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Formula optimization of functional beverage made from Carica seeds

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Formula optimization of functional beverage made from Carica seeds

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Abstract. Cheese Carica fruit *(Carica pubescens, Lenne)* is a geographical indication product of Wonosobo District. Usually, Carica fruit is processed into cocktails before consumption. Carica seeds are one of the wastes produced from the processing of cocktails. Research on processing Carica seeds has never been done. Carica seeds can be processed into ready-to-drink beverages through the fermentation process of seeds with commercial inoculums containing microorganisms and mixing with other ingredients such as jackfruit (in the form of dried jackfruit powder). This study was aimed to 1) determine the appropriate fermentation method to produce Carica seeds with high content of phenol and antioxidants; 2) optimizing the fermentation time of Carica seeds and the proportion between Carica seeds and jackfruit to produce a beverage that has a sensory responses, i.e. overall acceptability (maximum), fruity flavor (maximum), bitter taste (minimum) and after taste (minimum); 3) examine the sensory characteristics of product with optimum formula by quantitative descriptive analysis (QDA) used 15 cm unstructured scale; 4) examine the physicochemical properties of product with optimum formula. The study of determination of Carica seed fermentation methods and the physicochemical properties of the product was carried out 2 times and the data were analyzed by Ms. Excel 2010. Formula optimization was done by surface response methodology with a central composite design using Design Expert V.7 software (for trial). The sensory responses were tested by 10 trained panelists with rating method using 9 intensity scales. The results showed that: 1) Solid-state fermentation method with a proportion of 0.2% commercial inoculum resulted in Carica seed powder which had the highest phenol and antioxidant content compared to other methods (0.27 and 86.17%, respectively); 2) Formula with 0.9 of desirability value consisting of 75 hours of fermentation time, 72% of Carica seeds powder and 28% of jackfruit powder produce beverage with actual rating score (range = 1–9) of overall acceptability, fruity flavor, bitter taste, and after taste i.e 8.23, 7.76, 4.41, 3.79, respectively. Compared to control (a beverage made from 100% Carica seeds without fermentation), the optimum product has a stronger fruity flavor and sweet taste and a weaker in a bitter taste. Products with optimum formula preferred by 25 semi-trained panelists and have a higher content of antioxidant, phenol, vitamin C, sugar, potassium than control, i.e 89.21% db, 0.35% db, 135.25 mg/100 g db, 10.94% db, 90.65 ppm, and 51.66 ppm, respectively. (slightly favourable).

Keywords: Carica seeds, functional beverage, solid-state fermentation, formula optimization, quantitative descriptive analysis

1. Introduction
As a major commodity in Dieng, Central Java, Indonesia, Carica fruit contains high in vitamin C, vitamin K, flavonoids, antioxidants, and dietary fiber. Carica fruit which has a tough texture with acid, sweet, and fruity taste. It can only be consumed after processing. Carica fruit has been processed into cocktails by around 300 SMEs in Wonosobo. One of the side products of the Carica cocktail which until now has not been utilized is Carica seeds. Research on the processing of Carica seed has never been done. The preliminary study indicates that Carica seeds were rich in antioxidant content but bitter-astringent tastes that are less acceptable to consumers. The fermentation process using commercial inoculums containing microbes such as molds, lactic acid bacteria, and yeast could reduce the bitter taste of Carica seeds. Dried fermented Carica seeds can be used as functional drinks after being ground and mixed with other ingredients such as dried jackfruit in a certain comparison.

This study was aimed to 1) determine the appropriate fermentation method to produce Carica seeds with high content of phenol and antioxidants; 2) optimizing the fermentation time of Carica seeds and the proportion between Carica seeds and jackfruit to produce a beverage that has a sensory responses, i.e. overall acceptability (maximum), fruity flavor (maximum), bitter taste (minimum) and after taste (minimum); 3) examine the sensory characteristics of product with optimum formula by quantitative descriptive analysis (QDA) used 15 cm unstructured scale; 4) examine the physicochemical properties of product with optimum formula.

2. Experimental details

2.1. Ingredients
Carica fruit and jack fruit was obtained from Wonosobo district. Other ingredients (sugar, commercial inoculums) were obtained from a local market in Purwokerto central Java.

2.2. Methods
The stages of this research were: 1) determination of the fermentation method of Carica seeds to obtain dry seed powder which has a low intensity of bitter and astringent taste and high content of antioxidants and phenols. This stage includes the determination of the fermentation method and fermentation time; 2) Formula optimization. This stage includes a) determination of basic formula and process; b) Recruitment of trained panelists; c) Formula optimization; 3) Product characterization through quantitative descriptive analysis (QDA) and analysis of physicochemical properties of the product with optimum formula compared to control; 5) hedonic test by 70 untrained panelists.

2.2.1. The production of Carica seed powder. Carica seeds were obtained from the waste of cocktails production by SMEs in Wonosobo. Carica seeds were separated from fruit flesh by washing with running water. 3 kg of Carica seeds were soaked with 9 L of a citric acid solution of 0.20% w/v for 1 hour. After draining, Carica seeds were steamed for 1 hour over medium heat. For the application of the submerged fermentation method, after cooling, Carica seeds were immersed in 9 L of water containing 0.2% w/v of commercial inoculum (La Prima) for a certain time (2–10 days). For the application of the solid-state fermentation method, after being cooled, Carica seeds were mixed with 0.2% w/v commercial inoculum (La Prima), packed in perforated plastic (dimensions of length x width x height = 20 cm x 20 cm x 0.5 cm), then fermented for a certain time (1–5 days). Fermented Carica seeds were dried with a cabinet dryer (60°C of temperature). Dried Carica seeds were ground with a blender and sifted with a 60 mesh sieve to produce Carica seed powder.

2.2.2. The production of dried jack fruit. Jackfruits with medium maturity were separated between flesh and seeds. After washing, fruits were steamed for 4 minutes over medium heat. After being cooled, the fruits were dried with a cabinet dryer (60°C of temperature). Dried jackfruits were ground with a blender to produce jackfruit powders.

2.2.3. The production of Carica seed beverages. The main ingredients consisting of Carica seed powders and dried jackfruits in a certain proportion. Carica seed beverage was made by brewing 5% of the main ingredient with 2% sugar using boiling water. After the mixture was left for 5 minutes, the mixture was filtered to separate the liquid and solid parts.

2.2.4. The recruitment of trained panelists dan semi-trained panelists. The stages of recruitment of trained panelists i.e.:1) Selection of panelists: a) filling out the questionnaire, acuity test through i) introduction test of primary aroma and taste, intensity test of primary taste; ii) sensitivity test (taste, texture, color, and aroma); 2) Panelist training through rating and ranking test (3 x) of Carica seed beverage using hedonic scale 1–9 [1]. The panelists of sensory tests who passed in the selection and training stages were 10 people. They were students at Food Science and Technology study program, Jenderal Soedirman University that consisting of 6 women and 4 men with the age between 20–21 years old. In this study, they carried out intensity tests using 1 to 9 numerical scales in overall acceptability, fruit flavor, bitter taste, and after taste responses of 14 products at the formula optimization stage, including verification and validation stage. The recruitment of semi-trained panelists was carried out to run the hedonic test of optimum and control products. They were students at Food Science and Technology study program, Jenderal Soedirman University who have graduated in sensory evaluation courses that consisting of 15 women and 10 men with the age between 20–21 years old.

2.2.5. Formula optimization. Formula optimization was done by the response surface methodology (RSM). This method only requires experimental data in a small amount and a short time to be able to explain the effect of independent variables on the responses, get a mathematical model that explains the relationship between independent variables and responses and get the process conditions that produce the best responses. The stages of formula optimization include: 1) conducting preliminary research as the basis for determining the treatment to be optimized and the minimum and maximum values of each treatment; 2) enter the lower and upper limit values of each treatment to the optimization program used to obtain the recommended combination of formulas; 3) determine the optimization criteria for each response measured; 4) measuring the responses (non-independent variables) of each combination of formulas recommended by the optimization program; 5) determine the composition of the independent variables as a result of RSM recommendations based on the specified optimization targets, predictive mathematical models obtained with certain R^2 values, and the value of the desirability; 6) making product with the optimum treatment recommended by the program as many as 5 replications (verification stage 1); 7) measuring the responses of overall acceptability, fruity flavor, bitter taste, and after taste of the product with the optimum formula to obtain the actual response value (verification stage 2); 8) perform validation of the optimum formula based on the predicted interval value and the actual value of the responses that has been obtained in the previous stage [1].

In this study, there were 2 independent variables observed, namely fermentation time and the ratio of Carica seed powder: dried jackfruit. The lower and upper limits for fermentation time were 24 and 96 hours, the proportion of Carica seeds were 50 and 100%, the proportion of dried jackfruit were 0 and 50% (table 1). The responses optimized were sensory attributes, i.e. overall acceptability and fruity flavor with the optimization criteria were maximum; bitter taste and after taste with optimization criteria were minimum.

Table 1. The upper and lower limits in formula optimization.

Treatment	Unit	-alpha	- Level	0	+ Level	+ alpha
Fermentation time	hr	24	34.54	60	85.45	96
The proportion of dried Carica seed powder	%	50	57.32	75	92.68	100
The proportion of dried jackfruit	%	0	7.32	25	42.68	50

2.2.6. Quantitative descriptive analysis, hedonic test, and physicochemical properties test. Quantitative descriptive analysis, hedonic tests, and physicochemical properties tests were carried out on products with optimum formula and control (containing 100% dried Carica seed powder). The Quantitative descriptive analysis was carried out by 10 trained panelists through a rating test using a 15 cm unstructured line scale. Quality attributes in the quantitative descriptive analysis were determined by trained panelists through Focus Group Discussion-FGD [3–6]. The hedonic test was carried out by 25 semi-trained panelists based on appearance, texture, aroma, and taste attributes using 9 hedonic scales. The physicochemical properties analyzed were water, ash, protein, fat [7], total dietary fiber, soluble dietary fiber, insoluble dietary fiber [8], total sugar with Anthrone method [9], vitamins C, potassium, sodium, phenol, and antioxidant capacity.

2.3. Experimental design

The formula optimization stage with the response surface methodology was done using Design Expert V.7 software for a trial. The experimental design applied was a central composite design (CCD). There are 2 treatments that will be optimized with 2 replications so that there were 14 formula combinations from the Design Expert program. Formula variations in Carica seed beverage recommended by the Design Expert V.7 program could be seen in table 2.

Table 2. The formula recommended by the Design Expert V.7.

Formula	Fermentation time (hr)	The proportion of dried Carica seed powder (%)	The proportion of dried jackfruit (%)
1	34.54	92.68	7.32
2	85.46	92.68	7.32
3	60.00	75.00	25.00
4	34.54	57.32	42.68
5	60.00	75.00	25.00
6	60.00	75.00	25.00
7	85.46	57.32	42.68
8	60.00	75.00	25.00
9	60.00	50.00	50.00
10	96.00	75.00	25.00
11	24.00	75.00	25.00
12	60.00	100.00	0.00
13	60.00	75.00	25.00
14	60.00	75.00	25.00

2.4. Analysis of samples

The results of the measurement of all responses from all formula combinations were analyzed by ANOVA. The mathematical model obtained will show positive or negative constant values. The positive constant value of a model showed that the optimized response will change with changes in the independent variable, while for negative constant values, the optimized response will change in contrast to the change in the independent variable. The model used for response analysis was the one that results in significant value on ANOVA and not significant value in lack of fit. The significant value of ANOVA showed that the optimized treatment affects the measured responses. Non-significant value of the lack of fit showed that there was a match between the responses data as measured by the RSM prediction model obtained. The coefficient of determination (R^2) of the mathematical model obtained illustrates the quantity of response measurement data that can be explained by the mathematical model [9].

The contour graph showed how the combination of independent variables influences the optimized responses. Different colors in the contour chart showed the value of the responses measured. The red color showed the highest response value. Lines consisting of points on the contour graph showed a combination of all the independent variables with different quantities that produce the same response value. The surface shape of the interaction relationship between independent variables can be seen more clearly with three-dimensional graphs. The three-dimensional graph showed projections from a contour graph. Areas that high on the 3-dimensional graph showed high desirability values, while in the low area the opposite applies. The best condition that brings together all objective functions in RSM was illustrated by the value of desirability (0–1). The higher the desirability value (close to 1) means the program was more perfect in predicting optimum conditions based on the target response that has been designed [2]. The graph of a normal plot of residual which illustrates the accuracy of the predicted value of the responses from its actual value. Residual means the difference between the RSM prediction responses and the actual responses. If the residual follows the straight line, that means the predicted value of the responses from RSM approaches its actual value [11].

For analysis of fermentation methods, quantitative descriptive analysis, hedonic tests, and physicochemical properties of the product were carried out twice. The data obtained analyzed by T-test.

3. Results and discussion

3.1. Determination of fermentation conditions

The fermentation process of Carica seeds was mainly to reduce the bitter and astringent taste. The fermentation conditions applied were submerged fermentation for 2, 4, 6, 8, and 10 days and solid-state fermentation for 1, 2, 3, 4, and 5 days. Fermentation was done by adding commercial inoculum La Prima as much as 0.2% of the amount of water used for immersion in the submerged fermentation method and 0.2% of the number of Carica seeds used for the solid-state fermentation method. La Prima contains microbial Rhizopus oligosphorus. The analysis for fermented products conducted was phenol content, antioxidant activity, and rating test for bitter and astringent using an intensity scale of 1 to 9 by 10 trained panelists.

Table 3. The characteristics of fermented Carica seeds.

Treatment	Time (day)	Fenol (%)	Antioxidant (%)	Bitter taste	Astringent taste
Control	0	0.16^{c}	83.98^{bc}	6.82^{a}	7.84^{a}
Submerged fermentation	2	0.14^{cd}	84.05^{bc}	6.51^{ab}	7.58^{ab}
	4	0.12^{d}	84.12^{bc}	6.39^{ab}	7.16^{bc}
	6	0.12^{d}	84.26^{bc}	6.24^{ab}	6.91^{bc}
	8	0.11^{d}	84.31^{bc}	5.71^{bc}	6.67^{bc}
	10	0.10^{de}	84.35^{bc}	5.59^{bc}	6.59^{bc}
Solid-state fermentation	1	0.22^{ab}	85.67^{ab}	4.56^{d}	5.75^{c}
	2	0.24^{ab}	86.09^{a}	4.45^{d}	5.23^{cd}
	3	0.27^{a}	86.19^{a}	4.42^{d}	4.62^{de}
	4	0.28^{a}	86.21^{a}	4.54^{d}	4.74^{de}
	5	0.28^{a}	86.20^{a}	5.34^{cd}	4.51^{de}

[abcde]The different superscript in the same column means significantly ($p < 0.005$) different

The results showed that the solid-state fermentation method was able to reduce the intensity of bitter and astringent taste and increase in phenol content and antioxidant activity higher than submerged fermentation. With the submerged fermentation method, an increase in fermentation time causes the antioxidant activity to increases while the phenol content decreases. With solid-state fermentation method, an increase in fermentation time causes antioxidant activity and phenol content increases. Solid-state fermentation was caused by the growth of mycelia quickly with darker colors (blackish gray) in line with the increasing of fermentation time. Based on the results of the research at this stage, at the next research stage, the fermentation method that will be applied was solid-state fermentation with a maximum time of 4 days. This was because the fermentation time of 5 days causes an increase in a bitter taste, and visually, the fermented Carica seeds have blackish mycelia with over-fermented aromas which were less acceptable by trained panelists. The characteristics of fermented Carica seeds can be seen in table 3.

3.2. Formula optimization

The treatments observed in this study consisted of fermentation time and the proportion of Carica seed powder: dried jackfruit. In the use of the design expert software, the term Carica seeds refer to the treatment of the proportion of Carica seed powder: dried jackfruit, which shows that the increase of the proportion of Carica seed powder means the decrease in the proportion of dried jackfruit seeds, as shown in the formula in table 2.

Table 4. The results of measurements of the responses of each formula.

Formula	Overall acceptability	Fruity flavor	Bitter taste	After taste
1	6.4	5.6	6.6	5.4
2	7.4	7.1	4.3	3.5
3	7.8	7.3	4.7	3.8
4	6.8	5.9	5.5	4.6
5	8.1	7.5	4.4	3.7
6	8.3	7.8	4.6	4.1
7	7.3	6.2	3.9	3
8	7.9	7.6	4.5	3.6
9	7.1	6.5	4.1	3.1
10	7.6	6.8	4.2	3.3
11	6.9	5.4	5.9	4.8
12	6.6	5.7	6.2	5.1
13	8.3	7.4	4.6	4.1
14	8.1	7.6	4.4	3.8

The results of the optimization with response surface methodology showed that the mathematical equations obtained for all responses (overall acceptability, fruity flavor, bitter taste, and after taste) following the quadratic polynomial model. The results of ANOVA showed that the difference in fermentation time had a significant effect ($p < 0.05$) on all responses measured. The difference in proportions of Carica seed powder: dried jackfruit has a significant effect ($p < 0.05$) on all responses observed, except fruity flavor. The interaction between the treatment of fermentation time and the proportions of Carica seed powder: dried jackfruit had a significant effect ($p < 0.05$) on all responses measured except after taste. The lack of fit analysis for all responses optimized was not significantly different ($p > 0.05$). This showed the suitability between data from all responses measured by the prediction model obtained. The value of the coefficient of determination showed that 95% of the overall acceptability response can be explained by the mathematical equation model, while fruity flavor, bitter taste, and after taste responses were 94%, 93%, and 89%, respectively. Data from responses measured can be seen in table 4. Analysis of the model for all responses observed in the study can be seen in table 5.

Table 5. Mathematics models for each response tested.

No	Response	Criteria of response	Importance	mathematics models	Determinate coefficient
1	Overall acceptability	Maximum	5	$8.08 + 0.31A - 0.13B + 0.13\ AB - 0.44A^2 - 0.64B^2$	0.95
2	Fruity flavor	Maximum	4	$7.53 + 0.47A - 0.066B + 0.30\ AB - 0.69A^2 - 0.69B^2$	0.94
3	Bitter taste	Minimum	3	$4.53 - 0.79A + 056B - 0.17\ AB + 0.25A^2 + 0.30B^2$	0.93
4	After taste	Minimum	3	$3.85 - 0.70A + 052B - 0.075\ AB + 0.11A^2 + 0.14B^2$	0.89

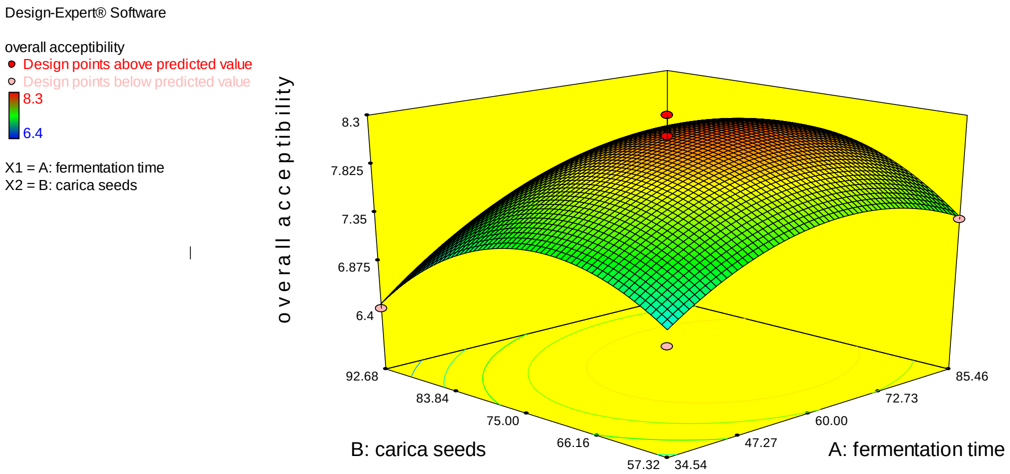

Figure 1. The three-dimensional contour of overall acceptability.

The description of the overall acceptability response in this study refers to the preference of Carica seed drinks based on the quality attributes of appearance, aroma, and taste. The target of overall acceptability response was maximal with 5 of importance value. The 3-dimensional contour graph for the overall acceptability response (figure 1) showed that the range of overall acceptability response from all formula combinations recommended by the optimization program was 6.4–8.3 (intensity scale ranging 1 to 9). The highest overall acceptability score of 8.3 (very like) was produced through 60 hr of fermentation time, 75% of Carica seed powder and 25% of dried jackfruit, whereas the lowest overall acceptability score of 6.4 (slightly like) was produced through 34.54 hr of fermentation time, 92.68% of Carica seed powder and 7.32% of dried jackfruit. Based on mathematical equations (table 5) and the 3-dimensional contour graph showed that the increase in fermentation time and the proportion of dried jackfruit causes an increase in product preference, while, an increase in the proportion of Carica seed powder causes a decrease in product preference. From the normal plot of the residual graph, it appears that for overall acceptability response following a straight-line pattern. It means that the predicted value of the response was close to its actual value (figure 2).

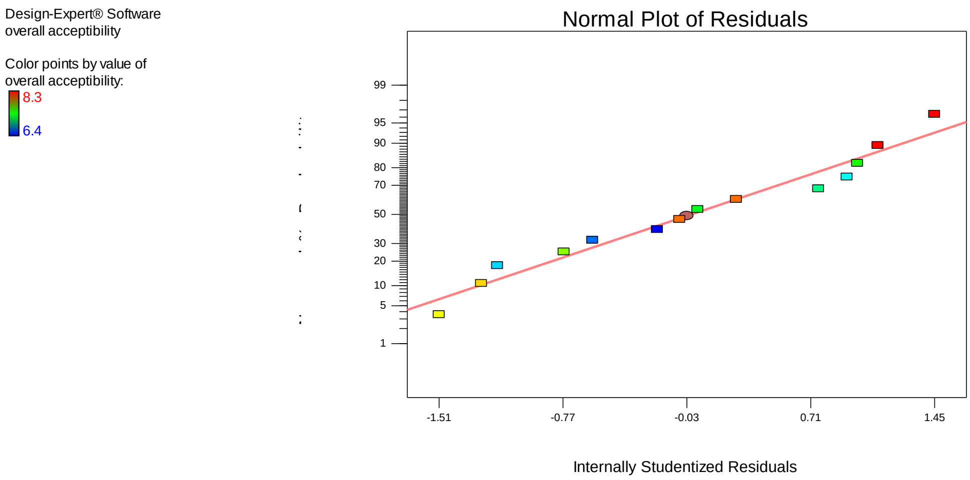

Figure 2. Normal plot of residuals of overall acceptability response.

The description of the overall acceptability response in this study refers to the preference of Carica seed drinks based on the quality attributes of appearance, aroma, and taste. The target of overall acceptability response was maximal with 5 of importance value. The 3-dimensional contour graph for the overall acceptability response (figure 1) showed that the range of overall acceptability response from all formula combinations recommended by the optimization program was 6.4–8.3 (intensity scale ranging 1 to 9). The highest overall acceptability score of 8.3 (very like) was produced through 60 hr of fermentation time, 75% of Carica seed powder and 25% of dried jackfruit, whereas the lowest overall acceptability score of 6.4 (slightly like) was produced through 34.54 hr of fermentation time, 92.68% of Carica seed powder and 7.32% of dried jackfruit. Based on mathematical equations (table 5) and the 3-dimensional contour graph (figure 1) showed that the increase in fermentation time and the proportion of dried jackfruit causes an increase in product preference, while, an increase in the proportion of Carica seed powder causes a decrease in product preference. From the normal plot of the residual graph, it appears that for overall acceptability response following a straight-line pattern. It means that the predicted value of the response was close to its actual value (figure 2).

The description of the fruity flavor response in this study refers to the aroma and taste of Carica fruit. The target of fruity flavor response was maximal with 4 of importance value. The 3-dimensional contour graph for the fruity flavor response (figure 3) showed that the range of fruity flavor response from all formula combinations recommended by the optimization program was 5.4–7.8 (intensity scale ranging 1 to 9). The highest fruity flavor score of 7.8 (strong to very strong) was produced through 60 hr of fermentation time, 75% of Carica seed powder and 25% of dried jackfruit, whereas the lowest fruity flavor score of 5.4 (neutral) was produced through 24 hr of fermentation time, 75% of Carica seed powder and 25% of dried jackfruit. Based on mathematical equations (table 5) and the 3-dimensional contour graph (figure 3) showed that the increase in fermentation time causes fruity flavor increase. From the normal plot of the residual graph, it appears that for fruity flavor response following a straight-line pattern. It means that the predicted value of the response was close to its actual value (figure 4).

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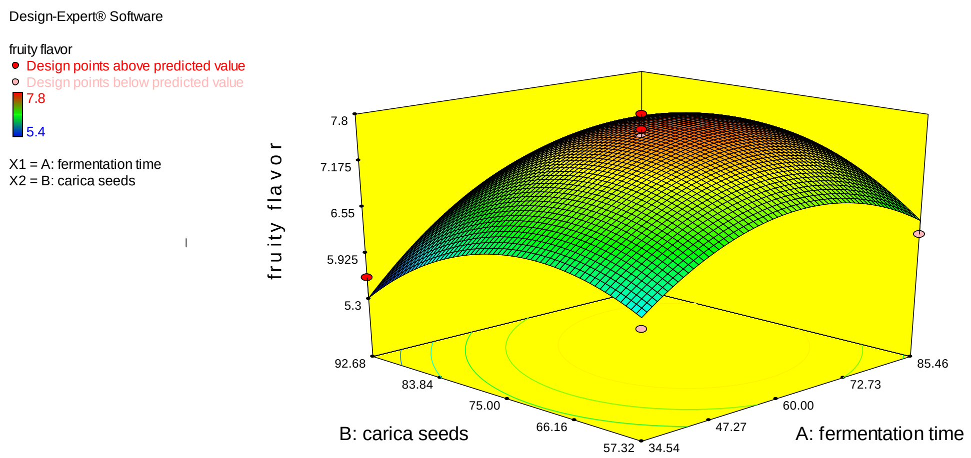

Figure 3. The three-dimensional contour of fruity flavour.

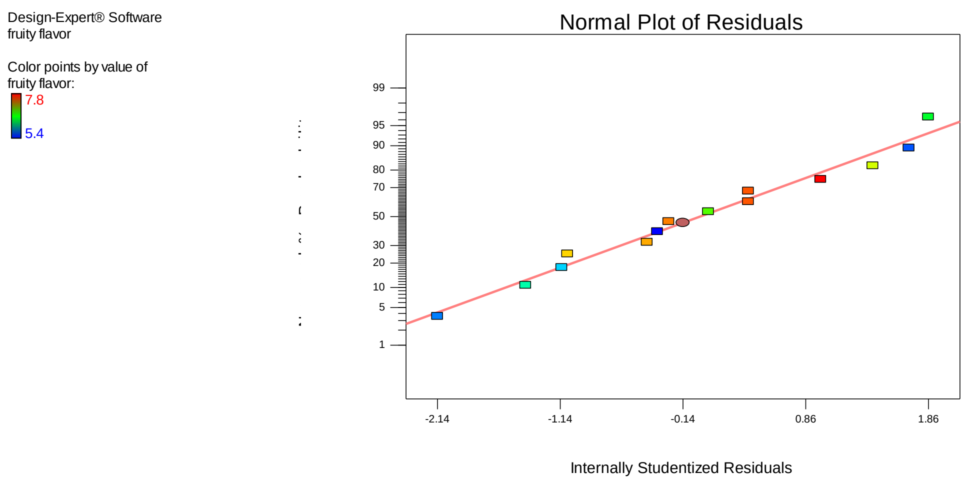

Figure 4. Normal plot of residuals of fruity flavor response.

The description of the bitter taste response in this study refers to taste detected by the tip of the tongue that comes from the compounds carrying the bitter taste, especially from Carica seeds. The target of bitter taste response was minimum with 3 of importance value. The 3-dimensional contour graph for the bitter taste response (figure 5) showed that the range of bitter taste response from all formula combinations recommended by the optimization program was 3.9–6.6 (intensity scale ranging 1 to 9). The lowest bitter taste score of 3.9 (slightly bitter) was produced trough 85.46 hr of fermentation time, 57.32% of Carica seed powder and 42.68% of dried jackfruit, whereas the highest bitter taste score of 6.6 (slightly bitter-bitter) was produced trough 34.54 hr of fermentation time, 92.68% of Carica seed powder and 7.32% of dried jackfruit. Based on mathematical equations (table 5) and the 3-dimensional contour graph (figure 5) showed that the increase in fermentation time and the proportion of dried jackfruit causes a decrease in a bitter taste, while, an increase in the proportion of Carica seed powder causes an increase in a bitter taste. From the normal plot of the residual graph, it appears that for bitter taste response following a straight-line pattern. It means that the predicted value of the response was close to its actual value (figure 6).

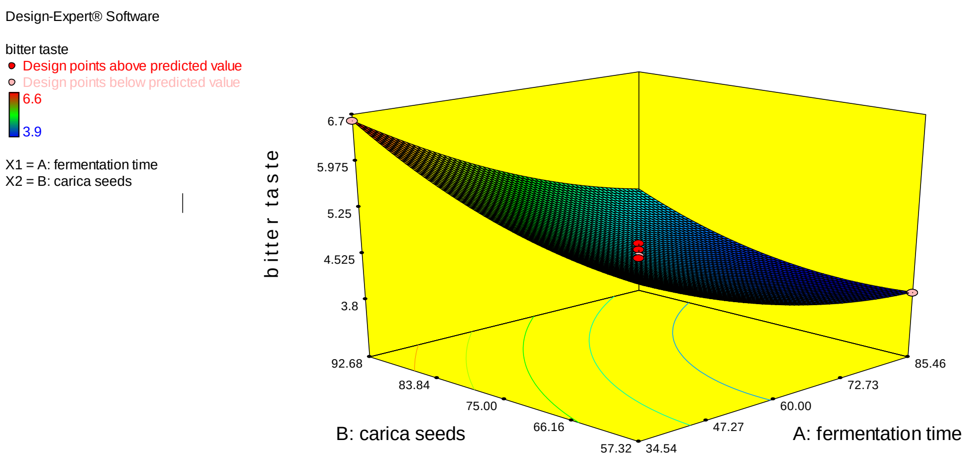

Figure 5. The three-dimensional contour of a bitter taste.

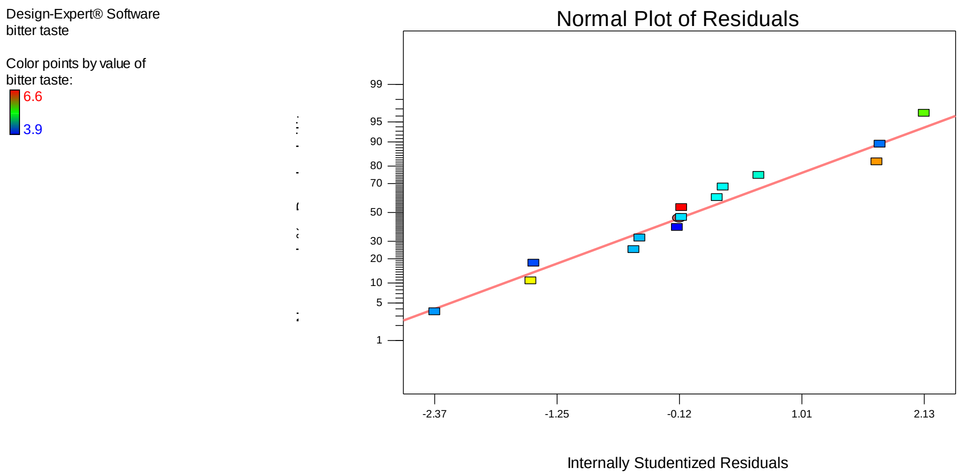

Figure 6. Normal plot of residuals of bitter taste response.

The description of the after taste response in this study refers to the taste that left on the tip of the tongue after the beverage was swallowed and goes into the esophagus. The taste identified was bitter-astringent, a distinctive taste of Carica seeds. The target of after taste response was minimum with 3 of importance value. The 3-dimensional contour graph for the bitter taste response (figure 7) showed that the range of after taste response from all formula combinations recommended by the optimization program was 3.0–5.4 (intensity scale ranging 1 to 9). The lowest after taste score of 3.0 (no- after taste) was produced through 85.46 hr of fermentation time, 57.32% of Carica seed powder and 42.68% of dried jackfruit, whereas the highest bitter taste score of 5.4 (neutral) was produced through 34.54 hr of fermentation time, 92.68% of Carica seed powder and 7.32% of dried jackfruit. Based on mathematical equations (table 5) and the 3-dimensional contour graph (figure 7) showed that the increase in fermentation time and the proportion of dried jackfruit causes a decrease in after taste, while, an increase in the proportion of Carica seed powder causes an increase in after taste. From the normal plot of the residual graph, it appears that for bitter taste response following a straight-line pattern. It means that the predicted value of the response was close to its actual value (figure 8).

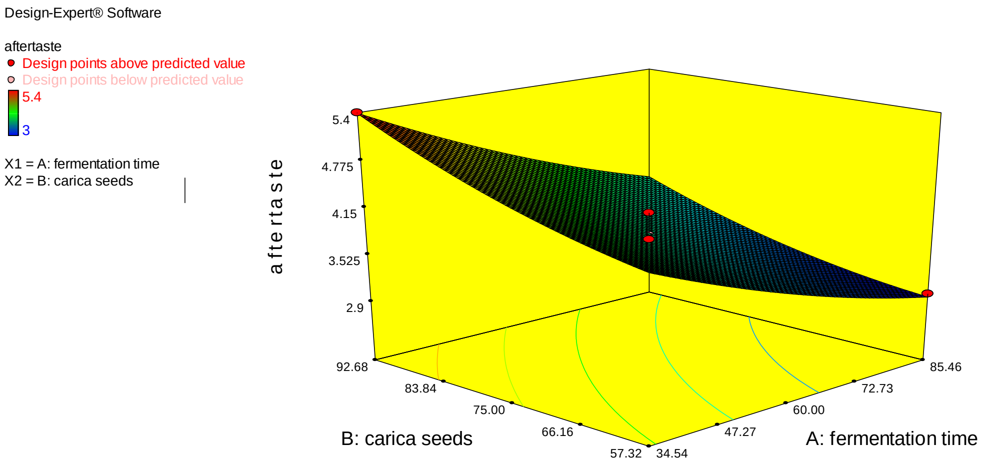

Figure 7. The three-dimensional contour of after taste.

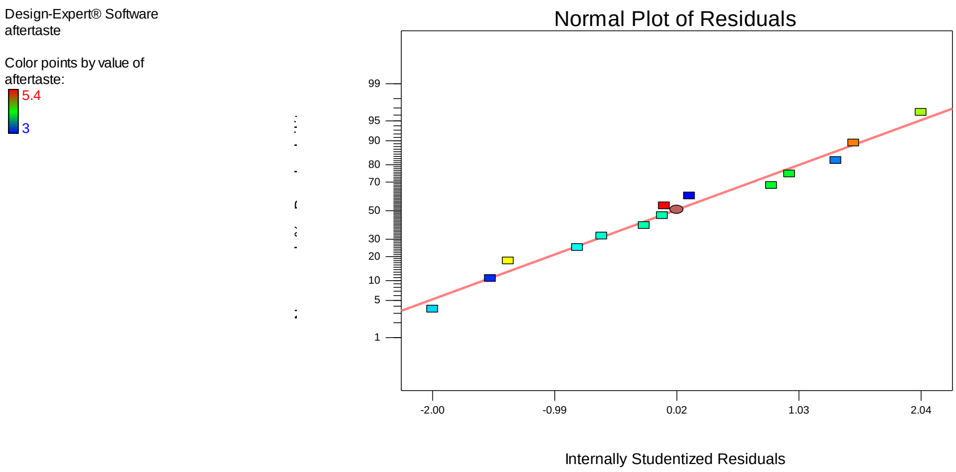

Figure 8. Normal plot of residuals of after taste response.

The optimization stage resulted in one recommendation for a formula combination. Formula with 0.9 of desirability value consisting of 75 hours of fermentation time, 72% of Carica seeds powder, and 28% of jackfruit powder produce beverage with prediction rating score (range = 1–9) of overall acceptability, fruity flavor, bitter taste, and after taste i.e 8.11, 7.54, 4.1, 3.4, respectively (figure 9 and 10).

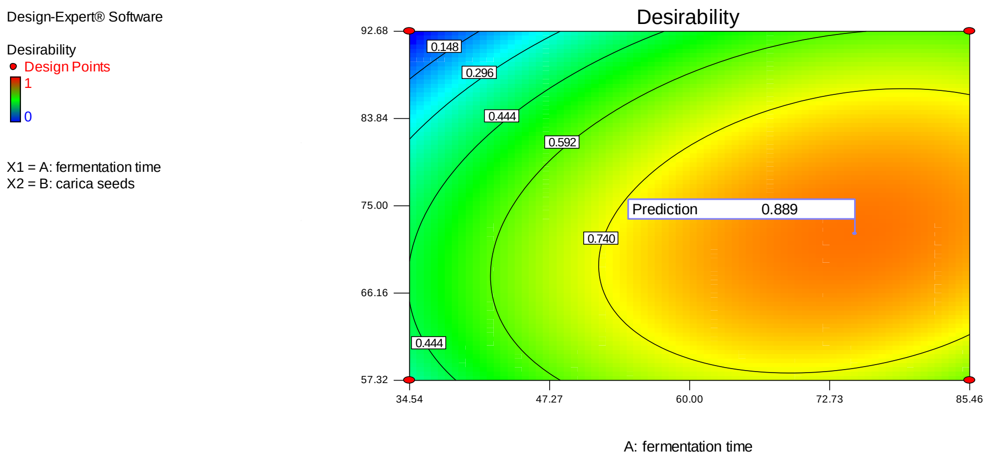

Figure 9. The desirability value of the optimum formula.

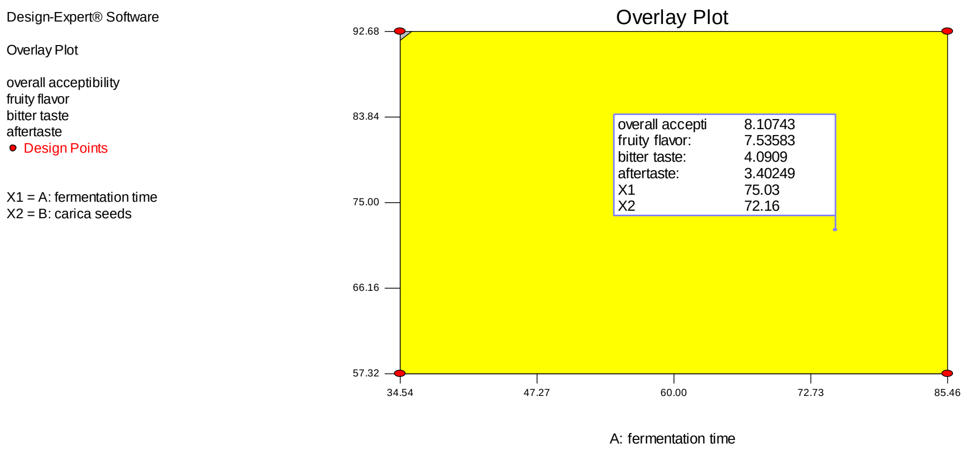

Figure 10. The composition of the optimum formula.

Table 6. The sensory score of optimum formula.

Response	Low PI	High PI	Prediction score	Actual score (Range 1–9)
Overall acceptability	7.61	8.60	8.11	8.23 ± 0.52
Fruity flavor	6.82	8.25	7.54	7.76 ± 0.41
Bitter taste	3.33	4.85	4.09	4.41 ± 0.37
After taste	2.57	4.24	3.40	3.79 ± 0.29

The product with the optimum formula was made 5 times. They were tested in the intensity of overall acceptability, fruity flavor, bitter taste and after taste through a rating test (intensity scale 1–9) by 10 trained panelists. From the data in table 6, it appears that all actual response values were at the prediction score interval recommended by the optimization program. This means that the optimum formula recommended by the expert design program was proven to produce the optimum response targeted in the study.

3.3. Product characterization with optimum formula

Characterizations include quantitative descriptive analysis, hedonic test, and physicochemical properties test. The analysis was carried out between products with optimum formula and control. Control was made from 100% Carica seeds without fermentation and without the addition of dried jackfruit.

3.4. Quantitative descriptive analysis

Quantitative descriptive analysis intended to quantify the intensity of all sensory attributes identified from products. The first stage of this test was to determine the description of all sensory attributes identified by 10 trained panelists through focus group discussions (FGD) led by a panel leader. The FGD results showed 2 sensory attributes identified in the appearance group, 5 in the aroma group, 5 in the taste group, and 2 in the after taste group. The appearance was detected by the organ of vision (eyes). The aroma was detected by the olfactory organ (nose). The taste was detected when the beverage is in the oral cavity (as a result of the product chemical reaction with the tongue), and after taste was the tastes left on the tip of the tongue when the beverage was swallowed into the stomach through the esophagus [1,12].

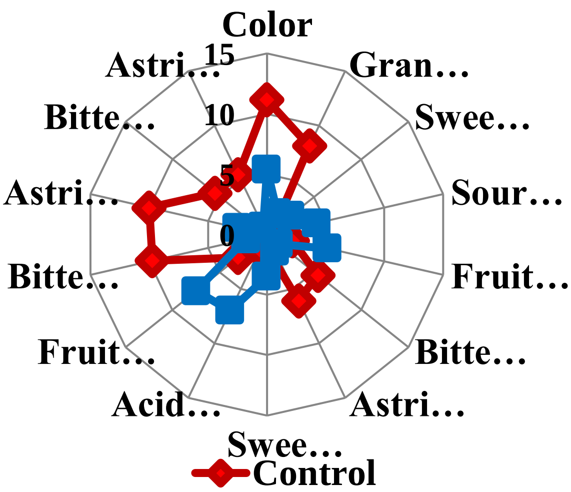

Figure 11. The spider diagram of sensory attributes resulted from quantitative descriptive analysis.

Sensory attributes identified i.e. appearance include blackish brown color and granule size; aroma includes sweet, sour, fruity, bitter and astringent; taste include sweet, acid, fruity, bitter and astringent; and after taste includes bitter and astringent. Blackish brown color refers to the distinctive color produced by Carica seeds and jackfruit. Granule size refers to the dimensions of Carica seed powder and jackfruit fruit used in the formula. The aroma and sweet taste refer to the aroma and taste produced from jackfruit and sugar added to the formula. Sour aroma, acid taste, fruity aroma, and fruity taste refer to the aroma and taste produced by Carica seeds and jackfruit. The aroma, taste and after taste of bitter and astringent refer to the bitter and astringent aroma, taste, and after taste produced by Carica seeds [1,12]. Based on the spider diagram (figure 11) appears that the control has a higher blackish brown color intensity, larger granule size, higher intensity of bitter and astringent aroma, taste, and after taste than the optimum product. The optimum product has a higher intensity of fruity, acid, and sweet aroma and taste than the control. Carica seeds contain alkaloid and saponin compounds that contribute to bitter and astringent flavors [1,13].

3.5. Hedonic test

The hedonic test by 25 semi-trained panelists showed that the optimum product had a higher hedonic intensity score (range 1 to 9) for appearance, texture, aroma, and taste, while the after taste was lower (table 7). If it was associated with the results of the quantitative descriptive test, it appears that panelist preference for products with optimum formula was caused by visually acceptable yellowish-brown color, a more homogeneous appearance and less sedimentation, and higher intensity in fruity, sour, and sweet aroma and taste, lower intensity in bitter and astringent aroma and taste. Control has a less favored was caused by its after taste (bitter and astringent).

Table 7. The hedonic score of Carica seeds beverage.

Attributes	Hedonic score	
	Control ± SD*	Optimum formula ± SD*
Appearance	5.15 ± 0.07	6.34 ± 0.08
Texture	5.26 ± 0.09	6.79 ± 0.06
Aroma	4.84 ± 0.06	6.78 ± 0.04
Taste	4.25 ± 0.09	7.39 ± 0.07
After taste	5.24 ± 0.08	7.62 ± 0.06

*S = standard deviation

3.6. The physicochemical properties

The results of ANOVA (table 8) showed that Carica seed beverage with the optimum formula had higher in vitamin C, phenol, sugar, sodium, and potassium content than controls, i.e. 0.35% db, 135.25 mg/100 g db, 10.94% db, 90, 65 ppm, and 51.66 ppm, respectively. Changes in formula at optimum product compared to control caused a significant increase in vitamin C, phenol, potassium, sodium, and sugar percentage, i.e. 25.29%, 392.21%, 56.72%, 37.70%, and 239.39%, respectively. Antioxidant capacity, total dietary fiber, soluble dietary fiber, insoluble dietary fiber, fat, protein, and ash were not significantly different between optimum products and controls.

Table 8. The physicochemical properties of Carica seeds beverage.

Composition	Control	Optimum Formula
Water (% wb)	4.14	6.49
Ash (% db)	3.75	3.98
Protein (% db)	27.99	24.41
Fat (% db)	1.19	1.08
Soluble dietary fiber (% db)	2.92	1.57
Non-soluble dietary fiber (% db)	43.40	43.37
Total dietary fiber (% db)	46.31	44.97
Potassium (ppm)	32.96 ^{b}	51.66 ^{a}
Sodium (ppm)	65.84 ^{b}	90.65 ^{a}
Sugar (% db)	3.22 ^{b}	10.94 ^{a}
Vitamin C (mg/100 gdb)	27.48 ^{b}	135.25 ^{a}
Phenol (%)	0.28 ^{b}	0.35 ^{a}
Antioxidant (%)	88.01	89.21

^{ab}The different superscript in the same line means significantly ($p < 0.005$) different

4. Conclusion

Solid-state fermentation method with a proportion of 0.2% commercial inoculum resulted in Carica seed powder which had the highest phenol and antioxidant content compared to other methods (0.27 and 86.17%, respectively); 2) Formula with 0.9 of desirability value consisting of 75 hours of fermentation time, 72% of Carica seeds powder, and 28% of jackfruit powder produce beverage with actual rating score (range = 1–9) of overall acceptability, fruity flavor, bitter taste, and after taste i.e 8.23, 7.76, 4.41, 3.79, respectively. Compared to control (a beverage made from 100% Carica seeds without fermentation), the optimum product has a stronger fruity flavor and sweet taste and weaker in a bitter taste. Products with optimum formula were preferred by 25 semi-trained panelists and have a higher content of antioxidants, phenol, vitamin C, sugar, calcium, potassium than control, i.e 89.21% db, 0.35% db, 135.25 mg/100 g db, 10.94% db, 90.65 ppm, and 51.66 ppm, respectively. From this study appears that Carica seed beverage that were engineered through the fermentation process of seeds with commercial inoculums containing Rhizopus oligosporus and the addition of dried jackfruit can be used as alternative functional beverage that was acceptable by panelist and rich in antioxidants and vitamin C. Further, research related to shelf-life product still needs to be done.

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