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Optimization of the Process of Reducing the Bitterness of Bitter Melon with Response Surface Methodology

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Abstract

Bitter melon fruit (*Momordica charantia*, Linn.) is rich in vitamins and minerals, antioxidants, and steroid saponins, which taste bitter and are not liked. Steroid saponin compounds have a good role in biological activities such as antidiabetic, anti-hypercholesterolemia, anti-obesity, anti-tumor, anti-inflammatory, analgesic, antiviral, and antidepressant. Currently, many efforts have been made to reduce the bitter taste, such as salting. To optimize the salting process, the response surface method with the developed model was employed. The design in this study was a 2k or partial factorial design with two levels for each variable coded -1 and +1 and expanded with a value of α, where α = 2k/4, and k = number of variables. To check the accuracy of the second-order polynomial model, the observations were repeated five times at the center point (X1 = 0 and X2 = 0). Bitter taste response parameters were measured using descriptive sensory tests and LC-MS chromatographic analysis of diosgenin compounds. The optimization results show that the quadratic polynomial regression equation is Y = 2.468 – 0.1053X1- 0.0176X2 + 0.0153X12 + 0.0178X22 + 0.085X1X2 where X1 = salt concentration and X2 = salting time. A minimal bitter taste response was obtained at a salt concentration of 5 % and 15 min of salting time with a desirability value of 94.1%. In the condition of a minimum bitter taste response of 2.145, the optimum point for each variable is a stationary point, considered the optimum response.

Keywords: Bitter gourd, Bitter cucumber, Reduce bitter taste, Herbal medicine, Medicinal vegetable, *Momordica charantia* L., Optimization decrease, Salting treatment

1. Introduction

The bitter melon plant belongs to the Cucurbitaceae family; and the distribution includes China, India, and Southeast Asia. Bitter melon or bitter gourd fruit (*Momordica charantia*, Linn.) is rich in nutrients, especially vitamins, minerals, and fiber, and contains many complexes of beneficial bioactive compounds, and antioxidants, among other alkaloids, terpenoids, steroids, tannins, and saponins (Nursal and Yeanny 2019), which contribute to extraordinary versatility in treating diseases. Several researchers (Asmawati *et al*., 2022; Damat *et al*., 2019, 2020, 2021; Gangakhedkar *et al*., 2021, Setyobudi *et al*., 2019; Sur and Ray, 2020; Ummi *et al*., 2019) stated that fiber and antioxidants have good roles in supporting biological activities and act as anti-diabetic, anti-hypercholesterolemic, anti-obesity, antitumor, anti-inflammatory, analgesic, antiviral, and antidepressant. The people of southern Japan use bitter melon as a laxative and anthelmintic (Shubha *et al*., 2018). Bitter melon extract in India is used as a diabetic drug, rheumatic drug, liver disease drug, and lymphatic disease drug (Ee Shian *et al*., 2015).

Bitter melon in Indonesia, apart from being known as a vegetable, is also traditionally used as a phlegm laxative, fever reducer, and appetite enhancer. Bitter melon leaves are used as a menstrual laxative, burn medicine, skin disease medicine, and worm medicine (Bahagia *et al*., 2018). Since it is known that the bitter melon plant is productive for health, several researchers have tried to identify and isolate the material that depends on the bitter melon plant. Various kinds of treatment can be done to reduce the bitter taste of bitter melon before the culinary/cooking process is carried out. One way is often done is by salting treatment (Ummi *et al*., 2019). Various methods of salting are carried out by the community, squeezing bitter melon fruit that has been sliced

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longitudinally and transversely to become bitter melon slices using fingers for a specific time and adding a certain amount of salt. Indonesia, an archipelagic country consisting of thousands of islands, has various tribes, languages , and cultures. Each region also has a distinctive food, processed using local ingredients and favored by the local community and then referred to as the area's traditional food. This food can change regarding ingredients and processing methods (Weaver, 2018). Even more interesting is consuming food with essential elements derived from plants and animals locally with natural processing (Wijaya, 2019). The problem is more in the way Indonesian people process their food. Almost all traditional Indonesian food is processed using heating until it is cooked. In addition, especially for vegetable processing, the vegetable ingredients are often washed, boiled, and the water removed, then the vegetables are kneaded before being mixed with spices and served. This way, some nutrients, such as vitamins and minerals that are soluble in water, will be wasted through washing, and some that are not heat resistant will be damaged during the cooking process (Reis *et al*., 2015; Xu *et al*., 2014). Setyobudi *et al*. (2021, 2022) reported a decrease in vitamin C and vitamin A levels when heating above 50 °C. This high temperature has a negative impact because these two vitamins are enchangers in the absorption of Fe non-heme nutrients in the human body. Another adverse effect is the damage to antioxidants. Therefore, this research was carried out to avoid damage to the valuable ingredients in bitter melon by developing a model for optimizing the salting process.

2. Material and Methods

Optimization of the bitter melon pre-treatment process by adding salt and pressing was carried out using the Response Surface Methodology (RSM). The research design using the RSM method, which many statisticians have developed to construct a second-order response surface design with two variables, is the Central Composite Design (CCD). The design of this study is a 2k factorial or partial factorial (fractional factorial) with two levels on each variable coded -1 and +1 and expanded with a value of α, where α = 2 k/4 and k = number of variables. The second-order polynomial model is tested for accuracy by making observations repeated five times at the midpoint ($X1 = 0$ and $X2 = 0$) (Kumari and Gupta, 2019). In addition, before compiling the primary research with a composite design, a preliminary study was carried out to determine the optimum point of each optimized variable. These points are considered process conditions that produce the optimal response.

3. Results and Discussion

3.1. Preliminary research

This preliminary study optimized the concentration of added salt (KK) and kneading time (KW). In addition, preliminary research was conducted to determine the optimum point of each optimized variable in the initial treatment of squeezing bitter melon slices that had been added with salt. This point is suspected to be a condition that produces an optimum response, namely a minimal bitter taste response (Y). The results of Y response data obtained from preliminary research from each optimization variable are presented in Figure 1 and Figure 2.

Based on Figure 1, it can be seen that the minimum score, which is the optimal value in the preliminary study of the salting optimization process, is found in the 7.5 % concentration treatment with a duration of 15 min with an average score of 2.4. This score shows the minimum bitter taste response score. Figure 2 shows that the minimum mean score of 2.4 is found in the 10 % salt concentration treatment and the salting time of 10 min. This minimum score is the optimum value at the preliminary research stage of the salting optimization process. This optimum value indicates a minimal bitter taste response. The optimum value at the initial research stage of the optimization of the salt kneading process is then used as the optimum level and the experimental area in the primary research stage of the optimization of the salt kneading process.

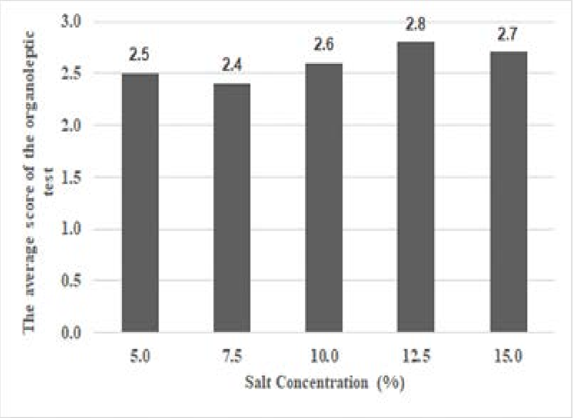


Figure 1. Histogram of preliminary research on the optimization of the squeezing process at 15 min

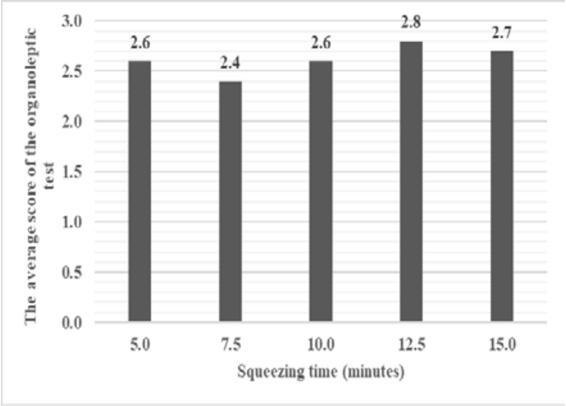


Figure 2. Histogram of preliminary research on process optimization salting at 10 % salt concentration

As a comparison material in the preliminary research on the optimization of the salting kneading process with this descriptive sensory test, a test was carried out by measuring the levels of bitter taste compounds in bitter melon fruit by LC-MS chromatographic method. Diosgenin compounds are known to have a bitter taste in bitter melon. Diosgenin is a steroidal saponin compound with a bitter taste (Joseph and Jini, 2013). The chromatogram of the results of the analysis of the diosgenin content of bitter melon in a preliminary study of optimization of the salting squeezing process of bitter melon before culinary processing is contained in the appendix. In comparison, the table of diosgenin levels on the optimization of salt concentration and salting time variables is presented in Figures 3 and Figure 4. The chromatography results of the diosgenin content in Figure 3 show that the combination treatment of 7.5 % salt concentration and 15 min of squeezing time resulted in the lowest diosgenin level of 0.461 mg g^{-1}. Meanwhile, the chromatography results of diosgenin levels on optimization of squeezing time with a salt concentration are presented in Figure 4.

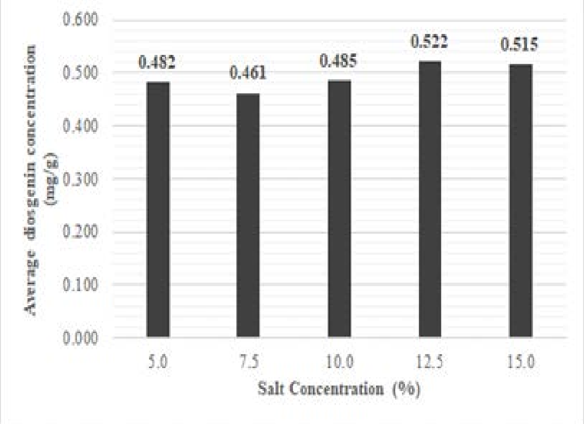


Figure 3. The levels of diosgenin compounds are corrected for the bitter taste in optimization of salt concentration with 15 min

The lowest diosgenin level shown in Figure 4 is 0.470, namely the 10 % salt concentration treatment with a kneading time of 10 min. Low diosgenin levels indicate a lot of diosgenin is wasted after squeezing bitter melon slices with added salt. In addition, low diosgenin levels indicate a minimal bitter taste response. The points of the lowest diosgenin levels in Figure 3 and Figure 4 are the points that are thought to produce an optimum response, namely a minimal bitter taste response (Y), while the level of treatment that will be the center point of the experiment in the main study is the level of treatment that produces a minimal bitter taste response in each optimized variable.

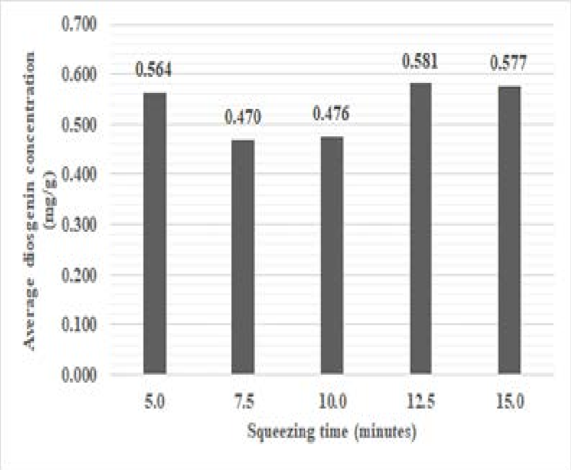


Figure 4. Levels of bitter-tasting compounds (diosgenin) on optimization of squeezing time with 10 % salt concentration

Based on Figure 1 and Figure 3, the minimal bitter taste response with a mean score of 2.4 and the lowest diosgenin level with a concentration of 0.461 mg g^{-1} was obtained at a salt concentration of 7.5 %, while Figure 2 and Figure 4 show a minimal bitter taste response with the same average score (2.4) and the lowest diosgenin level of 0.470 mg g^{-1} obtained at 10 min of squeezing. The variables with minimal bitter taste response and the lowest diosgenin levels were then used as the center point for the primary research with the coded variable (0,0). The treatment for coded variables with a central point (0.0) was expanded by taking two inflection points from each variable, namely at the point before the level (coded -1) and at the point after the level (coded +1) from the optimum level. Therefore, the two inflection points for the salt concentration variable are 5 % (code -1) and 10 % (code +1), while the two inflection points for the variable length of extraction time are 5 min (code -1) and 15 min (code + 1). These points are then used to develop experimental designs in the primary research.

3.2. Main research process optimization

The primary research was carried out using three levels on each variable which was assumed to be the optimum level and the experimental area obtained from the preliminary research. Determining the desired optimum point in the response surface analysis with a central composite design requires an experimental area around the center point. The experimental area consists of two levels of each variable coded with -1 and +1 and expanded with the value of α. The formula set for the value of α is where k is the number of variables being tested (Kumari and Gupta, 2019) so that the value of α is set at 1.414. Code variables -1 and +1 indicate the level of variables that are before and after the optimum point. The distance between the coded variable -1 and the optimum variable 0 is the same as the distance between the coded variable +1 and the optimum variable 0.

The experimental design used in this main study is the Central composite design with a combination treatment of the processes obtained from the introduction, namely the salt concentration of 7.5 % and the duration of 10 min repeated five times. Further expansion of the treatment was carried out by combining the points before and after the optimum conditions for the concentration variables of 5 % (code -1) and 15 % (code +1) and for the variable length of time 5 min (code -1) and 15 min (code +1). In determining the second-order equation, the treatment is further expanded by combining each optimum condition with points – α and – α. The point α for the concentration variable was determined at 3.965 % (code -1) and 11.035 (code +1), while for the time variable, it was determined for 2.93 min (code -1) and 17.07 (code +1). The composite design of this research center is presented in Table 1.

Table 1. Central composite design for pre-salting process conditions for Bitter melon

Condition	Original variable		Code Variable			
Process	Concentration (%)	Time (min)	X_1	X_2	Response of Bitter taste	
1	5	5	-1	-1	2.87± 0.06	bit bitter
2	5	15	-1	+1	2.1± 0.05	bit bitter
3	10	5	+1	-1	2.5± 0.03	bit bitter
4	10	15	+1	+1	2.47± 0.06	bit bitter
5	7.5	10	0	0	2.53± 0.08	bit bitter
6	7.5	10	0	0	2.5± 0.05	bit bitter
7	7.5	10	0	0	2.57± 0.05	bit bitter
8	7.5	10	0	0	2.47± 0.03	bit bitter
9	7.5	10	0	0	2.5± 0.07	bit bitter
10	3.965	10	-1.414	0	2.33± 0.05	bit bitter
11	11.035	10	+1.414	0	2.37± 0.03	bit bitter
12	7.5	2.93	0	-1.414	2.67± 0.03	bit bitter
13	7.5	17.07	0	+1.414	2.47± 0.03	bit bitter

Score description: 1 to 1.99: less bitter ; 2 to 2.99: slightly bitter ; 3 to 3.99: bitter ; 4: very bitter

3.2.1. Response surface methodology analysis (RSM)

3.2.1.1. Model selection analysis

Statistical model selection analysis was conducted to determine the appropriate model for describing the significance of the research results obtained. The model selection analysis was based on: i) the description of the sum of squares (Sequential Model Sum of Squares), ii) the inaccuracy test of the model (Lack of Fit Test), and iii) the summary of the statistical model (Summary of Statistics). Several models may be selected, including linear models, 2-factor interaction models, and quadratic and cubic models.

3.2.1.2. Model selection based on the number of squares

The selection of the model based on the number of squares is based on the highest value of the polynomial degree with the condition that the model is accepted if the p-value < 0.05 (the probability of error from the model is less than 5 %), which means the model has a significant effect on the response. The table of the model selection results based on the description of the number of squares is presented in Table 2. Based on the analysis of the Sequential Model Sum of Squares (Table 2), it is found that a model can be chosen to describe the phenomenon of the effect of salt concentration and to knead time on the resulting bitter taste. Therefore, the analysis results obtained that matches the selected model (Suggested) is of quadratic model design.

The model selection based on the number of squares starts from the linear model. The linear model has a p-value of 0.0856 (8.56 %), indicating that the probability of model error is more than 5 % (the p-value in the program has been set < 5 %), which means that the model is not significant (not significant) on the response. The next model observed is 2FI (interaction between two variables). Based on Table 2, it can also be seen that the p-value for the 2FI model is 0.0057 (0.57 %), which indicates the model error is less than 5 % which means that the 2FI model is significant to the response. The following model observation is the Quadratic form with a p-value of 0.0529 (5.29 %) – indicating that the probability of error from the model is close to 5 % – which means that the Quadratic model has a significant (significant) effect on the response, making it the best design and selected as "Suggested" referring to the sum of the squares description. The Cubic model has a p-value of 0.0121 (1.21 %), which indicates that the probability of error from the model is less than 5%, which means that the Cubic model has a significant effect on the response. The program declares the Cubic model "Aliased" (not recommended) because it is suspected that the model is too complex and impossible to use.

Table 2. The results of model selection based on the description of the sum of squares

Source Diversity	Number of Squares	db	Square Mean	F Count	P value Prob>F	Information Model
Mean vs Total	80.50	1	80.50			
Linear vs Mean	0.15	2	0.073	3.18	0.085 6	
2FI vs Linear	0.14	1	0.14	13.04	0.005 7	
Quadratic vs 2FI	0.054	2	0.027	4.61	0.052 9	Suggested
Cubic vs Quadratic	0.034	2	0.017	12.13	0.012 1	Aliased
Residual	0.006 97	5	0.001 39			
Total	80.88	13	6.22			

3.2.1.3. Model selection based on model inaccuracy test

The selection of the second model is based on the model inaccuracy test (Lack of Fit Test). Kumari and Gupta (2019) state that the main criterion for the accuracy of the model is based on the lack of fit test because a model is considered appropriate if the model inaccuracy test is not statistically significant and is considered inappropriate to explain a problem from an analyzed if the model's inaccuracy is statistically significant. In contrast to the previous model selection criteria, the model selection criteria based on the model's inaccuracy is determined by a p-value > 5 %, where the model is accepted if it has a p-value > 5 %, which means the model inaccuracy is not significant to the response. The calculation of the model selection results based on the model inaccuracy test is presented in Table 3.

Table 3. Results of model selection based on inaccuracy of the test model

Source Diversity	Number of Squares	db	Square Mean	F Count	P value Prob>F	Information
Linear	0.23	6	0.038	26.30	0.0035	
2FI	0.089	5	0.018	12.42	0.0151	
Quadratic	0.035	3	0.012	8.18	0.0351	Suggested
Cubic	0.001 25	1	0.001 25	0.87	0.4027	Aliased
Pure Error	0.005 72	4	0.001 43			

The p-value in the analysis of the model's inaccuracy in Table 3 for the Quadratic model shows a result of 0.0351 (3.51 %), which means that the inaccuracy of the Quadratic model has a significant effect on the response, and so the Quadratic model is not accepted. Still, compared with other values, the Quadratic model has a p-value close to 0.05, and so the test suggests using the Quadratic model (suggested). For the Cubic model, a p-value of 0.4027 (40.27 %) was obtained, which indicates that this model is not significantly different, but the Cubic model is not recommended (aliased). In the Linear and 2FI models, each has a p-value of 0.0035 (0.52 %) and 0.0151 (0.30 %), indicating a p-value of less than 5 %, and so the two models are significantly different and the model is conclusively incorrect.

3.2.1.4. Model selection based on statistical model summary

The selection of the third model is based on the summary statistical model analysis (model summary statistics), namely the calculation analysis of the conclusions from the previous calculations. According to Montgomery (2013), determining the best model is focused on the maximum adjusted R2 and Predicted R2 values. Furthermore, the model selection also focuses on the minimum PRESS (Prediction Error Sum of Squares) value (Arham *et al.*, 2013). The analysis of the model based on the complete summary of the statistical model is presented in Table 4.

Table 4. The results of the model selection based on the summary description of the statistical model

Source Diversity	Standard Deviation	R^2	Adjusted R^2	R^2 Prediction	PRESS	Information
Linear	0.15	-0.325 4	0.388 4	0.266 1	0.50	
2FI	0.10	0.312 7	0.750 2	0.667 0	0.26	
Quadratic	0.076	0.317 1	0.892 2	0.815 1	0.26	Suggested
Cubic	0.037	0.764 9	0.981 6	0.955 8	0.089	Aliased

The statistical parameter used to select the suitable model is the model with the lowest standard deviation and PRESS. The low standard deviation and PRESS indicate that the level of variance and prediction of the number of squares error is low. Based on Table 4, the lowest standard deviation is owned by the Cubic model, but the model has a high PRESS value, so the Cubic model cannot be selected. In addition, the complexity of the Cubic model makes this model Aliased status. The model that has the lowest standard deviation value is the Quadratic model. The quadratic model has a standard deviation of 0.076, and the lowest PRESS is 0.26, which makes this model a Suggested status (recommended to be selected).

The quadratic model has an R2 value of 0.3171, which indicates that the salt concentration factor, soaking time in salt solution in the study, has an effect of 31.71 % on the diversity of bitter taste responses while the remaining 68.29 % is influenced by other factors not included in the model. The adjusted R2 value is 0.892 2, which means the close relationship between salt concentration and soaking time in a salt solution to the bitter taste response is 89.22 %. The difference between the R2 and Adjusted R2 is thought to be caused by the emergence of insignificant additional variables in the development of the model. Montgomery (2013) states that a decrease in Adjusted R2 will occur if the variables added to the modeling have no effect.

3.2.2. Analysis of variance (ANOVA) of RSM on bitter taste response

From the results of the three model selection processes, the Quadratic model was chosen as the model used to explain the relationship between the variables X1 (salt concentration) and X2 (squeezing time) to the Y response (bitter taste). After selecting the model, an analysis of variance was carried out on the Quadratic model. The results of the study of variance for the Y response (ethanol content) with the complete Quadratic model are presented in Table 3. The analysis of variance (ANOVA) presented in Table 5 shows that the model significantly affects the response, where the p-value is less than 0.05 (5 %). A lack of fit or significant test inaccuracy of 0.0351 indicates that the model only matches some design values. This is because the p-value is smaller than 0.05. The analysis of variance showed that the soaking time, the interaction between the salt concentration and the soaking time, and the salt concentration (squared) had a significant (significant) effect on the response. Other factors, namely salt concentration (linear) and soaking time (squared), had no significant (not significant) impact on the response.

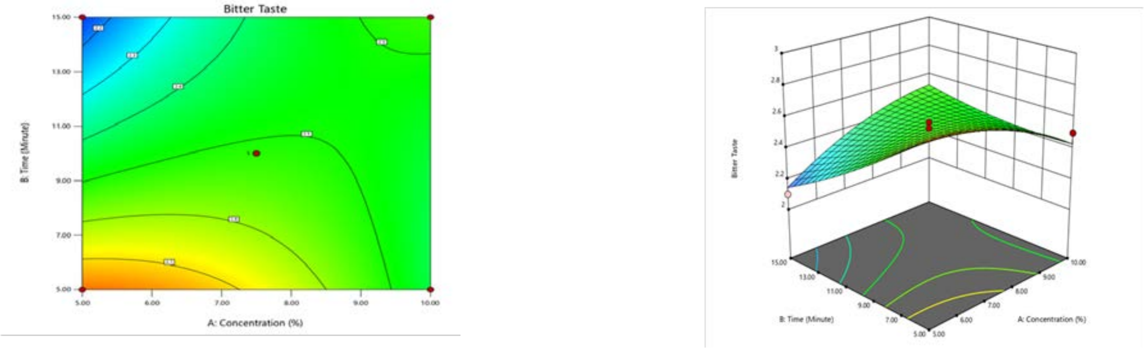
Table 5. Results of analysis of variance (ANOVA) of the quadratic model

Source Diversity	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Information
Model	0.34	5	0.068	11.58	0.002 8	significant
A-Concentration	4.000E-004	1	4.000E-004	0.069	0.800 9	not significant
B-Time	0.15	1	0.15	25.15	0.001 5	significant
AB	0.14	1	0.14	23.49	0.001 9	significant
A^2	0.040	1	0.040	6.85	0.034 6	significant
B^2	8.160E-003	1	8.160E-003	1.40	0.275 3	not significant
Residual	0.041	7	5.829E-003			
Lack of Fit	0.035	3	0.012	8.18	0.035 1	significant
Pure Error	5.720E-003	4	1.430E-003			
Cor Total	0.38	12				

Description: A = Variable X_1/Salt Concentration (% v v^{-1}); B = Variable X_2 (soaking time h^{-1}) , AB, A_2, B_2 = interaction between treatments

3.2.3. Response graph of the effect of salt concentration and salting time on the decrease in the bitter taste of bitter melon.

The response graph is used to facilitate the description in knowing the effect of the variable on the bitter taste response. The bitter taste response is depicted in 3-dimensional curves and plot contours. A contour plot is a 2-dimensional plot, a cross-section of a 3-dimensional curve. Contour plots help analyze the effect of interaction between factors on responses Oliveira (2019). Figure 5 depicts dimensional curves and plot contours for optimizing the process of squeezing bitter melon with the addition of salt. Each figure shows the effect of the two parameters on the fruit-squeezing process. The values listed in the boxes on the contour plots indicate bitter taste in the studied squeezing process conditions. Figure 5 shows that the shape of the saddle shape curve depicts the possibilities of the variables at the maximum and minimum points. Such a contour system is called a saddle or minimax system. The interaction between salt concentration and soaking time is shown in Figure 5. The x-axis and y-axis in Figure 5(a) show the optimized variables. The x-axis shows the salt concentration variable, while the y-axis shows the soaking time variable. Circular lines indicate the response. The optimal response is characterized by the presence of a flag in the middle of the contour, which shows the optimal point information located at the point (node) displayed on the flag. The contour plot indicates the optimum salt concentration at the point (node) at 5 % (v v^{-1}) and and 15 min.



(a) (b)

Figure 5. (a) Contour plot and (b) response surface curve (three dimensions) salt concentration and duration of concentration on bitter taste respons

3.2.4. Determination of optimum conditions

Software Design Expert 7.1.5 was used to identify the best combination of bitter taste reduction process parameters to optimize bitter taste response. A desirability is a tool used to explain how well the optimal solution offered follows the objectives of the response A desirability value of 1 indicates the perfect case, but a desirability value of 0 indicates the response must be discarded. In this study, the optimal solution offered by the model is a salt concentration of 5 % ($v\ v^{-1}$), and a soaking time of 15 min for predicting the bitter taste response is the same as the optimization, which is 2.145 with a desirability value of 0.941. The optimum point of each variable is a stationary point which is assumed to be the optimum response.

4. Conclusion

The optimum treatment for removing the bitter taste of bitter melon can be done by squeezing the bitter melon, which has been sliced lengthwise and crosswise for 15 min with the addition of 5% salt concentration. This treatment is optimal for salting bitter melon with a minimum bitterness response score of 2.145. The optimization results show that the quadratic polynomial regression equation is Y= 2.468–0.1053X1- 0.0176X2 + 0.0153X12 + 0.0178X22 + 0.085X1X2, where X1=salt concentration and X2=stirring time. This salting process changes several parameters of nutritional content and bioactive compounds, such as total carbohydrate, crude protein, fat, total sugar, pectin, soluble and insoluble food fiber, vitamin C, β-carotene, diosgenin, β-sitosterol, stigmasterol, and campesterol.

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