safe food production, which began to apply the analysis of the macro-economic environment to the real business of many food companies, especially healthy food.

In essence, the application of realistic analyzes of the macroeconomic environment to the real business of many food companies, especially healthy food, can serve as a method and way to realistically improve and raise the general level of business security in health food companies.

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MAKRO-EKONOMSKO UVAŽAVANJE HETEROGENIH FAKTORA OKRUŽENJA U KOMPANIJAMA KOJE SE BAVE PROIZVODNJOM ZDRAVSTVENO BEZBEDNE HRANE

Sažetak

Makro-ekonomsko uvažavanje brojnih heterogenih faktora okruženja koje je od uticaja na rad kompanijama koje se pretežno bave proizvodnjom zdravstveno bezbedne hrane zahteva od top menadžmenta u pomenutim kompanijama da uvede u svoju analizu čitav niz novih načina posmatranja pomenutih faktora. To je od važnosti za realno poslovanje pomenutih kompanija. Uvažavanje ekonomskog okruženja kompanija je od velike važnosti za rad i funkcionisanje kompanija koje se bave odgovornom društvenom proizvodnjom. Rezultati njihovih aktivnosti po pravilu mogu da utiču na veliki broj ljudi, pogotovo sa zdravstveno bezbednog aspekta, što može da ima implikacije na celokupno društvo u jednoj ekonomiji. Jedan od savremenih pristupa poslovanja u takvim kompanija je analiza ekonomskog okruženja kompanija koje se bave proizvodnjom zdravstveno bezbedne hrane u okviru svog ukupnog poslovanja. Cilj sprovođenja takvih aktivnosti je podizanje nivoa realnog sagledavanja uticaja okruženja na moguće poslovanje kompanija, pogotovo u okviru poslovanja kompanija koje se bave dominantno proizvodnjom zdravstveno bezbedne hrane.

Ključne reči: *proizvodnja zdravstveno bezbedne hrane, okruženje kompanije*



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Original Scientific paper

INDEPENDENT FORENSIC ACCOUNTING IN FOOD COMPANIES

Abstract

Independent forensic accounting in companies engaged in food production should be viewed as a practical discipline that can practically contribute to the improvement of work in companies that have introduced forensic accounting into their operations. Top management can establish a new specialized department in the company that can fundamentally improve the security of top management's financial reporting. In this way, the business of the entire company gains the safety and economy of making management decisions, which is of great importance in companies that do most of their business in food production. Essentially forensic accounting can contribute with its existence to improve the overall reporting in the company and essentially become one of the parts of the independent audit of financial statements.

Keywords: *forensic accounting, management, top management reporting*

INTRODUCTION

Forensic accounting is a relatively new practical discipline, which is introduced by a large number of companies in their regular operations. Essentially, it should be pointed out that the application of forensic accounting in companies in the global framework is more and more forthcoming, that is, its place and role in theory and practice is gaining more and more importance [1-5].

It is believed that independent forensic accounting in companies can be seen as a practical application of a new specialized service in a company that can fundamentally improve the security of top management's financial reporting, and that it is one of the parts of the independent audit of financial statements.

Forensic accounting in companies that have introduced it uses heterogeneous methods in its work to monitor and collect forensic evidence, in order to investigate possible crimes such as fraud and embezzlement in the financial statements of companies [6-9].

Forensic accounting in companies is carried out continuously, regardless of the time frame of the executed transactions, it focuses on finding facts and admissible evidence that may appear in the procedures for determining the overall validity of the operations of all parts, that is, all sectors within the company [9-11].

Before the start of the specific work that should be done by employees employed in forensic accounting jobs in companies, the head of forensic accounting makes a plan for performing forensic accounting jobs.

The possible layout of the plan of forensic accounting in companies is given in the form of tabular representation number 1, in which the possible interval of importance for the overall forensic plan by its parts or wholes is included.

PLANNING OF FORENSIC ACCOUNTING IN FOOD PRODUCTION COMPANIES

Forensic accounting planning in food production companies is done continuously in companies. The possible layout of the forensic accounting plan in the given companies was presented by the authors in the form of tabular presentation No. 1. Table No. 1 shows a presentation in which the possible importance interval for the overall forensic plan is included in its parts or wholes.

Table 1. Presentation of the plan of forensic accounting in companies and the possible interval of importance by plan segments

FORENSIC PLAN ACCOUNTING	Plan number
	Importance interval for the safety of the forensic accounting plan
The person who created the forensic accounting plan	1-10
Subject of forensic accounting	1-10
Key objectives of forensic accounting	1-10
Findings from previous forensic accounting reports	1-10
Objectives of forensic accounting	1-10
Scope of performing forensic accounting	1-10
Priorities of forensic accounting	1-10
Approaches of forensic accounting	1-10
Remarks on the forensic accounting report	1-10
Planned time frame for performing forensic accounting	1-10

Source: *Authors (2022)*.

TIME FRAME FOR FORENSIC ACCOUNTING PLAN IN FOOD PRODUCING COMPANIES

The timeline of the forensic accounting plan in food companies can be viewed operationally within the planning that is essentially carried out by the top management of the food company.

A possible layout of the time frame of the forensic accounting plan in companies is given in the form of tabular presentation number 2, which includes the time frame.

*Table 2. Presentation of the timeline of the forensic accounting plan
in companies*

TIME FRAMEWORK FOR FORENSIC ACCOUNTING PLAN			
Serial number	The content	Time frame for performing forensic accounting (coordinated between top management and head of forensic accounting)	Importance interval for the safety of the forensic accounting plan
1.	Introduction		1-10
2.	Recording of the existing state		1-10
3.	Establishing goals		1-10
4.	Identification		1-10
5.	Assessment of control		1-10
6.	Testing		1-10
7.	Making conclusions		1-10
8.	Forensic audit report		1-10

Source: *Authors (2022)*.

CONCLUSIONS

Forensic accounting in the company that introduced it achieves continuous cooperation with other auditors (internal, external, forensic who are engaged on a specific issue by the company or state authorities, banks, etc.), but also with other parts or sectors in the company, but before especially with the financial and accounting sector.

The goal of the introduction of forensics is the systematic detection of fraud by employees in the company, but also the processing thereof, the delivery of data and findings to the courts, etc.

Forensic audit is a special audit service, and the subject of interest of forensic auditors is financial statements, which are based on the application of accounting and auditing skills in the form of targeted, focused and detailed audit procedures. Therefore, a forensic audit should reveal illegalities, primarily in financial statements.

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Оригинални научни рад

НЕЗАВИСНО ФОРЕНЗИЧКО РАЧУНОВОДСТВО У КОМПАНИЈАМА ЗА ПРОИЗВОДЊУ ХРАНЕ

Сажетак

Независно форензичко рачуноводство у компанијама које се баве производњом хране треба посматрати као практичну дисциплину која практично може допринети побољшању рада у компанијама које су увеле у своје пословање форензичко рачуноводство. Топ менаџмент може да оснује нову специјализовану службу у компанији који суштински може побољшати сигурност финансијског извјештавања топ менаџмента. На тај начин пословање целе компаније добија на сигурности и економичности доношења управљачких одлука, што је од велике важности у компанијама које остварују већину свог пословања у производњи хране. Суштински форензичко рачуноводство може допринети својим постојањем да се побољша укупно извештавање у компанији и да суштински постане један од делова независне ревизије финансијских извјештаја.

Кључне речи: *форензичко рачуноводство, управљање, извештавање топ менаџмента.*



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Original Scientific paper

THE IMPORTANCE OF OPTIMIZING THE VALUE-ADDED TAX RATE IN PIGLET PRODUCTION

Abstract

The aim of the work is the analysis of the tax burden, that is, its impact on the volume of production and budget revenues. Through the projection, it is shown what happens when the government increases the tax rate (value added tax), that is, how it is reflected on the volume of piglet production on the one hand and how it is reflected on the amount of tax revenues. Fiscal policy is a complex area through which the state determines business conditions with various instruments. In the paper, on the example of piglet production, a simulation of tax rates and the effects they maintain on the produced quantities is made. By imposing high taxes, taxpayers feel less incentive to earn because everything they earn goes to the state, which is shown in the paper as a multiplier effect. A more detailed analysis was made on a sample of piglet production of 10,000 pieces. Some implications of established empirical relationships between variables are presented in the last part of the paper. Based on the obtained results, it was shown how much tax revenues change at different tax rates. A change in the tax rate of 8% causes an increase in tax expressed in money by 80%.

Key words: *VAT, tax revenues, tax collection efficiency, piglet production, branch offer*

INTRODUCTION

The production of piglets in the Republic of Croatia represents a very important segment in the balance of agricultural production. Various theoretical studies show that at a tax rate of zero percent and at a rate of one hundred percent, tax revenues are equal to zero. With an increase in the tax rate, tax revenues also increase, but after a certain point, the increase in the tax rate no longer fills the state budget (Baumol,

Blinder, 1991), but the other way around. This point of view is advocated by Pareto. In economic theory, efficient allocation is considered Pareto efficient allocation, which means that no movement of economic factors can improve the position of one person without worsening the position of another person. Namely, high tax rates discourage people from working and saving. They either move their economic activity to the area of the informal economy, or they decide to have more leisure and less work. By increasing the tax rate, the state did not reach for an acceptable fiscal policy measure: state revenues are lower, there is less work effort, lower investments and lower growth. When production factors become more expensive, domestic products are not competitive and the export of agricultural products decreases (Messere, 2009). The introduction of taxes leads to changed behavior on both the supply side and the demand side. Growing increases in allocative losses with each increase in the tax rate form the basis of arguments against high marginal tax rates. (Frank, Bernanke). Value added tax (hereinafter referred to as VAT) is a multi-phase sales tax that is calculated at each stage of the production-sales cycle, but only on the amount of added value formed in that stage, and not on the entire production value. Therefore, the aim of this paper is to show the importance of optimizing the value added tax on the example of piglet production.

MATERIAL AND METHODS

The calculation of the selling price of beef will be made on the basis of 10,000 piglets weighing an average of 20 kg, and fixed costs, variable costs, mixed costs and earnings will be calculated. Furthermore, the offer of the company and the offer of the branch will be analyzed. This analysis will be based on Hal R. Varian's microeconomic model. Final considerations will be made based on a comparison of the tax rate with the amount of tax revenue. This method will show the consequences for the revenue side of the budget if the tax rate varies, and how it affects the total volume of production. The data that is the subject of the analysis was obtained from the manufacturers: Belje d.d., Gavrilović d.o.o., Pivac d.o.o. and PIK Vrbovec and refer to average expenditures. In connection with this, the following methods were used during the research: analytical method and case method from practice. Prices are expressed in euros.

Table 1. Elements of piglet cost price formation in the Republic of Croatia

Elements of calculation	The price of a 20 kg fattener	Price per kg of meat
Fixed costs	12,95	0,65
Variable costs	21,93	1,10
Mixed costs	5,58	0,28
Cost price	40,47	2,02

* Made by the author according to the data obtained

The table shows the average costs for the production of piglets weighing 20 kg, and the calculated price per kilogram of piglets in euros.

The cost price plus tax constitutes the selling price. Given that there is no clear idea of an efficient VAT system, it is impossible to determine a precise measure of VAT efficiency. According to the authors Ebrill and Keen, one of the measures of VAT collection efficiency is the C-efficiency ratio (CER). In this context, the C-efficiency ratio (CER) can be expressed as:

$$CER = R/(FC*r)*100$$

where R is the total income from VAT, FC is the final consumption of households, and r is the standard rate of VAT.

It is important to point out that increasing the standard VAT rate in practice does not contribute to increasing efficiency if all circulation of money, goods and services is not in the fiscalization system. In order to determine how much certain factors contributed to the efficiency of VAT, i.e. to the growth/decrease of VAT revenue, it is necessary to perform a decomposition of VAT changes according to efficiency measures, changes in the tax base (personal consumption) and changes in the tax rate. The application of reduced rates and exemptions and the expansion of bases for the application of reduced rates leads to a loss of VAT revenue, and thus to a decrease in VAT efficiency (Ebril et.al., 2001; Copenhagen Economics, 2007 & 2013). However, according to the authors Bettendorf and Cnossen, if there is room to introduce a reduced rate of VAT for some products, the efficiency of collection should not go in a downward direction, because in this way an incentive would be given for the production of those products, provided that no they lose tax revenues. In practice, most EU countries have introduced and reduced tax rates for certain products in their tax system, which they introduced to protect their strategic resources.

RESULTS AND DISCUSSION

In the analysis of business costs, the calculation of the selling price of beef at different VAT rates is formed. It should be noted here that the legislation of the European Union allows each member state to apply reduced VAT rates to certain goods. By analyzing the production of 10,000 piglets with an average weight of 20 kg, a sales price calculation was made at different VAT rates. An overview is given in the following table.

From this analysis, it can be seen that at different VAT tax rates (10%, 13%, 15%, and 18%) the tax varies. The lowest tax is EUR 40,460.00, and the highest tax is EUR 72,828.00. The difference between the highest and the lowest tax amounts to EUR 32,368.00, i.e. 80%. Although the biggest difference compared to the smallest analyzed tax rate is 8%, the monetary amount of the tax is multiplied 10 times. From the data previously mentioned, considering that we are analyzing the production of cattle at four tax rates, the offer of the branch is formed.

Table 2. Calculation of the selling price of cattle

Elements of calculation	Calculation of the sales price at different VAT rates			
	10%	13%	15%	18%
Fixed costs	129.500,00	129.500,00	129.500,00	129.500,00
Variable costs	219.300,00	219.300,00	219.300,00	219.300,00
Mixed costs	55.800,00	55.800,00	55.800,00	55.800,00
Cost price	404.600,00	404.600,00	404.600,00	404.600,00
Amount of VAT	40.460,00	52.598,00	60.690,00	72.828,00
Sale price	445.060,00	457.198,00	465.290,00	477.428,00

* Made by the author according to the data obtained

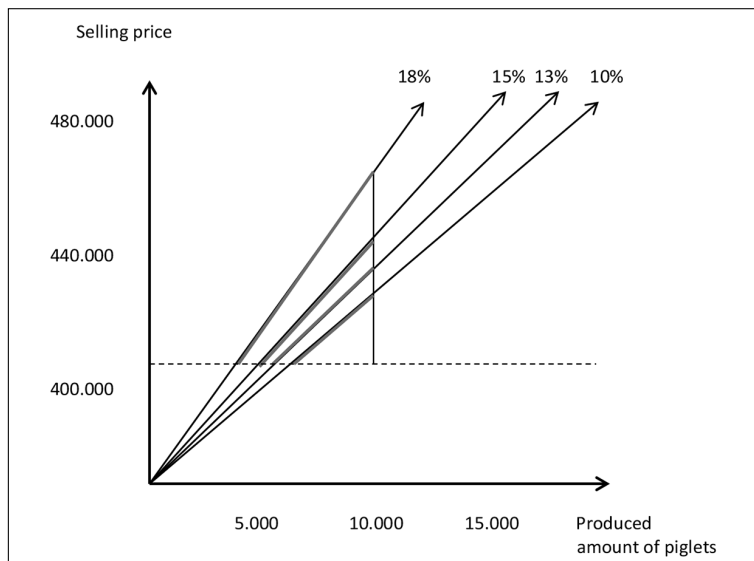


Figure 1. Branch offer

Figure 2 shows the supply of the branch that was created based on costs and different tax rates. The parts of the offer curve marked in red represent the taxable part. It can be seen that the segments where the tax rate is lower are flatter compared to those where the tax rate is higher. As the tax rate increases, the supply curve becomes steeper and vice versa, when the tax rate decreases, the supply curve becomes flatter. In an industry with free entry and exit of goods to the market, the long-run average cost curve would have to be a straight line at the price level equal to the minimum average cost. This is precisely the long-term supply curve of the company that has constant returns to scale (Varian, 2008). Given that our market is free, i.e. entry and exit from the market is free, in the short term the branch's supply curve will have a positive slope

(upward), while in the long term it becomes flatter at the price level with the same average cost (Barro, 1987). The introduction of a higher tax confronts final consumers with a higher price (Samuelson, 1992).

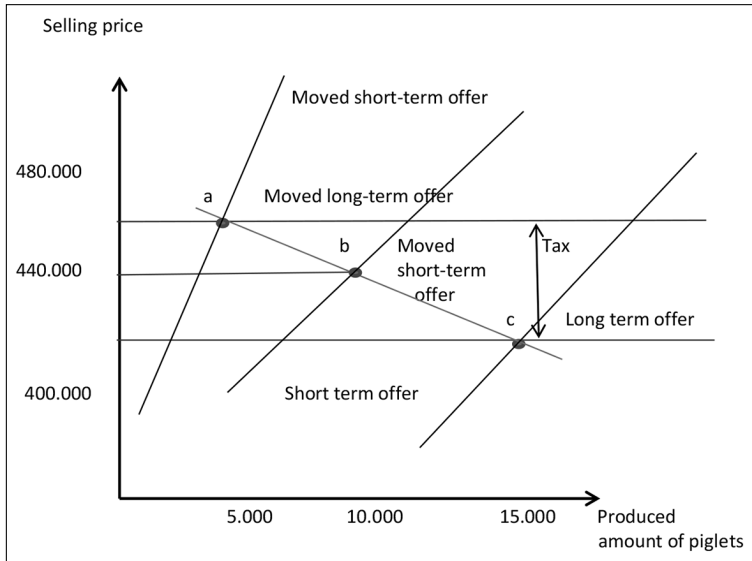


Figure 2. Changes in tax rates and the impact on the supply and demand of fattening animals

Figure 2 shows the behavior of the supply and demand curves due to changes in tax rates that are reflected in price changes. The blue horizontal lines represent long-run supply curves. The black vertical lines represent short-run supply curves. The red line represents the search curve. The introduction of taxes is represented by point c. At that point, a demand of 15,000 units of cattle is projected, and the short-term supply curve is under a slope of 45 degrees. Point b represents the profitability limit of cattle production, and shows that if a tax rate of 13% is introduced, the price is 457,198.00 eur, the demand decreases to 10,000 units of piglets, and the short-term supply becomes steeper. The more the price increases due to the introduction of a higher tax rate, the market suffers pressure, and the demand decreases to 5,000 piglets, the short-term supply curve is completely vertical, the long-term supply curve shifts upward and the intersection with the demand curve is at point a.

CONCLUSION

Therefore, when determining the tax rate, its creators must be very careful and well define the goals in the short and long term, so that the fiscal measures are stimulating and in the function of economic growth. The tax policy of the state is an instrument

used to determine market conditions. In the research part of the paper, it was presented that tax revenues vary with different tax rates. However, the state can also suffer negative consequences if it wants to raise the tax rate beyond realistic limits. Analyzing the relationship between supply and demand, long-term and short-term, the conclusions are reached that at a higher tax rate, the supply curve is upward and vertically positioned, and intersects the demand curve in the upper part of the graph. In the opposite case, when the tax rate is low, the short-term supply curve is flatter and intersects the demand curve in the lower part of the graph. Therefore, the VAT system should be set up in such a way that, on the one hand, the introduction of a reduced rate does not have a negative effect on tax revenues, and on the other hand, the level of the tax rate keeps domestic production competitive in the market.

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Original Scientific paper

ЗНАЧАЈ ОПТИМИЗАЦИЈЕ СТОПЕ ПОРЕЗА НА ДОДАТУ ВРЕДНОСТ У ПРОИЗВОДЊИ ПРАСАДИ

Сажетак

Циљ рада је анализа пореског оптерећења, односно његовог утицаја на обим производње и буџетске приходе. Кроз пројекцију се показује шта се дешава када држава повећа пореску стопу (порез на додату вредност), односно како се то одражава на обим производње прасиди с једне стране и како се одражава на висину пореских прихода. Фискална политика је сложена област кроз коју држава разним инструментима утврђује услове пословања. У раду је на примеру производње прасиди урађена симулација пореских стопа и ефеката које оне одржавају на произведене количине. Увођењем високих пореза, порески обвезници имају мање подстицаја да зараде јер све што зараде иде држави, што је у раду приказано као мултипликативни ефекат. Детаљнија анализа урађена је на узорку производње прасиди од 10.000 комада. У последњем делу рада дате су неке импликације утврђених емпиријских односа између варијабли. На основу добијених резултата показано је колико се мењају порески приходи по различитим пореским стопама. Промена пореске стопе од 8% изазива повећање пореза израженог у новцу за 80%.

Кључне речи: *ПДВ, порески приходи, ефикасност наплате пореза, производња прасиди, гранска понуда*



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Original Scientific paper

COMPARISON OF THE SYSTEM OF EU COMMUNITY TRADEMARKS AND HUNGARIKUMS

Abstract

The study aims to present the framework in which the European Union protects and supports the production of high-quality agricultural products and foodstuffs and their trade within the Union. A further objective is to introduce the relevant Hungarian quality control systems. A comparison of the Hungarian regulations concerning Hungarikums and Hungarian wine regions with EU Community Protection is made. It is shown that many high-quality Hungarian products with a national characteristic possess Community Protection and most of them are included in the Hungarian Repository of Values, but because of some reasons, Hungarian regulation declares fewer items as Hungarikum.

Key words: *Community Protection, Hungarikums, Agricultural products and foodstuffs*

INTRODUCTION

The study briefly details the European Union's Community Protection trademarks supporting the production and trade of high-quality agricultural products and trademarks, together with the groups of products. The number of trademarks registered by Member States (groups) is presented in the categories of Protected Designation of Origin, Protected Geographical Indication and Geographical Indication. Then the Hungarian regulation of the area is examined and compared to EU regulation in terms of specific items protected by either system.

EU REGULATION ON FOOD AND AGRICULTURAL PRODUCTS

In 1992 the European Union introduced trademarks in order to promote quality control of agricultural products and foodstuffs. In 2006 Council Regulation (EC) No 510/2006 laid down the regulation of the following labelling:

1. Protected Designation of Origin (PDO):
for food and wine products



2. Protected Geographical Indication (PGI):
for food and wine products



3. Geographical Indication (GI):
for spirit drinks and flavoured wines.



This labelling should apply only to those products for which there is an organic link between the product's characteristics and its geographical origin. An explicit objective of introducing protection is to enable producers of a product of given characteristics and quality to make a fair revenue and to make sure customers get well informed about the geographical origin and related specific attributes of the product when making a decision about purchasing it.

'Designation of Origin' means the name of a region, a specific place, or a country, used to describe an agricultural product or a foodstuff originating in that region, specific place or country, the quality or characteristics of which are essentially or exclusively due to a particular geographical environment with its inherent natural and human factors, and the production, processing and preparation of which take place in the defined geographical area. 'Geographical Indication' means the name of a region, a specific place or a country, used to describe an agricultural product or a foodstuff originating in that region, specific place or country, and which possesses a specific quality, reputation or other characteristics attributable to that geographical origin, and the production and/or processing and/or preparation of which take place in the defined geographical area (Council Regulation, 2006).

For better transparency, the Regulation introduced three main and several sub-categories for foodstuffs, wine, and spirit drink products:

1. Agricultural products and foodstuffs with 23 subcategories.
2. Wines (with no subcategories).
3. Spirit drinks with 45 subcategories

In these three categories, Member States possess 1391 protections for Agricultural products and foodstuffs, 1614 protections for Wines and 242 for Spirit drinks altogether (as of June 2022).

Figure 1 shows the number of foodstuff items with Protected Origin or Geographical Indication possessed by member states (or groups) of the European Union. It is clearly seen that most of the items belong to Italy and France.

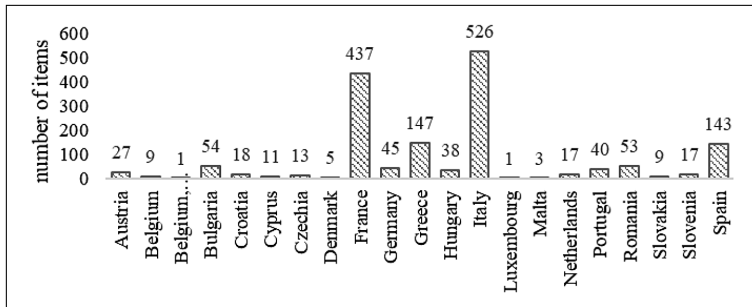


Figure 1: Number of foodstuff items with Protected Origin or Geographical Indication by member states (or groups) of the European Union. (Source: own compilation based on eAmbrosia)

Figure 2 shows the number of wine items with Protected Origin or Geographical Indication possessed by member states of the European Union. Again, most of the items belong to Italy and France.

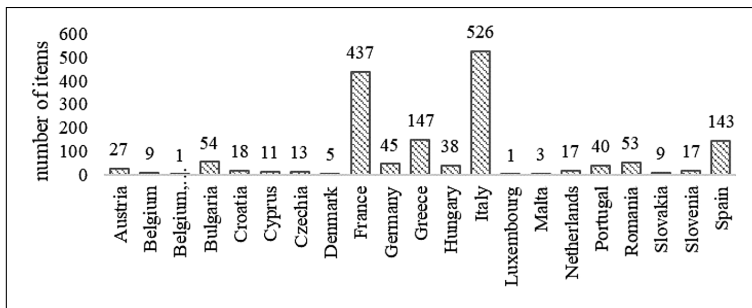


Figure 2: Number of wine items with Protected Origin or Geographical Indication by member states (or groups) of the European Union. (Source: own compilation based on eAmbrosia)

Figure 3 shows the number of spirit drink items with Protected Origin or Geographical Indication possessed by member states (or groups) of the European Union. Here Germany joins the top three along with Italy and France.

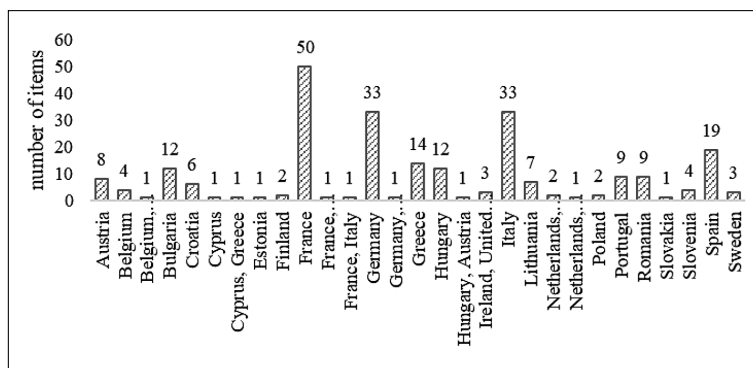


Figure 3: Number of Spirit Drink items with Protected Origin or Geographical Indication possessed by member states (or groups) of the European Union. (Source: own compilation based on eAmbrosia)

The regulation on EU Community Trademarks was created in order to protect and assist the producers by distinguishing them from other producers and to ensure the quality of the relevant products for the customers. Having a Community Trademark provides the producers with further benefits in terms of administrative and legal issues as with a single procedure the protection gets valid in all EU member countries. Additionally, the fee is lower than the total fee would be for separate protection in all countries. (Székelyhidi, et al., 2014).

Most probably, these advantages motivate EU member countries to take this opportunity – as it is also suggested by the high number of protected products.

HUNGARIAN REGULATION

Based on national regulation, products with a registered Community Trademark automatically make part of the Collection of Hungarian Values. Currently, 92 Hungarian products are protected by Community Trademarks or are registered as products with Protected Origin as Table 1 shows.

The purpose of Act XXX of 2012 on Hungarian national values and Hungarian values is – apart from enhancing agricultural product and foodstuffs quality assurance – to highlight and preserve national values. The fundamental point of view of the Act is that Hungarian National Values are unique values to be preserved, collected and documented in a researchable manner. The manmade and natural values shall be compiled in a comprehensive repository of values.

The Act does not contain a detailed description of the necessary parameters of Hungarian values. The inclusion of national values in repositories of values is a three-step procedure:

Application for inclusion in a local, county, regional or branch repository and for declaring the item a national value.

*Table 1: Number of Protected products by product category and protection type
(source: own compilation based on eAmbrosia)*

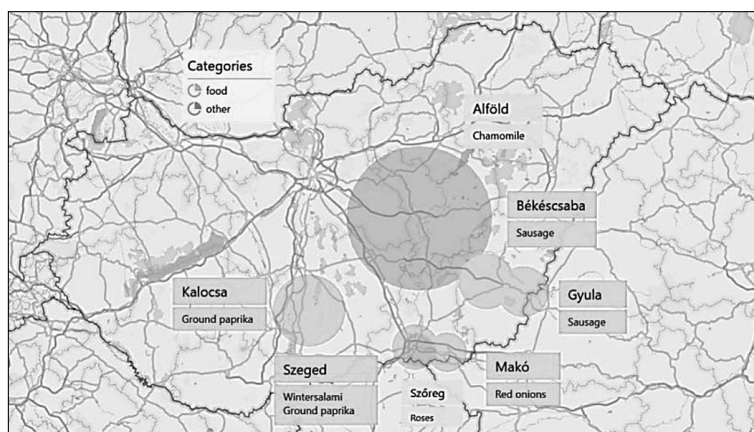
Type	GI	PDO	PGI	Sum
Foodstuffs	0	9	24	33
Wine	0	38	5	43
Spirit drink	16	0	0	16
Sum	16	47	29	92

Application for inclusion of the item in the Hungarian Repository of Values, i.e. for its classification as an outstanding national value.

Filing a request to the Committee for Hungarikums for classification of the item as Hungarikum (CNV a, 2022).

GEOGRAPHICAL LOCATIONS

The following section examines how many Hungarian products with EU Community Protection in each major category are registered as a Hungarikum.



*Figure 4: Hungarikum foodstuffs with Community Protection
(source: own compilation) Note: The size of the disks roughly indicates the size of the relevant geographical area*

Agricultural products and foodstuffs

Only 9 items are declared Hungarikum out of the 33 items with Community Protection, as shown on the map (Figure 4). As it is seen, these Hungarikums are related to locations in Mid- and Eastern Hungary (with the only exception being the Hungarian

grey cattle, which is produced at several places all over the country, so they are not shown on the map). The reason for this lies in the difference in the natural resources and the settlement structure between the various regions.

In the case of the following foodstuffs (Figure 5) with Community Protection, no application for registering them as a Hungarikum was filled yet – although their national characteristic, uniqueness and quality are unquestionable.

Fruits: <ol style="list-style-type: none"> 1) Black cherry from Szomolya 2) Apple from Tuzsér 3) Peach from Budaörs 4) Apple from Derecske 5) Apricot from Gönc 6) Apple from Szabolcs 7) Sour cherry from Újfehértó 8) White-heart cherry from Nagykorú 	Other plants: <ol style="list-style-type: none"> 1) Horse radish from Hajdúság 2) Carrots from Fertőd 3) Parsley root from Hegykő 4) Parsley root from Makó 5) Rice from Nagykunság 6) Grounded paprika from Szentés 7) Pumpkin seed oil from Őrség 	Fish: <ol style="list-style-type: none"> 1) Carp from Akasztó 2) Fish from Balaton 3) Mirror carp from Szeged 4) Trout from Szilvássvár
Mushroom: <ol style="list-style-type: none"> 1) Summer truffle from Jászság 	Cheese: <ol style="list-style-type: none"> 1) Csemege cheese form Győr-Moson-Sopron county 2) Lajta cheese 	Meat products: <ol style="list-style-type: none"> 1) Wintersalami from Budapest 2) Lamb from Kelemér

Figure 5: Non-Hungarikum foodstuffs with a Community Protection (source: own compilation)

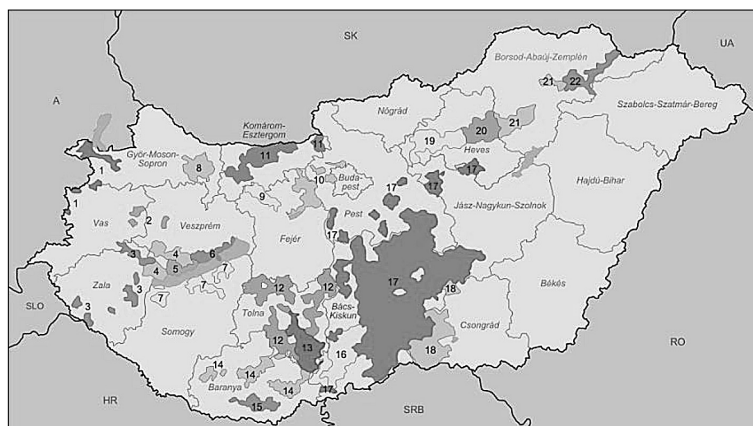


Figure 6: Wine-producing areas (numbered) in Hungary. (wikipedia, 2022) Colours indicate the production volume (the higher the darker)

Wines and spirit drinks

In the main category of wines only two products – Egri Bikavér and Tokaji Aszú – are Hungarikums, but Hungarian agricultural legislation considers the production of high-quality wines as a highlighted strategy objective. For this end Act XVIII of 2004

on viticulture and wine defined the list of wine-producing areas and regions in Hungary. According to the regulation, a wine-producing area is an area with similar climatic, geographical and soil characteristics with a specific set of grape varieties and wine production traditions. The wine region is a set of wine-producing areas with common geographical characteristics.

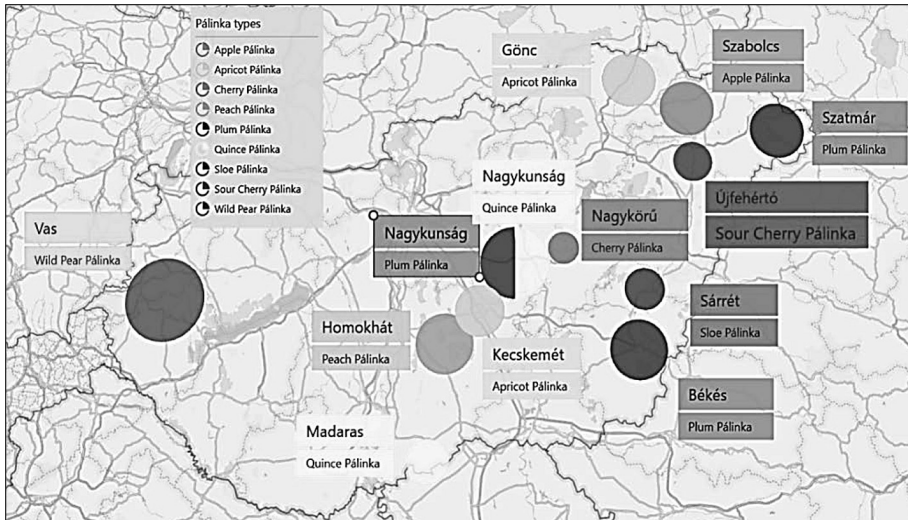


Figure 7: Hungarikum spirit drinks with Community Protection (source: own compilation) Note: The size of the disks roughly indicates the size of the relevant geographical area

For spirit drinks, currently, there is no Hungarian regulation to ensure their traditional and national characteristics and high quality. The Act on Hungarikums declared all Pálinka (including marc Pálinka) products as Hungarikums, but no regulation exists with detailed requirements on raw materials or production procedures. On the other hand, 16 Pálinka products from various fruits and geographical locations possess Community Protection, as shown on the map (Figure 7).

SUMMARY

The study briefly summarized the European Union’s quality control regulations concerning food and agricultural products and some statistics about the Member States. It detailed the Hungarian regulations and revealed the harmony between the two systems. Hungarian products with Community Protection are also included in the Hungarian Repository of Values, but they are not necessarily Hungarikums. This is explained by special legal and administrative features of the procedure to declare a

product as a Hungarikum. Furthermore, Hungarikums include a lot of items outside of the framework of food and agricultural products, from the domain of health and lifestyle, built environment, industrial and technical solutions, cultural heritage, sport, natural environment, tourism and hospitality (Hungarikum Committee, 2022).

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POREĐENJE SISTEMA ZNAKOVA HUNGARIKUMS ZAJEDNICE EU

Sažetak

Studija ima za cilj da predstavi okvir u kojem Evropska unija štiti i podržava proizvodnju visokokvalitetnih poljoprivrednih i prehrambenih proizvoda i njihovu trgovinu unutar Unije. Dalji cilj je uvođenje relevantnih mađarskih sistema kontrole kvaliteta. Napravljeno je poređenje mađarskih propisa koji se odnose na Hungarikume i mađarske vinske regije sa zaštitom EU zajednice. Pokazalo se da mnogi visokokvalitetni mađarski proizvodi sa nacionalnim obeležjem poseduju zaštitu zajednice i većina njih je uključena u mađarski repozitorijum vrednosti, ali iz nekih razloga mađarska regulativa proglašava manji broj artikala kao Hungarikum.

Ključne reči: *Zaštita zajednice, Hungarikumi, Poljoprivredni proizvodi i prehrambeni proizvodi*



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Original Scientific paper

ECONOMIC RESPECT FOR HETEROGENEOUS FACTORS IN COMPANIES ENGAGED IN THE PRODUCTION OF HEALTHY SAFE FOOD

Abstract

Economic appreciation of numerous heterogeneous factors in companies that are mainly engaged in the production of safe food requires the top management in these companies to introduce a whole range of innovations in business. This is of great importance especially because these companies are engaged in responsible production, which can affect a large number of people, especially their health. One of the modern approaches to doing business in such companies is the application of a higher level of standardization in the overall business, such as the application of international accounting standards within the standard business of these companies. The goal of implementing such activities is to raise the level of real financial reporting in companies, especially within the business of companies that are predominantly engaged in the production of safe food.

Key words: *health food production, international accounting standards*

INTRODUCTION

Top management in health food companies is constantly in need of finding new ways to improve the overall management of the companies in which it performs management functions.

One of the ways in which the application could improve the overall business of the company is management in companies where clear and precise accounting procedures have been established in all parts of management in the company. The authors point out that such management is necessary for all levels of management in the company, it needs to be practically adopted and changed by all employees in the company and there is a need for prior public disclosure of changes that may occur in the implementation of accounting policies.

This is especially important in companies that produce mainly food, where it is necessary to achieve a high level of standardization of all activities and at all levels within the business of the company itself. We also have such an observation in our works (Chen, 2019; Popović, 2014). Novaković et al., 2018).

The application of international accounting standards enables the improvement of the relevance and reliability, first of all, of the financial reporting of the top management, which is of great importance in the business of companies engaged in the production of safe food.

This means that the application of international accounting standards in companies engaged in the production of safe food can significantly contribute to the improvement of overall management in companies, because the real financial statements of companies are essentially used (Popović et al., 2015; Popović et al. 2018, Popović et al., 2020, Radović et al., 2019; Vitomir et al., 2019).

The practical improvement of the overall business in these companies can be defined as a substantial contribution of authors who expressed their views in emphasizing the application of international accounting standards, as part of improving and improving financial reporting in these companies, which mainly operate in food production, especially health. food.

SWOT ANALYSIS OF THE STATE OF COMPANIES OPERATING IN THE FIELD OF HEALTH SAFE FOOD AND REGULARLY APPLY INTERNATIONAL ACCOUNTING STANDARDS IN THEIR BUSINESS

Using already published papers (Popović et al., 2017) the authors presented the SWOT analysis in companies that apply the principles in the field of health safe food and regularly apply international accounting standards in their business. The presentation of the mentioned analysis was given by the authors in the form of tabular presentation number 1.

The Republic of Serbia has signed documents with the EU on the basis of which it intends to carry out the process of accession to the European Union. This essentially means adjusting to the standards that apply in EU countries.

The situation is similar with other countries in the region, which are essentially striving to join the EU, and are rapidly working on introducing standards in the business of their companies.

Table 1. SWOT analysis of the situation of companies operating in the field of health safe food and regularly apply international accounting standards in their business

Avalirale options	Weaknesses
<ul style="list-style-type: none"> • The existence of a signed Chapter 32 with the EU on financial control in the public sector, • The existence of positive attitudes of internal audit introduced in the public sector, • adopted internal audit procedures are compatible with internal control procedures, • Many years of tradition in applying audit procedures in the public sector 	<ul style="list-style-type: none"> • Lack of complete information on the importance of full implementation of internal control in the public sector, • Lack of interest of local self-government units to significantly increase the operational reliability of companies, • Insufficient interest of companies that have established local self-government units for internal control in terms of essential financial reporting to the founder, • The frequent change of the top management appointed by the local self-government causes discontinuity in the performance of commenced tasks in the introduction, control and implementation of internal control recommendations in enterprises
Chances	Threats
<ul style="list-style-type: none"> • Increase in commitments based on signed pre-accession chapters with the EU, • Increasing the safety of top management that has implemented internal control in the companies it manages, • Increasing the security of financial reporting to local government and state authorities, • Facilitates public-private investment activities in the public sector, • Internal control contributes to the increase in the level of local government investment in the primary enterprises due to the increased security of investment and the return of funds into the budget based on the generated profit of the company 	<ul style="list-style-type: none"> • Insufficient sanctioning of companies established by local self-government in terms of poor financial reporting, • Insufficient sanctioning of public utility companies established by local self-government in terms of poor implementation of business programs

The research was conducted in the Republic of Serbia, which essentially seeks to apply positive experiences from the EU and introduce its companies to regular business, which is of great importance especially in companies engaged in food production, especially health food, where it can be said that there is greater need to apply standard control methods.

In this practical presentation of the importance of applying international accounting standards in the business of companies primarily operating in the field of food production, especially health food, the authors stressed the importance of presenting the application of international accounting standards by presenting SVOT analysis. apply the standard in financial reporting of top management companies.

General model of implementation of international accounting standards in companies which operating in the field of health safe food and regularly apply international accounting standards in their business

The general model of implementation of international accounting standards in companies operating according to the principles in the field of health safe food and regularly apply IAS in their business, was presented by the authors in the form shown in Figure 1.

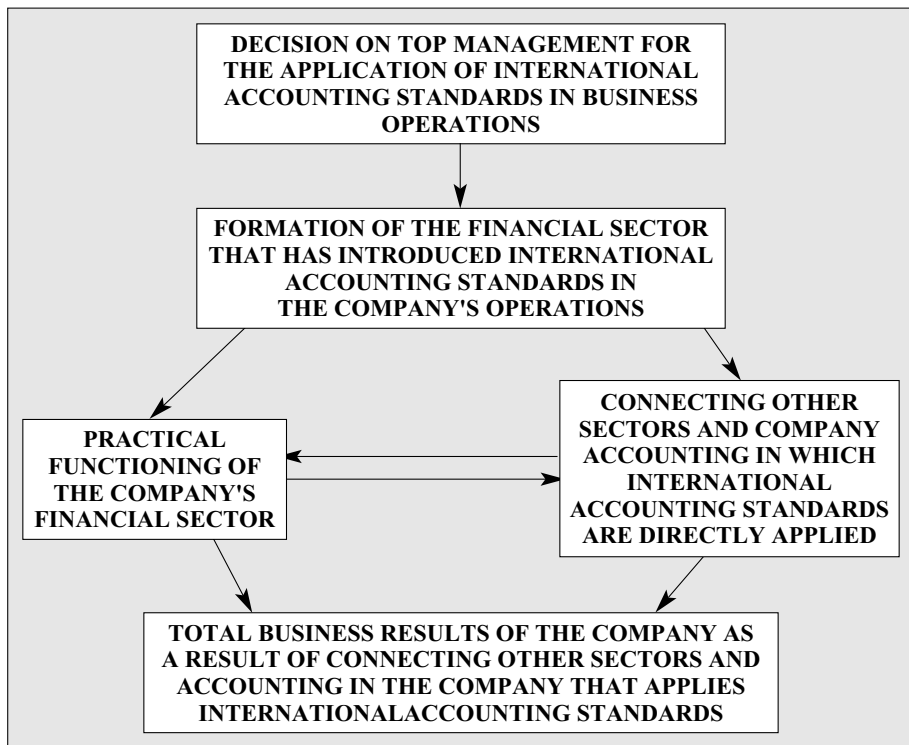


Figure 1. Overview of the general model of implementation of international accounting standards in companies which operating in the field of health safe food and regularly apply international accounting standards in their business

Based on the presentation of the general model, which shows the application and introduction of international accounting standards in the business of companies that are mainly engaged in the production of safe food, it can be seen that there are the following important characteristics:

1. The connection of all sectors in the company is great,
2. Connectivity and connections within all sectors in the company is strong,
3. The connection of all sectors and the connections that arise in the real business of the company are based on financial statements that should be considered by the top management of the company,
4. There is a strong impact of financial reporting on the business results of the company,
5. There is a strong influence of accounting policy on the financial reporting and operations of the company,
6. A decision and the will to implement the decision of the top management on the application of international accounting standards in the regular operations of the company is needed, in order to achieve significantly better overall business results.

CONCLUSIONS

The processes of real standardization in the business of a large number of companies cannot bypass the companies that produce health-safe food. The application of standards in production and organization of production is of great importance for the company's business. Application of principles and application of international business standards are just one of the ways in which it is possible to improve production.

One of the examples of positive application in business is the application of international accounting standards and such application may mean that in the work and operations of many companies that produce food and especially healthy food should be applied standardization, which can essentially begin with the application of international accounting standards. is to raise the general level of business security in the work of the companies themselves.

The authors came to these observations by observing the work and business of companies in the field of health safe food production, which began to implement the application of standards and the application of international accounting standards in their regular operations.

In essence, the application of international accounting standards can improve and raise the general level of business security in companies that produce health-safe food. This is an approach that has a practical application of a relatively new way of organizing work in the mentioned companies, especially in the Republic of Serbia. In this way, it is possible to change the business of a large number of companies that produce safe food, which is of great importance for the entire process of rapprochement, both countries and the Republic of Serbia and for companies that introduce one form of standard in their business. which already exist in the EU.

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EKONOMSKO UVAŽAVANJE HETEROGENIH FAKTORA U KOMPANIJAMA KOJE SE BAVE PROIZVODNOM ZDRAVSTVENO BEZBEDNE HRANE

Sažetak

Ekonomsko uvažavanje brojnih heterogenih faktora u kompanijama koje se pretežno bave proizvodnjom zdravstveno bezbedne hrane zahteva od top menadžmenta u pomenutim kompanijama da uvede čitav niz novina u poslovanju. To je od velikog značaja pogotovo jer se te kompanije bave odgovornom proizvodnjom, koja može da utiče na veliki broj ljudi, pogotovo na njihovo zdravlje. Jedan od savremenih pristupa poslovanja u takvim kompanija je primena većeg nivoa standardizacije u ukupnom poslovanju, poput primene međunarodnih računovodstvenih standarda i to u okviru standardnog poslovanja pomenutih kompanija. Cilj sprovođenja takvih aktivnosti je podizanje nivoa realnog finansijskog izveštavanje u kompanijama, pogotovo u okviru poslovanja kompanija koje se bave dominantno proizvodnjom zdravstveno bezbedne hrane.

Ključne reči: *proizvodnja zdravstveno bezbedne hrane, međunarodni računovodstveni standardi*



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Original Scientific paper

IMPLEMENTATION OF INTERNAL AUDIT IN COMPANIES MAINLY OPERATING IN THE FIELD OF HEALTHY SAFETY PRODUCTION

Abstract

The implementation of internal audit in companies that mainly operate in the field of safe food production is one of the opportunities available to the company's top management in order to improve the real business of the company. Essentially, its introduction can improve the overall management of the company. In the processes of real decision-making of top management, it is very important to make valid business decisions, and one of the most important is to introduce some form of control. The implementation of internal audit in companies that mainly operate in the field of safe food production can contribute to its activities to improve the security of business decisions by top management. Implementation of internal audit in companies that mainly operate in the field of health food production can increase the security of the entire business in the company, which refers specially to achieving prescribing procedures in food production, which is the main goal of introducing internal audit in these companies.

Key words: *internal audit, companies, production of safe food*

INTRODUCTION

The company's top management can implement internal audit in companies that mainly operate in the field of health food production because they look at one of the possible models and opportunities to increase the work and security of the overall business in the company. One of the ways to implement internal audit in companies that

mainly operate in the field of safe food production is the essential introduction of internal control and internal audit in the operations of these companies.

This is observed within the already adopted policy of implementing internal audit in companies that mainly operate in the field of safe food production, which is essentially a set of special principles, bases, agreements, rules and practices that the company already applies.

Such observation must be made in particular when compiling and presenting financial statements for the purposes of both top management and government reporting (Barker & McGeachin, 2013; Chen, 2019).

Financial reporting by top management is one of the most important reports in a company's business. Financial reporting is also important for other management bodies in the company and it is the next phase of financial reporting, and finally we can observe the third phase of actual reporting, i.e. reporting for government agencies by companies operating in the field of health food production.

In addition to the above three forms of dominant corporate reporting, reporting based on the existence of internal audit work can also be used for reporting purposes of majority owners, shareholders and others. relevant users of financial statements made by the company's top management for the needs of external reporting (Popović, 2014; Popović et al., 2015; Vitomir et al., 2019).

This view of the application of internal audit and reporting is of great importance for the establishment of a standard way of reporting (Popović et al. 2017; Popović et al. 2020).

The formation of accounting policies in companies operating on the principles of the green economy should take into account the following areas of application in companies:

- valuation of fixed assets,
- conducting a policy of intangible investments in the company,
- valuation of investment real estate in the company,
- selection of methods for calculating ascending stocks in the company,
- the chosen method regarding the estimated method as well as the participation in the capital of related legal entities, which relate to the significant operations of the company,
- determining the recording of interest expenses and other borrowing costs,
- consolidation of joint,
- Controlled legal entities and others.

THE PROCESS OF IMPLEMENTING INTERNAL AUDIT IN COMPANIES THAT MAINLY OPERATE IN THE FIELD OF HEALTH FOOD PRODUCTION

The top management of the company can implement internal audit in companies that mainly operate in the field of health food production should be seen as an extension of the establishment of a control function by the top management of the company.

Based on the implemented internal audit in companies that mainly operate in the field of health and food production, it can be said that there is an increase in occupational safety and business in the entire company that operates in the field of health and food production.

Implemented internal audit in companies that mainly operate in the field of safe food production should aim to achieve improvement and business security throughout the company, or in all its parts, in all sectors and departments.

In addition, the work of internal audit should contribute to the improvement of the overall decisions made, which are based on the existence and fulfillment of the standards of the audit profession. The introduction of internal audit can significantly improve the overall business of the company.

In its work, internal audit is dedicated to real business, as follows:

- independence in the work of internal auditors in relation to the entity in which the required audit work is performed;
- wide scope of work of internal audit in companies,
- the ability of internal auditors to make the results obtained by auditors such that they can become available to all interested parties in the company that has essentially commissioned the internal audit of a particular area.

Internal auditors should take into account heterogeneous requirements in their work, such as:

- analysis of the effectiveness (efficiency) of the internal control system;
- the audit also deals with aspects of correctness, as well as the structure that ensures economy, efficiency and effectiveness (effectiveness) in companies;
- consideration of legality / regularity of transactions;
- an analytical approach to the work, where the starting point should be an overview of the characteristics of the audited entity, the responsibilities and problems it faces, and the administration and organization of the audited entity;
- an active approach, through which audit work, where possible, is characterized by a constructive attitude that ensures change, which can be seen in maintaining constructive and adequate relations with employees, but also in the timing and approach applied in reporting audit findings.

USE OF SOFTWARE DURING INTERNAL AUDIT IN FOOD COMPANIES ACCORDING TO THE PRINCIPLES OF SAFE FOOD PRODUCTION

The authors presented the possible use of software during the work of internal audit in companies that produce food according to the principles of safe food production in the form of Figure 1 and Figure 2.

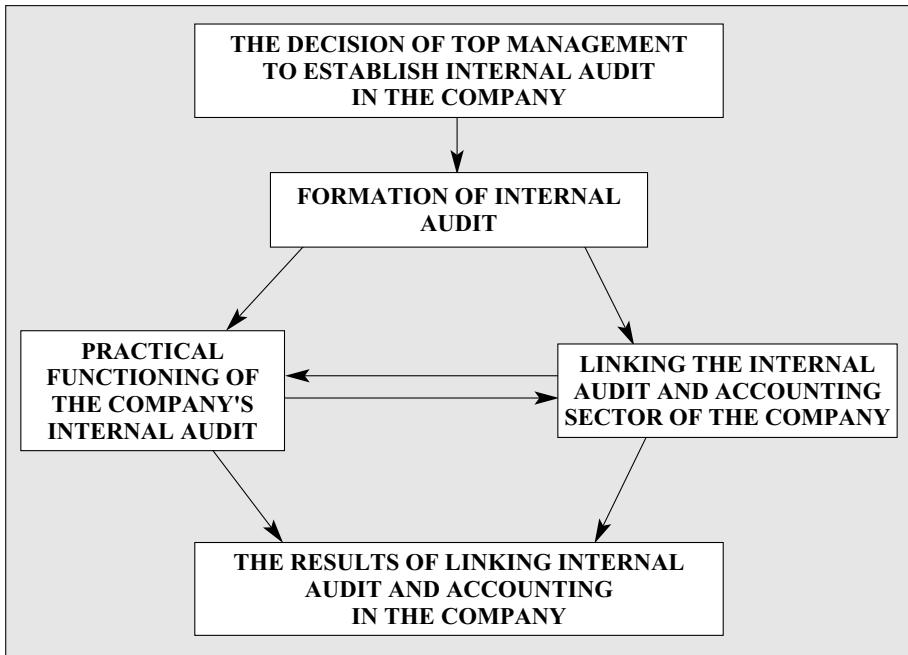


Figure 1. The general course of implementation of internal audit in companies that mainly operate in the field of safe food production

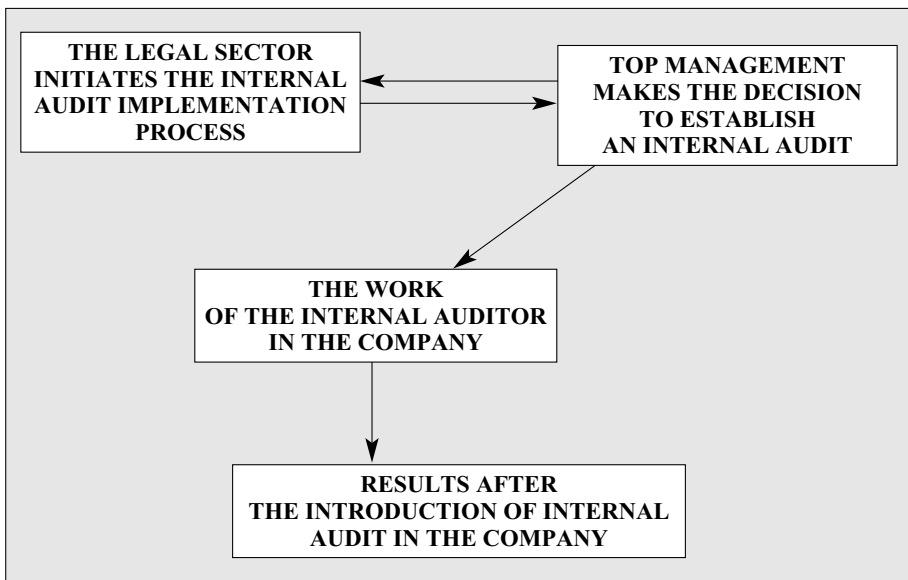


Figure 2. Launching an initiative for the operation of internal audit in the company

APPLICATION OF INTERNAL AUDIT IN THE OPERATIONS OF COMPANIES THAT BASICALLY HAVE THE PRODUCTION OF SAFE FOOD

The basic feature of the application of this approach remains the professional audit court formed by the internal auditor in his work on the basis of the authority given to him by the top management of the company.

Although it is important that auditors are thoroughly acquainted with all aspects of the real functioning of internal audit in companies. Some of the key messages, the author pointed out, such as:

1. Internal audit should be adjusted to the circumstances within the audited entity and should be based on an audit risk assessment,
2. giving an opinion on internal controls is not the only goal of the audit in companies,
3. examination of the structure of corporate governance in the audited entity and examination of the performance of management in the medium term should become equally important for the adoption of the position and opinion of the internal auditor,
4. Audit findings should be presented in a timely manner in internal audit reports to ensure that appropriate measures can be taken in a timely manner by the company's top management.

In addition, it should be emphasized that the external audit and the already formed internal audit in the companies that have introduced internal audit should be essentially linked.

With such an approach, the top management of the company has the opportunity to achieve the overall goal by obtaining the highest quality audit services from the aforementioned internal and external audit that they have engaged in the process of strengthening financial reporting in companies.

CONCLUSIONS

The implementation of internal control mechanisms in companies should be carried out continuously. One of the ways is the implementation of one or more forms of internal control mechanisms in the business of companies, and in this paper the authors drew attention to the work and implementation of internal audit. Internal audit aims to increase the security of financial reporting at all levels of management in these companies. In this way, the top management of the company has the opportunity to make valid business decisions within the regular business of the company. As a result of improvements in company management, a reduction in total operating costs in the regular operation of companies most often occurs.

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IMPLEMENTACIJA INTERNE REVIZIJE U PREDUZEĆIMA KOJA PRETEŽNO POSLUJU U OBLASTI PROIZVODNJI ZDRAVSTVENO BEZBEDNE HRANE

Sažetak

Implementacija interne revizije u kompanijama koja pretežno posluju u oblasti proizvodnje zdravstveno bezbedne hrane jedna je od mogućnosti koju ima na raspolaganju top menadžment kompanije sa ciljem da unapredi realno poslovanje kompanije. Suštinski njenim uvođenjem može da se unapredi ukupno upravljanje u kompaniji. U procesima realnog odlučivanja top menadžmenta veoma je važno da se donesu validne poslovne odluke, a jedna od bitnih je da se uvode neki od oblika kontrole. Implementacija interne revizije u kompanijama koja pretežno posluju u oblasti proizvodnje zdravstveno bezbedne hrane može da doprinese da se njenim delovanjem pospeši sigurnost donošenja poslovnih odluka od strane top menadžmenta. Implementacija interne revizije u kompanijama koja pretežno posluju u oblasti proizvodnje zdravstveno bezbedne hrane može se povećati bezbednost celokupnog poslovanja u kompaniji, što se odnosi naročito na postizanje propisivanja procedura u proizvodnji hrane, a što predstavlja i osnovni cilj uvođenja interne revizije u pomenutim kompanijama.

Ključne reči: *interna revizija, kompanije, proizvodnja zdravstveno bezbedne hrane*



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Original Scientific paper

THE ROLE OF THE BIOLOGICAL AND NUTRITIONAL VALUE OF THE DIET IN THE PREVENTION OF CORONAVIRUS INFECTION

Abstract

Deficiency of a number of nutrients in the diet can disrupt the chemical, structural and regulatory processes in the body, which negatively affects the state of the immune system. The purpose of the review article is to consider the role of nutrients and a number of biologically active substances in improving immunity for the prevention of COVID-19 in the population. As a result of the analysis of foreign and Russian scientific publications, it was found that such nutrients as complete protein, vitamins A, C, D, E, minerals – selenium, zinc, biologically active substances - resveratrol, turmeric flavonoids, melatonin play an important role in the prevention of coronavirus infection, having immunomodulatory, anti-inflammatory, antioxidant and antiviral properties.

Key words: *nutrients; immunity; COVID-19; coronavirus infection; biologically active substances*

INTRODUCTION

The disease caused by the new Severe Acute Respiratory Syndrome coronavirus, called SARS-CoV-2 (Severe Acute Respiratory Syndrome-related Coronavirus), has been named COVID-19. To cause disease, a virus must enter, replicate, and damage a human cell, escaping immunological defenses to infect neighboring cells. The entry gate for the causative agent of this disease is epithelial cells, on the surface of which there are receptors for angiotensin-converting enzyme type II - ACE-2. In addition to ACE-2, 2 more enzymes play an important role in the introduction of coronavirus into the cell: furin and 3-CL protease [14,17]. Increasing human innate immunity and influencing ACE-2, furin, and 3-CL protease with various dietary biologically active

substances is an alternative approach to the prevention of coronavirus diseases. To ensure the proper functioning of the immune system, it is necessary to consume a sufficient amount of biologically active nutrients that act on the immune system through gene expression and correction of intestinal microbiocenosis.

MATERIALS AND METHODS

The work used the method of literary research and analysis of modern domestic and foreign scientific literature on the research topic. The review analyzes the data of scientific electronic libraries eLIBRARY.RU, bibliographic databases of articles on medical sciences MEDLINE and PubMed-NCBI.

RESULTS AND DISCUSSION

For the prevention of infectious diseases, an adequate intake of nutrients plays an important role in maintaining immunity, increasing the functional reserves of the body and reducing the risk of infection. Improving immunity is the main link in the prevention of coronavirus infection [10]. This requires such components as an increase in a person's innate immunity before a disease, sufficient sleep (at least 7-8 hours a day), moderate physical activity and positive emotions. An increase in a person's innate immunity before a disease can be provided by the consumption of a normal amount of protein in the diet, vitamins C, D, A and E, minerals, especially zinc and selenium. Biologically active substances from curcumin, an aqueous extract of wormwood leaves, echinacea, propolis, garlic, soy, green tea, as well as probiotics, prebiotics, melatonin and other nutrients are important for improving immunity.

According to Methodological recommendations 2.3.1.0253-21 «Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation» [5], a person should receive proteins in an amount from 75 to 114 g/day for men and from 60 to 90 g/day for women, depending on the intensity of physical activity, of which 50% are animals. The lack of proteins in the diet leads to a decrease in the efficiency (effectiveness) of the functioning of the body's immune defense, since the synthesis of antibodies, lymphocytes, neutrophils, lysozyme, adrenal cortex hormones, biologically active substances and other participants in the body's defense reactions requires the entire spectrum of amino acids.

However, for people suffering from COVID-19, attempts to improve the body's immune function can be harmful. In COVID-19 infection, it is important to prevent overreactivity of the innate immune system and reduce inflammation. Therefore, an increased content of protein, especially of animal origin, can worsen the patient's well-being, since meat products are rich in tryptophan and arginine. The latter increase the production of Th-17 cells, which increases overall inflammation in the body. Another amino acid, leucine, is able to activate the mTOR protein (a special intracellular protein that regulates the development of muscle fibers), which also contributes to the growth

of the number of Th-17 lymphocytes. Decreased mTOR production by reducing inflammation, mTOR inhibitors found in coffee, extra virgin olive oil, and lack of amino acids in the diet.

More than 100 functions in the body are known for vitamin C, in which it takes part. Ascorbic acid is involved in the functioning of the body's immune system by enhancing the activity of the T-cell link of immunity, as well as stimulating the bactericidal activity and migratory ability of neutrophils; has a regenerating effect, increasing the level of cyclic adenosine monophosphate (3',5'-cAMP) in tissues by suppressing the activity of phosphodiesterase; participates in the synthesis of corticosteroids (anti-inflammatory action), as well as in the synthesis of connective tissue, etc [14]. The physiological need for adults is 100 mg/day, for children – from 30 to 90 mg/day, depending on age, an adequate intake level for adults is 70-100 mg/day, the upper acceptable intake level is 700 mg/day [MP 2.3.1.19150-04 «Recommended consumption levels of food and biologically active substances»].

Ergocalciferol (vitamin D2) is found in food, synthesized by ultraviolet irradiation from provitamin – ergosterol, which is part of plant tissues. Cholecalciferol (vitamin D3) is formed from 7-dehydrocholesterol contained in the skin under the influence of ultraviolet rays (up to 80% of the daily requirement for vitamin D), it is also found in animal products. Vitamin D synthesized by the skin circulates 2-3 times longer than vitamin D obtained from food.

Already by 2010, the results of numerous studies were obtained indicating the antibacterial and antiviral effects of vitamin D [12]. Vitamin D, modulating the activity of T-lymphocytes, mast cells, antigen-presenting cells, helps to reduce the excessive inflammatory response, increasing the levels of anti-inflammatory IL-10, reducing the levels of IgE, alarmin cytokines, IL-17, histamine, leukotrienes [14]. The discovery of calcitriol receptors in many cells of the immune system (on T-lymphocytes, macrophages, immature thymus lymphocytes and mature CD8 cells), as well as the ability of mononuclear phagocytes to produce 1,25-dihydroxyvitamin D3, proved the participation of vitamin D in functioning of the immune system.

All healthy people need a minimum physiological dose of vitamin D in the summer months of 500 IU/day, and from September to June – 1000 IU/day. In the clinical guidelines «Vitamin D deficiency in adults: diagnosis, treatment and prevention» developed by the Russian Association of Endocrinologists to maintain the level of 25(OH)D 30 ng/ml, the following regimen for taking Aquadetrim® is presented: 1000–2000 IU daily orally (2–4 drops per day); 6,000–14,000 IU once a week orally (15–30 once a week). Elimination of vitamin D deficiency is fundamentally important for maintaining innate antiviral immunity in all age groups [13], reducing the risk of a “cytokine storm” and compensating for comorbid diseases associated with vitamin D deficiency (type 2 diabetes mellitus, arterial hypertension, coronary artery disease, bronchial asthma, COPD), which aggravate the course of coronavirus infection. Karonova T.L., Andreeva A.T., Vashukova M.A. [3] also found that vitamin D deficiency is a risk of severe and fatal coronavirus infection.

Vitamin A plays an essential role in maintaining the integrity of respiratory epithelial cells [8,14]. Vitamin A has an immunostimulating effect by accelerating the

proliferation of lymphocytes and activating phagocytosis; is an antioxidant; necessary for the function of the eyes, the condition of the skin, and mucous membranes, the synthesis of chondroitin sulfates (“beauty vitamin”, “growth vitamin”). The active form of vitamin A, retinoic acid, has a protective effect in respiratory pathology [16].

Zinc has a multifaceted effect on all parts of the immune system [2,15]. It is necessary for the synthesis of T-helpers, the formation of antibodies, and the blast transformation of B-lymphocytes. Zinc has an immunostimulatory effect, increases the activity of T and B lymphocytes, the phagocytic activity of neutrophils. The biochemical basis of the activity of zinc is its ability to block the enzyme RNA polymerase, which is necessary for virus replication. 118 zinc-containing proteins are involved in human antiviral immunity, 11 of which are directly related to protection against single-stranded RNA viruses, which include SARS-CoV-2 [7].

The Zn-dependent protein TRIM5 α inhibits the release of viral RNA inside the cell. The Zn-dependent proteins TRIM22, ISG15 and the iron/folate (B9)-dependent protein viperine, whose gene expression is regulated by vitamin D, inhibit viral replication and virus budding from the plasma membrane. In addition, zinc is involved in the metabolism of vitamin A, is part of corticosteroids (protection against stress, anti-inflammatory effect), provides the function of assessing the taste of the papillae of the tongue, stimulating the synthesis of thickening by the parotid glands, is involved in spermatogenesis, testosterone synthesis. An increased concentration of zinc prevents the penetration of virions into cells and blocks its reproduction process. Zinc has become even more popular due to the development of the COVID-19 pandemic [6].

Zinc and selenium deficiency occurs in 30–40% of Russians. Among older people with comorbid pathology or alcohol abusers, the deficiency of these trace elements is observed in 60–80%. Given this situation, zinc and selenium preparations can be taken without examining their content in the body for no more than 3 months and in moderate doses: for zinc 5–10 mg / day, for selenium – 50 mcg/day. With a significant deficiency, the intake is needed longer, and the doses are larger. For zinc, this is 80 mg / day, for selenium – 100–200 mcg/day. With coronavirus infection, such doses can be taken for 3 weeks. The main dietary sources of zinc are seafood, meat, eggs, nuts, and legumes.

Selenium is an essential component of antioxidant protection. Actively participating in antioxidant protection, selenium exhibits synergism with respect to the action of tocopherols, and also ensures the reactivation of ascorbic acid as a result of its interaction with selenium-containing thioredoxin reductase. Selenoprotein P protects vascular endothelial cells from reactive nitrogen radicals. Epidemiological studies demonstrate that selenium deficiency alters immune responses and reduces resistance to viral infection.

Reliable sources of selenium are animal products (seafood, meat products, nuts), as they contain a stable amount of it. In plant products, the content of selenium directly depends on its concentration in the soil. Selenium is present in cereals, meat products and mushrooms in the form of selenomethionine and selenocysteine, in onions and garlic – in the form of selenocysteine oxide, in nuts - in the form of selenocystathionine. The assimilation of selenium from mixed diets is practically not inhibited and reaches 80% or more.

Administration of higher than recommended daily doses of nutrients, such as vitamins D, C, E, zinc, and ω -3 fatty acids, may have a beneficial effect in the setting of disease, potentially reducing SARS-CoV-2 viral load and length of hospital stay [4].

American scientists have discovered the anti-coronavirus properties of an aqueous extract of wormwood leaves. The results of the study are published in the bioRxiv online library. Wormwood can produce artemisinin, a substance with antiviral activity that helps to suppress the replication of the coronavirus even after it enters the cell.

To improve the performance of ACE-2, you can suggest regular aerobic exercise, a diet of whole foods of plant origin, the use of curcumin (a set of turmeric flavonoids) and resveratrol. Resveratrol is synthesized by some plants to protect them from bacteria or fungi. Inhibits the growth of the corona virus, reduces the inflammation caused by it. Resveratrol reduces the amount of free radicals and enzymes involved in inflammatory reactions and the formation of reactive oxygen species (including myeloperoxidase, NADPH oxidase). In animal and cellular studies, resveratrol increases levels of key antioxidant enzymes: mitochondrial superoxide dismutase-2 (SOD2) and Nrf2 (nuclear factor-2) protein, which are key components of antioxidant defense, as well as glutathione, catalase, and heme oxygenase 1. In a study on brain cells, resveratrol increased the activity of heme oxygenase 1, which protects the brain from damage. This effect is enhanced by melatonin.

To increase the bioavailability of resveratrol, it is necessary to combine resveratrol with other polyphenols or flavonoids. Thus, the counting of resveratrol, quercetin and genistein is highly synergistic. Low dosages of these natural compounds can provide similar benefits in the aggregate. In addition, you can increase the bioavailability of resveratrol by taking liposomal or nano-resveratrol, as well as along with foods rich in fats.

Curcumin is a plant-derived polyphenolic compound found in the rhizomes of *Curcuma longa*. Curcumin can be used as a biologically active food supplement with protective, in relation to free radical oxidation, and antioxidant properties [1]. According to the current legislation of the EAEU, the adequate daily dose of curcumin for a person is 50 mg, the upper allowable dose is 150 mg. The results of studies [11] demonstrated the health-promoting effects of curcumin in healthy middle-aged people. Materials published in the databases PubMed, Web of Science, Google Scholar, platform eLIBRARY.RU for 2008-2020 testify to the immunotropic, anti-inflammatory, antioxidant effects of curcumin.

Melatonin is a hormone produced by the pineal gland. It supports antiviral immunity and also helps control NLRP3 (cryopyrin). Melatonin is produced in the dark, mostly between 2-3 am. Melatonin synthesis decreases with age, which may be one of the factors influencing the outcome of COVID-19 in elderly patients. Melatonin is able to prevent infection with the SARS-CoV-2 coronavirus, the development of a severe form of COVID-19. Melatonin is found in cherries, cherries, and bananas.

As the pandemic continues, many people continue to work remotely and try to minimize social contact. In conditions of prolonged stay at home and a high probability of infection, general recommendations for maintaining a nutritious diet can be extrapolated to the entire population in order to maintain the immune system and body resistance to infection, as well as reduce the risk of forming incorrect food stereotypes [9].

CONCLUSION

Optimal nutrient intake in diets influences the immune system through gene expression, cell activation, and modification of signaling molecules. Various food ingredients are also determinants of the microbial composition of the gut, subsequently shaping the body's immune responses. Using the method of literary research, it has been established that vitamins C, D, A and E, minerals – selenium, zinc, biologically active substances – resveratrol, turmeric flavonoids, melatonin play an important role in the prevention of coronavirus infection. With immunomodulatory, anti-inflammatory, antioxidant, and antiviral properties, these nutrients and bioactives are promising alternative nutritional approaches to combat COVID-19.

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**ULOGA BIOLOŠKE I NUTRITIVNE VREDNOSTI ISHRANE
U PREVENCIJI INFEKCIJE KORONA VIRUSOM**

Sažetak

Nedostatak određenog broja hranljivih materija u ishrani može poremetiti hemijske, strukturne i regulatorne procese u organizmu, što negativno utiče na stanje imunog sistema. Svrha preglednog članka je da se razmotri uloga hranljivih materija i niza biološki aktivnih supstanci u poboljšanju imuniteta za prevenciju COVID-19 u populaciji. Kao rezultat analize stranih i ruskih naučnih publikacija, utvrđeno je da hranljive materije kao što su kompletni proteini, vitamini A, C, D, E, minerali – selen, cink, biološki aktivne supstance - resveratrol, flavonoidi kurkume, melatonin igraju važnu ulogu u prevenciji infekcije koronavirusom, i imaju imunomodulatorna, antiinflamatorna, antioksidativna i antivirusna svojstva.

Ključne reči: *hranljive materije; imunitet COVID-19; virus korona zaraza; biološki aktivne supstance*



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Original Scientific paper

CURRENT ISSUES ON LESSER-KNOWN FIELD CROPS FOR FOOD SAFETY PURPOSES

Abstract

Current agricultural systems stress the environment and degrade biodiversity, making it difficult to ensure safe food, human health, conservation, and climate change. Nevertheless, agriculture as a source of nutrients and income must not be interrupted and abandoned. Modern agriculture and food production have negative impacts on the environment and do not satisfy nutritional recommendations for a healthy diet. The challenge for modern civilization is to produce enough healthy food in a sustainable manner at affordable prices. In contrast, the use of lesser-known field crops has implications for diversified production. This enables a healthy diet, reduces poverty levels, increases income, and improves health.

Key words: *Agrobiodiversity, alternative crops, sustainable production, healthy food*

INTRODUCTION

FAO (Food and Agriculture Organization of the United Nations) recognizes that food production must take into account the conservation of natural resources in the countries in which it operates and therefore supports programs that promote conservation and assist in the development of codes of conduct for responsible fishing and farming practices that help streamline activities, access, and/or linkages to them (Food ..., 2020).

One of the challenges currently facing the entire planet is climate change, which affects sowing and harvesting times, and thus yields. Predicting the future development of agriculture will therefore be one of the most important areas of our work.

Current trends pose numerous challenges to safe food and agriculture. Resource-intensive agricultural systems that have led to widespread deforestation, water scarcity,

soil depletion, and high greenhouse gas emissions cannot ensure sustainable food and agricultural production. Innovative systems must be developed to protect and enhance natural resources while increasing productivity. This requires a transformation process toward "holistic" approaches such as agroecology, agroforestry, climate-smart agriculture, and conservation agriculture that also draw on indigenous and traditional knowledge. Technological improvements combined with drastic reductions in the use of fossil fuels in business and agriculture would help combat climate change and the increase in natural hazards that affect all ecosystems and all aspects of human life. Greater international cooperation is needed to prevent emerging transboundary threats to agriculture and food systems, such as pests and diseases (FAO, 2017).

Today's agriculture, including fisheries and forestry, is anything but sustainable from a global perspective. Much of human progress comes at a significant environmental cost. To produce more food and other agricultural goods, a combination of increased agricultural production methods and deforestation has damaged natural resources and contributed to climate change. If we continue to address these challenges with a "business as usual" approach, the future does not look promising. Sustainable food and agriculture systems cannot be achieved without significant additional effort and change. Nevertheless, there are ways to address these challenges, but they must be carefully considered. Food and agricultural systems can take alternative paths depending on the evolution of numerous factors, such as population growth, dietary habits, technological advances, income distribution, natural resource condition and use, climate change, and conflict prevention and resolution. These pathways can and will be influenced by strategic and policy decisions. Swift and focused action is needed to ensure the long-term sustainability of food and agricultural systems. The future is uncertain, but to act now, we need a good sense of what the world might look like along potentially different pathways (FAO, 2018).

It is therefore essential to shift agricultural practices to more sustainable ones (Adegbeye et al., 2020). Today, rice, wheat, and maize account for 50% of the calories consumed daily. 12 crops and 5 livestock species provide 75% of the world's food. Agriculture is largely evolving away from the diverse, nutrient-rich crops domesticated by earlier civilizations. The reasons for these changes are complex (Hunter et al., 2019).

Switching to healthier foods would help reduce pollution. Food diversity contributes to the natural wealth of a country of which we are not sufficiently aware (Hunter et al., 2019).

PROBLEMS IN THE CULTIVATION OF LESSER-KNOWN FIELD CROPS

A modified approach to plant production

Agricultural development and the industrialization of food production have reduced diversity on farms and in the food chain. This in turn has led to a decline in biodiversity and ecosystem services that are essential for food production. Specialization in the cultivation of a particular crop is not a new doctrine, it goes back to the earliest times

of species domestication. Each of the major agricultural revolutions has contributed to a reduction in the range of cultivated crops (Mehrabi et al., 2022). The diversity of cultivated crops in agriculture refers to the simultaneous cultivation of different plant and animal species on a given area. This system can be called agrobiodiversity, which has a positive impact on ecosystem services and reduces the need for external fertilizers or sprays. It relies on crop diversity as a mechanism to sustainably increase yields (Garibaldi et al., 2016). The decline in cultivated crops has led to a situation where food security depends on only a handful of cultivated crops (maize, rice, and wheat) that provide 50% of the world's caloric needs. Dependence on only a few crops leads to food shortages and threatens food security (Pirzadah and Malik, 2020).

The emphasis must return to improving the nutrient content of crops rather than maximizing yield. At the same time, greater diversity in production systems is needed. This will reduce pressure on the environment and improve food security (Hunter et al., 2019).

Modern biotechnology and the improvement of lesser-known crops can bring us closer to ensuring safe food. Currently, widespread production of lesser-known crops is hindered primarily by poor yields, ill-adapted farming methods, limited markets, and breeding strategies (Mir et al., 2022).

Globally, lesser-known crops are better adapted to local environmental conditions. As a result, they contribute to the diversification and resilience of local agricultural systems. At the same time, they tend to be less demanding in terms of fertilizer and pesticide use. Often, more physical labor is required (Hunter et al., 2019).

Sustainable food production requires a shift toward crops that require less water to grow, are more tolerant of abiotic stresses, and have good yields or favorable nutrient composition relative to inputs. By increasing demand, we encourage farmers to choose to grow a particular crop. In this case, they also make sure that they use high quality inputs (seeds, fertilizers, sprays). This also leads to higher yields and incomes (Hossain et al., 2022).

Seeds:

Seed production is quickly limited when governments do not encourage the cultivation of a particular crop. This puts lesser-known crops at even greater risk. Seed producers and consumers determine which crops and varieties are sold and developed and how they are adapted to local conditions. Currently, there is a global struggle to privatize seeds by large corporations. This prevents local people from producing their own seeds. Both natural and cultural diversity is being lost. This leads to even greater economic problems for farmers (Gerrano et al., 2022).

Limited production of lesser-known crops:

Among the lesser-known crops, some are also drought tolerant. Some even have favorable amino acid composition (Adegbeye et al., 2020). Lesser-known crops are grown in limited quantities due to lower yields, water scarcity in growing areas, lack

of hybrids and varieties with improved properties, poor soil fertility, and losses to pests and diseases (Gerrano et al., 2022).

Yields can be increased by breeding hybrids and precision farming systems. Better yields could also be achieved through improved knowledge that enables farmers to sow/plant their crops at the most appropriate time. Other measures could also be optimal soil conditions before seeding and adequate nutrient supply to the plants during the growing season. Optimal growth and yield could also be achieved by establishing irrigation systems and pest control. Of course, good agricultural practices such as crop rotation and optimal harvesting methods should not be forgotten. For all these measures, the farmer needs financial resources, which primarily means having access to affordable credit. Crop perishability is also an issue. Farmers do not want to produce perishable food if they do not have the possibilities to store it properly and sell it quickly (Gerrano et al., 2022; Pirzadah and Malik, 2020).

A cost effective biotechnological solution would be to breed hybrids that mature faster and consume fewer resources to do so. Locally adapted, specialized cultivation techniques for growing lesser-known crops would also have a global impact (Adegbeye et al., 2020).

Ensuring food security

Adequate intake of macro- and micronutrients is an important factor for good metabolism and health. Nutrient deficiencies lead to dysfunction and the development of diseases. The quantity and quality of food available for consumption is influenced by environmental factors (Mir et al., 2022). The number of undernourished people continues to increase. About two billion people do not meet their needs for micronutrients such as vitamin A and iron, which are necessary for optimal body development and functioning. On the other hand, the proportion of obese and over-nourished people is increasing as well. More than 600 million adults are overfed. No less than 3 out of 4 deaths are caused by non-contagious chronic diseases resulting from poor nutrition and unhealthy lifestyle. The current food production system produces a large amount of food that is insufficient to meet all nutritional needs. Traditional crops have a higher nutritional potential. By bringing these products back into the diet, we can reduce food insecurity and provide a local, sustainable, and culturally appropriate diet (Hunter et al., 2019). Lesser-known and native crops are also a good solution in years when yields of commercially attractive crops are reduced due to unfavorable environmental conditions (Gerrano et al., 2022).

In recent years, there has been a trend toward global standardization of eating habits that has led to a loss of diversity in food sources. Many nutrient-rich sources such as millet, sorghum millet, sweet potatoes, yams, and cassava have been pushed out of the modern diet at the expense of increasing production and consumption of wheat, maize, and rice. Lesser-known crops are those that are neglected by farmers, producers, policy makers, and researchers (Hunter et al., 2019).

Genetic diversity of foods helps address vitamin and mineral deficiencies in the diet. Lesser-known crops also contribute to the intake of complex carbohydrates, proteins, and plant metabolites. Secondary plant metabolites have health benefits as

they can lower blood pressure, are involved in cholesterol metabolism, and have anti-oxidant and antidiabetic effects (Mir et al., 2022). Therefore, global dietary guidelines recommend the consumption of a wide range of foods. However, it would be useful to include recommendations for the use of lesser-known crops in the dietary guidelines (Hunter et al., 2019).

Quinoa and amaranth are two of the lesser-known crops that have made a major breakthrough in recent years, primarily because they have an extremely favorable protein composition similar to that of cow's milk. At the same time, they are also free of gluten, which has been unnecessarily avoided by many in recent years (Mir et al., 2022).

Lesser-known crops, especially pseudocereals, have great potential for producing functional foods to combat "hidden hunger" or micronutrient deficiencies in the diet. Pseudocereals such as buckwheat, millet, quinoa, teff, and amaranth are rich in polyphenols, flavonoids, fiber, vitamins, minerals, antioxidants, and amino acids. Pseudocereals are also good for the diet because they contain less starch than conventional grains. Instead of starch, they provide energy in the form of protein and fat, which are essential for humans. They are well adapted to unfavorable conditions and are suitable for cultivation on less fertile soils. At the same time, growing pseudocereals would provide economic benefits and ensure food security in the face of climate change (Pirzadah and Malik, 2020).

In some parts of the world, lesser-known crops have been nicknamed "food for the poor." The unpleasant connotation and the rise in economic status are reasons for increasing reliance on animal foods. In particular, some legumes are also referred to as "nonprofit crops," although farm-gate market prices for legumes can be higher than those for grains. In several regions of the world, there is a trend toward substituting animal products, especially meat, for traditional foods. Some authors refer to this trend as the "livestock revolution." The question arises to what extent it will be possible to offer meat on the market. Meat production requires a lot of feed. It generates large amounts of greenhouse gasses. Excessive meat consumption contributes to non-contagious chronic diseases (Gerrano et al., 2022).

"Organic" products:

A study by Velez-Terreros et al. (2021) found that consumers perceive "organic" products as healthier and more environmentally friendly. The results of the study showed that there were no differences in health benefits between "organic" and conventional products. Labeling contributed to consumers' willingness to pay more for such products. It was also found that consumers attributed a better, fuller taste to "organic" products.

Insects:

High-quality protein can also be obtained from insects. Insects processed into feed are less harmful to the environment than whey, eggs or fish meal. Raising insects does not require large amounts of fertilizer, water or land. Insects can even be successfully grown on kitchen and agricultural waste (Adebeye et al., 2020).

Social aspects of using lesser-known crops for food

Population growth and climate change will increase the likelihood of insufficient food production and associated food and nutrient shortages. Lesser-known crops provide a nutrient-rich alternative to crops of commercial interest (Mir et al., 2022). Yield increases are not necessarily the solution to ending hunger and ensuring food access. Today, enough food is produced globally to feed everyone, but it is not adequately distributed. Interestingly, yields in areas most affected by nutrition problems have not changed significantly for more than half a century (Garibaldi et al., 2016).

Some of the reasons for the loss of food diversity can be attributed to the neglect of lesser-known crops by researchers and the interconnections between health, agricultural, and education sectors on this topic. Infrastructure and market interconnections for lesser-known crops are also often inadequate. Tariff restrictions and strict safety regulations hinder access of lesser-known crops and their products to the global market (Hunter et al., 2019). An even greater problem is the growing urban population and its rising economic status, which is changing people's food habits. It is evident that as economic status increases, more animal foods, especially meat, are consumed. The consumption of starchy foods is decreasing (Gerrano et al., 2022).

A revival and renewed interest in lesser-known crops could be stimulated by including these foods in school lunch programs and food procurement systems. This would support local agriculture, shorten supply chains, preserve cultural landscapes, and encourage decision makers to work to preserve diversity. Celebrity chefs could also play a role in promoting and publicizing lesser-known crops by preparing dishes using these foods in cooking shows or online publications. Events such as markets and stalls featuring local dishes and visits to growers also show good results (Hunter et al., 2019).

Lesser-known crops should be better marketed rather than just creating highly specialized markets for them (Hunter et al., 2019).

CONCLUSION

Legislation on lesser-known crops and legislative action to promote diversity should aim to increase the availability of lesser-known crops at a reasonable price. When an issue becomes part of the public policy debate, it helps raise awareness and promote the product. At the same time, policies should be developed that incorporate diversity into all aspects of nutrition, from education to the political agenda. Laws and policies should promote diversity already on the farm level. At the same time, a review of which crops are adapted to which biotic and abiotic stressors should be conducted, and the use of the most adaptable crops should be encouraged. A review of crop adaptations to stress would also facilitate the selection of crops with which to conduct biotechnological breeding processes (Hunter et al., 2019).

The successful use of lesser-known crops in nutrition would require an expansion of research in this area and its scope. The results should be publicized by educating school children and by including these crops in dietary guidelines. Policy guidelines should be developed to encourage the cultivation of lesser-known crops and increase

agricultural diversity. Locally grown and culturally appropriate lesser-known crops should then be included in school meals to ensure a sustainable and nutritious meal for school children.

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Оригинални научни рад

АКТУЕЛНА ПИТАЊА О МАЊЕ ПОЗНАТИМ ВРСТАМА ХРАНЕ У СВРХУ БЕЗБЕДНОСТИ ИСХРАНЕ

Резиме

Садашњи пољопривредни системи наглашавају животну средину и деградирају биодиверзитет, што отежава осигуравање безбедне хране, здравља људи, очувања и климатских промена. Ипак, пољопривреда као извор хранљивих материја и прихода не сме бити прекинута и напуштена. Савремена пољопривреда и производња хране негативно утичу на животну средину и не задовољавају нутритивне препоруке за здраву исхрану. Изазов за савремену цивилизацију је произвести довољно здраве хране на одржив начин по приступачним ценама. Насупрот томе, употреба мање познатих ратарских усева има импликације на разноврсну производњу. Ово омогућава здраву исхрану, смањује ниво сиромаштва, повећава приход и побољшава здравље.

Кључне речи: *Агробiodиверзитет, алтернативни усеви, одржива производња, здрава храна*

**NUTRITIONAL FOOD VALUE
AND QUALITY NUTRITION**



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Original Scientific paper

IMPORTANCE OF BROAD BEAN IN THE DIET – POSSIBILITY OF MORE RATIONAL USE OF MAIN AND BY-PRODUCTS

Abstract

Broad bean is rich in quality proteins, which is why we use it in the diet of humans and domestic animals. Ripe bean seeds can be used as a stew or ground, and flour mixed with grain flour in a certain percentage is an excellent raw material for making various bakery products. The aim of this study is to point out the benefits of improving broad bean production in the Balkans use of its main and by-products. In addition to the main product, mature grain, there are harvest residues that have multiple applications.

Key words: *biomass, broad bean, by - products, nutrition, climate change*

INTRODUCTION

Broad bean (lat. *Vicia faba* sect. *Faba* Cubero; *Faba vulgaris* Moench; eng. Broad bean; fra. *Fève*; rus. *Kormovie bobi*) is an annual plant from the legume family (*Fabaceae*), genus *Faba*, with large seeds used in the diet as well as beans. The genus *Faba* has one cultural species – *Faba vulgaris* – cultural broad bean and one wild species that grows in Algeria – *Faba pliniana* (Murat). According to archaeological data, the cultivation of this plant began about 8,800 years ago in the eastern

Mediterranean. The largest areas are in China (806,858 ha), followed by Ethiopia, Morocco, Peru, etc. In Serbia, broad bean is grown on small areas, mostly in backyards. In addition to the main product, which is usually mature grain, there is harvest waste that is used in a similar way as other grain legumes. For human consumption, unripe or ripe grain are used. The work of breeders has created several types of broad bean that differ in the way they are used (vegetable and livestock). With the introduction of beans into production, it was pushed to the narrower Mediterranean area, but also to northwestern Europe. Cultivated beans are divided, according to the size of the seeds, into three varieties: large-seeded beans – *Faba fulgaris* var. major, medium-sized (horse bean) – *Faba vulgaris* var. equina and small-seeded beans – *Faba vulgaris* var. minor. Large-seeded broad bean is more of a garden crop. The weight of 1000 grains is 800-1200 g, and sometimes up to 2400 g. Small-seed and horse beans are grown as field crops and are used to feed livestock. They differ in seed size. Small-seeded beans have a weight of 1000 grains, 300-650 g, and horse beans 650-800 g (Lakić et al., 2018; 2019). Broad bean is cultivated for seeds rich in quality proteins. We use it in the diet of humans and domestic animals. Broad bean seed is suitable for canning (Lakić et al., 2018). Broad bean is used as vegetables. Young legumes or seeds, rich in nutrients, are used in the diet. 100 grams of mature raw seeds contain over 25 grams of protein, carbohydrates, fiber and minerals. In folk medicine, broad bean seeds are used as a diuretic, expectorant or as a tonic. In addition to the great nutritional value, these seeds also contain a significant percentage of anti-nutrients that are decomposed by heat treatment. People who have problems with glucose-6-phosphate dehydrogenase (G6PD) deficiency, may have a broad bean seed allergy. Broad bean is rich in the amino acid L-dopa, which is a precursor of dopamine, so it is recommended for people with Parkinson's disease, because their body weakly produces dopamine. Harvesting leftovers can be used as coarse fodder. The grain of small-seeded varieties of broad bean is used mainly in the diet of ruminants and non-ruminants, then caged birds, and that without prior peeling. The harvest residues of broad bean are suitable for composting because they contain a higher percentage of nitrogen compounds. Chopped can be used as mulch in the fields or as a mat for domestic animals. From the agronomic point of view, the best way is to plow the crop residues, especially in conditions when farmers have less and less quality manure or compost at their disposal. Broad bean is also of great agro-technical importance, because, like all nitrogen-collecting plants, it enriches the soil with mineral nitrogen compounds. In recent times, it is increasingly serving as a raw material for the production of bio-fuels (Lakić et al., 2018). One of the significant possibilities of reducing the emission of greenhouse gases is the use of plant residues from agricultural production for energy purposes (Popović et al., 2020a, 2020b). Varieties of small-seeded and medium-seeded broad bean are grown as fodder plants and their seeds. We grow broad bean as a fodder plant, most often in combined sowing with millet-like cereals, and we make silage from above-ground biomass. Harvest residues of broad bean straw, have up to 10% of total protein and are suitable coarse food for domestic ruminants. In some countries, straw is briquetted after the broad bean harvest and used as a heating material. Broad bean is also of great agro-technical importance, is nitrogen-collecting plants, they enrich the soil with mineral nitrogen (Lakić et al., 2018).

The time of harvest determines the further procedure of using the grain. The broad bean reaches the stage of maturity, depending on the type of variety and the method of use, in 85 to 200 days from the emergence of plants. Broad bean grown in combined sowing with cereals is used to make silage and is mowed with forage harvesters when the lower pods on the trees are in the phase of milk maturity. Mown biomass is ensiled just after mowing. The broad bean ripens quite unevenly and the seeds from the lower pods are shed, so in some countries, to accelerate ripening, the crops are treated with desiccants at 10-15 days before the harvest. Dried seeds break a lot during threshing, despite the necessary adaptations that we perform on harvesters before harvesting. On small areas, broad bean can be harvested in two phases, first mowing the plants, drying them in the swaths and then threshing with harvesters (Lakić et al., 2018; Janković et al., 2019). The aim of this paper is to examine the possibility of more rational use of broad bean and harvest residues of it in order to protect the environment.

MATERIALS AND METHODS

Bean sowing is done in early spring because it tolerates low temperatures well. In our conditions, sowing should be done in mid-March. The amount of seeds depends on the size of the seeds and the number of plants per unit area. The number of plants depends on the variety (more in small seeds and vice versa), and especially on the amount of precipitation in the growing area. In drier regions, 20-40 plants per m² are sown, depending on the variety, and in wetter areas, the number of plants per m² increases to 40-60 plants. Based on the number of plants, the weight of 1000 grains and the use value, it is easy to calculate the amount of seeds in kg ha⁻¹. Sowing is done in a wide row, with a distance between rows of 50-60 cm. If the broad bean is used for silage, the width of the rows is smaller 30-40 cm. Depth of sowing depends on the type of soil and varies from 5-8 cm. The data presented in the paper are presented in Tables and Figures.

RESULTS AND DISCUSSION

Legumes include various types of vegetables such as beans, peas, broad bean, green beans, *Cicer arietinum* (chickpeas), peanuts, lentils (lentils) and soybean. They are nutritious and energetically strong, they are a source of protein and complex carbohydrates, they are rich in minerals such as iron, phosphate, potassium, calcium, magnesium and zinc, B vitamins, as well as fully or partially soluble fiber, which in intestines form a gel. Legumes are digested slowly and slowly turn into blood (Glamočlija et al., 2015). The main grain legumes (60), information on distribution and consumption are shown in Table 1.

Productivity of broad bean. The yields of mature broad bean are 3,000 to 3,800 kg ha⁻¹; the yields of aboveground biomass depend on the ratio of broad beans and sown cereals. In favorable conditions of the water regime, 50 to 80 t ha⁻¹ can be achieved (Lakić et al., 2018).

Table 1. The main grain legumes, with information on distribution and consumption, Ildikó Schuster-Gajzágó 2022, <http://www.eolss.net/sample-chapters/c10/e5-02-02.pdf>

Common name	Latin name	Distribution	Consumption
Soybean	<i>Glycine max</i> L.	USA, Brasil, China, Argentina, Japan	Human consumption, mainly processed products (soy meal, concentrate, isolate, soy milk, fermented products), animal feed
Faba bean	<i>Vicia faba</i> L.	Central Asia, South America, Europe, ...	Human consumption, and canning, freezing The dry harvested seeds are used as animal feed.
Common bean	<i>Phaseolus vulgaris</i> L.	India, Brazil, France, UK, Russia, German, Ukraine	Human consumption green in pods (canning, freezing) or dry seeds.
Cowpea	<i>Vigna unguiculata</i> L.	Mediterranean area, Africa, Asia	Human consumption, it is eaten as dhal made from soaked, dehulled seeds
Pea	<i>Pisum sativum</i> L.	Europe, North America	Human consumption, combining crop-animal feed

After harvesting, seeds should be dried to 10-14% of water and stored in warehouses where fumigation must be done, to protect them from barn pests. Malathion, Aluminum phosphide, Methyl bromide (Haltox preparation) or some other insecticide fumigant is used for fumigation. The yield of harvest residues of grain legumes, in addition to time and method of harvesting, is influenced by other factors, such as species, variety, method of cultivation, applying of agro-technics, agro-ecological conditions and so on (Lakić et al., 2018).

Chemical composition of broad bean seeds – Broad bean seeds contain 25-35% protein, depending on the variety and growing conditions. In addition to protein, it contains about 50% no-nitrogen extractives and up to 2.0% oil. Straw contains 7-20% protein. Broad bean is used for human consumption, for livestock nutrition and for vegetable fertilization. Mature grain is ground, and flour is used as a protein component in concentrated feed mixtures for cattle, especially for calves. Broad bean is grown as a side crop, but it can be grown in a mixture with corn for silage. Good silage is made from broad bean. The chemical composition of legumes is shown in Table 2. Similar to bean seed is bean seed which contains 22.6% protein, 42.2% starch and 1.8% oil, tab. 2. Broad bean has partially soluble fiber, and due to the combination of fiber and folic acid, it reduces the risk of stroke, as well as heart and blood vessel diseases. It is recommended for diabetics and pregnant women, but not for kidney patients. However, the shell contains pectin, which is difficult to digest, so broad bean is avoided in diseases of the digestive organs. This is one of the favorite foods of vegetarians because it contains a large amount of protein. It does not contain gluten, so it is also suitable for the diet of people with celiac disease. In many people, broad bean cause bloating and gas, so to alleviate this, it would be desirable to use spices such as cumin, mint leaves, ginger and garlic.

Table 2. Chemical composition of grains legume, %

No	Plants	Protein %	Oil %	Starch %	Cellulose %	Mineral salt, %
1.	Faba bean- <i>Vicia faba-F. vulgaris /bob</i>	25.1	2.0	50.1	6.3	1.5
	<i>Phaseolus vulgaris /obični pasulj</i>	22.6	1.8	42.2	23.0	4.1
2.	Pea – <i>Pisum sativum / grašak</i>	23.0	2.5	55.0	5.6	3.1
3.	Grass pea- <i>Lathyrus sativus / sastrica</i>	31.0	20.0	41.0	10.1	1.3
4.	Cowpea- <i>Vigna unguiculata / vigna</i>	25,5	1.7	40.5	4.3	3.5
5.	Lentil – <i>Lens culinaris / sočivo</i>	28.9	2.1	50.1	7.9	2.4
6.	<i>Cicer arietinum / Naut</i>	23.8	4.3	44.5	9.8	3.3
7.	<i>Lupinus luteus / žuta lupina</i>	42.0	4.5	42.5	6.0	3.0
8.	<i>Arachis hypogaea / peanut</i>	25.4	52.2	19.2		
9.	Common vetch- <i>Vicia sativa; grahorica/hay-dm</i>	24.0	1.2	37.0	4.5	3.4
10.	Soy – <i>Glycine max /soja</i>	38.5	20.1	32.5	6.7	4.1

Source: <http://www.feedipedia.org/node/4926>; Glamočlija et al., 2015.

Legumes are rich sources of protein as the seeds contain 200-250 g protein/kg. The protein content of cooked legume seed (70-100 g/kg cooked food) is similar to that of bread (80-90 g/kg), but still much higher than for potato (15-22 g/kg). Legume seeds are rich in lysine and poorer in sulfur-containing amino acids (methionine and cysteine) compared to cereals. Lysine is the first limiting amino acid so it is important that legumes complement cereals in lysine balance. Legume proteins are composed of several

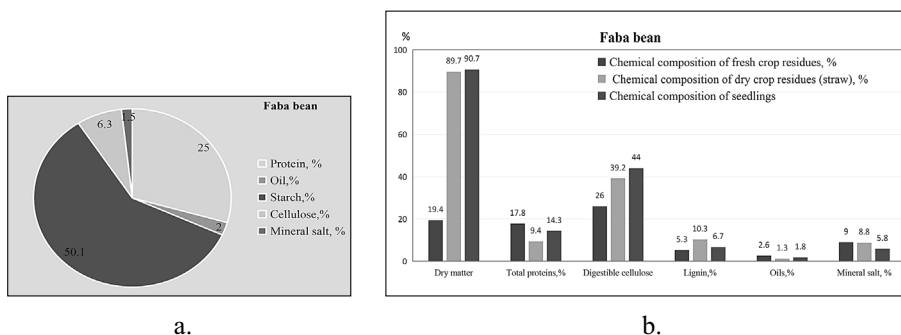


Figure 1. Chemical composition of Faba bean grain (a.) and of fresh and dry harvest residues (b.)

thousand specific proteins. About 70 to 80 % of the crude protein in legume seeds is storage protein. The non-storage proteins are enzymes, enzyme inhibitors, hormones, transporting, structural and recognition proteins (Ildikó Schuster-Gajzágó 2022).

The chemical composition of the harvested residues of grain legumes depends on the time of harvest, which, depending on the way the grain is used, may be in technological maturity (occurs at the time of pod formation), then in the doughy or full maturity phase. The yield of broad bean in the world is over 1.6 t / ha. The highest yields are in Europe and Asia (5-2.5 t / ha), and the lowest yields are in South and North America and Africa (600-800 kg / ha). Harvest biomass has the highest nutritional value during pod formation (Table 3, Fig. 1b).

Table 3. Chemical composition of fresh and dry harvest residues of Faba beans, %

Parameter	Dry matter	Total protein	Digestible cellulose	Lignin	Oils	Mineral salt
Faba beans bob	Chemical composition of fresh crop residues of legumes, %					
	19.4	17.8	26.0	5.3	2.6	9
	Chemical composition of dry crop residues of legumes, %					
	89.7	9.4	39.2	10.3	1.3	8.8
	Chemical composition of legume seedlinds, %, Constanza et al. (2012)					
	90.7	14.3	44.0	6.7	1.8	5.8

Source: <http://www.feedipedia.org/node/4926>

With the physiological maturation of plants, the nutritional value of harvest residues decreases. If the harvest is done in single-phase with harvesters in the phase of full maturity of seeds in pods, the harvest residues, as a whole, have the lowest nutritional value with increased content of non-nutrients (Table 3).

Remains in the field after harvesting legumes for grain have a great and diverse use value, which is shown by their chemical composition. Harvest residues of all legumes can be used as coarse fodder, but they do not have the same nutritional value.

Producers dry this biomass in the field, then bale it and use it as fodder, which does not lag behind alfalfa hay in terms of quality. Dry biomass of harvest residues of peas, fodder peas, lentil and broad bean grown for grain has a higher nutritional value, while in other legumes it is less usable due to the higher content of non-nutritious substances (lignins, hemicelluloses and silicates). If used for certain species of domestic animals (sheep or cattle) it should be treated with additives – ammonia or calcium hydroxide to increase the coefficient of digestibility. Harvest residues are suitable for composting because they contain a higher percentage of nitrogen compounds. Chopped can serve as mulch in the fields as a mat for domestic animals. At recent times, it is increasingly serving as a raw material for the production of bio-fuels. From the agronomic point of view, the best way is to plow the crop residues, especially in conditions when farmers

have less and less quality manure or compost at their disposal. This biomass in the country decomposes quickly due to the increased participation of organic nitrogen compounds.

Fresh harvest leftovers of broad bean. After the successive manual harvesting of unripe broad bean green pods, 20-30 t ha⁻¹ of vegetative biomass remains in the field, which consists of green trees, leaves and unharvested small, unripe pods. This biomass in total dry matter has 14-20% of total proteins (Alibes et al., 1990). The amount and digestibility of proteins depend on the share of leaves in the total yield of these harvest residues (McVicar et al., 2013). If it is planned to use harvest residues as fodder, the biomass should be cut as soon as possible after the pod harvest, because its nutritional value decreases every day. Mowing is best done with a forage harvester. Mowed biomass can be used fresh as fodder. However, the use of fresh biomass of crop residues in the diet of domestic ruminants reduced the utilization rate of dry matter, total protein and cellulose (Louw, 2009). Therefore, it is more expedient to use it to make silage. Baddeley and Walker (2014) point out that vegetative biomass of broad bean is low in sugars and in order to produce quality silage, it is necessary to mix it with grasses or biomass of some cereals (for example corn, sorghum or millet). This biomass can be used as mulch or used for ploughing in. Easily degradable organic compounds decompose rapidly in the soil and release significant amounts of nitrogen and potassium.

Leftovers of broad bean harvest. Broad bean is a legume that has multiple uses in the diet of domestic animals and humans. Legumes, unripe and ripe grain serve as food for humans, while the main product and all leftovers can be used in the diet of domestic animals, starting with the harvest residuals, ie straw. Residues remaining after machine harvesting of mature grain participate in the total biomass with more than 60%. According to chemical analyzes, they are rich in total proteins, digestible celluloses and mineral salts, but also a large amount of indigestible compounds that diminish the nutritional value of this bulky fodder. In order to increase the value of straw, as a bulky fodder, it should be chopped and treated with additives before use to neutralize hemicelluloses and tannins (McVicar et al., 2013). Other ways to use large biomass of crop harvested residues are to obtain bio-fuels. Above-ground biomass after pod harvesting is very suitable for use for bio-energy purposes. It is rich in celluloses which are technologically converted into soluble sugars, the basic raw material for the production of alcohol – ethanol or for the production of biogas. Petersson et al. (2007) state that the technology of obtaining bio-ethanol and biogas from lignocellulosic broad bean bio-mass, has been developed in Sweden. Harvesting residues are ploughing in, in order to increase soil fertility, and in some countries this plant is also grown as a cover crop or siderate to repair the structure and chemical composition of poor soils.

Residues after grain peeling. Edible broad bean grains are used to prepare basic food. However, they can be ground and the produced flour is mixed with wheat flour for baking bread and bakery products. In order to increase the digestible value of flour, it is necessary to prepare the grain beforehand, which means separating the seed coat. The grains have large amounts of oil. These compounds are first separated and then the remaining mass is ground to obtain defatted flour. The share of the seed coat in the total

mass of grain is different and depends on the type, variety, growing conditions and applied agricultural techniques. This by-product has significant amounts of polysaccharides, proteins and mineral salts. Therefore, it can be used as a concentrated fodder or as a raw material in further industrial processing. The variation in chemical composition is influenced by the more factors.

Seed coat of broad bean. The nutritional and digestible value of large-seeded broad bean grains is significantly reduced by the husk, which accounts for about 12% of the total mass. Before use in the diet of unripe or ripe (dry) broad bean, the wrappers are separated from the grains by soaking and rubbing. This results in a food product with more protein and starch, and less cellulose. The nutritional value of grain is highest in immature seeds (Crépon et al., 2010). Separated shells are rich in digestible celluloses (up to 50% dry matter) and digestible proteins (above 6%), but also tannins. With prior preparation, which includes the treatment of biomass with additives, they can be used in the diet of domestic ruminants. The grain of small-seeded varieties of beans is used mainly in the diet of ruminants and non-ruminants, then cage birds, without prior peeling (Aleksić et al., 1999).

CONCLUSION

Edible grains of broad bean are used for food preparation. The grain can be ground and the flour produced is mixed with wheat flour for baking bread and bakery products. Utilization of secondary residues of broad bean is of great importance. Biomass can be used for the production of compost or earthworms. Another way of using these secondary products is ploughing in during the basic tillage. The grain of small-seeded varieties of beans is used mainly in the diet of ruminants and non-ruminants, then caged birds, and without prior peeling.

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ZNAČAJ BOBA U ISHRANI – MOGUĆNOST RACIONALNIJE UPOTREBE GLAVNIH I SPOREDNIH PROIZVODA

Izvod

Bob je bogat kvalitetnim proteinima zbog čega ga upotrebljavamo u ishrani ljudi i domaćih životinja. Zrelo seme boba možemo koristiti kao varivo ili ga samleti, a brašno pomešano sa brašnom žita u određenom procentu predstavlja odličnu sirovinu za izradu različitih pekarskih proizvoda. Cilj ove studije je da se ukaže na prednosti unapređenja proizvodnje boba na području Balkana, kao i na mogućnosti racionalnije upotrebe glavnih i sporednih proizvoda boba. Osim glavnog proizvoda, zrelog zrna, ostaju žetveni ostaci koji imaju višestruku primenu.

Ključne reči: *biomasa, bob, sporedni proizvodi, ishrana, klimatske promene*



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Original Scientific paper

INFLUENCE OF GENOTYPES AND DIGESTATE ON THE PRODUCTIVITY OF THE MORPHOLOGICAL INDICATOR OF SILAGE-SORGHUM AS HIGH QUALITY FEED

Abstract

Fodder sorghum is used for preparing silage in the phase of milk-wax maturity, when sorghum contains 65-70% water. Sorghum silage is mostly used in the diet of dairy cows. Since sorghum contains a lot of sugar, silage is successfully prepared without the addition of additives. Sorghum forms a large biomass during the year. After each mowing the plants regenerate giving a new swath. The aim of this study is to examine the influence of digestate on sorghum productivity in the conditions of Vojvodina. Digestate had a great influence on plant productivity. Based on the analysis of variance, it is evident that there is a significant difference in the number of leaves per plant in relation to the variant ($F_{\text{ekp}} = 7.118$ *)

Key words: *fodder sorghum, fodder, number of leaves / plants, chemical traits*

INTRODUCTION

Fodder sorghum is an annual crop from the group of cereals, which is grown in Serbia on about 2000 ha. It belongs to the order *Poales*, family *Poaceae*, subfamily *Panicoideae*, genus *Sorghum* which contains 34 plant species. Fodder sorghum is characterized by high yields and high biomass production in our agro-ecological conditions. Most of these species are annual or perennial wild plant species, often

weeds in the fields. The most interesting for cultivation are two annual species and two interspecies hybrids: *Sorghum bicolor* (L.) Moench. – common sorghum, *Sorghum sudanense* L. – Sudanese grass, *Sorghum almum* Parodi – perennial alnum sorghum and *Sorghum bicolor* x *S. sudanense* – interspecies hybrid of common sorghum and Sudanese grasses (Lakić et al., 2018). It is characterized by high yields and high biomass production in our agro-ecological conditions. Green biomass yields range from 50 to 70 t ha⁻¹. It is resistant to drought, diseases and pests. In times of drought, it can stop development without harmful consequences, and as soon as moisture is provided, it continues to develop unhindered. The first swath for green mass, which gives the largest amount of green mass, arrives 50 days after germination, and the next one in 40 days. The yield of green biomass is from 50 to 70 t ha⁻¹, and even 100 tons. For the preparation of silage, sorghum is used in the phase of milk-wax maturity, when sorghum contains 65-70% water. Sorghum contains enough sugar, so silage is successfully prepared without the addition of additives. Sorghum silage is mostly used in the diet of dairy cows. Sorghum can also be used successfully as an energy crop.

The primary task of breeding is to create new inbred lines in order to obtain high-yielding and stable hybrids of good quality (Berenji, 1990). The goal of the selection is to create two types of sorghum hybrids: hybrids for animal feed and energy hybrids. The results of the analysis of yield and yield components in lines and hybrids are indicators used in selection and correctly direct the selection process towards obtaining hybrids of desirable quality, but also give a clear picture of the mode of inheritance (Debnath & Sarker 1989). Bangarwa et al. (1989) emphasize that selection for dry matter should be based on plant height and number of broad leaves, and it is necessary to pay attention to odder quality, because yield and quality determine the value of the genus *Sorghum* in ruminant nutrition. Domestic sorghum hybrids are mostly used for sowing in our country, but also hybrids of foreign seed houses. The Domestic Seed House Institute of Field and Vegetable Crops, has three varieties of fodder sorghum: Siloking, Titan and NS Džin with a biomass yield of over 100 t/ha in dry farming conditions (Glamočlija et al., 2015).

NS Džin is a new variety of fodder-sorghum created by crossing the lines of fodder sorghum and Sudanese grass. It is a multi-mowing variety (up to three mowing), with a high genetic potential for forage yield, over 100 t ha⁻¹ in dry farming conditions. It is mowing when the plants are 1.0-1.5 m tall. It is characterized by fast initial growth, excellent tillering and good regeneration after mowing. Siloking is a medium-late hybrid of sugar sorghum, long vegetation and high yields of green fodder and silage, suitable for late mowing and ensiling. It is resistant to economically significant diseases. The stem remains juicy until the end of vegetation, and is an excellent raw material for making silage in bio-digesters for biogas production. It achieves very high biomass yields, up to 110 t/ha. The domestic genotype Siloking has a fat content of 2.48%, a raw ash content of 11.60%, a cellulose content of 32.79% and BEM, and a dry matter content of 41.27% (Pataki et al., 2010). Titan is a new multi-mowing variety of fodder-sorghum, created by crossing the lines of fodder-sorghum and Sudan grass. The variety gives up to 3 mowing, high genetic potential for forage yield. In the conditions of dry farming, it produces up to 80 t ha⁻¹ green fodder. This variety rege-

nerates well after mowing, has a fast initial growth and excellent tillering. Thanks to the deep threadlike root of strong suction power, fodder sorghum can be successfully grown on different types of soil (salt marshes, sandy and heavier soils), which are less suitable for other plant species. Fodder sorghum tolerates drought and high temperatures well. The value of fodder sorghum will be even more significant if genotypes are found that will surpass silage corn in quality and yield. After mowing, sorghum regenerates from the ground nodes, so it can give a higher yield and better quality from two mowing, because the plants are used in the younger stages. It tolerates drought well, because it has a developed root system with strong suction power, and it uses water and nutrients from the soil, which are inaccessible or difficult to access for other cultivated crops. In the selection of parent lines for creating hybrids, those that have a succulent stem by the end of the vegetation are selected, and the seeds ripen on a green and succulent stem (Pataki et al., 2010).

We are witnesses of more frequent droughts and high daily temperatures (Popović et al., 2020a, 2020b). In such conditions, by sowing fodder sorghum, a high biomass yield is achieved, thus the possibility of obtaining the use of raw materials for bio-fuel. Due to the great importance of sorghum and its application, the aim of the study was to examine two energy hybrids in Vojvodina and the possibility of obtaining biogas and methane yields from sorghum biomass and to determine the impact of digestate, bio-stimulant nutrients on plant height, number of leaves, sorghum biomass and biogas yield.

MATERIALS AND METHODS

During 2021, experiment was set up as randomized block system in 12 repetitions with the size of basic plots of 10 m² (5 m x 2 m) in two variants, at the Ilandža site, on hydrogen-type marsh black soil. Standard cultivation technology for sorghum to obtain silage was used. For germination and sprouting of seeds, a minimum temperature of 8-10 °C is required, which is why sowing was done in the optimal time, at the end of April. Experiments with two genotypes G1-Bulldozer (KWS) and G2-Siloking (Institute of Field and Vegetable Crops, Novi Sad), were set up, in two variants: without digestate – control (C) and variant with digestate, which was introduced into the soil immediately before sowing sorghum – digestate (ADD). In both variants, 115 kgNha⁻¹ (250 kg / ha UREA with 46% N) was applied to the soil. Fodder sorghum hybrids, Bulldozer and Siloking were sown using a seeder for wide crops, at an optimal depth of 3 cm, density 30 kg of seeds ha⁻¹ (250,000 plants per ha), after quality pre-sowing soil preparations. Mowing of plants was performed at the beginning of the broom phase (second decade of July), for the analysis of morphological characteristics of the number of leaves, samples were taken from freshly mowed biomass. The results were analyzed by the method of analysis of variance of one-factor testing (ANOVA) using the statistical Program STATISTICS 12 and presented in tables and graphs. Significance of differences in mean treatment values was tested by LSD test.

RESULTS AND DISCUSSION

Meteorological conditions during the sorghum vegetation period

Depending on the climatic conditions, the variety has different plant production (Popovic et al., 2020a, Ikanović et al. 2013, 2020a, 2020b). Weather conditions for Ilandža were taken from the meteorological station in Vršac (Figures 1a and 1b). The average perennial temperatures, for the sorghum vegetation period, for Ilandža were 20.1 °C and the total vegetation precipitation was 290.2 mm. The vegetation season in 2021 is characterized by a higher amount of precipitation (334.4 mm) by 44.2 mm and lower average monthly air temperatures (18.9 °C) compared to the multi-year average for the Ilandža area (Fig. 1a and 1b).

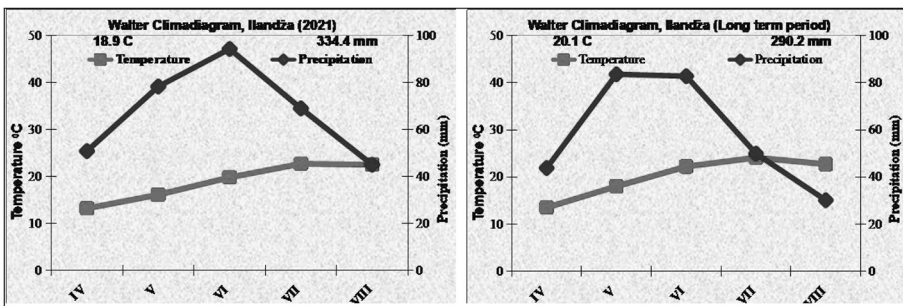


Figure 1. Mean monthly air temperatures (°C) and precipitation (mm) for the sorghum growing season in 2021, a), long term period b)

Morphological parameter: Number of fodder sorghum leaves per plant

The examined morphological-productive trait of sorghum genotypes is shown in Tables 1 and 2. Based on the analysis of variance, it is evident that there is a significant difference in the number of leaves per plant in relation to the variant ($F_{\text{ekp}} = 7.118^*$), (Table 1). Genotype and interaction of examined factors ($V \times G$) did not have a significant effect on the height of the obtained value of the number of leaves per plant ($p > 0.05$).

Table 1. ANOVA for number of leaves

Parameter	SS	Degr. of Freedom	MS	F	p
Intercept-blocks	2268.750	1	2268.750	1601.471	0.000000
Genotype – G	6.750	1	6.750	4.765 ^{ns}	0.060595
Variant – V	10.083	1	10.083	7.118*	0.028455
Genotype×Variant	0.083	1	0.083	0.059 ^{ns}	0.814467
Error	11.333	8	1.417		

The average value of the number of leaves of genotype G1 fodder sorghum varied from 13.66 in the control variant to 15.33 in the variant with digestate, while the average value in genotype G2 varied from 12.00 in the control variant to 14.00 in the variant with digestate. The average value for the number of leaves per plant for genotype G1 was 14.50, while genotype G2 had 13.00 leaves per plant. There were no statistically significant differences between the examined genotypes for the values of the number of leaves per plant, Table 2, graphs 3 and 4.

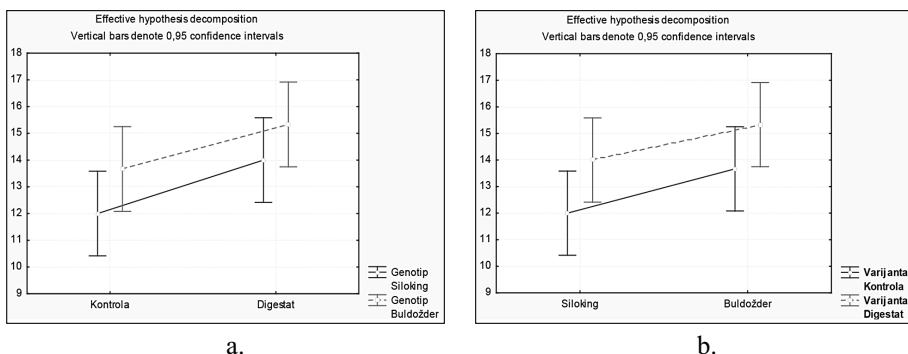
Table 2. Average values of examined morphological-productive parameters of fodder sorghum

Genotype	Year	Variant	Number of leaf
G1-Buldožder	2021	Control	13.66±0.57
		Digestate	15.33±0.58
Average			14.50±1.05
IV			1.67
Std. Error			0.43
G2-Siloking	2021	Control	12.00±2.00
		Digestate	14.00±1.01
Average			13.00±1.79
IV			2.00
Std. Error			0.73
Total Average			13.75±1.61
IV			1.50
Std. Error			1.60

Parameter	Genotype		Variant		G x V	
LSD	0.5	0.1	0.5	0.1	0.5	0.1
Number of leaf	1.585	2.306	1.584	2.305	2.703	3.261

Field trials should enable the selection of the most successful genotypes, which is critical due to the existence of genotype interaction with the environment. To assess this interaction, we are forced to conduct field research in several settings and / or years (Popović et al., 2011; 2013; 2019; Bojović et al., 2019; Lakić et al., 2018; 2019; 2020; Rakašćan et al., 2019; 2020; Mladenović-Glamočlija et al., 2020).

Fodder sorghum hybrids achieve high yields of green mass and dry matter and represent very high quality animal feed. In recent times, fodder sorghum is also used as a raw material for biogas production. In the area of eastern Croatia, in different agro-ecological conditions, fodder sorghum hybrids achieved a dry matter yield of 9.4 to 28.0 tha^{-1} (Gantner et al., 2015).



Graph. 3. Interaction $V \times G$ a.) and interaction $G \times V$ (b.) for number of leaves per plant of fodder sorghum

The nutritional composition of the fresh forage samples is in table 3. Forage sorghum (<https://dellait.com/nutritional-composition-of-sorghum-silage/>) had DM 28.1, starch 17.2%, protein content 5.0% and fat 2.0% prior to ensiling.

Table 3. Nutritional composition of Forage sorghum

No.	Parameter	Forage sorghum
1.	Dry matter (% AF)	28.1
2.	Protein (% DM)	5.0
3.	Neutral detergent fiber (% DM)	55.1
4.	Starch (% DM)	17.2
5.	Non-fibrous carbohydrates (% DM)	34.6
6.	Fat (% DM)	2.0

Neutral detergent fiber was 55.1% in Forage sorghum. Sorghum silage is high in water-soluble carbohydrates, which promote the growth of lactic acid bacteria, responsible for lowering the pH. In addition, sorghum silage has little buffering capacity (Fernandes et al., 2020), so pH reduction is often rapid in the early stages of the conservation process.

Mowed biomass can be stored as haylage, silage or dried. Fresh sorghum biomass can be used for domestic animals, after wizen for several hours to decompose harmful substances. Over 70 t ha⁻¹ of fresh sorghum biomass can be obtained during the year (Lakić et al., 2018).

CONCLUSION

The use of digestate on the soil has led to an increase in the number of leaves per plant. The obtained results showed a strong positive effect of digestate on the number of leaves per sorghum plant, while there was no statistically significant difference between the examined genotypes for the examined parameter. Future research on the morphological characteristics of sorghum is needed, as well as the use of more hybrids to determine which are the most productive and economical to produce.

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UTICAJ GENOTIPA I DIGESTATA NA PRODUKTIVNOST MORFOLOŠKOG POKAZATELJA SILAŽNOG SIRKA KAO VISOKOKVALITETNE STOČNE HRANE

Izvod

Krmni sirak se koristi za spremanje silaže u fazi mlečno-voštane zrelosti, kada sirak sadrži 65-70% vode. Silaža od sirka najviše se koristi u ishrani mlečnih krava. Pošto sirak sadrži dosta šećera, silaža se uspešno sprema bez dodavanja aditiva. Sirak formira veliku biomasu tokom godine, posle svake kosidbe biljke se regenerišu dajući novi otkos. Cilj ove studije je da se ispita uticaj digestata na produktivnost sirka u uslovima Vojvodine. Digestat je imao veliki uticaj na produktivnost biljaka. Pokošena biomasa može se spremati kao senaža, sinaža ili se suši. Sveža biomasa sirka za ishranu domaćih životinja može se koristiti kad provene nekoliko sati da bi se razložile štetne supstance. Tokom godine može se dobiti preko 70 t ha⁻¹ sveže biomase sirka.

Ključne reči: *krmni sirak, stočna hrana, broj listova/biljci, intreakcija godina genotip*



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Original Scientific paper

SOY HULL PECTIC FIBER – HIGHLY VALUABLE FIBER EXTRACTED FROM BY-PRODUCT OF SOYBEAN INDUSTRY

Abstract

Soy hull, a by-product of soybean processing, was investigated as a source of pectic fiber. Pectic fiber was obtained with yield of 5.72 g/100 g_{DW}. Additionally, pectic fiber was characterized and its galacturonic acid content was determined to be 44.32% (wt.) while its degrees of methoxylation and acetylation were low. Investigation of properties of pectic fiber from soy hull revealed considerable water and oil holding capacities as well as notable antioxidative activity. These favorable properties could indicate to a great potential for application in different food matrices.

Keywords: *pectic fiber, soy hull, waste valorization*

INTRODUCTION

Pectins are complex structural polysaccharides consisting mainly of α -(1–4)-D-galacturonic acid. Galacturonic acid in pectin backbone can be partially esterified with methoxyl or acetyl groups (Kalapathy and Proctor, 2001). Pectin is regularly used in food industry as stabilizing, thickening and gelling agent, although it keeps gaining more interest in applications such as functional foods, prebiotics, edible packaging, active substance carrier, etc. (Marić et al., 2018).

Common and the most exploited sources of commercial pectin are citrus peels and apple pomace (Monsoor and Proctor, 2001) but as the demand for pectin continually grows, new pectin sources need to be recognized and better utilized (Baississe and Fahloul, 2018). Soy hull, a by-product of the soybean industry available in large quantities, could be an alternative source of pectin rich fiber. Additionally, soy hull can

be easily stored and transported without further processing thus facilitating its handling while avoiding deterioration of the material (Kalapathy and Proctor, 2001).

The objective of this research was to evaluate soy hull as a potential source of pectin rich fiber. Furthermore, obtained fiber was characterized regarding its galacturonic acid content, degree of methoxylation and degree of acetylation. Lastly, pectin fiber properties relevant for its application in food matrices were evaluated.

MATERIALS AND METHODS

Soy hull was a kind gift from Sojaprotein, Bečej. Pectic fiber was extracted from soy hull in conventional hot-acid extraction, precipitated with ethanol and freeze-dried (Monsoor and Proctor, 2001). Galacturonic acid (GalA) was determined spectrophotometrically with m-hydroxydiphenyl method (Melton and Smith, 2005). The degree of methoxylation (DM) was assayed by spectrophotometric analysis described by Igarashi (1973). Degree of acetylation (DA) was assayed according to Aimanant and Ziemann (2013). The *in-vitro* antioxidant activity was evaluated with ABTS radical-scavenging activity assay (Re et al., 1999). Water holding capacity (WHC) was evaluated according to Bouaziz et al. (2016). Oil holding capacity (OHC) of soy hull pectic fiber was determined by previously described method (Tan et al., 2014).

RESULTS

Pectic fiber was extracted from soy hull and its yield is presented in Table 1. The obtained yield was 5.72 g from 100 g of soy hull dry weight.

Extracted soy hull pectic fiber was characterized regarding its galacturonic acid content, degree of methoxylation and degree of acetylation. Results are given in Table 1. GalA content amounted to 44.32% (wt.). Degrees of methoxylation and acetylation were low – DM of pectin fiber from soy hull was determined to be 6.94% (mol.) and DA was 0.82% (mol.).

Table 1. Extraction yield and characteristics of pectin fiber from soy hull

Yield (g/100 g _{DW})	GalA (% wt.)	DM (% mol.)	DA (% mol.)
5.72 ± 0.07	44.32 ± 1.19	6.94 ± 0.09	0.82 ± 0.01

Additionally, soy hull pectic fiber was evaluated in terms of its water holding capacity, oil holding capacity and *in-vitro* antioxidant activity. Obtained properties of soy hull pectic fiber are presented in Table 2. Both WHC and OHC were high for pectic fiber extracted from soy hull – 22.85 and 9.21, respectively. Antioxidant activity was assayed as ABTS radical-scavenging activity and expressed as trolox equivalents (mM TE/100 g fiber). Soy hull pectic fiber had considerable ABTS radical-scavenging activity of 650.07 mM TE/100 g fiber.

Table 2. Properties of pectin fiber from soy hull

WHC	OHC	ABTS (mM TE/100 g)
22.85 ± 0.67	9.21 ± 0.14	650.07 ± 17.89

CONCLUSION

Highly valuable pectic fiber was obtained from soy hull, a by-product of soybean industry. This fiber has exhibited low degrees of methoxylation and acetylation as well as high water and oil holding capacities and antioxidant activities. Therefore, pectic fiber from soy hull with these favorable characteristics and properties could be suitable for application in different food matrices.

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Originalni naučni rad

VISOKOVREDNO PEKTINSKO VLAKNO EKSTRAHOVANO IZ NUSPROIZVODA PRERADE SOJE

Sažetak

Sojine ljuske, koje predstavljaju nusproizvod prerade soje, su ispitane kao potencijalni izvor pektinskog vlakna. Pektinsko vlakno je ekstrahovano sa prinosom 5,72 g/100 g_{SM}. Ekstrahovano vlakno je zatim okarakterisano pri čemu je određeno da je sadržaj galakturonske kiseline bio 44,32% (m/m), dok su stepen metoksilacije i stepen acetilacije bili niski. Ispitivanjem svojstava pektinskog vlakna je utvrđeno da vlakno poseduje veliki kapacitet vezivanja vode i ulja, kao i visoku antioksidativnu aktivnost. Ova povoljna svojstva ukazuju na veliki potencijal primene ovog vlakna u različitim prehrambenim formulacijama i proizvodima.

Ključne reči: *pektinsko vlakno, sojine ljuske, valorizacija otpada*



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Original Scientific paper

GREEN TECHNOLOGY FOR ENHANCED RECOVERY AND PROPERTIES OF RuBisCO PROTEIN FROM PUMPKIN LEAVES

Abstract

The aim of this study was to evaluate potential of enzymes for the enhanced isolation of RuBisCO protein and upgrading of its biological properties. Pumpkin leaves present waste material that can be used for extraction of this protein. Results showed that applied green protocol, that implies usage of Viscozyme and Enzyme complex, enhanced recovery of protein by 50% and more than 200%, respectively. Enzymatic treatment with Enzyme complex improved antioxidant activity of isolated protein by 46%. Results showed that applied green technology enabled greatly enhanced recovery of protein with favorable biological properties suitable for advantageous incorporation into food matrix.

Key words: *pumpkin leaves, protein, RuBisCO, extraction, antioxidant activity*

INTRODUCTION

The importance of food proteins is well established – they are considered as the most valuable functional ingredients in food production. Over the last few decades growing global demand for food proteins has driven the search for new, sustainable protein sources based on plants able to potentially complement or replace animal proteins in various food applications (Rommi, 2016). Plant-based proteins represent a promising solution due to their long history of use and cultivation, lower cost of production, and easy access in many parts of the world. Plant proteins are also more environmentally sustainable (Willett et al., 2019). Beside their role in human diet, as

indispensable source of amino acids, plant proteins within their structure possess biological activity of importance to the health and well-being of the human organism.

While pumpkin is regarded as valuable vegetable that contains biologically active components and it is consumed all over the world, their leaves are usually considered as waste material. Pumpkin leaves are good source of crude protein, carbohydrates and minerals (Mashiane et al., 2021). Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is one of the most abundant proteins in plants.

During extraction of proteins, usage of enzymes helps disintegration of the plant cell wall that acts as a barrier for diffusion of protein into solvent, and consequently enhances protein extractability. Degradation of structural polysaccharides usually is achieved with carbohydrases (Rosset et al., 2014). Generally, enzyme-assisted extractions are recognized as eco-friendly technologies as they offer a possibility of greener chemistry in a search for cleaner routes in food industry.

MATERIAL AND METHODS

Pumpkin leaves were selectively harvested at suitable maturity from the fields where pumpkins were grown (JS&O, d.o.o. Novo Miloševo, Serbia). After harvest, leaves free from decay or damage were selected for the experiments. They were stored at $-18\text{ }^{\circ}\text{C}$ in the freezer for couple days prior to analysis.

Enzyme-assisted extractions were performed using enzyme preparations Viscozyme (Novozymes) and Enzyme complex (Novozymes) in previously determined dosages. Enzyme-assisted extractions were carried out at $45\text{ }^{\circ}\text{C}$ and pH 5.5 for 1 hour. After extraction, mixture was centrifuged and the contaminant proteins from green fraction were removed by thermal denaturation at $55\text{ }^{\circ}\text{C}$ for 30 minutes. Subsequently, supernatants from centrifugation were subjected to isoelectric precipitation at pH 4.5. After precipitation, protein curds were collected by centrifugation and freeze dried.

The antiradical scavenging activities of all isolated proteins were determined according to the method of Re et al. (1999).

RESULTS AND DISCUSSION

Results showed that green protocols with usage of enzymes enabled enhanced recovery of RuBisCO proteins from pumpkin leaves. Improvement with Viscozyme was approximately by 1.5 fold while treatment with Enzyme complex gave enhancement higher than 3 folds.

Table 1. Recovery of RuBisCO protein extracted from pumpkin leaves with and without enzymatic treatment

Enzyme	No enzymes	Viscozyme	Enzyme complex
Recovery of protein (% wt)	0.53±0.02	0.80±0.11	1.65±0.07

Moreover, it was confirmed that applied protocols affected antioxidant properties of isolated proteins. Treatment with Viscozyme and Enzyme complex improved antioxidant activity of proteins by 2 and 46%, respectively, compared to treatment without enzymes. It was revealed that isolated proteins exhibited superior antioxidant activity when they are extracted with the assistance of enzymes.

Table 1. Antioxidant activity of RuBisCo protein isolates obtained with and without usage of enzymes

Enzyme	No enzymes	Viscozyme	Enzyme complex
Antioxidant activity (mmol Trolox/g)	2093±35	2122±31	3055±21

CONCLUSION

Achieved considerable upgrade of recovery and the improvement of antioxidant activity of protein isolated by the assistance of enzymes indicated great potential of applied green method for the production of high-value proteins from waste pumpkin leaves.

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POBOLJŠANJE PRINOSA I SVOJSTVA RuBisCO PROTEINA IZ LISTA BUNDEVE PRIMENOM ZELENE TEHNOLOGIJE

Sažetak

Ova studija izučavala je mogućnost primene enzima za poboljšanu ekstrakciju RuBisCO proteina i unapređenje njegovih bioloških svojstava. Listovi bundeve predstavljaju otpadni materijal koji se može koristiti za ekstrakciju ove vrste proteina. Rezultati su pokazali da je primenjeni zeleni protokol, koji je podrazumevao upotrebu *Viscozyme* i *Enzyme complex* preparata, poboljšao ekstrakciju proteina za 50%, odnosno više od 200%. Enzimski tretman sa *Enzyme complex*-om poboljšao je antioksidativnu aktivnost izolovanog proteina za 46%. Rezultati pokazuju da je primenjeni zeleni protokol omogućio znatno poboljšanje prinosa proteina i unapređenje biološkog svojstva značajnog za njegovu inkorporaciju u prehrambene matrikse.

Key words: *lišće bundeve, protein, RuBisCO, ekstrakcije, antioksidativna aktivnost*



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Original Scientific paper

STUDENT'S PERCEPTION OF SOURCES AND SIGNIFICANCE VITAMINS A AND D IN THE DIET

Abstract

If someone would talk about the "fuel for the body", it would definitely be vitamins and minerals due to their huge impact on the development, function and overall health of the organism. Lack of vitamins in the body has the greatest impact to the immune system, since its deficiency causes decrease in immune system. In order to provide the body with the appropriate quantities of vitamins A and D, it is necessary regularly to consume food rich in these vitamins. The aim of this paper is to indicate the level of students' knowledge about the sources and application of vitamins A and D. The obtained results can contribute in raising the general awareness of students about the importance of these vitamins, as well as regular consumption of food rich in these vitamins.

Key words: *vitamin A, vitamin D, vitamin functions, vitamin sources, vitamin application.*

INTRODUCTION

Vitamins are essential for the normal functioning of the organism during life and for the optimal course of all biochemical processes which occur in the body.

Vitamin A is a basic liposoluble vitamin. Since it has a terpene chemical structure it belongs to the group of organic compounds called terpenes. Vitamin A can be present

in the body in various forms, so usually when we talk about Vitamin A we talk about a group of compounds with the properties of vitamin A. All together they belong to retinoids, i.e. retinoic acid derivatives (esters, ethers or alcohol derivatives). Vitamin A is also called retinol or vitamin A₁ (Gerster, 1997). Vitamin A is essential for the proper functioning of physiological and metabolic processes in the body, it is necessary for good eyesight, hematopoiesis, embryonic development, differentiation of skin cells, immune system function, etc. (Wolf, 1996).

Vitamin D belongs to a group of liposoluble secosteroids, which are responsible for the increase of calcium, magnesium and phosphate absorption in the intestines, and many other biological effects. In human body, the most important compounds are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). Vitamin D is primarily responsible for the metabolism of calcium and phosphorus in the human body. It provides their absorption in the small intestine and deposition in the bone tissue. (<https://link.springer.com/content/pdf/bbm%3A978-1-4615-2131-0%2F1.pdf>).

As the use of vitamins A and D has recently increased significantly, there is a great need to study these vitamins in detail and to find out the extent to which young people, especially students, are informed about the benefits and sources of these vitamins. Also, the authors wanted to find out whether they use them in adequate quantities through their daily diet or they use supplementation in order to provide adequate quantities of these vitamins. Although vitamins are normally present in food in extremely small quantities, they are essential for regulating bodily functions. The lack of certain vitamins, including vitamins A and D, causes certain negative effects in the body. The most common disorders caused by vitamin A deficiency are night blindness, keratomalacia, cell difference, and slow growth (Keith, 2003). When it comes to vitamin D, the most common disorders are rachitis, osteoporosis and susceptibility to viruses (Holick, 2007). In order to prevent this, it is necessary to supply the body with the certain amount of these vitamins. However, the ideal food in which all the vitamins are present in the right quantities does not exist. The best way to ensure adequate vitamin intake is to consume a varied and balanced diet. It should be noted that some of the vitamins present in food are not “real vitamins”. They are precursors, such as is the case with beta-carotene and vitamin A. Precursors are chemically altered in the body and converted into the active form of vitamins. To ensure a sufficient quantity of vitamins A and D intake in the body, it is necessary on regular basis to take foods rich in these vitamins. Moreover, it is important that food is fresh and unprocessed, in order to preserve vitamins as much as possible (Popov-Raljić, 2016).

Dietary sources of vitamin A are divided in two categories. The first refers to vitamin A (retinol), also known as formed vitamin A, and the second refers to the provitamin of vitamin A, i.e. carotenoid precursors that are biologically active as retinol. Retinol is found exclusively in foods of animal origin, while carotenoids are found in foods of plant origin (Gerster, 1997). Sources of provitamin A are dark leafy vegetables, algae, red and yellow vegetables, potatoes, red and orange fruits and fresh juices, red palm oil, carrots, etc. Sources of retinol are milk and dairy products, eggs, fish and fish oils, shellfish, liver, chicken. The recommended daily intake of vitamin A is 700 µg for women and 900 µg for men (Popov-Raljić, 2016).

However, it is important to keep in mind the fact that despite the great importance of vitamin A in the body, its excessive intake can cause serious problems that are manifested by disorders on the skin, bones, joints and digestion. It is recommended to take supplements in the form of provitamin of vitamin A (Novaković and Miroslavljev, 2002). Symptoms of increased vitamin A content in the body (hypervitaminosis) are: peeling skin (dry skin and mucous membranes), bone fragility, fatigue and insomnia, nausea and vomiting, anorexia, irritability and altered mental state, blurred vision, hair loss, muscle pain and weakness as well as pain in the abdominal area, edema (accumulation of fluid under the skin), yellowish skin color due to excess β -carotene that can not be converted into vitamin A.

Vitamin D is present in foods of animal origin such as cholecalciferol (vitamin D₃). Ergocalciferol (vitamin D₂) is found in some wild mushrooms, where it is converted from provitamin, ergosterol. Plants used as food may contain ergosterol, but ergosterol is not naturally converted into vitamin D₂. Vitamin D₃ is absorbed much more efficiently than vitamin D₂ in the gut (Alardt, 2006).

The recommended daily intake of vitamin D is 5 μ g for both adult males and females. (Popov-Raljić, 2016). Important sources of vitamin D are egg yolks, yeast, mushrooms, fish oil, eggs and butter, and fish such as eel, perch, herring, salmon and tuna. Daily needs are met through food intake and sunbathing (Tešanović, 2011).

Vitamin D can withstand various food processing procedures: smoking, pasteurization, sterilization and drying. For example, during processing and storage, milk should not be exposed to the sunlight, because due to oxidation process, vitamin D in milk can be transformed into an inactive form with the help of riboflavin molecules, which are sensitive to the action of oxygen. In addition to milk, it is very important to eat fish such as eels, perch, herring and salmon at least once a week (Popov-Raljić, 2016).

MATERIAL AND METHODS

The research was conducted in the period from April 1st 2022. to May 1st.2022. among students of the University of Novi Sad. The survey included a total of 242 students in five fields of study.

The research was conducted using a survey method. After data collection the results were processed by descriptive analysis.

The survey consisted of 4 parts and a total of 24 questions. The first set of questions related to the socio-demographic characteristics of the respondents. The second set of questions related to the respondents' sources of information about vitamins A and D. The third set of questions was aimed at showing the respondents knowledge of the basic characteristics of vitamins A and D, the recommended daily intakes, as well as the consequences of deficiency. The last set of questions gave an insight into whether the respondents during their daily diet take care to meet the daily needs for vitamins A and D, whether they use supplementation in the form of these vitamins and what are the reasons for using supplementation.

RESULTS AND DISCUSSION

ANALYSIS OF SOCIO-DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

Table 1. Socio-demographic characteristics of the respondents

Gender	Number	(%)
Men	93	38.4
Women	149	61.6
Total	242	100
Field of study		
Natural and mathematic sciences	54	22.3
Social sciences and Humanities	67	27.7
Medical sciences	38	15.7
Technical and technological sciences	60	24.8
Art sciences	23	9.5
The most common way of informing		
The media	107	44.2
Information via Internet	100	41.3
Other	35	14.5

As it can be seen from Table 1, that out of a total of 242 surveyed students, the female population was somewhat larger, 61.6%, and three areas of study were almost equally represented: 22.3% – natural and mathematic sciences, 24.8% – technical and technological sciences and 27.7% – social sciences and humanities. Students are most often and almost equally informed through the media and the Internet.

Results of student survey on knowledge about vitamins a and d and their importance

When it comes to the solubility of vitamin A only 113 respondents out of a total of 242 gave the correct answer, i.e. they said that vitamin A is soluble in fats, which presents less than 46.7%. Based on the obtained results, it can be concluded that less than half of the respondents gave the correct answer to this question. A more detailed analysis showed that the best results in this issue were achieved by the respondents who are studying the medical sciences, and the worst by the respondents who are studying the social sciences and humanities. This result is certainly justified, considering that medical science students constantly encounter all nutrients throughout their education, while social sciences and humanities students are not largely oriented towards this type of matter.

When it comes to the solubility of vitamin D, 100 respondents out of a total of 242 said that vitamin D is soluble in fats, which is 41.3%. The best results when it comes to this question were achieved by students of natural and mathematical sciences with 57.40% of correct answers.

Regarding the source of vitamin A, the most common answer of the respondents was carrots, which were marked by a total of 147 respondents, and presents 60.7%. After that, 105 respondents (43.4%) marked fish and fish oils, followed by 85 respondents who have chosen (35.1%) milk and dairy products. It is interesting that apricots were marked by 75 respondents (31%), while e.g. beef liver was marked by 68 subjects (28.21%), and has higher concentrations of vitamin A. That algae as a source of vitamin A was noted by 47 respondents (19.4%), shellfish were marked by 66 respondents (27.3%), chicken by 51 respondents (21.1%), dark leafy vegetables were marked as a source of vitamin A by 66 respondents (27.3%) and potatoes with 49 (20.2%) answers. Foods that are not sources of vitamin A, such as wheat, were labeled with 30 (12.4%) responses, followed by corn, which was labeled with 35 (14.5%) responses, and rye was labeled by 27 respondents (11, 2%).

The most common answer that the majority of respondents opted for was carrot, which was marked by 161 respondents (66.5%). As many as for 6% of respondents more, marked carrots as a source of beta-carotene, compared to the source of vitamin A, which is true statement. Interestingly, when asked whether vitamin A and beta-carotene are related, the following results were obtained: 115 respondents confirmed, which is 48.3%, then 85 respondents said they were not sure, which is 35.7%. If these answers are compared with the areas of study of the respondents, the results show that 68.51% of students from natural and mathematical sciences answered this question correctly. This could be attributed to the fact that students of natural and mathematical sciences, especially if they are students of chemistry, have a certain knowledge in this field.

The introductory part of the paper presents food which is considered as a source of vitamin D. The most common answer of the respondents, related to the knowledge of food as a source of vitamin D, was salmon, which was marked by a total of 125 (51.7%) of respondents. After that, 113 (46.7%) respondents marked cod liver oil, followed by 106 (43.8%) respondents who marked milk and dairy products. Herring was marked by 102 (42.1%) respondents, and perch was marked by 96 (39.7%) respondents. Only 83 (34.3%) respondents marked eggs as a source of vitamin D, and 57 (23.6%) marked soy milk. Foods that are not sources of vitamin D, such as cereals, were marked in 55 (22.7%) responses, followed by root porridge which was marked in 51 (21.1%) responses, pear was marked in 34 (14%) responses and apple in 24 (9.9%) responses.

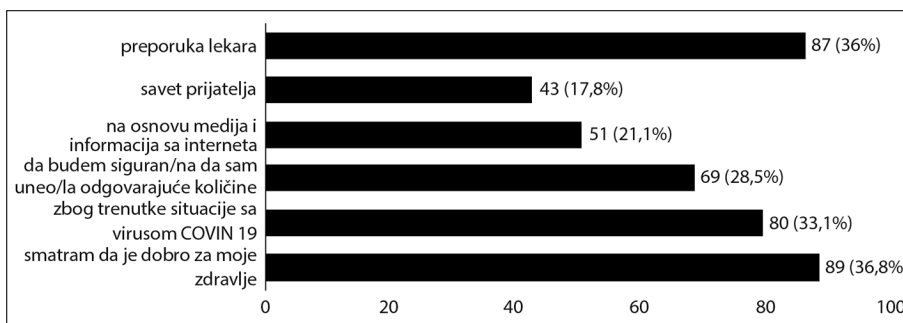
When it comes to the recommended daily intake of vitamin A, from a total of 242 respondents, 102 answered correctly to this question, i.e. that the recommended daily intake of vitamin A is 800 μg for adult women and men, which is 42.1%. The most correct answers to this question were given by students from natural and mathematical sciences. Regarding the recommended daily intake of vitamin D, the correct answer is 5 μg . Out of a total of 242 respondents, only 42 of them answered this question correctly, which is 17.4%. When it comes to this question, the students of medical sciences had the best results, of which 36.84% had a correct answer to this question. Summarizing these results, it can be said that the students showed poor knowledge of the recommended daily intake of these vitamins.

From the statistical analysis of the data, it can be concluded that all the consequences of the deficiency caused by vitamin A were marked by only 4 students, which

is 1.65%. Of these 4 students, 3 are students on basic academic studies of natural and mathematical sciences, while one respondent is on master studies in medical sciences. Nausea, which was one of the most common incorrect answers, marked by 51 (21.1%) respondents, occurs due to vitamin A surplus, but not during deficiency.

Apart from knowing the recommended amounts of daily vitamin intake, the answers to the question about the consequences of vitamin D deficiency is also interesting. The correct answers to the question were rachitis, psoriasis, osteoporosis, susceptibility to viral infections and multiple sclerosis. A completely correct answer to this question, which was a multiple choice question, was marked only by 3 students out of a total of 242 respondents, which is 1.23%. Headache, which was one of the most common incorrect answers, marked by 89 (36.8%) respondents, occurs due to vitamin D surplus, but not due to deficiency.

By processing statistical data, it was concluded that 77 students or 31.8% of a total of 242 respondents take into account the needs of the body for vitamins A and D during the daily diet. As many as 93 respondents achieve optimal intake by consuming foods rich in these vitamins, which is 38.4%. 88 respondents, or 36.4% combine intake through appropriate foods and the use of supplements, and 61 respondents, or 25.2% take into account about the intake of these vitamins only through supplementation.



Picture 1. Graphic overview of the reasons for using vitamin A and D supplementation

The most common answer was the reason that they think it is good for health, which was noted by 89 respondents, i.e. 36.8%. Then, a doctor's recommendation was cited as the reason for the same share of respondents, 87 respondents, i.e. 36%, and the third most common answer was due to the situation with the COVID-19 virus.

CONCLUSION

Based on the entire research, it can be said that the general knowledge of students about the sources and application of vitamins A and D in the diet is at a low level. As the key for a good health is in a rational and balanced diet, it would be desirable to

improve students' awareness of these vitamins, as well as all nutrients. Students represent the young population, which is mostly fed improperly. In the first place, it is fast food or ready-made products that are easy to prepare and are not time consuming, but also they are not nutritionally adequately balanced. A number of students eat in student canteens, but these balanced meals are mostly prepared by fast processing methods, such as deep fat frying, which negatively affects the nutrients in foods, especially vitamins, most of which are very sensitive. Another aspect that students look at when choosing food, in addition to the duration of preparation, is the price. The importance of balanced nutrition is not only to regulate body weight, but also to ensure the normal functioning of the organism. Improper diet also affects the mood and concentration that are most important for students. Based on the entire research, it was concluded that students are not sufficiently familiar with these micronutrients, and we believe that it is desirable to organize more lectures related to nutrition and nutrients, regardless of the field of study.

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ПЕРЦЕПЦИЈА СТУДЕНАТА О ИЗВОРИМА И ЗНАЧАЈУ ВИТАМИНА А И Д У ИСХРАНИ

Резиме

Уколико би организам имао своје “гориво”, то би били витамини и минерали који имају огроман утицај на развој, функцију и здравље организма. Недостатак витамина у организму највеће последице испољава у односу на имуни систем. Како би се витамини А и Д уносили у организам у одговарајућим количинама неопходно је редовно конзумирати намирнице богате овим витаминима. Циљ овог рада јесте да укаже на ниво информисаности студената о изворима и примени витамина А и Д. Добијени резултати могу допринети у подизању опште свести код студената о важности ових витамина, као и редовном конзумирању намирница богатим овим витаминима.

Кључне речи: *витамин А, витамин Д, функције витамина, извор витамина, примена витамина*



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Original Scientific paper

NUTRITIONAL VALUE OF REFRESHING NON-ALCOHOLIC BEVERAGES

Abstract

Refreshing non-alcoholic beverages (soft drinks) are liquid products, preserved by chemical means, obtained by a specific technological process from tap or mineral water, sugar, carbon dioxide and additives (acids, preservatives, natural or artificial flavors, vitamin C, dyes, caffeine for drinks such as cola and quinine hydrochloride for tonic drinks, etc.)

The aim of this paper is to review the nutritional value of the refreshing soft drink Multi Sola and determine the recommended daily dose for use. Excessive use causes numerous disorders in the body.

Key words: *refreshing soft drinks, nutritional value, vitamins, carbohydrates*

INTRODUCTION

Multi Sola is a non-carbonated, non-alcoholic refreshing soft drink with mixed fruit juice, with the minimum fruit content of 12%. The drink is pasteurized and without preservatives, and has the following ingredients: water, sugar, concentrated juices of orange, pineapple, grape, lemon and passion fruit, acid (citric acid), antioxidant (ascorbic acid), mash of apricot, banana, mango, guava and peach. Vitamin content per 100 ml is as follows: niacin – 7 mg or 15%; vitamin E-1.5 mg or 15%; pantothenic acid – 0.9 mg or 15%; vitamin B6-0.3 mg or 15%; vitamin A – 240µg or 30%; thiamine

– 0.21µg or 15%; folic acid-30µg ili 15%; biotin-0.02mg or 15%; vitamin B12-0.15µg or 15%; stabilizers pectin and carub gum, flavors. The total dry matter content is 8%.

VITAMINS

Vitamin B6 in food: bananas, whole grain cereals, legumes, chicken, legumes, egg yolks, most dark green leafy vegetables, most fish and shellfish, meat, offal, milk, nuts, peanut butter, potatoes, prunes, raisins, wheat , wheat germ, corn, avocado, black mola-sses sugar syrup, brewer's yeast.

The main vitamins for enriching drinks are antioxidant vitamins A, C and E. Ascorbic acid is easily oxidized and lost during storage of drinks, depending on storage conditions. This fact is of great importance for the consumer who must know how to store drinks and when to consume them in order to get the maximum benefit from the addition of vitamin C (Cvetković and Jokanović, 2009).

Despite the proven harmful effects on health, the consumption of soft drinks in the world and in our country is increasing every day. Additives are an indispensable ingredient in refreshing soft drinks. They are the only carriers of the sensory properties of these products. Of the total number of allowed additives, 2/3 can be used for refreshing non-alcoholic beverages, and for about 60 of them the use is accompanied by appropriate restrictions (Mališić et al., 2011).

Table 1. Recommended daily doses of vitamin B6

Age	Recommended doses of vitamin B6, mgd ⁻¹
Men	1,8-2,2
Women	1,8-2,0
Pregnant and breastfeeding women	2,3-2,4
Children from 2 months to 4 years old	0,7
Children aged 4 and over	2,0

CARBOHYDRATES

Carbohydrates are deposited in the human body in the form of glycogen. It is found in the liver and serves as a reserve of energy that the body can quickly use by converting it into glucose if needed (physical or intellectual effort). It is found in small amounts in the muscles.

Daily needs for carbohydrates depend on physical activity; the greater the muscle work the more carbohydrates are needed. On average, carbohydrates should provide 50-60% of energy in the total daily meal.

NUTRITION VALUE LABELING

Article 31.

Labeling of nutritional value is done in one of the following ways:

1. Energy value: – amounts of protein, carbohydrates and fats.
2. Energy value: – amount of protein, carbohydrates, fats, sugars, saturated fatty acids, fiber and sodium.

The nutrition declaration may include information on the amount of one or more of the following ingredients: starch, polyol, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, minerals and vitamins.

Table 2. Nutritional value

Calories	198 kJ, 46,5 kcal
Proteins	<0,1 g
Carbohydrates	11,1 g
Total sugars	11,1 g
Fat	<0,1 g
Saturated fatty acids	<0,1 g
Fibers	<0,1 g
Sodium	<0,03 g
Niacin	2,4 mg (15%) *
Vitamin E	1,8 mg (15%) *
Pantothenic acid	0,9 mg (15%) *
Vitamin B6	0,21 mg (15%) *
Vitamins (with provitamin A)	240 mg (30%) *
Tiamin	0,165 mg (15%) *
Folic acid	30 mg (15%) *
Biotin	7.5 mg (15%) *
Vitamin B12	0,375 mg (15%) *

* RDA% - recommended daily intake

Calories in 100ml: 46kcal

Carbohydrates: 11.1g x 4 = 44.4 kcal

Proteins: 0.1 g x 4 = 0.4 kcal

Fat: 0.1g x 9 = 0.9 kcal

Daily needs:

Energy value: 2228.91 kcal

Fat (30%) = 74,297g

Carbohydrates (55%): 278.61 g

Proteins (15%): 83,584g

In the period January-June 2002, a total of 85 samples of soft drinks (66 soft drinks and 19 syrups for soft drinks) were analyzed by HPLC, four of which were imported and the rest from domestic production. Increased content of preservatives (benzoate and sorbate) was found in 24 samples of soft drinks (36.36%) and in 15 samples of syrup (78.95%). Labels represented a special problem as they either did not state the presence of preservatives or showed preservatives that were not used in the product. There were 18 such refreshing soft drinks (27.7%) and one syrup for refreshing soft drinks (5.26) (Siriški et al., 2003). The control of the health correctness of the packaging should include a larger number of samples of the packaging, and it includes testing, ie. determination of migration of organic and inorganic substances, migration of dyes, phenols, formaldehyde, migration and content of heavy metals and metalloids (Miodragović-Milojević et al., 2003). Quality control of soft drinks according to the legal regulations, in addition to other parameters, includes determining the content of preservatives. Based on the obtained results, it was noticed that it is necessary to control the content of preservatives in soft drinks, with education of producers and mandatory control of imported raw materials – base for their production (Siriški et al., 2003). The resulting by-products of fruit processing are intensively studied as raw materials for the extraction of phenolic compounds, natural pigments, dietary fiber, protein isolates and oils, as well as for the production of supplements with potential health benefits (Denev et al., 2018).

CONCLUSION

The production of fruit juices is a very dominant and promising industry on a global scale. The largest producers are Germany (with an annual production of 2,800 billion liters), France (1,600), Great Britain (1,400) and Spain (1,300). Our country produces about 230 million liters of fruit juice a year. This puts us among the most serious producers in the region, given that Greece produces 187 million liters per year, Hungary 127, Romania 111, Bulgaria 106 million, and Slovenia 40.

They can in essence differ in terms of used raw materials, composition, quality, fruit content and packaging. According to the content of insoluble ingredients (suspensoids), fruits can be divided into three subcategories: clear, cloudy and mushy. Quality control of soft drinks according to the legal regulations, in addition to other parameters, includes determining the content of preservatives.

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Originalni naučni rad

NUTRITIVNA VREDNOST OSVEŽAVAJUĆEG BEZALKOHOLNOG PIĆA

Abstrakt

Osvežavajuća bezalkoholna pića su tečni proizvodi, konzervisani hemijskim sredstvima, dobijeni određenim tehnološkim postupkom od vode ili mineralne vode, šećera, ugljen-dioksida i aditiva (kiseline, konzervansi, prirodne ili veštačke arome, vitamin C, boje, kofein za pića tipa cola i kinin hidrohlorid za pića tipa tonic i sl.). Cilj rada je pregled nutritivne vrednosti osvježavajućeg bezalkoholnog pića Multi sola i određivanje preporučene dnevne doze za upotrebu. Prekomernom upotrebom nastaju brojni poremećaji u organizmu.

Ključne reči: *osvežavajuća bezalkoholna pića, nutritivna vrednost, vitamini, ugljeni hidrati*



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Original Scientific Paper

THE IMPACT OF DIET ON ELITE VOCAL PROFESSIONALS WITH SPECIAL REFERENCE TO STOMACH ACID REFLUX (GERD – GASTROESOPHAGEAL REFLUX DISEASE AND LPR – LARYNGOPHAGEAL REFLUX)

Abstract

Vocal professionals are exposed to great physical and mental strain and stress. They use their voice as one of the basic means of their professional activity. In the process of vocal education, they develop vocal technique, but also awareness of the anatomy and physiology of the vocal apparatus and vocal hygiene. Among other things, they often face problems of vomiting or reflux of gastric acid, which occurs in two forms: as gastroesophageal reflux disease (GERD) – damage to the oesophageal mucosa caused by pathological reflux of gastric contents into the oesophagus or as laryngopharyngeal reflux (LPR), so-called “silent reflux”- into the pharynx and then into the larynx. The paper points out the symptoms of these disorders and risk factors, among which the impact of diet is emphasized, and an appropriate way of eating and behaving is recommended.

Key words: *elite vocal professionals, diet, gastroesophageal reflux disease, laryngophageal reflux*

INTRODUCTION

Elite vocal professionals (opera singers, other singers and actors) are people who use their voice as the main means of their professional activity, and in whom a slight change in the voice can cause severe professional consequences. Since the daily phonatory requirements are higher than for the average speaker, vocal education and knowledge of vocal hygiene are the basis of the health of their speech apparatus, which ensures them a long and successful career. Care for the vocal apparatus, as well as personal health, is the basis of care aimed at preventing voice disorders that can

interfere with daily activities and affect the overall quality of life. The profession of elite vocal professionals depends on the coordination of the nervous, muscular, respiratory and, to some extent, skeletal system of the body, so it is important that the person who chooses this profession is physically and mentally healthy, and that their lifestyle is adjusted to their profession. Through vocal education, an individual develops awareness of their vocal abilities and the capabilities of a vocal professional, but in addition to developing vocal technique, one also acquires knowledge about the anatomy and physiology of the vocal apparatus, as well as vocal hygiene and risk factors that can lead to organ damage.

According to Murry and Rosen (2000), the basic elements of vocal education, without taking into account the general understanding of how the mechanism works, include: (1) voice hygiene; (2) understanding medical problems that can affect the voice; (3) smoking cessation; (4) the influence of alcohol, drugs, diet and hydration; (5) vocal stress; (6) vocal exercise; and (7) singing during illness. According to Mitrović (2011), the human voice is a complex psychophysical phenomenon, and professionals use it for a longer duration, with greater scope and intensity, under various psychophysical and microclimatic conditions. Because of that, damage to the voice and the system that creates it is much more common.

Elite vocal professionals and stomach acid reflux

Acid reflux, also known as stomach reflux, can be:

- gastroesophageal reflux disease (GERD) – damage to the oesophageal mucosa caused by pathological reflux of gastric contents into the oesophagus or / and
- laryngopharyngeal reflux (LPR), so-called “silent reflux” – the return of gastric contents through the oesophagus to the pharynx and then to the larynx.

In the area responsible for swallowing (oesophagus) and the area responsible for breathing and voice production (larynx with structure), as well as the area of the stomach, there are sphincters (rings). Sphincters are special, circular muscles that open and close certain parts of the body, and they usually regulate the passage of some type of fluid. Sphincters can act involuntarily, through the autonomic nervous system, or, sometimes, under certain voluntary control they can act through the somatic nervous system. If a sphincter loses muscle tone or has too much tone (spasticity), symptoms and illness may occur. At both ends of the oesophagus there is a ring of muscles (lower and upper oesophageal sphincter). Under normal circumstances, these sphincters hold the contents of the stomach where they are needed – inside the stomach, but, in the case of LPR, the sphincters do not work properly. Stomach acid returns to the back of the throat (pharynx) or to the speech box (larynx), and even to the back of the nasal airway. It can cause inflammation in areas that are not protected from exposure to stomach acid. If the "lower oesophageal sphincter" or simply the lower circular muscle of the oesophagus, the small circular muscle, loses its tone, and thus its effectiveness in preventing food from reaching the stomach, the food is returned to the oesophagus. Then we are talking about GERD.

The symptoms of GERD and LPR are similar, but by no means the same, nor do they have the same consequences for elite vocal professionals. “The link between gastrophageal reflux (GER) and laryngeal (laryngeal) disorders in adults was recognized in the late 1960s” (Young, Shav, Serl & Miner, 1996). In a 2002 article, Koufman described the differences between gastroesophageal reflux disease (GERD) and its related laryngopharyngeal reflux disease (LPR). GERD occurs when stomach acid is withdrawn into the oesophagus; LPR occurs when it is pulled further down the throat. Direct contact with gastric acid can injure the delicate mucous membrane of the larynx and pharynx, which surrounds the vocal cords (Sereg-Bahar, Janša & Hočevvar-Boltezar, 2005). Why are elite vocal professionals more prone to stomach acid reflux? In order to produce a sonorous voice that fills the space without sound in different spaces – scenes in which the acoustics are different, the voice that can withstand several hours of use, the voice that is expected to have a greater range, more intense physical and mental activity is necessary. The muscles of the inhalation and exhalation, and especially the diaphragm, are in constant tension, which over time weakens the lower sphincter, and allows the unwanted passage of stomach acid to the oesophagus, larynx and pharynx.

In addition, the larynx changes its position depending on the requirements of the composition, vocal readiness and abilities of the individual, which can also contribute to the weakening of the sphincter function, i.e., the creation of space for the passage, i.e., the return of stomach acid. Furthermore, travelling, a change of residence during the preparation for a concert or performance, leads to eating disorders, sleep disorders and increases stress. Also, rehearsals, concerts and performances are usually held in the evening, and most elite vocal professionals, just like athletes, do not consume food before the performance, but after it. Late meals, before going to bed, especially if they are large and heavy, contribute to the occurrence of gastric acid reflux. We must not forget the impact of stress, which is closely related to the profession. Stress caused by a sense of responsibility, constant exposure to the assessment of colleagues, critics, superiors and recipients, fear of possible failure, etc., can contribute to the emergence of GERD or LPR.

SYMPTOMS OF GERD AND LPR

The most significant and most common symptoms of GERD:

- heartburn – a burning sensation in the stomach and behind the sternum that can occasionally spread to the throat or oral cavity leading to a sour taste in the mouth; it most often occurs after a meal, at night, when lying down or bending
- regurgitation – the involuntary return of food or fluid from the stomach to the oesophagus
- dyspepsia – a feeling of discomfort or pain in the upper abdomen
- difficult / painful swallowing – it most often occurs when there are complications in connection with GERD
- respiratory symptoms – hoarseness, dry cough, a feeling of a lump in the throat or the entry of acid into the lungs and consequent pneumonia, which are also often caused by reflux of gastric contents into the throat.

Symptoms of LPR may not feel like acid reflux at all. The person will have no heartburn or chest pain.

Symptoms include:

- chronic cough
- sore throat
- postnasal drip or a feeling of mucus in the back of the throat
- hoarseness of voice
- a constant need to clear the throat
- red, swollen or painful vocal cords
- swallowing problems
- laryngopharyngeal reflux (LPR).

Risk factors:

Obesity, overeating, pregnancy, strenuous physical work (which includes singing), diet, time of the last meal, stress, smoking, alcohol, i.e., natural predispositions, lifestyle, diet and vocal hygiene (when it comes to elite vocal professionals) are the most common risk factors for the occurrence of GERD and LRP.

Diet

When we talk about nutrition and the impact of food on the human body, Jovanka Ninković (Ninković, 2009) analysing the production of healthy food in her final work at the Technical College of Vocational Studies in Zrenjanin, described them as follows: “The impact of food on the human body is multiple, very complex and continuously present throughout life from birth to death. It affects all the senses: smell, taste, sight. It also affects people’s emotions... Nutrition is a process that takes place from the moment of food intake to the full utilization of nutrients in the body.

Food that is taken into the body, among other things, should meet 3 conditions:

1. to be sufficient in quantity to provide the necessary energy for the work, growth and renewal of cells and tissues
2. to be diverse, i.e. to contain in the required amount all nutrients and other necessary ingredients such as proteins, fats, carbohydrates, minerals
3. to be health safe.” (Ninković, 2009: 1).

In the continuation of her text, the author states that the term "food", according to the European Consumer Centre in Ireland (Dublin), stands for any substance or product that is processed, partially processed or unprocessed, and is intended or may be intended for human consumption. Improper diet in terms of food choices, time and manner in which it is consumed can significantly affect the quality and damage of the vocal apparatus. Late and heavy meals that are common among vocal professionals

due to evening performances, concerts and plays, insufficiently chewed food, hastily eaten meals, fast and fried foods, alcohol, caffeinated beverages and others, favour the development of gastroesophageal reflux disease and laryngopharyngeal reflux (Bumber et al., 2004). Given that elite vocal professionals are more prone to GERD and LPR diseases, adjusting diet and eating habits can help reduce acid reflux symptoms.

The question is how to reduce and eliminate the occurrence of GERD and LPR. Some people have mild symptoms that can be successfully treated by lifestyle changes, such as: weight loss, consuming smaller portions during meals, avoiding lying down after meals, smoking cessation. Also, avoiding certain foods that cause symptoms can provide relief. It is recommended to eliminate foods with a high content of fats, sweets and sour drinks. In that sense, one should avoid: high fat dairy products, fried foods, greasy pieces of meat, caffeine, alcohol, carbonated drinks, onions, kiwis, oranges, lemons, grapefruit, pineapple, tomatoes and tomato-based foods, as well as chocolate, mint and spicy foods. It was found that these foods significantly weaken the oesophageal sphincter.

High-fat meals

High-fat foods generally reduce the pressure on the lower oesophageal sphincter (LES) and delay gastric emptying. The results of analyses by the US National Institute of Diabetes and Digestive and Kidney Diseases have shown that fatty foods can increase the risk of reflux symptoms, which is why a reduction in total fat intake is recommended. High-fat foods that should be avoided include: French fries, fried onion rings, chips, butter, high fat milk, cheese, ice cream, high-fat sour cream, high-fat salad dressings, creamy sauces, greasy pieces of meat.

Spicy food

Studies have shown that spicy foods can cause stomach pain and burning symptoms if a person has a functional gastrointestinal disorder. Spicy foods can irritate parts of the oesophagus, which can often cause reflux (return) of stomach acid. A 2017 Korean study (Choe JW et al., 2017) found that hot, spicy stews led to GERD symptoms in more than half of the evaluated cases.

Fruits and vegetables

Fruits and vegetables are an important part of our diet. However, certain types can cause reflux and worsen its symptoms, such as: pineapple, citrus fruits (oranges, grapefruit, lemon and lime), tomatoes (and tomato-based foods), garlic and onions.

Beverages

Some common drinks can also cause symptoms in people with reflux: alcohol, coffee and tea, carbonated drinks, citrus and tomato juices.

Food that should be preferred

Intake of foods rich in fibre, low-fat protein and vegetables is recommended. A 2004 study (Nilsson et al., 2004) showed that increasing the amount of fibre and limiting salt in the diet can protect against reflux symptoms. Therefore, it would be good to consume: lean meat, whole grains, bananas, apples, decaffeinated drinks, water, leafy green vegetables, legumes.

Recommendations

Some lifestyle changes contribute to maintaining optimal health and reducing reflux-related discomfort, so the following are recommended:

- diet modification
- smoking cessation
- avoiding food intake for at least two to three hours before bedtime
- exercising to maintain a healthy weight
- smaller portions of food
- chewing gum to increase saliva secretion and neutralize acid
- raising head to a higher position during sleep to prevent the symptom of reflux at night
- wearing loose clothing to prevent tightness and pressure in the abdomen
- maintaining a well-balanced low-fat diet.

There are many factors that influence the possibility of voice disorders, such as lifestyle, diet, stress, inadequate care and hygiene of the vocal apparatus, abuse and misuse of the voice, and others. Vocal disorders affect the daily life of a large part of humanity, but certainly the most affected are those whose work depends on speech and voice (Sataloff, 2005).

CONCLUSION

The activity of elite vocal professionals is accompanied by greater psychophysical tension, due to which the voice is consumed significantly more than in the conditions of spontaneous, everyday speech, so the vocal disorders are more frequent among them. Elite vocal professionals thus belong to the risk group, prone to conditions caused by reflux (gastric acid reflux), gastroesophageal reflux disease (GERD) and laryngopharyngeal reflux (LPR) – both because of the profession they practice and because of their lifestyle and diet. There are many factors that influence the possibility of voice disorders, such as lifestyle, diet, stress, et al. If you experience symptoms such as frequent morning hoarseness, a sensation of a lump in the throat, frequent coughing and the need to clear the throat, hoarseness and other symptoms listed in the text, it is necessary to consult a phoniatician and start prevention or therapy to prevent these conditions caused by return of stomach acid.

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**УТИЦАЈ ДИЈЕТЕ НА ЕЛИТНЕ ВОКАЛНЕ ПРОФЕСИОНАЛЦЕ
СА ПОСЕБНИМ ОСВРТОМ НА РЕФЛУКС СТОМАЧНЕ КИСЕЛИНЕ
(ГЕРД - ГАСТРОЕЗОФАГЕАЛНА РЕФЛУКСНА БОЛЕСТ И ЛПР
– ЛАРИНГОФАГЕАЛНИ РЕФЛУКС)**

Извод

Вокални професионалци су изложени великом физичком и психичком напрезању и стресу. Свој глас користе као једно од основних средстава своје професионалне делатности. У процесу вокалног образовања развијају вокалну технику, али и свест о анатомији и физиологији вокалног апарата и вокалне хигијене. Између осталог, често се суочавају са проблемима повраћања или рефлукса желудачне киселине, који се јавља у два облика: као гастроезофагеална рефлуксна болест (ГЕРБ) – оштећење слузокоже једњака узроковано патолошким рефлуксом желудачног садржаја у једњак или као ларингофарингеални рефлукс (ЛПР), такозвани "тихи рефлукс" – у фарингекс, а затим у ларинкс. У раду се указује на симптоме ових поремећаја и факторе ризика, међу којима се истиче утицај исхране и препоручује се одговарајући начин исхране и понашања.

Кључне речи: *елитни вокални професионалци, дијета, гастроезофагеална рефлуксна болест, ларингофагеални рефлукс*

**LEGAL ASPECTS OF HEALTHY SAFE FOOD
PRODUCTS PROTECTION**



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Original Scientific paper

PRODUCTION OF HEALTHY SAFE FOOD IN THE LIGHT OF LAND AND WATER PROTECTION

Abstract

In the paper presented to you we view the production of safe food in the light of the legal protection of land and water, as key conditions for such doing. We do this starting from the Constitution, as initiating point for its realization, then going legally-logically by pointing to generally accepted and codified elements of international public law that must be respected, and finally to the most important segments of domestic legislation that protect land and water and thus contribute to the healthy food producing.

Keywords: *Constitution, international public law, national legislation, essential elements*

INTRODUCTION

In the paper we present to you, we view the production of health-safe food in the light of the legal protection of land and water as the basic conditions for its realising. To achieve so it is logical to respect the fundamental methods of legal sciences [11] and to properly consider their legal force and mutual hierarchical relationship in normative works which are crucial to the topic of our researching and presentation. In our work, which respects the normative hierarchical method, we start from pointing out the most important elements of the highest national normative product – the Constitution [2], then focusing on a number of elements of international public law, finally pointing at the elements of Serbian legislation of the greatest importance for our topic and goal. We approach all this with one guiding idea: We must respect all those normative works that prevent endangerment and/or damage to land and water in order to preserve the conditions for the production of safe food, hence the health and lives of people. The most common sources of pollution that deserve special attention of lawyers are: energy,

oil industry, chemical and pharmaceutical industry, mining and geological activities, but also traffic, construction and waste management [12].

Respecting the above mentioned our presentation will be concise, having in mind that we have already published a number of texts on environmental protection, but also economic development, with respect for it [10] [13], which was done by other authors [5] [6], as well as institutions [8] [32].

1. Elements of the Constitution relevant to the production of safe food

“The Constitution of the Republic of Serbia” prescribes that “the rule of law ... is based on inalienable human rights (Art. 3, par. 1)” and “their guarantees (Art. 3, par. 2)”, as well as that “Everyone has the right to a healthy environment (Art. 74, par. 1)” and “timely and complete notification of its condition (Art. 74, par. 1)”, whereby “everyone ... is responsible ... for the protection of the environment (Art. 74, par. 2)”, and also is “obliged to protect and improve the environment (Art. 74, par. 3)”. On the basis of Art. 16 we are obliged to respect generally accepted rules of international law and ratified international legislative agreements, of course those that are environmentally oriented. Domestic laws and sub-laws must not be in conflict with ratified international agreements and generally accepted rules of international law (Art. 194). At the same it is important to know that are prescribed: Article 18 – Direct application of guaranteed rights, “Art. 24. – Right to life”, and “Art. 27. – The right to liberty and security”, which norms clearly stipulate that no human rights are allowed to be endangered or violated. Not even with some environmentally negative treatment! Any negative doing towards mentioned values and liabilities are subject to a fair trial (Art. 32 – Right to a fair trial) and any subject possesses “The right to equal protection of rights and a legal remedy (Art. 36)”, both before courts and other state bodies. All subjects have to respect Art. 51. (The right to information), right defined with the “Aarhus Convention” as well as the “Law on Ratification of the Aarhus Convention” [23] [29]. The Constitution also established “Art. 56. – The right to petition”, petition which also can be in relation to a project that could have an impact on any of the human rights (on: health, life, living conditions, work, maintaining the quality of the environment, property ...). The Constitution also protects property (Art. 58 - Right to property) and especially state property (Art. 87 - State property), with a clear obligation for “Health care (Art. 68)”. At the same time, based on “Art. 88. – Land (par. 2)”, Constitution clearly stated that “the law ... may limit the forms of use and disposal, i.e. prescribe conditions for use and disposal in order to eliminate the danger of harm to the environment or to prevent violation of the rights and legitimate interests of others.” Such constitutional restrictions are then more detailed and precisely prescribed by a number of laws, such as: “Law on Environmental Protection” [16], “Law on Nature Protection” [21], “Law on Planning and Construction” [22], “Law on Mining and Geological Researching” [19], “Law on Chemicals” [15], but also “Law on Health Care” [17], as well as a number of other law, and many of their accompanying by-laws.

When we think about possible economic activity, we point out that it is also important for its development in a specific area, and according to the defined and

approved project, the “Art. 89 of the Constitution – Preservation of Heritage (par. 1)”, which clearly stipulates that "everyone ... is obliged to preserve natural rarities and scientific, cultural and historical heritage, as goods of general interest, in accordance with the law. It is important to know that there is a “special responsibility for the preservation of heritage ... in the Republic of Serbia (Art. 89, par. 2)”.

In order to realize all the above mentioned, on the basis of “Art. 97 of the Constitution – Competence of the Republic of Serbia”, the Republic of Serbia is obliged to legislate and regulate: realization and protection of freedoms and rights of citizens (Art. 97, par. 1, item 1), constitutionality and legality; proceedings before courts and other state bodies (Art. 97, par. 1, item 2), liability and sanctions for violations of freedoms and rights of citizens established by the Constitution and for violations of laws, other regulations and general acts (Art. 97, par. 1, item 2), security of citizens, which includes: environmental security, but also health (Art. 97, par. 1, item 4), protection of all forms of property (Art. 97, par. 2). 1. item 7), sustainable development (Art. 97, par. 1, item 9), system of protection and improvement of the environment (art. 97, par. 1, item 9), protection and improvement of flora and fauna (Art. 97, paragraphs 1, 9), production, trade and transport of ... hazardous materials (Art. 97, par. 1, item 9), but also the protection of cultural property (Art. 97, par. 1, item 10), collection of data of general interest (Art. 97, par. 1, p. 11.); as well as the organization and use of space (Art. 97, par. 1, item 12).

2. Elements of international law relevant to the production of safe food

The Republic of Serbia has more than 90 ratified conventions of environmental importance within its legislation. Among them, the most hierarchically important act is the “UN Charter” [1], which establishes “International Economic and Social Cooperation (Chapter 9)” and prescribes respect for human rights [(Art. 55, (C.))], which includes the human right to health, life and a healthy (adequate) environment, while states are obliged (Art. 103) to treat and apply all principles and obligations arising from this Charter as hierarchically superior to norms of other international normative documents. There are few of these acts that deserve our special attention. Let us briefly explain them.

“The International Covenant on Economic, Social and Cultural Rights” [9] clearly obliges states to ensure: healthy and safe working conditions [(Art. 7 (b.)) as well as living conditions (Art. 11), which includes providing all necessary conditions for environmental hygiene and that in all areas of business [(Art. 12, par. 2, under (b.))]. The Covenant [Art. 7, (b)], also obliges to respect the principles of prevention, for the sake of safe and healthy working conditions.

“The Declaration of the United Nations Conference on the Human Environment” [3] stipulates, in Principle 11, that states must develop environmental policies in a way that will not adversely affect their current and future development potential or hinder the achievement of better living conditions for all with the highest requirements for the preservation of human health, well-being and the environment (Part: Rights and obligations, under: 1. - 8.).

“Rio Declaration” [33], Principle 10, says that environmental activities are best carried out with the participation of interested citizens at all appropriate levels, which also includes decision-making level! Moreover, as prescribed by Principle 11, States undertake to establish adequate legislation to that effect.

“The Vienna Declaration of Human Rights” says that development needs do not justify denying people internationally recognized rights, and that means a healthy environment – even more clearly: a.) Space, and b.) Its quality, c.) Without violating the rights of future generation of the same [31], with Article 11 specifically and explicitly talking about environmental issues.

“The Aarhus Convention” [30] points out in its introductory part that it is adopted in full respect of Principle 1 of the “Stockholm Declaration” and Principle 10 of the “Rio Declaration”. In its introductory part points that this act respect human right to life in a healthy environment and that respect this right as a right of present and future generations (Principle 1). This convention, to make this right a reality, enact that human rights have to be respected at all levels, at: 1. Access to environmentally important information, 2. Participation in the formation of decisions of environmental importance, as well as 3. Effective access to administrative and judicial proceedings wherever the public considers it for necessary (Rio Declaration, Principle 10).

3. Elements of national legislation important for the production of safe food

For our topic, at the level of domestic legislature, hierarchically are the most important, as fundamental: “Law on Environmental Protection” and “Law on Nature Protection”. The “Law on Environmental Protection” obliges that every resource must be treated in accordance with: the principle of sustainable development (Art. 11, par. 1), the obligation of adequate control and protection (Art. 14), the officially approved project of protection and rehabilitation (Art. 15), and by implementing the obligation to carry out remediation (Art. 16). All these obligations arise from the established duty to protect: land (Art. 22), water (Art. 23) and air (Art. 24). All the above leads to a general obligation to achieve:

1. Adequate planning and construction (Art. 33),
2. Assessment of possible environmental impacts (Art. 35 and 36),
3. Integrated control and prevention of pollution (Art. 37) and to achieve
4. All this without violating the regulations that determine the limits of various emissions (Art. 39).

Due to all the above, it is necessary to determine “Conditions for specific operation of the plant and performance of (economic) activities (Art. 40)”, as well as to comply with “Conditions for operation of the plant and performance of activities (Art. 41)”, also obligation to monitor pollution (Articles 60d and 70-72). All these previously mentioned obligations were then elaborated within a series of specialized laws and accompanying by-laws, which determine the conditions for our topic of supreme

importance: planning and construction, mining [20], as well as the use and protection of water [34] and land [14].

“Law on Mining and Geological Researching” with the Art. 1 stipulates the obligation for all subjects to apply adequate measures during geological researching, design, construction of facilities, use of resources and rehabilitation and reclamation of land, as well as mining waste management. In specially protected natural and cultural areas mining can be performed only on the basis of a specially obtained permit (Art. 6), with respect for environmental protection, as well as remediation of used land (Art. 21, par. 1). Each project is subject to approval or disapproval, as well as control (Art. 25, par. 1), while the requirements of environmental and cultural treasures protection must be respected (Art. 25, par. 2). The project contractor must respect the prescribed measures of importance for human health and the environment (Art. 50, par. 1, item 8). Permission to conduct specific research can be obtained only if the project in question is done in accordance with the conditions of the so-called approved project (Art. 109, paragraphs 1 and 2). This law also obliges subjects to obtain a special permit for the treatment of mining waste during exploitation (Art. 144) and after the suspension of mining (Art. 151, par. 1), whereby the obligation to rehabilitate the terrain is also prescribed (Art. 153).

“The Law on Planning and Construction” prescribes, by Art. 3, the protection of natural and environmental values created by work, based on the “Spatial Plan of the Republic of Serbia” (Art. 14), while Articles 15, 18 and 19 set out guidelines and measures to protect them from possible negative effects. In particular, the spatial plan must contain the planned protection, arrangement, use and development of natural and cultural assets and the environment (Art. 20, par. 1, item 7). In order to have an adequate attitude towards the environment and the objects that are built into it by work, specific building rules must be formed for each specific space, i.e. process and object of action. These rules are contained in particular in the: Spatial plan of the special purpose area, Spatial plan of the local self-government unit and the plans of general and detailed regulation (Art. 31, par. 1). These documents must be available to the public (Articles 41, 42 and 50).

“Law on Planning and Construction” explicitly obliges the subjects to prepare: Preliminary feasibility study (Art. 113), Feasibility study (Art. 114), General design (Art. 117), Conceptual design (Art. 117a), Preliminary design (Art. 118) and Project for construction permit (Art. 118a) in accordance with the requirements of various projects. For example, if you want any mining activities in a field, then in accordance with the requirements of the “Rulebook on the content of mining projects” [27], and if we look at energy, then we act in accordance with the “Energy Law [7]” and accompanying decree [4].

“The Law on Integrated Prevention and Control of Environmental Pollution” [18] regulates the conditions and procedure for issuing integrated permits for facilities and activities that may have negative impacts on human health, the environment or material goods (Art. 1) as well as activities that may produce emissions and pollution (Art. 2, par. 1, item 3). An Integrated permit may be issued but an application for this permit may also be rejected. The Government of the Republic of Serbia prescribes the types of activities and facilities for which an Integrated permit is issued.

“Law on waste management” determines (with the Art. 1, par. 1,) the subjects of waste management, then the conditions and procedure for issuing permits related to waste management, but also cross-border movement of waste, as well as obligations for:

1. Waste classification,
2. Organization of waste management, as well as
3. Implementation of such organized management, then
4. Management of special waste streams,
5. Formation of databases (on waste),
6. Waste reporting,
7. Financing of waste management,
8. Supervision, and
9. All other possible issues of importance for waste management.

Art. 25 of this law contribute to the prevention of environmentally negative effects of waste, while Art. 26, par. 1, point. 6 oblige the producer of waste to dispose of it in a manner that does not adversely affect human health and the environment, and Art. 42 prohibits disposal in places for which no special permit has been obtained (see, for example, Art. 42, par. 2.). “Law on Waste Management” follow a number of by-laws, among which, for example, are particularly important: “Ordinance on the conditions and manner of collection, transport, storage and treatment of waste used as a secondary raw material or for energy production” [25], “Ordinance on categories, testing and classification of waste“ [24], “Ordinance on permitted quantities of hazardous and harmful substances in soil and irrigation water and methods of their testing” [26], and “Ordinance on the manner of storage, packaging and labeling of hazardous waste” [28].

CONCLUSION

We emphasize that at the level of the Republic of Serbia there are all needed legal conditions at the level of: 1. Constitution, 2. Adopted elements of international public law, as well as 3. Domestic laws and accompanying bylaws important for protection of: land, water, air, other natural and labor-created values, as well as protection from inadequate economic treatment(s), which elements also created legal conditions for the production of healthy food, i.e. preventing disruption of this production or reducing the quality of this food. This means that the competent authorities, in accordance with all these norms, must perform their duties and thus provide the necessary protection. And as we can see, for example, on the basis of the Jadar Project (lithium mining), the state authorities of Serbia are not fulfilling these obligations in an adequate way. We ask them to respect the valid legal norms!

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**ПРОИЗВОДЊА ЗДРАВСТВЕНО БЕЗБЕДНЕ ХРАНЕ
У СВЕТЛУ ЗАШТИТЕ ЗЕМЉИШТА И ВОДА**

Извод

У раду који вам представљамо посматрамо производњу безбедне хране у светлу правне заштите земљишта и воде, као кључне услове за то. То чинимо почев од Устава, као полазне тачке за његову реализацију, затим идући правно-логички указујући на општеприхваћене и кодификоване елементе међународног јавног права који се морају поштовати, и на крају на најважније сегменте домаћег законодавства који штите земљиште. и воде и тиме доприносе производњи здраве хране.

Кључне речи: *Устав, међународно јавно право, национално законодавство, битни елементи.*



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Original Scientific paper

THE IMPORTANCE OF THE APPLICATION OF FOOD STANDARDS IN THE HOSPITALITY INDUSTRY

Abstract

The implementation of quality standards in hospitality is broad (such as standards related to human resource management, standards related to the safety of tourists and employees in tourism, environmental standards, etc.). Particularly important standards are those related to food and beverages, representing the essence of hospitality services. The paper highlights the importance of introducing and performing food standards in the hospitality industry by presenting the most frequently used standards in this area, such as ISO 22000, HACCP, Halal and Kosher standards.

Key words: *quality, hospitality, food and beverage, standards*

INTRODUCTION

The hospitality industry is part of the service industry that includes a combination of different services – accommodation, food and beverage, and other non-hospitality services. Food products represent a set of raw materials, semi-finished products, and products integrated into one final product by applying different mechanical and thermal processing methods in special production conditions, which are offered to the guest and shaped into a gastronomic product.

Food and beverage quality is often a decisive factor in evaluating the quality of the hospitality product, especially in the restaurant industry (Gagić et al., 2013). The characteristics and quality of gastronomic products depend on several factors that should be observed throughout the entire production process, through the quality of presentation to the consumer, its sustainability on the market, and the profit it brings.

Quality in all levels of business represents the basis for competitive advantage and the strategic orientation of all organizations. It is achieved by providing quality principles and standards and implementing a quality management system. By introducing quality standards, organizations ensure more rational use of resources, achieve goals more efficiently and effectively, and provide higher quality services and products.

The quality of gastronomic products is a complex and multidimensional concept. It represents the level at which the product's characteristics meet the needs of consumers and assumes five aspects of quality: technological, health-safety, nutritional, microbiological, and sensory quality.

Technological quality includes characteristics of inputs, procedures in preparation, norms, and the way of serving food and drinks. Safety standards mean that the products must not contain prohibited amounts of heavy metals, pesticides, radionuclides, and other harmful substances. The microbiological aspect of quality indicates whether there is a greater number of total microorganisms in food and drink than allowed and that there are no pathogenic and conditionally pathogenic organisms in the food that cause food spoilage, intoxication, and harmful infection, i.e., food poisoning. Sensory quality represents the overall sensory impression of the sample, which is evaluated for the appearance, consistency/texture, and aroma of food products using the human senses (Popov Rajčić et al., 2010). Also, it is essential to mention the market-consumer quality, which refers to supply, demand, price, religious commitment, customs, consumer habits, etc. However, this element does not constitute the essence of a gastronomic product (Gagić et al., 2014).

Organizations in the hospitality sector should provide the market with quality food with specific nutritional and sensory characteristics but at the same time hygienically appropriate and safe food, which will have a favorable effect on the health of consumers (Jovičić, 2015). To ensure the guaranteed quality of products and services, organizations in the hospitality industry introduce and apply various standards that provide organizations with lower costs, rationalization of business activities, and greater client satisfaction.

The paper aims to point out the importance of standardization and certification in the hospitality sector, as well as to point out the most commonly applied standards in business catering practice, which are related to food safety, such as ISO food safety standards, HACCP standard, Kosher, and Halal standard.

The importance of standardization and certification in the hospitality industry

Quality standards are defined as documents that provide requirements, specifications, guidelines, or characteristics that can be used consistently to ensure that all materials, products, and processes are fit for purpose (Institute for Standardization of Serbia, 2022). Standardization means defining rules in an area to ensure that products or services are delivered in adequate and expected quality. Based on the Law on Standardization (Official Gazette of the Republic of Serbia 36/2009 and 46/2015) and the Law on the health safety status of foodstuffs and general use items (Official Gazette of the Republic of Serbia, No. 92/2011), a series of quality regulations were adopted

in the Republic of Serbia, which prescribes the minimum conditions that must be met by foodstuffs in production and circulation.

In addition to the legal minimum, ISO standards are a voluntary and effective tool for the management of any organization that wants to improve the quality of products and services because it ensures more efficient and effective monitoring of processes, achievement of goals, and detection of bottlenecks in the system.

The top management decides to implement standards in its organization, and confirmation of successful implementation is obtained through the certification procedure (<https://www.srdjansimic.com/standardizacija-sertifikacija/>). The application and performance of the standard are voluntary, except in cases where the producer is called to the standard or specific technical regulations by contract with the end customer.

Certification represents the process of determining that a product, service, organization, or individual meets the requirements of the relevant standard and results in obtaining an official confirmation – certificate of conformity, and registration of the certificate holder in the appropriate register (<https://iso.org.rs/>).

The preparation process for certification includes the preparation of documentation and employees for certification and can be observed through several stages. The first stage consists of the preparation of documentation for the introduction of ISO standards. Then follows the preparation of the employees, which involves examining the employees about their knowledge regarding the application of procedures in the service or sector, the responsibilities of the employees, the documentation used, etc. In the last phase, after certification, the person in charge of implementing the standards is defined. This person manages and reviews the ISO system, removes non-conformities, undertakes control checks, and changes and improves the system. Preparing documentation and employees takes one to two months (Barjaktarović & Knežević, 2021).

Among the most important standards in the hospitality sector, the standards related to food safety stand out, which will be presented in the next part of the paper.

ISO 22000 Food Safety Management Systems

ISO 22000 is the first international standard for food safety management systems. The ISO 22000 standard covers all organizations in the food chain and defines the requirements of the food safety management system (ISO 22000:2018).

This standard's basis is the HACCP system's principles designed to provide consumers with a safe final food product. ISO 22000 is the first standard that is suitable for implementation by all organizations involved in the food supply chain, such as suppliers of non-food products and services, equipment and packaging manufacturers, logistics service providers, agricultural producers, food manufacturers, manufacturers of additives and auxiliaries in processing, suppliers, retailers, catering establishments and catering companies, etc.) (<https://haccp.rs/iso-22000-2005/>).

ISO 22000 can be implemented by organizations of various sizes, from bakeries with a few employees to large food manufacturers employing thousands of workers. As the implementation of HACCP is often complex for the performance of large

production and sales chains and as it requires the implementation of ISO 9001, the ISO 22000 standard represents a compromise solution because it integrates both the mentioned standards, along with the analysis and monitoring of trends of many factors from the environment that they can negatively affect food safety, production process, financial result, employees and more.

Introducing ISO 22000 improves the quality and safety of food products, creating trust and greater consumer protection, lower costs in the production and distribution chain, and more.

This standard combines recognized elements for food safety, such as interactive communication, management system, control of the process, HACCP principles, etc. (<https://haccp.rs/iso-22000-2005/>).

HACCP

HACCP (Hazard Analysis Critical Control Points) is a food safety system based on the analysis and control of potential biological/microbiological, chemical, and physical hazards to which raw materials are exposed, possible hazards during handling, production, distribution, and consumption of the final product (<https://haccp.rs/>). HACCP is a system that ensures food safety "from farm to fork."

Its application implies compliance with standard operating procedures and instructions that reduce food safety risks and ensure a safe product. Given that a significant part of the hospitality industry is related to food production and processing activities, it is clear how important it is to manage the quality control of food products. The application of the HACCP standard is often considered one of the indicators of service excellence.

The HACCP system is a model that contributes to the safety of guests, their health, and satisfaction. Implementing this system in hotels and restaurants aims to increase the quality of the processes. Therefore, in this way, sanitary correctness is ensured, and a better quality of catering service in general, and the method can be applied both to individuals and to a larger number of operations (Erdeji et al., 2012).

HACCP system certification goes through several stages that include the following activities:

- carrying out a hazard analysis,
- identification of critical points in the process where danger can be prevented, eliminated, or reduced to an acceptable level,
- establishing a critical limit for the control of preventive measures concerning each identified critical control point (CCP),
- establishment of CCP monitoring,
- establishment of corrective activities to be undertaken when monitoring indicates that there is a deviation from the established critical limits,
- establishment of effective records of documents of the HACCP system.
- establishing procedures for verifying that the HACCP system is appropriate.

Halal standard

The Halal standard represents a set of rules and guidelines for producing and preparing food by Islamic religious practices. Halal requirements come from the Koran and Sharia law, which prescribe what is halal, i.e., what is permitted, and what is haram – what is forbidden. Halal food is prepared according to Sharia law which most Muslims worldwide follow. Among other things, Halal prohibits the use of pork, blood, alcohol, the meat of dead animals, carnivores, donkeys, dogs, birds of prey; food prepared with wine, cakes with any alcohol, food containing emulsifiers based on pork or animal fat, etc. (Gagić et al., 2017).

Unlike other standards and the HACCP system, Halal certification is not part of the formal certification and accreditation system. Certificates are awarded by organizations authorized by the Islamic religious community. A company interested in introducing this standard must apply to the Islamic Community Agency. The certificate is valid for one year, and during the duration of the certificate, regular control is carried out by the Halal controller and a sampling of products from the market.

The Halal sign contributes to the recognition of Halal products on the market. In case of any deviation from the standard, the Agency revokes the certificate, the products are withdrawn from the market, and the appropriate procedure is initiated (<https://www.kvalitet.org.rs/infrastruktur/standardi/halal>).

Kosher standard

The Kosher standard initiates food production and preparation according to Jewish regulations and customs. Kosher certificate guarantees that a particular food product meets the requirements prescribed by the Torah. The Torah prescribes which products are acceptable for consumption and how they must be prepared to be allowed for consumption (Gagić et al., 2017).

Kosher foods are meat, goods, fruits, vegetables, cheese, fish, eggs, fruits, and vegetables that can be consumed together with dairy or meat products. In contrast, the Kosher standard prohibits meat and dairy products from being prepared and finished together. Also, meat, eggs, and organs from a non-Kosher animals are not allowed. Vegetable oils can be used in the preparation of dairy and meat products.

Thanks to the unique control prescribed by this standard, Kosher food is considered healthier. Considering that the Kosher certificate is increasingly recognized as a sign of healthy and quality food, in addition to members of the Jewish community, food with the Kosher label is also consumed by members of the Muslim faith, vegetarians, and people who are allergic to different types of food. Many multinational companies have long recognized the benefits of Kosher certification (Gagić, 2017).

This *certification* verifies that the products are by the Kosher requirements issued by an authorized Rabbi. In contrast, the ECK (European Central Kashrut) approves the Kosher certificate. In the case of technologically simpler products, the kashrut expert controls the product himself, while more technologically complex products are controlled by a representative of ECK (<https://www.kvalitet.org.rs/infrastruktura/standardi/koser>).

CONCLUSIONS

Food and beverage are essential services in the hospitality industry, and their quality level determines the guests' satisfaction. Hospitality organizations introduce quality standards presented in the paper, aiming to bridge the gap between users' perceptions and experiences about the quality of service, on the one hand, and the management and employees of the organization, on the other hand. This ensures a competitive advantage, securing higher revenues, and creates guests' satisfaction and loyalty.

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ЗНАЧАЈ ПРИМЕНЕ СТАНДАРДА У ВЕЗИ СА ХРАНОМ У УГОСТИТЕЉСКОМ СЕКТОРУ

Резиме

Примена стандарда квалитета у угоститељству је широка (стандарди који се односе на управљање људским ресурсима безбедност туриста и запослених у туризму, еколошки стандарди итд.). Посебно су важни стандарди који се односе на храну и пиће, који представљају суштину угоститељских услуга. У раду је истакнут значај увођења и спровођења стандарда за безбедност храну у угоститељству представљањем најчешће коришћених стандарда у овој области, као што су ИСО 22000, НАССР, Халал и Кошер стандарди.

Кључне речи: *квалитет, угоститељство, храна и пиће, стандарди*

**ECOLOGICAL MODELS AND SOFTWARE
IN THE PRODUCTION OF HEALTHY
SAFE FOOD.**



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Original Scientific paper

DESIGNING OF FORAGE SYSTEMS FOR IMPROVED SUSTAINABILITY OF CATTLE FARMING AND AGRICULTURE

Abstract

The majority of Croatian arable land is destined to fodder and forage production. Modern feeding of arable produced feeds enabled the probably greatest livestock productivity in history. However, arable farming consumes much fossil fuel from finite resources and ruins biodiversity, while heavy farm machinery causes soil compaction and amplified drought effects. The aim of the work is to answer if we can make our cattle farming and agriculture more sustainable by turning cattle farming into more grazing and less feeding of concentrates and silage maize?

Research has shown that a shift to grazing during the grazing season and feeding forages only during the off-pasture season would certainly improve the biodiversity in the agricultural area and would make our environment and landscape more beautiful. Improvements in soil quality are also expected. Since only about half of forages have to be conserved for the off-pasture season, the consumption of fossil fuels would also be decreased. Findings synthesized from the cited literature, and obtained from the authors' projections indicate that the shift to solely grazing during the grazing season and feeding solely on forages during the off-pasture season would require more than a double agricultural land resource if there is intended to maintain the contemporary production volume. The need for an increase in forage production area comes mainly because of greater total feed consumption per head, due to prolonged feeding of beef cattle and lesser milk yield per dairy cows, and somewhat lesser yields of perennial forages when compared to silage maize. However, an abundant and underutilized perennial grasslands area of Croatia could probably partially offset the needed increase in the forage production area. Therefore, appears that we could gain many benefits if the future forage systems are designed more rely on pastures and perennial forages on arable land instead of contemporary annual forages and grains.

The main findings derived from the presented synthesis are partially based on the literature review of published field research and partially based on the authors' theoretical projections and therefore cannot be regarded as reliable without field testing and measurements on live animals in Croatian conditions.

Key words: *forage system, agriculture, sustainability, livestock farming*

INTRODUCTION

Sustainable society development is closely related to conservation or even improvement of the environment, biodiversity, soil fertility, and even independence of fossil energy resources.

However, the production of annual arable crops is associated with the consumption of fossil fuels from distant and finite resources, loss of soil organic matter, soil compaction, and detrimental effects to biodiversity and ruined beauty of the landscape. Namely, arable soils have almost no vegetation cover during the shift between crops in their temporal sequence and during the early phases of crop development, therefore looking like deserts. A desert-like landscape on arable land is very often longer than mentioned because of the lack of cover-cropping practices between the harvest of fore-crops and the establishment of subsequent crops. Considering biodiversity, Mueller et al. (2014) have found that meadows and pastures were the least harmful to biodiversity, followed by permanent crops, whilst arable land had the poorest biodiversity.

According to the Croatian Bureau of Statistics (CBS, 2018), of the total arable area in Croatia (about 850,000 ha), crops destined for feeding farm animals occupy about 52 %, of which about 48 % belongs to annuals (mainly maize, soybean, and barley) and about 4 % to perennials (mainly lucerne and clover). According to the same source, there is being fed about 445,000 heads of cattle (including calves), about 1,150,000 pigs (including piglets), about 620,000 sheep (including lambs) and 10,000 poultry, which indicates that principal herbivores and grazers (cattle and sheep) take up the majority of the livestock sector. Despite the pasture being the most natural feed for grazers, and grazing is there the most natural behavior, there largely prevail confinement feeding operations with TMR ratios in modern cattle farming in Croatia (as well as in the majority of countries with industrialized economies). Therefore, appears an issue: can we make our agriculture more sustainable by a shift of the cattle farming to more grazing and less concentrates and silage maize feeding?

Findings from this research can impact the farm managers and decision-makers to consider more grazing and less concentrated production and consumption for the future design of forage systems.

DAIRY CATTLE PRODUCTIVITY ISSUES

The standard lactation period of dairy cows lasts for 305 days, and with divided into three phases: the 1st third or maximum lactation, mid-lactation, and end-lactation with minimum productivity. Modern dairy cows are very productive due to contemporary feeding with TMRs rich in concentrates (maize or barley grain and soybean meal) and energy-rich forage (whole-crop maize silage). Average milk production during the standard lactation in Croatia for the cows of the Simmental breed is about 5,500 kg/head/305 days and of the Holstein breed about 8,300 kg/head/305 days (CAAF, 202), which response to the average daily milk yield of 18 and 27 kg/head/day, respectively. However, maximum daily milk yields with TMR feeding can easily reach

the levels between 40 and 50 kg/head/day for the Holstein breed in Croatia. For dairy cows when fed solely the pasture, daily milk yield can reach up to 30 kg/head/day, but only if it is a high-quality and lush pasture, which can be found mainly in spring, and during the period of maximum lactation. In the research of Kolver and Miller (1998) in Pennsylvania (USA), Holstein cows on perennial ryegrass + white clover pasture yielded 29.6 kg/head/day during the period of maximum lactation. However, in reality there often do not prevail perfect conditions, so the daily milk yield can be lower during the maximum lactation period, like in the study of O'Neill et al. (2011) in Ireland, who obtained 21.1 kg/head/day with Holstein cows that grazed perennial ryegrass. When fed stored forages only, cows can yield less milk since the storage forages usually have lower quality than fresh herbage in the same maturity stages. Brown Swiss and Holstein cows in Switzerland, fed mature hay (from predominantly perennial ryegrass sward) and second-cut silage (from the same sward), without concentrates, yielded only about 10 kg/head/day milk at the end of their lactation (Hindrichsen et al., 2006). For the east Croatian semiarid conditions, there would be more applicable data about dairy cows' productivity when grazed on drought-resistant forages like lucerne and its mixtures with perennial grasses. In the research of Smith et al. (2013) in New Zealand, Fresian×Jersey on pure lucerne pasture yielded an average of 25.3 kg/head/day of milk during the early lactation, which indicates that drought-tolerant lucerne can provide for good productivity of dairy cows. Probably would be the same when grazed on lucerne-grass mixtures. During the lactation period, daily DM consumption of highly productive and TMR-fed dairy cows varies between 3 and 4 % of the actual cow's LBM, whilst during the 60 days' dry period it is about 2.5 % of the LBM. Considering the pasture DM consumption, it is expected about 2.5 % relative to the cow's LBM, as in the study of O'Neill et al. (2011). However, for high-yielding cows, the pasture DM consumption can be about 3 % of the cow's LBM or even greater (Kolver and Miller, 1998), but only when the pasture is of high quality and the offered herbage allowance is greater enough than the targeted DM consumption.

YIELD OF FORAGES AND GRAIN FEEDS

According to Gantner et al. (2021), whole-crop maize silage is the most used forage in the TMR-fed cattle because of its high energetic value and its exceptionally high herbage DM yield – about 20 t/ha on fertile soils of continental Croatia, which response to about 60 t/ha of the silage as-it-is. However, in drought conditions, and on poorer soils, actual DM yields can be much lesser, often halved. In semiarid regions and on fertile soils, the second forage is usually lucerne due to its appreciable protein content, drought resistance and fine stems that provide long fibre. On poorer soils and more humid regions, as a fine-stemmed and protein-rich forage there prevail red clover, cool-season perennial grasses or grass-clover mixtures. These fine-stemmed forages on fertile soils of continental Croatia usually yield about 10 t/ha of forage DM, which is usually harvested, stored and fed as hay, haylage or silage. On poorer soils and in drought conditions yields are usually much lesser, and can be less than half of the presented.

Considering the grain yields, on fertile soils of continental Croatia, maize yields about 10 t/ha of grain, but in the drought years, it can be less than a half. Barley yields between 5 and 9 t/ha and is less affected by drought because it ends its vegetation before the onset of summer drought. Soybeans yield about 4 t/ha on fertile soils, while on poor soils and in drought conditions yield can be less than a half.

The turn to more grazed and hayed perennial forages would provide somewhat lesser yields because the whole-crop maize silage yields about double or even more. But, maize silage is not the only component of TMR, and the TMR's components' weighted average yield is decreased by lower yields of grain crops (maize, barley, soybean). More reliable projections there would be required a more accurate estimation of the weighted average yield of TMR components and comparison with yields of perennial forages. Also, there is an apparent need to check what would be the yields of perennial forages like lucerne and lucerne-grass mixes when utilized by grazing. Namely, the absence of heavy machinery and its soil compaction could provide for a higher forage yield, better soil water holding capacity and lesser drought impacts, but it could also lead to a lesser utilization rate of forage yield, and surface soil compaction if grazing is conducted on wet soil, what in turn again leads to lesser yields. There should also be studied various grazing methods and skills to derive the maximum from grazed areas.

SHIFT EFFECTS ON CARRYING CAPACITY AND NEEDS FOR ADDITIONAL PERENNIAL FORAGE CROPS AREA

The carrying capacity of productive agricultural land (head/ha) depends on the ratio of annual feed DM production (t/ha/year), annual feed DM consumption (kg/head/year) and utilization rate (%). Annual consumption per animal or head would probably be similar between the modern system and the proposed shift to grazing with no concentrates, so this would not seriously change the carrying capacity. But, as presented above, a shift of beef fattening solely to grazing and concentrate-less feeding leads to a decrease in ADGs from about 1.3 kg/head/day to about 0.7 kg/head/day, i.e. ADGs are near halved, which implies that the fattening period should be doubled for the same final LBM (2 years instead of 1 year). This elongation would require doubling the total feed DM consumption, which has to be considered in the estimation of the required area of perennial forage crops. The milk yield of dairy cows is expected to be halved also on an annual basis when compared to the modern TMR-fed cows, which implies that the same milk production there would be required two cows instead of one modern TMR-fed cow. Therefore, there would be needed to double forage production upon the shift to grazing and solely forages feeding during the off-pasture season.

Somewhat lesser forage yields of arable perennial forage crops (lucerne, lucerne-grass-mixes, clover-grass mixes) than yields of the weighted average yield of TMR components would require an additional area under the forages production. Croatian extensive permanent grasslands occupy about 1.5 million hectares (CBS, 2003) and present a huge and underutilized resource for forage production. The annual production

of hay equivalents is estimated at 700,000 tons (CBS, 2003). If we assume that a herbivore livestock unit (LU) of the 500 kg LBM equivalent consumes 2.8 % of LBM daily, this gives an annual consumption of 5,110 kg/LU/year of forage DM. If the utilization rate of the forage from Croatian permanent grasslands (as pasture and hay) is about 70 %, then the carrying capacity of these grasslands would be near 100.000 LU. Some improvement of grasslands (fertilization and better utilization) could likely enhance their productivity and raise their carrying capacity.

SHIFT EFFECTS TO BIODIVERSITY, SOIL QUALITY AND LANDSCAPE BEAUTY

Shift to perennial forages on arable land used for grazing and haying or silage-making would certainly improve the biodiversity of agricultural land since Mueller et al. (2014) found that meadows and pastures were the least harmful to biodiversity, followed by permanent crops, whilst arable land mostly affected biodiversity. Considering the soil quality, perennial forages do not require soil tillage for many years thus conserving and building the soil's organic matter and fertility. Improvements in soil quality upon the conversion of arable into perennial forage crops were documented in Germany (Ajayi and Horn, 2016). In the cases where livestock can be grazed the whole year, it would be possible to completely exclude the heavy farm machinery from the agricultural land, thus enabling the recovery of nowadays compacted arable soils. Perennial forage crops on arable land cover the soil the whole year, and if they comprise the mixes of grasses with legumes, they usually rarely have a bare soil area. An increase in the arable area permanently covered with plant cover makes our environment and landscape more beautiful than bare arable soils. This would allow for a greater presence of game, wildlife, birds, butterflies and other insects in our agricultural area, and a more attractive landscape for the urban people.

CONCLUSIONS

Shift to grazing during the grazing season and to forage-only feeding during the off-pasture season would certainly improve the biodiversity in the agricultural area and would make our environment and landscape more beautiful. Improvements in soil quality are also expected. Since only half of the forages have to be conserved for the off-pasture season, the consumption of fossil fuels would be also decreased. Findings synthesized from the cited literature and upon the presented authors' projections indicate that the shift to solely grazing during the grazing season and feeding solely on forages during the off-pasture season would require more than a double agricultural land resource if there is intended to maintain the contemporary production volume. The need for an increase in forage production area comes mainly because of greater total feed consumption per head, due to prolonged feeding of beef cattle and lesser milk yield per dairy cows, and somewhat lesser yields of perennial forages when com-

pared to silage maize. However, an abundant and underutilized perennial grasslands area of Croatia could probably partially offset the needed increase in the forage production area. Therefore, appears that we could gain many benefits if the future forage systems design greatly relies on pastures and perennial forages on arable land instead of contemporary annual forages and grains.

The main findings derived from the presented synthesis are partially based on the literature review of published field research and partially based on the authors' theoretical projections and therefore cannot be regarded as reliable without field testing and measurements on live animals in Croatian conditions.

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ПРОЈЕКТОВАЊЕ СИСТЕМА ЗА ЗА СТОЧНУ ХРАНУ ЗА УНАПРЕЂЕЊЕ ОДРЖИВОСТИ СТОЧАРСТВА И ПОЉОПРИВРЕДЕ

Сажетак

Највећи део хрватских ораница намењен је производњи сточне хране и сточне хране. Савремена исхрана сточне хране омогућила је вероватно највећу сточарску продуктивност у историји. Међутим, ратарска пољопривреда троши много фосилног горива из ограничених ресурса и уништава биодиверзитет, док тешка пољопривредна механизација узрокује збијање тла и појачане ефекте суше. Циљ рада је да одговоримо да ли можемо да учинимо наше сточарство и пољопривреду одрживијим претварањем сточарства у више испаше и мање исхране концентрата и силажног кукуруза?

Истраживања су показала да би прелазак на испашу током пашне сезоне и исхрану сточне хране само током ванпашњачке сезоне свакако побољшао биодиверзитет у пољопривредном подручју и учинио нашу околину и пејзаж лепшим. Очекује се и побољшање квалитета земљишта. С обзиром да се само око половине сточне хране мора сачувати за сезону ван пашњака, смањила би се и потрошња фосилних горива. Налази синтетизовани из цитиране литературе, а добијени из пројекција аутора указују на то да би прелазак на искључиво испашу током пашне сезоне и исхрану искључиво сточном храном током сезоне ван пашњака захтевао више него дупло већи ресурс пољопривредног земљишта ако се намерава одржати савремени обим производње. Потреба за повећањем површина за производњу сточне хране настаје углавном због веће укупне потрошње сточне хране по грлу, због продужене исхране јунади и мањег приноса млека по музним крвама, и нешто нижих приноса вишегодишњих крмних биља у односу на силажни кукуруз. Међутим, обилна и недовољно искоришћена вишегодишња пашњачка површина у Хрватској би вероватно могла делимично да надокнади потребно повећање површине за производњу крмне хране. Стога се чини да бисмо могли да добијемо много користи ако се будући системи сточне хране ослане више на пашњаке и вишегодишњу сточну храну на обрадивим површинама уместо на савремену једногодишњу сточну храну и житарице.

Главни налази произашли из приказане синтезе дијелом су засновани на прегледу литературе објављених теренских истраживања, а дијелом на теоријским пројекцијама аутора и стога се не могу сматрати поузданим без теренских испитивања и мјерења на живим животињама у хрватским увјетима.

Кључне речи: *систем сточне хране, пољопривреда, одрживост, сточарство*



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Original Scientific paper

STUDY ON POTENTIAL CYTOTOXIC AND CLASTOGENIC EFFECTS OF *ROSA CENTIFOLIA* L. WASTEWATER

Abstract

A considerable amount of wastewater is produced during water-steam distillation of rose oil. This represents an environmental problem that should not be neglected. However, since this wastewater contains many bioactive substances, other targeted uses should be considered. Indeed, it should be checked whether this wastewater is harmless. In this sense, the aim of the present study was to investigate the cytotoxic and genotoxic potential of *Rosa centifolia* wastewater. For this purpose, we used a battery of test-systems, including higher plant, rodents, and cultured human lymphocytes, to obtain detailed information. Classical cytogenetic methods were used. A cytotoxic effect was found in mice and lymphocytes. No effect on mitotic activity in barley root meristems has been noted. Only a weak genotoxic activity of *Rosa centifolia* wastewater was detected in all test systems. Our data are promising for further use of this product in human life.

Key words: *R. centifolia* wastewater, cytotoxicity, genotoxicity, test-systems

INTRODUCTION

Given the extremely low cost of agricultural bio-waste and the significant waste generated by the use of technological processes, it is clear that the wastewater potential is not to be discarded. Increasingly, this material is being investigated for the presence of bioactive substances (Celano et al., 2017; Musto et al., 2022). Waste products from

rose oil distillation have recently gained particular interest (Rusanov et al., 2014). The wastewater (ww) as liquid aqueous phase after dry distillation of *R. damascena* Mill. rose petals contains three groups of polyphenolic compounds - quercetin glycosides, kaempferol glycosides and gallic acid glycosides (Dragoev et al., 2021). In the ww of Narcea rose, a natural hybrid of *R. gallica* and *R. centifolia*, the followed phenolic compounds were identified – total quercetin derivates ($1176.90 \pm 45.52 \mu\text{g/g}$), total kaempferol derivates ($154.70 \pm 14.71 \mu\text{g/g}$) and gallic acid $726.96 \pm 23.47 \mu\text{g/g}$ (Boso et al., 2022). Georgieva et al. (2021) determined tannins, flavonoids, and total polyphenols in rose ww's. *R. centifolia* L. ww contains $2.47 \pm 0.05 \text{ mg/mL}$ tannins, $0.61 \pm 0.04 \text{ mg/mL}$ total flavonoids and $7.8 \pm 0.22 \text{ mg/mL}$ total polyphenols. This logically raises the question of whether products made from biowaste, or the use of biowaste itself, are safe for biological systems or even for human use?

Limited studies exist about the cytotoxic/genotoxic activity of rose wastewaters. *R. alba* L. ww showed a negligible genotoxicity, but a weak antiproliferative effect in laboratory animal test model (Gerasimova et al., 2022). The wastewaters of Bulgarian *R. damascena* Mill. and *R. alba* L. released after water-steam distillation of essential oils did not show strong cytotoxic/genotoxic effects and could decrease DNA damage, induced by proven mutagen in different experimental test-systems. (Gateva et al., 2022).

Up to now, no data on the possible genotoxicity of *R. centifolia* ww are available in the literature. Thus, the aim of the present work was to evaluate the cytotoxic and genotoxic potentials of the ww using three different experimental test-systems - higher plant *in vivo*, ICR mice *in vivo* and human lymphocytes *in vitro*. Relevant endpoints for cytotoxicity and genotoxicity, namely mitotic index (MI), chromosome aberrations (CA) and micronuclei (MN) were evaluated.

MATERIAL AND METHODS

Preparation of wastewater

The ww was collected after the distillation of rose essential oil, in the semi-industrial installation of the Institute for Roses and Aromatic Plants, Kazanlak. The process parameters were as follows: raw material 8 - 10 kg; hydro module 1:4; flow rate 16-20 ml/min; duration 150 min. The ww was stored under cool conditions until use.

Chemicals

The standard mutagen N-methyl-N'-nitro-N-nitrosoguanidine MNNG ($50 \mu\text{g/mL}$) was used as a positive control for all three test-systems. A parallel experiment with 0.9% NaCl solution was used as a negative control in the experiments with ICR strain mice.

Test-systems and experimental design

Plant test-system – Hordeum vulgare (barley). Seeds of the reconstructed karyotype MK 14/2034 of barley were presoaked for 1 h in tap water and germinated for 17 h in Petri dishes on moist paper at 24°C. The root meristems were exposed (4 hrs) to *R. centifolia* ww at 6, 14 and 20%. After the recovery times of 18, 21, 24, 27 and 30 h, the root tips were treated with 0.025% colchicine in a saturated solution of α -bromonaphthalene (2 h), fixed in ethanol: acetic acid (3:1), hydrolyzed in 1 N HCl at 60 °C (9 min), Feulgen-stained, macerated in 4% pectinase in distilled water for 12 min and squashed on slides for scoring of chromosome aberrations (Jovtchev et al., 2002). For scoring of micronuclei (MN), the root tips were fixed after 30 h recovery time without any colchicines treatment.

ICR strain albino mice. Eight-week-old male and female mice (20.0±1.5g b.w.), were kept for several days at temperature 20-22°C, photoperiod 7 am to 7 pm, free access to standard for laboratory animal's food and water. The experiments were performed in accordance with Bulgaria's Directorate of Health Prevention and Humane Behavior toward Animals.

For scoring chromosome aberrations, the *R. centifolia* L. ww, the animals were administered with a single dose of 11% or 20% ww solution by intraperitoneal (i.p.) injection, for 24h or 48h. The positive control group was treated i.p. with MNNG - 50 µg/ml (0.01 mg/mL). For the micronucleus test, blood samples were obtained from the tail veins of mice, before ww solution supplementation (t0) and 48 (t1) h post-ww solution injection. The detailed methodology can be found in Gerasimova et al. (2022).

Human lymphocytes in vitro. Peripheral venous blood of healthy donors (33 to 40 years) was used to prepare lymphocyte cultures, which contained RPMI 1640 medium, 12% calf serum, 40 mg/ml gentamycin and 0.1% phytohemagglutinin (PHA). The method of Evans, 1984 was used to assess the chromosome aberrations. The lymphocytes were treated with ww in concentrations of 6, 11 and 14% (4 hrs). After the treatment, the cells were washed in fresh medium and cultured at 37 °C. At the 72-nd hour of cultivation, 0.02% colchicine was added to each sample, followed by 0.56 % KCl, fixation in methanol: acetic acid (3:1, v/v), and stained in 2% Giemsa. For scoring the micronuclei, cytochalasin-B (6 µg/ml) was added to each culture at the 44th hour after PHA stimulation (Fenech, 2007). All procedures were conducted according to the Declaration of Helsinki.

Untreated cells were used as a negative control for barley and human lymphocytes. The negative control group for animals received only 0.9 % NaCl.

Cytotoxic/genotoxic endpoints

Cytotoxic effect was assessed by the value of mitotic index (MI=A/1000, A-number of dividing cells) in the three test-systems.

Genotoxic effect was evaluated by frequencies of chromosome aberrations (CA) and micronuclei (MN). The percentage of metaphases with chromosome aberrations (MwA% ± SD) was calculated for each experimental variant. Percentage of micro-

nuclei (MN% \pm SD) per each sample was calculated. Micronuclei were easily distinguishable in peripheral blood polychromatic erythrocytes (PCE) of ICR mouse with acridine orange staining, which emit red fluorescence (Hayashi et al., 1983).

Statistical analysis Data were statistically evaluated for their significance by analysis of variance using two-tailed, two-sample Student's t-test. Statistical significance is expressed as $p < 0.001$; $p < 0.01$; $p < 0.05$; $p > 0.05$; – (not significant).

RESULTS

Cytotoxicity

The results obtained for cytotoxicity, evaluated by MI values, showed that *R. centifolia* ww did not exhibit any reduction of mitotic activity in barley (Fig. 1A), whereas it was significantly suppressed in bone marrow cells of ICR mice (Fig. 1B) and in human lymphocytes (Fig. 1C) in a concentration dependent manner compared to the negative control. A dose-dependent effect ($p < 0.05$) – 20 % rose ww solution significantly inhibited the bone marrow cells proliferation compared to the 11 % dose at 24 h as well as 48 h after ww administration. The values of MI in the groups of animals, treated with ww, were relatively close to the MNNG values. The similar result was obtained for lymphocytes, which is an undoubtedly evidence of the ww cytotoxic properties in both test-systems. On the other hand, MI values in ww-treated barley showed no changes in mitotic activity compared to the control (Fig. 1A).

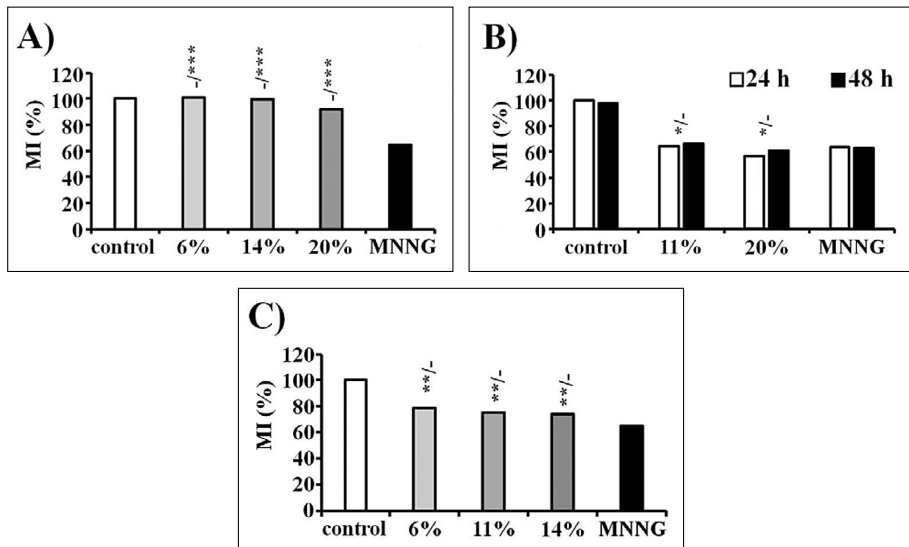


Figure 1. Mitotic activity observed after application of different concentrations of *R. centifolia* wastewater in: A) *H. vulgare*; B) ICR strain mice; C) human lymphocytes in vitro, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, - not significant, compared with the negative control (under slash), compared with MNNG (over slash)

Genotoxicity

Chromosome aberrations. Wastewater enhanced CA frequencies ($p < 0.001$) compared to untreated control samples both in plant and in lymphocyte test-systems (Fig. 2A, C). No dependence on the concentration applied was observed. In animal test-system *in vivo* all treated with the rose by-product experimental groups showed a low percentage of damaged cells, and the data were comparable ($p > 0.05$) with the untreated control group values (Fig. 2B). The lowest percentage of chromosomal aberrations ($0.50\% \pm 0.92$) was obtained in the group, which received *R. centifolia* ww (11 %, 24 h after a single i.p. supplementation) and the highest one was calculated in the group, directly treated with the ww (11 % – 1.75 ± 1.67) at the 48th hour after exposure. Nevertheless, there was no dose-dependent or sampling time-dependent increasing trend in the mean chromosomal aberrations values after treatment with ww solution. No significant differences were observed between results for males and females mice; consequently, data for both sexes were combined.

The mean aberrant metaphases in the MNNG treated plant cells, animal groups and lymphocyte cells were observed to be statistically higher ($p < 0.001$) as compared to rose wastewater treated samples and animal groups in all concentrations and time intervals investigated by our team. (Fig. 2A, B, C).

This result indicates that *R. centifolia* ww induced a genotoxic effect in the concentrations tested (6%, 11%, 14% and 20%) in higher plant and in human lymphocytes and did not induce such effect in 11% and 20% in animal test-system assessed by CA assay.

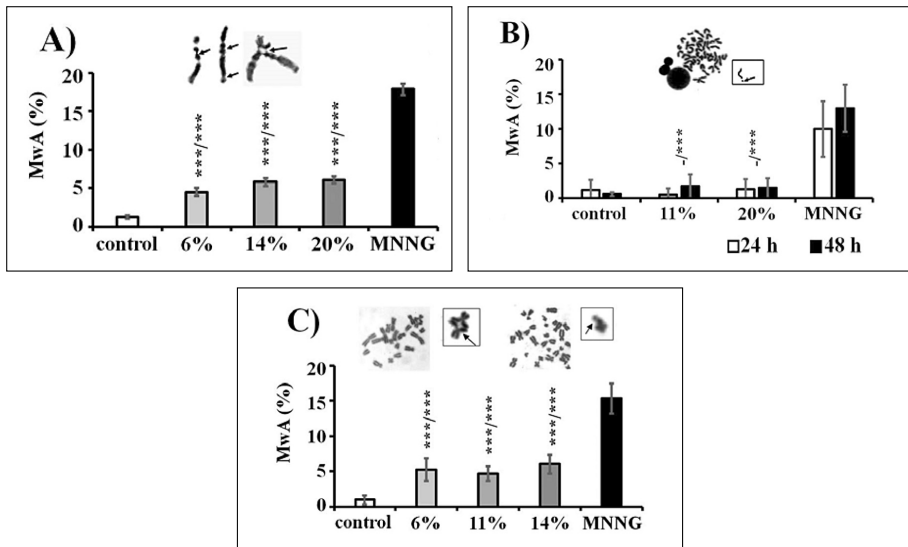


Figure 2. Chromosome aberrations induced after application of different concentrations of *R. centifolia* wastewater in: A) *H. vulgare*; B) ICR strain mice; C) human lymphocytes *in vitro*, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, – not significant, compared with the negative control (under slash), compared with MNNG (over slash)

Micronuclei. When the genotoxic effect of rose ww was evaluated by MN induction, the higher values of damages were obtained after treatment with all concentrations of ww in both barley and human lymphocytes (Fig. 3 A, C). In the plant test system MN frequencies varied in a dose dependent manner. The range of induced MN by concentration of 6% were $0.20\% \pm 0.05$, by 14% – 0.43 ± 0.08 and by 20% – $0.47\% \pm 0.08$, respectively. In human lymphocytes the values of MN increased with enhancing the concentration, but this was not statistically proven. The frequency of MN was in range from 0.8 ± 0.15 for 6% to 1.05 ± 0.12 for 14%. The MN was observed in animal test-system after a single i.p. application of rose ww, where the blood collection was performed 48 h after treatment. There was not any increase in frequency of MN in PCE from treated groups, when compared to saline group ($p > 0.05$). It can be noted that for both doses of ww (11% and 20%), the total number of micronucleated polychromatic erythrocytes is almost the same as that of the saline group.

As with the test for induction of chromosome aberrations as well with that for micronuclei, MNNG induced a significant increase ($p < 0.001$) in the frequency of MN compared with that induced by the rose ww in all test-systems (Fig. 3A, B, C).

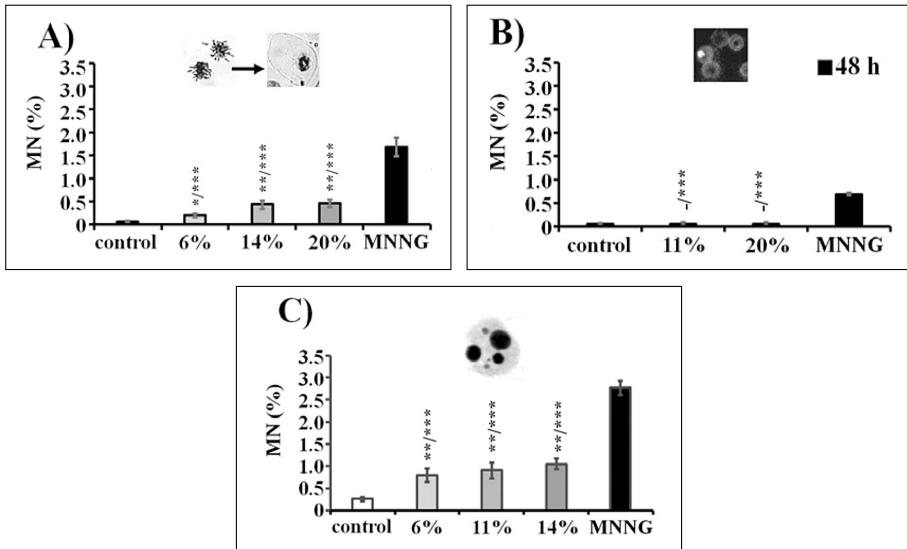


Figure 3. Micronuclei induced after application of different concentrations of *R. centifolia* wastewater in: A) *Hordeum vulgare*; B) ICR strain mice; C) human lymphocytes in vitro, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, – not significant, compared with the negative control (under slash), compared with MNNG (over slash)

DISCUSSION

In this study, the possible cytotoxic and genotoxic effects of *R. centifolia* ww were investigated using MI, CA and MN assays in different test-systems. These tests on plant root meristem cells, mammalian bone marrow cells and human lymphocytes are

widely used to provide information on potential cytotoxicity, clastogenicity of chemicals both in *in vivo* and *in vitro* (Rothfuss et al., 2011; Yon et al., 2018; Jovtchev et al., 2002; Kwasniewska and Bara, 2022).

The significant reduction of bone marrow cell population and human lymphocytes mitotic activity obtained by us for the studied concentrations indicated that *R. centifolia* L. ww influenced the replicative capacity of the cells. Rose petals are described to contain compounds with potential antiproliferative activity (Wedler et al., 2016), and the chromatographic profile of rose constituents are of interest to be separately investigated (Ren et al., 2018). Our results obtained for the antiproliferative effects of *R. centifolia* ww are probably due to the certain flavonoids contain, in particular quercetin, which is known for its inhibitory effect on cell division (Delgado et al., 2014; Klimaszewska-Wiśniewska et al. 2017). Antiproliferative activity of geraniol in cancer cells has also been reported by Carnesecchi et al., (2004). The polyphenol-enriched fraction of *R. damascena* ww induced significant cytotoxicity *in vitro* at a concentration of 100 µg/ml or higher (Sabahi et al., 2020). Contrary to the present results with *R. centifolia* ww concerning mammalian, Gerasimova et al. (2022) showed a slight antiproliferative effect and a negligible genotoxic effect in ICR mice after application of *R. alba* ww solution in the concentrations. Gateva et al., (2022) reported a lack of cytotoxicity in barley after treatment with *R. alba* and *R. damascena* ww's. All concentrations tested did not affect the mitotic index values, whereas in lymphocyte cultures a significant reduction was noted. The effect is probably due to the quantitative and qualitative difference in the chemical composition of the wastewaters.

The results of our investigations showed that in ICR mice, *R. centifolia* ww did not increase the amount of CA and the mean number of MNs in the PCE of ICR mice at concentrations of 11% and 20% and treatment periods (24h and 48h post ww supplementation). Even more, the data in experimental animal groups were close to the negative control values. On the other hand, rose ww did not induce a strong but statistically proven genotoxic effect in barley and human lymphocytes. Similar data on genotoxicity have been reported following administration of *R. damascena* and *R. alba* ww (Gateva et al., 2022). The obtained data unequivocally confirm that the effect clearly depends on the test-system used. The metabolic biotransformation processes in *in vivo* animal test system are probably essential for the observed genotoxicity results in ICR mice.

CONCLUSION

All test systems showed different sensitivity to *R. centifolia* ww as measured by the mitotic index value. Barley meristem cells were not influenced by ww in comparison to the negative control, whereas the mammalian bone marrow cells and human lymphocytes show a notable cytotoxic effect. The genotoxic effect of rose ww was weak, but statistically significant in higher plant and lymphocyte test system; whereas no signs of genotoxicity were detected in the animal test system assessed by CA and MN assays.

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Оригинални научни рад

СТУДИЈА О ПОТЕНЦИЈАЛНОМ ЦИТОТОКСИЧНОМ И КЛАСТОГЕНОМ ЕФЕКТУ ОТПАДНИХ ВОДА *ROSA CENTIFOLIA L.*

Резиме

Током водено-парне дестилације ружиног уља настаје значајна количина отпадних вода. Ово представља еколошки проблем који не треба занемарити. Међутим, пошто ова отпадна вода садржи много биоактивних супстанци, треба размотрити друге циљане употребе. Заиста, треба проверити да ли је ова отпадна вода безбедна. У том смислу, циљ ове студије је био да се испита цитотоксични и генотоксични потенцијал отпадних вода *Rosa centifolia L.* У ту сврху користили смо батерију тест система, укључујући више

биљке, глодаре и култивисане људске лимфоците, да бисмо добили детаљне информације. Коришћене су класичне цитогенетске методе. Цитотоксични ефекат је пронађен код мишева и лимфоцита. Није забележен утицај на митотичку активност у меристемима корена јечма. У свим тест системима откривена је само слаба генотоксична активност отпадних вода *Rosa centifolia*. Наши подаци обећавају даљу употребу овог производа у људском животу.

Кључне речи: *отпадна вода, Rosa centifolia, цитотоксичност, генотоксичност, тест-системи*

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