Optimization of Karonda (Carissa carandas L.) Bread Spread Formulation Using Box–Behnken Response Surface Methodology and Its Nutritional Evaluation

Amit Kumar Singh
amitsinghbckv@gmail.com

Department of Post Harvest Technology, College of Horticulture Banda University of Agriculture and Technology, Banda-21001, India

Sameer Ahmad
Department of Bioengineering, Integral University, Lucknow, Uttar Pradesh,India

Gitanjali Singh Maurya
Department of Post Harvest Technology, College of Horticulture Banda University of Agriculture and Technology, Banda-21001, India

Deepak Kumar Verma
Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur 721302, West Bengal, India

Vigya Mishra
Department of Post Harvest Technology, College of Horticulture Banda University of Agriculture and Technology, Banda-21001, India

Bijendra Kumar Singh
Department of Fruit Science, College of Horticulture Banda University of Agriculture and Technology, Banda-21001, India

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Amit Kumar Singh*[1], Sameer Ahmad[2], Gitanjali Singh Maurya[3], Deepak Kumar Verma [4], Vigya Mishra [5],Bijendra Kumar Singh[6]**

[1]Corresponding Author: amitsinghbckv@gmail.com
Department of Post-Harvest Technology, College of Horticulture
Banda University of Agriculture and Technology, Banda-21001, India
*Corresponding Author

[2] Department of Biotechnology IILM University greater Noida, Uttar Pradesh, India

**Corresponding Author

[3] Department of Post-Harvest Technology, College of Horticulture
Banda University of Agriculture and Technology, Banda-21001, India

[4]Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur 721302, West Bengal, India

[5]Department of Post Harvest Technology, College of Horticulture
Banda University of Agriculture and Technology, Banda-21001, India

[6]Department of Fruit Science, College of Horticulture
Banda University of Agriculture and Technology, Banda-21001, India

Abstract:

Karonda is an underutilized indigenous berry fruit possessing high nutritional value, a distinctive sour taste, and adaptability to semi-arid regions. However, its short shelf life and lack of consumer awareness have limited its utilization and commercial importance. The present study aimed to develop and optimize a karonda-based bread-spread using Response Surface Methodology and a Box–Behnken Design. Three independent variables, namely aonla pulp (50–70 g), sugar (45–55 g), and citric acid (0.5–1.0 g), were optimized through 15 experimental runs generated using Design Expert® software version 13. Physicochemical, nutritional, and color parameters including total soluble solids (TSS), total sugars, reducing sugars, titratable acidity, ascorbic acid, flavonoids, antioxidant activity, β-carotene, total phenolic content (TPC), soluble protein, viscosity, and colour values (L*, a*, b*) were evaluated. Second-order regression models adequately fitted all responses. The optimized formulation consisted of 56.613 g aonla pulp, 54.607 g sugar, and 0.815 g citric acid, with an overall desirability score of 1.000. The optimized bread-spread exhibited maximum values of 66.10% TSS, 52.68% total sugars, 12.89% reducing sugars, 1.44% titratable acidity, 25.80 mg/100 g ascorbic acid, 10.40% flavonoids, 30.18% antioxidant activity, 4921.84 IU/100 g β-carotene, 107.37 mg GAE/100 g TPC, 0.991% soluble protein, and 1204.34 P.a viscosity. The colour values recorded were L* 12.75, a* 16.25, and b* 13.55. Experimental values showed close agreement with predicted values, confirming the suitability of the optimization model. The study demonstrated the potential of karonda fruit for the development of a nutritious and value-added bread-spread product.

Keywords: Karonda bread-spread; BBD-RSM; Functional food; optimization; TPC; Antioxidant activity; flavonoids

1. Introduction

Bread-spread is a medium-moisture food product prepared by boiling fruit pulp with sugar (sucrose), pectin, acid, and other ingredients to obtain a desirable gel-like consistency while retaining the characteristic texture and flavour of the fruit (Basu et al., 2013; Belovic et al., 2017). Traditionally, bread-spread is formulated using approximately 45% fruit pulp and 55% sugar, and the mixture is heated until it reaches about 65% total soluble solids. According to the European Union Council guidelines (2004), bread-spread or jam is defined as a mixture of sugars and pulp or puree of one or more fruits processed to achieve a suitable gel consistency (Abid et al., 2018). Bread-spreads are widely consumed as breakfast accompaniments and ingredients in bakery, dairy, and confectionery products (Igual et al., 2014). In recent years, increasing awareness regarding health, nutrition, obesity, metabolic disorders, and diabetes has shifted consumer preference toward nutrient-rich and functional food products (Belovic et al., 2017).

Karonda is an underutilized berry fruit of Himalayan origin, commonly grown in rural and sub-urban regions of India as a protective hedge plant because of its dense foliage and thorns (Saroj et al., 2022). The fruit possesses bright pink edible berries and has long been recognized in traditional medicinal systems such as Ayurveda, Unani, and Homoeopathy. Karonda is rich in bioactive compounds including anthocyanins, quercetin, tannins, flavonoids, phenolics, and vitamin C, which contribute to its antioxidant, anti-diabetic, hepatoprotective, and immune-boosting properties (Singh and Saxena, 2019; Nourmohammadi et al., 2021). Nutritionally, karonda fruits contain appreciable amounts of energy 42.5kcal, protein 0.39-1.1g, fat 2.5-4.63g, carbohydrate 0.51-2.9g, fiber 10-15g, calcium 21mg, phosphorus 28mg, vitamin A1619 IU, and ascorbic acid 11mg per 100 g of fruits, making them a valuable component of a healthy diet (Malik et al., 2010). In addition, karonda is naturally rich in pectin and exhibits excellent gelling properties, indicating its strong potential for use in bread-spread and jelly preparation as a substitute for commercial pectin. Despite these advantages, the industrial and commercial utilization of karonda remains limited.

Process optimization is essential for developing food products with desirable quality characteristics such as texture, spreadability, colour, flavour, and consistency. In bread-spread production, factors including fruit pulp concentration, sugar level, and citric acid concentration significantly influence the physicochemical and sensory properties of the final product. Conventional optimization approaches based on one-factor-at-a-time experiments are often time-consuming and fail to identify interactions among variables (Chettri et al., 2024). In contrast, Response Surface Methodology provides a systematic and efficient statistical approach for evaluating the combined effects of multiple variables and optimizing product formulations. Among RSM techniques, the Box–Behnken Design is widely used because it requires fewer experimental runs while providing reliable and precise optimization results (Chahdoura et al., 2024).

Therefore, the present study was undertaken to optimize the formulation parameters for the preparation of karonda fruit bread-spread using Box–Behnken Design under RSM. The effects of pulp concentration, sugar content, and citric acid concentration on the physicochemical, nutritional, rheological, and colour characteristics of the bread-spread were investigated. The study aims to promote the utilization of underexploited karonda fruit in value-added products and provide a scientific basis for its potential commercial-scale production in the food processing industry.

2. MATERIALS AND METHODS

2.1 Raw Materials. Freshly harvested ripe Karonda fruits were collected from the University Farm of Banda University of Agriculture and Technology. The fruits were thoroughly washed to remove dirt and dust and to maintain aseptic conditions. Subsequently, the fruits were peeled, and the peel, seeds, and core were removed prior to processing. Commercial table sugar and citric acid used in the preparation of bread-spread were procured from the local market.

2.2 Karonda fruit bread-spread preparation. The bread-spread was formulated using pulp from fresh Karonda, sugar, and citric acid in optimized proportions. The extracted karonda pulp was blended to obtain a smooth consistency and then transferred into a cooking vessel. Sugar was added in a predetermined ratio and the mixture was heated on low flame with continuous stirring until it reached approximately 65 °Brix. Citric acid was subsequently incorporated to improve taste, enhance gel stability, and act as a preservative by maintaining an appropriate pH for safe storage. The mixture was further cooked on medium heat with continuous stirring for about 1 minute to ensure uniform consistency and proper gel formation. The prepared bread-spread samples were then allowed to cool and subjected to further physicochemical, nutritional, and quality analyses.

2.3 Physicochemical analysis

2.3.1 Total Soluble Solids. To measure the total soluble solids (TSS) a digital refractometer (HANNA HI96801, Romania) was used and expressed in ^{O}Brix/% scale. A drop of bread-spread was placed on the prism of the digital refractometer after reset to zero by distilled water, and the total soluble solids were read at 20°C. Each sample was measured in three repetitions.

2.3.2 Viscosity. The viscosity of each sample was measured using a rotational viscometer (Anton PaarVisco QC 300). Measurements were performed at 25 ^{O}C using a spindle-type RS5 at room temperature (29^{O}C ± 0.1).

2.3.3 Titratable acidity. The titratable acidity (TA) was estimated by titrating the sample against 0.1N NaOH as per the standard procedures (AOAC, 2000).

2.3.4 Total and reducing sugars. The sugar content of the Karonda bread-spread was estimated using Fehling's solution by the Lane and Eynon method (1923). For total sugar determination, 5 g of the prepared sample was crushed in 10 mL of distilled water, followed by the addition of 2–3 drops of concentrated hydrochloric acid (HCl) to facilitate acid hydrolysis. The mixture was then heated until a brown colour developed, after which it was cooled and neutralized. The volume was made up to 50 mL with distilled water. The prepared solution was titrated against Fehling's solution A and B using methylene blue as an indicator. The endpoint was identified by the appearance of a brick-red precipitate, and the total sugar content was calculated accordingly.

For reducing sugars, the same procedure was followed; however, acid hydrolysis was omitted to ensure that only naturally present reducing sugars were estimated without conversion of non-reducing sugars. The titration was carried out similarly against Fehling's solution to determine the reducing sugar content.

$$Total\ sugars\ or\ reducing\ sugars\ (\%) = \frac{factor\ X\ volume\ made\ up\ X\ 100}{titer\ value\ X\ weight\ of\ the\ sample}$$

2.3.5 Soluble protein. The soluble protein content of the Karonda bread-spread was determined using the Lowry method (Lowry et al., 1951). For analysis, 0.5 g of the sample was homogenized in 10–30 mL of buffer solution and allowed to stand at room temperature for 1 hour to ensure proper extraction of soluble proteins. After incubation, 0.1 mL of the supernatant was taken and mixed with 0.9 mL of distilled water, followed by the addition of 5 mL of reagent C. The mixture was incubated for 10–15 minutes at room temperature. Thereafter, 0.5 mL of Folin–Ciocalteu reagent was added, and the reaction mixture was kept in darkness for 30 minutes to allow colour development. The absorbance was recorded at 666 nm using a UV–Visible spectrophotometer. The soluble protein content was calculated using a standard calibration curve and expressed as mg/100 g of sample or percentage.

2.3.6 Colour. The colour characteristics of the Karonda bread-spread were evaluated using a Hunter Lab colour system. The instrument operates as a filter colorimeter that expresses colour in a three-dimensional space based on human visual perception. The colour parameters were recorded in terms of L*, a*, and b* values, where L* represents lightness ranging from dark to light, a* represents the red–green spectrum, and b* represents the yellow–blue spectrum. This system provides an objective and quantitative measurement of colour, closely aligning with the way the human eye perceives colour variations in food products.

2.4 Biochemical Analysis

2.4.1 Total Phenolic Content. Total phenolic content (TPC) of the extract was determined using a modified Folin–Ciocalteu (FC) method as described by Tripathy and Srivastav (2023a). Briefly, 0.2 mL of sample was taken in a test tube, followed by the addition of 2.8 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent (10-fold diluted). Subsequently, 0.75 mL of sodium carbonate (Na_2CO_3, 20% w/v) solution was added. The mixture was thoroughly mixed and incubated in the dark for 90 min. Absorbance was measured at 760 nm using a UV–Vis spectrophotometer. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g dw), using gallic acid as the standard.

2.4.2 Flavonoid Content. Flavonoid content was estimated using the method of Joy et al. (2015). Briefly, 0.5 g of sample was homogenized with distilled water and the volume was made up to 1 mL. Subsequently, 5 mL of cinnamaldehyde reagent solution (HCl:CH_3OH:cinnamaldehyde, 1:3:0.004 v/v/v) was added. The absorbance was measured at 640 nm using a UV–Vis spectrophotometer.

$$TFC\left(\frac{MgGAE}{100g}\right) = \frac{Conc.of\ standard}{OD\ of\ standard} X\ OD\ of\ Test\ X\ \frac{Valume\ made}{Volume\ taken} X \frac{1}{Dry\ Weight} X \frac{1}{1000}$$

2.4.3 β-Carotenoid Content. The β-carotene content of karonda bread-spread was determined using the method described by Doka et al. (2015). The absorbance of the sample solution was measured at 450 nm using a UV–Vis spectrophotometer.

2.4.4 Antioxidant Activity. Antioxidant activity was determined using a slightly modified DPPH assay as described by Tripathy and Srivastav (2023). Briefly, 0.2 mL of sample was mixed with 0.95 mL of DPPH solution and 2.85 mL of methanol in a test tube. The reaction mixture was incubated in the dark for 30 min. Absorbance was then measured at 517 nm using a UV–Vis spectrophotometer, with methanol used as the blank. Antioxidant activity was expressed as mg gallic acid equivalents antioxidant capacity (GAEAC) per g dry weight (mg GAEAC/g dw), using gallic acid as the standard. The following equation was used for quantification:

$$GAEAC\ (mg\ GAEAC/g\ dw)\ = \frac{\Delta Abs_{sample}}{\Delta Abs_{standard}} \times C \times \frac{V}{W}$$

Where, C is the concentration of GA in mg/mL, V is the sample volume in mL and W is the weight of the dried sample taken in g.

2.4.5 Ascorbic Acid Content. Ascorbic acid content of the concentrated samples was determined using a modified 2,6-dichlorophenolindophenol (DCPIP) method as described by AOAC (2000). The results were expressed as mg ascorbic acid per 100 g fresh weight (mg/100 g FW), using a standard calibration curve of L-ascorbic acid.

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

2.5 Optimization of the process parameters. To obtain the optimal combination and improve consumer acceptability of karonda bread-spread, Response Surface Methodology (RSM) using a Box–Behnken design was employed. The independent variables selected for optimization were karonda pulp (A, 50-70g), sugar (B, 45-55g), and citric acid (C, 0.5–1g). The experimental design was generated using Design-Expert software (Version 13), resulting in a total of 15 experimental runs, including three centre points per block. The responses evaluated included total soluble solids, total sugars, reducing sugars, viscosity, titratable acidity, ascorbic acid, flavonoids, soluble protein, β-carotene, total phenols, antioxidant activity, and colour values (L*, a*, and b*).

The relationship between independent variables and responses was described using a second-order polynomial equation in terms of coded factors. By convention, the high and low levels of the factors were coded as +1 and −1, respectively (Table 1). The coded model facilitates evaluation of the relative influence of each factor through comparison of regression coefficients. Model development involved fitting experimental data to the quadratic equation given below:

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta11X11 + \beta22X23 + \beta33X33 + \beta12X12 + \beta13X13 + \beta23X23$$

Where $\beta0$, ($\beta1$, $\beta2$, $\beta3$) and ($\beta11$, $\beta22$, $\beta33$, $\beta12$, $\beta13$, $\beta23$) are the constants, linear and quadratic regression coefficients respectively and Xi's are the coded independent variables. The statistical significance was assessed at a significance level of $p = 0.05$. The objectives were subsequently integrated into a comprehensive desirability function to address the issue of responses.

Table 1: Process parameters for the optimization of Karonda-bread-spread

Process Parameters	**Unit**	**Coded levels**		
		-1	**Center**	**+1**

A: Karonda Pulp	**g**	50	60	70
B: Sugar	**g**	45	50	55
C: Citric acid	**g**	0.5	0.75	1

2.6 Statistical analysis

In this study, Design-Expert 13 (Stat-Ease, Inc., Minneapolis, MN, USA) was used to analyze the data and optimize the bread-spread production process. The correlation coefficient and the lack- of- fit criterion were used to determine the significance of regression equation models (Table 3). Analysis of variance (ANOVA) was performed on linear or single second- order regression coefficients, main interaction, and double effects. The significance of all terms in a polynomial was determined statistically by calculating the value of P at the probability level of 0.05.

3. RESULTS AND DISCUSSION

3.1 Optimization of Response Surface

3.1.1 Model Fitting

Table 2 displays the response data for each experimental run. The quadratic polynomial regression analysis was conducted using Design-Expert 13 software on the data presented in Table 3. This study resulted in the development of the following fourteen quadratic regression equations:

$TSS = 66.33 + 1.24A + 0.2525B + 0.0250C + 0.1875AB + 0.0225AC + 0.0075BC + 0.3838A^2 – 0.0412B^2 + 0.0237C^2$

$\text{Total sugars} = 56.00 + 9.59A + 0.9425B + 0.1725C + 0.7875AB + 0.0025AC – 0.0025BC - 3.16 A^2 -0.3829B^2 - 0.2229C^2$

$\text{Reducing sugars} = 13.52 + 2.40A + 0.2225B + 0.0175C + 0.0225AB – 0.0025AC + 0.0025BC + 0.1187A^2 - 0.362B^2 - 0.0262C^2$

$\text{Titratable acidity} = 1.42 + 0.0391A + 0.0024B + 0.1170C – 0.0003AB + 0.0205 AC + 0.0000BC + 0.0069 A^2 – 0.0016B^2 + 0.0241C^2$

$\text{Ascorbic acid} = 0.2742 + 6.22A + 0.2675B + 0.3312C + 0.0225AB – 0.3000AC + 0.0375BC – 0.5075A^2 + 0.2200B^2 + 0.0375C^2$

Flavonoids = 10.98 + 2.48A + 0.0289B – 0.1189C – 0.0037AB + 0.7527AC + 0.0340BC + 0.8290A^2 + 0.3093B^2 – 0.4613C^2

Antioxidant activity = 31.90 + 6.18A + 0.0475B + 1.17C – 0.0150AB + 0.3950AC – 0.0350BC + 1.85A^2 – 0.1742B^2 – 0.0342C^2

β-Carotene = 5097.67 + 602.62A + 53.50B + 11.63C + 6.50AB + 12.25AC - 1.00BC – 27.46 A^2 – 19.71 B^2 – 11.46C^2

Total phenol content = 111.86 + 15.85A – 0.1325B + 2.24C – 0.6800AB + 0.5250AC + 0.1200BC – 2.06A^2 + 0.5700B^2 – 0.2400C^2

Soluble protein = 1.03 + 0.1607A + 0.0226B + 0.0026C – 0.0052AB – 0.0008AC – 0.0005BC – 0.0187 A^2 – 0.0110B^2 + 0.0045C^2

Viscosity = 1240.00 + 256.25A + 45.00B + 6.25C + 0.0000AB – 2.50AC + 0.0000BC + 28.75A^2 + 6.25B^2 -11.25C^2

Colour L* value = 12.94 + 1.03A + 0.1412B + 0.0275C + 0.0050AB – 0.0175AC – 0.0025BC + 0.0483A^2 + 0.0133B^2 – 0.0042C^2

Colour a* value = 16.42 + 1.61A + 0.4575B + 0.1250C + 0.2500AB + 0.0500AC + 0.0150BC + 0.1258A^2 – 0.0242B^2 + 0.0408C^2

Colour b* value = 13.87 + 1.11A + 0.0887B + 0.0137C – 0.0125AB – 0.0025AC + 0.0000BC – 0.3133A^2 + 0.0042B^2 – 0.0108C^2

Table-2: BBD design of the experimental plan of the optimization of Karonda bread-spread

	Process Variables			Responses													
Run	**Karonda Pulp (g)**	**Sugar (g)**	**Citric Acid (%)**	**TSS (%)**	**Total Sugars (%)**	**Reducing Sugars (%)**	**Titratable Acidity (%)**	**Ascorbic Acid (mg/100g)**	**Flavonoids (%)**	**Antioxidant Activity (%)**	**β-carotene (IU/100g)**	**Total Phenols (mg GAE/100g)**	**Soluble Protein (%)**	**Viscosity (m.Pa.s)**	**Colour L* Value**	**Colour a* Value**	**Colour b* Value**
1	60	55	0.50	66.42	56.26	13.67	1.346	28.07	10.541	30.29	5119	110.17	1.045	1270	13.09	16.66	13.94
2	60	55	1.00	66.47	56.29	13.71	1.547	28.24	11.197	33.21	5128	114.84	1.051	1280	13.13	16.89	13.97
3	50	45	0.75	65.32	42.75	11.03	1.404	21.04	10.009	27.38	4407	94.08	0.811	960	11.85	14.63	12.36
4	70	55	0.75	68.4	63.74	16.22	1.448	33.27	14.214	39.74	5707	125.29	1.187	1590	14.17	18.92	14.73
5	60	45	0.50	66.17	54.5	13.21	1.340	27.19	10.521	30.11	5003	109.78	1.003	1190	12.77	16.02	13.75
6	70	50	0.50	68.00	62.29	16.02	1.356	33.01	14.005	38.69	5634	122.29	1.177	1500	14.00	18.04	14.65
7	60	50	0.75	66.33	55.97	13.54	1.417	27.46	10.988	31.85	5105	111.59	1.037	1240	12.97	16.51	13.89
8	60	50	0.75	66.31	56.00	13.50	1.421	27.42	10.974	31.89	5097	112.15	1.034	1245	12.91	16.33	13.87
9	50	50	0.50	65.41	42.91	11.17	1.281	19.66	9.748	27.05	4448	92.29	0.859	1000	11.91	14.84	12.41
10	60	50	0.75	66.35	56.04	13.52	1.425	27.38	10.969	31.97	5091	111.84	1.031	1235	12.95	16.43	13.84
11	50	50	1.00	65.43	42.94	11.21	1.507	21.49	7.179	27.95	4459	95.77	0.864	1020	12.01	15.04	12.44
12	70	50	1.00	68.11	62.33	16.05	1.664	33.64	14.447	41.17	5694	127.87	1.179	1510	14.03	18.44	14.67
13	70	45	0.75	67.28	60.15	15.75	1.445	33.11	14.194	39.69	5594	127.81	1.148	1490	13.91	17.26	14.59
14	50	55	0.75	65.69	43.19	11.41	1.408	21.11	10.044	27.49	4494	94.28	0.871	1060	12.09	15.29	12.55
15	60	45	1.00	66.19	54.54	13.24	1.541	27.21	11.041	33.17	5016	113.97	1.011	1200	12.82	16.19	13.78

The analysis of variance (ANOVA) was conducted to illustrate the statistically significant impact of the independent variables on the dependent variables. Figure 1, 2 and 3 displays the response surface plots for all dependent variables in a three-dimensional representation. The model suitability was evaluated using various statistical measures, including *p* values, lack of fit, R^2 and adjusted R^2 values. These measures are presented in Table 3. The suitability of the model may be determined based on several criteria. Firstly, the *p* value should be less than 0.05, indicating statistical significance. Additionally, the lack of fit should be non-significant. Furthermore, the R^2 value should exceed 0.8, indicating a strong fit of the model to the data. Lastly, the adjusted R^2 value should be comparable to the R^2 value, as suggested by Tripathy and Srivastav (2024). Factor A was the most influential variable across almost all responses. Where, factor B significantly affected sugar-related properties such as viscosity, and colour. Factor C mainly influenced titratable acidity, antioxidant activity, β-carotene, and phenolics contents in karonda bread-spread. Most interaction effects were non-significant, indicating limited synergistic interactions among variables. Significant quadratic terms revealed nonlinear behavior and existence of optimum processing conditions. It was observed from Table 3 that the linear terms A and B are significant for all the regression models except for reducing sugars, titratable acidity, ascorbic acid, flavonoids, antioxidant and total phenol contents. The interactive term AC is statistically significant for reducing sugars, titratable acidity, ascorbic acid, flavonoids, antioxidant and total phenol contents. The quadratic term B^2 has statistical significance in all regression models except for ascorbic content, TA, and viscosity. Similarly, the quadratic term A^2 exhibits statistical significance in ascorbic acid, TSS, and viscosity content. Based on the ANOVA table (Table 3), it was determined that all of the examined models exhibited statistical significance ($p < 0.05$), indicating their appropriateness for prediction. Based on the data presented in Table 3, the R^2 values for the variable TSS, total sugars, reducing sugars, titratable acidity, ascorbic acid, flavonoids, antioxidant activity, carotenoid content, TPC, soluble protein, viscosity, colour value L*, a* and b* were found to be 0.9882, 0.9998, 0.9999, 0.9613, 0.9962, 0.9550, 0.9974, 0.9998, 0.9987, 0.9995, 0.9984, 0.9995, 0.9932, and 0.9998, respectively. These results suggest that the created model exhibits a high level of desirability (1.000). Nevertheless, the strong correlation between R^2 and modified R^2 provides further evidence of the experimental consistency, the ability to anticipate data accurately, and the potential use of the current model for predicting the design space (Li et al., 2021). All the responses demonstrated a lack of fit that was not statistically significant, suggesting that the models adequately fit the data.

Table-3: Estimated coefficients and corresponding ANOVA data representing the relationship between process parameters and response variables

Sources	Coefficient for the responses													
	TSS (%)	Total Sugars (%)	Reducing Sugars (%)	Titratable Acidity (%)	Ascorbic Acid (mg/100g)	Flavonoids (%)	Antioxidant Activity (%)	β-carotene (IU/100g)	Total Phenols (mg GAE/100g)	Soluble Protein (%)	Viscosity (m.Pa.s)	Colour L* Value	Colour a* Value	Colour b* Value
Intercepts	+66.33	+56.00	+13.52	+1.42	+27.42	+10.98	+31.90	+5097.67	+111.86	+1.03	+1240.00	+12.94	+16.42	+13.87
A	+1.24	+9.59	+2.40	+0.0391	+6.22	+2.48	+6.18	+602.62	+15.85	+0.1607	+256.25	+1.03	+1.61	+1.11
B	+0.2525	+0.9425	+0.2225	+0.0024	+0.2675	+0.0289	+0.0475	+53.50	-0.1325	+0.0226	+45.00	+0.1412	+0.4575	+0.0887
C	+0.0250	+0.0175	+0.0175	+0.1170	+0.3312	-0.1189	+1.17	+11.63	+2.24	+0.0026	+6.25	+0.0275	+0.1250	+0.0137
AB	+0.1875	+0.7875	+0.0225	-0.0003	+0.0225	-0.0037	-0.0150	+6.50	-0.6800	-0.0052	+0.0000	+0.0050	+0.2500	-0.0125
AC	+0.0225	+0.0025	-0.0025	+0.0205	-0.3000	+0.7527	+0.3950	+12.25	+0.5250	-0.0008	-2.50	-0.0175	+0.0500	-0.0025
BC	+0.0075	-0.0025	+0.0025	+0.0000	+0.0375	+0.0340	-0.0350	-1.00	+0.1200	-0.0005	+0.0000	-0.0025	+0.0150	+0.0000
A^2	+0.3838	-3.16	+0.1187	+0.0069	-0.5075	+0.8290	+1.85	-27.46	-2.06	-0.0187	+28.75	+0.0483	+0.1258	-0.3133
B^2	-0.0412	-0.3829	-0.0362	-0.0016	+0.2200	+0.3093	-0.1742	-19.71	+0.5700	-0.0110	+6.25	+0.0133	-0.0242	+0.0042
C^2	+0.0237	-0.2229	-0.0262	+0.0241	+0.0375	-0.4613	-0.0342	-11.46	-0.2400	+0.0045	-11.25	-0.0042	+0.0408	-0.0108
P_{model}	0.0003	0.0001	0.0001	0.0050	0.0001	0.0071	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P_{lof}	Significant	Significant	NS	Significant	Significant	Significant	Significant	NS	NS	NS	NS	NS	NS	NS
F value	46.70	3612.45	5398.17	13.80	144.31	11.79	212.11	2953.53	436.77	1072.26	346.46	1124.15	81.32	2799.43
R^2	0.9882	0.9998	0.9999	0.9613	0.9962	0.9550	0.9974	0.9998	0.9987	0.9995	0.9984	0.9995	0.9932	0.9998
Adj. R^2	0.9671	0.9976	0.9997	0.8916	0.9893	0.8740	0.9927	0.9995	0.9964	0.9986	0.9955	0.9986	0.9810	0.9994

Table-4: Experimental and predicted values of extract yield, total phenolic content (TPC), and DPPH with the percentage of error

Name	Goal	Lower Limit	Upper Limit	Predicated Value	Actual Value	% Error
A: Karonda Pulp	is in range	50	70	56.613	60	-
B: Sugar	is in range	45	55	54.607	47.5	-
C: Citric acid	is in range	0.5	1.0	0.815	0.75	-
TSS (%)	is in range	65.32	68.40	65.5316	66.53	0.2114
Total Sugars (%)	is in range	42.75	63.74	47.5281	53.99	0.1825
Reducing Sugars (%)	is in range	11.03	16.22	11.7617	13.55	0.0365
Titratable Acidity (%)	is in range	1.281	1.664	1.40323	1.44	0.0374
Ascorbic Acid (mg/100g)	is in range	19.66	33.64	23.0171	27.29	0.5767
Flavonoids (%)	is in range	7.179	14.447	9.82756	11.34	0.8519
Antioxidant Activity (%)	is in range	27.05	41.17	28.4949	32.78	0.4891
β-carotene (IU/100g)	is in range	4407	5707	4625.61	5066.40	12.3601
Total Phenols (mg GAE/100g)	is in range	92.29	127.87	100.606	110.93	0.8541
Soluble Protein (%)	is in range	0.811	1.187	0.887842	1.02	0.0055
Viscosity (m.Pa.s)	is in range	960	1590	1047.95	1252.67	15.5628
Colour L* Value	is in range	11.85	14.17	12.1696	12.97	0.0345

Colour a* Value	is in range	14.63	18.92	15.1441	16.50	0.2076
Colour b* Value	is in range	12.36	14.73	12.9041	13.70	0.0238

3.1.2 Effect of process variables on TSS content

Due to the interaction between different independent variables the TSS values varied from 65.32 to 68.40% (Table 2). The quadratic model of F-value (46.70) implies the model is significant for TSS. Two process parameters *i.e.* sugar (g) and citric acid (g) had a significant effect on TSS at the linear level, further, pulp and citric acid also had a significant effect at the interactive level. The sugar also had a significant effect on TSS at the quadratic level ($p<0.05$). The regression equation (R^2) accounts for 98.82% of the total variation in TSS (Table 3). Further, from Figure 1(A), it is very clear that there was an increment in TSS with the addition of fruit pulp and sugar, while, the increase in citric acid from 0.5 to 1.0g had a significant negative effect on TSS. The increment in TSS content might be due to the contribution of soluble solids by the addition of sugar and dragon fruit pulp (Sen & Baruah, 2024). Sugar is the most significant constituent of fruits which acts as a preservative and has an effect on the TSS value which is important for the quality of the jam during storage (Ayub et al., 2010).

3.1.3 Effect of process variables on total sugars content

The total sugars content of bread-spread was in the range of 42.75 to 63.74% (Table 2). The quadratic model of F-value (3612.45) implies the model is significant for total sugars. Two process parameters *i.e.* pulp (g) and sugar (g) had a significant effect on total sugars at the linear level, further, pulp and citric acid also had a significant effect at the interactive level. The sugar also had a significant effect on TSS at the quadratic level ($p<0.05$). The regression equation (R^2) accounts for 99.98% of the total variation in total sugars (Table 3). Further, from Figure 1(B), it is very clear that there was an increment in total sugars with the addition of fruit pulp and sugar, while, the increase in citric acid from 0.5 to 1.0g had a significant negative effect on TSS. The increment in total sugars content might be due to the contribution of soluble solids by the addition of sugar and karonda fruit pulp (Sen & Baruah, 2024). Sugar is the most significant constituent of fruits which acts as a preservative and has an effect on the TSS value which is important for the quality of the jam during storage (Ayub et al., 2010).

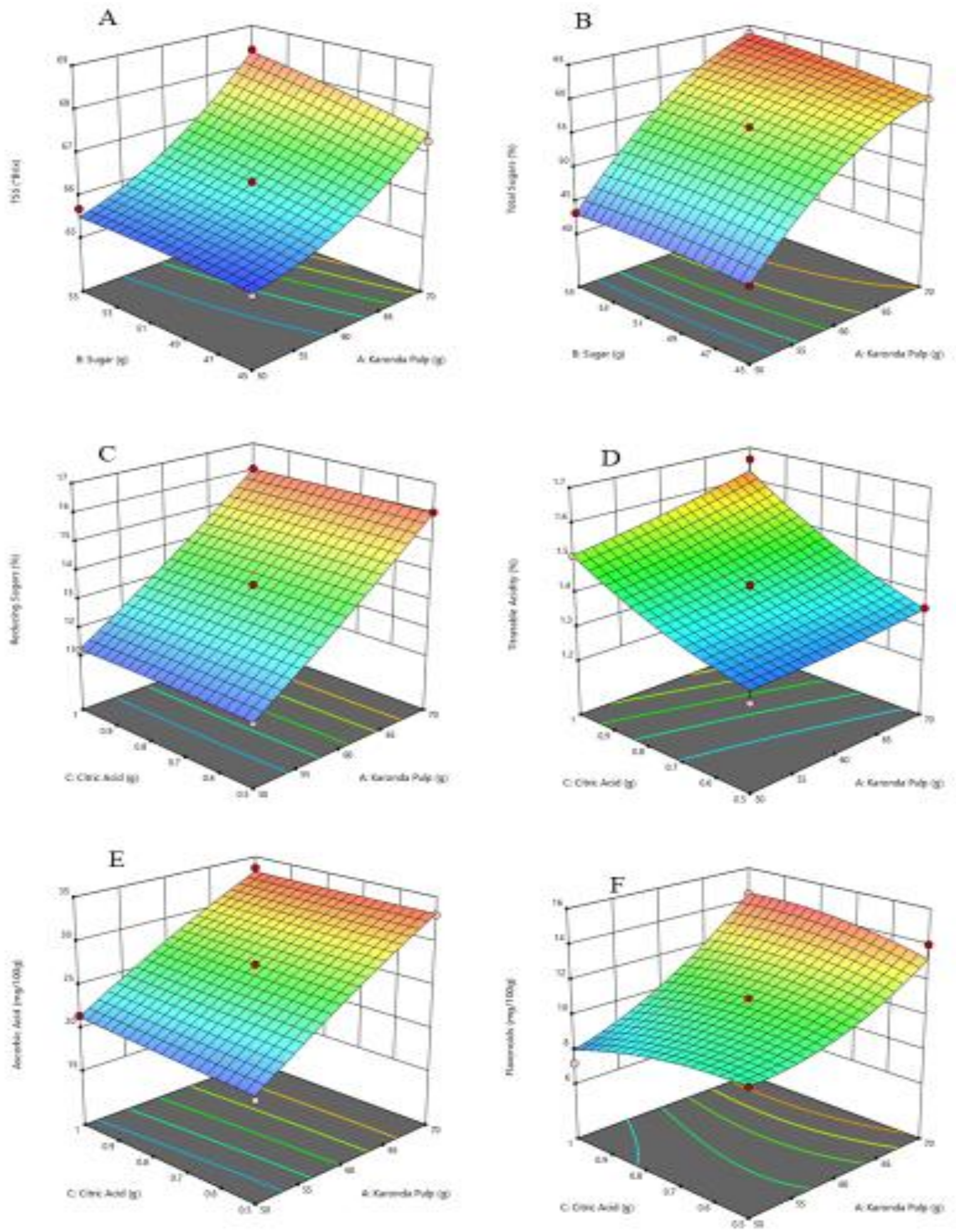

Figure 1: Response surface plots for the effect of (A) Karonda pulp and sugar for TSS, (B) karonda pulp and sugar for total sugars, (c) karonda pulp and citric acid for reducing sugars, (D) karonda pulp and citric acid for titratable acidity, (E) karonda pulp and citric acid for ascorbic acid, (F) karonda pulp and citric acid for flavonoids

3.1.4 Effect of process variables on reducing sugars content

The reducing sugar content ranged from 11.03 to 16.22% across the different experimental runs (Table 2). At the linear level, karonda pulp, sugar, and citric acid exhibited a positive effect on reducing sugars, whereas citric acid showed a significant negative effect. At the quadratic level, all variables demonstrated a negative correlation with reducing sugar content (Table 3). A similar trend is illustrated in Figure 1(C), where reducing sugar content increased with increasing karonda pulp (50-70g) and sugar (45-55g), while a significant decrease was observed with increasing citric acid concentration (0.5-1.0g). Fruit pulp serves as the primary source of reducing sugars in fruit-based systems; therefore, increasing pulp concentration enhances the overall reducing sugar content due to the natural abundance of simple sugars in fruit skin and flesh, thereby contributing to higher levels in the final product (Yucetepe et al., 2024). The regression model explained 99.99% of the total variation in reducing sugars content. The observed increase in reducing sugars may also be attributed to the addition of fruit pulp and sugar during formulation. Similar findings have been reported by Sabale et al. (2024) in karonda fruit syrup.

3.1.5 Effect of process variables on titratable acidity

Titratable acidity (TA) was significantly influenced by fruit pulp, sugar content, and citric acid concentration ($p < 0.05$). The TA values ranged from 1.281 to 1.664% (as citric acid). As shown in Table 3, sugar and citric acid exhibited a significant effect on TA at the linear level, while karonda and citric acid, along with sugar, showed a significant effect ($p \leq 0.05$) at the quadratic level. The coefficient of determination ($R^2 = 96.13\%$) indicated a strong agreement between experimental and predicted values, demonstrating good model performance. However, the lack-of-fit was significant, suggesting some inadequacy of the model in fully capturing the variability of the response. As illustrated in Figure 1(D), increasing karonda pulp (50-70g), sugar (45-55g), and citric acid (0.5-1.0g) significantly increased the titratable acidity of the bread-spread. This increase may be attributed to the presence of natural organic acids in fruit pulps; therefore, higher pulp addition contributes to greater acid content in the final product (Shi et al., 2022). Similar trends have also been reported by Jaiswal et al. (2015) during jam preparation from jamun fruit.

3.1.6 Effect process variables on ascorbic acid

Ascorbic acid content in the bread-spread ranged from 19.66 to 33.64 mg/100g across the experimental runs (Table 2). Fruit pulp had a significant effect ($p < 0.05$) on ascorbic acid content at the linear, interactive, and quadratic levels (Table 3). As shown in Figure 1(E), increasing sugar concentration from 45 to 55g initially enhanced ascorbic acid content; however, a subsequent decline was observed at higher sugar levels. This reduction may be attributed to the Maillard reaction, which can occur at elevated sugar concentrations during processing or storage. Under such conditions, ascorbic acid may react with reducing sugars, leading to its degradation (Agcam, 2022). The interaction between citric acid and sugar did not show a significant effect on ascorbic acid content. Additionally, the increase in sugar concentration was associated with a darker product appearance, which may be linked to pigment degradation. In general, colour changes during thermal processing may result from the degradation of carotenoids and non-enzymatic browning reactions, including the Maillard reaction and oxidation of ascorbic acid (Jabbari et al., 2018).

3.1.7 Effect of process variables on Flavonoids

The flavonoid content ranged from 7.179 to 14.447% across the different experimental runs (Table 2). At the linear level, karonda pulp, and sugar showed a positive effect on flavonoid content, whereas citric acid exhibited a significant negative effect. At the quadratic level, all variables showed a positive correlation with flavonoid content (Table 3). A similar trend is illustrated in Figure 1(F), where flavonoid content increased with increasing pulp concentration (50-70g), while a decrease was observed with increasing citric acid concentration (0.5-1.0g). Fruits are the primary source of flavonoids in fruit-based systems; therefore, increasing pulp content enhances flavonoid concentration due to their natural abundance in the skin and flesh of fruits, thereby contributing to higher flavonoid levels in the product (Yucetepe et al., 2024). The regression model explained 95.5% of the total variation in flavonoid content, indicating good model fitness. The observed increase in flavonoids may be attributed to the incorporation of karonda and sugar in the range of 50-70g and 45-55g, respectively. Similar findings have been reported in karonda syrup by Sabale et al. (2024) and in cashew apple–açaí blended nectar by Pinheiro et al. (2022).

3.1.8 Effect of process variables on antioxidant activity

The DPPH assay is widely used to quantify the antioxidant capacity of bioactive compounds and it ranged from 27.05 to 41.17%. The mean experimental values and ANOVA results are presented in Table 3. Antioxidant activity was significantly influenced by all three independent variables. Among the interaction terms, AB showed a significant effect on DPPH scavenging activity. The interactions between variables are illustrated in the three-dimensional response surface plot shown in Figure 2(A). The plot indicates that antioxidant activity increased with increasing pulp and sugar concentrations. In contrast, higher citric acid levels resulted in a reduction in antioxidant activity. This decrease may be attributed to the acid-induced lowering of pH, which can destabilize antioxidant compounds, particularly phenolics and ascorbic acid (Rady et al., 2023). The regression model explained 99.74% of the total variation in antioxidant content, indicating good model fitness.

3.1.9 Effect of process variables on β-carotene

The model exhibited an F-value of 2953.53 with a corresponding p-value of 0.0001 for β-carotene content, indicating the statistical characteristics of the fitted model. Based on the coefficient estimates and associated p-values, the linear terms A, B, and C were identified as the most influential factors affecting the response variable. Although the model showed a high level of fit, as indicated by an R^2 value of 0.9998, the p-value suggests that the model was not statistically significant at the 0.05 level. As shown in Figure 2(B), β-carotene content increased with increasing sugar concentration, whereas karonda pulp and citric acid had a comparatively minimal effect on the response. The increase in β-carotene with higher sugar levels may be attributed to the formation of a viscous protective matrix around carotenoid molecules, which reduces their exposure to oxygen, light, and heat—major factors responsible for carotenoid degradation (Lavelli & Sereikaite, 2022).

3.1.10 Effect of process variables on total phenols content (TPC)

Table 3 presents the mean response values of TPC in the bread-spread across 15 experimental trials. The TPC values ranged from 92.29 to 127.87 mg GAE/100g. A negative linear correlation was observed between TPC and both sugar content and citric acid concentration, as shown in Figure 2(C) and Table 3. In contrast, interaction terms exhibited a statistically significant effect on total phenolic content. An increasing trend in TPC was observed with higher pulp concentration. This may be attributed to the fact that phenolic compounds are naturally present in the plant matrix; therefore, increased pulp addition provides a greater reservoir of phenolics for extraction (Martins et al., 2022). These compounds, particularly phenolic acids such as chlorogenic and caffeic acids, play a key role in the antioxidant activity of karonda by scavenging free radicals and minimizing oxidative damage (Al-Busaidi, 2025). The variation in total phenolic content in karonda bread-spread may also be attributed to oxidation, degradation, and polymerization of phenolic compounds with proteins, or their condensation into brown pigments during processing (Sabale et al., 2024).

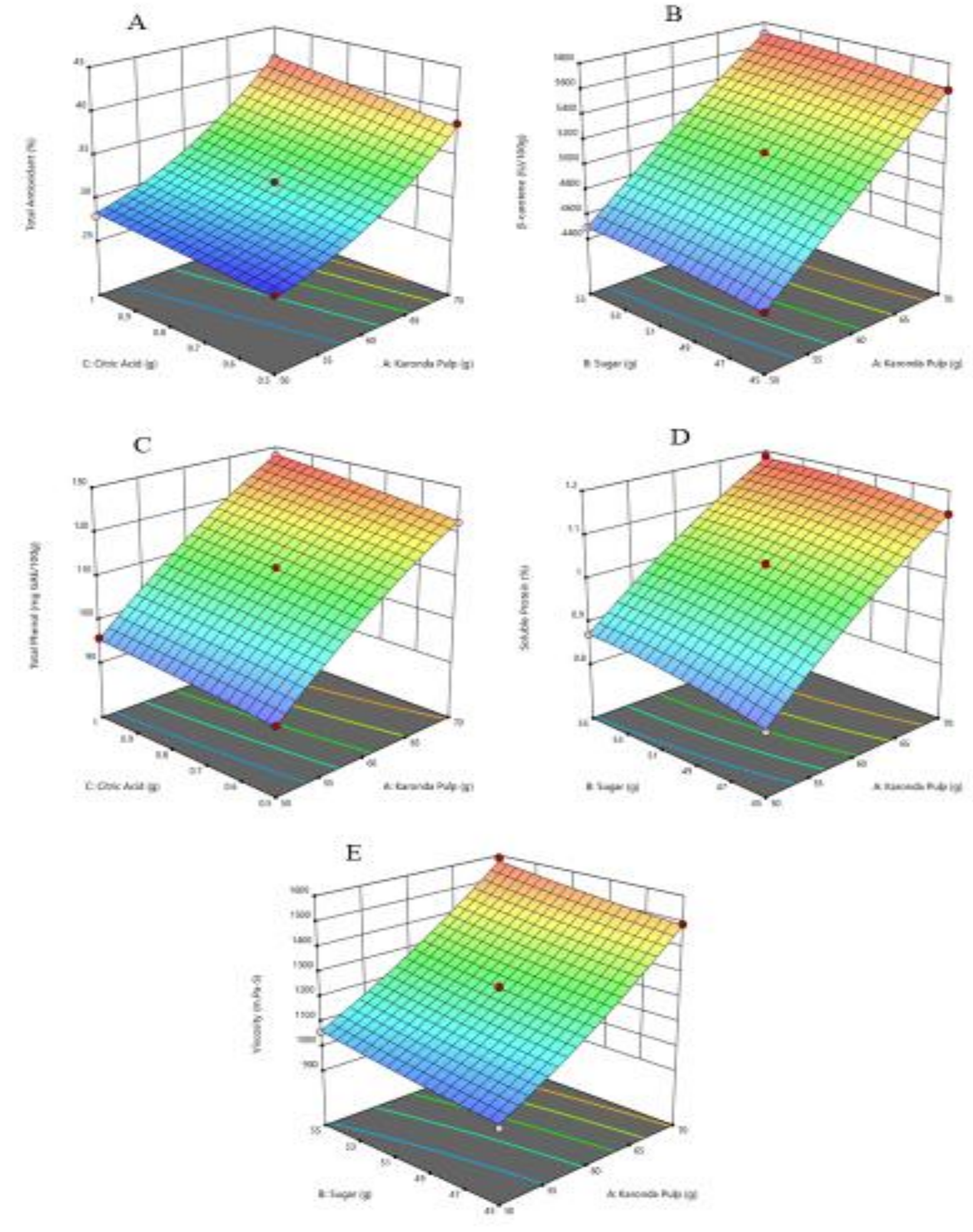

Figure 2: Response surface plots for the effect of (A) karonda pulp and citric acid for antioxidant activity, (B) karonda pulp and sugar for β-carotene, (C) karonda pulp and citric acid for TPC, (D) karonda pulp and sugar for soluble protein, (E) karonda pulp and sugar for viscosity

3.1.11 Effect of process variables on soluble protein

The soluble protein content in the prepared bread-spread ranged from 0.811 to 1.187% (Table 2). The quadratic model showed an F-value of 1072.26, indicating that the model was not significant for soluble protein prediction. At the quadratic level ($p < 0.05$), karonda and sugar exhibited a significant effect on soluble protein content. The regression model accounted for 99.95% of the total variation in soluble protein, as shown in Table 3. As illustrated in Figure 2(D), soluble protein content increased with the addition of fruit pulp and sugar, while it remained relatively constant with increasing citric acid concentration. The higher soluble protein content in the bread-spread may be attributed to the presence of fruit-derived nutrients and added sugar (Nur et al., 2023). Similar observations have been reported by Garg et al. (2019) in nutritionally enriched jams prepared from blends of Indian blackberry and other fruits, where variations in pulp combinations influenced the nutrient composition of the final product.

3.1.12 Effect of process variables on viscosity

The viscosity of karonda bread-spread ranged from 960 to 1590 cP across the suggested experimental runs (Table 2). At the linear level, karonda pulp, sugar, and citric acid exhibited a positive effect on viscosity, whereas the interaction terms showed a negative effect (Table 4). The increase in karonda pulp, along with sugar concentration, contributed to higher viscosity values in the bread-spread. The coefficient of determination (R^2 = 99.84%) indicated a strong correlation between the experimental and predicted values, demonstrating excellent model fit. As shown in Figure 1(E), increasing karonda pulp (50-70 g), and sugar (60–70 g) significantly enhanced the viscosity of the bread-spread. This increase may be attributed to the higher content of fibrous materials, pectin, and natural sugars present in fruit pulps, which contribute to the structural matrix of the bread-spread. Pectin, a natural gelling agent in fruits, forms a network that entraps water molecules, thereby increasing viscosity (Alam et al., 2024). Furthermore, higher sugar concentrations increase soluble solids and reduce free water availability due to the hygroscopic nature of sugar, thereby promoting gel formation and increasing viscosity (Ding & Yang, 2021). Similar observations have been reported in karonda syrup by Swami et al., (2013).

3.1.13 Effect of process variables on colour value L*

Colour is expressed in terms of three parameters, where L* represents lightness (0 = black, 100 = white), a* represents redness (positive values) to greenness (negative values), and b* represents yellowness (positive values) to blueness (negative values). The L* value ranged from 11.85 to 14.17 across the 15 experimental runs (Table 2). At the linear level, karonda pulp and sugar showed a positive effect on L*, whereas citric acid exhibited a significant negative effect. At the quadratic level, all variables showed a negative correlation with L* values (Table 3). A similar trend is illustrated in Figure 3(A). The L* value increased with increasing karonda pulp concentration (50-70 g), while a significant decrease was observed with increasing citric acid concentration (0.5-1.0 g). Fruit pulp serves as a major contributor to colour in fruit-based systems; therefore, increasing pulp concentration enhances colour intensity due to the presence of natural pigments in the fruit skin and flesh, thereby influencing L* values (Yucetepe et al., 2024). The regression model accounted for 99.95% of the total variation in L* values, indicating excellent model fit. The increase in L* value was primarily attributed to the incorporation of karonda pulp.

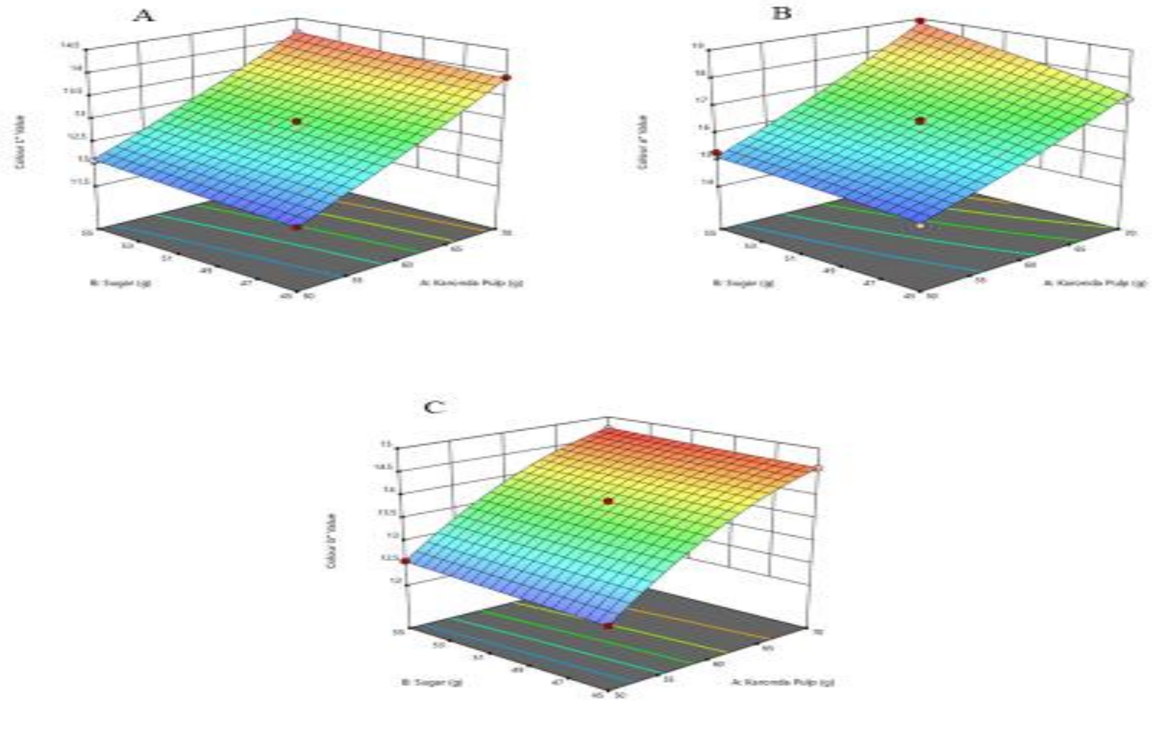

Figure 3: Response surface plots for the effect of (A) karonda pulp and sugar for colour L* value, (B) karonda pulp and sugar for colour a* value, (C) karonda pulp and sugar for colour b* value.

3.1.14 Effect of process variables on colour value a*

The a* colour value ranged from 14.63 to 18.92 across the 15 experimental runs (Table 2). At the linear level, karonda pulp and sugar exhibited a negative effect on a* values, while sugar and citric acid also showed a significant negative influence. At the quadratic level, all variables demonstrated a negative correlation with a* values (Table 3). A similar trend is illustrated in Figure 3(B). The a* value increased with increasing sugar (45-55 g) and citric acid concentration (0.5-1.0 g), whereas a significant decrease was observed with increasing karonda pulp (50-70 g) and sugar (45-55 g). Fruit pulp acts as a major source of natural pigments in fruit-based systems; therefore, increasing pulp concentration enhances colour intensity due to pigment abundance in fruit skin and flesh, thereby influencing a* values (Yucetepe et al., 2024). The regression model explained 99.32% of the total variation in a* values, indicating excellent model fit. The observed changes in a* values were primarily associated with the incorporation of karonda pulp and sugar content.

3.1.15 Effect of process variables on colour value b*

Table 3 presents the mean response values of the b* colour parameter for the bread-spread across 15 experimental trials. The b* values ranged from 12.36 to 14.73. A negative linear correlation was observed between b* values and both sugar content and citric acid concentration, as shown in Figure 3(C) and Table 3. In addition, interaction terms exhibited a statistically significant effect on the b* colour parameter. An increasing trend in b* values was observed with increasing pulp concentration. This may be attributed to the fact that fruit pulp contains natural pigments within the plant matrix; therefore, higher pulp levels provide a greater reservoir of colour compounds contributing to the b* value.

3.1.16 Numerical optimization of process parameters

Optimization plays a crucial role in food process engineering, as it is essential for achieving efficient process performance and ensuring the development of food products with superior quality. However, during optimization, multiple factors influence product quality simultaneously. The simultaneous optimization of several responses can be effectively achieved using Response Surface Methodology (RSM) combined with a desirability function approach (D) (Singh et al., 2025). The primary objective of the present study was to determine the optimal levels of karonda pulp, sugar, and citric acid. Accordingly, optimization was performed by considering target criteria such as maximization, minimization, and values within a specified range, depending on the response variables. Table 4 presents the comparison between predicted and experimental values, demonstrating the adequacy and reliability of the developed model. The optimized conditions obtained for the present study were 56.613 g karonda pulp, 54.607 g sugar, and 0.815 g citric acid. These optimized conditions were slightly adjusted for validation purposes (Table 4). The desirability value under optimal conditions was 1.000, indicating an ideal solution. Furthermore, the deviation between predicted and experimental values was less than 10%, and no significant differences were observed, confirming the validity and reliability of the optimization model.

4. Conclusion

The karonda fruit bread-spread demonstrated that fruit pulp, sugar, and citric acid played significant roles in process optimization. The addition of karonda pulp, and sugar exerted a positive influence on most response variables, including total soluble solids, viscosity, titratable acidity, ascorbic acid, flavonoids, soluble protein, β-carotene, total phenols, DPPH radical scavenging activity, total sugars, reducing sugars, and colour parameters (L*, a*, and b*). In contrast, citric acid significantly affected total soluble solids, viscosity, titratable acidity, ascorbic acid, β-carotene, total phenols, DPPH activity, and colour attributes (L*, a*, and b*). Based on the Box–Behnken design, the factor loadings indicated that fruit pulp and sugar had a predominantly positive effect on the evaluated responses, whereas citric acid exhibited a comparatively lower influence. The numerically optimized formulation obtained from the study was 56.613 g karonda pulp, 54.607 g sugar, and 0.815 g citric acid. This combination exhibited the highest desirability value and can therefore be recommended for the preparation of karonda fruit bread-spread.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personnel relationship that could have appeared to influence the work reported in this paper.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Declarations Ethics approval

Ethics approval and consent to participate -Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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