Svetoslav Dimitrov Todorov
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Editors

Tropical Fruits– From Cultivation to Consumption and Health Benefits

Food Science and Technology

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TROPICAL FRUITS — FROM CULTIVATION TO CONSUMPTION AND HEALTH BENEFITS

PAPAYA

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PAPAYA

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FOREWORD

Within the tropical fruits, the papaya, *Carica papaya* L. (family Caricaceae Dumort.), is presented as the main representative being cultivated in tropical and sub-tropical areas mostly in developing countries. Papaya's nutritional value, beneficial to health, as well as various industrial applications of their products, led to be economically important for both developing and developed countries. Within this broad field of knowledge, this book aims to contribute to better understanding of the topic.

The authors wish the book to be cohesive and readable, herein the reader will learn about papaya and it's microbiota. The botany, taxonomy, breeding and applications of papaya could be seen in the Chapters 1 to 4, and the microbiological aspects, which involves from diseases affecting papaya to relevant aspects to human welfare, are showed in chapters 5 to 8.

The organizations of the chapters and sections is also straightforward, in the chapter 1 are presented what papaya is (*Carica papaya* L.), its taxonomy, distribution, origin and morphology. Closing the first part, the Chapter 3 and 4 shows the nutritional and medical values, discussing about vitamins, minerals and dietary fibers, the industrial applications of using papaya and various parts of the plant, as a source of proteolytic enzymes and some active compounds reported to antimicrobial, anticancer, amongst other properties, illustrating the fatty acid composition, triacylglycerol profile and papaya seed oil of malaysian papaya fruits.

The second part of the book, the readers should find the relevant aspects of papaya microbiology related to fresh fruits quality and safety and the beneficial effects of microorganisms isolated from papaya, such as some Latic Acid Bacteria strains that have been proposed to be potentially probiotics, as shown in chapter 5 and 6. Finally the book addresses the importance of Integrated Management of the Papaya Ringspot Virus, which is transmitted by several aphid species and could commit 100% of the crop as described in chapter 7 and the biotechnological strategies for control of papaya virus diseases as show in chapter 8. The authors' expertise brings to us valuable information about microorganisms from papaya. Enjoy!

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EDITORIAL

A long time ago, in the ancient world, Hippocrates had a dream that one day our food would be medicine and medicine would be food. Our ancestors had no knowledge on plant taxonomy, enzymes, antioxidates, and microbiology, they even had no idea about the existence of microbes with all these molecules. They had a one very powerful piece of knowledge, knowledge of traditional know how. Based on the personal experience and the knowledge transferred from parents to children through the centuries, they knew about beneficial properties of fruits, vegetables, and medical plants. The longest part of this history was based on empirical knowledge, gained by experience without former knowledge of either mechanisms or scientific basis.

If we re-evaluate our experience in fermentation processes and traditional medicine, we will be surprised to discover that only since the last century we have tried to find answers on the questions about the scientific basis of these phenomena. If we look back in history, we can find the uses of various fruits, vegetables, and medical plants in the treatment of numerous diseases, and how they were appreciated for their nutritional value or used in everyday domestic processes. Based on empiric experience, a high number of fruits have been used in traditional medicine. Empiric knowledge, frequently transferred from one generation to the next, was the only basis for the preparation and application of these products.

Papaya, the fruit of the Carica papaya, is native to Mexico, Central America, and northern South America. It has been said that the inhabitants of the ancient Mayan civilization honored the papaya tree as their sacred "Tree of Life" because the fruit (especially the ripe or fermented fruit) was used in various traditional medicines. Green (or unripe) papaya has many described benefits as do their seeds, such as their ability to kill parasites, lower blood pressure, reduce inflammation and pain. They also possess aphrodisiacs, and have spermicidal properties.

From the view of the 21st century scientist, we have sufficient knowledge to address the various beneficial properties of the Carica papaya plant. Nowadays, application of various parts of the papaya plants are used in preparation of numerous bioactive molecules, including enzymes, antibacterial proteins, antioxidates, various extracts with application in modern medicine, the food industry, etc. In this book we have tried to collect materials covering some aspects from the characterization and place of the papaya plant, into the taxonomical position of the plants. We also try to summarize information about the application of the fruits and other parts of the plant; to cover some aspects of the agro technical production of papaya fruits; present some points on the problem of diseases attacking the plants, and aspects of microbiology accompanying the production of the fruits.

PART I – BOTANY, TAXONOMY, BREEDING AND APPLICATIONS OF PAPAYA

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Chapter 1

PAPAYA: BOTANY AND TAXONOMY

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ABSTRACT

The species *Carica papaya* L. (papaya) native to Central America is one of the major fruit crops cultivated in tropical and sub-tropical zones of the world. The plant is an important source of vitamins for people and industrially important proteolytic enzymes such as papain and chymopapain. Nowadays it is placed in the dicotyledonous family Caricaceae Dumort. (order Brassicales) which comprises 6 genera and 35 latex containing species. Papaya is a fast-growing tropical tree, with single, straight, and hollow stem. It forms several types of flowers – typical female, smaller trumpet shaped male, and shortly tubular hermaphrodite with well developed ovary. The trees can be monoicous or dioicous. The fruits of papaya are fleshy and resemble melons, reaching a weight of 9 kg. The mature fruit is juicy, sweetish, containing grey-black ovoid seeds and rich in natural vitamins, minerals, ascorbic acid. Papaya trees are usually grown from seeds and bear fruits throughout the year.

Keywords: *Carica papaya*, taxonomy, distribution, origin, morphology, fruit

Papaya, *Carica papaya* L., (family Caricaceae Dumort.) is one of the major fruit crops cultivated in tropical and sub-tropical zones of the world. The largest production is in Central and South America - mainly in Brazil, in Asia and in Africa. World production of papayas in 2009 was over 10 million tons, which was almost 1.5 times higher than that in 1999. Papaya is mainly grown and consumed in developing countries but it has become an important fruit crop worldwide as a fresh fruit or in processed products. The United States, Singapore, Canada, the Netherlands and Germany are the major importers (Lobo, Pastor, 2012). The plant is an important source of vitamins for people in the tropics, and two industrially

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important proteolytic enzymes, papain and chymopapain, are obtained from the milky latex extracted from unripe fruits. Green fruits are generally better sources, containing more papain than ripe fruits (Krishnaiah et al., 2002; Scheldeman et al., 2011). The latex is used also for treatment of gangrenous wounds or burns (Hewitt et al., 2000) and in cosmetic products (Knight, 1980).

The classification of papaya has changed over the years. The genus *Carica* was placed in different plant families, including Passifloraceae, Cucurbitaceae, Bixaceae and Papayaceae. Nowadays, it is placed under Caricaceae, a dicotyledonous plant family belonging to order Brassicales (APG III, 2009) and incorporating 35 latex-containing species, including one formally named hybrid (Carvalho, Renner, 2012).

The family Caricaceae currently comprises six genera of trees, shrubs and one climber, of which *Carica* is represented with the only species *C. papaya*, native to Central America from Mexico to Panama (Badillo, 1993, 2000). The genus *Cylicomorpha* that is indigenous to Africa is represented by two species in west and east Africa. The monotypic genus *Horovitzia*, endemic to Mexico, is a small tree (Lorence, Colin, 1988; Badillo, 1993). The genus *Jarilla* comprises three species of perennial herbs (McVaugh, 2001), while *Jacaratia* has eight tree species found in tropical America from southern Brazil to Mexico. The last genus *Vasconcellea* consists of 20 species, 19 of them trees or shrubs and one a climber, concentrated in the northwestern Andes. Several species that were formerly assigned to genus *Carica* were classified in the genus *Vasconcellea* on the basis of molecular evidence indicating genetic distances between papaya and other related species (Jobin-Décor et al., 1996; Kim et al., 2002; Carvalho, Renner, 2012).

The closest relative family is the Moringaceae, comprising 13 species of trees and shrubs from the dry habitats in the Horn of Africa, Madagascar, southwest Africa and tropical Asia (Olson, 2002).

On the basis of molecular analyses the family Caricaceae has been proposed to have originated in Africa where nowadays two species occur about 65 million years ago in the Early Paleocene. The ancestral area reconstruction suggests dispersal from Africa to Central America c. 35 million years ago possibly via a floating island carried by ocean currents from the Congo delta via the North Atlantic Equatorial Current (Renner, 2004; Carvalho, Renner, 2012). Caricaceae members reached South America from Central America between 27 and 19 million years ago which matches recent geological evidence, suggesting that the formation of the Isthmus of Panama already began 23–25 million years ago, earlier than previously suggested (Farris et al., 2011).

The history of papaya was documented for the first time by Oviedo, the Director of Mines in Hispaniola (Antilles) from 1513 to 1525. The Spaniards gave it the name ‘papaya’. The Spanish and Portuguese introduced the seeds, which remain viable for several years when dried, in the Philippines, Malaysia, Pacific islands, India, and Africa (Nakasone, Paull, 1998). In Europe it is only cultivated in the Canary Islands (Spain) under mesh greenhouses (Rancel Delgado et al., 2007). The fruits have other common names such as ‘paw paw or papaw’ in the United Kingdom and North America, ‘papaye’ in France, ‘meloenboom’ in The Netherlands, ‘melonenbaum and papaya’ in Germany. The fruit is also known as ‘kapaya’, ‘kepaya’, ‘and ‘papyas’ in the Philippines, or ‘gedang castela’ or ‘Spanish Musa’ in Bali. In Venezuela it is known as ‘lechosa’, as ‘maman’ in Argentina, and ‘fruta bomba’ in Cuba. In the Portuguese-speaking countries (Portugal, Brazil, Angola, Mozambique, etc.) the fruit is known as ‘mamão’ or ‘mamoeiro’. In Australia, red and pink-fleshed cultivars are called

"papayas" to distinguish them from the yellow-fleshed fruits known as 'papaw' or 'paw paw' (Lobo, Pastor, 2012).

The place where papaya originated through natural hybridization between *Carica peltata* and another wild species (Purseglove, 1968) was the Caribbean coast of Central America. Then it spread to South America (Argentina and Chile) and to southern Mexico (Manshardt, 1992).

Information on the morphological description of papaya is presented in a number of publications (Morton, 1987; Du Puy, Telford, 1993; OECD, 2005, etc.). Papaya is a fast-growing tropical tree. In all parts of the plant, including the unripe fruits are found latex vessels. The stem is single, straight, hollow and contains prominent leaf scars. The green or deep purple trunk can grow up to 10 m and can be 30–40 cm thick at the base, thinning to 5–7.5 cm at the crown. The leaves appear directly from the upper part of the stem in a spiral on nearly horizontal petioles 25–100 cm long and form a loose open crown. The leaf blade is divided into 5 to 9 main lobes, each cut pinnately, with yellowish ribs and veins. The life of a leaf is from 2.5 to 8 months, new leaves appear at the rate of 1.5–4 per week (Sippel et al., 1989). Several types of flowers are found in papaya. The typical female flowers, 3-5 cm long, have free petals, the ovary is 2-3 cm long formed by five carpels. The female flowers are single, or in small groups in the leaf axils, sometimes emerging directly from the stem in its upper part on short petioles. The male flowers are smaller, trumpet shaped with 10 stamens, found on long hanging panicles. There are also hermaphrodite flowers which are shortly tubular, with larger petal lobes, with either 5 or 10 stamens and well developed ovary. Different types of hermaphrodite flowers may occur on the same tree, depending on the season or on the age of the tree. The trees can be monoicous or dioicous.

The fruits of papaya are classified as fleshy berries (Villegas, 1997), sometimes called pepo-like berries, since they resemble melons. They are oval to nearly round, or elongated club-shaped, 15-50 cm long and 10-20 cm thick, reaching a weight of 9 kg. The immature fruit is rich in white latex, and the skin is green and hard. With ripening papaya fruit develops a light- or yellow-orange coloured skin while the thick wall of succulent flesh becomes yellow-orange and aromatic. It is already juicy, sweetish and somewhat like a cantaloupe in flavor but some types are quite musky. The mature fruits contain numerous grey-black ovoid seeds attached to the flesh by soft fibrous tissue (Morton, 1987). Papaya fruits consist mostly of water and carbohydrate and are rich in natural vitamins (A and C in particular), minerals, ascorbic acid and potassium (Chan, Tang, 1979).

Papayas are usually grown from seeds and germination occurs within 2-4 weeks after sowing. It grows best in a well drained, well aerated and rich organic matter soil with pH 5.5-6.7 (Morton, 1987). Waterlogging of soils often results in the death of trees within 3-4 days (Storey, 1985). The plants can only be grown between latitudes 32′ N and S, with optimal growth at 22-26°C and an evenly distributed rainfall of 1000-1500 mm (Litz, 1984). Vegetative propagation of papaya is possible but is not widely practiced except in South Africa where rooting of cuttings is used to eliminate variability in some papaya varieties. As papaya trees are fast-growing the first fruit is expected in 10-14 months from germination and in general the fruit takes about 5 months to develop.

Fruit production may occur following either self-pollination or cross-pollination and is affected by pollinator efficiency or abundance (Garrett, 1995). The morphology of the flowers plants suggests insect pollination by honey bees, wasps, midges, thrips, surphid flies, and

butterflies (Lobo, Pastor, 2012) but some authors consider that wind pollination may also be important (Nakasone, Paull, 1998).

Female plants produce medium to large round-shaped fruit of good quality with a large seed cavity, hermaphrodite plants produce small to medium elongated fruit of good quality but with a smaller seed cavity and male plants with male flowers produce a few, elongated, fruit of low commercial value (Crane, 2008).

Papaya trees bear fruits throughout the year but yields decline as the trees age and picking becomes difficult. For those reasons fields of papaya are usually replanted or abandoned after 3 or 4 years in commercial production (Lobo, Pastor, 2012).

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Chapter 2

PAPAYA FRUIT AROMA COMPOUNDS – STATE OF THE ART RESEARCH

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ABSTRACT

This work presents an overview on fundamental and applied aspects related to production of aroma volatile compounds in papaya fruit. Using different analytical techniques (distillation, extraction, headspace and sorptive), the volatile compounds have been analyzed in conjunction mainly with gas chromatography-mass spectrometry. In papaya fruit, more than 380 volatile compounds have been reported, but only few of these volatiles are considered important contributors to the flavor. Volatile compounds in papaya have been reported to be influenced by various factors including the cultivars, geographical location, fruit maturity at harvest, processing, storage, and ripening conditions.

Keywords: Aroma volatile production in papaya fruit, distillation, extraction, headspace, sorptive, gas chromatography-mass spectrometry

INTRODUCTION

Papaya (*Carica papaya* L.) is a native fruit of tropical America, but it is currently disseminated throughout the tropical and subtropical regions of all World. It is a very popular fruit with consumers, appreciated for its high content of sugar, vitamin C and carotenoids, as well as for its pleasant flavor [1]. Field ripe fruits are best for immediate consumption, and it

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is only necessary to remove the peel; while surplus ripe fruits are peeled, sliced and canned in syrup, or made into preserves (jam, marmalade, jelly) or nectar.

The first mention of the existence of the papaya plant was recorded in 1535 by the Spanish author, G. H. de Oviedo, in his book, "La natural hystoria de las Indias" in which he informed the King of Spain of the discovery of papayas growing between the south of Mexico and the north of Nicaragua. It is believed that from this region that the first seeds were taken and spread to Panama, Santo Domingo, certain Caribbean islands, and parts of South America [2].

Some authors support believe that this species is originated from the south of Mexico and Nicaragua [3], while others suggest an origin related to the northwest of South America [4]. After the discovery of the New World, the papaya tree was spread widely throughout the tropical regions. Papaya is grown in nearly all countries of the tropical Americas (Central and South America and the US state of Hawaii). It is also extensively cultivated in India, Sri Lanka, various other Asian countries, as well as in the Antilles and tropical Africa [3].

Papaya is an elongated berry of various sizes with a smooth thin skin and a greenish-yellow color. Its flesh is thick with a color ranging from yellow to red and offers a pleasant, sweet, mellow flavor [5]. This species requires cultivation temperatures between 21 and 33 °C and does not tolerate cold weather [5-7], while prolonged dry periods reduce crop output [8].

Worldwide papaya production has increased dramatically during the last decades, with a majority of the increase related to American orchards. At present time, Brazil stands out as the world's biggest producer, supplying 25% of the world demand, followed by Mexico at 14%, Nigeria at 11%, India and Indonesia at 10%. Other papaya growing producers include Venezuela, China, Peru, Congo and Ethiopia, however, at present all of which contribute less than 3% of the papaya supply [9, 10].

Papaya crops require year-round labor, which has made it an excellent choice from a socio-economic perspective in countries that produce it. The quality of the papayas grown can be compromised by conditions and practices adopted during commercialization [11,12]. Papaya is a climacteric fruit with a typical rise in respiration as a consequence of the autocatalytic production of ethylene during ripening. This ethylene increase accounts for several changes in sensorial attributes, such as pulp firmness, color, and taste. In addition, the amount of compounds beneficial to human beings, such as carotenoids, can be adversely affected by the supplementation or depletion of ethylene [13]. Papaya, like many climacteric fruits, undergoes a variety of physical and chemical changes after harvest. First of all, the stage of ripeness determines the fruit's final quality. Physiological disorders can at times result in restrictions against exportation of the fresh fruit and cause production losses and, in turn, a negative financial impact throughout the chain of papaya production [14].

Many cultivars of papaya are grown in various parts of the world and are known to vary markedly in their flavor characteristics. Cultivars 'Solo' and 'Taiwan' are common in Brazil [15], 'Maradol' (also known as 'Maradol Roja') in Cuba, Mexico and Colombia [16], the 'Sekaki' (also known as 'Hong Kong') and 'Eksotika' in Malaysia, and 'Khack Dum' cultivated in Thailand [3].

Analytical research on the aroma compounds of papaya fruit was carried out for 40 years and was summarized and reviewed by different authors [17-22]. As a result, many hundreds of volatile constituents have been identified in fresh fruit, however, only limited numbers of them have been recognized important as papaya flavor contributors.

In this chapter, the aroma composition of papaya fruit is summarized, but this contribution is not limited to a compilation of reported compounds. Each section presents an overview of important publications and a description of the characteristic features of the volatile composition of the papaya fruit.

CONSTITUENTS INFLUENCING PAPAYA FLAVOR

Flavor consists mainly of lipophilic volatile compounds, but low and nonvolatile chemical structures also play an important part of the overall sensation. As been recorded for many other fruits, papaya flavor is a combination of volatile compounds perceived by the human olfactory system and nonvolatile components (mainly sugars and acids) recognized by tongue sensors. In fact, flavor is a combination of both, taste and odor [23].

The olfactorically evaluation by perfumers of pulp samples of ripe and unripe fruits showed that ripe fruit has exotic fruity (papaya, apricot, banana and pineapple notes) with floral side-notes, while the unripe fruit was described as exotic fruity (papaya, apricot, banana and pineapple notes) some weaker than ripe fruit, floral and green side-notes, in the background pungent-sour aroma [24]. Generally, the flesh of ripe papaya is pale orange–red with a sweet taste.

The flavor of ripe papaya is a blend of a number of volatile and nonvolatile constituents, present in small amounts and in complex mixtures. Many of these compounds have been identified and reported by several authors from fresh fruit and processed products. However, comparison among these reported results is difficult, since different cultivars have been used, results are given in different units and bases, and isolation techniques vary among reported studies. A summary of papaya constituents identified by several researchers from 1977 to 2014 is presented in Table 1. There are more than 380 identified compounds including hydrocarbons, esters, lactones, alcohols, aldehydes, ketones, acids, furans, phenols, oxides, S- and N-compounds, but qualitatively and quantitatively the most important of these are esters and terpenes.

Numerous publications have investigated on identification of volatile components in papaya so far, but only few studies have made efforts to evaluate the aroma contribution of these volatile compounds.

It is important to identify the trace compounds contributing significantly to a food aroma. For this purpose, it is necessary to achieve appropriate isolation and identification methods for detection of odor-contributing constituents in combination with sensory evaluation of the fruit and its individual components.

It has been shown for a considerable number of food products that all the present volatiles compounds are not able to interact with human olfactory receptors. Instead, only a smaller number of the so-called key odorants is obviously detected by the human odorant receptors and, consequently, participate in the creation of the respective aroma impression in the brain [25].

Well accepted approach to separate odor-active volatiles from the bulk of odorless food volatiles is GC–Olfactometry (GC–O) on serial dilutions of aroma distillates, by, for example, aroma extract dilution analysis (AEDA).

Table 1. Volatile compounds reported in papaya fruit

Compound	Reference
Hydrocarbons	
n-hexane	[48]
n-heptane	[48]
n-octane	[38]
n-nonane	[38]
n-decane	[48]
n-tetradecane	[48]
n-pentadecane	[76]
n-hexadecane	[38]
methylcyclohexane	[26,48]
propylcyclohexane	[48]
iso-propylcyclohexane	[48]
butylcyclohexane	[48]
methylbenzene	[26,27,38,48]
ethylbenzene	[27,38,48]
vinylbenzene	[26]
propylbenzene	[48]
iso-propylbenzene	[48]
butylbenzene	[48]
1,2-dimethylbenzene	[38]
1,3-dimethylbenzene	[27,38]
1,4-dimethylbenzene	[27,38,48]
1-ethyl-2-methylbenzene	[48]
1-ethyl-3-methylbenzene	[48]
1-ethyl-4-methylbenzene	[48]
1-iso-propyl-4-methylbenzene	[24,27,30,38,43,48]
1,2,3-trimethylbenzene	[48]
1,2,4-trimethylbenzene	[48]
1,3,5-trimethylbenzene	[48]
1,4-dimethyl-2-vinylbenzene	[38]
p-1-menthene	[38]
p-4(8)-menthene	[38]
α-pinene	[24,27,30]
β-pinene	[24,48]
camphene	[24,38]
α-ocimene	[48]
cis-β-ocimene	[24,26,38,43,76]
trans-β-ocimene	[24,38,43,76]
cis-neo-allo-ocimene	[38,43]
trans-neo-allo-ocimene	[38,43]
myrcene	[24,27,30,38,43,76]
α-phellandrene	[38,76]
β-phellandrene	[38,43,76]

Compound	Reference
sabinene	[38,43]
α-terpinene	[24,38,43,76]
γ-terpinene	[24,27,30,38,76]
terpinolene	[24,38,43,76]
limonene	[24,27,30,38,43,48,76]
β-caryophyllene	[24,27,30,76]
α-humulene	[24,27,30]
germacrene D	[76]
Alcohols	
methanol	[48]
ethanol	[24,48]
1-propanol	[27,30,48]
2-propanol	[27,48]
2-methyl-1-propanol	[27,30,38,43,48]
3-methoxypropanol	[24]
1-butanol	[27,30,38,42,43,48]
2-butanol	[24,27,30,48]
2-methyl-1-butanol	[27,30,38,43,48]
3-methyl-1-butanol	[24,27,30,38,43,48]
2-methyl-2-butanol	[38,48]
2-methyl-3-buten-2-ol	[38,43]
1-pentanol	[48]
2-pentanol	[24,27,30,38,43,48]
3-pentanol	[38,43,48]
(Z)-2-penten-1-ol	[27,30,38,43]
(*E*)-2-penten-1-ol	[27,30,38,43]
1-penten-3-ol	[27,30,38,43,48]
1-hexanol	[24,27,30,38,43,48]
2-hexanol	[48]
3-hexanol	[48]
(Z)-2-hexen-1-ol	[27,30,38,43]
(*E*)-2-hexen-1-ol	[27,30,38,43,48]
(Z)-3-hexen-1-ol	[24,27,30,38,43,48]
(*E*)-3-hexen-1-ol	[27,30]
(*E*)-1-hexen-3-ol	[24]
2-ethyl-1-hexanol	[38,43]
1-heptanol	[24,38,43,48]
2-heptanol	[24]
3-heptanol	[38,43]
2-methyl-2-heptanol	[38,43]
1-octanol	[24,27,30,38,43,48]
3-octanol	[24,26]
1-octen-3-ol	[24]
2-ethyl-1-octanol	[38]
3,7-dimethyl-1,5,7-octatrien-3-ol	[38]

Table 1. (Continued)

Compound	Reference
2,6-dimethyl-1,7-octadiene-3,6-diol	[37]
2,6-dimethyl-3,7-octadiene-2,6-diol	[37]
3,7-dimethyl-1-octene-3,6,7-triol	[37]
1-nonanol	[24,38,43]
1-decanol	[24,38,43]
1-undecanol	[38,43]
2-undecanol	[38,43]
2-tridecanol	[27,30]
1-nonadecanol	[38,43]
benzyl alcohol	[24,26,27,30,38,42,43,48]
2-phenylethanol	[26,27,30,38,43,48]
p-cymen-8-ol	[24]
nerol	[38]
geraniol	[24,38,42,43,48]
linalool	[24,26,27,30,38,42,43,48,76]
geranyl linalool	[27,30]
(*Z*)-carveol	[27,30]
cis-8-hydroxylinalool	[37]
trans-8-hydroxylinalool	[37]
iso-borneol	[38]
terpinen-4-ol	[24,27,30,38,43,76]
α-terpineol	[24,27,30,38,43,48]
(*E*,*E*)-farnesol	[27,30]
Aldehydes	
acetaldehyde	[27,30,48]
(*Z*)-2-propenal	[27]
butanal	[27,30]
2-methylbutanal	[26]
3-methylbutanal	[27,30]
2-methyl-2-pentenal	[24,48]
hexanal	[27,30,76]
(*E*)-2-hexenal	[27,30]
heptanal	[76]
octanal	[24,76]
nonanal	[24,27,30,38,76]
decanal	[24,76]
benzaldehyde	[24,26,27,30,38,43,48,76]
phenylacetaldehyde	[26,27,30]
Ketones	
acetone	[27,30,42]
2-butanone	[27,30,48]
3-hydroxy-2-butanone	[27,30,38,42]

Compound	Reference
2,3-butanedione	[26,27,30,38]
2-pentanone	[24,27,30,48]
1-penten-3-one	[27,30]
(*E*)-3-penten-2-one	[27,30]
4-methyl-2-pentanone	[38,43]
4-hydroxy-4-methyl-2-pentanone	[38,76]
1-hexen-3-one	[27,30]
2,3-pentanedione	[27,30]
2,4-pentanedione	[76]
5-methyl-2-hexanone	[27,30,38,43]
2-heptanone	[38,43,48,76]
4-heptanone	[48]
5-methyl-3-heptanone	[38,43]
6-methyl-5-hepten-2-one	[27,30,38,43,76]
2-octanone	[27,30,48]
3-octanone	[24]
2-tridecanone	[27,30]
2-pentadecanone	[27,30]
carvone	[27,30]
geranylacetone	[27,30,76]
cyclohexanone	[38,43]
3,5,5-trimethyl-2-cyclohexanone	[27,30]
2,6,6-trimethyl-2-cyclohexene-1,4-dione	[27,30]
iso-phorone	[38,43]
acetophenone	[38,43]
2-methylacetophenone	[38,43]
3-methylacetophenone	[38,43]
(*E*)-β-ionone	[27,30,48]
Acids	
formic	[38]
acetic	[24,27,30,38]
hydroxyacetic	[44]
propanoic	[27,30,48]
2-methylpropanoic	[48]
2-hydroxypropanoic	[44]
3-hydroxypropanoic	[44]
2-hydroxy-2-methylpropanoic	[44]
butanoic	[24,38,42,44,48]
2-butenoic	[48]
2-methylbutanoic	[24,44,45]
3-methylbutanoic	[24,44,48]
3-hydroxybutanoic	[44]
pentanoic	[42,44,48]
2-methylpentanoic	[44]
4-methylpentanoic	[44]

Table 1. (Continued)

Compound	Reference
4-oxopentanoic	[44]
hexanoic	[24,26,38,42,44,48]
2-hexenoic	[48]
2-methylhexanoic	[24]
2-ethylhexanoic	[44]
3-hydroxyhexanoic	[44]
heptanoic	[44,48]
2-ethylheptanoic	[44]
3-hydroxyheptanoic	[44]
octanoic	[26,27,30,38,42,44,48]
2-methyloctanoic	[44]
3-hydroxyoctanoic	[44]
nonanoic	[38,44,48]
3-hydroxynonanoic	[44]
decanoic	[27,30,38,42,44,48]
3-hydroxydecanoic	[44]
trans-5-hydroxydec-7-enoic	[44]
undecanoic	[44]
dodecanoic	[27,30,38,44,48]
3-hydroxydodecanoic	[44]
tridecanoic	[27,30,44]
tetradecanoic	[27,30,38,44]
pentadecanoic	[27,30,38,44]
hexadecanoic	[30,38,44]
(*Z*)-9-hexadecenoic	[27,44]
(*E*)-9-hexadecenoic	[27]
heptadecanoic	[27,30,44]
octadecanoic	[27,30,38,44]
(*Z*)-9-octadecenoic	[44]
(*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic	[27]
trans-geranic	[44]
succinic	[44]
2-methylsuccinic	[44]
glutaric	[44]
adipic	[44]
pimelic	[44]
suberic	[44]
azelaic	[44]
malic	[44]
benzoic	[44,48]
2-methylbenzoic	[44]
2-hydroxybenzoic	[44,48]

Compound	Reference
p-1,8-menthadienoic	[44]
phenylacetic	[44,48]
3-phenylpropanoic	[44]
Esters	
ethyl formate	[27,30]
ethyl acetate	[24,27,30,38,43,48,76]
1,2,3-propanetriol triacetate	[76]
butyl acetate	[38,43]
iso-butyl acetate	[48]
pentyl acetate	[24]
iso-pentyl acetate	[24,27,48]
hexyl acetate	[24]
(*E*)-2-hexenyl acetate	[24]
(*Z*)-3-hexenyl acetate	[24,27,30]
linalyl acetate	[24]
geranyl acetate	[24]
methyl propanoate	[27,30,48]
ethyl propanoate	[27,30]
propyl propanoate	[48]
iso-propyl propanoate	[38]
(*Z*)-3-hexenyl propanoate	[24]
methyl 2-methylpropanoate	[48]
ethyl 2-methylpropanoate	[48]
iso-pentyl 2-methylpropanoate	[48]
ethyl 2-hydroxypropanoate	[27,30]
methyl butanoate	[26,27,30,38,48]
methyl (*Z*)-2-butenoate	[27]
methyl (*E*)-2-butenoate	[27,30]
ethyl butanoate	[27,30,48,76]
ethyl (*E*)-2-butenoate	[27,30]
benzyl (*E*)-2-butenoate	[30]
propyl butanoate	[48]
iso-propyl butanoate	[76]
butyl butanoate	[48]
iso-butyl butanoate	[24]
pentyl butanoate	[24]
iso-pentyl butanoate	[24,27,30]
(*Z*)-2-pentenyl butanoate	[24]
(*E*)-2-hexenyl butanoate	[24]
(*Z*)-3-hexenyl butanoate	[24,27,30]
benzyl 2-butenoate	[26,27]
methyl acetylbutanoate	[27]
methyl 2-hydroxybutanoate	[27,30]
methyl 3-hydroxybutanoate	[27,30]

Table 1. (Continued)

Compound	Reference
ethyl 3-hydroxybutanoate	[27,30]
methyl 2-methylbutanoate	[27,30]
ethyl 2-methylbutanoate	[48]
iso-butyl 2-methylbutanoate	[48]
methyl 2-hydroxy-2-methylbutanoate	[27,30]
methyl 3-methyl-2-butenoate	[27,30]
ethyl 3-methylbutanoate	[27,30,48]
(*Z*)-3-hexenyl 3-methylbutanoate	[24]
methyl 2-hydroxy-3-methylbutanoate	[27]
methyl pentanoate	[48]
methyl 3-hydroxypentanoate	[27]
methyl hexanoate	[26,27,30,48,76]
ethyl hexanoate	[27,30]
butyl hexanoate	[76]
iso-butyl hexanoate	[24]
pentyl hexanoate	[24]
iso-pentyl hexanoate	[24,30]
hexyl hexanoate	[42]
methyl (*E*)-2-hexenoate	[26,27,30]
ethyl (*E*)-2-hexenoate	[27,30]
ethyl 2-hydroxyhexanoate	[27]
methyl 3-hydroxyhexanoate	[27]
ethyl 3-hydroxyhexanoate	[27,30]
ethyl 5-hydroxyhexanoate	[27]
methyl 2-hydroxy-3-methylpentanoate	[27,30]
methyl 2-hydroxy-4-methylpentanoate	[27,30]
methyl heptanoate	[48]
ethyl heptanoate	[27,30]
methyl octanoate	[26,27,30,76]
methyl (*E*)-2-octenoate	[27,30]
methyl 4-oxooctanoate	[27]
ethyl octanoate	[24,27,30,76]
ethyl (*E*)-2-octenoate	[27,30]
allyl octanoate	[27]
methyl 3-hydroxyoctanoate	[30]
methyl decanoate	[26,27,30,48]
ethyl decanoate	[27,30]
methyl dodecanoate	[26,27,30]
ethyl dodecanoate	[27,30]
methyl tetradecanoate	[26,27,30]
ethyl tetradecanoate	[27,30]
iso-propyl tetradecanoate	[38]

Compound	Reference
methyl 2-hydroxytetradecanoate	[38,43]
ethyl pentadecanoate	[27,30]
methyl hexadecanoate	[38,43]
methyl (*Z*)-9-hexadecenoate	[27]
ethyl hexadecanoate	[27,30]
ethyl (*Z*)-9-hexadecenoate	[27]
ethyl (*E*)-9-hexadecenoate	[27]
methyl octadecanoate	[38,43]
methyl (*Z*)-9-octadecenoate	[27]
ethyl (*Z*)-9-octadecenoate	[27]
methyl (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoate	[27]
ethyl (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoate	[27]
allyl cyclohexylpropanoate	[27]
methyl geranate	[76]
methyl benzoate	[24,26,27,30,48]
ethyl benzoate	[27,30,48,76]
butyl benzoate	[48,76]
iso-butyl benzoate	[24,48]
pentyl benzoate	[24]
iso-pentyl benzoate	[24,76]
benzyl benzoate	[38,43]
methyl 2-hydroxybenzoate	[24,30,76]
iso-butyl 2-hydroxybenzoate	[24]
pentyl 2-hydroxybenzoate	[24]
iso-pentyl 2-hydroxybenzoate	[24]
ethyl phenylacetate	[38,43]
Lactones	
4-hydroxy-4-methyl-5-hexenoic acid lactone	[38,43]
4-hydroxyhexanoic acid lactone	[27,30]
4-hydroxyheptanoic acid lactone	[38,43,48]
4-hydroxyoctanoic acid lactone	[27,30,38,43,48,76]
5-hydroxyoctanoic acid lactone	[26,38,43,48]
4-hydroxynonanoic acid lactone	[27,30,48]
4-hydroxydecanoic acid lactone	[48]
5-hydroxydecanoic acid lactone	[38]
4-hydroxydodecanoic acid lactone	[38]
4-hydroxytridecanoic acid lactone	[43]
Nitrogen compounds	
pyrazine	[38,43]
methylpyrazine	[27,30,38,43]
2,3-dimethylpyrazine	[38,43]
2,5-dimethylpyrazine	[27,30]
2,6-dimethylpyrazine	[27,30]
methyl nicotinate	[26]
benzyl isocyanate	[24]

Table 1. (Continued)

Compound	Reference
phenylacetonitrile	[27,30]
Sulfur compounds	
methyl propyl sulphide	[27]
dimethyl disulfide	[27,30]
2-(methylthio)ethanol	[27,30]
(methylthio)acetic acid	[44]
methyl (methylthio)acetate	[26]
methyl thiocyanate	[30,38,43,48,76]
ethyl thiocyanate	[38,43]
methyl methylthiocyanate	[27]
benzyl isothiocyanate	[24,26,27,30,38,42,43,48,76]
methyl (methylthio)acetate	[27,30]
ethyl (methylthio)acetate	[27,30]
2-methylthiophene	[38,43]
benzothiazole	[38,43,48]
thiobenzoic acid	[27]
Acetals	
1-ethoxy-1-methoxyethane	[48]
1,1-diethoxyethane	[48]
1,1-diethoxy-2-methylpropane	[48]
(Ep)oxides	
1,8-cineole	[27,30,38,43]
6,9-epoxy-*p*-1-menthene	[38]
anhydrolinalool oxide	[38,43]
(*Z*)-linalool oxide (pyranoid form)	[26,27,30,38,42,43,48]
(*E*)-linalool oxide (pyranoid form)	[26,27,30,38,42,48]
(R*,R)-6,7-epylinalool	[37]
(R*,S)-6,7-epoxylinalool	[37]
(R*,R,R)-linalool oxide 1,2-epoxide	[37]
(R*,R,S)-linalool oxide 1,2-epoxide	[37]
(R*,S,R)-linalool oxide 1,2-epoxide	[37]
(R*,S,S)-linalool oxide 1,2-epoxide	[37]
2,2,6-trimethyl-6-vinyl-tetrahydro-3-pyrone	[38,43]
Phenols	
thymol	[38]
carvacrol	[38]
1,4-dimethoxybenzene	[38,43]
4-vinyl-2-methoxyphenol	[27,30]
Furans	
2,2-dimethyl-5-(1-methyl-1-propenyl)tetrahydrofuran	[38]
(*Z*)-linalool oxide (furanoid ring)	[24,26,27,30,37,38,42,43,48,76]
(*E*)-linalool oxide (furanoid ring)	[24,26,27,30,37,38,42,43,48,76]

Compound	Reference
δ-jasmin lactone	[24]
2-furfural	[27,30]
2-furancarboxylic acid	[44]
furfuryl alcohol	[27]
methyl 2-furoate	[26,27]

Dilution-based odor threshold techniques, such as AEDA, are useful methods for the screening of important odorants in food products, but these methods neither permit a study on the influence of the food matrix on odorant binding nor on the interactions of odorants when matching the overall odor impression of the food. These limitations are resolved when the concentrations of the individual odorants are correlated with the respective odor thresholds using the odor activity value (OAV) concept [25].

The first application of odor assessment to papaya volatiles was performed on fruits cultivated in Sri Lanka, which has a pronounced pervading sweaty note to the aroma [26]. The perceived odor quality of methyl butanoate suggests that this ester is mainly, if not exclusively, responsible for the characteristic odor of the Sri Lankan papaya fruit. Another aldehyde, 2-methylbutanal, might also contribute somewhat to that odor [26].

GC–O approach was use in the evaluation of volatiles isolated from the Cuban cv. Red Maradol and the esters from short-chain acids were found to contribute to the fruit aroma [27].

Using the same analytical technique the volatile profile from unripe and ripe fruits grown in Cameroon were analyzed [24] and it was determined that a combination of high concentration of linalool and short-chain alcohols and esters (exotic fruity, floral odor is additionally known from linalool and its derivatives), plus some C6 compounds (e.g., (*E*)-3-hexen-1-ol) in medium or low amounts (green-notes of unripe fruit), while a pungent-sour odor impression, found in the unripe fruit, is due to benzyl iso-thiocyanate (mustard-oil-aroma) in high amounts. The aroma of diluted benzyl iso-thiocyanate was described as fruity and papaya-like [28].

The volatiles from four Indonesian and one Brazilian papaya cultivars were analyzed by gas crhomatography-mass spectrometry (GC–MS) and GC–O and found that character impact odorants were hexanal (herbaceous), (*Z*)-2-penten-1-ol (chemical), nonanal (herbaceous), (*Z*)-linalool oxide (floral), linalool (flowery), dimethyl sulfoxide (sweetish), butanoic acid (stinky), verbenone (flowery), phenylmethyl butanoate (sweetish, floral), δ-octalactone (flowery), and benzyl iso-thiocyanate (smokey) [29].

Recently, investigations on cv. Red Maradol papaya flavor reported the identity of potent odorants that are responsible for the overall aroma of this papaya cultivar by application of aroma extract dilution analysis and odor activity values [30]. Twenty-five odorants were considered as odor-active compounds, from which ethyl butanoate (fruity), benzyl iso-thiocyanate (papaya-like), 1-hexen-3-one (herbaceous), (*E*)-β-ionone (woody) and methyl benzoate (fruity, sweet) were the most odor-active compounds [30].

ANALYTICAL METHODOLOGIES APPLIED TO PAPAYA AROMA RESEARCH

Flavor research has largely meant studying the volatile compounds in a food products. Without aroma, it is very difficult to identify the flavor of a food product. The task of identifying volatile flavor constituents (aroma compounds), particularly in a food matrix, is one of the most formidable tasks faced by an analytic chemist. The first obstacle is that instruments are less sensitive to many aromas than the human nose, and this requires necessity off isolation and concentration of these compounds. Because the aroma components of food products are distributed in its matrix, the procedures of isolation and concentration of them are extremely complicated and complex approach. Most of the techniques used in aroma isolation take advantage of either solubility or volatility of the aroma compounds. The analysis of aroma compounds has been the subject of important specialized treatises [23, 31−36] and the present section will focus only on those methods applied in papaya aroma research.

The extraction step may lead to artifacts or to produce losses, and the total volatile content in most cases is very difficult to relate to a sensory profile determined by a panel or experienced by a consumer. There are two clear examples about the occurrence of these problems in papaya studies. The first one is related to the great variation in the content of linalool oxides depending on sample preparation [26]. The content of linalool oxides increased during storage of the fruit pulp and after heating. Later, two diastereoisomers of 6,7-epoxylinalool were identified as labile natural precursors of the isomeric linalool oxides [37]. In addition to 6,7-epoxylinalool, further oxygenated linalool derivatives were identified, indicating various different metabolic pathways of linalool in papaya [37]. The second example is the occurrence of various monoterpene hydrocarbons in fruits from var. Solo [38], which was found to be due to an acid-catalyzed degradation of linalool during sample preparation [39]. The results of model experiments achieved with this monoterpene alcohol at pH 5.6 (the natural pH of papaya fruit) showed a similar qualitative and quantitative profile with the reported monoterpene hydrocarbon composition in the fruit [38].

The methods most commonly used for the isolation of volatile compounds from papaya included vacuum distillation [37, 38, 40–45], simultaneous distillation-extraction [26, 30, 46], solvent extraction [29, 37, 47], conventional headspace analysis [48], stir bar sorptive extraction [29], and headspace solid-phase microextraction [24, 30, 49].

As no exists universal extraction method, it is essential to choose a method that yields an extract as representative as possible of the sensory properties of the fruit.

GLUCOSINOLATES AND GLYCOSIDICALLY-BOUND AROMA COMPOUNDS

Papaya is very interesting related to that it contains of benzylglucosinolate, and hence produces, by enzyme action, benzyl isothiocyanate among its volatiles. Glucosinolates are thioglucosides mainly located in the botanical family Cruciferae, but *Carica papaya* is one of the few known examples of a noncruciferous glucosinolate-containing species [13, 50–55]. Benzylglucosinolate has also been detected in other *Carica* species [51].

Glucosinolates found in plants have potential benefits for human health and controlling agricultural practices. Although glucosinolates themselves are stable and inactive, the products of their breakdown, especially iso-thiocyanates, are bioactive compounds acting as insect repellents, bactericides, nematicides [56–58], and putative anticarcinogenic compounds in humans [59–64]. Iso-thiocyanate is produced from the unstable aglycone moiety of the glucosinolate following glucose release by the action of β-thioglucosidase (EC 3.2.1.147) or myrosinase. This enzyme plays an important role in the production of the bioactive iso-thiocyanate molecule. In normal plant tissues, the enzyme is brought into contact with its substrate mostly after physical damage to the tissue by mechanical action, pests, mastication, freeze-thawing, etc. However, although the enzyme is concentrated in the myrosin cells, the production of iso-thiocyanates under conditions of preservation of cellular integrity is possible by symplastic transportation of the glucosinolates to the myrosin cells [65].

In papaya, the occurrence of benzyl iso-thiocyanates was first reported in the seeds [50] and pulp [26, 52] of the fruit. The precursor benzylglucosinolate was detected in all of the plant tissues [39, 51, 53], being the only glucosinolate found in this species [53]. Some studies had attributed a protective effect of benzylglucosinolate and benzyl iso-thiocyanate against the deposition and viability of fruit fly eggs and larvae [66, 67]. Glucosinolate production seems to be responsive to several biotic and abiotic stresses in plants, and also to molecules that are usually involved in signaling for disease resistance, such as salicylic acid, jasmonic acid, and ethylene [68–71]. The importance of glycosidically-bound volatile constituents and their contribution to fruit flavor has been receiving increasing attention by many workers, because of the increase in processed fruit products. These glycosidically-bound volatiles are present in fruits and plant tissues (subsequent to their release by enzymatic and/or chemical hydrolysis) and they could increase the yield of flavor compounds and serve as controlled release mechanism in the liberation of free volatiles (aglycones) during free ripening and climacteric, and also, glycosides serve as precursors to free fruit volatiles and flavor [72]. Besides the volatile compounds identified in free form in papaya fruits, volatile compounds have also been found in the glycosidically-bound form [47, 73, 74,]. A β-glucosidase (EC 3.2.1.21) was isolated from papaya fruit pulp [75]. Analogously, benzyl iso-thiocyanate has been recognized for a long time to be formed by enzymic splitting of glucosinolates during disruption of cell tissue in papaya, e.g., during fruit processing [52]. Looking for additional volatiles that might be produced as a result of cell structure disruption, the quantitative composition of papaya flavor was isolated by three different methods of sample preparation: (*i*) with addition of NaN_3 (*ii*) with addition of Hg^{2+}, and (*iii*) without any enzyme inhibitor during fruit homogenization [47]. As expected, the concentration of benzyl iso-thiocyanate was markedly suppressed by enzyme inhibition. As to the linalool concentration, enzymatic inhibition with Hg^{2+} was even more effective. Only 1% of the amount determined in the no inhibited experiment was found with Hg^{2+} inhibition. In contrast, NaN_3 addition did not influence the concentration of linalool in the fruit pulp. Furthermore, the formation of linalool oxides and of monoterpene hydrocarbons was also influenced by enzymatic inhibition during sample preparation. The concentrations of other flavor compounds, such as α-terpineol, benzyl alcohol, and γ-octalactone, were not changed by Hg^{2+} or NaN_3 treatments. The identification of bound volatiles from the fruit was achieved after isolation of an extract and simultaneous enzyme catalysis extraction using glycosidase (emulsin) and acid phosphatase [74]. Aromatic compounds such as benzaldehyde, benzyl

alcohol, 2-phenylethanol, and benzyl iso-thiocyanate as well as (*E*)-3,7-dimethylocta-2,6-dienoic acid, were liberated by phosphatase activity. As precursor of phosphate-bound 2,6-dimethyloct-7-ene-2,3,6-triol, the phosphorylated 6,7-epoxylinalool was proposed [39].

CHANGES IN PAPAYA FLAVOR PROFILE

Aroma of papaya fruit has been reported to be influenced by various factors, including fruit maturity stage [7, 13, 16, 76], physiological disorders [77–80], genetic modifications [49] and processing conditions [46, 81]. It might be included the variety of the fruit as another factor, but this is difficult to analyze due researchers had been used different isolation technique for volatile compounds and in those cases the comparison is not always adequate.

Influence of Maturity Stage

The volatile emission from ripening papaya var. Solo was the first study related to the influence of maturity stage in the quality of papaya [76]. Linalool, benzyl iso-thiocyanate, and phenylacetonitrile were released in significant amounts at all four ripeness stages, but linalool production increased dramatically as the fruit progressed from one-fourth to full ripeness. Free benzyl iso-thiocyanate levels also increased with fruit ripening, but phenylacetonitrile release fluctuated across the four fruit stages, showing no clear correlation between ripeness and this volatile compound. Numerous esters and monoterpenes were only detected in emissions from fully ripe fruit [76].

The effect of ripening on the chemical composition of papaya var. Maradol Roja cultivated in Cuba, with special focus on volatile components, was investigated in four ripening stages [16]. Alcohols, e.g. 1-butanol, 3-methylbutanol, benzyl alcohol and α-terpineol showed an increase in area ratios at the third day and diminished after. At the third day the fruit was ready for consumption. These effects could be used as maturity criteria for this fruit. No significant differences were observed in linalool oxides during ripening. On the other hand, dodecanoic acid showed a significant change up to the third day and continues increased to fifth day. Methyl butanoate and ethyl hexanoate increased their concentrations in the fruit during the five days of the study [16]. Benzyl iso-thiocyanate concentration decreased during ripening, which was in agreement with previous reported data [52].

Benzylglucosinolates and benzyl iso-thiocyanates levels were quantified in peel, pulp, and seeds of papaya fruit var. Golden from Brazil during fruit development and ripening [13]. Benzyl iso-thiocyanates were also verified in the internal cavity of the fruit during ripening. The influence of the ethylene in benzyl glucosinolates and benzyl iso-thiocyanates levels and mirosinase activity was tested by exposing mature green fruits to ethylene and 1-methylcyclopropene. The highest benzyl glucosinolates levels were detected in seeds, followed by the peel and pulp being decreased in all tissues during fruit development. Similarly, the levels of benzyl iso-thiocyanates were much higher in the seeds than the peel and pulp. The levels of benzyl glucosinolates for control and ethylene-treated fruit were very similar, increasing in the pulp and peel during late ripening, but not changing significantly in seeds. On the other hand, fruit exposed to 1-methylcyclopropene showed a decrease in benzyl

glucosinolates amount in the pulp and accumulation in seed. The treatments did not result in clear differences regarding the amount of benzyl iso-thiocyanates in the pulp and peel of the fruit. According to the results, ethylene does not have a clear effect on benzyl iso-thiocyanates accumulation in ripening papaya fruit. The fact that benzyl glucosinolates levels in the pulp did not decrease during ripening, regardless of the treatment employed, and that papaya is consumed mainly as fresh fruit, is argument in favor of this fruit as a good dietary source for glucosinolate and iso-thiocyanates.

The papaya var. Pluk Mai Lie is a promising cultivated fruit in Thailand for use in fresh and processed products due to its firm flesh, but the aroma it releases is flat [7]. Changes in quality and volatile profiles were analyzed during on- and off-tree fruit ripening. Detached fruit ripened faster than attached fruit, accumulating high internal ethylene levels. Aside from peel color, which was redder in on-plant ripened fruit, most quality attributes changed similarly in the two ripening situations. A slight increase in total soluble solids was measured from the onset of the preclimacteric stage, whereas titratable acidity remained stable throughout the development. Whereas 2-ethyl-1-hexanol was found specifically in green fruit, ethyl octanoate emerged only in fully-ripe fruit. Furthermore, benzyl iso-thiocyanate was the most abundant volatile and was present in fruit at every stage except full ripening. The production of total esters, highly correlated with a loss of firmness and an increase in cavity ethylene accumulation, was about 10-fold higher in off-tree ripened fruit. The levels of methanol and ethanol sources in fruit increased steadily throughout ripening, with esters formed from ethyl alcohol predominating from the half-ripe through the senescence phases. The alcohol dehydrogenase activity in the mesocarp increased dramatically during the early ripening stages, whereas alcohol acetyltransferase was active throughout ripening. No difference in volatile profiles was found in the papaya fruit during on-tree and postharvest ripening.

Influence of Physiological Disorders

Among the most important physiological disorders of the papaya are [12]: (1) skin freckles, characterized by the appearance of blemishes on the fruit while still in its growth stage, which seem to be related to latex leaking, (2) pulp flesh translucency, possibly promoted by a reduction in the entrance of water in the vacuoles and accumulation of water in the apoplast, (3) pulp softening, which occurs in response to Ca^{2+} deficiencies in the development of the fruit, and (4) hard lumps in the pulp, caused by the inactivation of cell wall hydrolases enzymes as a result of stress brought on by high temperatures. These disorders contribute negatively to crop development, diminish the quality of the fruit, and reduce their shelf life, but they are not directly related to changes in volatile composition.

Influence of Genetic Modifications

Papaya fruits suffer from a serious disease caused by papaya ringspot virus (PRSV), a major limitation to papaya production worldwide. This virus induces plant stunting and drastically reduces the yield and quality of papaya fruits. Efforts to develop PRSV-resistant transgenic papaya were initiated in 1985, with the first commercialized transgenic papayas

genetically modified with coating protein were SunUp and Rainbow in Hawaii (USA) [82]. Transgenic papaya was produced with the introduction of replicase gene for resistance to papaya ringspot virus (PRSV) [49]. In order to investigate the potential unintended compositional changes in transgenic papaya, profiles of volatile organic compounds, sugar/polyals, organic acids, carotenoids and alkaloids in transgenic and non-transgenic papaya were obtained respectively by HPLC, GC–MS and LC–MS, and compared mutually by multivariate statistical methods. The profiles of volatile compounds from papaya samples showed that 14 volatiles were identified from cv. Mei Zhong Hong and 15 volatiles identified from cv. Sui Zhong Hong. The detected compounds consisted of six main groups according to their diverse functional groups, namely esters, alkenes, alcohols, alkanes, aldehydes and other organic compounds. From the semi-quantitative results of volatile compounds determined by GC–MS, the volatile compositions showed great similarity between the transgenic and non-transgenic papayas collected during the two harvesting times, although the contents of some volatile compositions were found to have changed at different harvesting times. 3,7-Dimethyl-1,6-octadien-3-ol, benzyl iso-thiocyanate, myrcene and 3-carene were the characteristic compounds identified in transgenic cv. Mei Zhong Hong and the non-transformed counterparts, while 3,7-dimethyl-1,6-octadien-3-ol, benzyl iso-thiocyanate, methyl butanoate, methyl benzoate and methyl salicylate were characteristic compounds in the transgenic cv. Sui Zhong Hong and the non-transformed counterparts. In both cultivars, there were no significant compositional differences between transgenic and non-transgenic papayas. Results showed that the composition in transgenic papayas exhibited great similarity to non-transgenic counterparts for measured components. The contents of important nutrients of β-carotene and vitamin C were also similar between transgenic papayas and non-transgenic counterparts for these components. The variation of composition in papaya caused by genetic effect was slight during two harvesting times during reported work. The authors consider that this study could provide some reference value for a safety evaluation of transgenic papaya from the compositional point of view, and could also propose a method for discrimination of transgenic food from non-transgenic counterparts.

Influence of Processing Conditions

The development of off-odors and off-flavors in commercially frozen papaya puree prepared by non-heat treatment was studied in early seventies [41]. These defects have been described as “sulfury, butyric, acrid, pungent, sour, amine-like, and bitter”. Off-odor and off-flavor development in papaya puree can be of either microbial or enzymatic related. Acidification of papaya puree to pH 3.5 was beneficial in retaining quality by reducing microbial growth and thus, acidification is recommended as part of an improved processing method. The unpleasant odorous compounds, e,g butyric, hexanoic, and decanoic acids and their methyl esters, were present in purees with off-odors and off-flavors and they were not present in purees prepared by the improved processing method.

Studies were conducted to monitor the volatile aroma compounds emanating from fresh-cut papaya cv. Solo over a 3-day storage period at 20-22°C [81]. The most intense aroma-active compounds during the first two days of storage were linalool (sweet + flowery) and benzaldehyde (almond). Also contributing to the fruity aroma were smaller quantities of *cis*- and *trans*-linalool oxides, cyclohexane, hexanoic acid, and benzenemethanol. The relative

amounts of these compounds changed significantly at a third day with linalool decreasing by approximately 50% and benzaldehyde, linalool *cis*-linalool oxide, and cyclohexane being absent altogether. Benzyl acetate was the dominant aroma volatile at this storage interval and together with the earthy + flowery odor of *trans*-linalool oxide and the unpleasant coffee-like aroma from tetrahydro-3-furfuryl-1-furan, accounted for the less acceptable odor at the third day compared to the earlier sampling dates.

Among the newer non-thermal methods for post-harvest preservation of food, processing using gamma radiation/electron beam occupies a unique position. Recently, impact of radiation processing on the volatiles profile of papaya was investigated [46]. Gamma-radiation processing (0.05–3.00 kGy) resulted in the appearance of phenol as a new detected compound. Further, once released, the content of phenol remained unchanged during the entire storage period suggesting its possible use as a means of detecting irradiated food even under extended storage. The observed dose dependent increase in phenol content suggested possible use of this compound as a marker for radiation processed papaya.

PRODUCTION OF PAPAYA WINE

In the tropics, there is an abundant supply of exotic fruits to be consumed freshly or used by the food industry. However, large quantities are still wasted during peak harvest periods due to the rapid post-harvest deterioration resulting from high heat and humidity, poor handling, poor storage procedures and microbial contaminations/infestations. Selection and utilization of these fruits for wine-making offer an alternative means for utilizing of these fruits [83−86]. However, there are other tropical fruits such as papaya that have not been fully explored. Till now, only a few studies have been conducted on the use of papaya for wine-making [87−94], which provide a basis for further exploring into papaya wine fermentation to meet the consumers' demand for newer styles of fruit wine. Wine fermentation is a complex process characterized by a succession of different yeasts (*Saccharomyces* and non-*Saccharomyces* yeasts). Several authors claim that non-*Saccharomyces* yeasts used in mixed starter cultures may enhance the organoleptic characteristics of wine due to higher production of important metabolites, such as glycerol, esters and higher alcohols [95,96]. In this way, there are several interesting results using mixed culture of *S. cerevisiae* and *Williopsis saturnus* [89–91, 93]. These studies suggest that papaya juice fermentation with a mixed culture of *S. cerevisiae* and *W. saturnus* may be able to result in the formation of more complex aroma compounds and higher ethanol level than those using single yeasts. The potential of papaya juice fermentation and the kinetic changes of volatile compounds produced during papaya juice fermentation by three commercial wine yeasts was evaluated recently [92]. During fermentation, the three wine yeasts grew actively and showed similar growth patterns. A range of volatile compounds were produced during fermentation including fatty acids, alcohols, acetaldehyde, esters and acetoin. Esters were the most abundant volatile compounds produced. Some volatiles indigenous to the papaya juice such as benzaldehyde, β-damascenone and benzyl iso-thiocyanate were consumed during fermentation. Some volatiles increased initially, and then decreased during fermentation. Overall, the profiles of volatile compounds changes during fermentation were similar among the three yeasts with some differences observed. The volatile compounds of papaya wine produced by yeast (*S.*

cerevisiae) fermentation were isolated to determine aroma profile and the odor-active compounds [94]. A total of 118 volatile constituents were detected, sixty-two of them not previously found in papaya fruit. Six odorants were considered as odor-active volatiles: ethyl octanoate (fruity), (*E*)-β-damascenone (cooked apple), 3-methylbutyl acetate (banana), benzyl iso-htiocyanate (papaya-like); ethyl hexanoate (fruity) and ethyl butanoate (fruity).

FINAL REMARKS

Papaya consumption occurs worldwide, with the USA and Europe constituting the main consumer markets. The fruit is cultivated in various tropical countries of the world. Ripe fruits are best for eating fresh, but many processed products can be prepared, including wine. Among its notable nutritional characteristics, elevated levels of vitamins are commonly cited, as well as being an excellent source of sugars. More than 300 volatile constituents have been identified in fresh and processed papaya. Thus, papaya flavor consists of a huge variety of volatile compounds. Among them, twenty-five odorants were considered as odor-active compounds, from which ethyl butanoate, benzyl iso-thiocyanate, 1-hexen-3-one, (*E*)-β-ionone and methyl benzoate were the most odor-active compounds. However, factors such as fruit maturity stage, physiological disorders, genetic modifications, and processing conditions, can directly affect the flavor profile. Moreover, the information is scarce about its impact aroma compounds and how they change during the action of these factors. Studies in this subject are still very limited, and more efforts should be made not only to determine the influence of these factors on the odor-active compounds, but also to study changes during processing and storage, as well as pre- and postharvest practices.

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Chapter 3

PAPAYA AND ITS APPLICATIONS

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ABSTRACT

Papaya fruit, otherwise scientifically known as *Carica papaya* Linn, has been widely acknowledged to be packed with numerous health promoting nutrients. It is rich in vitamins such as A, B, and C, as well as minerals and dietary fibers. Papaya also formed an excellent source of two main proteolytic enzymes, papain and chymopapain, which are involved in protein digestion. Many research investigations conducted throughout the world revealed that all the plant's parts (leaves, seeds, latex, peel, root, and bark), exhibit medicinal properties, adding to the benefits of the fruit. The active compounds present in various parts of the plant are reported to show antimicrobial, anticancer, antisickling, antihelmentic, antihyperlipidemic, anti-diabetic, antioxidant, antihypertensive, and wound-healing properties. This valuable neutraceutical fruit has also found its application in food industries. The papain enzyme extract that is primarily obtained from the fruit is commercially used as a meat tenderizer, to de-gum natural silk, as well as in chewing gum industry. The present work deals with nutritional values, medical and industrial applications of using papaya and various parts of the plant, and also in terms of a safety assessment of papaya.

1. INTRODUCTION

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Papaya, the common man's fruit, is a member of the small family Caricaceae. The genera associated with this family include *Carica* (1 species), *Jarilla* (3 species), *Horovitzia* (1 species), *Jacaratia* (7 species), *Vasconcellea* (21 species), and *Cylicomorpha* (2 species) (Carvalho and Renner, 2013; Ming et al., 2008). *Carica papaya* Linn is the most important member of this family, and is extensively cultivated due to its great commercial interest in food industry. Besides being consumed fresh, the fruit is also processed into products such as jams, jellies, juices and dried slices. There are various prevailing opinions concerning the origin of papaya, due to limited availability of archaeological evidence. This plant's origin has widely been postulated to be from the Northwest of South America. However, it is increasingly accepted to be native to south of Mexico and Nicaragua (Fuentes and Santamaría, 2014; de Oliveira and Vitória, 2011). Papaya now thrives in more than 60 countries (FAOSTAT, 2014) with tropical or subtropical climates, and its distribution is restricted by frost sensitivity (Campostrini and David, 2007; Veena and Dinesh, 2013).

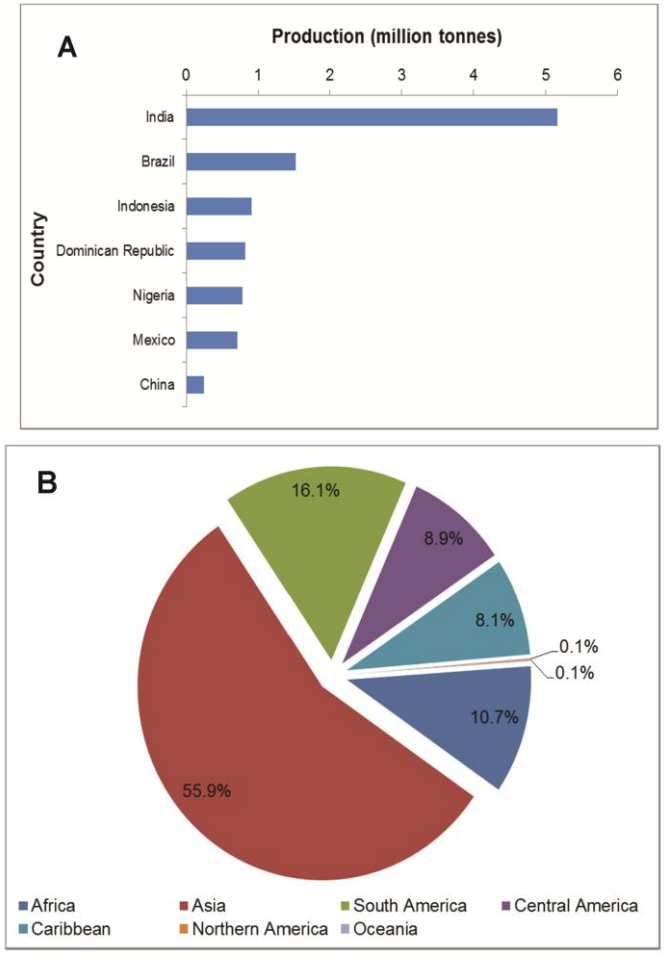

Figure 1. World papaya production (A) and leading papaya producing countries (B) in 2012 modified from FAOSTAT (2014).

Global papaya production in 2012, at 12.41 million tonnes, has shown a 71% increase from 2000 (FAOSTAT, 2014). Striking parallel with increasing concern about the consumption of natural products by worldwide consumers, papaya is an excellent choice from a socio-economic aspect, as it requires year round labor due to no seasonality limitations (de Oliveira and Vitória, 2011). India leads the world with 41.6% of production, followed by Brazil at 12.2% and Indonesia at 7.3%. Other important papaya growing nations include the Dominican Republic (6.6%), Nigeria (6.2%), Mexico (5.7%) and China (2.0%) [Figure 1(A)]. Asia stands out as the prominent papaya producing region, accounting for 55.9% of global production, followed by South America (16.1%) and Africa (10.7%) [Figure 1(B)] (FAOSTAT, 2014).

The growth and development of a papaya plant is strongly dependent on the climatic factors, predominantly temperature and light intensity (Campostrini and David, 2007). The ideal temperature range is 21-33ºC with relative humidity of 60-85%. Temperatures below 10°C can adversely affect the plant growth, flowering as well as fruit development and maturation (Allan, 2002; Allan, 2005; de Almeida et al., 2003). The nutritional quality of papaya fruits produced is also regulated by soil type, mulching, irrigation, and fertilization. Papaya plants are intolerant of waterlogging, which often leads to strict stomatal regulation, cavitation repair, and intense osmotic adjustment (Campostrini and David, 2007; Jiménez et al., 2014). Commercially planted papaya performs best in highly fertile and well-drained soils and pH within 6-7 (Veena and Dinesh, 2013). The economic life cycle of the plant is normally 2-3 years, though it may live up to 20 years, and productivity declines with age (Jiménez et al., 2014). From its initial flowering, the plant will repeatedly flower and produce fruit throughout the year (Ming et al., 2007).

Papaya is usually a single stemmed, semi woody and fast growing perennial herb having 5-10 m height with a short juvenile phase (3-8 months). Fruit bearing trees are usually less than 15 months old. Large (0.6 m^2) and deeply palmately-lobed leaves, with 5-9 pinnate lobes emerges from the upper part of the stem and arranged in a spiral pattern. The genome of the plant occupies an intermediate position between herbs and trees due to distinct features of the major gene groups involved in cell size and lignification, carbohydrate economy, photoperiodic responses, and secondary metabolites (Ming et al., 2008). In nature, papaya plants are either dioecious (with individual male and female plants) or gynodioecious (with hermaphrodite and female plants) (Agrawal and Konno, 2009; Moore, 2014).

The presences of biologically active phytochemicals in different parts of the papaya plant, from fruit to the leaves, are currently being investigated worldwide for various medicinal properties. Papaya is classified as lactiferous as it contains specialized cells called laticifers, which are responsible for secreting a substance known as latex. Copious amounts of latex are found in the leaves, stems and unripe papaya as these cells are distributed throughout the plant (Agrawal and Konno, 2009; Macalood et al., 2014). Latex is a complex mixture of at least four distinct cysteine proteinases, with papain and chymopapain present in abundance (Robinson, 1975). The proteolytic activity of latex from unripe papaya has been exploited to aid protein digestion in dyspeptic patients. Ripe papaya is known to offer numerous health benefits such as increasing resistance to coughs and colds due to good supply of vitamin A and C, prevention of diabetes mellitus, and rheumatism. Since acts as a laxative, consumption of ripe papaya is also associated with regular bowel movements. The young leaves of papaya are cooked as vegetables in some Asian countries. The leaves extract have been documented to possess wound-healing properties and anti-inflammatory activities for arthritis,

rheumatism, and asthma while used to combat dengue fever (Aravind et al., 2013; Krishna et al., 2008). The black seeds of papaya are edible, though quite pungent and peppery, making it a good substitute for black pepper (Aravind et al., 2013). Significant research has shown the seeds to be effective male contraceptive agent and possess anti-bacterial properties against several enteropathogens. The root extract of papaya tree has demonstrated both anti-hypertensive and diuretic properties. The stem bark extracts are identified as remedies for jaundice and diabetes mellitus (Aravind et al., 2013; Krishna et al., 2008). Table 1 summarized the phytochemical composition and some of the medicinal uses of different parts of papaya plant. The present chapter deals with nutritional values, medical, and industrial applications of papaya and various parts of the plant. Also, a safety assessment of papaya is given.

1.1. Nutritional Values

Papaya is a climacteric fruit, known as pepo-like berries due to having a central seed cavity that resembles melon. The fruits are borne axillary on the main stem, usually singly but sometimes in small clusters. It is pear-shaped of various sizes (7-35 cm long). Smaller papayas typically reach approximately 0.25 kg while the larger varieties can grow up to 10 kg or more. The surface of papaya fruit is covered with a smooth thin skin that is greenish to yellowish in color depending on the degree of ripeness. The inner thick flesh, with colors ranging from yellow to red, has a melon-like texture with a pleasant sweet taste. An individual papaya matures within 5-9 months (Milind and Gurditta, 2011; de Oliveira and Vitória, 2011).

Papaya is attractive due to its high nutritive value as well as being reasonably priced. The fruit is an excellent source of vitamins and minerals (Table 2) (Krishna et al., 2008). However, nutritional composition may vary depending on the varieties, growing conditions and degree of the ripeness (de Oliveira and Vitória, 2011). Regular consumption of papaya may strengthen an individual health against coronary heart disease and prevent early age blindness in children as it is uniquely rich in vitamin A and C. Also, the presence of pronounced amount of dietary fiber makes papaya a popular choice for ensuring normal bowel movements, parallel to prevention treatment for constipation (Krishna et al., 2008). Glucose, sucrose and fructose have been determined to be the major component of carbohydrates in the ripe papaya with the percentage of total sugar varying between 10 and 13%, while glucose is present predominantly in green papaya (de Oliveira and Vitória, 2011; Zhou and Paull, 2001). The nutritional values of green and ripe papaya have been reported to be in the range of 27 to 32 kcal per 100 g of fresh fruit. The comparatively low calorie content of papaya are grabbing the attention of those who are into weight management program. Mature papaya fruit is rich in carotene, which is crucial to prevent damage caused by free radicals associated with some forms of cancer. In contrast, green papaya does not contain carotene (Krishna et al., 2008). Apart from that, papaya has been identified as a good source of serotonin, which is important to mediate reflex activity in gut and also to decrease the risk of thrombosis (Santiago-Silva et al., 2011). As mentioned, consumption of green papaya that consists of latex pertaining to different types of proteolytic enzymes may help in protein digestion.

Table 1. Phytochemical composition and some of the medicinal uses of different parts of papaya plant modified from Krishna et al. (2008)

Part	Constituents	Medicinal uses
Fruits	Protein, fat, fiber, carbohydrates, mineral: calcium, phosphorous, iron, vitamin C, thiamine, riboflavin, niacin and carotene, amino acids, citric and malic acids (green fruit)	Ripe fruit: Stomachic, digestive, carminative diuretic, dysentery and chronic diarrhoea, expectorant, sedative and tonic, relieves obesity, bleeding piles, wound of urinary tract, ringworm and skin disease psoriasis. Unripe fruit: Laxative, diuretic, dried fruit reduces enlarged spleen and liver, use in snakebite to remove poison, abortifacient, anti- implantation activity and antibacterial activity
Seeds	Fatty acids, crude protein, crude fiber, papaya oil, carpaine, benzylisothiocynate, benzylglucosinolate, glucotropacolin, benzylthiourea, hentriacontane, β-sitosterol, caricin and enzyme myrosin	Carminative, emmenagogue, vermifuge, abortifacient, counter irritant, as paste in the treatment of ringworm and psoriasis, anti-fertility agent in males.
Root	Carposide and enzyme myrosin	Abortifacient, diuretic, checking irregular bleeding from the uterus, piles, anti-fungal activity
Leaves	Alkalodis carpain, pseudocarpain and dehyrocarpaine I and II, choline, carposide vitamin C and E	Young leaves as vegetable, jaundice (fine paste), urinary complaints and gonorrhoea (infusion), dressing wound (fresh leave), antibacterial activity, vermifuge, in colic, fever, beriberi, abortion (infusion), asthma (smoke)
Bark	β-sitosterol, glucose, fructose, sucrose, galactose and xylitol	Jaundice, anti-haemolytic activity, STD, store teeth (inner bark), anti-fungal activity
Latex	Proteolytic enzymes, papain and chemopapain, glutamine cyclotransferase, chymopapains A, B and C, peptidase A and B and lysozymes.	Anthelmintic, relieves dyspepsia, cures diarrhoea, pain of burn and topical use, bleeding haemorrhoids, stomachic, whooping cough

Table 2. Nutritive value of 100g of ripe and unripe papaya (Krishna et al., 2008)

Chemical composition	Ripe papaya	Unripe papaya
Protein	0.6g	0. 7g
Fat	0.1g	0.2g
Fiber	0.8g	0.9g
Carbohydrates	7.2g	5.7g
Minerals	0.5g	0.5g
Energy	32 Kcal	27 Kcal
Total carotene	2,740µm	0
Beta carotene	888µm	0

Additionally, extensive investigation revealed that close to four hundred fruit volatile compounds mostly esters, hydrocarbons, benzylisothiocynate, terpenes, aldehydes, ketones, alcohols and organic acids have been detected with various papaya cultivars (Almora et al., 2004; Flath and Forrey, 1977; Fuggate et al., 2010). Papaya is one of the many fruits containing structurally diverse ester. Abundance of butyl acetate is identified in *C. pubescens* (Idstein et al., 1985) while highest concentration of methyl butyrate has been detected in *C. papaya* of the Solo group varieties from Sri Lanka (MacLeod and Pieris, 1983). Low molecular weight esters play an important role in the modulation of aroma and flavor of papayas (de Oliveira and Vitória, 2011).

Fermented papaya preparation is a promising dietary supplement as an antioxidant produced using a biotechnological process. It has been shown to improve antioxidant defense in elderly patients, even without any overt antioxidant deficiency state, at a dose of 9 g/day orally (Marotta et al., 2006). Dried fruits contribute towards reduction of food wastage while improving shelf life. Dried papaya skin has been reported as a potential source of dietary ingredient for broiler chickens resulted in similar food consumption, food conversion efficiency, survivability and meat yields with respect to a control diet when used up to 120 g/kg of diet (Kamaruzzaman et al., 2005).

1.2. Papaya Enzymes

Latex, the milky fluid from unripe papaya is a rich source of cysteine proteinases variants. Papain and chymopapain are the most widely studied enzymes of papaya latex, representing 5% and 27% of the protein constituents (Robinson, 1975). Papain (EC 3.4.22.2), is the first cysteine proteinase to be crystallized by Balls et al. (1937). The isolation of papain was achieved using ammonium sulfate precipitation. Refinement of the isolation procedure led to the discovery of chymopapain (EC 3.4.22.6) (Jansen and Balls, 1941), which has high solubility in salt solutions with remarkable stability at acidic pH values as low as 2 (Baines and Brocklehurst, 1982). The stem ‘chyme’ refers to the thick semi-fluid mass of partially digested food (milk) that is being broken down by gastric secretion in the stomach. Thus ‘chymo’ is used as a term for an enzyme having a higher ratio of milk-clotting to hemoglobin-digesting activity than the existing enzyme (Storer and Ménard, 2013).

Papain and cyhmopapain belong to the family CI. These enzymes show proteolytic activity towards proteins, short chain peptides, and amino acid esters. A review of the various aspects of papain is available elsewhere (Amri and Mamboya, 2012). Papain is a single polypeptide chain made up of 212 amino acids with three disulfide bridges and a sulfhdryl group necessary for the enzyme activity (Kamphuis et al., 1984). Papain is one of most versatile proteases, showing broad specificity. It degrades protein substances more extensively than pancreatic proteases. The enzyme has been determined to be stable under a wide range of reaction conditions and is known to be active in organic solvents. The optimum pH value for papain activity is in the range of 3.0-9.0 which varies according to the substrate and is able to retain activity in 8 M urea. The enzyme exhibits high activity when the temperature is maintained around 65-80°C (Amri and Mamboya, 2012; Storer and Ménard, 2013)

Papain is a globular protein consisting of two distinct structural domains forming the active site cleft, with a molecular weight of 23,406 Da. The existence of three disulfide bonds is important in stabilizing the tertiary structure of papain by creating a strong interaction among the side chains. The hydrophobic and hydrophilic interaction of amino acids in the side chain seems to be the major driving force for the protein folding. The catalytically important residues for papain are located in the following positions: Glutamine-19, Cysteine-25, Histidine-158 and Histidine-159 (Amri and Mamboya, 2012; Menard et al., 1990). The biological role of papain remains ambiguous but considering the location, pronounced quantity of enzyme, and its promiscuous specificity, it has been presumed to play a defensive role, guarding the plant against attack by pests such as insects and fungi (Storer and Ménard, 2013).

Chymopapain is made up of a single non-glycosylated polypeptide chain of 218 amino acids, with a molecular weight of 23 650 Da. The sequence analysis of chymopapain is 58% identical to that of papain. Chymopapain is acknowledged to catalyze the breakdown of all the bonds that were cleaved by papain but at slower rates. It is able to catalyze reaction in wider range of pH and requires reducing conditions. Cystatins are specific inhibitors of chymopapain, and its inactivation by E-64 resembles the characteristics of most members of family CI. Heterogeneity and similar physicochemical properties of chymopapain to the other cysteine endopeptidases are known to complicate purification from papaya latex. High purity chymopapain free from glycyl endopeptidase contamination may be obtained, following findings that this enzyme is highly stable at acidic pH. Though the biological roles of the enzyme are not fully understood, it is anticipated to be part of the plant defense mechanism (Buttle, 2013).

1.2.1. Mechanism of Functions

Papain has been widely engaged as a model enzyme to delineate the features of the catalytic mechanism for cysteine peptidases (Storer and Ménard, 2013). The basis of papain reaction mechanism involves deprotonation of Cys-25 in the catalytic triad by His-159. Subsequently, Cys-25 performs a nucleophilic attack on the carbonyl carbon of the peptide backbone, freeing the amino terminal of the peptide followed by formation of a covalent acyl-enzyme intermediate. The intermediate then reacts with a water molecule (deacylation) to release the carboxyl terminal portion of the peptide simultaneously regenerating the free enzyme molecule. Asparagine-175 favorably orientates the imidazole ring of His-159 to facilitate deprotonation reciprocally, allowing formation of the thiolate-imidazolium ion pair. The interaction among these three amino acids that are spaced far apart within the chain is possible due the folding pattern that brought them in close proximity (Amri and Mamboya, 2012; Storer and Ménard, 2013). It has been established that papain activity may be efficiently inhibited by peptidyl or non-peptidyl N-nitrosoanilines (Guo et al., 1996). The formation of a stable S-NO bond in the active site of papain (*S*- nitroso-Cys^{25}) results in the inactivation of the enzyme (Xian et al., 2000).

2. APPLICATIONS

2.1. Medical Applications

2.1.2. Antimicrobial

A study was conducted by Dawkins et al. (2003) on the antibacterial effects of ripe and unripe *Carica* (C.) *papaya* on common wound organisms using *in vitro* disc diffusion method. The authors found that the seed extracts of immature, mature, and ripe fruits were shown to inhibit the growth of *Bacillus cereus*, *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Proteus vulgaris* and *Shigella flexneri* while no inhibition effect was observed for epicarp and endocarp extracts. This indicated that the *C. papaya* seeds possess antibacterial properties and the inhibition activity on these Gram-positive and Gram-negative bacteria was independent of fruit maturity stage. This beneficial effect might play an important role in treating chronic skin ulcers. Akujobi et al. (2010) also reported that extracts from seeds (epicarp and endocarp) of both ripe and unripe *C. papaya* were able to inhibit the growth of pathogenic bacteria, where the highest antibacterial activity was observed for the extract from endocarp of unripe fruit. It was suggested that the high amount of papain present in the papaya seed could be the bactericidal compounds that are responsible for this activity. The authors found that the antibacterial activity of the extracts was maintained from 30°C to 50°C but showed a decreased in activity at 60°C and above. Moreover, Baskaran et al. (2012) performed a study on efficacy of various solvent extracts of *C. papaya* leaves on pathogenic bacteria. Among the solvents (ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane and water) used, chloroform extract of *C. papaya* leaves demonstrated the highest antibacterial activity against *Micrococcus luteus* with a 15.17±0.29 mm inhibition zone.

In another study, Zakaria et al. (2006) investigated the antibacterial effect of methanol and ethanol extracts of *C. papaya* flowers on *Corneybacterium diptheriae*, *Staphylococcus aerues*, *Streptococcus pneumonia*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris*. The authors reported that both methanol and ethanol extracts were able to inhibit the growth of selected Gram-positive bacteria, but were not effective against selected Gram-negative bacteria. Methanol extract of *C. papaya* flowers was found to inhibit the growth of *S. Aureus* and *S. pneumonia*, while the ethanol extract inhibited the growth of *C. diptheriae*, *S. Aureus* and *S.pneumoniae*. The same authors also revealed that the methanol extract of *Carica papaya* flowers exhibited higher antibacterial activity compared to that extracted from ethanol. Another study performed by Anibijuwon and Udeze (2009) on antibacterial activity of bioactive compounds extracted from leaf and root of *C. papya* using water and organic solvents against human pathogenic bacteria. The authors found that the organic solvent root extracts and leaf extracts were more effective against Gram-positive bacteria than Gram-negative bacteria. This might be due to the presence of lipopolysaccharide (LPS) in the Gram-negative bacteria that interact with the antibacterial peptides and subsequently reduce the effectiveness of the peptides to inhibit the bacteria growth (Zakaria et al., 2006).

Furthermore, Nirosha and Mangalanayaki (2013) reported that *C. papaya* leaves and stem extracts showed higher antibacterial activities against Gram-negative bacteria (*Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeroginosa*) compared to the Gram-positive

bacteria (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Bacillus cereus*), with highest activity demonstrated (18 mm zone of inhibition) against *Salmonella typhi*. The authors also reported that the minimum inhibitory concentration (MIC) of the extracts ranged between 50-200 mg/ml, including alkaloids, tannins, saponins and phenols. Same bioactive compounds were reported by Anibijuwon and Udeze (2009), where the leaf and root extracts containing alkaloids, tannins, saponins, glycosides and phenol, with minimum inhibitory concentration (MIC) ranged between 50-200 mg/ml. Past studies have shown that leaves of papaya contain papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates. Flavonoids are phenolic compounds that possess antimicrobial and antiviral properties. Additionally, antibacterial properties have been reported for alkaloids isolated from plants (Baskaran et al., 2012).

2.1.2 Antisickling

Sickle cell incidence has been found in African, the Mediterranean countries, India, and the Middle East; however, it seldom occurs in Europe countries (Mojisola et al., 2008). Sickle cell disorder (SCD) is a hereditary blood disorder that affects red cell hemoglobin which includes sickle thalassaemia and sickle cell anemia (HbSS). Sickle cell anemia is a genetic disease where the 'SS' individual contains an abnormal beta-globin gene. This occurrence is due to the replacement of beta-6-glutamic acid by valine in the gene encoding the human beta-globin (Imaga and Adepoju, 2010). Sickled red blood cells (crescent-shaped) tend to block the capillaries which cause stasis and starve organs from nutrient and oxygen which subsequently leads to organ damage (Imaga et al., 2009). A decrease in solubility of deoxy HbSS might occur due to low concentration of oxygen, low pH and/or high temperature, which subsequently leads to polymerization into fibers that distort its shape and function (Iweala et al., 2010).

The membranes of sickle cell hemoglobin (HbSS) are also more fragile compared to those normal red blood cells which are easily destroyed and removed from circulation in the spleen. In addition, the SS patients have a short life span of red blood cells (about 2 weeks) than those of normal people which is 120 days (Mojisola et al., 2009). Sickle cell diseases are associated with symptoms such as joint pain, acute chest syndrome, malfunctioning of organs (spleen, heart and brain), anemia, fever, paleness, shortness of breath and jaundice (Iweala et al., 2010). The management of SCD involves gene therapy, bone marrow transplantation and antisickling drugs (piracetam, tucaresol and hydroxyurea) or agents that are able to inhibit the polymerixation of sickle cell hemoglobin and also increase the oxygen affinity of hemoglobin (Afolabi et al., 2012).

It has been reported that papaya possesses an antisickling effect and may reverse sickling due to the presence of flavonoids, carotenoids, glycine, phenylalanine and tryptophan and organic acids that were produced after hydrolysis of corresponding esters (Iweala et al., 2010; Mojisola et al., 2009). An *in vitro* study evaluated the antisickling properties and membrane stabilizing activity of methanolic papaya leaf extracts on HbSS red blood cells of non-crisis state sickle cell patient was performed by Imaga et al. (2009). The authors found that there was a reduction in the hemolysis and increased in the red blood cell membrane integrity under osmotic stress conditions in the presence of methanolic papaya leaf extract. Imaga and Adepoju (2010) also studied the antisickling effect of *C. papaya* dried leaves on both sickle thalassaemia and sickle cell anemia (HbSS) red blood cells obtained from non-crisis state sickle cell patients of both sexes. The authors reported that the SS cell suspensions treated

with *C. papaya* leaf extracts inhibit the hemoglobin polymerixation in the red blood cell suspensions and subsequently inhibited the time course for sickling of HbSS cells. The SS cell suspensions treated with crude extracts showed only 0-5% of sickle cells at 60 min while there was more than 90% of sickle cells in untreated SS cell suspensions (treated with normal saline). However, the papaya leaf extracts were unable to prolong the delay time and the antisickling activity was dose dependent where 10 mg/ml of extract showed the highest activity compared to 5 mg/ml of extract.

Another study was conducted by Oduola et al. (2006) to investigate the antisickling and reversal of sickling activities of an aqueous extract of unripe *C. papaya* on blood samples of sickle cell patients. The authors found that the unripe papaya extract was able to prevent the sickling of HbSS red cells and reversed sickled HbSS red cells in 2% sodium metabisulphite, and that the antisickling agent was found in the ethyl acetate fraction of the extract. The authors also reported that 1 g/ml of unripe papaya extract was the minimum concentration needed for the maximum antisickling activity. In addition, Afolabi et al. (2012) carried out a comparison study on the antisickling effects of *C. papaya* seed oil and *Vernonia amygdalina*, in male and female human sickle cell blood sample. The authors reported that there was a significantly ($P<0.05$) reduced number of sickle blood cells that were treated with papaya seed oil or *Vernonia amygdalina* compared to that of untreated sample. However, the number of red blood cells in papaya seed oil-treated sample was higher than the *Vernonia amygdalina*-treated sample. In addition, papaya seed oil was found to reduce lactate dehydrogenase activity and facilitated biochemical changes that improved the oxidative interactions in HbSS blood. These results indicated that the papaya extract has not only expressed antisickling properties but also helps in alleviation of stress in HbSS patients.

Furthermore, Mojisola et al. (2008) evaluated the antisickling effects of unripe fruit pulp in aqueous, methanol and chloroform on 2% sodium metabisulphite sickled red blood cells. It was concluded that the highest antisickling (87%) and reversal (74%) activities were observed from aqueous extract of papaya pulp that was fermented for 5 days at concentration of 2.5 mg/ml while methanol extract showed 64% of antisickling and 55% of reversal activities. However, no inhibitory or reversal activity was reported from chloroform extract. This indicated that the potential antisickling agents are relatively polar substances which are mostly soluble in water. It has also been observed that methanol and aqueous papaya leaf extracts showed the highest antisickling activity over time, followed by ethyl acetate extract and butanol extract. Different solvents used to extract the bioactive compounds from *C. papaya* also showed different antisickling activity where the highest activity of butanol extract and ethyl acetate was observed at concentration of 10 mg/ml and 5mg/ml, respectively (Imaga and Adepoju, 2010).

In line with this finding, Oduola et al. (2008) carried out a study to evaluate the kidney functions of sickle cell patients (2 to 12 years old and above) upon consumption of unripe *C. papaya* extract. The authors revealed that the plasma levels of sodium, potassium, chloride, bicarbonate urea, creatinine, uric acid and calcium upon consumption of aqueous extract of papaya were comparable to those before consumption of extract and were maintained in the reference range. This indicates that the aqueous extract of papaya possess no harmful effect on the functions of kidney. Moreover, a study was conducted by Iweala et al. (2010) using new drug preparation, Ciklavit®, as the positive control to investigate the antisickling effect of *Carica papaya* extracts on sickle cell trait (HbAS) and sickle cell disease (HbSS) blood samples. Results showed that the alkaline and alcoholic papaya extracts were able to reduce

the number of sickle cells significantly ($P<0.05$). Same observation was also observed with the use of Ciklavit®, with a significant decrease in the number of sickle cells in both HbAS and HbSS blood samples. This suggested that the antisickling properties of papaya extracts were comparable to the new drug preparation, Ciklavit®.

2.1.3. Anthelmintic

High occurrence of intestinal nematode infections in developing countries has gained increasing attention, due to the severe consequences for the health of man and domestic animals. Intestinal disorders, discomfort and loss of productivity via direct or indirect interference with host metabolism and nutrition are the main causes of these infections (Satrija et al., 1995). There are three types of parasites that infect livestock, namely round worms (nematodes), tape worms (cestodes), and flatworms (trematodes) (Ameen et al., 2012). While synthetic drugs have been found to control the parasite infections effectively, they are too costly and come with side effects. Hence, most of farmers in developing countries are unable to afford the costly synthetic drugs for their livestock. This infection causes anaemia, hydreamia, oedemas, general weakness and emaciation (Shaziya and Goyal, 2012). Therefore, interest has been growing in a cheaper and simpler way with no side effects to control the nematodes infections.

Anthelmintic properties of papaya have been reported in *in vitro* and *in vivo* studies. Adu et al. (2009) conducted a study involving 120 cockerels to investigate the anthelmintic activity of oven dried ground latex of *C. papaya*. Results showed that birds treated with higher dose of latex (400 mg/dose) decreased the egg counts by 77.8% while birds treated with 300 mg/dose showed 26.9% reduction and only 8.3 % reduction was observed for untreated group. This was also supported by Ameen et al. (2012) who examined the anthelmintic activity of powdery form and aqueous extract of *C. papaya* seeds in 40 nematodes infected-Isa Brown commercial layers. The birds were randomly divided into both experimental and control groups (10 birds per group). The experimental groups were assigned to three groups: one group fed with anthelmintic, piperzine (322 mg/kg body weight/day), one group fed with powdery form of papaya seeds (300 mg/day/bird), and one group fed with aqueous extract of papaya seeds (1:10 ml water required/day) for 14 days. Results showed that birds fed with powdery form and aqueous extract of papaya seeds caused a significant ($P<0.05$) increase in packed cell volume, number of red blood cells, and lymphocytes. They also showed a significant ($P<0.05$) decrease in incidence of eosinophils. In addition, the aqueous extract of papaya seeds was found to be more effective in reduction of the fecal egg counts compared to that of powdery form of papaya seeds. This reduction in egg counts might be due to the destruction of parasitic cells by proteolytic activity of papain from papaya seeds.

On the other hand, Rupa and Jayanta (2013) conducted a comparative study on anthelmintic properties of *Carica papaya* seed and *Cucurbita maxima* (pumpkin) seed extracts against *Pheretima posthuma* adult earthworms, with the use of albendazole as a standard reference. The authors reported that both extracts were able to exhibit the wormicidal activities at concentration of 60 mg/ml. *Carica papaya* seeds extract was found to be a better anthelmintic compared to that of *Cucurbita maxima* due to the shorter paralysis time (1.88 min) and shorter death time (3.45 min). It has been suggested that the tannins from papaya extract can bind to the glycoprotein on the parasite cuticle that subsequently led to the death of *Pheretima posthuma*. Kanthal et al. (2012) also reported that 100% of *C. papaya* latex exhibited shorter paralysis time (24.5 min) and death time (56 min) of *Pheretima*

posthuma compared to standard reference, piperazine citrate (10mg/ml) and control (distilled water). Kanthal et al. (2012) also reported that 100% of *C. papaya* latex exhibited shorter paralysis time (24.5 min) and death time (56 min) of *Pheretima posthuma* compared to the standard reference, piperazine citrate (10mg/ml) and control (distilled water).

In a comparison study, Buddhachat et al. (2012) found that aqueous extract of papaya seeds was more pronounced in killing the worms, *Stellantchasmus falcatus*, compared to the bitter cucumber fruits (*Mormordicacharantia* Linn.). The scanning electron microscopic results showed that both extracts caused the changes on the tegumental surface where dead worms showed curving at the edge of the spines, blebbing, and rupturing at the body surface. In additional, loss of spine around the oral sucker and posterior region was observed for worms that were treated in papaya seed extract. Tegumental surface of the helminth is required for adhesion and absorption of nutrient from the host. There are several postulated mechanisms for anthemintic activity. Damage of tegument is one of possible mechanism to cause detachment of worms from host epithelial tissue. In addition, this damage may subsequently affect the worm's defense system making it susceptible to the host immune system. Moreover, Kermanshai et al. (2001) performed a study to evaluate the anthelmintic effect of papaya seeds on *Caenorhabditis elegans* using a viability assay. The authors found that the anthelmintic activity of papaya was positively correlated to benzyl isothiocyanate, which might be the sole anthelmintic agent.

Moreover, Adiwimarta et al. (2010) developed a trial involving 18 female Bligon goats to evaluate the anthelmintic effect of cassava and *C. papaya* leaf. The goats were divided into three groups where experimental group I was fed on a mixture of 70% grass and 30% cassava leaf, experimental group II was fed on a mixture of 70% grass and 30% papaya leaf, while the control group was fed on 100% grass for six weeks. The authors reported that the feeding of *C. papaya* leaf caused a significant reduction in the number of worm eggs and *Coccidia oocytes* by slope of regressions -170.0 ($P<0.05$) and -714.2 ($P<0.05$), respectively. Same observation was observed for group I, where cassava leaf reduced the counts of worm eggs and *Coccidia oocytes* by slope of regressions -291.7 ($P<0.05$) and -325.0 ($P<0.05$), respectively. The anthelmintic effect might be due to the tannins content in the cassava and papaya leaf. *Carica papaya* extract (0.2 ml/mouse) was also found to exhibit anthelmintic activity in *Ancylostoma caninum* infected-Swiss albino mice (500 *Ancylostoma canimun*) with a decrease in number of worm burdens and larval recovery, an increased in number of mucosal mast cells on day 16, and significantly ($P<0.05$) reduced eosinophil levels in 24 days after infection (Shaziya and Goyal, 2012). This study has strongly indicated that papaya extract may be a potential anthelmintic against gastrointestinal nematodes. The anthelmintic activity involves the expulsion or destruction of the gastrointestinal nematodes, either by starving them to death or paralyzing them. These parasites must absorb nutrients to meet their metabolic needs due to the lack of energy storage. Death may also be due to paralysis caused by temporarily lost of adherence ability to maintain in the gut. Similar observation was reported by Ameen et al. (2010) that the egg counts of *Haemonchus contortus*, *Trichostrongylus* spp., *Strongyloides* spp. and *Ostertagia* spp. were significantly ($P<0.05$) decreased in West African Dwarf sheep, upon oral administration of crude extract and aqueous extract of *C. papaya* seeds for 2 weeks.

Furthermore, Pone et al. (2011) used a placebo-controlled design trial to evaluate the ovicidal and larvicidal properties of aqueous and ethanolic extracts of papaya seeds on the eggs and first stage larvae of *Heligmosomoides bakeri*. The results showed that the extracts

inhibited embryonic development, egg hatching, and larval survival by destroying their blastomer as compared to the placebo and negative control (0.05% ethanol) group. In addition, increasing the concentration of extracts was found to be more effective against eggs and larvae. Same authors also reported that aqueous extract was found to be more effective in preventing embryonnation (only 8% of eggs embryonnated) at concentration of 2.75 mg/ml. This is due to the hydrophobic surface of egg shell which facilitates the penetration of aqueous extract compared to ethanol extract. However, ethanolic extract was found to be more efficient in inhibiting larval development (96% mortality) due to the presence of alkaloid that form an alkaline condition that are unfavorable for the growth of larvae. Same observation was reported by Satrija et al. (1995) that the anthelmintic activity was observed in four groups of BALB/C mice (10 mice per group) that were infected with 100 *Heligmosomoides polygyrus* infective larvae/mouse after administration of 2, 4, 6 and 8 g of papaya latex/kg body weight, respectively, for 3 days. The authors also found that the highest concentration of papaya latex used (8g of papaya latex/kg body weight) showed the highest antiparasitic activity, which is 84.5% compared to that of control (non-treated mice), which is most likely due to the presence of proteolytic enzymes (papain, chymopapain and lysozyme).

It has also been reported that *H. polygyrus* adult worms were dead in the presence of cysteine proteinases (CPs) (Shaziya and Goyal, 2012). Cysteine proteinases can attack the protein on the cuticle surface, weakening the cuticle and enabling the internal hydrostatic pressure to disrupt the body wall and cause the release of internal tissues. Loss of internal tissues may subsequently lead to the death of the worm (Buttle et al., 2011). In an *in vitro* study, Stepek et al. (2005) found that the CPs from papaya was able to attack and digest the cuticle of *Heligmosomoides polygyrus* adult male and female worms with a 2 h incubation period, which subsequently led to the decreased number of *Heligmosomoides polygyrus*. The composition of nematode cuticle is made from collagens cross-linked by disulphide bonds and it is sensitive towards digestion of cysteine preoteinase. The cuticle is acting as a support and leverage point for movement in order to survive in gastrointestinal tract. Luoga et al. (2012) also found that CPs extracted from papaya latex not only exhibited anthelmintic effect on *Heligmosomoides bakeri* in C3H mice, but also helped in reducing stress in the fasting model.

2.1.4. Anti-Diabetic

Several anti-diabetic mechanisms have been proposed, which include delaying the digestion of glucose, inhibiting the α-amylase and α-glucosidase enzymes and also regulating the intestinal brush border transport f glucose. Saponins and flavonoids are the phytochemicals that are found in papaya and they have been found to promote β-cell regeneration. Thus, the restoration of pancreatic islet cell function could be one of the possible anti-diabetic mechanisms for papaya extract (Omonkhua Akhere et al., 2013). Venkateshwarlu et al. (2013) conducted another placebo-controlled study to evaluate the effects of *Carica papaya* seeds on blood glucose levels in 30 steptozotocin-nicotinamide induced diabetic rats (male Sprague Dawley rats). The authors reported that the feeding of papaya seeds extracts caused a reduction in the blood glucose levels (41.8%) at concentrations of 100 mg/kg while the blood glucose level was reduced by 44.9% at 200 mg/kg after 14 days of the feeding trial. The authors postulated that a reduction in blood glucose level might be attributed to the increased tissue uptake of glucose by increasing the sensitivity of insulin or increased secretion of insulin from the beta cells of pancreas. It was

observed that ethyl acetate, alcohol, and aqueous extracts of papaya leaves inhibited alpha amylase activity by 85.5%, 79.3% and 88.2%, respectively (Jiju et al., 2013). The authors suggested that all extracts could be used as the potential alpha amylase inhibitors in managing hyperglycemia patients. Alpha amylase inhibitors are the agents that reduce and/or delay the digestion of carbohydrate and absorption of glucose by reducing the amylase activity (Jiju et al., 2013).

In another study, Juarez-Rojop et al. (2012) used a placebo-controlled design trial involving 42 streptozotocin-induced diabetic rats (adult male Wistar rats) to examine the anti-diabetic effect of *C. papaya* leaf extract. The authors reported that diabetic rats fed with papaya leaves extract (0.75 g, 1.5g and 3.0 g/100 ml) for 30 days showed a significant ($P<0.05$) reduced in blood glucose level by 29.6%, 29.4% and 42.4%, respectively, compared to the control that was not fed the papaya leaves extract. In addition, the authors found that papaya leaf extract could facilitate the regeneration of pancreatic islet cells in diabetic rats and also prevent the accumulation of glycogen and lipids. Same observation was reported by Adeneye and Olagunju (2009) where a lower concentration of fasting blood sugar was observed in male Wistar rats fed with papaya leaves extract for 30 days, compared to that of the control and glibenclamide treated rats.

Additionally, Maniyar and Bhixavatimath (2012) evaluated the antihyperglycemic effect of aqueous extract of papaya leaves in 36 alloxan-induced diabetic albino rats. The authors found that the oral administration of aqueous extract of papaya leaves (400 mg/kg body weight) for 21 days significantly reduced ($P <0.01$) the blood glucose level in alloxan-induced diabetic albino rats. Results showed that aqueous extract of papaya leaves contains alkaloids, tannins, saponins, flavonoids, anthraquinones, anthocyanosides and reducing sugars which might be the potential active compounds in regulating the glucose concentration. In a 24 weeks of long term study, a total of 24 adult male and female Wistar rats were randomly assigned to three groups: healthy rats (normal control), streptozotocin-induced diabetic rats (diabetic control) and papaya leaves extract treated diabetic rats. The diabetic rats were induced with streptozotocin (65mg/kg body weight) for 7 days through intraperitoneal injection. The authors found that diabetic rats fed with papaya leaves extract for 2 weeks significantly decreased fasting blood sugar compared to diabetic control group and achieved normoglycemia in week 8 and sustained until week 24 (Omonkhua Akhere et al., 2013).

2.1.5 Antihyperlipidemic

It has been reported that diabetic subjects typically present with high cholesterol levels due to the increase mobilization of free fatty acids from peripheral fat deposits. This mobilization of free fatty acids mainly occurs due to the inhibition of hormone sensitive lipase production by insulin (Venkateshwarlu et al., 2013). However, high nutritional values of papaya are found to be able to prevent cholesterol oxidation and improve cardiovascular system. Papaya contains fibrin which is rarely found in the plant world. Fibrin has been found to prevent blood clots, improve the quality of blood cells, maintain the smooth blood flow in the circulatory system and also helps in preventing stroke (Aravind et al., 2013).

Iyer et al. (2011) reported that the oral feeding of *C. papaya* extract (200 mg/kg body weight) and water fraction (400 mg/kg body weight) caused a reduction in total cholesterol, triglycerides, low-density lipoproteins level and increased the high-density lipoprotein level in olive oil-induced hyperlipidemic rats. The authors revealed the presence of tannins, alkaloids (carpain and carpasemine) and glycosides in the water fraction, which might be the

components responsible for the antihyperlipidemic effect of papaya. Maniyar and Bhixavatimath (2012) observed a significant ($P<0.01$) decrease in total cholesterol and triglycerides levels of alloxan-induced diabetic albino rats fed with aqueous extract of papaya leaves (400 mg/kg body weight) on day 21. Omonkhua Akhere et al. (2013) also reported that the serum total cholesterol (at 4 and 12 weeks) and LDL-cholesterol (at 12, 16 and 20 weeks) were significantly ($P<0.05$) reduced in papaya leaves extract treated diabetic rats, accompanied by an increase in HDL-cholesterol at week 4 and l6. Results also showed a significant reduction ($P<0.05$) in atherogenic and coronary risk indices.

Similar observations were reported by Venkateshwarlu et al. (2013), that a 7.5% reduction in total cholesterol and a 11.0 % reduction in triglycerides levels in streptozotocin-nicotinamide induced type II diabetic rats (male Sprague Dawley rats) after administration of 100 mg/kg of papaya seeds extracts for 14 days, while a higher reduction of 29.9% in total cholesterol and 17.3% reduction in triglycerides levels were observed for rats fed with 200 mg/kg of papaya seeds extract. In another study, Adeneye and Olagunju (2009) evaluated the effects of *C. papaya* seeds extract on cholesterol metabolism in 30 male Wistar rats. The authors found that the supplementation of papaya seeds extracts (100, 200 and 400 mg/kg) for 30 days not only reduced total serum cholesterol, very low density lipoprotein, low density lipoprotein and triglycerides levels but also increased the high density lipoprotein level compared to the control and glibenclamide treated rats (positive control). Additionally, the papaya seeds extract also lowered atherogenic and coronary artery indices. This was supported by Kantham et al. (2011), who studied the effect of aqueous and ethanol extracts of papaya fruits on the hyperlipidemic activity in 42 Wistar albino adult male rats fed with cholesterol (500 mg/kg body weight). The authors found that rats administrated with 25, 50 and 100 mg/kg body weight of papaya extracts for 30 days reduced the lipid parameters compared to that of cholesterol-treated rats. There was also a significant ($P<0.05$) increased in high-density lipoprotein level with the feeding of 100 mg/kg body weight of papaya extracts compared to the standard drugs, lovastatin and guggul (standard drug from plant source). The authors suggested that the antihyperlipidemic effect may be due to the presence of oleanolic acid in the papaya extracts, and it has been reported to possess a potential antihyperlipidemic effect.

2.1.6 Antioxidant

Reactive oxygen species (ROS) are the products expressed from oxygen energy metabolism in the body. Over expressed of ROS due to environmental stress could lead to oxidative stress, which involves the damage of cell structure. The presence of oxidants could cause oxidative stress due to imbalance metabolic condition between the oxidant and antioxidant systems in our body (Maccio and Madeddu, 2012). ROS are likely to attack unsaturated lipids, amino acids and DNA nucleotides (Somanah et al., 2014). Damage of DNA may also contribute to the progression of dysplastic lesions to precancerous lesions, and subsequently to the development of anaplastic cancerous and metastatic dissemination (Marotta et al., 2006). This oxidative stress can be countered by the presence of antioxidant. In addition, excessive levels of serum iron can surpass the binding capacity of transferrin and form non-transferrin bound that subsequently leads to increased cellular unbound labile iron pool (LIP). This LIP is active in production of the reactive oxygen species (ROS) and increased ROS contents results in oxidative stress and toxicity to the liver, heart, and other organs (Prus and Fibach, 2012).

Numerous antioxidants are available in most of the plant products (Ozkan et al., 2011). Antioxidants can be divided into endogenous antioxidants (NADPH, NADH, glutathione, uric acid, bilirubin and metalloenzymes), dietary antioxidants (vitamin C, vitamin E and carotenoid), metal binding protein (albumin, transfeein and ferritin) and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) (Rahmat et al., 2006). Mechanisms of antioxidants involve the enzymatic degradation of free radicals, chelation of metals to prevent the generation of free radicals and also free radicals scavenging activity (Mehdipour et al., 2006). It has been suggested that papaya contains most of the compounds with hydroxyl groups (glucose, saccharose, citric acid, and malic acid) involved in free radical scavenging, and β-carotene is known as an oxidant stress suppressant (Somanah et al., 2014).

Ozkan et al. (2011) carried out an *in vitro* study to investigate the antioxidant activities of papaya juices from three different cultivars (Sunrise Solo, Red Lady and Tainung). All juices (fully ripen fruits) were found to exhibit free radicals (1,1-diphenyl-2-picrylhydrazyl, DPPH) and active oxygen species (superoxide anion and hydroxyl radicals) scavenging activities, with higher antioxidant activity observed from Sunrise Solo, and followed by Red Lady and Tainung. This higher antioxidant capacity was closely related to the high concentration of total phenolic in the papaya juices. Phenolic compounds possess the ability to scavenge free radicals, singlet oxygen, superoxide free radicals and hydroxyl radicals. In addition, the high concentration of total chlorophyll a and b found in Sunrise Solo might further help in free radical scavenging activity. This was supported by Mehdipour et al. (2006) that studied the antioxidant effect of dried papaya juice in 30 adult male Wistar rats. The authors found that rats administrated different doses of papaya juice (100, 200 and 400 mg/kg) daily for 2 weeks significantly ($P<0.01$) increased the blood total antioxidant power by 11.1%, 23.6% and 23.1%, respectively, and was comparable to that of standard antioxidant, vitamin E (18.4%). In addition, the supplementation of papaya juices also reduced blood lipid peroxidation, while the highest antioxidant activity (80%) was observed at 17.6 mg/ml of papaya juice in *in vitro* experiment.

β-sitosterol from papaya has also been reported to modulate the antioxidant enzymes that resulted in decreased production of ROS. The importance of antioxidant enzymes is to maintain the equilibrium of cellular redox and aid in ROS scavenging activity (Oloyede et al., 2012). Zhou et al. (2011) reported that ethyl acetate and *n*-butanol fractions of papaya seeds possessed the scavenging activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+), superoxide anion and hydrogen peroxide radicals. The authors found two potential antioxidants, *p*-hydroxybenzoic acid and vanillic acid, in ethyl acetate fraction, and these might contribute to the scavenging activities. Same observation was reported by Oloyede et al. (2011) who observed a significant ($P<0.05$) increase in antioxidant enzymes activities of glutathione reductase, glutathione transferase and metabolizing enzyme glucose-6-phosphate dehydrogenase in Albino mice but a reduction in glutathione peroxidase (GPx) activity was found in rat's kidney after consumption of 100 mg/kg of ethyl acetate fraction of unripe papaya fruits for 7 days. Quercetin and β-sitosterol were identified and isolated from ethyl acetate fraction at concentration of 120.2±0.16 mg/g and 279.1±0.09mg/g, respectively. These compounds might enhance the antioxidant enzymes activities through induction or activation of enzyme synthesis.

Indran et al. (2008) revealed that *C. papaya* leaves extract reduced the gastric ulcer index and plasma lipid peroxidation in alcohol induced acute gastric damage and blood oxidative

stress rats, and was attributed to the increased GPx activity. Moreover, Sadek (2012) evaluated the antioxidant effect of *Carica papaya* fruit aqueous extract in 60 male Wistar acrylamide (ACR)-intoxicated rats. The authors found that the supplementation of papaya extract (250 mg/kg) for 40 days not only reduced concentrations of malondialdehyde in the stomach (13.5%), liver (19.3%) and kidney (20.2%) of ACR-intoxicated rats, but also significantly ($P<0.05$) reduced catalase, superoxide dimutase and reduced glutathione compared to the ACR-intoxicated rats fed without papaya extract. The depletion of antioxidant enzymes might be due to an increased utilization to offset the increased ROS content.

Recently, Somanah et al. (2014) observed that the FPP (6g/day) reduced the susceptibility of human erythrocytes to undergo free radical-induced hemolysis after 14 weeks. This was attributed to the increased integrity of erythrocytes and also increase in total antioxidant upon consumption of FPP. Fibach et al. (2010) also reported that FPP not only increased the number of reduced glutathione in red blood cells but also decreased the ROS contents, membrane lipid peroxidation and externalization of phosphatidylserine in β-thalassemia and E-β-thalassemia patients. This showed the improvement of oxidative status with no impact on the hematological parameters. Prus and Fibach (2012) examined the antioxidant effect of FPP on liver-and heart-derived cells, and also red blood cells that were treated with non-transferrin bound iron. The authors found that FPP reduced the LIP and ROS, which points to the involvement of iron chelation as antioxidant mechanism.

2.1.7. Wound Healing Activity

Prevalence of burns and trauma wounds in the developed and developing countries remains high and resulted in financial burden, especially in developing countries. Therefore, the use of papaya as a healing remedy has gained increasing public interest, due to its ability to heal burn wounds, soft tissue wounds, skin infections, and other skin disorders (Gurunga and Skalko-Basnet, 2009). Regeneration of dermal and epidermal tissues is the essential process to repair the wound. The process involves separate inflammatory, proliferative, collagen synthesis and tissue remodeling (Anuar et al., 2008). Additionally, the wound contraction could be affected by the restorative ability of the tissue, type and extent of the damage and also general state of the health of the tissue (Gurunga and Skalko-Basnet, 2009). Thus, the possible wound healing mechanism of papaya might be due to the antioxidant effect of flavonoids, anti-inflammatory effect of sterol and triterpene derivatives and antimicrobial activity of alkaloids (carpaine), glycosides (glucotropaelin) and benzyl-isothiocyanate that shorten the inflammatory phase, which subsequently leads to an increased rate of wound closure (Nayak et al., 2012).

Past studies have demonstrated that papaya could promote wound healing in animal models. Anuar et al. (2008) reported that aqueous extracts of green papaya epicarp induced the complete healing in 13 days compared, to ripe papaya epicarp, sterile water, and Solcoseryl ointment, which required 17, 18 and 21 days, respectively. The authors suggest that these observations might be due to different compositions in green and ripe papaya. Green papaya consists of high amount of chymopapain, papain and carpaine, which helps in preventing the bacterial infection, reducing inflammation and risk of oxidative damage to the tissues. Unripe papaya fruits are also known to promote desloughing (fibrinolytic properties), granulation and healing of wound, and also reducing the offensive odor associated with chronic skin ulcers (Mahmood et al., 2005). In addition, papaya has been found to exhibit the

softening and disintegrating properties through alkaline combination with borax or potassium carbonate in treating the warts, corns, sinuses, eczema, cutaneous tubercles and other skin hardness to avoid skin irritation (Chidan Kumar et al., 2012).

Considering that collagens are the major proteins of the extracellular matrix that release free hydroxyproline, the increased concentration of hydroxyproline would serve as an indicator for increased collagen synthesis that subsequently leads to increased would healing (Gurunga and Skalko-Basnet, 2009). Nayak et al. (2012) reported that administration of ethanol extract of papaya seed (50 mg/kg) to wound-induced Sprague-Dawley rats for 13 days decreased the wound area by 89% and increased the hydroxyproline content in the granulation tissue. Deposition of well-organized collagen in the granulation tissue was observed via histological analysis. In another study, Gurunga and Skalko-Basnet (2009) evaluated the healing potential of papaya latex on burn wounds induced in 40 Swiss albino mice. They were randomly divided into 5 groups, each treated with Carbopol 974P NF gel, Carbopol gel containing 1% dried papaya latex, Carbopol gel containing 2.5% dried papaya latex, standard drug (silver sulphadiazine and chlorhexidine gluconate cream) as positive control, and untreated mice as negative control. Treatments with different percentage of dried papaya latex were found to increase the wound contraction percentage, hydroxyproline content and shorten epithelialization time on the wound-induced mice. Diabetic wounds are normally hard to manage and the wound might require weeks to recover. However, the administration of aqueous extract of papaya fruit at 100 mg/kg for 10 days decreased the wound area by 77% in streptozotocin-induced diabetic rats compared to that of the control (fed without papaya fruit extract). This was attributed to the faster epithelization and increased hydroxyproline content (Nayak et al., 2007).

2.2. Industrial Applications

The term papain not only stands for purified enzyme, but is also a commercial name given to the spray-dried powder of latex obtained by tapping the green fruits or stems and trunk of papaya (Caro et al., 2000). Papain is extensively used in industrial applications, and is best recognized as a meat tenderizing agent. Meat tenderization is usually achieved through the combined action of proteolytic activity by papain, cyhymopapain and other enzymes. The mechanism can be described by relative hydrolysis of collagen, the major structural protein of connective tissues as well as break down of myofibrillar proteins mainly actomyosin (Amri and Mamboya, 2012; Khanna and Panda, 2007). Chymopapain is the major constituent of enzyme in the mixture compared to papain, with higher thermostability and exhibited favorable activity at the meat's natural pH. It is speculated to be primarily corresponded for the tenderization. The tenderizing effects notably expressed during initial stages of cooking with inactivation eventually takes place at high temperatures concurrently eliminating any undesirable side effects that can be associated with residual protease activity (Rathi and Gadekar, 2007). Papain has been employed for boiling off cocoons and degumming of silk. Cocoon cooking is prerequisite for the reeling of silk thread from cocoon spun by the silkworm. At the same time, raw silk must be degummed to remove sericin, a proteinaceous substance that binds to the fibroin strands (Devi, 2012; Mahmoodi et al., 2010). Formerly, papain was used for shrink proofing of wool fabrics to furnish silky luster quality to the material. The process was evaluated based on the partial hydrolysis of the scale tips.

Nevertheless, treatment irregularity and extensive weight loss of the fiber made the method not industrially feasible (Araújo et al., 2008). However, papain has been successfully applied as a biological detergent to remove protein stains (Grzonka et al., 2009).

Papain is a common ingredient in the brewing industry and acts as a beer clarifying agent. Beer contains complexes of proteins that may precipitate at low temperatures, forming insoluble colloidal particles known as chill haze. Papain is used to hydrolyze high molecular weight proteins that are involved in the haze formation to amino acids and smaller peptides, thus providing a simple and inexpensive mechanism to improve the beer stability when chilled. Nevertheless, there have been growing concerns over the health related issues due to retention of papain activity in the beer even after going through the pasteurization process. Besides, papain is fairly unspecific in the proteolytic activity and acts on both haze- and foam-active proteins resulting in undesirable progressive loss of head retention (Rehmanji et al., 2005). Several studies have been dedicated to improve protein digestibility in animal feeds through supplementation of papain which would apparently lower the production cost of the feed by increasing nutrients availability (Grzonka et al., 2009; Wong et al., 1996).

Papain contributions for medical applications are also greatly recognized. This enzyme is used for wound debridement which is essential for the removal of necrotic or nonviable tissue from the wounded area to facilitate healing (Grzonka et al., 2009). In addition, it has a long history of being used to treat sports injuries, other causes of trauma and allergies (Deitrick, 1965). Papacárie, a gel based on papain, is an inexpensive Brazilian formulation used for chemomechanical caries removal. It acts by specifically removing dental caries from affected tissues, with no harmful effects pertaining to sound tissues close to the lesion (Lopes et al., 2007). Likewise, papain is involved in the preparation of tetanus vaccines and immunoglobulin samples for intravenous injections as well as synthesis of tyrosine derivatives for the treatment of Parkinsonism (Grzonka et al., 2009).

3. Safety Assessment of Papaya

Papaya, when used in right proportion, may serve as a natural hair conditioner. However, the release of latex from unripe papaya may cause skin irritation and provoke allergic reactions in some people (Aravind et al., 2013). Pregnant women are strictly prohibited from eating ripe and unripe papaya for fear of its teratogenic and abortifacient effects (Adebiyi et al., 2002). Oxytocin is commonly employed for labour induction (Chard, 1989) while analogues of prostaglandins have been noted to cause induction of abortion via different routes of administration in both rodents (Elger et al., 1981; Lau et al., 1975) and human subjects (Karim et al., 1971). Papaya latex has been shown to exhibit oxytocic properties similar to the *in vitro* effects of oxytocin and prostaglandin $F_{2\alpha}$ in pregnant and non-pregnant rat uterus. Hence, consumption of papaya that is highly associated with latex may cause marked uterine contractions, as well as severe complications during pregnancy, and may ultimately lead to miscarriage (Adebiyi et al., 2002). Excessive ingestion of papaya can cause carotenemia, a clinical entity characterized by yellowing of the skin and elevated beta-carotene levels in the blood. The yellowish-red to orange hue pigmentation is mostly visible at areas having a thick stratum corneum, such as soles and palms than other parts of body. Appropriate dietary modification, with cessation of papaya ingestion, can help to resolve the

yellow discoloration of the skin (Arya et al., 2003). Apart from that, excessive intake of papaya may result in gastrointestinal disturbances and severe gastritis together with some other symptoms consistent with hay fever or asthma, including wheezing, breathing difficulties and nasal congestion (Aravind et al., 2013). The black seeds of papaya are known to possess carpine which can lower the pulse rate and depress the nervous system when taken in excess. Carpine and papain have been reported to exhibit anti-fertility properties, with complete loss of fertility observed in various animal models, linking the possibility that consumption of papaya seeds can affect human males or other male mammals' fertility (Ayotunde et al., 2010).

CONCLUSION

The low price, high nutritional value, and widespread availability of papaya makes the fruit popular globally, especially among consumers that are passionate about natural products. In addition, many studies have generated various experimental evidences that prove papaya possesses potential health benefiting properties that can be useful for mankind. Also, enzymes from the papaya have been widely exploited for industrial applications. However, excessive intake of papaya has been associated with adverse effects which require further evaluation to better understand the exact underlying mechanisms.

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Chapter 4

CHARACTERIZATION OF PAPAYA SEED OIL FROM TWO MALAYSIAN PAPAYA FRUIT VARIETIES

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ABSTRACT

The present chapter investigated the fatty acid composition, triacylglycerol profile, thermal behaviour and physicochemical properties of papaya seed oil from two Malaysian papaya varieties (i.e. *Sekaki* and *Eksotik*). Results depicted that the seeds of both papaya varieties had almost similar oil content (30.6%). GC analysis showed that the predominant fatty acids in *Sekaki* and *Eksotik* papaya seed oils were oleic acid (C18:1, 70.5-74.2 %), palmitic acid (C16:0, 14.9-17.9 %), stearic acid (C18:0, 5.21-4.5 %) and linoleic acid (C18:2, 3.5-4.6 %), respectively. The present study revealed that the fruit variety significantly affected the fatty acid composition, triacylglycerol profile (TAG), thermal behaviour, colour intensity and oxidative stability of papaya seed oil. It was indicated that *Eksotik* seed oil had higher saturated fatty acids (SFA) than *Sekaki* seed oil (23.1%>20.7%). Moreover, it had higher melting point, lower crystallization point and darker color than *Sekaki* seed oil.

1. INTRODUCTION

Papaya (Caricaceae family) is originally from tropical and subtropical America and Africa (Yon, 1994). However, it is widely cultivated in all tropical countries nowadays. *Carica papaya Linn* is the most common cultivar in horticulture as it is easily grown in home gardens and commercial farms. Different papaya varieties provide different fruits in terms of size, shape and flavor (7-60 cm, length, up to 9 Kg, weight) (Yon, 1994; Chan, 2008). Traditional papaya varieties in south Asia have large fruits with 1-3 Kg weight (Yon, 1994);

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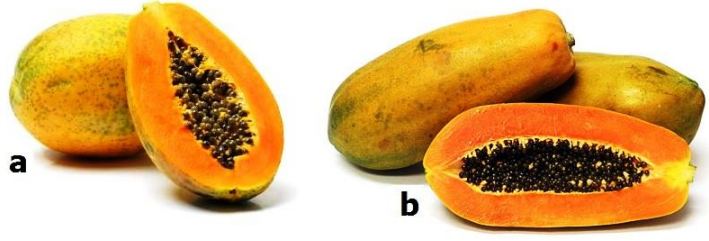
while some of papaya varieties (e.g. pyriform) have small fruits. *Kaegdum*, *Kaegnuan*, *Koko*, and *Sainampeung* are commercial papaya varieties in Thailand. They have also large papaya fruits with the weight of 0.6-2 Kg (Chan, 2008; Yon, 1994). In Indonesia, the main papaya varieties are *Dampit*, *Jingga* and *Paris* which are different in shape and skin color (Chan, 2009; Yon, 1994). *Cavite Special* is a commercial papaya in Philippine (Chan, 2009). *Exotic* and *Sekaki* are the most commonly cultivated and utilized papaya varieties in Malaysia (Samaram, 2013). *Eksotik* papaya variety has pear shaped fruits with the weight of 0.6 to 1 Kg. *Sekaki* papaya is a locally distributed variety with larger fruits (1.5-2 Kg) than *Eksotik* papaya (0.6-1 Kg) (Figure 1). Papaya peels and seeds are the biomass waste in food industry. Dried papaya peel is a low calorie by-product mainly used in poultry diet (Krishna et al., 2008). Papaya seeds have been also used as an inexpensive dye adsorption for removing methylene blue from aqueous solution. Moreover, papaya seed is a potential source of protein, fiber and oil (Samaram, 2013). As reported by previous researchers (Puangsri et al., 2005; Sammarphet, 2008; Samaram et al., 2013), papaya seeds from different varieties had 30-34% oil. Papaya seed oil has the reddish yellow color containing high amount of monounsaturated fatty acids. Likewise, it is highly stable oil to oxidation due to its considerable antioxidant activity (Malacrida et al., 2011). Papaya seed oil contains anti-carcinogenic compounds such as benzyl-isothiocyante (BITC) (Rossetto et al., 2008; Lee et al., 2011). In tropical countries like Malaysia, papaya seeds are massively remained as the biomass waste of fruit processing units (Hameed, 2009); therefore it can be utilized as an inexpensive raw material for production of commercial papaya seed oil.



Source: Samaram et al., 2014; www.specialtyproduce.com; www.mercucita.com.

Figure 1. Malaysian papaya fruit varieties (a: *Eksotik*; b: *Sekaki*).

The objective of this chapter was to investigate the oil content, fatty acid composition, triacylglycerol profile, thermal behavior, oxidative stability, color intensity, iodine value (IV), saponification value (SV) and unsaponifiable matters of the seed oil from two Malaysian papaya varieties (*Sekaki* and *Eksotik*).

2. MATERIALS AND METHODS

2.1. Materials

Two different varieties of papaya (i.e. *Sekaki* and *Eksotik*) were purchased from a hypermarket in Selangor Malaysia. Aluminum pans were supplied by Perkin-Elmer (Norwalk, CT, USA). The pure mixed standard of fatty acid methyl esters (FAME) was

purchased from Sigma-Aldrich (St. Luis, MO, USA). N-hexane (Reagent grade and HPLC grade), petroleum ether and methanol (reagent grade), 2-propanol, acetone and acetonitrile (HPLC grade) were supplied by Fisher scientific (Pittsburgh, PA, USA). Other chemicals (such as ethyl alcohol 96%, potassium persulfate, potassium hydroxide, acetic acid, chloroform, potassium iodide, sodium thiosulfate, P-anisidine, starch indicator and phenolphthalein) were provided by Merck (Darmstadt, Germany).

2.2. Sample Preparation and Oil Extraction

Papaya fruits (*Sekaki* and *Eksotik*) were chosen based on their maturity stages (Yon, 1994). Fruits were cleaned and cut into halves in order to collect the seeds. The seeds were washed and dried at 45°C in oven for 2 days. Dried papaya seeds were grinded and packed for further extraction (Samaram et al., 2013). The seed powder was subjected to Soxhlet extraction (SXE) according to AOCS Official Method (Am 2-93, 1993). Extraction was performed in triplicate for each treatment.

2.3. Analytical Methods

2.3.1. Oil Content

The oil content was calculated by dividing the amount of the extracted seed oil to the initial amount of seed powder. A 0.0001 g analytical balance (Mettler Toledo GmbH, Greinfensee, Switzerland) was applied. The extraction yield was calculated from the following formula (Bimakr et al., 2012; Samaram, 2013):

$$\text{Yield } \% = [m_{(oil)} / m_{(sample)}] \times 100$$

2.4.2. Fatty Acid Profile

Fatty acids methyl esters (FAME) were prepared by 2 M methanolic KOH and hexane (AOCS Official Method, Ce 2-66, 2009). Fatty acid composition of papaya seed oil was analyzed by an Agilent gas chromatography (GC) 6890N (Palo Alto, CA, USA). GC was equipped with a flame ionization detector (FID) and a DB-23 capillary column (60 m × 0.25 mm × 0.15 µm) (J&W Scientific, Folsom, CA, USA). For GC analysis, a liner (0.75 mm) (Supelco, Bellefonte, PA, USA) was placed inside GC injector to minimize peak widening (Cheong et al., 2011). The analysis was operated under the following experimental condition: injection volume 0.5 µL, inlet temperature 250 °C and split ratio (1:20). Helium was used as a carrier gas with a flow rate of 0.7 ml/min. Oven temperature was set at 50 °C and held for 1 min at 50 °C. Then, the temperature was raised to 175 °C with a flow rate of 25 °C/min. In the last step, the temperature reached to 230 °C with the flow rate of 4 °C/min and held for 5 min at 230 °C. Detector temperature was set at 280 °C. Hydrogen gas and air were employed as detector gases with the flow rate of 40 and 450 ml/min, respectively (David et al., 2005). The experiment was carried out in duplicate for each sample (Samaram et al., 2013).

2.4.3. Triacylglycerol (TAG) Profile

Quantitative analysis of triacylglycerol (TAG) was carried out according to AOCS method (Ce 5b-89, 2009) with minor modification to achieve proper peak separation. For TAG analysis, a high performance liquid chromatography (HPLC) (Waters 600, Waters, Milford, MA, USA) was applied. HPLC system was equipped with a Waters 600 pump, a differential refractometer (RI) detector (Waters 410) and a reverse phase C18 symmetry column (15cm × 3.9 mm × 5µ). In this experiment, 20 µL of the diluted oil (with acetone, 5% W/V) was manually injected into HPLC injector. A mixture of acetonitrile and acetone (20:80) was used as the mobile phase. Total run time was set for 50 min (Puangsri et al., 2005; Samaram et al., 2013). TAG analysis was performed in duplicate for each sample.

2.4.4. Thermal Behavior

Thermal behavior of papaya seed oil was analyzed by using a Perkin Elmer differential scanning colorimeter (DSC 7) with Pyris analysis software (Perkin Elmer Corp., Norwalk, CT, USA). For thermal analysis, 5 mg of the oil was weighted in the specific aluminum pans. Pans were sealed tightly to tolerate the internal pressure during analysis. Two empty sealed pans were used to take the baseline in the adjusted temperature program. The temperature program of the cooling process was started from 60 °C to -60 °C. The sample was kept for 5 min in both temperatures to obtain complete crystallization curve. Melting curve was obtained by heating the oil from -60 °C to 60 °C in the same condition as cooling curve. Thermal behavior was analyzed in duplicate for each sample (Puangsri et al., 2005; Lim et al., 2010; Samaram et al., 2014).

2.4.5. Physicochemical Analysis

Iodine value (IV) of papaya seed oil was determined from its fatty acid profile according to AOCS official method (Cd 1c-85, 2009). Unsaponifiable matters were also measured according to AOCS official method (Ca 6a-40, 2009). Color measurement was performed by using a Lovibond visual colorimeter (Tintometer Ltd, Amesbury, UK) and 1 inch optical glass cell according to the AOCS official method (Cc 13e-92, 2009). Peroxide value (PV) and p-anisidine value (AV) were determined based on AOCS official methods (Cd 8-53, 2003; Cd 18-90, 2009). The physicochemical tests were carried out in triplicate for each sample. TOTOX value (TV) was calculated based on the following equation (Serjouie et al., 2010; Samaram, 2013): $TV = 2PV + AV$

2.5. Statistical Design and Data Analyses

A completely randomized design (CRD) was considered to prepare different experimental treatments. The independent variables were papaya varieties (i.e., *Sekaki* and *Eksotik*). The response variables were oil content, fatty acid compodition, TAG profile, thermal behavior, iodine value (IV), peroxide value (PV), p-anisidine value (AV), TOTOX value (TV), unsaponifiable matters and color. The univariate one way analysis of variance (ANOVA) was used to analyze the data. MINITAB software (version 14, Minitab Inc., State College, PA, USA) was used to create the proper experimental design and further data analysis (Mirhosseini et al., 2008; Samaram et al., 2014).

3. RESULTS AND DISCUSSION

3.1. Oil Content

Results indicated that both *Sekaki* and *Eksotik* seeds had almost equal oil content (30.6 %). Previous researchers (Puangsri et al., 2005; Malacrida et al., 2011; Sammarphet, 2008) also reported that papaya seeds from different varieties had relatively high oil content (29.2-34.7%) (Table 1).

Table 1. Oil content of papaya seeds

	Papaya variety					
	Sekaki	*Eksotik*	*Batek Batu*	*Formosa*	*Kaeg-dum*	*Hawaii*
Oil content	30.6	30.6	30.7	29.2	34.7	31.0

Sekaki, *Eksotik* (current chapter); *Batek Batu* (Puangsri et al., 2005); *Formosa* (Malacrida et al., 2011); *Kaeg-dum*, *Hawaii*, (Sammarphet, 2008).

3.2. Fatty Acid Profile

The current study revealed that the main fatty acids of *Sekaki* and *Eksotik* seed oils were oleic acid (C18:1, 70.5-74.2 %), palmitic acid (C16:0, 14.9-17.9 %), stearic acid (C18:0, 5.21-4.5 %) and linoleic acid (C18:2, 3.5-4.6 %) (Table 2; Figure 2). *Eksotik* seed oil had significantly ($p < 0.05$) lower contents of stearic acid (C18:0) and oleic acid (C18:1) than *Sekaki* seed oil; while it showed higher levels of palmitic acid and linoleic acid than *Sekaki* seed oil (Table 2). Total saturated fatty acid (SFA) of *Eksotik* seed oil (23.1%) was relatively higher than that of *Sekaki* seed oil (20.7%). The minor fatty acids of papaya seed oil were myristic acid (14:0), palmitoleic acid (16:1), linolenic acid (18:3), arachidic acid (20:0) and eicosenoic acid (20:1), which were less than 0.5% in papaya seed oil. *Eksotik* seed oil had significantly ($p < 0.05$) higher content of myristic acid and palmitoleic acid than *Sekaki* papaya seed oil (Table 2). However, *Eksotik*- and *Sekaki* seed oils did not have significant ($p > 0.05$) different linolenic and arachidic acid contents.

The fatty acid compositions of *Sekaki* and *Eksotik* papaya seed oils were slightly different from that of reported by previous researchers (Table 3). This indicates the significant ($p < 0.05$) effect of papaya variety on fatty acid composition of the seed oil. The difference could be due to the utilization of different varieties and maturity stages of papaya fruits applied in these studies (Table 3) (Puangsri et al., 2005; Sammarphet, 2008; Lee et al., 2011; Malacrida et al., 2011; Samaram, 2013). Moreover, the fatty acid composition of papaya seed oils was very similar to that of olive oil. Both olive oil and papaya seed oil are rich in oleic acid. Oleic acid is beneficial for the human health and it is an indicator of high stable oil (Abdulkarim et al., 2007; Huertas, 2010). Many fruit seed oils are rich in polyunsaturated fatty acids (PUFAs) mainly linoleic acid (C18:2). Polyunsaturated fatty acids are highly prone to oxidation. This is mainly due to the presence of double bonds in their molecular structure (Abdulkarim et al., 2007). Linoleic acid is the abundant fatty acid in cooking oils (such as sunflower oil and soybean oil). Different levels of linoleic acid have been detected in grape seed oil (50-78%),

orange seed oil (34.5-39.3%), apple seed oil (48.2-64.1 %), pumpkin seed oil (55.6%) and watermelon seed oil (59.6%) (Yu et al., 2006).

Table 2. Fatty acid composition of papaya seed oils from two different papaya varieties

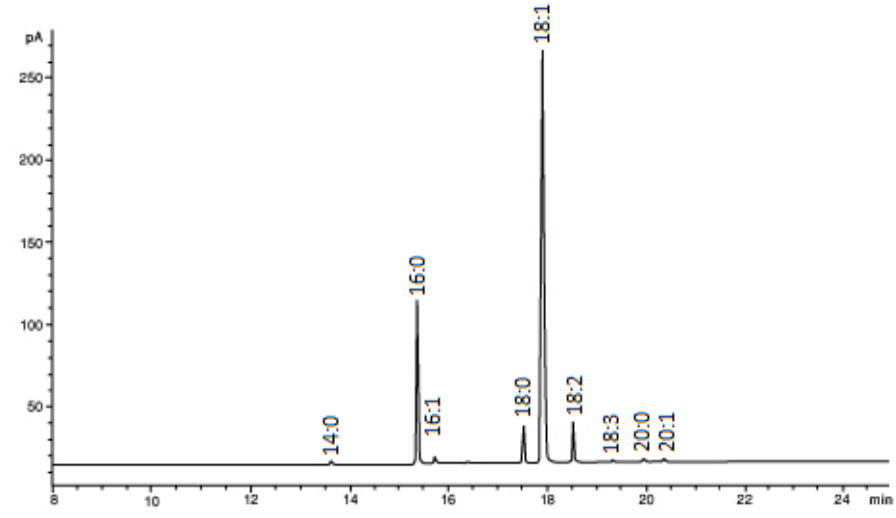
Papaya variety	Fatty acids								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1
Sekaki	0.21 ± 0.02[a]	14.9 ± 0.05[a]	0.27 ± 0.04[a]	5.21 ± 0.01[a]	74.2 ± 0.21[a]	3.50 ± 0.03[a]	0.17 ± 0.01[a]	0.38 ± 0.01[a]	0.42 ± 0.03[a]
Eksotik	0.29 ± 0.00[b]	17.9 ± 0.03[b]	0.67 ± 0.04[b]	4.50 ± 0.01[b]	70.5 ± 0.11[b]	4.6 ± 0.02[b]	0.18 ± 0.01[a]	0.36 ± 0.01[a]	0.38 ± 0.01[b]

[a, b], indicated the significant difference at the confidence level of $P < 0.05$. (Mean ± SD, n = 2); 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid; 20:1, eicosenoic acid.

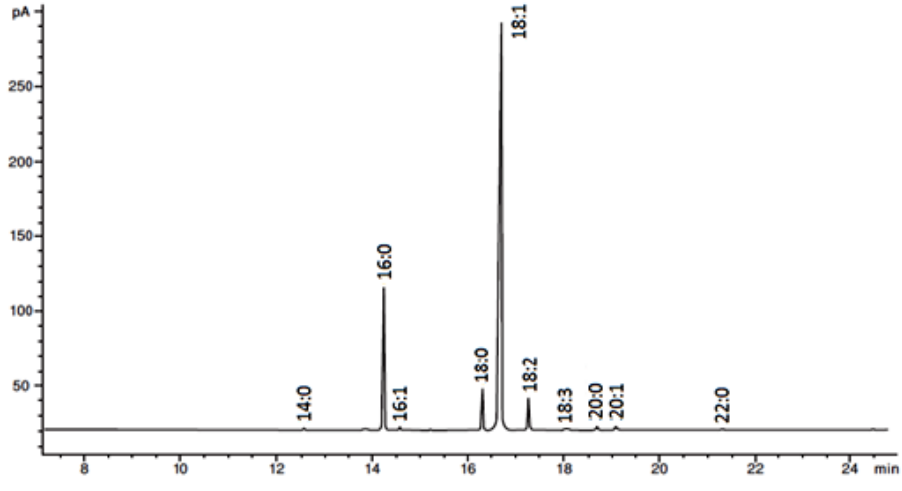
Table 3. Fatty acid profile of papaya seed oils from different papaya varieties

	Papaya varieties						
Fatty acids	*Sekaki*[*]	*Eksotik*[*]	*Batek Batu*[*]	*Formosa*[*]	*Kaeg-dum*[*]	*Hawaii*[*]	*Tainoung No. 2*[**]
14:0	0.21	0.29	0.20	0.20	-		0.72
16:0	14.9	17.9	13.9	16.2	17.2	18.0	19.7
16:1	0.27	0.67	0.20	0.27	-	-	0.36
18:0	5.21	4.50	4.90	4.73	3.19	2.82	6.68
18:1	74.2	70.5	76.8	71.3	77.6	76.1	66.7
18:2	3.50	4.60	3.00	6.06	2.03	3.08	3.17
18:3	0.17	0.18	0.20	0.22	-	-	0.17
20:0	0.38	0.36	0.40	0.38	-	-	0.38
20:1	0.42	0.38	0.30	0.32	-	-	0.46
PUFA	3.67	4.78	3.20	6.28	2.03	3.08	3.34
MUFA	74.9	71.5	77.3	71.8	77.6	76.1	67.6
SFA	20.7	23.1	19.4	21.4	20.4	20.8	29.1

Sekaki, *Eksotik* (present chapter); *Batek Batu* (Puangsri et al., 2005); *Formosa* (Malacrida et al., 2011); *Kaeg-dum*, *Hawaii*, (Sammarphet, 2008); *Tainoung No. 2* (Lee et al., 2011); PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; [*] Solvent extracted papaya seed oil (soxhlet); [**] Screw pressed extracted papaya seed oil; 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid; 20:1, eicosenoic acid.



a: Eksotik



b: Sekaki

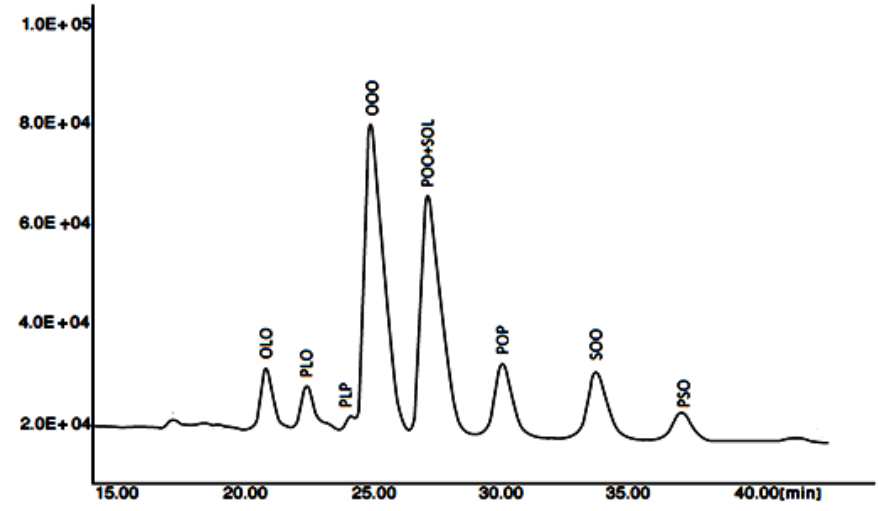
Figure 2. Fatty acid profile of papaya seed oil from two different Malaysian papaya varieties ; 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid; 20:1, eicosenoic acid.

3.3. Triacylglycerol Profile

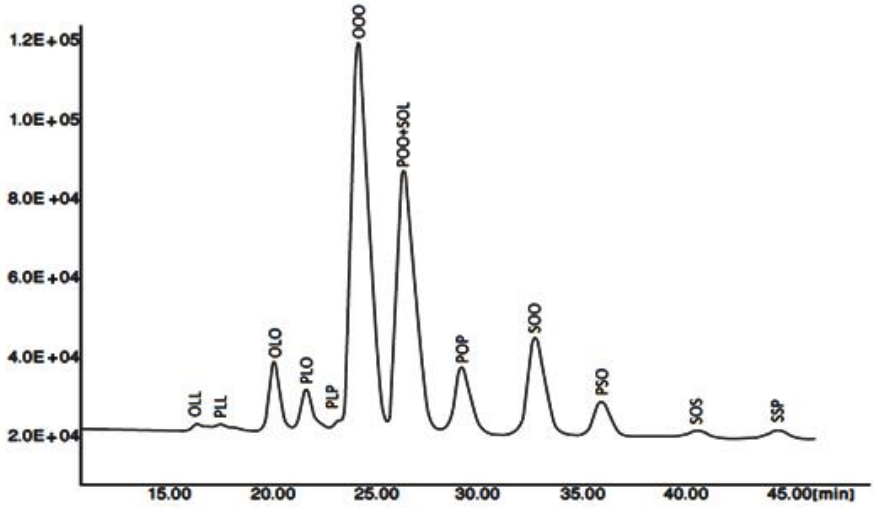
Triacylglycerol (TAG) profiles of *Eksotik* and *Sekaki* papaya seed oils are displayed in Figure 3. Results indicated that the most abundant TAGs in papaya seed oil were triolrin (OOO), dioleoyl palmitin (POO), and stearoyl oleoyl linolein (SOL) (Table 4). OOO is the main TAG in extra virgin olive oil (23-48%), which attributes for high stability and health benefit of the high oleic oil (Piravi-Vanak et al., 2009; Huertas, 2010). Papaya seed oil may

not be edible; therefore a further study is recommended to investigate its toxicity and other safety issues.

Results showed that fruit variety significantly ($p < 0.05$) affected the type and content of the main triacylglycerols in papaya seed oil. *Eksotik* seed oil had significantly ($p < 0.05$) lower OOO (37.0 ± 0.42) than *Sekaki* seed oil (41.3 ± 1.84). Moreover, it contained significantly ($p < 0.05$) lower level of SOO (8.30± 0.14) than *Sekaki* seed oil (9.70 ± 0.42). However, *Eksotik*- and *Sekaki* seed oils did not have significant different contents of SOO + SOL, POS and OOL (Table 4). As mentioned earlier, *Eksotik* seed oil had higher levels of palmitic acid and linoleic acid than *Sekaki* seed oil. TAG analysis also revealed that *Eksotik* seed oil contained significant higher contents of PPO and POL than *Sekaki* papaya seed oil.



a: Eksotik



b: Sekaki

Figure 3. TAG profile of papaya seed oil from two different varieties of papaya. OOL, dioleoyl linolein; POL, palmitoyl oleoyl linolein; OOO, triolein; POO, dioleoyl palmitin; SOL, stearoyl oleoyl linolein; PPO, dipalmitoyl olein; SOO, dioleoyl stearin; POS, palmitoyl oleoyl stearin.

Table 4. TAG profile of PSO from two different varieties of papaya

Papaya varieties	TAG area%						
	OOL	POL	OOO	POO+SOL	PPO	SOO	POS
Sekaki	4.40± 0.14[a]	2.80± 0.14[a]	41.3± 1.84[a]	27.7± 1.84[a]	6.15± 0.21[a]	9.70± 0.42[a]	3.15± 0.10[a]
Eksotik	4.6± 0.14[a]	3.80± 0.10[b]	37.0± 0.42[b]	29.7± 0.42[a]	8.10± 0.14[b]	8.30± 0.14[b]	3.15± 0.10[a]

[a, b] indicated the significant difference at the confidence level of $P < 0.05$. (Mean ± SD, n=2); OOL, dioleoyl linolein; POL, palmitoyl oleoyl linolein; OOO, triolein; POO, dioleoyl palmitin; SOL, stearoyl oleoyl linolein; PPO, dipalmitoyl olein; SOO, dioleoyl stearin; POS, palmitoyl oleoyl stearin

Previous researchers (Puangsri et al., 2005; Lee et al., 2011) investigated TAG profile of papaya seed oil extracted by different methods (i.e. solvent extraction, aqueous enzymatic extraction and screw pressing extraction) from different varieties. As reported by Puangsri et al. (2005), papaya seed oils from different extraction methods (i.e. solvent extraction and enzymatic extraction) did not have significant ($p \geq 0.05$) different TAG composition. Table 5 shows the different TAG compositions of papaya seed oils from different varieties (i.e. *Sekaki*, *Eksotik*, *Batek Batu* and *Tainoung*) (Puangsri et al., 2005; Lee et al., 2011).

This difference might be due to the substantial effect of fruit variety and maturity degree on TAG composition.

Table 5. TAG levels in different varieties of papaya seed oils

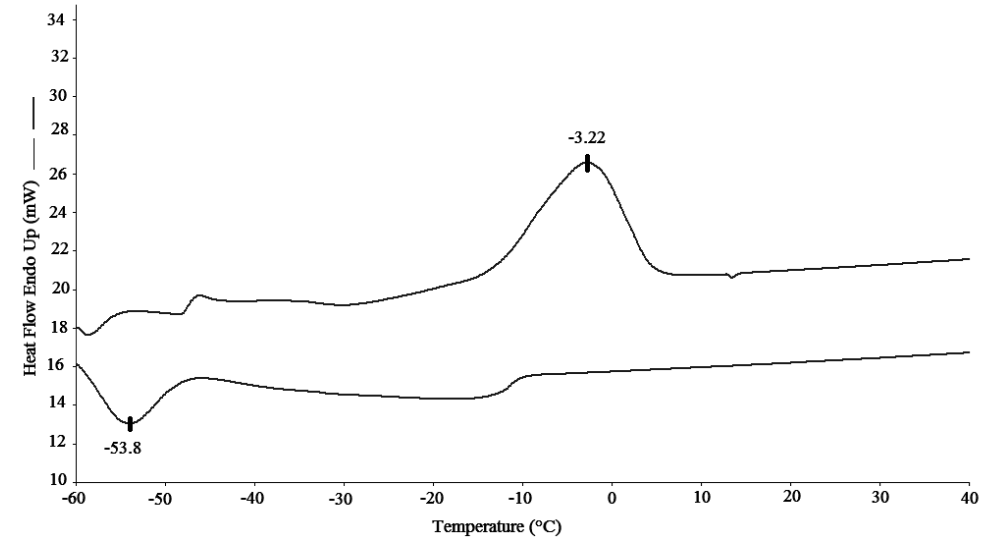
TAG	Papaya varieties			
	Sekaki[1]	*Eksotik*[1]	*Batek Batu*[2]	*Tainoung No. 2*[3]
OOL	4.40	4.60	3.70	2.54
POL	2.80	3.80	2.30	1.72
OOO	41.3	37.0	44.6	43.8
POO + SOL	27.7	29.7	30.5	33.8
PPO	6.15	8.10	5.10	6.19
SOO	9.70	8.30	9.80	8.37
POS	3.15	3.15	3.80	2.41

[1] soxhlet extracted papaya seed oil (present chapter); [2] solvent extracted papaya seed oil (Puangsri et al., 2005); [3] screw pressing extracted papaya seed oil (Lee et al., 2011); OOL, dioleoyl linolein; POL, palmitoyl oleoyl linolein; OOO, triolein; POO, dioleoyl palmitin; SOL, stearoyl oleoyl linolein; PPO, dipalmitoyl olein; SOO, dioleoyl stearin; POS, palmitoyl oleoyl stearin.

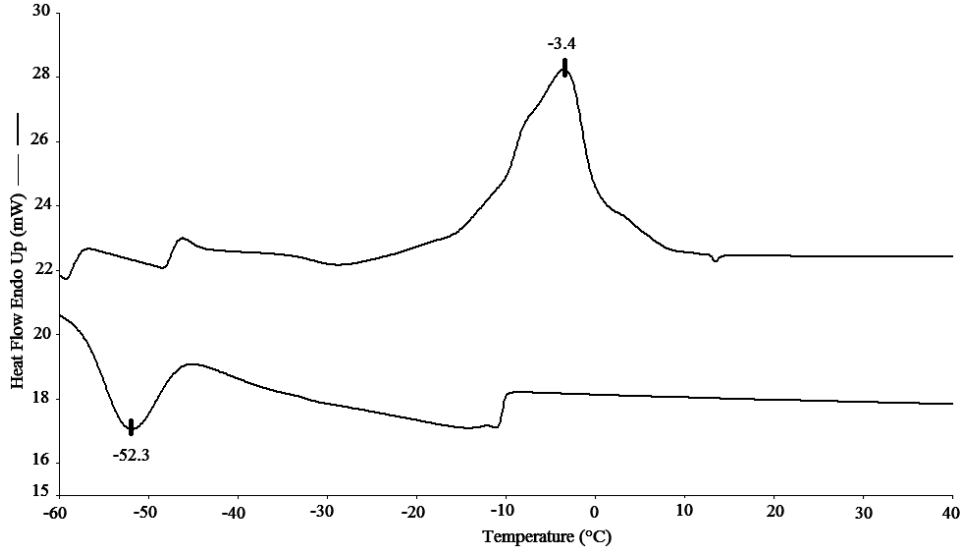
3.4. Thermal Behavior

Figure 4 displays the thermal behavior of *Sekaki* and *Eksotik* papaya seed oils. Figure 5 also demonstrates the peak temperature of melting- and crystallization behaviors. As shown in Figure 5, melting curve is consisted of a single peak during the melting process. The melting of papaya seed oil was started at -17°C (T_{on}) for both *Eksotik* and *Sekaki* seed oils. Most of TAGs were liquid in the peak point (-3.2 to -3.4 °C); while both *Eksotik* and *Sekaki*

seed oils were fully liquid at 7 to 9 °C (T_{off}). There was no significant ($P \geq 0.05$) difference between the melting peak temperatures of papaya seed oils from two different varieties (Figure 5a). However, the melting end point (T_{off}) of *Sekaki* papaya seed oil was significantly ($p < 0.05$) lower (7 ± 0.2 °C) than that of *Eksotik* seed oil (9 ± 0.5°C). This difference could be explained by the fact that *Eksotik* seed oil had higher content of saturated triacylglycerols than *Sekaki* seed oil, thus having higher melting point. Moreover, the presence of impurities in the crude papaya seed oil might also affect its melting and crystallization behaviour (Samaram et al., 2014).



a: Sekaki



b: Eksotik

Figure 4. Thermal behavior of Papaya Seed Oil from two different varieties of papaya; a: *Sekaki*; b: *Eksotik.*

As shown in Figure 4, the crystallization behavior of papaya seed oil has a dual peak (Figure 4). A shallow peak was observed at -10°C by which saturated TAGs should be crystallized. While, the presence of 80% unsaturated fatty acids in papaya seed oil led to accomplish full crystallization at the second deep peak (-52 °C to -54 °C). Results indicated that *Sekaki* seed oil was crystallized at lower temperature (-53.8 ± 0.6 °C) than *Eksotik* seed oil (-52.3 ± 0.4 °C) (Figure 5b). The different crystallization point might be due to the presence of higher content of unsaturated TAGs in *Sekaki* seed oil than *Eksotik* seed oil. Puangsri et al. (2005) investigated the thermal behavior of papaya seed oil from *Batek Batu* variety (Table 6).

The results revealed that both *Sekaki* and *Eksotik* seed oil had significant different melting and crystallization point compared to *Batek Batu* seed oil (Table 6). This confirmed that the thermal behavior of papaya seed oil was significantly influenced by its variety.

3.5. Physicochemical Properties

Table 7 displays physicochemical properties of *Sekaki* and *Eksotik* seed oils. Results showed that *Eksotik* seed oil had significantly lower iodine value (IV) than *Sekaki* papaya seed oil. This indicated that *Eksotik* seed oil had more saturation degree than *Sekaki* seed oil (Table 2). This was comparable with IVs reported by previous researchers for the seed oils from different papaya varieties. Puangsri et al. (2005), Sammarphet (2008), Lee et al. (2011) and Malacrida et al. (2011) reported IVs of 66.0 (g I_2/100 g oil), 64.1 (g I_2/100 g oil), 72.5-74.9 (g I_2/100 g oil) and 79.95 (g I_2/100 g oil) for papaya seed oil from *Batek-Batu*, *Tainoung*, *Kaeg-dum*, *Hawaii* and *Formosa* varieties, respectively. This confirms the considerable effect of fruit variety on the saturation degree of papaya seed oil.

Table 6. Thermal behavior of papaya seed oil from two different papaya varieties

Papaya Varieties	Melting Temperatures (°C)				Crystallization Temperatures (°C)			Source
	T_{on}	Peak Temperature	T_{off}		T_{on}	Peak Temperature	T_{off}	
Batek Batu	- 14.0	- 2.5	10.5		- 34	- 2.5	- 45	Puangsri et al., (2005)
Eksotik	- 17.0	- 3.4	9.00		- 44	- 52.3	- 59	Current chapter
Sekaki	- 17.0	- 3.2	7.00		- 46	- 53.8	- 60	Current chapter

There was no significant difference ($p \geq 0.05$) in saponification value (SV) of *Eksotik* seed oil (193.2 ± 0.02 mg/g) and *Sekaki* seed oil (192.6 ± 0.04 mg/g) (Table 7). Moreover, the unsaponifiable matters of *Eksotik* seed oil was significantly lower than that of *Sekaki* seed oil. This proves the substantial effect of variety on the unsaponifiable matters in papaya seed oil. Both *Eksotik*- and *Sekaki* seed oils had the comparable saponification value to the seed oils from *Batek-Batu* variety (1.39 ± 0.17%) (Puangsri et al., 2005) and *Formosa* variety (1.35 ± 0.14%) (Malacrida et al., 2011).

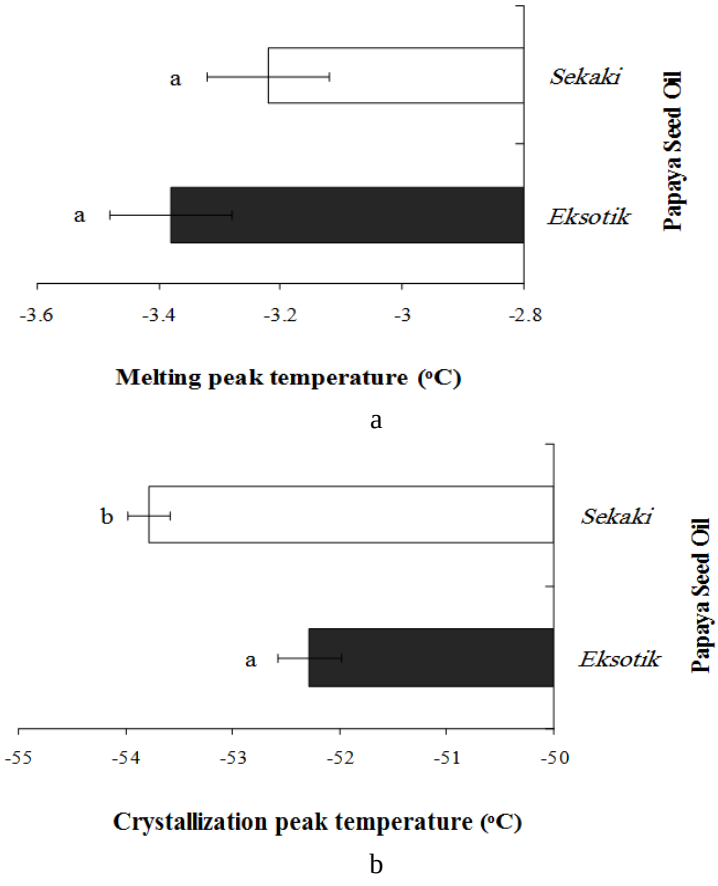


Figure 5. Melting and crystallization peak temperatures of papaya seed oils from two different varieties of papaya (i.e. *Sekaki* and *Eksotik*).

Table 7. Physicochemical properties of papaya seed oil from two varieties of papaya

Parameters		Papaya Seed Oil	
		Eksotik	*Sekaki*
Extraction yield (W %)		30.6 ± 0.23[a]	30.6 ± 0.34[a]
IV (mg I_2/100g)		70.1 ± 0.03[a]	71.18 ± 0.007[b]
SV (mg/g)		193.2 ± 0.02[a]	192.6 ± 0.035[a]
Unsaponifiable matters (%)		1.30 ± 0.06[a]	1.60 ± 0.03[b]
PV(meq/kg)		0.80 ± 0.04[a]	1.26 ± 0.15[b]
AV		1.45 ± 0.07[a]	1.65 ± 0.21[a]
TV		3.04 ± 0.17[a]	4.04 ± 0.08[b]
Color	Red	10.0 ± 0.00[a]	8.0 ± 0.00[b]
	Yellow	72.5 ± 3.50[a]	50.0 ± 0.00[b]
	Blue	3.50 ± 0.70	-

[a, b] indicated the significant difference at the confidence level of ($P < 0.05$), (mean ± SD, n=3 or n=2); IV, iodine value; SV, saponification value, FFA, free fatty acid; PV, peroxide value; AV, p-anisidine value, TV, TOTOX value.

The current research revealed that the seed oils from both varieties were highly stable to oxidation. This stability could be due to the presence of high level of monounsaturated fatty acid in papaya seed oil (Table 2).

On the other hand, this might be also because of benzyl-isothiocyanate and other natural antioxidants (such as squalene and l-(+)-ascorbic acid 2,6 dihexadecanoate) present in the seed oils from *Sekaki* and *Eksotik* varieties (Samaram, 2013). Moreover, *Eksotik* seed oil showed significantly lower PV and TV than *Sekaki* seed oil, showing its higher oxidative stability (Table 7). Samaram (2013) revealed that the antioxidant activity of *Eksotik* papaya seed oil (IC_{50} of 56.8 ± 2 ppm) was considerably higher than *Sekaki* papaya seed oil (IC_{50} of 77.8 ± 4.0 ppm). Bouanga-Kalou et al. (2011) reported a relatively low PV (0.05 ± 0.24 meq/g) for papaya seed oil from an African papaya variety.

The difference between the oxidative stability of the seed oils from Malaysian and African papaya varieties reflects the significant effect of fruit variety on the oxidative stability of papaya seed oil. The color measurement indicated that *Eksotik* seed oil had significant ($p < 0.05$) colour intensity (i.e. red, 10 ± 0; yellow, 72.5 ± 3.5) than *Sekaki* papaya seed oil (Table 7). Color analysis revealed the presence of blue color in *Eksotik* seed oil. This could be due to the presence of chlorophyll pigments in *Eksotik* seed oil.

The current study revealed the significant effect of fruit variety on the color intensity of papaya seed oil.

CONCLUSION

Papaya seed is a potential source of the oil containing high content of monounsaturated fatty acid. The predominant fatty acids in papaya seed oil were oleic acid (18:0), palmitic acid (16:0), stearic acid (C18:0) and linoleic acid (C18:2) respectively. Moreover, triolein (OOO), dioleoyl palmitin (POO), stearoyl oleoyl linolein (SOL) and dioleoyl stearin (SOO) were the main triacylglycerols (TAGs) in papaya seed oil. The present study revealed that fruit variety (*Sekaki* and *Eksotik*) significantly affected the fatty acid composition, triacyglycerol (TAG) profile, thermal behavior, color intensity and oxidative stability of papaya seed oil. Papaya seed oil can be considered as a source of high oleic oil like olive oil; however it is recommended testing the toxicity and safety issues of papaya seed oil.

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PART II – MICROBIOLOGICAL ASPECTS

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Chapter 5

RELEVANT ASPECTS OF PAPAYA MICROBIOLOGY

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ABSTRACT

The recent increase in market demand for healthy foods, associated with the globalization of food trade and the improvement of food chain technologies contributed to an increase in the consumption of fresh fruit around the world. This increase called attention to issues related to fresh fruit quality and safety. Since microorganisms are responsible for the great majority of post-harvest losses and are one of the main hazards found in these products, the knowledge about the aspects and conditions which may influence the presence of undesirable microorganisms is of great value when trying to guarantee fruit quality and safety. Papaya is a much appreciated tropical fruit with exquisite flavor and high levels of nutrients and antioxidant compounds, however it is a highly perishable produce and the post-harvest losses may range from 40% to 100%. Besides, it presents very good conditions for microbial growth (low acid pH, high water activity and nutrient availability) and, if not produced in appropriate conditions, may allow the growth of both spoilage and pathogenic microorganisms. This chapter discusses fresh papaya microbiology, the main microbial contaminants and the consequences of the contamination, including plant-disease, spoilage and pathogenic microorganisms, both in the field and during its processing. The chapter also covers the major sources of microbial contamination and the mechanisms by which microorganisms can internalize fruit tissue. Finally, it presents the influence of papaya processing on microbial populations and the strategies adopted to avoid and/or control microbial growth.

1. OVERVIEW

Fresh fruits are an essential component of a healthy diet and represent an important raw material for food industries. The consumer's demand for natural, fresh and healthy foods

contributed to the increase in sales of fresh fruits observed in the last decades. Besides, advances in food chain technology (agronomic, processing, preservation, distribution, and marketing) have enabled the produce industry to supply many types of high-quality fresh fruits to consumers around the world, leading to an increase in the international trade and per capita consumption. However, some of these same technologies have also led to an increase in the risk of contamination with both spoilage and pathogenic microorganisms. The presence of spoilage and pathogenic bacteria, yeasts and molds on fresh produce has been recognized for many years and, due to some of its intrinsic characteristics (living organs with high water activity and available nutrients), the presence of undesirable microorganisms may cause quality loss, spoilage and damages to human health. Therefore, understanding the conditions that influence the presence of microorganisms in these products, the expected population, as well as the changes that they may cause in these foods are very important to guarantee fresh fruit quality and safety. Nevertheless, it is not an easy task. The microbial ecology of a produce will be influenced by many conditions, such as type, species and cultivars of fruits and vegetables, the environment under which it is cultivated, the stage of physiological maturity, the practices applied during cultivation, harvesting, processing, storage, distribution and consumption, etc. The major populations present in different produce may be investigated under general aspects, but it is important to consider that variations may occur, due to specific characteristics of each product.

This chapter will discuss the pathogenic and spoilage microorganisms that can be found in fresh papaya, as well as their main contamination sources and ways to avoid and/or minimize the microbiological problems resulting from the growth of these microorganisms.

2. PAPAYA MICROBIAL ECOLOGY

2.1. Sources of Contamination

Papaya (*Carica papaya* L.) is a crop of nutritional and economic relevance that is cultivated in tropical and subtropical countries, such as Brazil, Mexico, Peru, Nigeria, Jamaica, Indonesia, China, India, etc. According to the Food and Agriculture Organization, the worldwide approximate production of papaya reached 11.2 million tons in 2010. It is a very appreciated fruit due to its flavor and high nutritive value, and considered a good source of vitamins A, B and C, carotenoids, proteins, carbohydrates (cell wall polysaccharides and soluble sugars) and proteolytic enzymes (papain and chymopapain). Papaya is mainly consumed fresh, but the ripe fruit can also be processed in a great variety of products (jam, juice, puree, pulp, etc.), while the unripe (green) ones can be used as vegetables. Like many other climacteric fruits, it undergoes a variety of physical and chemical changes after harvest. The source of the microorganisms present in the plant's surface may be the seed itself or initial contact with soil, irrigation water and air. The major determinants influencing the microbial communities present in papaya are the genotype of the plant and stimuli from the environment. For instance, bacteria usually colonize leaf areas that retain water and are protected from solar UV radiation. Also, papaya physicochemical properties vary according to type of fruit and maturation degree. In general, fresh ripe fruits present high water activity (<0.99), low acid pH (4.5-6.0), positive Eh and high nutrient content (vitamins, minerals and

sugars), therefore supporting the growth of a great variety of microorganisms, including bacteria, fungi, virus and parasites. Due to the optimal conditions for their growth, the microbial populations that are present and/or predominant in papaya will depend on the sources of contamination, type and conditions of processing, transportation and storage. As many other fruits and vegetables, papaya presents natural barriers for microbial infection. The peel constitutes a physical barrier that hinders fruit infection, and other substances as tannin, enzymes (papain, lysozyme, etc) and phenolic compounds can inhibit microbial growth. Thus, the internal tissues of intact and healthy fruits are expected to present low numbers of microorganisms. However, all plants have natural openings, such as fruit and steam junctions, stomata, lenticels, trichomes, and cracks. Physical and mechanical injuries, such as wounds, clefts, scars, and cracks, may also occur due to insects or incorrect practices of harvest and processing. These openings may allow contamination of internal tissues and, if growth conditions are present, the internal populations may increase to levels capable of provoking spoilage and/or representing a risk for the product consumption.

The initial microbiota of papaya comes from the environment, harvesting procedures and post-harvesting practices. In the field, soil, air, rain and irrigation waters, dust, fertilizers, insects, domestic and wild animals, as well as human manipulation are the main sources of microbiological contamination.

Usually, fresh produce contain populations of 10^4 to 10^6 CFU.g^{-1}, when they arrive at the packinghouse or processing plant. The processes of harvesting, cleaning, sorting, packing, and initial storage of fruits usually have little impact on the initial microbiota. Dry or wet cleaning processes, when performed in agreement with good manufacturing practices, may contribute to remove some of the microorganisms, but it is not enough to eliminate all contamination.

2.2. Mechanisms of Microbial Internalization

Even though the microorganisms present in the environment may contaminate the fruit's surface, the major problems occur when they reach internal tissues, since this part of the plant has more available nutrients and less natural defenses. Microorganisms present in plant tissue may be defined as internalized, i.e. breaking through the surface barrier and settling inside the plant. In this sense, these microorganisms cannot be removed by washing or sanitizers and are protected against environmental stress. Inside the plant, most microorganisms are located in intracellular spaces, while plant viruses and other pathogens are located inside the host cell. In this context, to survive the internalization process, microorganisms must avoid or neutralize the host's defenses. Phytopathogens, which naturally cause damage to plant tissues, have developed ways to cope with host defenses; on the other hand, other microorganisms do not harm living tissue and consequently do not seem to stimulate plant defenses. Furthermore, the absence of tissue damage reduces the likelihood of non-plant pathogens being exposed to pre-formed plant antimicrobial compounds, which could be compartmentalized in the cytoplasm or in specialized cells.

Internalized microorganisms are part of the complex microbial ecosystem of plants, which is composed of epiphytic microorganisms that are able to survive and multiply on the plant's surface, endophytic microorganisms that colonize the interior of plants without

causing significant damage, and phytopathogens, which are endophytic microorganisms that cause injury to tissue, regardless their location in the plant.

Plants are covered by a protective layer (cutin) that is relatively impermeable to water and gas exchange, but since gases are essential for vital metabolic and photosynthetic processes, plants present natural openings on their surface (stomata and lenticels). These same openings, however, may allow the internalization of some microorganisms. There are two mechanisms whereby microorganisms penetrate through the plant's surface, the active and the passive ways. In active internalization, microorganisms penetrate the surface of the plant directly through the cuticle (mechanism adopted by some phytopathogens) or indirectly through the lenticels, stomata, and wounds caused by insects, aerosols, water, or even during normal fruit development. Passive internalization occurs when a contaminated vehicle (objects, water, aerosol, etc.) carries microorganisms into the plant's tissue.

2.3. Influence of the Producing Chain on Papaya Microbial Populations

The harvest can be seen as the first in a series of events that lead to contamination. Harvest causes physiological changes in the plant, associated with the maintenance of homeostasis, injury repair and prevention of infections caused by opportunistic microorganisms. Post-harvest practices are applied to plants in order to delay the quality decay that arises from physiological changes triggered by harvest, but it may also play an important role in produce contamination. The main microbial contaminants of fruits and vegetables are part of the natural microbiota of the soil. These microorganisms may reach the produce through soil particles, airborne spores and irrigation water.

Soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms present on harvesting and handling equipment, packinghouses, storage facilities, and on food contact surfaces throughout the distribution chain. Therefore, early intervention measures (during crop development) and observation of good agricultural practices (GAP) during harvest will provide dramatic reductions in yield loss due to spoilage at all subsequent steps in the farm-to-fork continuum.

Since papaya is a perishable fruit and post-harvest practices may influence its contamination with undesirable microorganisms, studies intending to improve storage, transport, and distribution are essential to minimize the losses.

2.4. Pathogenic and Spoilage Microorganisms in Papaya

The most common microbial populations in papaya are spoilage fungi and bacteria, however, pathogenic microorganisms may also be present. Some pathogens, such as mycotoxigenic fungi, *Listeria monocytogenes*, and spore-forming bacteria (*Bacillus cereus*, *Clostridium botulinum* and *Clostridium perfringens*) are wide spread in nature and may contaminate fruit surfaces in the field. The contamination with enteric pathogens, such as *Salmonella* spp, *Shigella* spp, *Escherichia coli*, *Vibrio cholerae*, hepatitis virus, and parasites, may also occur, but only in case of contact of the fruit with human or animal fecal material, indicating failure in the good agricultural practices (utilization of inappropriate fertilizers,

lack of equipment hygiene, etc.). Table 1 shows the main sources related with pathogenic bacteria contamination of fresh produce.

Due to vegetables low acidity, the great majority of foodborne outbreaks linked to consumption of contaminated produce refers to these products; however, fresh fruits also play an important role in this scenario. Since papaya presents low acidity, it may be a source of pathogenic microorganisms, especially when we consider that, in many cases, fresh papaya is sold for direct consumption after extended storage periods under temperature abuse conditions (for minimally processed fruits) or even at room temperature (for whole fruits). These conditions may favor microbial growth and, if present, pathogen populations may reach sufficient number to cause illness.

Some authors have already demonstrated that pathogenic bacteria such as *Listeria monocytogenes*, C*ampylobacter jejuni*, *Salmonella* spp. and *Shigella* spp. are able to survive and grow in fresh papaya. Two foodborne outbreaks linked to the consumption of contaminated papaya have already been reported in literature, but this numbers must be much higher if we consider the lack of information about foodborne outbreaks in many countries (especially in developing ones, where papaya is most consumed). Gibbs et al. (2009) reported an outbreak caused by consumption of papaya contaminated with *Salmonella* Litchfield in Western Australia, affecting 26 individuals between October 2006 and January 2007. In 2011, the Center for Disease Control and Prevention (CDC, USA) reported a multistate outbreak of human *Salmonella* Agona linked with whole, fresh papayas imported from Mexico, it affected 106 individual from 25 states between January 1st and August 25th, 2011.

The main cause of papaya contamination with pathogens is the failure of good agricultural and manufacture practices, which can occur throughout the whole production chain (growing, processing, storage, and distribution). Figure 1 presents some mechanisms by which fresh produce may become contaminated with pathogenic microorganisms.

Table 1. Sources of pathogenic microorganisms on fresh produce and conditions that influence their survival and growth (adapted from Beuchat, 1996)

Preharvest	Post-harvest
Feces; Soil; Irrigation water; Green or inadequately composed manure; Air (dust); Wild and domestic animals; Human Handling.	Feces; Human handling (workers, consumers); Harvesting equipment; Transport containers (field to packing shed); Wild and domestic animals; Air (dust); Wash and rinse water; Sorting, packing, cutting and further processing equipment; Transport vehicles; Improper storage; Improper packing; Cross-contamination; Improper storage and/or display temperature; Improper handling after wholesale or retail purchase.

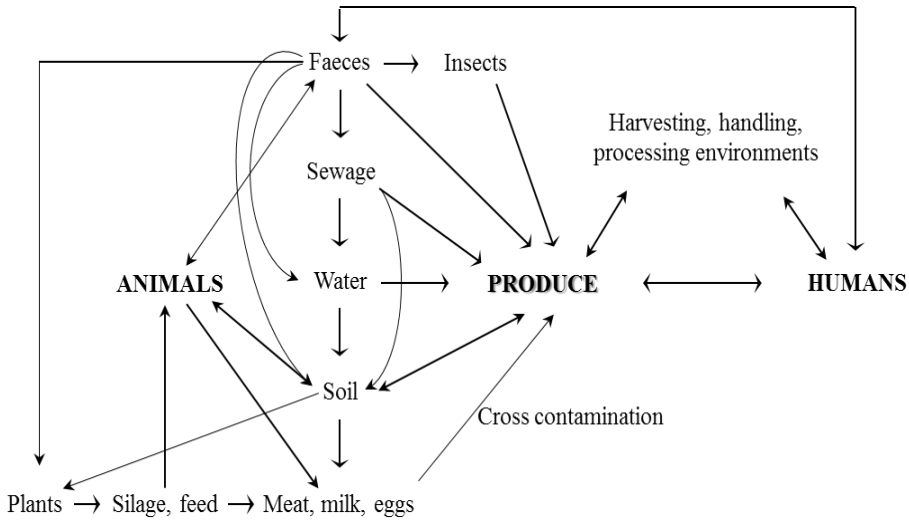

Figure 1. Mechanisms by which fresh produce may become contaminated with pathogenic microorganisms and serve as vehicle of foodborne human disease (Beuchat, 1996).

Use of improper fertilizers, contaminated water and/or soil will result in the presence of pathogenic microorganisms on the surface of the fruits, from where they can access internal tissue and multiply, posing a threat to human health. Therefore, the elimination of pathogens from the surface might contribute to minimize the risk of foodborne outbreaks. Washing intact fresh fruits in water can effectively remove sand, soil, and other debris from the surface, but should not be relied upon to completely remove microorganisms, since only up to 2 log of the populations are reduced this way. The use of antimicrobial compounds such as chlorine and ozone in wash water will further reduce microbial populations, but, depending on the initial microbial load, it may not be sufficient to eliminate all the pathogens. During processing, poor sanitary conditions of workers and inadequate sanitation in the processing environment may increase microbiological safety risks related to fresh papaya. The environment where the fruit is processed must be properly sanitized, since it presents excellent sources of undesirable microorganisms (processing equipment, floors, walls, ceiling, drains etc), including pathogenic bacteria. Besides, accidental damage of the fruit, cross contamination, improper packing, and temperature abuse conditions will certainly contribute to higher microbial loads and are also important points that should be observed when trying to avoid papaya contamination.

As for the spoilage microbiota of papaya, environment is the main source of contamination. Once more, soil, air, waters, insects, and animals may harbor undesirable microorganisms and/or its spores, which may contaminate fruit surfaces by contact. Spoilage bacteria and fungi may also be present in the seeds, infecting even young fruits in the field, or be added through the contact with contaminated surfaces during improper handling, transportation, processing and storage. Papaya is a highly perishable fruit, which presents a short post-harvest shelf life. Its exposure and susceptibility to biotic and abiotic stress contribute to rapid quality decay. Since it is a living organ, metabolism continues after harvest in a natural process of ripening and senescence. Respiration and ethylene production (climacteric fruit) induces the synthesis of secondary metabolites that may lead to the

deterioration of chemical and physiological properties, resulting in weight loss, membrane disruption, pulp softening, color and flavor changes, etc. Besides, chilling injury, mechanical damage, and microbial growth may contribute to and/or accelerate papaya deterioration. It is important to highlight that all these events can contribute to the loss of the fruit's natural barriers to microbial contamination and to the liberation of nutrient juices, enhancing the ability of microorganisms to grow, facilitating internalization and accelerating papaya spoilage, which may lead to its complete disintegration. Fungal and bacterial spoilage of papaya are more common in ripe fruits and are externally visible, but in some cases the deterioration may reach internal tissues. In the case of virus infection, the symptoms may appear in very young plants and, if so, usually fruits cannot develop. The primary mechanisms that cause post-harvest spoilage of produce are linked to the ability of microorganisms to produce amylases and pectinases, breaking the cell wall structures and reaching internal tissues. After the external barriers are disrupted, non-pectinolytic opportunistic microorganisms have access to nutrients inside the plants and may provoke further spoilage like sugar fermentation (mainly by lactic acid bacteria) with acid production and nitrogen compounds degradation (by proteolytic microorganisms) with the release of volatile ammonium compounds.

Table 2. Examples of fungi, bacteria, and viruses that cause papaya diseases and spoilage

Microorganism	Type of disease or spoilage
Fungi	
Alternaria alternata, *Alternaria solani* and other *Alternaria* sp	*Alternaria* black rot: black spots and dark lesions
Asperisporium caricae, *Cercospora papayae* and *Phomopsis caricae-papayae*	Black spot
Colletotrichum gloeosporioides	Anthracnose: dark spots and sunken lesions
Corynespora casiicola, *Cercospora* spp and *Helminthosporium* spp	Brown spot and greasy spot
Monilinia spp	Brown rot: Brown spots with white molds in concentric circles
Phoma caricae-papayae, *Ascochyta caricae* and *Mycosphaerella caricae*	Dry rot
Fusarium solani and *Fusarium* spp	*Fusarium* fruit rot
Guignardia spp	*Guignardia* spot
Phomopsis caricae-papayae, *Lasiodiplodia theobromae*, *Botryodiplodia theobromae*, *Mycosphaerella* spp and *Phytophthora palmivora*	Stem-end rot: begins or affects the tissue where the stem or pedicel attaches to the fruit
Cladosporium spp, *Fusarium* spp and *Penicillium* spp	Internal blight: fungus invades the seed cavity causing it to become dark and dry
Phytophthora spp	*Phytophthora* blight and *Phytophthora* fruit rot

Table 2. (Continued)

Microorganism	Type of disease or spoilage
Sclerotium rolfsii and *Athelia rolfsii*	*Sclerotium* blight
Rhizopus spp	Soft, very wet rot with the development of profuse mycelium and black spore heads appearance
Stemphylium lycopersici	*Stemphylium* rot
Bacteria	
Erwinia herbicola	Purple stain
Erwinia spp	Canker, blak rot
Enterobacter clocae	Internal yellowing disease
Virus	
Papaya ringspot virus (*Potyvirus*)	Fruits from infected trees may have bumps and often present green 'ringspots', which remain green after ripening. A severe infection may induce systemic necrosis and wilting along with mosaic and chlorosis.

Infection of papaya with undesirable microorganisms may be caused by extended wet periods and lack of an integral hygiene control in the field (removal of fallen fruit and leaves), or to mishandling during post-harvest operations, thereby facilitating the entrance of the inoculum and hastening disease symptoms. Some authors comment that papaya losses due to deterioration after harvest may range from 40% to 100%, depending on the production zone, and that 93% of this losses are due to plant infection with microorganisms that cause rots, hinder quality, interfere in cell wall integrity, decrease nutrient value and may facilitate contamination with opportunistic microorganisms. Post-harvest rots can be superficial, peduncular and internal (in this case the fruit can deteriorate completely).

The most common papaya disease is anthracnose, caused by *Colletotrichum gloeosporioides*, a widespread microorganism found in many tropical areas where papaya is cultivated; it may provoke quiescent infections and post-harvest diseases and its presence causes an important shelf life limiting deterioration, characterized by the appearance of dark spots and sunken lesions on the skin. Other important fungi spoilage in papaya is the end-stem rot, a very characteristic deterioration caused, both in the field and during storage, by *Phomopsis caricae-papayae*, *Lasiodiplodia theobromae*, *Botryodiplodia theobromae*, *Mycosphaerella* spp. and *Phytophthota palmivora*. Other microorganisms, as fungi, bacteria, and viruses, have also been reported to cause papaya diseases and spoilage. Table 2 brings some examples of these microorganisms and the diseases they may cause.

3. Strategies for Control of Papaya Microbial Contamination

To meet consumer's demand for more convenient products the market of minimally processed fresh-cut fruits has increased in the last decades, and many types of produce,

including papaya, may be found as a ready-to-eat fresh-cut product. Generally, minimal processing increases the conditions for microbial growth, since it removes some of the natural barriers of the plant, expose internal tissues, and may cause accidental injuries that lead to the release of nutrient exudates. The quality of raw material, ripening stage, and hygiene during handling and processing are of utmost importance to avoid fruit contamination and microbial growth, ensuring the final product's quality and safety. Typical processing involves the following steps: harvesting, receiving, pre-cooling, washing and disinfection, peeling, trimming, deseeding, cutting to specific sizes, sorting for defects, dipping (sprinkle of antimicrobial, anti-browning, and texture-preserving agents), drying, packaging, storage, and distribution. All these steps must be carried out carefully and in accordance with good manufacture practices, otherwise, instead of increasing product quality and convenience, the processing will contribute to quality decay, contamination with spoilage and pathogenic microorganisms, and may lead to loss of the fruits. While whole fruits may be stored for a short period under room temperature, fresh-cut products must be kept under refrigeration (4 ºC), since its internal tissues are completely exposed to the environment. The utilization of modified atmosphere for packing can positively contribute to minimize microbial growth, but it is important to highlight that the quality of raw material and hygiene during handling and processing have a great influence on the microbial load of the products. Fresh-cut fruits should present lower numbers of microorganisms when compared with whole fruits, since the step of disinfection (washing in water added of antimicrobials) is expected to reduce microbial populations. However, slicing, dicing, and shredding procedures and storage under abuse temperatures may introduce undesirable microorganisms to the product and/or facilitate their growth. Microorganisms impact the economic value of fresh-cut papaya by spoiling the fruit and decreasing its shelf life. Furthermore, it can pose a risk to public health, acting as a vehicle of foodborne pathogens. Hence, the effect of processing and storage conditions in fresh-cut produce is a quality and safety concern.

Contamination of fresh produce with pathogenic agents may occur during harvesting, packing, processing, distribution, or marketing. In this sense, all fresh commodities need to be free from disease agents, insects, and synthetic chemicals. Besides, they should also be cleaned from dirt or dust before being sent to the market. Food industries adopt different technologies to control microbial growth: chilling, freezing, water activity reduction, nutrient restriction, acidification, modified atmosphere packaging, fermentation, non-thermal physical treatments, or the use of antimicrobials. For a long time, chemical treatments (mainly application of fungicides) were the main technology used to ensure post-harvest quality. However, consumer's demand for safe, good-quality, and nutrient-rich foods has prompted researches focused on the discovery and evaluation of novel antimicrobial agents to be applied as an alternative to the usual post-harvest technologies. Even though government agencies regulate the use of chemical preservatives in food, interest in the use of chemical-free technologies is rising. Irradiation, modified atmosphere packaging, storage at low temperatures, and use of Generally Recognized as Safe (GRAS) compounds are some of the chemical-free technologies that can be used to keep the post-harvest quality of papaya. Maintenance of the cold chain during papaya post-harvest processing is of utmost importance, since this fruit is susceptible to rapid ripening and disease infection, leading to discoloration and deterioration in a few days. However, some precautions must be observed, since papaya also is very sensitive to chilling injury (CI), what limits storage temperature to 8 ºC or above.

In order to extend shelf life and control diseases it is important to prevent contamination, eradicate infection, or at least to postpone symptoms. Therefore, a number of treatments may be employed for controlling and managing post-harvest diseases of fruits; they are divided into physical, chemical, and biological control.

Chemical Control

The main fungicides used to control post-harvest diseases in papaya belong to the benzimidazole (Thiabendazole and Benomyl), Imidazole (Phrochloraz), ethylene, bisdiothiocarbamate (EBDC), strobulins (Azoxystrobin), and the benzonitrile (Chlorothlonil) compound groups. The effectiveness of these fungicides is influenced by the applied dose, fruit-ripening stage, sensitivity response of the target fungi, and application time, among others factors. However, the use of synthetic fungicides has become a controversial issue due to global concern for their carcinogenic potential and the hazard they present to the environment. Thereby, non-chemical alternatives or the combination of non-chemical treatments with fungicides at low concentrations could be an efficient strategy to control post-harvest diseases and pathogenic bacteria in fruits.

In this sense, gaseous chemical disinfectants are extremely advantageous because these compounds can spread through spaces and penetrate into places that would be inaccessible to liquid or solid pesticides. Besides, they continue to act and spread through tissues even after the exposure period, leaving little or no residue.

The main gaseous chemical disinfectants used for fresh fruits include chlorine dioxide gas (ClO_2), ozone, hydrogen peroxide vapor, acetic acid vapor, and allyl isothiocyanate. The use of some of them has already been reported for papaya, and may be a potential substitute for commercial fungicides in the control of pathogen-related rots.

Chlorine dioxide (ClO_2)

Chlorine dioxide (ClO_2) is a neutral compound of chlorine in the +IV oxidation state. It has received attention as a disinfectant for fruits and vegetables, largely because pH and organic matter do not strongly impact its efficacy, and it does not react with ammonia to form chloramines, as do liquid chlorine and hypochlorites. This chemical compound is a strong oxidizing agent; since the metabolism of microorganisms and, consequently, their ability to survive and/or grow, are influenced by the oxidation-reduction potential of the medium in which they live, ClO_2 has a high biocidal effectiveness. Its efficiency for fruit decontamination depends on gas concentration, exposure time, relative humidity, temperature, surface integrity of plants, and microbial inoculation sites.

The mechanisms for microbial inactivation are not completely understood. While some authors suggested that the primary inactivation mechanism is the disruption of protein synthesis (Bernard et al., 1967), others reported an increase of cell membrane permeability (Aieta and Berg, 1986; Ghandbari et al., 1983). On the other hand, Berg et al., (1986) studied ClO_2 mode of action on *E. coli* and suggested that the microbial death was due to the loss in the control of potassium efflux from the bacterial cells. Consequently, the effectiveness of ClO_2 in killing specific pathogenic and spoilage microorganisms on specific types of fruits deserves further research. It is important to notice that chlorine and fungicides have limited ability to destroy microorganisms on the surface of fruit, as bacteria and fungi have become more resistant. Furthermore, environmental organizations have shown concern related to the arising of residual by-products, such as trihalomethanes and other persistent chemical wastes.

Therefore, ozone has been suggested as an alternative to replace chlorine compounds and fungicides.

Ozone

The interest in the utilization of ozone as preservation technology is based on its high biocidal efficacy, wide antimicrobial spectrum, safety, biodegradability, and non-toxicity. Within the food industry, ozone-based gaseous treatment has been used routinely for washing and storage of fruits and vegetables. In 2011, U.S. Food and Drug Administration (FDA) declared ozone to be a GRAS substance for commercial use as a disinfectant and sanitizer in food handling. The lethal effect of ozone is a consequence of its strong oxidizing power on polyunsaturated fatty acids, membrane-bound enzymes, glycolipids, and glycoproteins, leading to a decrease in cell permeability and disruption of the normal cellular activity. Other mechanisms of bacterial inactivation reported in the literature are related to inactivation of cellular enzymes and destruction of genetic material, making ozone effective in controlling bacteria, mould, protozoa, and viruses. Ozone low concentrations (< 100 ppm) associated with long exposure time (more than one day) have been used for growth inhibition and inactivation of spoilage microorganisms in fresh fruits (Linton et al., 2006).

As mentioned earlier in this chapter, anthracnose is considered one of the major problems of post-harvest decay in papaya, and some studies have reported the use of ozone in the control of this disease. Ong et al. (2013) reported that ozone exposure at the concentration of 1.6 and, to a lesser extent, 4 ppm for 96 h were efficient to control antrachnose in papaya, since it inhibited mycelia growth and spore germination effectively and delayed the decay onset of artificially inoculated fruits. Figueira et al. (2011) reported a quicker ripening of papayas treated with a continuous flow of ozone (6 ppm) for 24 h, in comparison to untreated samples, and attributed this fact to the degradation of clorophylls and wax layers, which contribute to undesirable physiological changes. Ozone has also been used to prevent other papaya diseases; Bataller et al. (2012) showed that gaseous and aqueous ozone was effective in the papaya post-harvest control of pathogenic fungi, such as *Fusarium*, *Aspergillus*, *Sclerotium rolfsii*, *Curvularia*, *Phoma*, *Gliocladium,* e *Rhizoctonia*.

This sanitizer is not only used to control post-harvest disease, but also to extend the shelf life of minimally processed fruits. Yeoh et al. (2014) verified that, when fresh-cut papaya was subjected to 30 min of ozone (9.2 µL/L) treatment, the total mesophilic bacteria were significantly reduced by 0.33 $\log_{10}$ CFU g^{-1} and the coliform populations were decreased significantly by 1.12 $\log_{10}$ CFU $^{g-1}$. It is important to notice that ozone antimicrobial properties depend on the nature and composition of fruit surfaces and the type and load of microbial contaminants that can affect bacteria attachment and, consequently, the overall performance of the treatment.

Hydrogen peroxide vapor

Hydrogen peroxide (H_2O_2) is an extremely efficient sanitizer, due to its sporicidal properties against *Bacillus* spp. spores and other spoilage bacteria, especially at elevated temperatures and concentrations. According to Wang and Toledo (1986) vapor-phase hydrogen peroxide (VPHP) has been shown to be effective in inactivating spores of *Bacillus subtilis* var. *Niger*, reaching a 99.9999% destruction in <10 min, when exposed to 1.131 mg VPHP/liter at 40 ºC.

Acetic acid vapor

Acetic acid is an acidulant and preservative widely used in the food industry to control microbial growth. It is considered a GRAS compound and is comparable to hydrogen peroxide, bicarbonate, carbonate, and other GRAS compounds. Its mode of action is attributed to direct pH reduction, leading to the depression of the internal pH of microbial cells by ionization of the undissociated acid molecule, or disruption of substrate transport by alteration of cell membrane permeability.

Allyl isothiocyanate gas

It is well known that spices and essential oils contain phenolic compounds that have antimicrobial activity, but as they are volatile, the majority of these compounds are effective only in high concentrations, which adversely affects the sensory quality of food. On the other hand, some studies demonstrated that allyl isothiocyanate (AIT), a volatile and aliphatic sulfur-containing compound, inhibits a variety of pathogens, even when used in low concentrations.

Allyl isothiaocyanate is the main component in the essential oils found in stems, leaves, and roots of cruciferous plants such as horseradish, black and brown mustard, cabbage, brussels sprouts, broccoli, cauliflower, kohlrabi, kale, turnip, rutabaga, watercress, wasabi, radish, and papaya seeds. Since it is a natural compound, its use as a food preservative is allowed in Japan, and as a GRAS flavoring agent in the US. AIT is a specific compound from the isothiocyanate group that presents bactericide, bacteriostatic, and fungicide activity and is also harmless to the environment.

Like several other antimicrobial compounds, its mode of action is not completely understood, but it is known that AIT provokes nonspecific inactivation of enzymes through cleavage of proteins disulphide bonds, and interference in specific enzymes in the electron transport chain (cytochrome C oxidase).

Studies with papaya demonstrated that AIT was very efficient to control *C. gloesporioides*, completely inhibiting its growth in this fruit (Ramos-García et al., 2010). In further experiments, papaya treated with AIT (0.5 µL mL^{-1}) showed lower percentage of infection (33%) than the control samples (50%), which were water-treated (Ramos-García et al., 2012).

Physical control

Heat treatments

Post-harvest heat treatments are the main physical control technologies used in fresh produce preservation. Hot water dips, vapor heat, hot dry air, and short hot water rinse and brush are the main heat treatments used to control decay and pest infections during fruits storage and commercialization. These technologies are largely applied in papaya preservation, and its efficiency depends on some parameters, such as the condition of the fruit prior to treatment, temperature, duration of treatment, and mode of heat application. The main advantage of hot treatments over chemical sanitizers is the inactivation of bacteria and spores even below the fruit surface, delaying conidia germination, growth, and sporulation.

Initially, hot water treatment was used to control fungal disease in citrus fruit, but its use has been extended to manage insect infestation and alleviate physiological disorders (as

chilling injury) in many fruits, thereby maintaining fruit quality and prolonging storage. Since the 1960s hot water treatment has been used to control stem-end rot and anthracnose, as their causing agents can infect young papaya and remain latent during fruit growth in the field. The presence of latent phytopathogens make it difficult to prevent these diseases, and it may significantly reduce quality and commercial value of the fruit.

Chávez-Sánchez et al. (2013) reported that hot water treatment (55 ºC/3 min) delayed and reduced papaya decay without affecting softening and quality of fruit. Li et al. (2013) commented that the treatment with 54 ºC for 4 min could maintain the quality of papaya and induce resistance to post-harvest disease.

Some studies have demonstrated that this technology combined with fungicide successfully reduces post-harvest disease. Pérez-Carrilo and Yahia (2004) concluded that post-harvest dry (50% RH) hot air at 48.5 ºC for 4 h, alone or in combination with TBZ, was effective in decreasing chilling injury and fungi development without causing negative effects on fruit quality. Others reports have demonstrated that forced hot air treatments (47.5 and 48.5 ºC) associated with the fungicide prochloraz not only controlled stem-end, but also body rots (Lay-Yee et al., 1998).

The mechanism by which hot water treatment controls post-harvest decay could be the induction of fruit defense responses, preventing pathogens from spreading throughout the tissues. Also, this technology melts the wax arrangement on the surface of papaya, providing a mechanical barrier against wound pathogens; cuticular fractures, microwounds, and most stomata are partially or completely filled with molten wax, and early-germinated spores may be encapsulated and inactivated as well.

However, according to Rawson et al. (2011), severe heat processing may induce several chemical and physical changes that impair the organoleptic properties of fruit and reduce the content or bioavailability of some bioactive compounds.

In this context, non-thermal preservation techniques such as ozone, high hydrostatic pressure, irradiation, and biocontrol became an interesting alternative for their ability to destroy all the microorganisms while causing minimal changes to foods.

High Hydrostatic Pressure

High hydrostatic pressure (HHP) is a non-thermal food processing technology that practically does not change the treated fruit, maintaining the nutritional and sensory quality while rendering it free of viable microorganisms, extending the shelf life of the product, and improving its safety.

This technology subjects food to pressures between 100 and 800 Mpa, with exposure times ranging from a millisecond pulse to over 20 minutes, although commercial treatment usually lasts less than seven minutes. The temperature during processing may vary (below 0 ºC or above 100 ºC), but current commercial HPP uses room temperature.

Since the covalent bonds of low molecular weight compounds such as flavoring agents, pigments, and some vitamins are not affected by pressure, only minimum chemical changes occur in HPP-treated fruit. Quality retention and inactivation of yeasts, molds, and most vegetative bacteria (pathogenic and spoilage) depend on time, temperature, and pressure treatments.

Pressure interferes with cell structure and cell division, affecting the arrangement of the cell wall, membrane fluidity, and several intracellular organelles. The cytoplasmic membrane has been pointed out as the key target of HHP cellular inactivation.

Palhano et al. (2004) verified that HPP was very efficient to reduce post-harvest losses in papaya, successfully inhibiting *C. gloeosporioides* spores when a pressure treatment of 350 Mpa for 30 min was applied.

Irradiation

Ionizing irradiation (^{60}Co or ^{137}Cs) is another method for preserving many types of foods. It is a well-established technology used to extend shelf life and, being a cold process, it reduces the decay caused by indigenous microflora and post-handling contaminants, as well as the presence of foodborne pathogens, without adversely affecting the nutritional and sensory qualities of the product. Nowadays irradiation is widely used to inhibit tuber sprouting, delay fruit and vegetable ripening, and control insects in fruits. Its efficacy is based on the high penetration power that enables elimination of high microbial loads even inside tissues. For this reason, ionizing radiation has been widely used on fresh produce to guarantee bacterial reduction and longer shelf life.

However, this method for controlling post-harvest disease is still under experimentation and is a little controversial. While Pimentel and Walder (2004) observed no effect of irradiation (0.75 kGy) in papaya regarding disease reduction, weight loss, occurrence of diseases, chroma of flesh color, pH, and total soluble solids contents, Cia et al. (2007) reported that 0.75 and 1 kGy doses reduced anthracnose incidence and severity, but did not reduce them when the fruit were inoculated after irradiation.

Modified Atmosphere

In order to preserve quality and reduce post-harvest losses of tropical fruits during transport and storage, a modified or controlled atmosphere may be used, generally consisting of less O_2 and more CO_2 than air (air can be approximated as < 0.1% CO_2, 21% O_2, 78% N_2).

Metabolic processes such as respiration and ripening are sensitive to temperature, and according to biological reactions generally increase two- to three-fold for every 10 ºC rise in temperature. Therefore, temperature control is vitally important for a Modified Atmosphere Packaging (MAP) system to work effectively.

Carbon dioxide at concentrations ranging from 15-20% is an effective fungistatic, and atmospheres containing <1% O_2 and/or > 60% CO_2 have been reported to be lethal to several insects. However, according to Kader (1986) off-flavors can develop in any fresh fruit or vegetable if it is exposed to O_2 and/or CO_2 levels that result in anaerobic respiration and formation of ethanol and acetaldehyde. Low concentrations of O_2 may induce physiological disorders such as impaired ripening of climacteric fruits, internal browning, external brown discoloration, and surface pitting, hence, controlled atmosphere condition of 2-5% of O_2 and 5-8% of CO_2 is recommended to extend the storage life of papaya. Yahia et al. (1992) mention that papaya can only tolerate insecticidal atmospheres (0.17% to 0.35% O_2) for <3 days at 20 ºC, without the risk of significant fruit injury, since longer exposure can induce off-flavors and fruit decay. Maharaj and Sankat (1990) reported effective control of post-harvest rots and significantly less weight loss for the cv. 'Tainung 1', after CA storage for 35 days at 16 ºC. Rohani and Zaipun (2007) observed a 4-week increase in papaya storage life when stored at 10-12 °C under modified atmosphere condition (4-5% CO_2 and 2-3% O_2). The post-harvest quality of papaya was enhanced significantly by combining methyl jasmonate (MJ) treatments and MAP (Gonzalez-Aguilar et al., 2003). Use of MJ at 10^{-5} M with MAP is beneficial to maintaining post-harvest quality of papaya during low-temperature storage and

shelf-life period. Cenci et al. (1997) reported that at 10 °C papaya could be stored for 36 days in 8% CO_2 and 3% O_2 followed by another 5 days at 25°C to improve retail market conditions. On the other hand, Fonseca et al. (2004) showed that controlled atmosphere containg 3% of O_2 and 6% of CO_2 generated more losses due to the increase of post-harvest diseases: anthracnose, chocolate spot, stem-end rot, and, mainly, black spot; this probably occurred because the increased CO_2 concentration promoted injury in superficial cells, infection, and tissue colonization.

Biocontrol

We have already seen that chemical compounds, thermal, and nonthermal treatments can help to reduce pathogens load and decay losses, and that implementation of GAPs and maintaining the commodity at storage conditions that optimize host resistance will also aid in suppressing disease development after harvest. But, among all the alternative methods that have been pursued for the control of various post-harvest diseases, biological control is the one that deserved the most attention.

Microbial antagonists can be used for controlling post-harvest diseases in fruits and vegetables in two different ways: (1) use of microorganisms that already exist on the produce itself, promoting and/or managing their growth, or (2) artificially introducing microorganisms that act against post-harvest pathogens.

For an antagonistic microorganism to be considered an effective control agent, it must protect the fruit against new incoming inoculums, as well as already established ones. Thus, an antagonistic microorganism must operate on different control mechanisms, which may include mycoparasitism, antibiosis, competition for space and nutrients, and ability to induce resistance in their hosts.

Several biocontrol agents such as bacteria and yeast have been tested on various post-harvest papaya fungi. Gamagae et al. (2003, 2004) reported that use of 2% sodium bicarbonate incorporated wax coating with *Candida oleophila* is a commercially viable alternative to disease control in papaya during storage. Sanchez et al. (2005) reported that *Bacillus* spp. could control *Penicillium* infection on papaya during in-vivo studies and decrease blue mold rotting of fruit.

Tasiwal et al. (2009) revealed that *Trichoderma* spp., specifically *T. virens*, significantly reduced the growth of *C. gloesporioides* in papaya. Shi et al. (2011) reported that endophyte *Pseudomonas putida* MGY2 was efficient on reducing anthracnose caused by *C. gloeosporioides* infection in harvested papaya, by decreasing disease index, disease incidence, and lesion diameter in treated fruit when comparing to control ones. Capdeville et al. (2007) isolated, selected, and tested the ability of epiphytic microorganisms, isolated from fruit and leaf surfaces, in controlling antrachnose, and concluded that among all the isolates, the yeast *Cryptococcus magnus* CEN63 was the most effective for the development of a commercial product.

Burkholderia cepacia strain B23 supplemented with 0.75% chitosan and 3% calcium chloride was effective to control anthracnose and extend the storage life of papaya due to its strong antagonistic activity towards *Colletotrichum gloeosporioides*. Furthermore, this combination also delayed climacteric ethylene evolution, reduced respiration rate, retained fruit firmness, and decreased weight loss during storage at 14 °C and 95 % RH for 28 days (Rahman et al., 2009).

Sharma and Srivastava (2013) showed the effectiveness of *Debaryomyces hanseni* Zopf isolated from the fructoplane of apples as biocontrol agent against soft rot of papaya caused by *Ulocladium. chartarum* (Pr.) Simm.

FINAL REMARKS

Even though this chapter is focused on the microbial ecology of fresh papaya, it is important to highlight that this fruit can be processed in a great variety of ways, to allow its utilization as ingredient of other food preparations (e.g. juices, jams, purees, fruit compotes, yogurts and other dairy products, etc), as well as to increase its shelf life and/or to improve its microbiological safety. It is very well known that the type of processing exerts a very important role in the microorganisms present in any kind of food. As for papaya, some treatments such as pasteurization, high pressure, irradiation, freezing, drying, and canning can be applied to whole, minimally processed, and pulped fruit and generally contribute to reduce microbial load. However, the effect of those treatments on the sensory characteristics and nutrient content of papaya should be taken into account when choosing the best process to be used. Changes in the integrity of tissues, decrease in vitamin content, and appearance of off-flavors have already been reported as consequences of freezing and heating treatments of papaya. These changes can depreciate the product's quality and lead to consumer rejection. Besides, in spite of the reduction in microbial populations achieved with those treatments, the quality of the raw material is of utmost importance for the quality of the final product, since any changes provoked by the growth of spoilage microorganisms that might have occurred before the treatments will not be reversed.

At last, but not least, it is important to highlight that, as any other produce, papaya is a living organ, which undergoes complex metabolic reactions from the moment of harvesting until its consumption, and all these processes affect the microbial populations present on the fruit, as do the steps of processing chain. Thus, the microbial ecology of papaya will depend on many factors, such as fruit physiology, phytopathology, and microbiology, sources of contamination, and practices applied from farm-to-fork. Besides, in the context of a globalized world, with international trade rising every year, it is essential to guarantee food safety and quality, a fact that will only be achieved with the application of Good Agricultural and Manufacturing Practices, as well as efficient microbial control technologies.

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Chapter 6

BENEFICIAL EFFECTS OF MICROORGANISMS ISOLATED FROM PAPAYA

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ABSTRACT

This Chapter will describe the current knowledge regarding the beneficial effects of microorganisms that have been isolated from papaya which is the fruit of *Carica papaya*. There have been some studies where specific strains of lactic acid bacteria (LAB), which represent a heterogeneous group of microorganisms that are naturally present in many foods and possess a wide range of therapeutic properties, have been identified on papaya and that some of these possess the ability to produce beneficial compounds such as bacteriocins amongst others. Some LAB isolated from papaya have even been proposed to be potentially probiotic, meaning that they not only can survive in the harsh conditions of the gastrointestinal tract, but also can provide health benefits to the consumers. Also a review of the microbiota involved in natural papaya fermentation will be discussed. This is important because it has been reported that fermented papaya can have beneficial effects against certain types of diabetes and possess antioxidant properties. Thus microorganisms present on papaya could be used in the food industry to develop new products with beneficial effects on consumers.

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INTRODUCTION

Papaya, the fruit of *Carica papaya* is native to Mexico, Central America and northern South America. It has been said that the habitants of the ancient Mayan civilization honored the papaya tree as their sacred "Tree of Life" because the fruit (especially the ripe or fermented fruit) was used in various folk medicines. Green (or unripe) papaya has many described benefits as do their seeds such as their ability to kill parasites, lower blood pressure, reduce inflammation and pain, possess aphrodisiac and spermicidal properties [1].

Although there is an increase in scientific evidence that now supports most of these popular beliefs, these will not be the focus of this Chapter.

In this Chapter, the focus will be placed on the scientific evidences that have demonstrated some of the many proposed beneficial effects of fermented papayas such as its increased antioxidant, anti-inflammatory, modulation of certain immune parameters that could have beneficial effects in the treatment of chronic disease such as diabetes, cancer, cardiovascular and neurological disorders. Besides describing some of these claims, the microorganisms that are present during spontaneous fermented papaya preparations or used to prepare these products will be discussed as will the use of specific beneficial strains that have been isolated from this fruit. This information is essential to understand the mechanisms by which microorganisms can be used to produce some of the beneficial effects of fermented papaya, either due to their intrinsic (probiotic) properties or to their ability to produce biologically active compounds that make the products very interesting functional foods.

Definitions

In April 2014, if one performed a MEDLINE search using the keyword "fermented papaya" the total hits would be just 42 articles. From these, almost 50% (20) were published since 2010 showing that although relatively few studies have been performed on this beneficial product, there is a growing interest in the scientific community to understand the mechanisms of action of their traditional medicinal claims. As stated earlier, the objective of this Chapter is to give an overview of what is known about the microbial composition (microbiota) of fermented papaya and how these microorganisms could be the causing agents of most of the health promoting properties of this functional food.

When speaking about beneficial microorganisms, there is one term that is commonly used to describe them, probiotics. The most commonly accepted definition of probiotics was published by the World Health Organization/Food and Agricultural Organization in 2001 that states that probiotics are "*live microorganisms which when administered in adequate amounts confer a health benefit to the host*" [2]. However, according to the International Scientific Association for Probiotics and Prebiotics (ISAPP), a non-profit scientific organization dedicated to advancing the science of probiotics and prebiotics, the term probiotic is commonly misused both commercially, when the term is featured on products with no substantiation of human health benefits, and scientifically, when the term has been used to

describe bacterial components, dead bacteria or bacteria with uncharacterized health effects in humans [3]. The ISAPP does not provide a new definition for probiotics, it simply points out the important elements that are contained in the FAO/WHO definition. This being said, they clarify that a probiotic must: i) be alive when administered; ii) have undergone controlled evaluation to document health benefits in the target host; iii) be a taxonomically defined microbe or combination of microbes (genus, species and strain level); and iv) be safe for its intended use.

Furthermore, beneficial foods are often referred to as being functional foods which are foods that can be incorporated as a part of a normal diet and that provides the consumer with a beneficial effect often related to health-promotion or disease prevention, in addition to the intrinsic nutritional value. Functional foods were defined by the Institute of Medicine of the US National Academy of Sciences as "*foods that encompass potentially healthful products, including any modified foods or food ingredients that may provide a health benefit beyond the nutrients it contains*" [4]. A more specific definition has been adopted by the Functional Foods Center (Dallas, TX, US) that states that a functional food is a "*natural or processed food that contains known biologically-active compounds which when in defined quantitative and qualitative amounts provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age*". In this last definition, it is clear that the foods must contain beneficial biologically-active compounds, which for the scope of this Chapter will include nutraceuticals and/or probiotics. The term "nutraceutical" was coined from joining the words "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice, MD, founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford, NJ [5]. According to DeFelice, a nutraceutical can be defined as "*a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease*". However, the term nutraceutical as commonly used in marketing has no regulatory definition [5]. When a functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) (except anemia), it is because it contains or is considered as being a nutraceutical [6]. In this Chapter, the presence of probiotic microorganisms and the capacity of fermentative microorganisms to produce or release beneficial compounds (nutraceuticals) will be discussed in order to demonstrate that fermented papaya should be considered a functional food.

BENEFICIAL EFFECTS OF FERMENTED PAPAYA

Traditionally, in order to make fermented papaya preparations, fresh papaya leaves and fruit are aged slowly over a few months, during which autochthonous fermentation occurs. The fermented substance is then dried and ground into a fine powder, which can be consumed by itself or added to food and provide beneficial health effects as shown on Japanese consumers [7]. In the Philippines, fermented papaya has been produced commercially since 1969 [8]. In addition to the high nutrient content of the fruit, fermentation of papaya is able to increase the levels of beneficial compounds or improve their flavor (through the production of volatile compounds). In vitro bacterial fermentation of fruits such as papayas in conditions that stimulate the gastrointestinal tract has shown that digestible carbohydrates can be removed preventing the production of gas, and short chain fatty acids (SCFA) concentrations

can be increased [9]. The SCFA produced during fermentation can significantly improve colonic health of consumers by reducing the risks of developing gastrointestinal disorders, cancer and cardiovascular disease [10]. In another study, it was shown that the fermentation of papaya juice by a mixed culture of *Saccharomyces cerevisiae* var. *bayanus* R2 and *Williopsis saturnus* NCYC2251 produced a range of volatile compounds including fatty acids, alcohols, aldehydes and esters [11]. These authors concluded that these selected yeasts were able to produce more complex aroma compounds than those found in unfermented papaya juice and even compared to the fermentation with a unique strain, showing that the addition of complementary cultures is an interesting alternative to improve the technological and organoleptic properties of fermented papaya. This same group recently published a report where they showed that the yeast ratio is a critical factor for the adequate fermentation of papaya juice and that modifying the ratio of the fermenting strains can modulate the flavor of the final product [12]. These results show the importance of the relative quantities of starter cultures for the development of fermented foods with acceptable aromas, and also implies that other compounds, which can be potentially beneficial to the consumers could also be modulated by the adequate selection of starter cultures.

In 2012, a review of the past research of fermented papaya preparation was published [13]. The authors stated that functional foods represent an emerging opportunity and that nutritionists are responsible for increasing the attention on the development of new innovative solutions that act on improving the overall health conditions through diet. In this review, the authors cite a report published in Nature were the Japanese were already exploring in 1993 the limits between food and medicine [14]. They emphasize the importance of the food industry to develop new effective products which not only meet consumer requests, but also have been validated by robust scientific research that are recognized by non-profit non-governmental international associations. The use of fermented papaya preparations as a novel functional food is thus promising due to the emergence of reports by groups from around the world that are evaluating the effects that the indigenous microbiota could play directly on human health or by enhancing or producing health promoting substances.

Antioxidant Properties

Some of the first scientific studies on the beneficial effects of fermented papaya preparations were performed to demonstrate that these products are high in antioxidants which help to prevent damage done to cells by free radicals in a variety of situations.

Antioxidants donate an extra electron to the free radical, which helps stop the domino effect and prevent further free radical damage. It has been demonstrated that free radicals contribute to the aging process and the development of many different diseases and it has been proposed that eating a diet high in antioxidants, including fermented green papaya, can help to prevent or slow down free radical damage. One of the first published reports on papaya fermentations showed that this product has a very important free radical scavenging activity that can affect lipid peroxide levels and superoxide dismutase activity using a rat model [15]. In their article, the researchers state that free radicals have been related to aging and diseases such as cancer, diabetes and especially in neurological disorders such as Parkinson's or Alzheimer's diseases and that diets rich in antioxidant foods (including fermented papaya preparation) could help prevent these health problems.

The free radical scavenging activity of the fermented papaya preparation, which was prepared by yeast fermentation of *Carica Papaya* Linn, was examined using an electron spin resonance spectrometer. The fermented papaya preparation scavenged 80% of hydroxyl radicals (˙OH) generated by Fenton reagents. These promising *in vitro* results prompted the researchers to further their studies to evaluate the antioxidant effect using a rodent model. They showed that the oral administration of the fermented papaya preparation for 4 weeks decreased the elevated of lipid peroxide levels in the ipsilateral 30 min after injection of iron solution into the left cortex of rats [15]. The fermented papaya preparation also increased superoxide dismutase activity, an important antioxidant enzyme, in the cortex and hippocampus of these rodents. They concluded that their results suggest that the fermented papaya preparation has antioxidant actions and that it may be prophylactic food against the age related and neurological diseases associated with free radicals.

Following these promising results, others studied the antioxidant properties on other biological mechanisms such as cancer prevention. In one such study, the antioxidant effect of fermented papaya preparation on iron-mediated damage to DNA and proteins was evaluated since it is known that the carcinogen ferric nitrilotriacetate (Fe-NTA) catalyzes hydrogen peroxide-derived production of free radicals and possibly acts through a mechanism involving oxidative stress. In this study, fermented papaya protected supercoiled plasmid DNA against Fe-NTA and H_2O_2 that both induce single and double strand breaks [16]. The protective effects of this product were also demonstrated when human T-lymphocytes were challenged with Fe-NTA/H2O2 and DNA damage was determined using the Comet assay. In this same study Fe-NTA/H2O2 was used to induce fragmentation of bovine serum albumin *in vitro* which also depleted cellular glutathione levels in lymphocytes, both effects were dose-dependently counteracted by the fermented papaya preparation [16].

More detailed studies demonstrated that antioxidant properties of the fermented papaya product were related to both hydroxyl scavenging as well as iron chelating properties [16].

In a more recent article, it was demonstrated that the antioxidant effect of fermented papaya involves iron chelation [17]. These authors stated that iron-overload is a major clinical problem in various diseases since serum iron surpasses the binding capacity of transferrin and is present as non-transferrin bound iron and cellular unbound labile iron pool (LIP) is increased. The LIP in turn is involved in the generation of free radicals, including reactive oxygen species (ROS) that can results in oxidative stress and toxicity to the liver, heart and other tissues, causing serious morbidity and eventually mortality. In this study, liver- and heart-derived cells, and red blood cells were exposed to non-transferrin bound iron and the effect of fermented papaya on the LIP content and ROS generation was measured by flow-cytometry. Their results indicated that fermented papaya preparations reduce LIP and ROS, and suggest that its antioxidant mechanism is related, at least in part, to iron chelation [17].

Oxidative DNA damage, an indicator of the potential for carcinogenesis, also occurs as an early event in hepatitis C virus (HCV) infections. The antioxidant/immunodulatory effects of fermented papaya preparations and alpha-tocopherol (vitamin E) were evaluated in patients with HCV-related cirrhosis with transaminase values less than two-fold increased (alanine aminotransferase < 80 IU/L) [18]. Patients were randomly allocated into two groups and then given either vitamin E (900 IU/day) or 9 g/day of a commercial fermented papaya preparation (Immun-Age, Osato Research Institute, Gifu, Japan), and monthly tests were performed to evaluate their health, redox status and circulating cytokine levels in plasma during 6 months. Patients with cirrhosis showed a significant imbalance of redox status (low antioxidants/high

oxidative stress markers) and a significant improvement of redox status was obtained by both regimens. Only the fermented papaya preparation significantly decreased 8-hydroxy-deoxy-guanidine, and also significantly improved the cytokine balance compared to the vitamin E treatment [18]. The authors conclude that even though fermented papaya seem to have a potential supportive role as antioxidants/immunomodulators in HCV patients, more studies are needed to substantiate their effect on the natural history of the disease.

Since many aspects of beta-hemoglobinopathies, such as beta-thalassemia and sickle cell anemia, are mediated by oxidative stress, fermented papaya preparations were evaluated in these diseases *in vitro* using blood from patients and *in vivo* using a rodent model. Using a cell-free system, it was shown that this product decreased the oxidation in both spontaneous and hydrogen peroxide-induced 2'-7'-dichlorofluorescin-diacetate [19]. Using flow cytometry, it was shown that *in vitro* treatment of blood cells from beta-thalassemic patients with a commercial fermented papaya preparation increased the glutathione content of red blood cells, platelets and polymorphonuclear leukocytes, and reduced their reactive oxygen species, membrane lipid peroxidation and externalization of phosphatidylserine [19]. These effects resulted in a reduced thalassemic red blood cell sensitivity to hemolysis and phagocytosis by macrophages; improved polymorphonuclear leukocytes ability to generate oxidative burst - an intracellular mechanism of bacteriolysis, and reduced platelet tendency to undergo activation, as reflected by fewer platelets carrying external phosphatidylserine [19]. Oral administration of the fermented papaya preparation (50 mg/mouse/day) for 3 months to beta-thalassemic mice and to patients (9g given 3 times daily) during for 3 months, reduced all the above mentioned parameters of oxidative stress both in mice and in patients [19].

These results demonstrate that the antioxidant properties of fermented papaya could also alleviate symptoms associated with oxidative stress in severe forms of thalassemia.

The antioxidant effect of fermented fruit from *Carica papaya* was also studies in patients suffering from hereditary spherocytosis (HS), where red blood cells have a shortened survival rates due to primary deficiency in membrane proteins. Using flow cytometry, it was shown that red blood cells from HS patients generate more reactive oxygen species, membrane lipid peroxides, and less reduced glutathione than normal; however, when the same blood was incubated with fermented papaya, the oxidative stress markers were significantly reduced [20]. When HS patients consumed the fermented papaya preparation during 3 months, a decreased tendency to undergo hemolysis was observed [20].

The hemoglobin levels increased, the mean corpuscular hemoglobin concentration decreased as did the reticulocyte count. Also lactic dehydrogenase activity decreased causing an indirect bilirubin reduction. These results demonstrated that oxidative stress plays an important role in the pathophysiology of HS which can be ameliorated by an antioxidant such as fermented papaya preparation.

From these studies it is clear that the fermentation of papaya is capable of improving the antioxidant potential of this traditional food and opens the door for its use in the prevention and treatment of many diseases that are caused by an imbalance of the redox status.

Diabetes Prevention

It has long been suggested that fermented papaya may help to prevent diabetes Mellitus. In this section, a review of studies that have demonstrated that this hypothesis is true and the

use of fermented papaya preparations could prevent or treat diabetes through different mechanisms, such as antioxidant mechanisms, improvement of lipid profiles and that in turn can cause a decrease in blood glucose concentrations.

Peripheral blood mononuclear cells (PBMC) from type II diabetes Mellitus patients elicit a compromised respiratory burst activity resulting in increased risk of infections for the diabetic patients. It was shown that when PBMC from diabetic patients are stimulated with phorbol 12-myristate 13-acetate, the production of reactive oxygen species was markedly compromised compared to that of the PBMC from non-diabetic donors [21]. When the PBMC were treated *ex vivo* with a fermented papaya preparation, there was an improved respiratory burst since the product significantly increased phosphorylation of the p47phox subunit of NADPH oxidase [21]. In addition, the protein and mRNA expression of Rac2 was potently up-regulated after fermented papaya supplementation. The proximal human Rac2 gene promoter is G-C rich and contains consensus binding sites for Sp1 and AP-1. While the fermented papaya had no significant effect on the AP-1 DNA binding activity, the Sp1 DNA binding activity was significantly up-regulated in PBMC after treatment of the cells with FPP [21]. This work provided first evidence that compromised respiratory burst performance of PBMC from diabetic patients may be corrected by a nutritional supplement.

Using adult obese diabetic (db/db) mice, it was shown that fermented papaya blunted the gain in blood glucose and improved the lipid profile after 8 weeks of oral supplementation with 0.2 g/kg body weight [22]. This supplementation was effective in correcting wound closure and improved respiratory-burst function as well as inducible NO production in viable macrophages isolated from the wound site [22]. Diabetic mice supplemented with fermented papaya showed a higher abundance of CD68 as well as CD31 at the wound site, suggesting effective recruitment of monocytes and an improved pro-angiogenic response [22]. This work provides the first evidence that diabetic-wound outcomes may benefit with fermented papaya supplementation by specifically influencing the response of wound-site macrophages and the subsequent angiogenic response.

In 2012, the results of a randomized controlled clinical trial conducted at the Cardiac Centre, SSRN Hospital, Pamplemousses, (Mauritius) to determine the effect of a short term supplementation of a fermented papaya preparation on biomarkers of diabetes and antioxidant status in a multi-ethnical neo-diabetic population was published [23]. Supplementation of 6g of a commercial fermented papaya preparation/day for a period of 14 weeks improved the general health status of several organs targeted by oxidative stress during diabetes [23]. Compared to the control groups, C-reactive protein levels significantly decreased, LDL/HDL ratio was considerably changed, and uric acid levels were significantly improved. The authors conclude that fermented papaya may present a novel, economically feasible nutraceutical supplement for the management of diabetes and for those at risk for cardiovascular disease, neurological disease and other conditions worsened by overt inflammation and oxidative stress.

It has been proposed that erythrocytes and their membranes are good models to study the relationship between diabetes and susceptibility of erythrocytes to oxidative stress damage. Low doses of fermented papaya significantly reduced the susceptibility of human erythrocytes to undergo free radical-induced hemolysis in a multi-ethnical pre-diabetic population [24]. The intake of 6g of a commercial fermented papaya preparation per day during 14 weeks significantly reduced the rate of hemolysis and accumulation of protein carbonyls in the blood plasma of pre-diabetics [24].

Also, fermented papaya consumption on a daily basis strengthened the antioxidant defense system *in vivo* as was clearly demonstrated by the marked increase of total antioxidant status.

In a human trial, 25 patients affected by type-2 diabetes Mellitus under treatment with the oral antidiabetic drug glybenclamide and 25 clinically-healthy subjects were given 3g of commercial fermented papaya daily during two months. It was shown that fermented papaya can induce a significant decrease in plasma glucose levels in both healthy subjects and type 2 diabetic patients [25]. This hypoglycaemic effect, associated with clinical signs, allowed the diabetic patients to reduce the dosage of their antidiabetic oral therapy; thus the authors proposed that fermented papaya administration could be used as an adjuvant drug together with oral antidiabetic therapy in type 2 diabetes Mellitus.

Other Benefits

Besides all the above mentioned beneficial properties, it has been demonstrated that fermented papaya preparations may have other potential health benefits which will be discussed in more detail in the following paragraphs.

In a recent study, the effect of fermented papaya preparation on respiratory tract infection was evaluated [26] because of its known antioxidant properties and the fact that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in vital replication and virus-induced lung damage. In a placebo controlled cross over clinical trial, GSTM1-positive healthy individuals, that possess the gene coding for glutathione S-transferases (GST) which is a crucial enzyme involved in detoxification by protecting cells against reactive oxygen metabolites by means of the conjugation of glutathione with electrophilic compounds, were supplemented with fermented papaya product (FPP) during 6 weeks followed by a 30 day washout period. Subjects treated with FPP showed a significantly higher level of salivary IgA and lisozyme production. FPP treatment brought about a significant up-regulation of all phase II enzyme and superoxide dismutase gene expression tested in nasal lavage cells. The biological significance of these promising effects, i.e. the capacity to reduce respiratory oxidative stress in the human airway and, hopefully, the incidence and/or severity of respiratory tract infections remains to be demonstrated in longer clinical trials.

Non-alcoholic steatohepatitis (NASH) can progress to cirrhosis or hepatocellular carcinoma because of oxidative stress. It was shown that plasma biochemical parameters and lipid peroxidation in the liver were elevated in NASH rats, that their livers showed fibrosis, mitochondrial dysfunction and over-expression of microsomal CYP2E1, and that myeloperoxidase activity and nuclear factor-kappaB activation were also markedly increased [27]. The oral administration of FPP reverted these changes in NASH rats suggesting that this fermented product could halt NASH progression through its anti-oxidative and anti-inflammatory properties.

In an attempt to determine anticancer activities of fermented papaya preparation, rat pheochromocytoma (PC12) cells were pre-treated with the FPP prior to incubation with hydrogen peroxide (which causes oxidative DNA damage). The FPP significantly increased viability and maintained the morphology and shape of these cells [28]. The same fermented papaya product also prevented DNA oxidative damage in human hepatoma (HepG2) cells showing great promise for this product as a cancer preventing agent [28].

In a follow up article, it was stated that studies in chronic and degenerative disease conditions such as Alzheimer's, thalassemia, cirrhosis, diabetes and aging showed that FPP favorably modulates immunological, hematological, inflammatory, vascular and oxidative stress damage parameters [29]. These authors showed that FPP reduced the extent of the H_2O_2-induced DNA damage, an outcome corroborated by similar effects obtained in benzo[a]pyrene treated cells as shown by the modulation of the H_2O_2-induced ERK, Akt and p38 activation with the reduction of p38 phosphorylation induced by H_2O_2.

In a different study, it was demonstrated that cyanocobalamin (vitamin B12) absorption, that is normally altered in alcoholics, is significantly improved after oral administration of a demonstrated antioxidant fermented papaya preparation (Bionormalizer) [30]. In this study, 30 alcoholics and 24 control patients were examined before and after consuming a commercial fermented papaya preparation during 1 week. Plasma malonyldialdehyde level and lipid hydroperoxides concentration as well as malonyldialdehyde and xanthine oxidase concentration in the gastric mucosa were significantly higher in alcoholics than in controls and despite unchanged alcohol consumption, all of these biological markers were significantly decreased after fermented papaya supplementation. Gastric mucosal glutathione concentrations that were decreased in alcoholics were at least partially recovered after fermented papaya supplementation. Although the alcoholics showed a normal intrinsic factor secretion in the gastric juice, they exhibited a markedly depressed intrinsic factor-cobalamin binding and nearly 23% of them had an abnormal Schilling test. Both these impairments reverted to normal after supplementation with fermented papaya. Based on these and previous results, the authors conclude that the antioxidative potential of fermented papaya, possibly due to its availability substrates for glutathione synthesis as well as to its effects on local oxidative burst from neutrophils, is able to recover a normal cobalamin absorption.

The mechanisms of action of fermented papaya on the immune system were also evaluated using RAW 264.7 macrophages where it was shown that this product was able to exert both an immunomodulatory and antioxidant effect on this cell line [31]. In this study, low (LMF) and high molecular weight fractions (HMF) of FPP showed different activities. FPP fractions alone did not affect nitric oxide (NO) production which contributes to the host immune defense against viruses and bacteria. However, in the presence of IFN-gamma, both LMF and HMF significantly increased iNOS activity and nitrite as well as nitrate accumulation. TNF-alpha, one of the central cytokines in macrophage antimicrobial activity synergizes with IFN-gamma in the induction of NO synthesis, was enhanced by HMF [31]. Moreover, LMF displayed a stronger superoxide anion scavenging activity than HMF. These results show that not only one molecule is necessary for the bioactivity of fermented papaya and that the product as a whole should be consumed to increase its potential beneficial health-promoting effects.

PROBIOTICS ISOLATED FROM PAPAYA

As stated earlier, the most studied microorganisms in commercial fermented papaya preparations are yeasts. However, a wide range of microorganisms exist on the fruit and are thus potential candidates for beneficial properties that have been attributed to spontaneously fermented papaya preparations.

In a study performed in Brazil, one strain of *Lactobacillus plantarum,* denominated ST16PA was isolated from papaya [32]. This important lactic acid bacterium was shown to produce a 6.5 kDa bacteriocin which is active against different species from genera *Enterobacter*, *Enterococcus*, *Lactobacillus*, *Pseudomonas*, *Streptococcu*s and *Staphylococcus* and different serotypes of *Listeria* spp. The peptide is inactivated by proteolytic enzymes, but not when treated with α-amylase, catalase, lipase, Triton X-100, SDS, Tween 20, Tween 80, urea, NaCl, EDTA or after 2 h at pH values between 2.0 and 12.0, and after treatment at 100 °C for 120 min or 121 °C for 20 min. Its mode of activity against *Lactobacillus sakei* ATCC 15521, *Enterococcus faecalis* ATCC 19443 and *Listeria innocua* 2030C was bactericidal, resulting in cell lysis and enzyme-leakage. This was the first report on detection of *L. plantarum* in papaya and since the identified strain produced an active bacteriocin, it was proposed that it could be used as a biocontrol agent by inhibiting the growth of potentially pathogenic microorganisms [32].

Because of the very important bacteriocin production by *L. plantarum* ST16PA, these authors then decided to evaluate the probiotic potential of this strain and evaluate the effect of encapsulation on the survival of this isolate in conditions stimulating the gastrointestinal tract of humans [33]. Good growth of *L. plantarum* ST16Pa was recorded in growth medium with initial pH values between 5.0 and 9.0 and good capability to survive in pH 4.0, 11.0 and 13.0. This strain was also able to grow well in the presence of bile salts at concentrations ranging from 0.2 to 3.0%. The level of auto-aggregation was 37%, and various degrees of co-aggregation were observed with different strains of *L. plantarum, Enterococcus* spp., *L. sakei* and *Listeria*, which is an important feature for probiotic activity. Although growth was affected negatively by several medicaments used for human therapy, mainly anti-inflammatory drugs and antibiotics, *L. plantarum* ST16Pa was able to adhere to Caco-2 cells within the range reported for other probiotic strains. Studies in a model simulating the transit through the GIT indicated that encapsulating *L. plantarum* ST16Pa in 2, 3 and 4% alginate protected them from the acidic conditions in the stomach [33]. This is the first report of a bacteriocinogenic LAB isolated from papaya that presents application in food biopreservation and may be beneficial to the consumer health due to its potential probiotic characteristics.

CONCLUSION

In this Chapter it has been shown that papaya contains a wide range of microorganisms that have great potential to improve the health of consumers. Some beneficial strains were isolated and could be used in the preparation of novel foods or be used as probiotic microorganisms that can provide a health-promoting effect on the consumers of such products. Also, it was shown that fermented papaya has antioxidant properties, can stimulate the immune system and help in the treatment of diabetes. The mechanisms of action of these beneficial properties have been discussed in detail in this Chapter and these are the scientific basis for the traditional beneficial properties that have been attributed to fermented papaya consumption. The exploitation of this knowledge is essential in continuing the studies that have been performed in demonstrating that the microorganisms present in fermented papaya preparations are responsible for most of their beneficial effects.

The long term consumption of fermented papaya could be helpful in the prevention or treatment of many neurological, immunological and cardiac diseases that affect millions of people around the world.

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Chapter 7

INCIDENCE OF THE PAPAYA RINGSPOT VIRUS (PRSV-p) AND MANAGEMENT IN THE STATE OF GUERRERO, MEXICO

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ABSTRACT

The papaya cv. 'red Maradol' is one of the most popular tropical fruits, nationally and internationally. In Mexico approximately 21,000 ha^{-1} of land is used in cultivating this fruit but despite its popularity in the producing areas, the crop has been limited by the papaya ringspot virus (PRSV-p), which is transmitted by several aphid species. In response, the Interdisciplinary Papaya Group (GIP), evaluated technology of Integrated Management PRSV-p (MIPRSV-p), the objective of this technology is to delay and reduce the damage of the disease and increase crop productivity. As in 2004, 2005 and 2007 parcels of papaya cv 'red Maradol', to assess MIPRSV-p in the state of Guerrero this management design consisting in the protection of the seedlings with a polypropylene mesh was established, the density of plants was increased (2,700 plants / ha^{-1}), plants with initial symptoms of PRSV-p removed physical barriers of *Hibiscus sabdariffa* and three rows of papaya created around the plantation and a row of corn

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inserted. Incidence of PRSV-p, and population dynamics of winged aphids was recorded on a weekly basis throughout the cycle, in addition fruit harvest number and yield in kg / ha was recorded.

The results were a five month delay of PRSV-p damage with an incidence of 30 % unlike the Regional Management (MR), which in the same period came to 100 % infection, the best health allows the greatest number of fruits per plant (MIPRSV-p = 15.3, MR = 10.2), which was reflected in the yield of 78 ton/ha, unlike MR yields of 48 ton / ha^{-1}. From a total of 621 types of aphids identified, was 74.4 % of the species *Aphis gossypii*.

The greatest catch in Coastal Area occurred during the months October, November, December and January, while for the Iguala Valley the greatest were in the months of January, February and March.

Keywords: Integrated Management of the Papaya Ringspot Virus (PRSV-p), red Maradol, aphids

INTRODUCTION

Papaya (*Carica papaya* L.) is a widely distributed crop in tropical regions. World production of papaya fruit during 2008 was 9732, 158 t (SIAP, 2010). Its use is mainly as fresh fruit because it contains vitamins A and C; it is also preserved or used for extraction of pectins and papain (Agustí, 2004). In the last decade, Mexico has been one of the leading producers of papaya (*Carica papaya* L.) in the world along with Indonesia, India and Brazil; Mexico is the first exporter, by more than 10 years (FAO, 2011).

14 000 hectares of this fruit is planted in the nation under irrigation and in the rainy season, an increasing trend in states such as Michoacán, Chiapas, Oaxaca, Guerrero and Nayarit, which together with Veracruz, represent the major producing states (SAGARPA, 2010).

Although the use of this cultivar has spread in papaya producing areas, it has been limited by plant health problems, the most problematic disease of viral type is "Papaya Ringspot Virus" (PRSV-p) (Mora et al., 1992, Hernandez-Castro et al., 2007). The PRSV-p, is one of the most destructive diseases that occur in cultivation worldwide. This disease can cause yield losses of between 5% to 100% depending on the age at which the plant is infected (Hernández-Castro, et al., 2004).

This virus (PRSV-p), belongs to the potyvirus, is not transmitted by seed but from infected papaya plants to healthy plants by sucking insects of the aphid group, being the most common: *Myzus persicae, Aphis gossypii, A. neeri, A. citricola* and *A. spiraecola* (Nieto et al., 1990, Villanueva-Jiménez and Peña, 1991).

In Mexico 205 species of aphids, 25.85% of agricultural importance are known; given the great diversity of climates and vegetation in the country it is estimated that there are approximately 500 species (Peña-Martínez, 1999).

Faced with this problem the Interdisciplinary Papaya Group (GIP), is working in the dissemination and transfer of technology for integrated management of the disease in the state of Guerrero, México, the technology aims to delay and reduce damages by PRSV-p and thereby increase crop productivity.

MATERIALS AND METHODS

The work took place in the grounds of cooperating producers, the selected localities had a" climate or Aw (w) (i ') g, the driest of the warm subhumid with summer rains and annual average temperature of 27.9^{0}C and annual rainfall of 797 to 1313.5 mm (García, 1973 and Gob. Edo. Guerrero, 1990). The validation plots with the papaya plants were established from December 2003 to December 2005 in the Coastal Region of Guerrero and the last plot in December 2006 to January 2007, in the Northern region of the state, as shown in Table 1, with a total of eight plots of 1 ha-1 surface. Four were set up with the technology of the Integrated PRSV-p Management and four with traditional management of producers of the region (MR).

Table 1. Distribution of plots *Carica papaya* L., and the assessment the PRSV-p, in the State of Guerrero, Mexico

Locality	Evaluation period	Region of the State of Guerrero
El Papayo (Loc. 1)	2003 - 2004	Coastal region
Coyuca de Benítez (Loc. 2)	2004 – 2005	Coastal region
San Marcos (Loc. 3)	2004 – 2005	Coastal region
Iguala (Loc. 4)	2006 - 2007	Northern region

The MIPRSV-p technology consisted in application of the following measures: protection of seedlings with polypropylene mesh, which prevents them from being infected by aphids to ensure plant health at the time of cultivation; high plant density (2,700 pl/ha^{-1}); the practice of eradication of diseased plants with initial symptoms of the disease of PRSV-p was performed (visual symptoms of PRSV-p began to appear in the first plants approximately four months after the transplant until fruit set), a corn barrier was planted on the same date around of cultivation of papaya and also intercropped every three rows, the VS -525 maize variety was used. A distance of 1.5 m was established between individual papaya plants and 2.10 m between rows.

On the other hand in the MR which served as controls, the following practices were performed: a density was established of 2,000 plants ha^{-1}, removal of weeds, intensive application of insecticides and application of chemicals to soil and foliar fertilizer.

The plots were established with furrow irrigation, four applications of chemical fertilizer with the formula 120-30-120 gr/plant, divided into four applications; the first 20 days after transplantation and the remainder (3) every 30 days after each, weed control was performed manually.

Variables

To meet the objective the following variables were considered:

- Incidence of PRSV-p disease. To assess the incidence of virus, records were made every five days throughout the cycle. On each date both healthy plants and plants that

had any of the following symptoms caused by the virus were detected: Chlorosis, mosaic, distortion, stunting and oily spots on flowers, petioles and fruit. In addition the incidence of disease was determined by the following formula

$$\%I = \frac{ni * 100}{Ni}$$

where:

I = Disease incidence percentage.
ni = number of diseased plants at the time i.
Ni = total number of plants evaluated.

- Population dynamics and identification of species of captured aphids was evaluated for which yellow plastic trays of 30x23x13 cm containing water and detergent to break the surface tension were used as traps. The traps were placed at the height of the papaya canopy and moved according to the growth of the plants. Four traps, one at each cardinal point at the edge of the area evaluated of the established plot. Review of trays and collection of aphids was performed twice a week. Aphids were separated from other insects, counted and placed in a glass jar containing 70% ethyl alcohol, properly labeled with the date of data collection, the cardinal point and corresponding number of aphids. Collected aphids were taken to the laboratory for identification.
- At first cut the number and weight of fruit and the plant height was taken, taking an average of 100 plants at random from each of the plots established

Data Analysis

With the data obtained from the incidence and aphid population dynamics, graphics were made, to explain the development process of the disease, the period of greatest amount of aphids per month per species. And finally a comparison of the data including plant height, number and weight of fruit, and the final yield of each of the evaluated plots was performed.

RESULTS AND DISCUSSION

Incidence of the Papaya Ringspot Virus, was detected from 45 days after transplanting (about a month and a half) in the MR plots, whereas plots with MIPRSV-p, the incidence was recorded starting 135 days after planting (four and half months), as seen in Figure 1, in the case of MIPRSV-p infected plants of plots presenting the early symptoms, were eliminated.

The progress of the disease coincides with that reported in the paper entitled Incidence of the Papaya Ringspot Virus and capture of winged aphids in Tabasco, Mexico (Cortez-Madrigal and Mora-Aguilera, 2008), which at 150 days after transplantation (approximately five months) the incidence reached a 32.2% infection while in the area evaluated by this time has less than 20% incidence when the plants is bearing fruit.

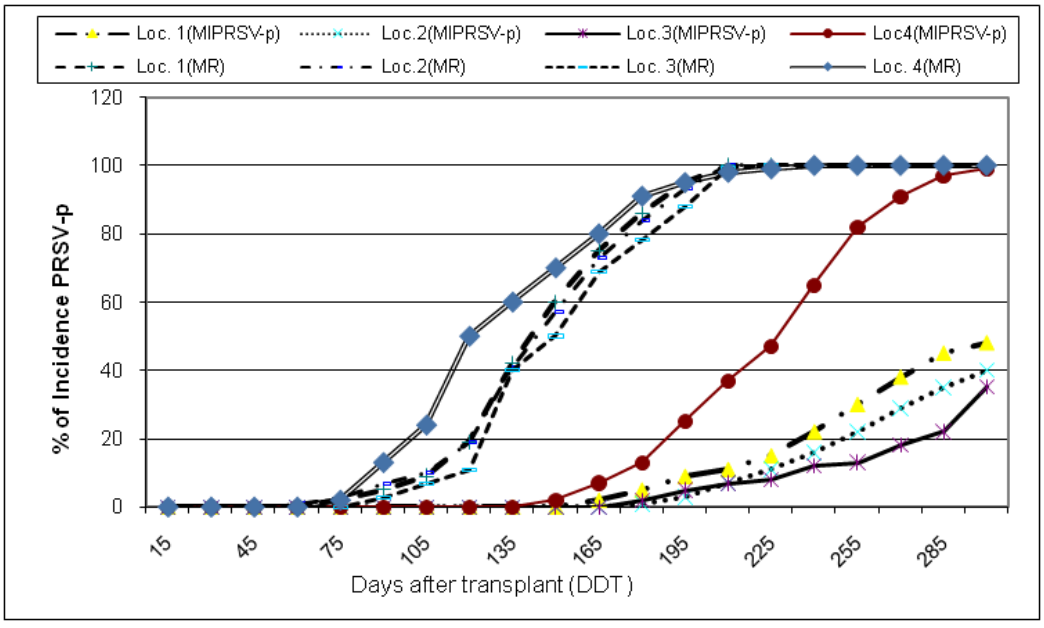

Figure 1. The progress of disease PRSV-p, in test plots in the state of Guerrero, Mexico.

Moreover plots with MR, in the same time reached 80% incidence of PRSV-p, these results have been encouraging, as it has managed to delay the damage caused by the disease for up to six months on average in all plots, having an incidence of less than 10%, while in the same period the witness (MR), came to 85% infection (Figure1); time the flowering and fruit set is given.

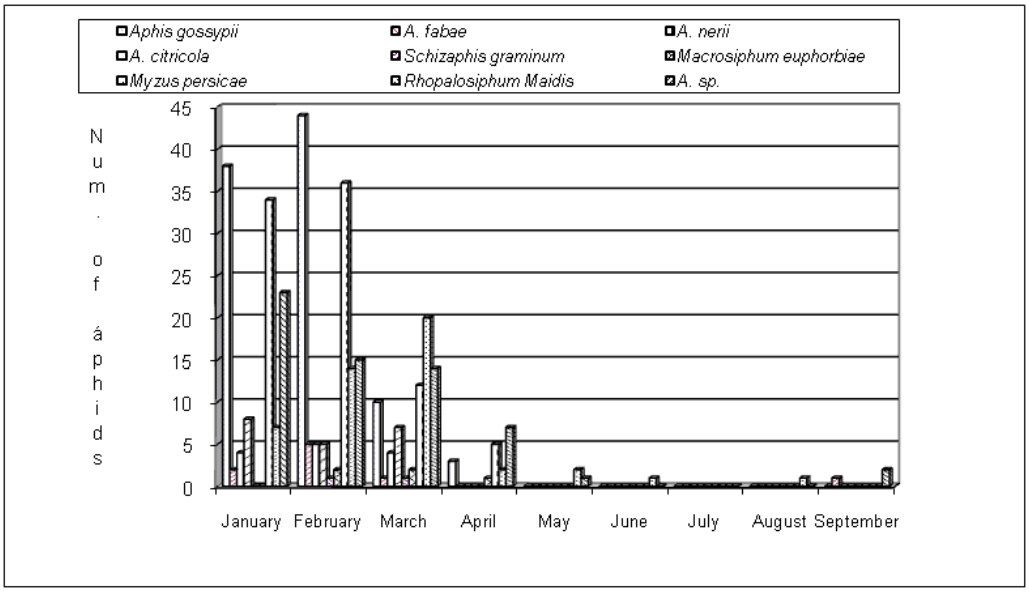

Figure 2. Population dynamics and diversity of aphid species, in plantings of papaya in the Coastal Zone of state Guerrero, Mexico.

The presence of aphids in the papaya plots was detected 30 days after transplantation, with an average of five individuals in each tray, populations increased during the next two months and fell from the fourth month after transplantation (Figure 2 and 3) this is because it is the start of the hottest months of the year. These results agree with those reported by Villanueva (1990), which work in papaya cultivation on College of Postgraduates, Veracruz, Mexico, by reporting high incidence peaks of winged aphids in the months of September to February. Similar results were found by Nieto et al., (1990) and Téliz et al., (1991).

These increases in aphid population are influenced by favorable climatic conditions for development, such as low temperatures, similar results are reported by Holmes & Hassan (1948) where since 1948 in Hawaii it is mentioned that the dispersion of PRSV-p in a population of papayas, was faster during the winter and spring months, and this was associated with high aphid vector populations.

The species most prolific in papaya plantations was *Aphis gossypii* (Figure 2 and 3) and along with *Myzus persicae*, were found in the work done in Coastal of Guerrero (Hernandez-Castro et al., 2007), while in Veracruz and Michoacan *A. gossypii* and *A. spiraecola* were found to have greater species abundance (Hernández 1998. Rivas-Valencia et al., 2008), and Tabasco to *A. spiraecola* (Cortez-Madrigal and Mora- Aguilera 2008), all these species are reported as the most efficient transmitters of virus in papaya cultivation (Villanueva and Peña 1991, Hernández 1994).

The months with the highest number of aphids were February and March (Figure 2 and 3), which coincides with that reported in research in Tabasco (Cortez-Madrigal and Mora-Aguilera 2008), where these researchers reported two distinct peaks: February-March and March-April.

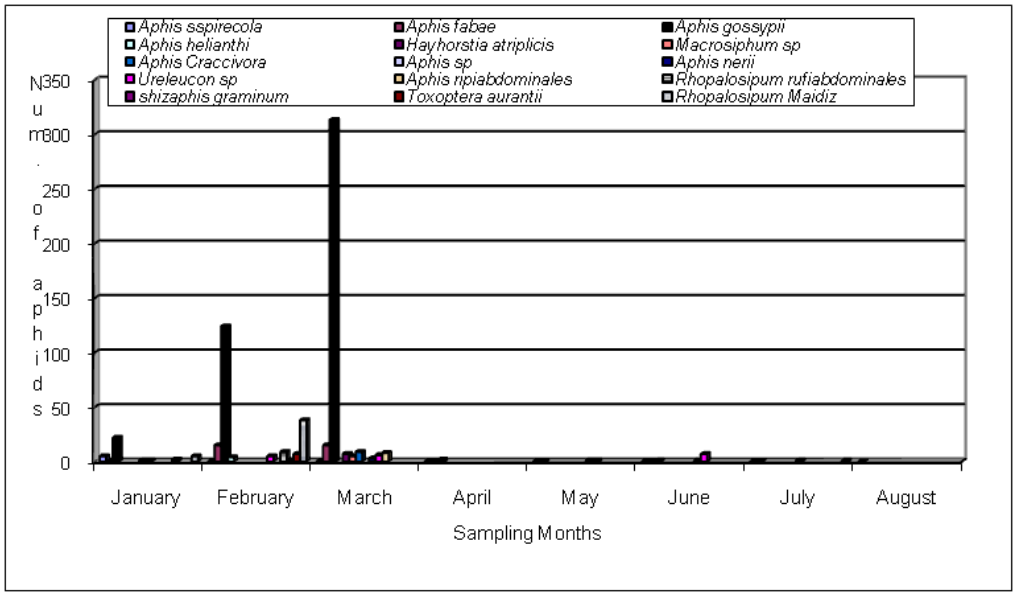

Figure 3. Population dynamics and species diversity of aphids in papaya plantation in the Northern Zone (Iguala) from state Guerrero, Mexico.

Other research indicates that the greatest catches of aphids in the tropics are recorded in winter and lowest in summer (Mora and Teliz 1987 Teliz et al., 1991), about Hernandez-

Castro et al., (2007) notes that the Coastal Guerrero defined two peaks from October to November and December to February are reported and also can see, that the greatest catches coincided with the period of lowest rainfall.

This lower incidence of plants viruses helped to obtain a yield in MIPRSV-p managed plots of up to 50 ton / ha, unlike the average yields of the plots with the regional management (MR) of approximately 22 ton / ha (Table 2.), these results are similar to those obtained in an experiment in the state of Veracruz, Hernandez et al., (2000) where they obtained yields of 30 ton / ha, various practices integrating PRSV-p management with cv. 'red Maradol', making it clear that such strategies offer better results than regional management.

Table 2. Effect MIPRSV-p in the production of cv. 'red Maradol' in plots established in the North and Coastal of Guerrero, Mexico

Technology	Average plant height (m)	Average No. of fruits / plant	Average fruit weight (Kg)	Final Yield (ton / ha)
Loc. 1 (MIPRSV-p)	1.48	8.0	3.2	52.3
Loc. 2 (MIPRSV-p)	1.47	7.6	3.5	49.2
Loc. 3 (MIPRSV-p)	1.53	6.9	3.8	49.7
Loc. 4 (MIPRSV-p)	1.50	7.5	3.3	50.3
Loc. 1 (MR)	1.40	4.1	3.0	22.2
Loc. 2 (MR)	1.41	4.0	2.9	19.8
Loc. 3 (MR)	1.52	4.8	3.2	21.8
Loc. 4 (MR)	1.49	5.3	3.1	22.7

CONCLUSION

The application of PRSV-p integrated management technology retards infection and lessens the severity, allowing greater production of fruit and therefore increased yield.

It is important to monitor aphids in papaya plantations for times of migration of these insects, that aphids do not colonize papaya plants, however many of these species are transmitting the Papaya Ringspot Virus and short flights migrations within the crop can occur.

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Chapter 8

BIOTECHNOLOGICAL STRATEGIES FOR CONTROL OF PAPAYA VIRUS DISEASES

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ABSTRACT

Papaya (*Carica papaya*) viruses diseases bring serious reductions of crop yield in different regions of the world, reaching to a total destruction of the affected orchards. Virus control is still a challenge, requiring an efficient and at the same time durable strategy.

There is no resistant papaya cultivar to several viruses, therefore the destruction of the infected plants is the main control method. The development of transgenic papayas in Hawaii, expressing the coat protein of *Papaya ringspot virus*, led to virus resistance with excellent fruit quality.

Therefore, it represents a promising strategy for controlling plant viruses. However, the production and consumption of transgenic plants still have many barriers in several countries.

Alternatively, the production of genetically modified plants, especially for the RNA silencing pathway, are becoming increasingly important and represent the best possible control strategy.

This chapter analyzes the main biotechnological strategies currently used in papaya virus diseases control and discuss other strategies for the future.

Keywords: Papaya (*Carica papaya*) viruses, *Papaya ringspot virus*, plant viruses, genetically modified plants, papaya virus diseases control

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INTRODUCTION

Plant viruses cause many diseases of international importance. Plant diseases are responsible for huge losses of crop production and quality in all parts of the world. The production is impaired due to the susceptibility of plants to viruses.

Papaya viruses are the biggest obstacle to the production of quality fruits for the international market. The diseases caused by *Papaya mosaic virus* (PapMV), *Papaya ringspot virus* (PRSV), *Papaya lethal yellowing virus* (PLYV), *Papaya meleira virus* (PMeV) and *Papaya leaf distortion mosaic virus* (PLDMV) can lead to total destruction of the affected plantations because the viruses control is not always effective. So, it is for the best to prevent infection. Some conventional methods have been used to this purpose, such as crop rotation and vector control. Another control method is the eradication (*rouging*) of plants with disease symptoms. However, to reduce crop losses, the use of cultivars with high levels of resistance[1] is the most viable strategy [1].

Like other viruses, the plants viruses are obligate intracellular parasites and therefore do not have the molecular machinery required to replicate their genome without the host cell. They have small genomes with limited genes number encoding for few viral proteins. The vast majority of plant viruses have positive RNA[2], though there are also negative RNA[3] and double-stranded RNA (dsRNA) genomes. Other plant viruses have single-stranded (ssDNA) or double-stranded (dsDNA) genomes. All plant viruses have their genome protected by a coat protein (CP).

During the infection process the plant viruses replicate their genome and form new particles, then they move from one cell to another, and between the plant tissues. During replication, these viruses synthesize viral proteins from messenger RNA (mRNA) with the aid of RNA or DNA polymerase (Figure 1).

Viruses have developed strategies to overcome the plant resistance during the evolutionary process. However, genetic engineering offered new perspectives on resistance against viruses.

Resistance genes present in different wild varieties of plants have been used in breeding programs in order to protect plants against virus attack. Nevertheless, these resistance genes have not overcome the several plant viruses mutations in the field. This is a especially important issue in cultivated crop such as papaya.

Understanding the mechanism of viral infection and the plant natural defense system has resulted in increasingly specific strategies designed to effectively limit the diseases caused by virus [2-4]. Genetically engineered plants expressing a specific viral protein are resistant to virus; for example, plants expressing the CP from *Tobacco mosaic virus* (TMV) are resistant to TMV.

Thus, various genetically modified plants resistant to viruses were developed using different types of viral genes.

[1] Genetic resistance can be defined as the ability of the plant to prevent or delay the establishment of the pathogen in their tissues in a highly coordinated and dynamic process.

[2] Positive RNA: RNA in 5'-3'orientation, which can be directly translated into viral proteins. The viral genome is a viral mRNA.

[3] Negative RNA: RNA in 3'-5'orientation, complementary to viral mRNA. The negative RNA must be converted to positive (mRNA) by a polymerase then to be translated.

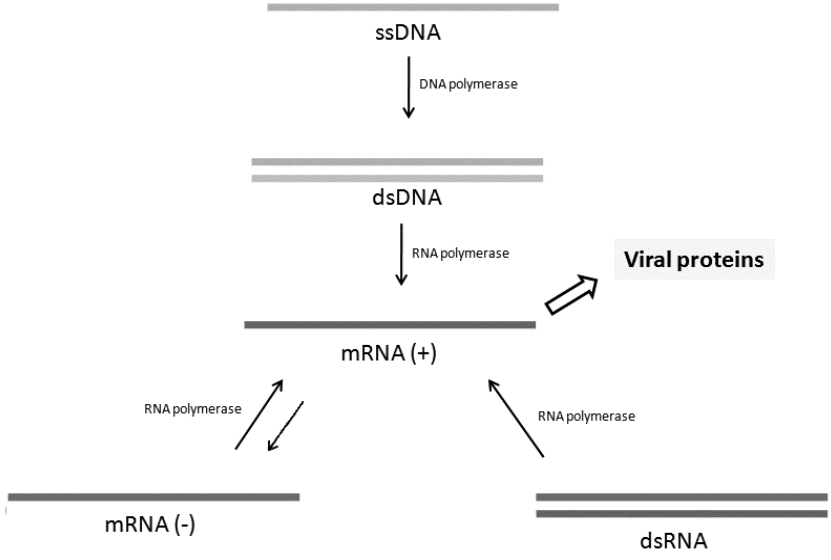

Figure 1. Scheme of the viral protein synthesis during replication process. The viral proteins are synthesize from positive mRNA. The polymerase enzyme, DNA or RNA polymerase, assists the mRNA synthesis.

In papaya, the development of resistant transgenic plants expressing the CP of PRSV opened new possibilities for solving the problem of viral control. Nevertheless, today the market still lacks resistant papaya cultivars to specific viruses.

IMPORTANT PAPAYA VIRUSES

The most important viral diseases of papaya, that cause serious damage to the papaya crop in many producing areas of the world, are the ones triggered by the *Papaya ringspot virus* (PRSV), the *Papaya meleira virus* (PMeV), the *Papaya lethal yellowing virus* (PLYV), the *Papaya mosaic virus* (PapMV) and the *Papaya leaf distortion mosaic virus* (PLDMV).

Papaya ringspot, caused by PRSV, is the most destructive viral disease of papaya crop. PRSV has been found in many tropical and subtropical areas where papaya is grown, including the USA, South America, Africa, India, Thailand, Taiwan, China and the Philippines, Mexico, Australia, Japan, French Polynesia, and the Cook islands [5]. *Papaya ringspot virus* is grouped into two types, PRSV-P and PRSV-W. PRSV-P infects both cucurbits and papaya while PRSV-W only infects cucurbits. PRSV belongs to the genus *Potyvirus*, a large and economically important group of viruses that infect plants, within the family *Potyviridae*. PRSV has aphid vectors that transmit in a non-persistent, non-circulative manner. There is no evidence that PRSV can be transmitted through seeds from infected papaya or cucurbits [6]. The *Potyviridae* family is organized into six genera (*Bymovirus, Ipomovirus, Macluravirus, Potyvirus, Rymovirus and Tritimovirus*), according to the vector and genome organization. Viruses in all genera except *Bymovirus* have a single molecule of

positive sense, ssRNA, of 9.3-10.8 kb in size. Virions[4] are flexuous filaments with no envelope. The *Potyvirus* genus has over 140 species described. Together, these species infect a wide range of monocotyledonous and dicotyledonous plants in different climatic regions, causing enormous economic damage in various cultures [7].

The PRSV has elongated and flexuous particles, measuring 780x12 nm, with a single-stranded positive sense RNA of 10,326 nucleotides (nt) as its genome. The 5' terminal is protected by a covalently binding VPg protein while the 3' terminal has a polyadenylated tail (Figure 2). The virions are composed by identical subunits of about 2,000 capsid protein which has a molecular weight of approximately 34 kDa (Figure 2A) [6].

The genomic RNA has two overlapping open reading frames (open reading frame, ORF) located between the two noncoding regions 5'NTR and 3'NTR. An ORF starting at nucleotide 86 and ending at nucleotide 10,120 encodes a polyprotein of 3,344 amino acids from which all virus proteins are derived. The different functional proteins are formed by a series of site-specific cleavage events carried out by three virus-encoded proteases, P1, HC-Pro, and NIa [8]. The mature proteins are: P1, HC-Pro, P3, CI, 6K, NIa, NIb and CP. The possible functions of the PRSV genome encoded proteins are listed in Figure 2B [9].

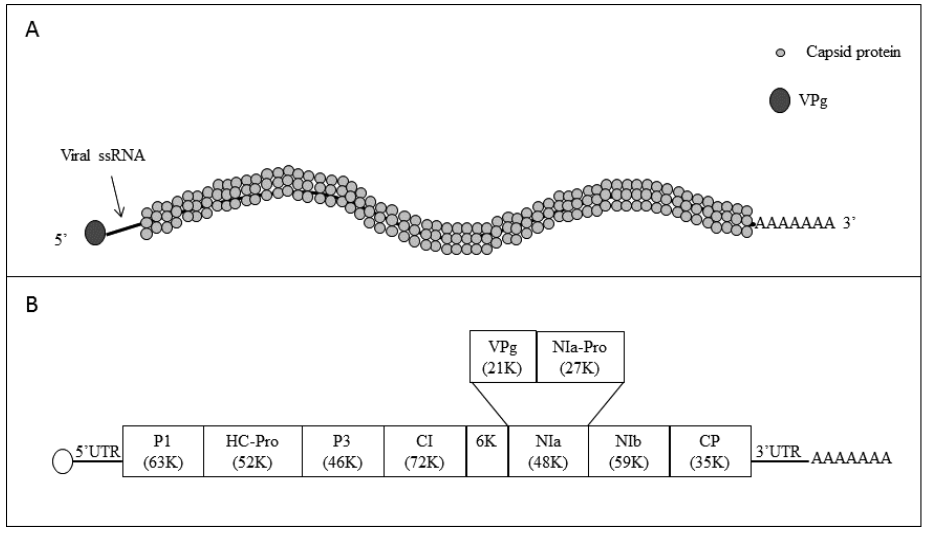

Figure 2. PRSV genome map. A) A model representing the virus particle. B) The viral genes in the PRSV genome. Molecular weights of the individual viral proteins are shown below the viral protein name in parentheses. Functions associated with these products are: P1, proteinase; HC-Pro, vector transmission, proteinase, suppressor of RNA silencing and cell-to-cell movement; P3, RNA replication; CI, RNA helicase; 6K, induce virus replication vesicle; VPg, primer for initiation of RNA synthesis; NIa-Pro, major proteinase; NIb, RNA-dependent RNA polymerase (RdRp) and CP, RNA encapsidation and vector transmission.

The consequence of this gene expression mechanism is that all viral proteins derived from the polyprotein are produced in stoichiometric amounts. The excess of produced proteins accumulate in the cytoplasm of infected cells and form inclusions bodies [6]. The

[4] Virion is an entire virus particle. A virion is the extracellular infective form of a virus. It consists of an RNA or DNA genome surrounded by a protein capsid.

second ORF referred to as PIPO (Pretty Interesting Potyviridae ORF) is located within the coding region of P3 protein, but in another reading frame [10].

PRSV-P infection symptoms is typically characterized by intense mosaic and leaf distortion (Figure 3 A), and also by the production of ringspots on fruit (Figure 3 B) of infected papaya trees [11]. In addition to ringspots, PRSV produces other symptoms such as water-soaked oily streaks on petiole and on the upper part of the trunk. Younger leaves show clearing along the veins that gives an appearance of flecks (Figure 3 A). Plants that are infected at a young stage remain stunted and do not product fruits [12].

The first papaya ringspot disease symptoms are the appearance of oily streaks and the clearing along the veins on the younger leaves. Experienced observers can detect symptoms on papaya at a very early stage, before the development of intense mosaic and chlorosis symptoms. These early symptoms are used to detect infected plants when rouging is used for disease management [13].

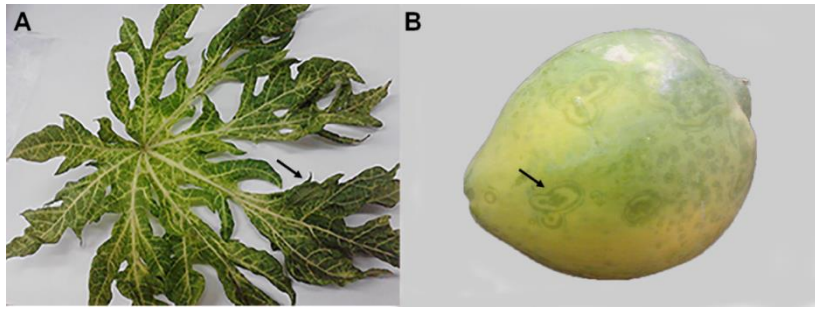

Figure 3. Symptoms of PRSV on papaya. (A) PRSV-infected papaya tree show intense mosaic and distortion of young leaves and (B) ringspot symptoms on fruit.

"Papaya sticky disease" or "meleira", caused by PMeV, was reported in 1980's in the south of Bahia and north of Espírito Santo States, Brazil. In few years, the disease spread rapidly, reaching the states of Pernambuco and Ceará. Currently it occurs in the states of Espírito Santo, Bahia, Pernambuco, Ceará, Rio Grande do Norte and Minas Gerais [14]. Outside Brazil, Mexico is the only other country where the disease was reported [15].

Initially, symptoms of the disease were attributed to a disturbance in calcium and boron absorption, resulting from water stress or imbalance of these elements in the soil [16]. In addition to abiotic factors, the involvement of microorganisms was suggested after the isolation of a bacteria of the genus Bartonella from diseased plants [17].

In preliminary studies, healthy papaya seedlings injected with latex obtained from diseased plants developed the anticipated typical symptoms as early as 45 days after inoculation, suggesting that the causative agent was present in the plant latex [18]. Transmission electron microscope studies of the diseased plant leaves and fruits latex indicated the presence of large number of isometric particles of about 50 nm in diameter. Ultrafine tissue sections reveled that these particles are restricted to lactiferous vesicles. The double-stranded RNA (dsRNA) with approximately 12,000 base pairs (bp) could be extracted from diseased plants and visualized in an agarose gel [19]. Finally, the viral etiology of the disease was confirmed after the purification of viral particles present in the latex, followed by inoculation in healthy papaya seedlings, which developed typical meleira symptoms (20). A model representing the virus particle is presented in (Figure 4).

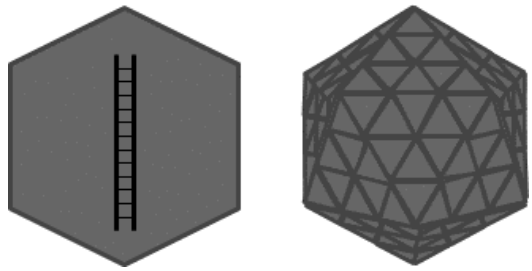

Figure 4. PMeV virion. A model representing the virus particle. PMeV is an isometric virus particle of approximately 50 nm in diameter. The virus genome is composed of a unique fragment of double-stranded RNA (dsRNA) of 12 Kb.

Table 1. Plant virus families and genera. The families and genera are listed according to the nature of the genome and morfology particle

Genoma	Virus Taxa Infecting Plants	Morphology	Genome configuration
ssDNA	Geminiviridae	Isometric	1 or 2 circular
	Nanoviridae	Isometric	6-9 circular
dsDNA-RT	Caulimoviridae	Isometric/ Bacilliform	Circular
ssRNA (-)	Rhabdoviridae	bullet-shaped, bacilliform	1 segment
	Ophioviridae	Filamentous	3/4 segments
	Tenuivirus	Filamentous	4-6 segments
	Bunyaviridae	Spherical	3 segments
	Varicosavirus	rod-shaped	2 segments
ssRNA-RT	*Pseudoviridae*	Spherical	1 segment
	Metaviridae	Spherical	1 segment
dsRNA	*Endornaviridae*	None	1 segment
	Partitiviridae	Isometric	2 segments
	Reoviridae	Isometric	10-12 segments

Genoma	Virus Taxa Infecting Plants	Morphology	Genome configuration
ssRNA	*Bromoviridae*	Isometric, bacilliform	3 + segments
	Closteroviridae	filamentous	1/2 + segments
	Comoviridae	*Isometric*	*2+ segments*
	Luteoviridae	Isometric	1 + segment
	Potyviridae	filamentous	1 / 2 +segments
	Secoviridae	isometric	1/2 + segment
	Sobemovirus	Isometric	1 + segment
	Tombusviridae	isometric	1 / 2 + segments
	Umbravirus	RNP complex	1 + segment
	Virgaviridae	rod-shaped	1-3 + segments
	Benyvirus	rod-shaped	4/5 + segments
	Bromoviridae	Isometric, bacilliform	3 + segments
	Idaeovirus	isometric	2 + segments
	Tymoviridae	Isometric	1 + segment
	Flexiviridae	Filamentous	1 + segment

Viruses with a genome of dsRNA is unusual among plant viruses, which have, in most cases, a genome consisting of single stranded RNA (ssRNA) (Table 1). dsRNA viruses represent a small group among plant viruses, grouped in the Endornaviridae, Partitiviridae and Reoviridae families.

Although PMeV is considered one of the major viruses infecting papaya in Brazil, knowledge on the sequence and genomic organization of this virus is poor. PMeV has not been sequenced nor classified by the International Committee on Taxonomy of Viruses (ICTV). A comparative analysis of a ~ 560 bp fragment amplified from the PMeV replicase gene from the major Brazilian papaya-producing states isolates indicated that PMeV has

similarity with mycoviruses of the family Totiviridae [14, 21]. However, a conclusive taxonomic position, will only be possible when the full genome is sequenced.

Unusually for a plant virus, PMeV appears to reside primarily in lactifers, where it modifies potassium levels and the osmotic balance, leading to rupture of cells [22]. Thus, papaya sticky diseased plants are characterized by spontaneous exudation of fluid and aqueous latex from the fruit and leaves. The latex oxidizes after atmospheric exposure, resulting in small necrotic lesions on the edges of young leaves and a sticky substance on the fruit, from which the name of the disease originates (Figure 5) [23]. In advanced stages of the disease, irregular light-green areas are observed on the surface of infected fruits.

Results obtained from electron microscopy and molecular analyses indicate that the viral particles are localized on and linked to the polymers present in the latex, perhaps acting as a protective mechanism or to assist the viral transport [22].

Figure 5. Papaya (*Carica papaya*) exhibiting symptoms caused by *Papaya meleira virus*. A) small necrotic lesions on the edges of young leaves; B, C) Spontaneous exudation of fluid and aqueous latex is observed in fruits surface.

The disease also interferes with the natural resistance of papaya fruits to fruit flies (Diptera: Tephretidae), which are significant pests of fruit production worldwide with a quarantine importance [24].

A study performed in 2003 with different papaya genotypes did not identify the existence of a resistant cultivar to PMeV. Thus, rouging of infected plants is the best strategy to control this viral disease [25, 26]. Symptoms are triggered only after flowering and, therefore, an infected plant without fruit or symptoms can remain unnoticed for months in the field, acting as an inoculum source until it is finally detected and eliminated [21].

The transmission of the virus by seeds has not been proven [27], but the recent detection of the virus in Mexico [15] suggests that PMeV can be transmitted by seeds.

Papaya lethal yellowing disease, caused by PYLV, is restricted to Brazil and it was described in the early 1980s in the state of Pernambuco, followed by Bahia, Rio Grande do Norte, Ceará and Paraíba. The disease has become a serious problem for papaya producers because of its serious damage in crop production and its increasing spread in the states of Ceará and Rio Grande do Norte reaching high incidence rates [14].

The symptoms begin with yellowing of the top third of the stem on partially expanded young leaves. Subsequently, the stem becomes twisted and the leaves, chlorotic (Figure 6A). With the disease progression to more severe symptoms, such as wilting and leaf senescence, eventually leading to plant death. Greenish circular spots appear on the fruits which turn yellowish when the fruits are ripening (Figure 6B, C). Fruits from infected plants have hard pulp and slower maturation, making unfeasible for consumption [28, 29].

PLYV does not have a known natural biological vector, but it is readily transmitted by human actions, including contaminated hands, agricultural tools, soil, and irrigation water [29, 30].

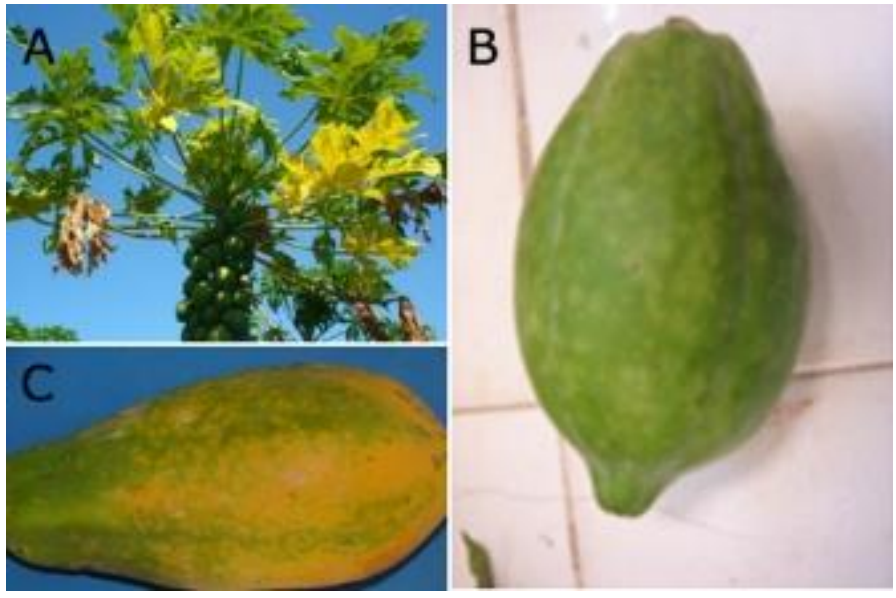

Figure 6. Papaya (*Carica papaya*) exhibiting symptoms caused by *Papaya lethal yellowing virus* (PLYV). A) A plant exhibiting the first symptoms with a progressive leaf yellowing in the third superior part of the plant canopy. B) Green fruits with greenish circular spots C) Mature fruits with greenish circular spots. Courtesy of Prof. Lima, J. Albersio de Araújo, Federal University Ceará, Brazil.

The presence of large numbers of isometric particles with approximately 30 nm in diameter can be observed by electron microscopy in the cytoplasm and vacuoles of leaves and fruits cells of lethal yellowing disease symptomatic plants. The virus genome consists of single-stranded RNA and its coat protein is formed by a single protein component of approximately 35 kDa [31]. A model representing the virus particle is presented in Figure 7.

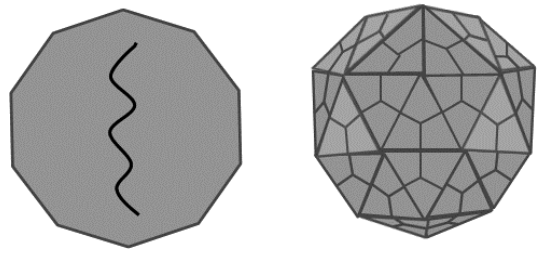

Figure 7. PLYV virion. A model representing the virus particle. PLYV particles are isometric with approximately 30 nm in diameter. The virus genome is composed of a unique fragment of single-stranded RNA (ssRNA) of 4145 nt, and its coat protein is formed by a single protein component of 34.7 kDa.

These characteristics suggested that PLYV could be included in the family *Tombusviridae*, genus *Carmovirus*. Nevertheless, molecular studies revealed 51% homology with polymerase and VPg nucleotide sequences of *Sobemoviridae* family viruses and only 39% with *Tombusviridae* family viruses, indicating that this virus might be included in the family *Sobemoviridae*, genus *Sobemovirus* [32]. In 2012, the sequence of the full-length genome of a PLYV isolate was reported, determined by deep sequencing. The virus genome is 4,145 nucleotides long and contains four ORFs, with an arrangement identical to that of sobemoviruses. Moreover, the coat protein encoded gene showed significant sequence identity with the corresponding protein of other sobemoviruses. Pairwise comparisons and phylogenetic analysis based on complete nucleotide sequences confirm the classification of PLYV in the genus *Sobemovirus* [33].

Papaya mosaic disease, caused by PapMV, was first reported in 1962 in Florida (USA). The disease has spread to other countries, reaching Bolivia, Peru, Venezuela and Mexico. Although PapMV has been found infecting papaya in different producing regions, it seems to be of little economic importance [34]. *Papaya mosaic virus* (PapMV) and *Papaya ringspot virus* (PRSV) were identified infecting papaya plants at the same time and region. The two viruses were differentiated using the length of particles, serology, host range and transmission by insects [35, 36].

PapMV is a member of the genus *Potexvirus* within the *Flexiviridae* family. It has a filamentous and flexuous particle of 530 nm long, with a positive sense single-stranded RNA genome comprising 6,656 nt with six open reading frames [6]. PapMV cause mild mosaic in papaya leaves and stunting. No symptoms appear on petioles, stems or fruit. Stunting is only apparent when healthy plants are present for comparison [35]. Approximately 5 days after inoculation, young greenhouse-grown seedlings show vein-clearing and downward cupping of leaves. Leaf mosaic develops after 15-20 days [37]. Papaya seems to be the only natural host; but it was reported, in host range studies, that other plants could be experimentally infected. PapMV is transmitted by mechanical inoculation and, until today, it has not been proven transmission by vector nor by seeds [35, 38].

In Mexico, PRSV and PapMV occurred in single or mixed infections of papaya and other host species that could function as virus reservoirs. In mixed infections, a synergistic interaction between the two independent viruses in the same host can occur and lead to increased symptoms and virus accumulation [34].

Papaya leaf distortion mosaic disease, caused by PLDMV, was first reported in 1954 on the island of Okinawa. Based on a ringspot symptom observed on fruits and the presence of flexuous filamentous particles, the virus was initially considered to be PRSV. However, no serological relationship was found between the virus and PRSV, which was later named PLDMV. Both viruses belong to the genus Potyvirus, and have similar characteristics, such as induced symptoms in papaya and host range [39]. The PLDMV also has two strains: PLDMV-P infects papaya while PLDMV-C is unable to infect papaya but infects cucurbits [40]. PLDMV emergence in PRSV-resistant papaya transgenic lines was considered as an emerging threat to papaya culture in China [41].

BIOTECHNOLOGICAL STRATEGIES TO PLANT VIRUSES CONTROL

Cross-Protection

The possibility that plants could be actively immunized against pathogens has attracted the attention of plant pathologists. In 1929, McKinney [42], demonstrated that tobacco plants infected with a strain of *Tobacco mosaic virus* (TMV) causing green mosaic could not subsequently be infected with a strain causing a bright yellow mosaic. This type of protection, later known as cross-protection, is a natural phenomenon whereby plant tolerance to a severe virus strain is induced by systemic infection with a mild strain of the same virus (Figure 8) [43].

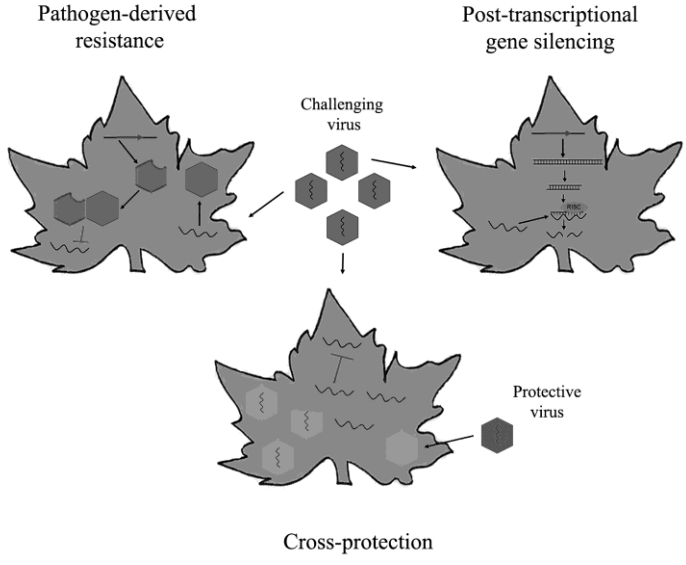

Figure 8. Strategies of plant virus resistance. In cross protection, plants that are systemically infected with a mild strain of a virus are protected against the infection effects by a more virulent related strain. In protein-mediated resistance, the insertion of pathogen (virus) genes, such as the coat protein (CP) gene, triggers the host defense response. In RNA-mediated resistance, the insertion of a gene fragment encoding a dsRNA conduct to cleavage of specific viral RNA molecules, preventing the synthesis of new viral proteins, and thus, the infection progress.

The key component in cross-protection is the availability of a mild strain that induces mild or no symptoms on the host and, at the same time, effectively protects against the target virus [44]. Severe strain infection induced lesions are often absent, or fewer and of reduced size, in mild strains pre-immunized plants when compared with symptoms generated by severe virus in unprotected plants [45].

The strategy of cross-protection is used in many countries in crops of economic importance, such as tomatoes, citrus, papaya and pepper [46].

Many decades later this finding formed the basis for the first demonstration of the use of transgenic plants to protect from virus infections. The development of genetically modified plants resistant to viruses may occur by a pathogen-derived resistance (PDR) or RNA-mediated resistance, which are based on post-transcriptional gene silencing (PTGS). Nowadays this is the most important strategy in the development of plant resistance to virus.

Pathogen-Derived Resistance

The concept of pathogen-derived resistance (PDR) strategy is based on the insertion of pathogen (virus) genes that trigger the host defense response (Figure 8). Thus, host transgenic plants that express genes encoding for viral coat protein (CP), movement protein (MP) or replicase subunits are resistant to these viruses [47].

Transgenic expression of CP is based on blocking the invading virus reassembly [48]. CP mediated resistance (CPMR) has been achieved by the expression of the CP from *Tobacco mosaic virus* (TMV) [49] in *Nicotiana tabacum*, *Solanum* tuberosum and *Carica papaya*, respectively. CPMR can provide either broad or narrow protection; for example, the CP of TMV provides effective levels of resistance to closely related strains of TMV and decreasing levels of resistance to Tobamoviruses that share less CP sequence similarity [50]. In papaya, the CPMR against PRSV occurs only for a single viral strain [51].

The replicase-mediated resistance (Rep-MR) genes that encode complete or partial replicase proteins can confer near immunity to infection that is generally, but not always, limited to the virus strain from which the gene sequence was obtained. Rep-MR to TMV was first described in transgenic plants that contain a sequence encoding a 54 kDa replicase fragment of [52]. The mechanisms that are involved in Rep-MR are not known, although it was shown that plants exhibiting Rep-MR can strongly repress replication, and, in many cases, are resistant to high levels of challenge inoculum. It is proposed that the transgene produced protein interferes, in some manner, with the function of the virus produced replicase, perhaps by binding to host factors or virus proteins that regulate replication and virus gene expression [47]. Generally, this is a resistance limited to viral strain that obtained the replicase [47].

In the MP mediated resistance (MPMR), as the transgenic and the viral MP have the same protein domain, they compete to interact with cellular factors that promote and interfere with the virus movement in the plant. Thus, the transgene interferes with the viral MP interaction with the host cell factors, hampering the viral cell-cell movement [53]. TMV infection, for example, is controlled when the TMV MP is expressed transgenically in plants, showing that the viral cell-cell movement stopped [54].

Post-Transcriptional Gene Silencing by siRNAs and miRNAs

A phenomenon known as RNA silencing occurs in many living organisms. In plants this phenomenon is known as post-transcriptional gene silencing (PTGS) and is a natural defense mechanism against virus. Basically, the plant identify dsRNA created during viral replication and destroys the specific viral RNA molecules, preventing the synthesis of new viral proteins, and, thus, the infection progress [54-57] (Figure 9).

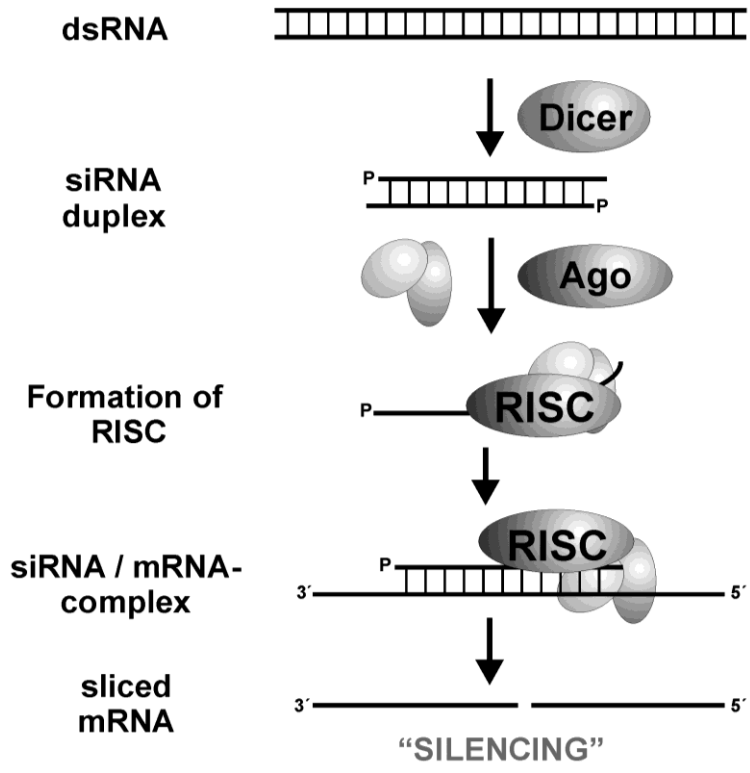

Figure 9. Mechanism of RNA silencing. In antiviral immunity DICER processes viral dsRNA in siRNAs. One strand of siRNA is incorporated in into an RNA-induced silencing complex (RISC) together with Argonaut (AGO) proteins. The AGO protein cleaves the target mRNA preventing viral infection progress. Available in http://www.gene-quantification.de/siRNA-mechanism.png.

The cell, perceiving the dsRNA, destroys RNA molecules whose sequences are of the same dsRNA. Initially, these molecules are cleaved by DICER proteins in small interfering RNAs (siRNA) with 21-26 nucleotides. One strand of siRNA is incorporated in a multiprotein complex, the RNA-induced silencing complex (RISC). RISC uses the siRNA as a template for recognizing complementary mRNA. When it finds a complementary strand, it activates Argonaute (a protein within RISC) and cleaves the RNA (Figure 9). This process is important in defense against viral infections, which often use dsRNA as an infectious vector. For example, dsRNA causes specific inhibition of *Pepper mild mottle virus* (PMMoV) infection in *Nicotiana benthamiana*. Sense RNA and antisense RNA corresponding to part of the readthrough domain of the replicase gene of PMMoV were transcribed in vitro and

annealed to each other to produce the dsRNA (54-kDa-protein dsRNA). Plants coinoculated with PMMoV plus 54-kDa-protein dsRNA were protected against infection, since they did not display disease symptoms [57].

Other small RNAs are important in pathogen and host interaction. Many micro RNAs (miRNAs), 21-24 nucleotides molecules, synthesized by virus were identified [58]. These miRNAs regulate many genes involved in viral replication and in the host defense response (58-63). In plants, miRNAs regulate mRNA that code for important regulatory factors of development, for stress response and for pathogens defense [64, 65].

Basically, primary miRNAs (pri-microRNA) are processed by DICER in precursor miRNAs (pre-microRNAs) [66]. In cytoplasm, pre-microRNAs are processed in mature miRNAs, with 21-25 nucleotides, which are incorporated in RISC complex with AGO proteins. This complex is targeted to mRNA through the miRNA seed region and, when annealed, induces either complete mRNA degradation or the alteration of translation [67, 68] (Figure 10).

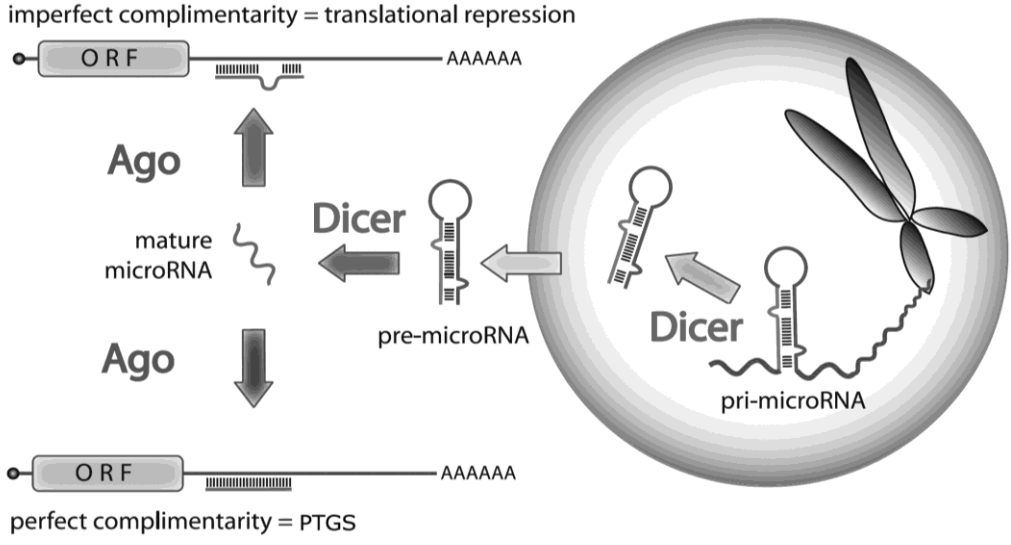

Figure 10. MicroRNA synthesis and the mechanism of messenger RNA regulation. MiRNAs are expressed in nucleus as primary miRNA (pri-microRNA), which are processed by Dicer in precursors miRNA (pre-microRNA). It is transported to cytoplasm by proteins present in nuclear membrane. In cytoplasm DICER processes pre-microRNAs in mature miRNAs, with 21-25 nucleotides. The mature miRNAs are incorporated in RISC complex with AGO proteins. This complex is targeted to mRNA through the miRNA seed region and, when annealed, induces either complete mRNA degradation (by perfect annealing) or the alteration of translation (with imperfect miRNA/mRNA annealing). Available in http://www.microrna.ic.cz/obr/image023.png.

Plant miRNAs can have viral genomes as target and thus contribute in the antiviral defense mechanism. Bioinformatic analyzes showed many miRNAs target viral genomes infecting plants [69]. These results showed that viral genes that code, for example, to the CP are targeted by plant miRNAs. Thus, the miRNA pathway is a support mechanism in antiviral defense triggered by siRNA [69]. The genetic engineering is an effective alternative to control virus diseases by the use of PTGS. Nevertheless, there is great public concern about the safety of the use of genetically modified plants in agriculture [1].

So today, different methodologies have been used in order to achieve resistance without plant genetic modification. This resistance might be achieved by transient expression[5] of interest exogenous genes. In this case, the plant cells are bombarded with gold or tungsten particles carrying dsRNA or siRNA molecules. As an example, the delivery of cognate dsRNA into single epidermal cells of maize, barley or wheat by particle bombardment interferes with the function of the endogenous genes. In these bombarded cells the accumulation of red anthocyanin pigments is reduced [70]. Another method use VIGS (virus-induced gene silencing), vectors constructed *in vitro* with the viral RNA complete sequence. When this vector is used to infect plants, the insert sequence induce and is targeted by PTGS [71]. Also, viral dsRNAs artificially introduced in plants by mechanic inoculation trigger a defense response protecting the plant against the viral infection [56, 57]. This same defense response can be achieved in plants sprayed with a solution containing viral dsRNA [56, 72]. Likewise, *Nicotiana benthamiana* and *Zea mays* sprayed days before viral inoculation with, respectively, *Pepper mild mottle virus* (PMMoV) [56] and *Sugarcane mosaic virus* (SCMV) [72] dsRNA, are protected against viral infection.

Although this plant response based on transient silencing of specific genes last a few days, it is believed that the siRNAs produced during the PTGS move through plasmodesmata and/or phloem triggering a systemic silencing.

BIOTECHNOLOGICAL CONTROL OF PAPAYA VIRUSES

Efforts to control papaya virus diseases are old. Many methods are currently used, such as the vector control, the use of papaya tolerant to certain viruses and cross-protection. Chemical or biological vector control can be very effective where the vectors need to feed for some time on a crop before the virus is transmitted, but are of much less value where transmission occurs very rapidly and may already have taken place before the vector succumbs to the pesticide. The use of tolerant papaya to certain viruses is marketed in some countries. These papaya plants develop only mild symptoms when infected and are able to produce respectable crops. However, it is still an inoculum source in the field and fruit quality is generally marginal, which in turn makes this technology not widely used [73].

PRSV-P mild strains development and selection through mutagenic treatments have been successfully done and used for cross protection. The PRSV HA 5-1 and PRSV HA 6-1 strains were selected after nitrous acid treatment of PRSV-HA, a severe strain from Hawaii, infected plants leaf extracts. Greenhouse experiments showed that both strains were mild on papaya and afforded protection against PRSV HA [44].

In 1980's, a series of field experiments was conducted with over three million papaya plants (Solo cultivars). Unfortunately, these studies showed that mild strain PRSV HA 5-1 did not confer complete protection against the severe strain in the field, but did show a delay of the severe virus symptoms. PRSV HA 5-1 produced noticeable symptoms on leaves and fruit, with a degree of symptom severity markedly dependent on the cultivar [44, 74, 75]. Cross protection has not been widely adopted on Hawaii and Taiwan because of the adverse effects

[5] Transient expression is one in which a given interest gene is expressed for a time short period in the cell, without any plant genetic manipulation. Thus, the transgene is not integrated into the chromosome and therefore is not passed to the next generations as in transformation by genetic engineering.

and instability of mild strains, allowing breakdown in the resistance. Plants that had a delay in the onset of symptoms experience severe symptoms of the disease [74].

Despite efforts for development of stable and protective PRSV mild strains in Brazil, Taiwan and the United States, practical results are not consistent. Some mild strains that were considered promising in greenhouse and in field experiments were stable for only a short period after the inoculation in commercial orchards [74].

Considering the lack of effectiveness of the methods mentioned above, the development of resistant papaya plants to viruses is the main goal for some groups in the world and virus-resistant transgenic plants is currently the most effective control of viral diseases.

The development of resistant transgenic papaya to PRSV in Hawaii opened new perspectives to papaya viruses control. PRSV GM papaya is produced and marketed in Hawaii since 1998. The studies began in 1985 using the concept of pathogen-derived resistance. The approach was to develop transgenic papaya with the PRSV CP gene. Briefly, the research to obtain the GM papaya involved isolating and sequencing the PRSV CP gene from the Hawaiian virus isolate, transforming embryogenic calli of nontransgenic Sunset papaya, a commercial papaya in Hawaii, selecting and regenerating plants transformed with the PRSV CP gene, screening transformed plants for resistance to PRSV, field trials, deregulation, and commercialization. In 1991, a PRS-resistant genetically engineering papaya line designated 55-1 was identified and subsequently field tested for resistance. Papaya line 55-1 was used to create the cultivars SunUp and Rainbow. This allowed the rapid development of cultivars that saved the Hawaiian papaya industry and that currently represent over 70% of the planted papaya acreage [73].

Unlike, in Brazil, both PRSV and PMeV are controlled through removal of plants with symptoms (rouging). In the states of Espírito Santo and Bahia, the main papaya producers in Brazil, rouging is an essential agronomic practice to papaya cultivation and it is governed by Normative Instruction number 17, May 27, year 2010, of the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA). Daily, inspections are performed in the entire culture crop and plants with symptoms similar to those caused by PRSV and PMeV are removed.

As mention before, one way of inducing viral resistance in plants is to introduce artificially viral dsRNA molecules, which are able to trigger the PTGS [56, 57]. PMeV infected papaya plants inoculated with dsRNA viral genome show a delay in the infection process, suggesting that the plant defense has been elicited, therefore inhibiting viral replication (Figure 11) [76]. These results is a prospectus for sticky disease control.

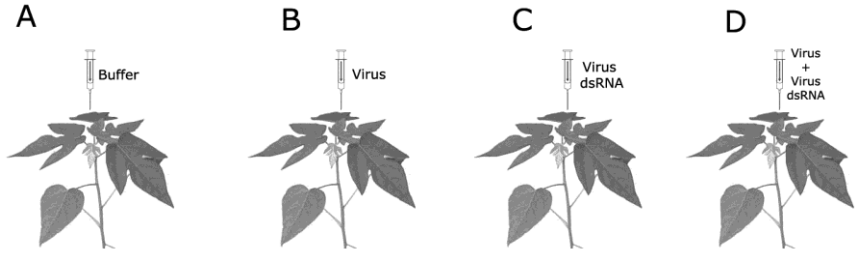

Figure 11. Induced resistance in papaya by viral dsRNA inoculation. Papaya seedlings were inoculated with A) buffer (control); B) PMeV; C) PMeV dsRNA and D) PMeV and PMeV dsRNA. A delay in the infection process occurred in plants inoculated by PMeV and PMeV dsRNA simultaneously.

NEW BIOTECHNOLOGICAL STRATEGIES FOR PAPAYA VIRUSES CONTROL

Since the discovery of RNA silencing pathway, degradation of specific RNA molecule is a potential mechanism for producing transgenic plants resistant to certain virus [77]. With the advent of next-generation sequencing and rapid discovery of miRNAs in different plant species, the study of virus-host interactions and their effects on the miRNA expression pattern has been used for the development of new strategies to virus control.

The expression of artificial miRNAs sequences (amiRNAs) that target specific viral gene generates virus resistant plants. For example, tobacco plants expressing amiRNA that target silencing suppressor 2b of *Cucumber mosaic virus* (CMV) are resistant to CMV [78].

The use of amiRNA is a good strategy for virus control in plants because it is possible to silence specific tissues genes and/or use several amiRNAs against different viruses or one amiRNA that target genomic regions of the same virus.

In papaya, it is feasible to express amiRNAs that target, for example, viral mRNA sequences encoding gene silencing suppressors and, as a result, get a specific viral resistant papaya. This resistance has been achieved in transgenic *Arabidopsis* plants expressing two amiRNAs targeting viral mRNA sequences encoding two gene silencing suppressors, P69 of *Turnip yellow mosaic virus* (TYMV) and HC-Pro of *Turnip mosaic virus* (TuMV) [79].

Virus resistant papaya can also be achieved by plant transformation with dsRNA or hairpin RNA viral fragment (Figure 12). Tobacco plants expressing simultaneous sense and antisense RNA to viral protease (Pro) of *Potato virus Y* is virus immune [80].

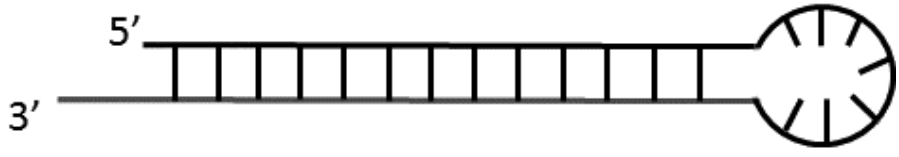

Figure 12. Hairpin RNA structure. Sense and antisense sequences are separated by a loop sequence. In plants, hairpin RNA can be used in PTGS pathway to silence target genes.

The success of GM papaya depends on the continued stability of transgenic resistance, evolution of virus population and the desirable horticultural papaya characteristics. Thus, it is important that researchers monitor the viral population and its diversity to ensure successful disease management.

Sequence diversity among virus isolates and their distribution are important for establishing virus origin, dispersion and disease etiology, in the pursuit of virus disease effective control. The genetic diversity in coat protein gene sequence of PRSV was observed in different regions of the world and it depends on virus geographical location. The transgenic papaya incorporating CP gene (HA 5-1) isolated from USA is resistant to infection by the severe USA PRSV isolate (HA) but did not show resistance against the Australian or Thailand isolates [75, 81].

Broad spectrum resistance against different PRSV isolates depends on the different PRSV strains genetic divergence which are correlated with their geographical distribution and also with the viral target genes transgene homology [82]. This strain-specific resistance limits

the application of the transgenic lines in other areas of the world. Thus, PRSV resistant GM papaya varieties must be developed individually for each different viral strain for the various papaya growing areas.

The development of new methodologies for fast and accurate detection of plant viruses is crucial for effective disease control. The accurate diagnosis of viruses that attack papaya is essential to help to reduce not only the economic loss on the papaya industry but also to reduce risk of introduction of other PRSV isolates from abroad.

The different methods to detect PRSV using RT-PCR targeted the coat protein gene, a conserved region of the nuclear inclusion protein gene or the 3' untranslated region (3' -UTR) [34, 83, 84]. However, many countries have used these genes from different isolates of PRSV to develop resistant transgenic varieties. Therefore, detection by RT-PCR using primers for these genes may fail to distinguish between the PRSV-resistant transgenic papaya and PRSV-infected papaya.

In 2014, Shen and colleagues [85] developed a RT-LAMP assay to detection of PRSV and to distinguish among transgenic, PRSV-infected and PLDMV-infected papaya. A set of four RT-LAMP primers was designed based on the region of the P3 gene of PRSV. The P3 gene includes a highly conserved region that had not been used for the development of PRSV-resistant transgenic papaya. This method will be useful for the early warning of PRSV in the papaya industry and for plant quarantine by relevant governments.

Next-generation sequencing (NGS) is arguably one of the most significant technological advances in the biological sciences of the last 30 years. NGS technologies have progressive advantages in terms of cost-effectiveness, unprecedented sequencing speed, high resolution and accuracy in genomic analyses. These high-throughput sequencing technologies have been comprehensively applied in a variety of ways, such as whole genome sequencing, gene expression profiling and small RNA sequencing, to accelerate the development of more effective control management [86, 87].

This advancement has considerably influenced plant virology in the field of diagnostics and host virus interaction. NGS technology has made it possible to directly detect, identify and discover novel viruses in several plants in an unbiased manner without antibodies or prior knowledge of the virus sequences. Entire viral genome could be sequenced from symptomatic or asymptomatic plants through next generation sequencing of total nucleic acids including small RNAs [88, 89].

The diagnosis of novel unidentified viral plant diseases can be problematic. Maize samples showing a range of leaf symptoms including spotting, streaking and necrosis on the margin were negative on ELISA test, whilst inoculation on other cereal species identified the presence of an unidentified sap transmissible virus. Deep-sequencing technology applied on RNA recovered from these maize plants showing symptoms allowed the identification of a two different virus on a mixed infection. Database searching of the resulting sequence identified the presence of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* [90]. This work shows that next-generation sequencing is a rich technique for rapidly identifying plant viruses.

The advent of high-throughput sequencing based methods has changed the way in which transcriptomes are studied. RNA sequencing (RNA-Seq) involves direct sequencing of complementary DNAs (cDNAs) followed by the mapping of the sequencing reads to the genome. RNA-Seq has been used successfully to determine non-coding RNAs and small RNAs, the transcriptional structure of genes, confirm or revise previously annotated 5 and 3

ends of genes, and map exon/intron boundaries and to quantify the changing expression levels of each transcript during development and under different conditions [91].

The study of virus-host interaction by RNA-seq allows the quantification of expression levels of each transcript during infection. Understanding the responses of plant hosts to viral infection is important for developing strategies for disease control.

Chen et al. (2013) [92] used RNA-seq to compare transcriptional changes in a *Tomato yellow leaf curl virus* (TYLCV)-resistant (R) tomato line and a TYLCV-susceptible (S) tomato line in response to TYLCV infection. Some sets of defense related genes encoding for WRKY transcriptional factors, R genes, protein kinases and receptor (-like) kinases which exhibited a dramatic down-regulation in the S line were up-regulated or not differentially expressed in the R line. The up-regulated differentially expressed genes in the R tomato line revealed the defense response of tomato to TYLCV infection was characterized by the induction and regulation of a series of genes involved in cell wall reorganization, transcriptional regulation, defense response, ubiquitination and metabolite synthesis. This study helps in the identification of important defense-related genes in tomato for TYLCV disease management. Understanding the transcriptome is essential for interpreting the functional elements of the genome and revealing the molecular constituents of cells and tissues, and also for understanding development and disease.

PRSV resistant gene is available in some wild varieties related to the *Carica* species. But the development of PRSV-resistant varieties through conventional breeding methods has been complicated due to the sexual incompatibility of wild species and cultivated papaya [93].

The transcriptome analysis of wild varieties related to the *Carica* species can help in the identification of defense related genes in *Carica* to PRSV. Transgenic papaya varieties resistant to PRSV could be obtained after the introduction of these resistance genes in cultivated papaya.

Plants have multiple mechanisms for adapting to biotic and abiotic stresses. Research on plant responses to these stresses has been focused on the gene regulation of transcriptional level. The interest in developing mechanisms to activate the resistance of the plant itself, for the control of diseases has increased in recent years [94].

It is well known that salicylic acid (SA) plays a very important role in plant defense response (95). Moreover, a number of studies have confirmed that exogenous treatments with SA induce resistance to different pathogens in different species [96, 97].

Several evidences indicate a possible overlap between RNA silencing and signal transduction pathways governed by SA. In *Nicotiana tabacum*, RNA dependent RNA polymerase 1 (RdRP1) activity has been found to increase after SA treatment and TMV infection [98].

In addition to the SA, it was shown that treatment with gentisic acid (GA) also induces resistance to RNA viruses. Treatments with SA or GA induced systemic resistance to *Tomato mosaic virus* (ToMV) in tomato plants. Tomato plants previously treated with SA and GA showed a lower accumulation of capsid protein and lower expression of symptoms. In tomato plants infected with ToMV or treated with SA and GA was observed the induction of silencing-related genes, like DCL1, DCL2, DCL4, RDR1 and RDR6. Therefore, this results suggest that the observed delay in the RNA pathogen accumulation could be due to the pre-induction of RNA silencing-related genes by SA or GA [99].

These methods, therefore, represent new possibilities for developing resistant transgenic papaya resulting worldwide in a best management of this crop.

CONCLUSION

Papaya is produced in about 60 countries, with the vast majority being grown in developing economies. Global papaya production was over 10 million (M) metric tons (t) in 2012 (FAOSTAT 2012) [100].

Virus diseases are significant threats to modern agriculture and their control remains a challenge to the management of cultivation. Wild varieties that possess natural resistance could be used in breeding method to produce the resistant plants. However, it is usually costly and time consuming work and features such as crop quality and quantity may be compromised by breeding for resistance.

In contrast, genetic manipulation is a relatively rapid method to introduce the virus resistance. Since the discovery of RNA silencing pathway, degradation of specific RNA molecules is a potential mechanism for producing transgenic plants resistant to certain virus. Transgenic plants expressing the dsRNA or hairpin RNA viral fragment have been shown to efficiently resist viral infection. Two of the major challenges for the successful use of RNAi are the availability of sequence information and the identification of the appropriate target genes. An increasing understanding of RNAi will open to new possibilities for controlling plant viruses. Several countries have already developed genetically modified papaya cultivars and are working on regulatory issues to release the new PRSV resistant cultivars, while other countries are still in the field testing stage of new PRSV-resistant genetically modified papaya cultivars. However, the production and the consumption of transgenic plants still have many barriers in several countries.

Thus, methods that induce viral RNA-silencing without altering the plant genome like transient expression of dsRNA viral fragment might be a best method to control plant viruses.

Knowledge of the plant-virus interaction improves a efficiency of the current approaches and allow the development of new strategies. In this chapter has been presented the biotechnological strategies for control of papaya virus diseases.

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