Optimizing Conditions for The Production Process of Cashew Apple Juice (Anacardium Occidentale L.) From Binh Phuoc (Viet Nam) Using Response Surface Methodology (RSM)

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Abstracts: Cashew apple is one of the promising food material sources because this fruit contains high nutrtional values. This study aims for developing cashew apple juice into a new beverage and contributing to increase economic value for the byproduct of cashew industry. In this study, by using Reponse surface methodology (RSM), four different factors (juice concentration, %acid, %aroma, Brix) in mixing cashew apple juice product were examined to determine their optimum value in order to obtain the highest acceptance of the final product. The results of performed experiments showed that the combination of juice concentration 36%, acid 0.32%, aroma 0.017% and 13.7oBx gave the highest acceptance (6.925/9).

Keywords: Cashew Apple, Juice, Optimum, Reponse Surface Methodology (RSM).

1. INTRODUCTION

Cashew tree is native of Brazil, it was introduced into India and Africa and then spread over South-east Asia. Cashew nut is the main product used wisely and applied in various food products. The cashew market size is forecasted to reach 8.91 billion USD by 2028 (1). In 2022, total cashew nut production worldwide reached more than 3.753 million tonnes according to Faostat, while the production volume of cashew nuts in Viet Nam was 335.5 thousand tonnes (2) and is mainly cultivated from Binh Phuoc province.

The byproduct of this promising industry is cashew apple and this fruit is mainly discarded. There are 10-15 tonnes cashew apple disposed when a tonne of nut produced (3). Consequently, it has negative impacts on environment such as increasing greenhouse gas and pollute the surrounding area, soil and groundwater. However, cashew apples possess a wide range of health advantages as they are rich in nutrients (vitamin C, sugars, polyphenols,..)(4–6). The principle reason why this fruit is still underutilized is its astringency caused by high tannin content (193.29±7.65 mgTA/100mL) (7). Therefore, developing drink from cashew apple would reduce the detrimental effects on environment, add value to cashew industry's byproduct and increase the diversity of plant-based food products.

In the current market, there are several products from cashew apple as a powder for supplement in biscuit-type cookies (8), cashew apple bagasse as a source of sugars for ethanol and tannase production (9), livestock feed and vermin composting (10).

To improve the variety of food products from cashew apples, some methods have been applied to eliminate tannins – the main drawback of this fruit. They include three main types, which are physical, chemical and enzymatic method. Microfiltration, heat treatment(11), (12), polysaccharides(13), protein-based materials(14) and enzymes(15) are used wisely in researches aimed for reducing astringency in cashew apple. All of these methods have shown the efficiency in tannin removal and therefore increased sensory value. The study of Hanh NT et al., indicated that using the combination of pectinase 0.8% and tannase 0.2%, 80 minutes of incubation, 35^{o}C at juice pH (4.61) can remove tannin up to 56.09% and the enzymatic-treated cashew apple juice and some nutritional values still remains high (7).

However, there is a limitation in the research into developing the final product of cashew apple after detannying step. Beverages from cashew apple juice usually are produced by mixing the treated juice with water, sugars, citric acid and preservatives. Another method is adding other juices and then producing ready-to-serve drinks. These products are increasing popularity over the world. The research of Vajira P. Bulugahapitiya et al., showed that mixing tannin-removal cashew apple juice with sugar syrup (85g sugar and 0.5g citric acid in 100mL boiled water), then filtrating by muslin cloth gave the stable sensorial properties (color, flavor, texture) and high acceptance, that enables consumers to enjoy cashew apple products throughout the year (16). Besides, Francisco Fábio de Assis Paiva suggested that cashew apple juice, after being treated and filtrated, was added 0.1% sodium benzoate or benzoic acid, 0.1% sorbic acid or sorbate and 0.02% sulfur dioxide, then following by pasteurization at 60^{o}C in 1 minute, could be preserved within 2 months in refrigerator (17). Beverages have been developed by adding the mixture of 200g sugar, 5g citric acid in 1L water into 200mL clarified cashew apple juice and 100mL other juice (18). Other studies about mixing cashew apple juice with juice obtained from pineapple, Ama, papaya, coconut or passion fruit have shown the positive effect on sesorial aspect and have high acceptance (19).

Respone surface methodology (RSM) decreases the quantity of experiments required to evaluate the process parameters and the interactions between them (20). Therefore, this method helps to reduce the time consumption compared to other methods. In this study, the optimizing experiments were conducted by using cashew apple harvested from Binh Phuoc, Viet Nam to improve the sensory quality and acceptance by adjusting juice concentration, sugar, aroma and acid content.

2. MATERIALS AND METHODS

2.1. Materials

Raw organic cashew apples (from Binh Phuoc, Viet Nam) cultivated and removed nuts were transported in refrigerating condition to the laboratory of Hanoi University of Food Technology (HUST) within 24–36 hours.

All chemicals used were of analytical grade. Enzyme Pectinex Ultra SP-L and Tannin acyl hydrolase (EC 3.1.1.20) from Kikkoman (Japan) originating from Aspergillus Oryzae were used in the study.

2.2. Sample Preparation

Treatment of Cashew Apple Juice

Cashew apples were cleaned and added with 0.01% enzyme preparation Pectinex Ultra SP-L in 2 hours to increase juice yield. The juice was collected by juicer model Sharp KS-888, followed by clarifying to obtain a clear juice before the combination of pectinase 0.8% and tannase 0.2% was added into the juice within condition: pH 4.61, 80 minutes of incubation, 35^{o}C (7).

Sample Preparation

After the enzymatic-treating step, cashew apple juice was mixed with sugar syrup (fructose ^{o}Bx = 50), aroma, citric acid (10%) and water at different ratios. Subsequently, the mixture was heated at 80-85^{o}C in 2-3 minutes before filling into sterilized containers. After that the sample was pasteurized at 90-95^{o}C, 15 minutes and cooled down by water, preserved at room temperature.

2.3. Experimental Design

The target response was the panelists' acceptance which is affected by the percentage of treated juice, aroma, acid and Brix to the sample. The effects of the four independent variables namely juice concentration (X_1, %v/v), acid (X_2, %w/v), aroma (X_3, %v/v) and total soluble solids - ºBrix (X_4, %w/v) were investigated using response surface methodology (RSM) (21). Firstly, the range of variables (X_1: 25, 30, 35, 40; X_2: 0.2, 0.3, 0.4, 0.5; X_3: 0.005, 0.01, 0.015, 0.02; X_4: 10, 12, 14, 16) were narrowed by using hedonic rating test. Each variable was examined in the mentioned range meanwhile others were at specific level (X_1: 35, X_2: 0.3, X_3: 0.015, X_4: 12).

Box-Behnken design (BBD) was then employed. Each independent variable had 3 levels which were -1, 0, +1. This design requires an experiment number according to N = 2k(k-1) + C_0 where k is the factor number and C_0 is the replicate number of the central point (22). The total number of experiments in this study was 25 based on 3 levels and a four-factor experimental design, with 1 point at centre (shown in table 1 and 2). Design expert (V.13.0.5.0) was used for experiment design, data analysis and regression modelling.

Experimental data from the Box-Behnken design was analysed and fitted to a second-order polynomial model: $Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i \neq j}^{k} X_i X_j + \varepsilon$. Where *Y* is the predicted response; β_0 is the model intercept; β_i and β_{ii} are the regression coefficients for the linear and quadratic effects of the model, respectively; X_i and X_j are the factors and *k* is the number of factors (23).

Table 1. The level of experimental variables chosen for the Box–Behnken design

Variable	Symbol	Low	Center	High
		-1	0	+1
Juice concentration	X_1	30	35	40
Acid	X_2	0.2	0.3	0.4
Aroma	X_3	0.015	0.0175	0.02
ºBrix	X_4	12	13	14

Table 2. Experimental design

No	Coded variable				Actual variable			
	X_1	X_2	X_3	X_4	**Juice**	**Acid**	**Aroma**	**ºBrix**
1	0	-1	0	+1	35	0.2	0.0175	14
2	+1	-1	0	0	40	0.2	0.0175	13
3	0	+1	-1	0	35	0.4	0.015	13
4	0	-1	-1	0	35	0.2	0.015	13
5	+1	0	+1	0	40	0.3	0.02	13
6	-1	+1	0	0	30	0.4	0.0175	13
7	0	0	+1	-1	35	0.3	0.02	12
8	0	0	-1	+1	35	0.3	0.015	14
9	-1	0	0	-1	30	0.3	0.0175	12
10	0	+1	0	+1	35	0.4	0.0175	14
11	0	+1	0	-1	35	0.4	0.0175	12
12	+1	0	-1	0	40	0.3	0.015	13
13	-1	0	0	+1	30	0.3	0.0175	14
14	0	0	0	0	35	0.3	0.0175	13

15	0	0	+1	+1	35	0.3	0.02	14
16	0	-1	+1	0	35	0.2	0.02	13
17	0	+1	+1	0	35	0.4	0.02	13
18	+1	+1	0	0	40	0.4	0.0175	13
19	+1	0	0	-1	40	0.3	0.0175	12
20	+1	0	0	+1	40	0.3	0.0175	14
21	-1	0	+1	0	30	0.3	0.02	13
22	-1	-1	0	0	30	0.2	0.0175	13
23	0	0	-1	-1	35	0.3	0.015	12
24	-1	0	-1	0	30	0.3	0.015	13
25	0	-1	0	-1	35	0.2	0.0175	12

2.4. Sensory Evaluation

Using the hedonic rating test (24) to choose the range for 4 independent factors in optimizing experiments: juice concentration (25 – 40%), total soluble solids - ^{o}Brix (10 – 16), aroma (0.005 – 0.02%) and acid (0.2 – 0.5%). A panel (60 individuals) participated in an sensory experiment to select the range of four factors and then determine optimum parameters for production process. They are from 18 to 30 years old, have good health condition and familiar with using fruit-flavor beverage. The 9-point hedonic scale is applied in sensory analysis, from extremely dislike to extremely like (1 to 9).

From the selected range of each factor, conducting the optimizing experiments which were designed by Box-Behnken designs.

2.5. Total Titratable Acidity, Total Soluble Solids (Bx)

Total titratable acidity as percent of acid malic was determined by the AOAC 942.15 method (25). Total soluble solids were recorded by a portable refractometer.

2.6. Data Analysis

Using Analysis of Variance (ANOVA) single factor by Microsoft Excel to calculate F standard in order to determine the statistic significance in panellist's acceptance between samples. If there is a statistic significance, Least Significance Difference (LSD) is used at $p = 0.05$.

3. RESULT AND DISCUSSION

3.1. Examine the Effects of Several Factors On the Sensorial Quality of Cashew Apple Juice

Table 3. The result of the hedonic rating test on juice concentration, ºBrix, aroma and acid range

Criteria		Acceptance
Aroma types	H1	6.6^{a}
	H2	4.8^{b}
	H3	6.1^{a}
Aroma concentration	0.005%	5.4^{b}
	0.01%	5.5^{b}
	0.015%	7.5^{a}
	0.02%	7.4^{a}
Acid concentration	0.2%	5.4^{ab}
	0.3%	6.4^{a}
	0.4%	5.9^{a}
	0.5%	3.9^{b}
Juice concentration	25%	5.0^{b}
	30%	6.5^{a}
	35%	6.9^{a}
	40%	7.1^{a}
Total soluble solids (ºBx)	10	4.3^{c}
	12	6.5^{a}
	14	6.6^{a}
	16	5.6^{b}

Values are demonstrated in means. Mean followed by difference lowercase superscripts in a column are significantly different at $p<0.05$.

Regarding aroma types, samples flavored with H1 and H3 were at the same acceptance and they have higher acceptance than H2. Therefore, H1 was selected to conduct further experiments. In terms of aroma concentration, samples at 0.015% và 0.02% were more preferred than those at 0.005% and 0.01%. Meanwhile, there was no statistic significance between the acceptance of 0.015% and 0.02% sample. Acid concentration at 0.3% and 0.4% have higher hedonic point than the sample at 0.5%, whereas there was no significant difference between 0.2% and other samples.

There was a similar acceptance in juice concentration at 30%, 35% and 40% and these samples are more preferred than the sample having 25% clarified cashew apple juice. The total soluble solids (Brix) is preferred the most at 12ºBx and 14ºBx, while 10ºBx had the lowest hedonic point. Consequently, four independent factors range was: juice concentration from 30% to 40%, acid 0.2-0.4%, aroma 0.015-0.02%, Brix 12-14ºBx.

3.2. Optimize independent variables of sensorial quality of cashew apple juice

The acceptance was represented in Table 4.

Table 4. The acceptance by hedonic rating test

No	Acceptance	No	Acceptance	No	Acceptance
1	6	11	5.2	21	5.3
2	5.4	12	5.5	22	5.3
3	5.6	13	6.1	23	5.1
4	5.3	14	6.7	24	5.3
5	5.8	15	6.3	25	5.2
6	5.3	16	5.4		
7	5.2	17	5.6		

8	6.2	18	5.8		
9	5.2	19	5.2		
10	6.5	20	6.4		

3.2.1. Analyze regression modelling

Table 5. Analyze of variance (ANOVA) the quadratic model of acceptance obtained from the experimental results

Source	Sum of Square	Degrees of Freedom	Mean Square	F value	P value	
Model	5.52	14	0.3946	118.38	< 0.0001	Significant
X_1	0.2133	1	0.2133	64	< 0.0001	
X_2	0.1633	1	0.1633	49	< 0.0001	
X_3	0.03	1	0.03	9	0.0133	
X_4	3.41	1	3.41	1024	< 0.0001	
X_1X_2	0.04	1	0.04	12	0.0061	
X_1X_3	0.0225	1	0.0225	6.75	0.0266	
X_1X_4	0.0225	1	0.0225	6.75	0.0266	
X_2X_3	0.0025	1	0.0025	0.75	0.4068	
X_2X_4	0.0625	1	0.0625	18.75	0.0015	
X_3X_4	0	1	0	0	1	
X_1^2	1.07	1	1.07	322.12	< 0.0001	
X_2^2	1.07	1	1.07	322.12	< 0.0001	
X_3^2	1.07	1	1.07	322.12	< 0.0001	
X_4^2	0.3796	1	0.3796	113.88	< 0.0001	
Residual	0.0333	10	0.0033			
Cor total	5.56	24				

F value of the model is 118.38 implies the model is significant with 99.99% confidence level ($p < 0.001$). p value < 0.05 indicated that the model terms are significant. In this case, X_1, X_2, X_3, X_4, X_1X_2, X_1X_3,X_1X_4, X_2X_4, X_1^2, X_2^2, X_3^2, X_4^2 are significant model terms; X_2X_3, X_3X_4 are not significant ($p > 0.05$) therefore eliminating out of the model. Therefore, the regression model as in the following equation:

$$Y = 6.7 + 0.1333X_1 + 0.1167X_2 + 0.05X_3 + 0.5533X_4 + 0.1X_1X_2 + 0.075X_1X_3 + 0.075X_1X_4 + 0.125X_2X_4 - 0.6167X_1^2 - 0.6167X_2^2 - 0.6167X_3^2 - 0.3667X_4^2 \quad (*)$$

Therefore, conducting the experiments according to Box-Behnken design has provided empirical values of Y, these values that helped build the regression model (*) through determining and evaluating the significance of coefficients. From this model we can calculate the value of the function Y if we know in advance a set of values of Xi, which means that for a set of values of Xi we have two values of Y: the value obtained from experiment and the value obtained by calculating according to model (*). These two values of Y always have deviations. If that deviation is small, we say the found model is significant, that is, the model correctly reflects the law of variation of the data in the experimental matrix. In contrast, if the deviation is large, the model does not correctly reflect the law of variation of the data in the experimental matrix, then we say the model is not significant. We perform this assessment using Design Expert 13.0.5.0 software, the analysis results are as follows:

Table 6. R^2, Adjusted R^2, and Adeq Precision value of the model

Std. Dev.	0.0577	R^2	0.994
Mean	5.64	Adjusted R^2	0.9856
C. V. %	1.02	Adeq Precision	35.2181

The confidence level of the model was expressed through the regression coefficient $R^2 = 0.995$. In addition, the value of Adjusted $R^2 = 0.9856$ proved that the model was highly significant at the 95% confidence level. Similarly, the standard deviation was low at 0.0577. Consequently, it can be seen that the model was significant.

The model (*) showed that the coefficients of variables and interactive coefficients of variables > 0 were: X_1, X_2, X_3, X_4, X_1X_2, X_1X_3, X_1X_4, X_2X_4. That means in the experimental scheme, in order to move the variables towards the optimal point for the the highest value of response, it is necessary to move the factors from the primary level (X_1: 35, X_2: 0.3, X_3: 0.0175, X_4: 13) gradually to the upper bound until meeting unchanged Y.

$\beta_4 = 0.5533 > \beta_1 = 0.1333 > \beta_2 = 0.1167 > \beta_3 = 0.05$ indicated that Brix had highest impact on the acceptance of sample, following by juice concentration, acid and aroma. The interactions between juice concentration versus acid, juice concentration versus aroma, juice concentration versus Brix, acid versus Brix significant affect the acceptance of panellists. All of the interactive coefficients were higher than 0 that means by increasing simultaneously 2 factors from primary level to specific values which in the identified range, the acceptance rises continuously.

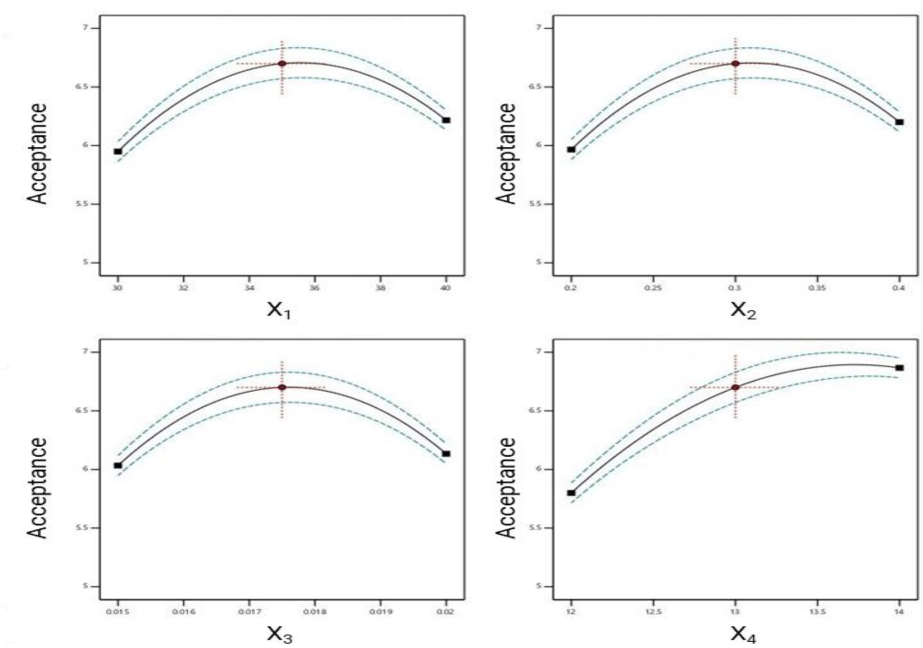

Figure 1. Effect of each factor (X_1: % juice, X_2: % acid, X_3: % aroma, X_4: ^{o}Brix) on the acceptance.

Consider the influence of each factor on the acceptance for the product (when other factors are kept at primary levels) (Figure 1). The acceptance increases when the juice concentration increased from 30% to 35.6%; acid increased from 0.2% to 0.31%; aroma increased from 0.015% to 0.0176%; Brix level increased from 12 to 13.7^{o}Bx.

In that range, the higher the juice concentration, the more the typical sensory properties of cashew fruit (mildly astringent taste, cashew aroma) became more evident, creating a feeling of excitement for the panelist. Increased acid content heightened the harmony of sweet and sour taste. The increased proportion of additional aroma harmonized the scent mixture of the sample. Increasing the Brix level increased the sweetness, created the balance between sweet - sour and astringent taste, increasing the acceptance for the sample.

The acceptance began to decrease when the juice concentration was greater than 35.6%; acid greater than 0.31%; aroma greater than 0.0176%; Brix degree greater than 13.7. The more fruit juice added, the higher astringent taste of the product; the higher the acid and Brix caused lost the balance between sourness and sweetness. Especially when the acid content was too high, the astringent taste increased in the cashew juice; the higher aroma was added, the higher the smell imbalance was and the typical aroma of cashew fruit was lost.

Figure 2 to figure 5 showed the relationship between the response Y to each pair of two experimental variables (the other two variables are kept at primary level). For each pair of values of two variables, a response value point was obtained.

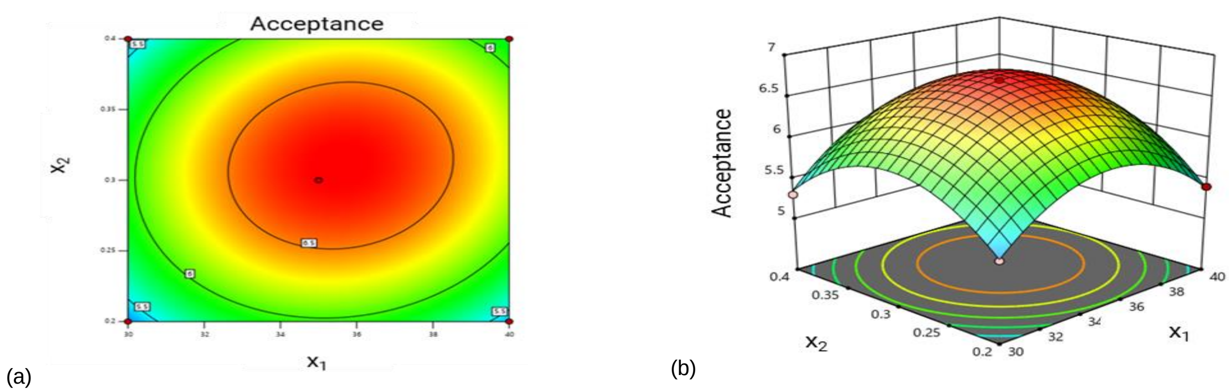

Figure 2. Contour plots (a) and response surface (b) showing the effects of factors % acid (X_2) and % juice (X_1) ($^oBx = 13$, aroma 0.0175%)

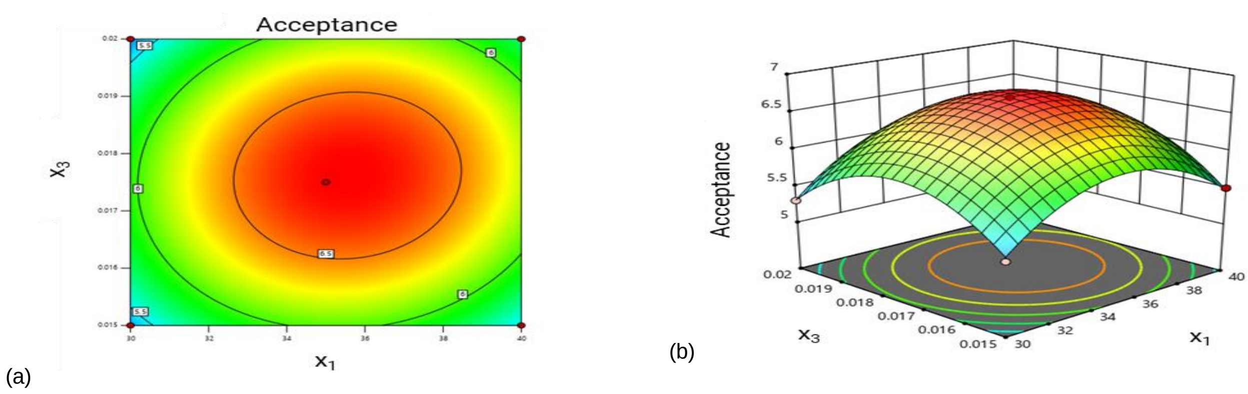

Figure 3. Contour plots (a) and response surface (b) showing the effects of factors % aroma (X_3) and % juice (X_1) ($^oBx = 13$, acid 0.3%)

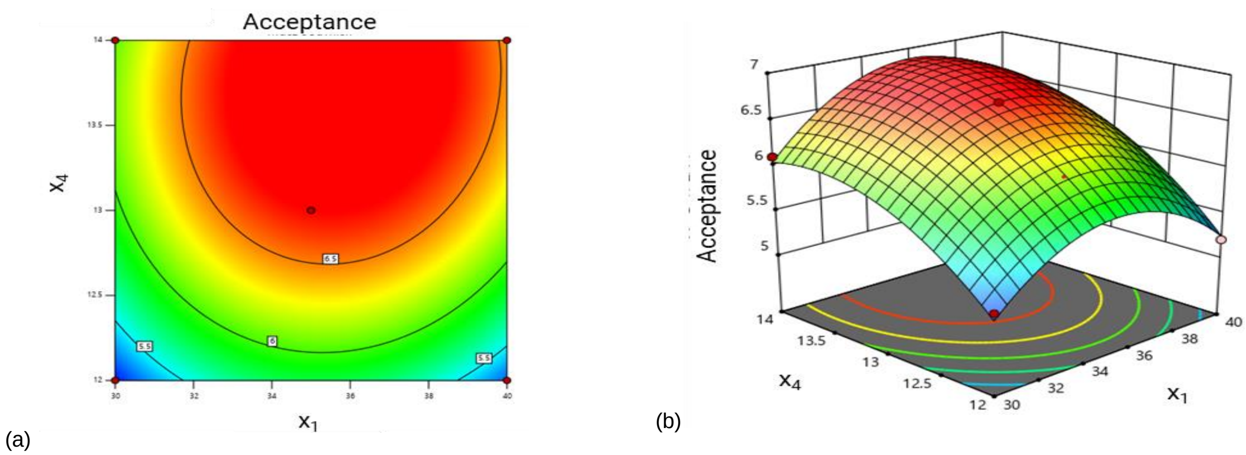

Figure 4. Contour plots (a) and response surface (b) showing the effects of factors Brix (X_4) and % juice (X_1) (aroma 0.0175%, acid 0.3%)

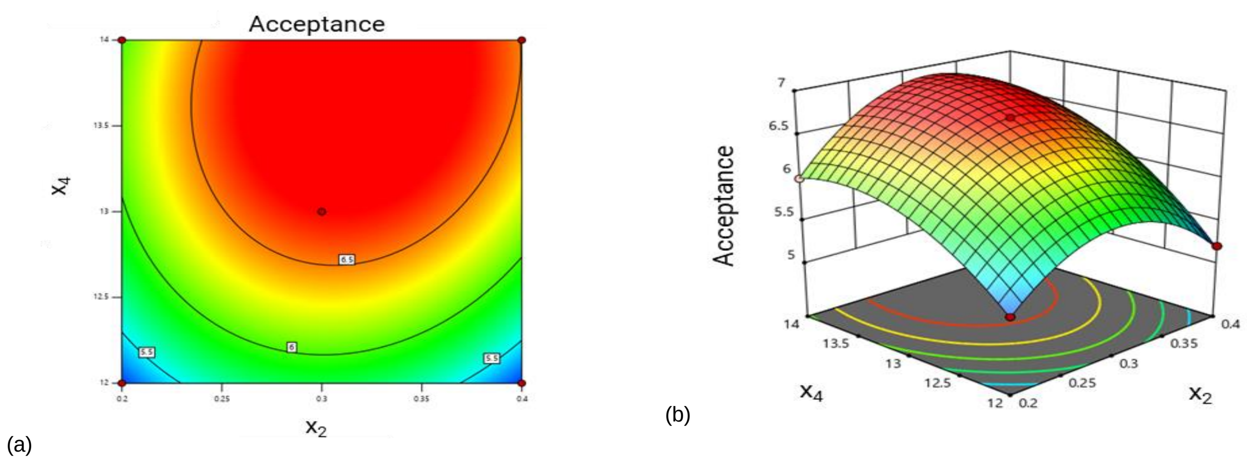

Figure 5. Contour plots (a) and response surface (b) showing the effects of factors Brix (X_4) and % acid (X_2) (aroma 0.0175%, juice 35%)

By using Design Expert 13.0.5.0 software, the optimum values of 4 factors are: juice concentration 36%, acid 0.32%, aroma 0.017% and 13.7^{o}Bx where the acceptance reached its highest point at 6.925.

CONCLUSION

The different factors (juice concentration, %acid, %aroma, Brix) for mixing cashew apple juice product showed the significant impact on the acceptance of final product. Using BBD helped in comprehending that effect in order to identify the optimum value of mentioned parameters in juice production process: juice concentration 36%, acid 0.32%, aroma 0.017% and 13.7^o Bx helped to reach highest acceptance (6.925/9).

ACKNOWLEDGEMENT

This research is funded by Hanoi University of Science and Technology (HUST) under project number T2022-PC-098 and ĐTĐL.CN - 57/22

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DOI: https://doi.org/10.15379/ijmst.v10i2.3341