Process Optimization of Vacuum Concentration of Karonda Fruit Juice Using Response Surface Methodology: Effects on Antioxidant Activity, Iron Content and Gcms Profile

Chinmayi A. S.
chinmayias9171@gmail.com
University of Horticultural Sciences Bagalkote
Suresha G. J.
University of Horticultural Sciences Bagalkote
Rosemary M. X.
University of Horticultural Sciences Bagalkote
Sadananda G. K.
University of Horticultural Sciences Bagalkote
Venugopalan R.
Indian Institute of Horticultural Research
Vasudeva K. R.
University of Horticultural Sciences Bagalkote
Suresha K. B.
University of Agricultural Sciences, Bangalore
Swamy G. S. K
University of Horticultural Sciences Bagalkote

Research Article

Keywords: Karonda, Vacuum concentration, RSM, Anthocyanins, Antioxidant activity, Fruit juice concentrates, GC-MS

Posted Date: April 5th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4138325/v1

Additional Declarations: No competing interests reported.

Abstract

In the realm of fruit processing, karonda juice stands out for its elevated moisture levels, posing challenges in storage and transportation. This study presents a pioneer effort for concentration of karonda juice through optimizing the process parameters *viz.*, temperature and time using central composite design (CCD) of response surface methodology (RSM) within rotary evaporator setup. Through ten experimental runs, variations were introduced, adjusting temperatures from 45 to 55°C and duration from 90 to 210 minutes. The results showcased the efficacy of vacuum concentration, reducing moisture content to 16.43% and significantly elevating total soluble solids (TSS) from 9.02 to 89˚B. Moreover, key nutrients experienced substantial increase: ascorbic acid surged from 3.79 to 14.66 mg per 100g, total phenolic content (TPC) soared from 100.74 to 386.97 mg GAE per 100g, total antioxidant activity (TAA) (FRAP) escalated from 76.74 to 328.10 mg AAE per 100g, anthocyanin content increased from 10.9 to 129.34 mg per 100g, and iron content rose from 39 to 150.54 mg per 100g. GC-MS profile elucidated compounds like Tetrahydrofuran, 3-Hydroxy-3-methylvaleric acid, 3-Hexen-2-one, Hydroperoxide and Octane. The optimization process, guided by RSM revealed the ideal parameters: 55°C for 150 minutes, marking a significant advancement in karonda concentration techniques.

INTRODUCTION

Fruits, being short-lived, are consumed either as fresh or processed within a limited time frame to savour their delightful flavours. However, the sheer volume of production during peak seasons often exceeds direct consumption capacity, necessitating transportation to geographically distant areas (Berk, 2016). To address this challenge and make nutritionally rich juice products available across the map, fruits are processed through a method known as "fruit juice concentration." In this process, a significant portion of the naturally occurring water content is physically extracted (Jeong et al., 2004). This concentration is essential for practical packaging, storage, transportation and preservation from an economic standpoint. Traditional techniques for juice concentration commonly utilize heat, a method that, while successful in lowering moisture content, may unintentionally result in flavour deterioration, nutrient depletion, and changes in colour and aroma. On the contrary, vacuum concentration offers a more delicate approach, preserving the unique nuances that define karonda juice. By working under reduced pressure, this method minimizes exposure to high temperatures, reducing the likelihood of thermal damage and ensuring the final product retains its authentic taste and nutritional qualities.

Karonda (*Carissa carandas L.*), also known as the 'Christ Thorn Tree,' belongs to the *Apocynaceae* family. The fruits of karonda are characterized by their sour and astringent taste, ranging from acidic to sweet, accompanied by a distinctive aroma. Despite their unique qualities, Karonda fruits are not widely consumed fresh due to their high pectin content and latex exudation. In the processing industry, ripe karonda fruits find utility in the preparation of preserves. These fruits are noteworthy for their richness in iron (150 mg 100 g^{-1}) and vitamin C (17.94 mg 100g^{-1}) Consequently, they possess antiscorbutic properties, making them beneficial in preventing anaemia, especially as the ascorbic acid content enhances the bioavailability of iron (Bose et al., 2011). Karonda is also known for its reported efficacy in

alleviating stomach-aches and acting as an anthelmintic (Itankar et al., 2011). In traditional medicine such as Ayurveda, unripe karonda fruits are employed for their astringent, appetizer, antipyretic, and antidiabetic properties. Additionally, root extracts are used to address conditions like lumbago, chest complaints, and venereal diseases.

Karonda fruits, when ripe, can be enjoyed as a dessert or utilized in the preparation of various processed products, including candy, jelly, squash, and chutney. Despite its versatility and nutritional benefits, the storage life of karonda is limited due to its soft flesh and high moisture content. Vacuum concentration emerges as a pragmatic solution to meet the rising consumer demand for convenient and less bulky and natural products. In response to the growing preference for beverages devoid of artificial additives and preservatives among health-conscious consumers, the vacuum concentration of karonda juice aligns seamlessly with this trend. This method offers a means to deliver a pure and unadulterated product.

The primary goal of the current study is to optimize process variables, temperature and time, in the vacuum concentration process of karonda juice. By focusing on these key factors, the research aims to enhance the efficiency and effectiveness of the concentration process, ensuring the production of a high-quality, natural beverage that resonates with the preferences of health-conscious consumers.

MATERIAL AND METHODS

Preparation of samples

Karonda var. Konkan bold was procured from the fruit orchard of Regional Horticulture Research and Extension Centre (RHREC), University of Horticultural Sciences Campus, Bengaluru, Karnataka. Fully matured and ripe fruits were harvested that are of uniform size, shape and free of visible damages. Fruits were washed with running tap water and the seeds were removed manually. After removing the seeds, the remaining fruit portion was hand crushed and used for further processing.

Karonda juice concentrating process

The concentration process of 250 mL Karonda juice commenced by placing it in a round-bottomed flask and subjecting it to a rotary vacuum evaporator (HS-2005 V, Hahnshin Scientific, Korea). This process entailed varying the heating temperature of the water bath across three settings (45, 50, and 55°C) and adjusting the evaporation time to three different duration (90, 150 and 210 minutes). Subsequently, karonda juice within the flask was immersed in a water bath to facilitate the vacuum concentration process. The selection of maximum and minimum heating temperatures, as well as evaporation times, was based on preliminary experimentation to optimize the concentration process systematically.

Experimental design

Response surface methodology (RSM) in conjunction with central composite design (CCD), was employed to optimize the process parameters *i.e.*, concentration temperature and evaporation time by generating a set of experimental runs. A calculation of experimental runs and optimum yield was

performed using SAS version 9.3 statistical software. A CCD design approach was adopted incorporating two independent factors (Table 1) and six responses (TSS, moisture (%), TPC, total antioxidant activity (TAA), total anthocyanin content (TAC) and iron content). Response values were analysed by fitting the data in a second order polynomial model. The generalized second order polynomial model proposed for predicting response variables is given as,

Table 1
Independent variables and their coded and actual values used for optimisation.

Code	Independent factors	Units	Level		
			-1	0	+1
X_1	Temperature	°C	45	50	55
X_2	Time	min	90	150	210

$$y = b_0 + b_1X_1 + b_2X_2 + b_1b_2X_1X_2 + b_1^2X_1^2 + b_2^2X_2^2$$

In this equation, y represents the dependent variable (the estimated response), X_1 represents concentration temperature and X_2 represents evaporation time. Coefficients of the polynomial were represented by b_0 (constant term), b_1 and b_2 (interactive coefficients). Model adequacy was tested by ANOVA. The fitness of the models was further affirmed based on statistical parameters such as coefficient of determination (R^2), F test value and lack of fit. Interaction effects of independent variables were pictorially represented by 3-D response surface graphs for better depiction of results.

Physicochemical parameters

TSS (˚Bx)

TSS of samples were determined by digital refractometer at room temperature (28 ± 2˚C) (Erma Japan). It was expressed in °Brix.

Moisture content (MC) (%)

The moisture content of the sample was determined by using an electronic moisture analyser (Sartorius MA 35) and it was expressed in per cent.

Ascorbic acid content (mg $100g^{-1}$)

Ascorbic acid content of concentrated samples was determined by modified method using 2, 6-dichlorophenol indophenol sodium salt described by AOAC, 2006. The results are expressed as mg ascorbic acid equivalent per 100g fresh weight using a standard curve of L-Ascorbic acid.

$$\text{Ascorbic acid (mg } 100g^{-1}) = \frac{\text{Titre value} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{Weight of sample} \times \text{aliquot of sample}}$$

Total phenol content (TPC)

TPC was determined by the spectrophotometric method, using Folin Ciocalteu Reagent (FCR) (Singleton and Rossi, 1965), and expressed in mg gallic acid equivalents in 100g of fresh samples using following formula,

$$\text{Total phenol content (mg GAE } 100g^{-1} \text{ FW)} = \frac{OD_{700nm} \times \text{Std. value } (\mu g\ OD^{-1}) \times \text{Volume made up (50 mL)} \times 100}{\text{Assay volume (mL)} \times \text{Weight of sample (g)} \times 100}$$

Total antioxidant activity (TAA) (FRAP index) (mg AAE $100g^{-1}$)

The antioxidant activity of the concentrated juice was determined using ferric reducing antioxidant potential (FRAP) assay and expressed in ascorbic acid equivalents (AAE) in mg 100 g^{-1}(Benzie and Strain, 1996).

$$\text{TAA (mg AAE } 100g^{-1}) = \frac{\text{Standard value X Total volume X 100}}{\text{Assay volume (mL) x Weight of sample (g) X1000}}$$

Total anthocyanin content (TAC) (mg $100g^{-1}$)

Anthocyanin estimation was done as per the procedure of Fuleki and Francis (1968) and expressed as mg per 100g.

$$\text{Total OD } 100g^{-1} = \frac{\text{OD X Volume made up (mL)}}{\text{Weight of sample (g)}} \text{X100}$$

$$\text{TAC (mg } 100g^{-1}) = \frac{\text{Total OD } 100g^{-1}}{87.3}$$

Iron content (mg $100g^{-1}$)

Iron content of karonda vacuum concentrated juice was estimated as per the procedure given in AOAC (AOAC, 1990) using atomic emission or adsorption spectrophotometer. Iron content was expressed as mg per 100g.

GC-MS profiling

The hexane extract of vacuum concentrated karonda juice was analysed using GC-MS employing a Shimadzu QP2020 series gas chromatograph with an SH-Rxi-5Sil column. Helium served as the carrier gas at a constant flow rate of 1.20 mm min^{-1} under 68.3 kPa pressure, with an injection volume of 1.0 µL and a split ratio of 1:50. Detection was conducted using a flame ionization detector at 320˚C. The temperature program for the oven included initial holding at 50℃ for 2 minutes, followed by ramping up to 220℃ at 10°C per minute, and maintenance at 310℃ for 5 minutes at a ramp rate of 15°C per minute. All samples were analyzed and results documented by comparing the mass spectrum with a library through computer analysis.

Statistical analysis

Each experimental run was triplicated. Combined ANOVA processing by SAS version 9.3 statistical software. The optimal concentrate parameter of karonda juice predicted with significance level below 5%.

RESULTS AND DISCUSSION

The observed values of response variables are given in Table 2. The generalized second-order polynomial model proposed for predicting response variables were generated. The models were compared based on their coefficient of determination (R^2), adjusted coefficient of determination (R^2 -adj), predicted coefficient of determination (R^2 -pred), and the probability (*p)* of lack of fit.

Table 2
Central composite design with experimental and predicted values for TSS, MC, Ascorbic acid, TPC, antioxidant activity, TAC and Iron

Runs	Independent variables		Responses						
	X_1	X_2	TSS	MC	Ascorbic acid	TPC	FRAP	TAC	Iron
	˚C	min	˚B	%	mg $100g^{-1}$	mg GAE$100g^{-1}$	(mg AAE $100g^{-1}$)	mg $100g^{-1}$	mg $100g^{-1}$
1	55	90	32	26.03	8.53	225.60	212.25	12.12	85.80
2	50	210	78	11.28	9.77	257.97	219.27	82.57	97.50
3	50	150	36.9	67.62	12.09	319.60	271.15	78.37	123.76
4	50	150	28	68.11	11.53	304.45	258.40	53.17	121.62
5	45	90	32	83.35	4.52	119.58	101.15	18.23	45.65
6	50	90	30.7	88.79	4.68	123.76	104.55	11.36	47.54
7	45	210	34	53.76	6.44	170.24	144.50	12.03	66.39
8	45	150	49.6	55.43	13.49	355.90	301.75	27.59	141.67
9	55	210	28.5	74.09	8.75	231.34	196.61	16.70	90.09
10	55	150	89	16.43	14.66	386.97	328.10	129.34	150.54
Note: TSS – Total soluble solids, MC- moisture content, TPC – Total phenol content, and FRAP- Ferric reducing antioxidant power, TAC- Total anthocyanin content									
Units- TSS, MC, TPC and FRAP in ˚Bx (degree brix), %, mg gallic acid equivalent per 100g, and mg AAE $100g^{-1}$ (milligrams of ascorbic acid equivalent (AAE) per 100 gram) respectively. Ascorbic acid, TAC and iron content expressed in mg$100g^{-1}$.									
X_1 and X_2 represents the independent variables temperature and time respectively.									

Effect of vacuum concentration process parameters on total soluble solids and moisture content

The ANOVA results from Table 3 indicate that the RSM model's effect is not significant for both TSS and MC, suggesting that the response surface model adequately explain the variability in the experiment, yet indicating its fit is inadequate. The RSM equation describing the effects of process variables on total soluble solids and moisture content of vacuum concentrated karonda juice in terms of actual levels of variables is given as:

Table 3
ANOVA of the second order polynomial model for the various responses for vacuum concentration of karonda juice

ANOVA	TSS	MC	Ascorbic acid	TPC	FRAP	TAC	Iron
Mean of response	43.87	54.48	9.44	249.54	213.77	44.14	97.05
RSME	29.28	30.07	1.5991	42.1	38.18	40.921	15.61
R^2	0.21	0.47	0.91	0.91	0.90	0.55	0.92
Adj – R^2	-0.78	-0.18	0.79	0.79	0.76	-0.01	0.82
p value	0.94^{NS}	0.64^{NS}	0.03^{S}	0.03^{S}	0.04^{S}	0.52^{NS}	0.02^{S}
Lack of fit	0.13^{NS}	0.007^{S}	0.15^{NS}	0.16^{NS}	0.15^{NS}	0.27^{NS}	0.06^{S}
Note: TSS – Total soluble solids, MC- moisture content, TPC – Total phenol content, and FRAP- Ferric reducing antioxidant power, TAC- Total anthocyanin content							
RMSE - Root mean squared error, R^2- Coefficient of determination and Adj-R^2- Adjusted R^2, *p* value – probability value							
RSM, *p*-value of lack-of-fit, if > 0.05 (not significant) means that the model fits well.							
Note – ^{S}- Significant, ^{NS}- Non significant							

$$Y_{(TSS)} = 81.43 + 67.70X_1 - 2.34X_2 - 6.01X_1X_2 + 34.39X_1^2 - 18.97X_2^2$$

$$Y_{(MC)} = -17.96 - 174.13X_1 + 51.94X_2 + 84.93X_1X_2 - 102.75X_1^2 + 8.93X_2^2$$

Figure 1. **a** and **1. b** illustrates the 3D relationships among concentration, temperature, and evaporation time on TSS and moisture content, respectively. For TSS, higher temperature levels positively impacted TSS, while prolonged evaporation time led to a decrease, exacerbated by the interaction between temperature and time. Conversely, moisture content exhibited a negative correlation with temperature and time. Notably, TSS in vacuum-concentrated karonda juice at 50℃ for 150 minutes was 28°Bx whereas when concentrated at 55℃ for 150 minutes resulted in highest TSS i.e., 89°Bx, aligning with findings in litchi juice by Rani et al. (2021), indicating increased TSS with temperature. The decrease in moisture content alongside increased TSS suggests moisture evaporation during concentration. The profound effect of concentration time on moisture content, rather than temperature, is highlighted, possibly due to water's viscosity dependency on temperature. Leong and Chua (2020) similarly observed these dynamics in pineapple juice concentration.

Effect of vacuum concentration process parameters on ascorbic acid

The ANOVA analysis (Table 3) affirms a well-fitted model for ascorbic acid, with a significant model *p*-value of 0.033, indicating a strong fit. Additionally, the model displays promising predictive capabilities, boasting an impressive R^2 value of 0.91 and an adjusted R^2 of 0.79. The RSM equation describing the effects of process variables on ascorbic acid content (Y) of vacuum concentrated karonda juice in terms of actual levels of variables is given as:

$$Y_{(\text{ascorbic acid content})} = 12.50 + 1.25X_1 + 1.20X_2 - 0.42X_1X_2 + 0.87X_1^2 - 5.97X_2^2$$

Figure 1. **c** illustrates the three-dimensional relationships among temperature, and time on ascorbic acid, as per the generated model. Notably, the linear model showcases a positive effect, resulting in an increase in ascorbic acid levels with higher temperatures and time. However, the quadratic model of concentration and time, along with the interactive effects of concentration, temperature, and evaporation time, demonstrate a notable negative effect, leading to a decrease in ascorbic acid. Interestingly, the quadratic model for temperature also shows a significant effect on the concentration process. Across various experimental combinations, a wide range of ascorbic acid concentrations, ranging from 4.52 to 14.66 mg $100g^{-1}$, was observed. The minimum value of 4.52 mg $100g^{-1}$ was obtained with concentration temperature of 45˚C and concentration duration of 90 minutes. Notably, the maximum value (14.66 mg $100g^{-1}$) was attained with a concentration temperature of 55°C and a concentration duration of 150 minutes, likely attributable to water removal during the concentration process.

Effect of vacuum concentration process parameters on TPC

Based on the proposed model, the linear and quadratic effects of concentration, temperature, evaporation time, and their interactive effect had a significant impact on TPC. The analysis (Table 3) revealed a model *p*-value of 0.032, indicating a well-fitted model. Moreover, the model demonstrated strong predictive abilities with an R^2 of 0.91 and an adjusted R^2 of 0.79. The RSM equation describing the effects of process variables on total phenolic content (Y) of vacuum concentrated karonda juice in terms of actual levels of variables is given as:

$$Y_{(\text{TPC})} = 548.07 + 509.59X_1 - 93.82X_2 - 49.13X_1X_2 + 282.67X_1^2 - 246.08X_2^2$$

Figure 1. **d** illustrates the three-dimensional relationships among concentration, temperature, and time on TPC, as per the generated model. TPC of concentrated samples fell within the range of 119.58 to 386.97 mg GAE $100g^{-1}$. It was observed that at lower temperatures (45°C) and times (90 minutes), the concentrated TPC was lower (119.58 mg GAE $100g^{-1}$). Notably, when karonda juice was concentrated at 55°C for 150 minutes, the highest amount of TPC was recorded, reaching 386.97 mg GAE $100g^{-1}$. This increase can be attributed to the evaporation of water during processing, resulting in a smaller diminution

of bioactive compounds, decreased activity of polyphenoloxidase, concentration of polyphenols, and stabilization of anthocyanins. However, with a duration increase to 210 minutes, there was a decrease in TPC, likely due to the degradation of bioactive compounds when exposed for longer duration at higher temperatures, as observed in pomegranate by Orak (2009) and Saenz et al. (2010) in pomegranate juice concentrate.

Effect of vacuum concentration process parameters on TAA

The Ferric Reducing Antioxidant Potential (FRAP) assay evaluates sample antioxidant activity and can indicate phenolic content. Results of ANOVA indicate a well-fitted model for TAA (*p*-value: 0.042), with strong predictive ability (R^2: 0.90, adjusted R^2: 0.76). The RSM equation describing the effects of process variables on total antioxidant activity (Y) of vacuum concentrated karonda juice in terms of actual levels of variables is given as:

$$Y_{(TAA)} = 486.61 + 490.96X_1 - 98.01X_2 - 64.52X_1X_2 + 275.30X_1^2 - 203.97X_2^2$$

Figure 1. **e** illustrates the impact of concentration, temperature, and time on TAA. Linear and quadratic effects of concentration and temperature positively influence activity, contributing to the observed peak activity at 55°C for 150 minutes. Conversely, negative effects, such as those from prolonged evaporation time or temperature-time interaction, result in decreased activity, as evidenced by the decline to 196.61 mg AAE $100g^{-1}$ at 210 minutes, likely due to phenolic compounds degradation. Understanding these positive and negative effects is crucial for optimizing conditions to maximize total antioxidant activity in vacuum-concentrated juice, in line with the findings of Nhi et al. (2020) in vacuum-concentrated pomelo juice.

Effect of vacuum concentration process parameters on TAC

Results of ANOVA (Table 3) indicate that the RSM model effect is not significant, with a *p*-value of 0.5265, suggesting adequate explanation of variability in the experiment, supported by a maximum R^2 value of 0.97. The RSM equation describing the effects of process variables on anthocyanin content (Y) of vacuum concentrated karonda juice in terms of actual levels of variables is given as:

$$Y_{(TAC)} = 41.23 - 165.83X_1 - 4.86X_2 + 11.80X_1X_2 - 158.70X_1^2 - 69.46X_2^2$$

Figure 1. **f** illustrates the 3D relationship between concentration, temperature, and time on anthocyanin content, revealing negative linear and quadratic effects of these factors, yet a significant positive interactive effect between temperature and time. The anthocyanin content of vacuum-concentrated karonda juice was ranging from 11.36 mg $100g^{-1}$ at temperature of 50°C for 90 minutes to 129.34 mg $100g^{-1}$, peaking at 55°C for 150 minutes, likely due to low temperature and rapid mass transfer via the vacuum process. These findings align with Mahmoud et al. (2017)'s work on concentrated pomegranate

juice, though a significant decrease in anthocyanin content occurred after exposure to 55°C for 150 minutes, possibly due to pigment polymerization, consistent with Jiratanan and Liu (2004)'s findings in beets.

Effect of vacuum concentration process parameters on iron content

From the results of ANOVA presented in Table 3, the RSM model effect is significant (p 0.005) *i.e.*, 0.025, which implies that the response surface model has explained adequately the variability present in the experiment conducted and max R^2 value is 0.99, which ensures RSM fit is adequate. The RSM equation describing the effects of process variables on iron content (Y) of vacuum concentrated karonda juice in terms of actual levels of variables is given as:

$$Y_{(\text{iron content})} = 217.20 - 209.07X_1 - 37.14X_2 - 17.99X_1X_2 + 119.18X_1^2 - 99.78X_2^2$$

Iron content was negatively affected by the concentration time and positively affected by evaporation temperature in the linear model, while temperature was positively affected and time was negatively affected in the quadratic model. For interactive effects concentration temperature and time had negatively affected the iron content. Figure 1. **g** shows the 3D graph for the effect of concentration temperature and time on iron content based on generated model.

The values of treatment combination of temperature for vacuum concentration was 45, 50 and 55℃, time was 90, 150 and 210 min and the corresponding iron content range happens to be 45.65 to 150.54 mg $100g^{-1}$ (Table 2). As concentration temperature was increased to 55℃ and duration to 150 min, the iron content increased to 150.54 mg $100g^{-1}$, the moisture content decreased thereby increasing the concentration of iron content in the concentrated samples.

GC-MS analysis of fresh karonda juice and vacuum concentrated karonda juice

The GC-MS chromatograms comparing fresh juice with juice concentrated at 55°C for 150 minutes are depicted in Fig. 2. **a** and **b**, respectively. From the total compounds, only the five major volatile compounds present in vacuum-concentrated karonda juice are listed in Table 4. Among these, the predominant volatile compound identified in both fresh and vacuum-concentrated juice was hydrocarbon tetrahydrofuran, consistent with findings by Pino et al. (2004) on Karanda fruit. Tetrahydrofuran is recognized for its antioxidant properties against oxidative stress-related ailments, as noted in studies by Fujimoton et al. (1988) and Vo et al. (2023). Vacuum concentration at 55°C for 90 minutes revealed oxirane (15.42%) as a major volatile compound, known for its reported anticancer, anti-inflammatory, immunosuppressive, and antitumor activities by Ram *et al.* (2019). Additionally, the second highest volatile compound identified was 3-Hydroxy-3-methylvaleric acid (9.04%), which aids in muscle growth promotion and reduces muscle breakdown. Conversely, vacuum concentration at 45°C for 150 minutes yielded acetoacetate (24.32%) as the major volatile compound, known for its neuronal cell protection

from oxidative glutamate according to Noh et al. (2006). The second highest volatile compound identified was sotolone (11.67%), recognized for its anti-virulence properties, corroborating findings by Aldawsari et al. (2021).

CONCLUSION

Present study investigated the effects of concentration temperature and evaporation time on TSS, moisture content, ascorbic acid, TPC, total antioxidant activity, TAC, iron content and GC-MS profile of volatile compounds of karonda juice concentrate. Temperature and time showed significant changes in quality parameters of concentrated samples. In conclusion, the vacuum concentration technique applied to karonda juice emerges as a promising and efficient method for enhancing its overall quality. Through meticulous experimentation and analysis, this research has demonstrated the significant impact of vacuum concentration at 55℃ for 150 min on the concentration of valuable bioactive compounds. The concentrated karonda extract showcased enhanced antioxidant properties, indicative of its potential for diverse applications in the food and beverage industry.

Declarations

This manuscript adheres to the following declarations:

Funding: PG grants by University of Horticultural Science, Bagalkot

Conflicts of Interest/Competing Interests: Not applicable

Ethics Approval: Not applicable

Consent to Participate: Not applicable

Consent for Publication: Not applicable

Availability of Data and Material: Not applicable

Code Availability: Numerical optimization of concentration parameters was conducted using SAS statistical software package version 9.3 (SAS Institute, Cary NC)

Authors' Contributions:

1. Chinmayi A. S. - Conceptualization, Methodology, Formal analysis, Writing - original draft preparation, writing review and editing,
2. Suresh G. J.- Writing - review and editing, Supervision
3. Rosemary M. X - Writing - review and editing
4. Sadananda G. K. – Investigation, Visualisation
5. Venugopalan R. - Methodology, Data analysis, Statistical interpretation
6. Vasudeva K. R. - Investigation, Visualisation

7. Suresha K. B. – Investigation and data curation
8. Swamy G. S. K. - Investigation, Data curation, Visualization

Acknowledgements

No acknowledgements are included in this manuscript. All information that could reveal the authors' identity has been omitted in adherence to the double-blind review process mandated by FABT.

References

1. Aldawsari, M. F., Khafagy, E. S., Saqr, A. A., Alalaiwe, A., Abbas, H. A., Shaldam, M. A., Hegazy, W. A., & Goda, R. M., (2021). Tackling virulence of *Pseudomonas aeruginosa* by the Natural Furanone Sotolon. *Antibiotics*, 10(7): 871. https://doi.org/10.3390/antibiotics10070871
2. AOAC., (1990). Official methods of analysis, (*15th Edition)*, Association of official analytical chemists, Washington, DC.
3. AOAC., (2006). Official methods of analysis, (*21th Edition)*, Association of official analytical chemists, Washington, DC.
4. Benzie, I.F, and Strain, J.J. (1996).The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal. Biochem. 239(1), 70-76. https://doi.org/10.1006/abio.1996.0292
5. Berk, Z. (2016). *Production of citrus juice concentrates. Citrus Fruit Processing*. Academic Press, San Diego, CA, (Chapter 9).
6. Bose, U., Saha, R., Hossain, L., & Rahman, A. A. (2011). Neuropharmacological and diuretic activities of Carissa carandas L. leaf, Pharmacology Online, 2: 320-327.
7. Fujimoto, Y., Eguchi, T., Kakinuma, K., Ikekawa, N., Sahai, M., & Gupta, Y. K., (1988). Squamocin, a new cytotoxic bis-tetrahydrofuran containing acetogenin from *Annona squamosa*. *Chemical and pharmaceutical bulletin*, 36(12): 4802-4806. https://doi.org/10.1248/cpb.36.4802
8. Fuleki, T., & Francis, F. J., (1968). Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science*, 33(1):72-77. https://doi.org/10.1111/j.1365-2621.1968.tb00887.x
9. Harsimrat, K. B., & Dhawan, S. S., (2003). Evaluation of methods of preparation of guava juice concentrate. *Crop Research.*
10. Itankar, P. R., Lokhande, S. J., Verma, P. R., Arora, S. K., & Sahu, R. A., (2011). Antidiabetic potential of unripe *Carissa carandas* L. fruit extract. *Journal of Ethnopharmacol.*,135: 430-433. https://doi.org/10.1016/j.jep.2011.03.036
11. Jeong, S. M., Kim, S. Y., Kim, D. R., Jo, S. C., Nam, K., & Ahn, D., (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. Journal of Agricultural Food Chemistry. 52 (11): 3389-3393. https://doi.org/10.1021/jf049899k

12. Jiratanan, T., & Liu, R. H., (2004). Antioxidant activity of processed table beets (*Beta vulgaris var. conditiva*) and green beans (*Phaseolus vulgaris* L.). *Journal of agricultural and food chemistry,* 52: 2659–2670. https://doi.org/10.1021/jf034861d
13. Leong, C. Y., & Chua, L. S., (2020). Optimization of concentrating process using rotary vacuum evaporation for pineapple juice. *Chemical Engineering*, 78.
14. Mahmoud, M. H., Seleet, F. L., & Foda, M. I., (2017). Effect of different concentration techniques on some properties of fresh and stored pomegranate juice. *Asian Journal of Scientific Research*, 10(4): 290-298. https://doi.org/10.3923/ajsr.2017.290.298
15. Nhi, T. T. Y., Phat, D. T., Quyen, N. N., Cang, M. H., Truc, T. T., Bach, L. G., & Muoi, N. V., (2020, December). Effects of vacuum concentration on color, polyphenol and flavonoid contents and antioxidant activity of pomelo *Citrus maxima* juice. In *IOP Conference Series: Materials Science and Engineering.* 991(1).
16. Noh, H. S., Hah, Y. S., Nilufar, R., Han, J., Bong, J. H., Kang, S. S., Cho, G. J., & Choi, W. S., (2006) Acetoacetate protects neuronal cells from oxidative glutamate toxicity. *Journal of neuroscience research*, 83(4): 702-709. https://doi.org/10.1002/jnr.20736
17. Orak, H. H., (2009). Evaluation of antioxidant activity, colour and some nutritional characteristics of pomegranate (*Punica granatum* L.) juice and its sour concentrate processed by conventional evaporation. *International journal of food sciences and nutrition,* 60(1): 1-11. https://doi.org/10.1080/09637480701523306
18. Ram, V. J., Sethi, A., Nath, M., & Pratap, R., (2019). The chemistry of heterocycles: nomenclature and chemistry of three to five membered heterocycles. *Elsevier.*
19. Rani J., Shelke A., Bansal B., & Gautam V., (2021 November). Concentrate development from Litchi Juice and quality evaluation during storage. In *Journal of Physics: Conference Series*, 2070(1): 012072. IOP Publishing
20. Saenz, C., Seguel, J., Gorena, T., & Sepulveda, E., (2010, October). Effect of the concentration temperature on some bioactives compounds and rheological properties of pomegranate juices. *In: International Conference Food Innovation, Uni. Politecnica De Valencia. Food Innova*: 1-4.
21. Singleton, V.L., and Rossi J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enology and Viticulture, 16(3), 144-158.
22. Vijayanand, P., Kulkarni, S. G., & Prathibha, G. V., (2010). Effect of pectinase treatment and concentration of litchi juice on quality characteristics of litchi juice. *Journal of Food Science Technology*, 47(2): 235-239. https://doi.org/10.1007/s13197-010-0023-3
23. Vo, Q. V., Hoa, N. T., Thong, N. M., Nam, P. C., Quang, D. T., Hien, N. K., & Mechler, A., (2023). The antioxidant activity of tetrahydrofuran lignans from *Anogeissus rivularis*: theoretical insights into the radical scavenging activity and enzyme inhibition. *New Journal of Chemistry*, 47(37):17314-17322. https://doi.org/10.1039/D3NJ03344A

Tables

Table 4 is available in the Supplementary Files section.

Figures

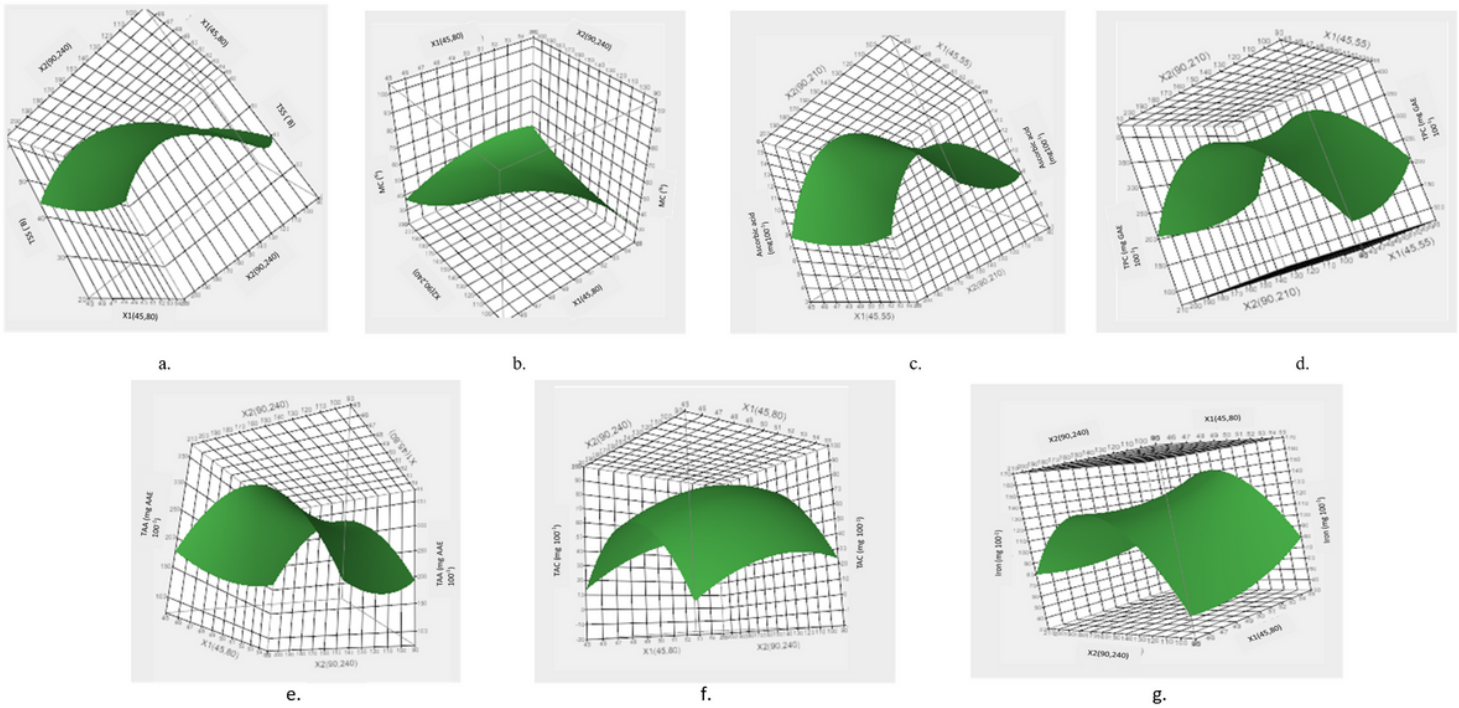

Figure 1

Response surface for a. TSS (˚B), b. MC (%), c. Ascorbic acid ($mg100^{-1}$), d. TPC (mg GAE 100^{-1}), e. TAA (mg AAE 100^{-1}), f. TAC ($mg100^{-1}$) and g. Iron ($mg100^{-1}$) of concentrated karonda juice as a function of (X_1) temperature (45-55˚C) and (X_2) time (90-210 min.)

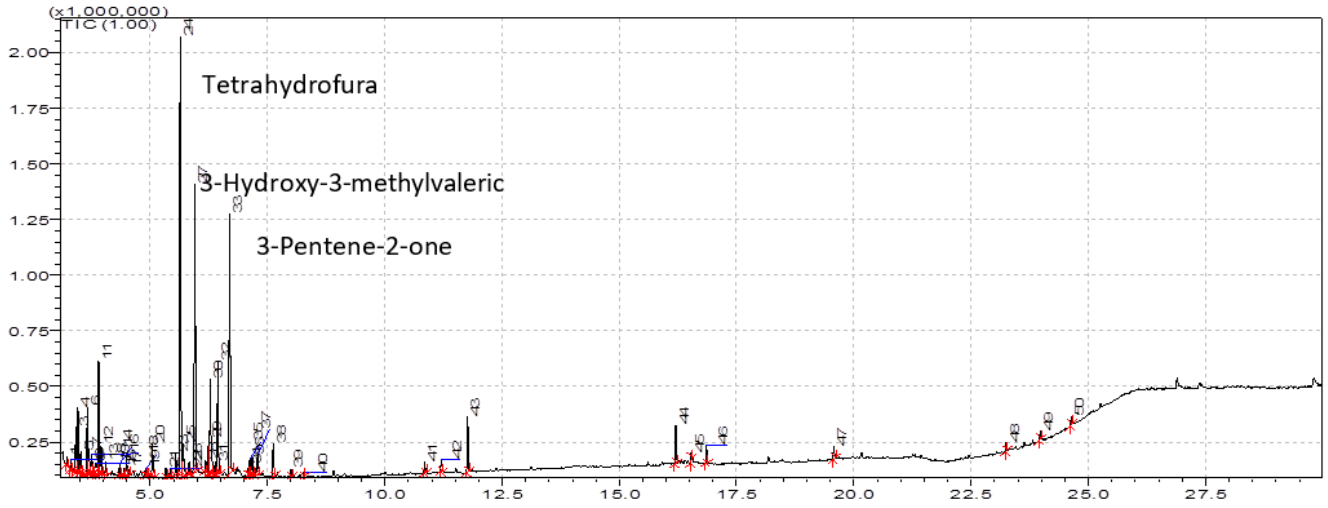

Figure 2

GC-MS Chromatograms of a. fresh karonda juice and b. vacuum concentrated karonda juice at 55°C for 150 min

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table4.docx