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Optimization of ingredient levels of reduced-calorie blackberry jam using response surface methodology

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ABSTRACT

In a developed society, health problems such as the risk of weight gain, diabetes, and heart diseases are often associated with a high-sugar diet. Food product reform is seen as one of the tools to promote healthier eating. Calorie-reduced product processing not only targeted diabetics but now also focuseds on human health. The experiments were designed based on the response surface methodology using the three-level-three-factor Box–Behnken design with total soluble solid (TSS) (40%–50%), low methoxyl pectin (LMP) (0.1%–0.3%), and kappa-carrageenan (*k*-carrageenan) (0.1%–0.3%) at a constant fruit batch of 10 kg. In the evaluation of jam, the viscosity, water activity, and bioactive compounds (anthocyanin, total polyphenol, and vitamin C) were determined and optimized in order to produce reduced-calorie blackberry jam (filling type) with favorable viscosity, water activity, and bioactive compounds. The results showed that the model fit was significant ($p < 0.05$); a satisfactory correlation between the experimental and the predicted values was found (high coefficient of determination R^2). All three factors affect the quality of the products, with sugar showing significant influence on physical characteristics, maintaining bioactive compounds and overall acceptability of the product. The overlay contour plot of quality attributes showed that the reduced-calorie blackberry jam should use 0.18% LMP , 0.22% *k*-carrageenan, and 45% TSS. Thus, the reduced-calorie jam produced from these optimum levels had better characteristics than the others.

1. INTRODUCTION

Possessing high levels of bioactive compounds such as phenolic compounds, ascorbic acid, anthocyanins, and carbohydrate [1], blackberries play an important role in human nutrition. These fruits were used to process jams by a traditional method, without the addition of pectin [2]. Jam is a product made by boiling the fruit pulp with a sufficient quantity of sugar to a reasonably thick consistency, firm enough to hold the fruit tissues in position. As pointed out from the literature, fruit jam is full of natural fibers, vitamins, minerals, amino acids, and other nutrients [3], and are fat- and cholesterol-free for health. The amount of pectin in the jam helps reduce the risk of cancer and may promote skin and bone health. Not only do the shorter heating periods reduce the formation of harmful compounds, but also the obtained jam could be with higher content of antioxidants as flavonoids [4].

Hydrocolloids/gelling agents were used in various food formulations such as sauces, soups, gel desserts, jam, and jellies as food additives in order to obtain the desired viscosity and mouthfeel. Pectin consists of a chain of D-galacturonic acid units which are linked by α-(1-4)-glycosidic bonds and are used for jam making. Among the hydrocolloids used for jam production, kappa-carrageenan (*k*-carrageenan) exhibits superiority with high stability. It is usually not easily degraded when the product is stored for a long time. *k*-carrageenan was also commonly used in the food industry as a thickener, gelling agent, stabilizer, and emulsifier [5,6]. Due to these characteristics along with the lower cost compared to other gelling agents, *k*-carrageenan can be considered as one of the gelling agents that can partially replace pectin in jam production [7]. Pectin and carrageenan

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are dispersed in water to produce a thickening or viscous effect. Considering their role in regulating the viscosity and texture of jam making, in this study, jam products were formed with the criterion of moderate product viscosity, suitable for cake decoration and later processing of mixed drinks. However, due to its high sugar content, jam can affect health such as causing tooth decay, weight gain, and the risk of obesity, so it should only be eaten in moderation. Accordingly, not only is reduced-calorie jam making targeted for diabetics but also it is now also targeted towards health purposes.

The response surface methodology (RSM) has been successfully used for development, process improvement, and optimization [8]. This technique has also been widely applied to optimize jam production processes [9–11]. To obtain the optimal value of ingredients (total soluble solids (TSS), low- methoxyl pectin (LMP), and *k*-carrageenan) for reduced-calorie blackberry jam processing, the RSM was also used. The RSM allows reducing the number of tests required to evaluate multiple parameters and their interactions, thus requiring less time and effort. However, limited information is available on reduced-calorie blackberry jam, and so this study was carried out to determine the optimal parameters from the designed ingredients in order to produce a low-calorie jam with a harmonious viscosity, low water activity, high content of bioactive compounds, and high acceptance by panelists.

2. MATERIALS AND METHODS

2.1. Materials

The blackberry fruits were collected from Da Lat, Vietnam. LMP (with DE < 50%) and *k*-carrageenan originated from Louis Francois (France). Sugar was purchased from the local market. All the materials were of food grade.

2.2. Reduced-Calorie Blackberry Jam Preparation

Ten-kilogram batches of jam were prepared with the ratio of blackberry to tap water (65:35) with the TSS ranging from 40 to 50°Brix by using formula (1).

$$\text{The desired TSS} = \frac{a + x}{100 + x} \quad (1)$$

Where a is the measured TSS in the batch, x is the added sugar (g/100 g of batch).

The pH value was controlled at 3.5 by citric acid. LMP was 0.1%, 0.2%, 0.3% *w/w* and *k*-carrageenan 0.1%, 0.2%, 0.3% *w/w* per batch. The jam was cooked in a vacuum evaporator with a vacuum of 600 mmHg and a holding time of 3.5 minutes (with an evaporating steam temperature of 60°C–66°C) [12]. The results of the final TSS for the different combinations ranged from 55° to 57°Brix. After sufficient cooking time, the hot jam was poured into glass containers that were washed and sterilized, covered with lids, and cooled down to 37°C–39°C. The products were then pasteurized at 90°C for 5 minutes.

2.3. Box–Behnken Design (BBD)

According to the preliminary study, the BBD with three factors and three levels was applied to evaluate the optimal combination of LMP, *k*-carrageenan, and TSS for the production of low-calorie blackberry jam. LMP (A), *k*-carrageenan (B), and TSS (C) were the independent variables selected to be in this experimental design. The viscosity, water activity, and bioactive compounds (anthocyanin, total polyphenol, and vitamin C) were selected as the responses for the combination of the independent variables. The factors and levels are coded and given in Table 1. The design consists of 18 runs with 6 center points (Table 2).

2.4. Water Activity and Viscosity Measurement

The water activity of the samples was measured by water activity measurement instruments (Novasina, Sweden). The viscosity of the jam was measured at 25°C by a Brookfield viscometer.

Table 1: Factor and coding levels of the Box–Behnken-centered combination experiment design.

Factors	Coding level		
	−1	0	1
A. LMP (%)	0.1	0.2	0.3
B. *k*-carrageenan (%)	0.1	0.2	0.3
C. TSS content (°Brix)	40	45	50

Table 2: Response surface and response value by the BBD.

Run	LMP (%)	*k*-Carrageenan (%)	TSS (°Brix)	Run	LMP (%)	*k*-Carrageenan (%)	TSS (°Brix)
1	0.2	0.2	45	10	0.2	0.1	50
2	0.2	0.2	45	11	0.1	0.3	45
3	0.2	0.2	45	12	0.1	0.1	45
4	0.2	0.2	45	13	0.3	0.3	45
5	0.2	0.2	45	14	0.3	0.2	50
6	0.2	0.2	45	15	0.2	0.1	40
7	0.1	0.2	40	16	0.3	0.2	40
8	0.1	0.2	50	17	0.3	0.1	45
9	0.2	0.3	40	18	0.2	0.3	50

2.5. Bioactive Compounds Analysis

The ascorbic acid content of the product was analyzed according to Association of Official Analytical Chemists standards [13]. TSS (°Brix) was defined using a refractometer (Model Atago Digital DBX-5). pH was measured with a digital pH meter (Model PHS-2F). The total anthocyanin content was determined by the pH-differential method [14] and the total polyphenol content (TPC) was analyzed by the Folin–-Ciocalteu method [15].

2.6. Sensory Evaluation

Sensory evaluation quantitative descriptive analysis (QDA) was applied for the blackberry jam's sensory evaluation by 20 fully trained panelists [16]. The participants tested selected samples to distinguish different intensities of attributes (color, odor, taste, and texture) with an intensity rating scale from 0 to 5 (where 0 is undetected and five is attribute extremely strong).

2.7. Data Analysis

By using the multiple regression analysis, the obtained data of water activity, viscosity, and bioactive compounds were analyzed. Statgraphics was used to analyze and fit the model to the experimental data. The model was proposed for each response (Y) as as Equation (2)

$$Y = b_0 + \sum_{n=1}^{3} b_n X_n + \sum_{n=1}^{3} b_{nn} X_n + \sum_{n \neq m = 1}^{2\ 3} b_{nm} X_{m'} \quad (2)$$

where b_o is the Y-intercept (constant), b_n is regression coefficient for the linear effect of X_n on Y, b_{nn} and b_{nm} are regression coefficients for quadratic effect on Y, and X_n and X_m are independent values. The selected equation was used to fit the obtained data based on the coefficient of determination R^2 value obtained from the multiple regression analysis.

3. RESULTS AND DISCUSSION

3.1. Some Chemical Components of Blackberry

Blackberry is a good source of antioxidants with high content of vitamin C, anthocyanin, and total polyphenol. The TSS was also rather high (10.2 ± 0.05°Brix) (Table 3). A higher anthocyanin level was found, representing 67% of the total phenolic compounds. The anthocyanin concentration is within the published values, 146–2,199 mg/100 g fresh weight [17]. The total phenolic content in blackberry obtained from this study seems to be similar to the analysis results of Yilmaz *et al.* [18] with the total phenolic content in blackberry cultivars and wild genotypes reported from 230 to 978 milligrams of gallic acid equivalents per 100 g fresh weight basis (mg GAE/100 g).

Table 3: Some characteristics of blackberry.

Characteristics	Content
Anthocyanin (mg/100 g)	166.99 ± 8.35
Vitamin C (mg%)	16.54 ± 0.12
Total phenolic content (mg GAE/100 g)	249.07 ± 2.26
pH	3.09 ± 0.02
TSS (°Brix)	10.2 ± 0.05

Mean value ± standard deviation of pulp weigh; $n = 3$.

3.2. Modeling the Effects

3.2.1. Water Activity and Viscosity

The results of water activity and viscosity of blackberry jam for the different combinations ranged from 0.799 to 0.89 and 1,255.67 to 10,579.67 cP, respectively (Table 4). It was observed that pectin and carrageenan concentration together with TSS significantly ($p < 0.05$) affected the water activity and viscosity.

The water activity of the treatment with 0.2%, 0.3%, and 40°Brix (pectin, carrageenan, and TSS, respectively) was the highest (0.89). The lowest a_w value (0.799) was obtained with the treatment using 0.2%, 0.3%, and 50°Brix compared to the other combinations. Water activity has been shown to be useful in predicting the growth of bacteria, yeasts, and molds. When food is stored at room temperature, the product needs to be controlled for both pH and water activity, which facilitates safe food storage [19].

Food can be safely preserved by lowering the water activity to a certain level that does not allow the growth of dangerous pathogens such as *Clostridium botulinum* and *Staphylococcus aureus*. The risk of food poisoning usually occurs for foods with water activity higher than 0.86 and pH > 4.5. With a controlled pH value of 3.5 for the entire product and the measured water activity shown in Table 4, our products are virtually safe from the growth of the majority of bacteria [20]. However, this product was used next to decorate cakes and prepare drinks, so it should continue to be pasteurized at the final stage to ensure product quality and safety requirements.

Moreover, sugar is also one of the important ingredients determining the rheological properties of jam. It stabilizes the shelf life of the product, enhances the taste, improves the texture of the product. In addition, sugar is essential to create the ideal jam texture, appearance, flavor, yield and also reduces the stability of the system by removing water from the pectin particles and affects the strength of acid. During cooking, the formation of a pectin-sugar gel occurs due to the precipitation of a portion of the pectin present in the solution. Precipitation takes place in such a way as to develop high binding forces at the surface. They keep the solution of the other ingredients strong enough to give the entire system the stiffness and texture associated with the jam.

A combinationof LMP and k-carrageenan is necessary to formulate reduced-calorie blackberry jam. In addition, LMP can be used for thosethat want to decrease the amount of sugar used in making jam. Sucrose is considered a better choice for jam than glucose because of its low recrystallization tendency [21]. From the obtained data, the results showed that the viscosity gradually increases with the increase in sugar concentration, indicating that sugar concentration plays an important role in maintaining the viscosity. Jam containing 40°Brix had 1,255.67 cP, while an increasing trend in viscosity was observed in a sample containing 50°Brix where it showed 10,579.67 cP.

Table 4: Water activity and viscosity of reduced-calorie blackberry jam at different levels of pectin, carrageenan, and TSS.

LMP pectin (%)	*k*-Carrageenan (%)	(°Brix)	a_w	Viscosity (cP)
0.2	0.2	45	0.869[a] ± 0.007[b]	1,985.33 ± 14.85
0.2	0.2	45	0.868 ± 0.001	1,957 ± 40.15
0.2	0.2	45	0.873 ± 0.009	1,973 ± 36.37
0.2	0.2	45	0.866 ± 0.004	1,979.667 ± 13.24
0.2	0.2	45	0.869 ± 0.009	1,922.333 ± 4.51
0.2	0.2	45	0.868 ± 0.000	1,971 ± 15.39
0.1	0.2	40	0.887 ± 0.000	1,255.67 ± 4.16
0.1	0.2	50	0.845 ± 0.001	4,851.67 ± 44.41
0.2	0.3	40	0.891 ± 0.007	1,953.67 ± 44.02
0.2	0.1	50	0.858 ± 0.002	5,600 ± 34.15
0.1	0.3	45	0.861 ± 0.003	5,223.33 ± 22.68
0.1	0.1	45	0.869 ± 0.000	1,266 ± 9.54
0.3	0.3	45	0.857 ± 0.002	10,573.67 ± 12.75
0.3	0.2	50	0.823 ± 0.015	10,908 ± 12.02
0.2	0.1	40	0.885 ± 0.004	1,554 ± 47.32
0.3	0.2	40	0.877 ± 0.002	1,954.33 ± 9.814
0.3	0.1	45	0.883 ± 0.002	5,554.33 ± 33.23
0.2	0.3	50	0.799 ± 0.000	10,579.67 ± 1.53

[a]Mean value of three replications.

[b]Standard deviation of the mean values.

Pectin is the most common gelling and thickening agent [22] and viscosity change is due to the presence of sugar [21]. In addition, one of the important physical properties of *k*-carrageenan is its gel-forming ability known as gel strength [23]. The *k*-carrageenan gel is characterized as strong, brittle, and subject to syneresis. The obtained results demonstrated that an increase in LMP and *k*-carrageenan concentrations had a significant effect ($p < 0.05$) on the viscosity of jam. Pectin is used to control the viscosity or characteristics of gel solutions of fruits [24]. The role of pectin is known to network and thicken jams during processing. The higher the pectin content, the higher the product consistency. However, a high-viscosity jam is not suitable for this product. A combination of LMP and *k*-carrageenan is necessary to formulate reduced-calorie blackberry jam. In addition, LMP can be used for those that want to decrease the amount of sugar used in making jam or jelly. This product creates a gel in the absence of sugar and acid for a quality product. As mentioned above, the aim of this work was to reduce the stickiness and the combination of LMP and *k*-carrageenan is often used for the desirable texture of this product. It is shown that the gel-sol conversion occurring in jam is clearly affected by the presence of sugar. As the temperature increases during cooking, the gel-sol transition occurs, then the hydrocolloid molecules become of continuous phase (disperse and liquid phase), and the hydrocolloid gel changes to hydrocolloid sol [25]. In this study, sugar affected the viscosity of pectin and *k*-carrageenan gels. Therefore, the hydrogen bonds between the sugar-OH groups and polymer molecules caused a change in the solvent structure in the aqueous solvent [26].

From all obtained data, the regression equations describing the relationship between water activity (a_w)/viscosity and independent variables for the Box–Behnken model are established (as mentioned above). The analysis of variance for a_w and viscosity were analyzed. In all cases, almost all effects have p values less than 0.05, indicating that they are significantly at the 95.0% confidence level. The quadratic models for a_w and viscosity of reduced-calorie blackberry jam are presented in Equations (3) and (4)

$$a_w = 0.0006 + 0.0556A + 1.4374B + 0.0379C - 0.4183AB - 0.0325BC - 0.0004C^2 \quad (3)$$

$R^2 = 92.17\%$; R^2 (adjusted for d.f.) = 91.17%, and SEE = 0.005,

$$\text{Viscosity (cP)} = 113{,}618 - 177{,}567A - 169{,}793B - 4{,}224.37C + 180{,}504A^2 + 26{,}550AB + 2{,}678.38AC + 198{,}446B^2 + 2{,}290BC + 42.0917C^2 \quad (4)$$

$R^2 = 98.12\%$, R^2 (adjusted for d.f.) = 97.75%, SEE = 413.86,

where A, B, and C are pectin concentration, carrageenan concentration, and TSS, respectively, used in jam making, as independent values. The high R^2 values of these models indicated a good fit of the data. The correlation between experimental and predicted values was found (Figs. 1 and 2).

This implied that the water activity of jam could be estimated by pectin, carrageenan concentration, and TSS. In most cases, the results of the analysis of variance showed that the pectin, carrageenan, and TSS significantly affected the physical properties (water activity and viscosity) of jam (as mentioned above), e.g., in

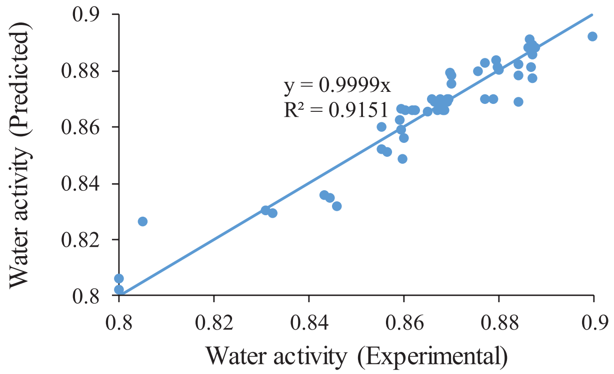

Figure 1: Correlation between the experimental and estimated a_w using the model described in (2).

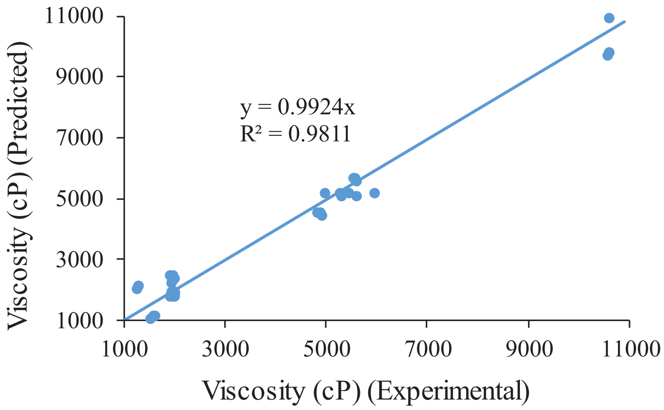

Figure 2: Correlation between the experimental and estimated viscosity using the model described in (3).

the cases of these gelling agents used together with TSS content. The water activity and viscosity are shown in Figures 3 and 4 with plots of the estimates in a decreasing order of importance.

The results in Table 5 showed the combination of factor levels which optimizes a_w and viscosity of blackberry jam in the cases of three factors over the indicated region, which maximize the desirability function. It also showed the combination of the factors in which that optimum is achieved (Fig. 5).

3.3. Bioactive Compounds

Evaluation of the influence of pectin, carrageenan concentration, and TSS on nutritional value, especially the content of biological compounds, was also carried out. The model describing the correlation between anthocyanin, vitamin, and TPC content in the product with independent variables (concentration of LMP, *k*-carrageenan, and TSS) was established in Equation (5), (6), and (7). The obtained values of determination coefficients from the analyzed model were higher than 80%. In addition, the lack-of-fit test was analyzed with the obtained *p*- value of greater than 0.05, which showed that the achieved model could fully predict the influence of pectin, carrageenan content, and TSS on these bioactive compounds of reduced-calorie blackberry jam.

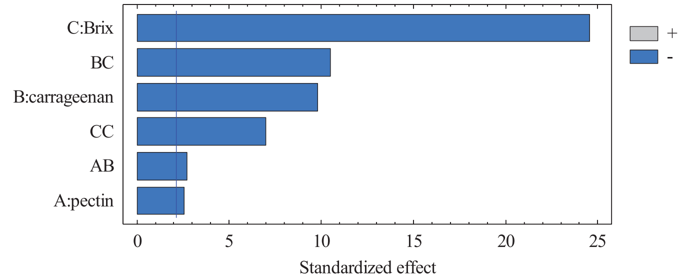

Figure 3: Standardized Pareto chart for a_w

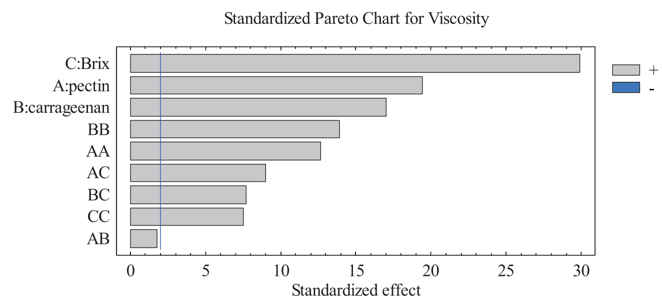

Figure 4: Standardized Pareto chart for viscosity.

Anthocyanin (mg/100 g) $= -446.66 + 244.55A + 209.29B + 23.24C - 113.04A^2 - 193.33AB - 4.035AC - 72.13B^2 - 3.46- - 0.24C^2$ (5)

$R^2 = 95.00\%$, R^2 (adjusted for d.f.) $= 93.98\%$, and SEE = 1.12,

Vitamin C (mg%) $= -10.07 + 3.66A + 3.22B + 0.66C - 11.39A^2 - 8.81B^2 - 0.007C^2$ (6)

$R^2 = 85.01\%$, R^2 (adjusted for d.f.) $= 82.32\%$, and SEE = 0.67,

TPC (mg GAE/100 g) $= -248.12 + 260.82A + 383.78B + 17.18C - 6.13AC - 8.53BC - 0.16C^2$ (7)

$R^2 = 89.94\%$, R^2 (adjusted for d.f.) $= 87.95\%$, and SEE = 1.35.

It can be seen that independent variables affect the content of biological compounds in blackberry jam. In particular, the content of soluble solids was remarkably related to the content of biological substances in the product (data was not shown). The sugar and pectin content used in jam processing contributed to the protection of bioactive compounds during heat treatment. They can reduce the adverse reactions occurring during jam cooking and the mechanism of interaction with functional components through hydrogen or hydrophobicity bonding. In some studies, the stability of bioactive compounds has been shown to be related to TSS content, pH, and gelling agents [27].

Overlay plots in multiple regression statistics help show and divide the level of desirability area based on the response surface plots that have been observed. Moreover, the advantage of using an overlay plot is that it is possible to find the optimal point and region containing the optimal point. The results showed that the highest concentrations of bioactive compounds were obtained

Table 5: Optimized response (a_w) and viscosity of jam with LMP , *k*-carrageenan, and TSS used.

Factor	Low	High	Optimum
Pectin (%)	0.1	0.3	0.18
Carrageenan (%)	0.1	0.3	0.22
TSS (°Brix)	40.0	50.0	44.96
Responses		Optimum	
a_w		0.868	
Viscosity		2,000 cP	

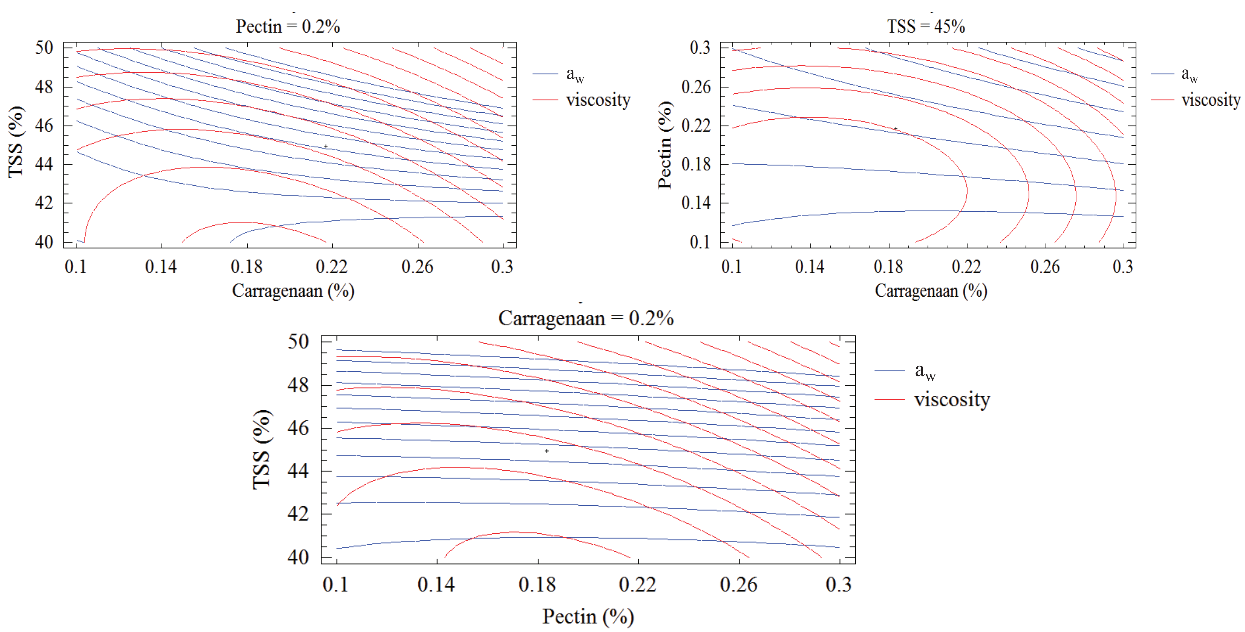

Figure 5: Overlay contour plot of the predicted water activity (a_w) and viscosity based on pectin concentration, carrageenan concentration, and TSS.

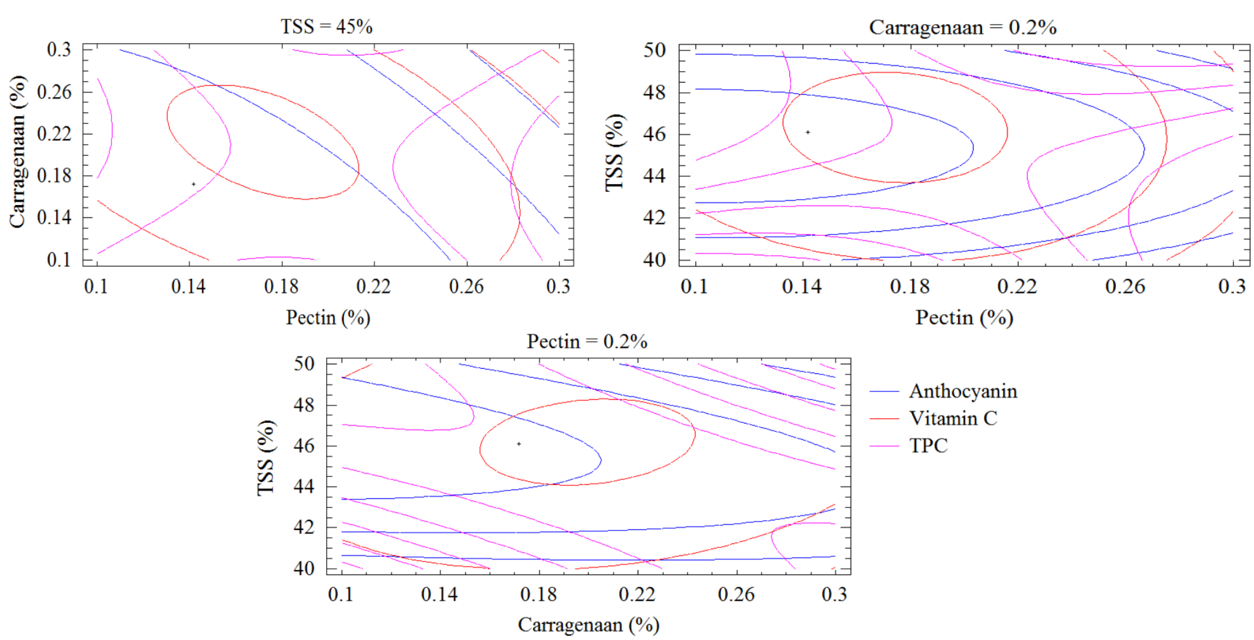

Figure 6: Overlay plot for bioactive compounds in reduced-calorie blackberry jam.

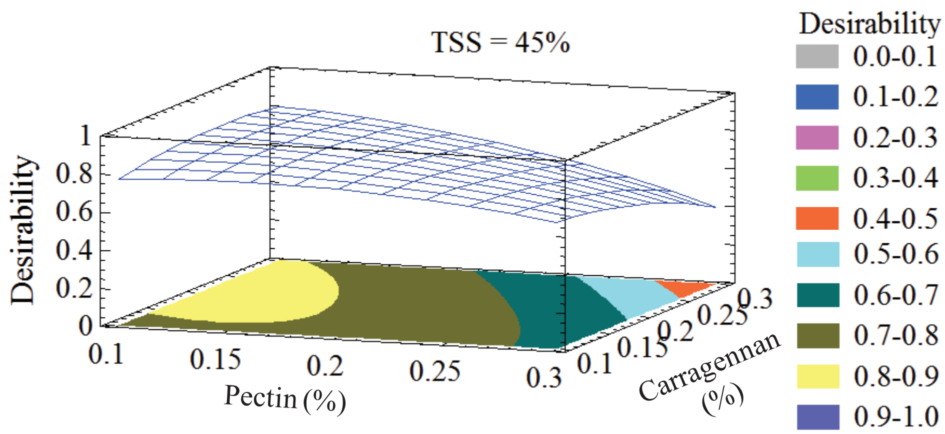

Figure 7: Estimated response surface for desirability.

when using pectin, carrageenan concentrations, and TSS in the ranges of 0.1–0.2%, 0.15–0.25, and 44–46°Brix, respectively (Figs. 6 and 7). Combined with the previous data about the change of water activity and viscosity, this confirmed that the appropriate pectin, carrageenan, and TSS content for blackberry jam processing were 0.18%, 0.22%, and 45°Brix, respectively. At this processing condition, the average sensory scores for color, odor, taste, texture, and overall acceptability were 4.3, 4.5, 4.7, 4.7, and 4.8, respectively, according to the QDA method.

4. CONCLUSION

The results obtained from the study confirmed that three levels of the three factors significantly influenced the physicochemical parameters, bioactive compounds, and sensory values of the jam produced. The RSM has been successfully applied to optimize the ingredient levels based on the desired quality. Multiple regression analysis of empirical data could allow the generation of useful equations for general use to predict the system's behavior under different combinations of factors in food processing. The optimal values could be utilized in low-calorie blackberry jam preparation with good quality and higher nutritive value.

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