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EFFECT OF ENZYME TREATMENT ON GUAVA JUICE PRODUCTION USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Response surface methodology (RSM) was used to investigate the effects of enzyme concentration (500-900 ppm) and incubation time (30-90 min) on viscosity of guava puree and pH, titratable acidity, clarity, yield, total soluble solid (TSS) and ascorbic acid of guava juice. In addition, the numerical optimization was conducted to find the best enzyme condition. The result indicated that the enzyme treatment reduced guava puree viscosity, promoted juice clarification and increased values for titratable acidity, yield, TSS and ascorbic acid of guava juice than that without enzyme. Only regression models of guava puree viscosity, yield, TSS and ascorbic acid with coefficient of determination (R^2) of 0.9706, 0.9785, 0.9678 and 0.9687, respectively were significant, reliable and suitable to describe the experimental data. Increasing of enzyme concentration and incubation time decreased puree viscosity and increased yield, TSS and ascorbic acid. Enzyme concentration was the most significant variable affecting these properties. The optimal enzyme treatment recommended to add 869.36 ppm pectinase in guava mash and incubated for 71.27 min before filtration process. Under this condition, predicted puree viscosity, yield, TSS and ascorbic acid were 160.07 cps, 85.10%, 5.60 °Brix and 54.27 mg/100 ml, respectively. In conclusion, the development of guava juice quality can be achieved by pectinase application. The RSM successfully revealed that increasing of enzyme concentration and incubation time related to decreased guava puree viscosity and increased yield, TSS and ascorbic acid of guava juice. The usage of proper pectinase concentration and incubation time would be an approach to enhance guava juice characteristics.

Key words: Guava juice processing, enzyme treatment, pectinase, response surface methodology.

INTRODUCTION

Guava (*Psidium guajava* L.) is a tropical fruit which usually consumed as fresh. It is rich in lycopene and ascorbic acid, especially it contains ascorbic acid (100-200 mg/100 g) higher than a fresh orange juice (60-80 mg/100 ml) (Sidhu, 2006; Chopda and Barrett, 2001). In addition, it is a good source of vitamin A, omega-3 and -6 polyunsaturated fatty acids, dietary fiber, potassium, magnesium and antioxidant pigments such as carotenoids and polyphenols (Mahattanatawee *et al*., 2001). As the ripened guava is highly perishable when kept at ambient temperature, it is processed in various commercial guava products including puree, paste, canned slices in syrup and juice. Among these products, the guava juice has become economically important in the market. The consumption of tropical fruit juice like guava juice has been increasing currently because it is natural, high in nutritional values and used as an alternative to other beverages such as soft drinks, tea and coffee.

The conventional guava juice processing can be made by mechanical pressing of guava mash. The obtained juice is cloudy and low in ascorbic acid due to a high content of ascorbic acids remains in the pomace (Kuar *et al.*, 2009). The use of enzyme in a mash treatment is now essential in juice industry and it shows increases in yield and ascorbic acid and also promotes juice clarification in a short processing (Sarioglu *et al.*, 2001: Demir *et al*., 2004). The enzymes including pectinase, cellulase and/or arabinase assist in the hydrolysis of pectic substances, pectins, celluloses or hemicelluloses. Consequently, it is advantageous to facilitate the subsequent filtration process and increase juice yield (Kuar *et al.*, 2009). The time to add enzyme is dependent on the type of fruits used in juice processing. Generally, the pectinase is applied during the maceration pretreatment for reducing the viscosity of fruit mash and the juice produces high yield and nutritive values (Sun *et al*., 2006).

The achievement of enzyme treatment in fruit juice processing is influenced by several process variables such as enzyme concentration, incubation time, incubation temperature or these interactive effects (Rai *et al.*, 2004; Lee *et al.*, 2006). Most studies reported on the optimal enzyme conditions where one process variable was varied in different levels while keeping the others at a constant level. There is no result of interaction effects among the variables and it does not depict the net effect of various parameters on the reaction rate (Rai *et al.*, 2004). Response surface methodology (RSM) is an effective tool which uses quantitative data in an experimental design to optimize a process (Vieira *et al*.,

2012). A central composite rotatable design (CCRD) is an experimental design to define empirical models or equations for describing the effect of test variables and their interactions on the responses (Sun *et al.*, 2006). RSM has been used for optimizing processes in fruit and vegetable juice production (Rai *et al.*, 2004; Sin *et al.*, 2006; Sun *et al.*, 2006). Kaur *et al*. (2009) revealed that the variation of guava juice yield was a function of enzyme hydrolysis pretreatment conditions where the independent variables including enzyme concentration, temperature and incubation time were established using RSM. However, other quality parameters such as clarity, TSS and ascorbic acid have not been investigated.

The objective of this study was to investigate the effect of enzyme concentration and incubation time on viscosity of guava puree and physicochemical properties of guava juice such as pH, titratable acidity, clarity, yield, TSS and ascorbic acid using RSM. The optimizing enzymatic condition for guava juice production was also determined.

MATERIALS AND METHODS

Fruit: Ripened guavas (*Psidium guajava* L.) with 80-90% maturity and free from visual blemishes and bruises were purchased from a local market. The firmness of guavas was measured and guavas with peak force values between 16.5 and 18.5 N were used in this study.

Enzyme source: Pectinex Ultra SP-L (Novozymes, Denmark), produced from *Aspergillus aculeatus*, contains different pectinolytic and cellulolytic enzyme (endo-polygalacturonase (EC 3.2.1.15; C.A.S. No. 9032-75-1), endo-pectinlyase (EC 4.2.2.10; C.A.S. No.9033-35-6) and pectin esterase (EC 3.1.1.11; C.A.S. No. 9025-98-3), and other activities, such as β-galactosidase, chitinase and transgalactosidase. The enzyme has its activity of 26,000 PG per ml (polygalacturonase activity per ml), optimum pH at 3.5-6.0 and optimum temperature below 90 °C (Abdullah *et al.*, 2007).

Guava juice preparation: Ripened guavas were washed with tap water, trimmed to remove blemishs (if any), cut in halves and deseeded. The guava halves were sliced into about 2 cm thickness and blended with appropriate amount of added water using a Waring® blender (700G, Waring Laboratory, Torrington, CT, USA) for 3 min. The guava puree was filtered through a cheese cloth to obtain the juice.

Enzyme treatment: For each experiment, 200 g guava puree was subjected to different enzyme treatment conditions, as given in Table 1. The reaction was carried out in a water bath shaker (30 ± 2 °C) with a constant stirring rate of 100 rpm, and then heated at 90 °C for 5 min in order to inactivate enzyme activity. The guava puree was filtered through a cheese cloth to obtain the juice.

Physicochemical properties

Puree viscosity: The viscosity was measured by a Brookfield viscometer (Model RVDV-II, Brookfield Engineering Laboratory, Stoughton, MA, USA) equipped with a spindle no.02 at 100 rpm. Each 200 ml sample was prepared in a 250 ml-beaker and the measurement was made at room temperature (28 ± 2 °C).

pH: The pH-meter (Model 320, Mettler-Toledo Ltd., Essex, UK) was used to measure pH of each sample according to AOAC (2005) procedure.

Titratable acidity: A 10 ml juice sample was diluted with 40 ml water, and then titrated with 0.1 N sodium hydroxide until reaching the end point at pH = 8.2, as described by Barrett *et al.* (2007). The total acidity was expressed as citric acid.

Clarity: Clarity was determined by measuring the absorbance at 660 nm using a spectrophotometer (Spectronic 21, Bausch & Lomb, USA) according to the method of Sin *et al.* (2006). Distilled water was used as a reference.

Yield: The juice yield was estimated as a percentage of weight of the juice obtained to the initial puree. The formula is:

$$\text{Yield} = \frac{\text{Weight of juice} - \text{Weight of added water}}{\text{Weight of guava}} \times 100\%$$

Total soluble solid (TSS): The guava juice was measured for TSS by an Abbe refractometer (Shanghai Precision & Scientific Instrument Co., Shanghai, China) at room temperature (28 ± 2 °C).

Ascorbic acid: Juice sample was determined for ascorbic acid by titration with a 2,6-dichlorophenolindophenol sodium salt solution as reported in AOAC (2005).

Firmness: Firmness was determined by a texture analyzer (Model LRX, Lloyd Instruments, Hampshire, UK). The maximum force (N) required to penetrate each sample up to 4 mm from the outer surface using a flat test cell (10 mm diameter) was recorded.

Experimental design: Two independent variables; enzyme concentration (500-900 ppm) and incubation time (30-90 min) were investigated for their influences on viscosity of guava puree and physicochemical properties of guava juice. A central composite rotatable design (CCRD) for a two-variable, five combinations coded -1.41, -1, 0, 1, 1.41 was employed to study the combined effect of these independent variables. Experimental design and actual values for guava production are shown in Table 1. This design required thirteen sets of randomized experiments, which included four factorial

points, five central points and four extra axial points, as evidence in Table 2. The model proposed for the response is

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

Where Y is the response calculated by the model; X_1 and X_2 are the coded enzyme concentration and incubation time, respectively, and b_1 and b_2 are linear, b_{11} and b_{12} are quadratic, and b_{12} is interaction coefficient, respectively (Anderson and Whitcomb, 2005).

Statistical analysis: The production of guava juice was carried out in duplicate and all analyses were carried out in triplicate. The data was subjected to analyze for analysis of variance (ANOVA) and multiple regression using the Design-Expert® Trial version 8.0.2 software (State-Ease Inc., Minneapolis, Minnesota, USA) (Anderson and Whitcomb, 2005).

RESULTS AND DISCUSSION

Enzyme treatment on guava puree and juice: Table 2 shows guava puree viscosity and physicochemical properties of guava juice with and without enzyme treatment. The addition of pectinase caused a decrease in puree viscosity, thus resulting in an ease of juice filtration process. The obtained guava juice was also high in titratable acidity, yield, TSS and ascorbic acid, but low in pH and absorbance values. This can be explained that pectinase, which include pectin methyl esterase and polygalacturonase, assist in pectin hydrolysis. Their reactions cause a release of carboxylic acids and galacturonic acids. This leads to a decrease in puree viscosity and pH of juice, but a significant increase in titratable acidity and juice yield (Tadakittisarn *et al.*, 2007). Besides these enzymes, arabinase and cellulase can convert araban and cellulose to soluble sugars, resulting in an increase of TSS in guava juice. In addition, the enzymes react on the guava peel which is rich in ascorbic acids (Chopda and Barrett, 2001), thus an increment of ascorbic acid would be due to the pectin breakdown from the peel. The pectinase-treated guava juice also demonstrated a lower absorbance value in relation to that without enzyme treatment, indicating that the juice was more clear. This was possibly due to the agglomeration of degraded products from pectinase hydrolysis of pectin, followed with the precipitation of fine particles as the time increased (Sin *et al*., 2006; Tadakittisarn *et al*., 2007). This finding was also confirmed by the work of Abdullah *et al*., (2007) who studied on the enzymatic clarification of carambola fruit juice.

Statistical analysis on model fitting: The experimental responses as a function of enzyme concentration (X_1) and incubation time (X_2) on guava juice production are summarized in Table 2. The values of puree viscosity (cps), pH, titratable acidity (%), clarity (Abs), yield (%), TSS (°Brix) and ascorbic acid (mg/100 ml) were within the ranges of 160.07-197.67, 3.70-3.73, 0.60-0.67, 1.716-1.779, 75.23-85.14, 4.75-5.65 and 45.95-55.36, respectively. Regression analysis and ANOVA results in Table 3 shows that the calculated F-values of four responses such as puree viscosity, yield, TSS and ascorbic acid were significant at $p < 0.001$. At the same time, they possessed non-significant lack-of-fit ($p>0.05$). These values indicated that the models were fitted and reliable. However, the adequacy of the model needed to be further checked by the coefficient of determination (R^2). The closer the value of R^2 to 1, the better correlation between the experimental and predicted values. From Table 3, R^2 values for guava puree viscosity, yield, TSS and ascorbic acid were 0.9706, 0.9672, 0.9678 and 0.9687, respectively. These models showing R^2 greater than 0.8 implied that each model indicated a good fit (Sin *et al*., 2006). Nevertheless, some researchers suggested that a large value of R^2 does not always imply that the regression model is a good one. Increasing R^2 can be obtained by adding a variable to the model. Thus, it is preferred to use an adj–R^2 to evaluate the model adequacy and it should be over 0.8 (Koocheki *et al.*, 2010). As seen in Table 3, adj–R^2 values of puree viscosity, yield, TSS and ascorbic acid were also high to advocate for a high significance of the model. Moreover, other parameters, namely pred–R^2 which should be closer to 1 and adeq–precision which should be greater than 4, of these models in Table 3 are supportive of the significance of the models. From Table 3, the coefficient of variation (CV) of yield was the lowest, indicating that this response had better precision and reliability of experiments as compared with other responses. In conclusion, these models adequately represented the real relationship between the variables chosen.

Effect on guava puree viscosity: Guava puree viscosity has been considered as an important quality parameter related to the juice filtration or pressing, namely a relative low viscosity leads to a better filtration. The regression model of puree viscosity as a function of enzyme concentration and incubation time can be described by the following equation after removing non-significant terms:

$$\text{Guava puree viscosity} = 23.68 + 0.54\ X_1^{***} + 0.26\ X_2^{***} - 4.11\ X_1^{2\,***} \quad (2)$$

where X_1 = enzyme concentration (ppm) and X_2 = incubation time (min).

***Significant at 0.001 level.

From equation 2, the model indicated that the variation in puree viscosity was significantly affected by positive linear ($p<0.001$) and negative quadratic ($p<0.001$) terms of enzyme concentration, followed by

the positive linear ($p<0.001$) term of incubation time. There was no interaction effect of the variables. Fig.1a shows the response surface plot generated from the fitted model. The puree viscosity slightly increased with increasing enzyme concentration and then greatly decreased by the second order parameter with negative effect. The lowest viscosity was obtained when enzyme concentration (> 860 ppm) and incubation time (> 80 min) were applied. The reduction of puree viscosity probably due to the commercial pectinase contains multienzymes such as endo-polygalacturonase, endo-pectinlyase and pectin esterase. These enzymes can hydrolyze protopectins and pectins to smaller chains like galacturonic acid (Sun *et al*., 2006), leading to a reduction of water holding capacity. As a consequence, free water was released to the system caused a reduction of viscosity (Lee *et al*., 2006).

Effect on yield: The quadratic polynomial for yield without non-significant terms was presented as the following equation:

$$\text{Yield} = 15.79+0.13\ X_1^{***}+0.32\ X_2^{***}-7.75E\text{-}005\ X_1^{2\ **}-1.95E\text{-}003\ X_2^{2\ *} \quad (3)$$

where X_1 = enzyme concentration (ppm) and X_2 = incubation time (min).
*Significant at 0.05 level, **Significant at 0.01 level, ***Significant at 0.001 level.

It may be observed that guava juice yield related to positive linear ($p<0.001$) and negative quadratic ($p<0.001$) terms of enzyme concentration, followed by positive linear ($p<0.001$) and negative quadratic ($p<0.05$) terms of incubation time. No interaction between the variables was found. Similar results were supported by the study of Diwan and Shukla (2005) and Kaur *et al*. (2009). The response surface plot in Fig.1b reveals that the enzyme concentration had a greater effect on juice yield. The increment of enzyme concentration led to a significant effect of incubation time on yield of guava juice. The pectinase usage showed a potential to hydrolyze soluble polysaccharides (high viscosity) to soluble sugars and short chain molecules (low viscosity) (Abdullah *et al*., 2007). It promoted the reduction of waste loss because the less viscous puree was easier for the filtration, showing a significant increase in juice yield (Sato *et al*., 2006). The highest yield was observed where enzyme concentration (>780 ppm) and incubation time (> 70 min) were used. Apparently, increasing of enzyme concentration and incubation time upon the optimal condition may slightly increase juice yield. This may be due to the concentration of substrate would be low for enzyme molecules.

Effect on TSS: Predicted response for TSS of guava juice as a function of enzyme concentration and incubation time can be expressed by the following equation:

$$\text{TSS} = 7.08-9.25E\text{-}003\ X_1^{***}+0.02\ X_2^{***}+7.30E\text{-}006\ X_1^{2\ **} -1.31E\text{-}004\ X_2^{2\ *}+2.5E\text{-}006 X_1X_2^{*} \quad (4)$$

where X_1 = enzyme concentration (ppm) and X_2 = incubation time (min).
*Significant at 0.05 level, **Significant at 0.01 level, ***Significant at 0.001 level.

As shown in equation 4, the linear effects of enzyme concentration and incubation time on TSS were significantly negative at $p<0.001$ and positive at $p<0.001$, respectively. Whilst, the quadratic effects of enzyme concentration and incubation time were positive at $p<0.01$ and negative at $p<0.05$, respectively. However, the interaction between enzyme concentration and incubation time was significant ($p<0.05$), and its effect was positive on TSS. This indicated that the action of pectinase was dependent on the incubation time during the enzyme treatment of the guava juice. This effect was evident when a high level of the two variables was used, which can be seen on the shape of the response surface plot in Fig.1c. In addition, enzyme concentration showed a greater effect on TSS than incubation time. The highest TSS was obtained when enzyme concentration and incubation time were used at least 850 ppm and 56 min, respectively.

Effect on ascorbic acid: The regression analysis revealed a relationship between enzyme concentration and incubation time as shown in the following equation:

$$\text{Ascorbic acid} = 93.29 -0.14\ X_1^{***}-0.13\ X_2^{**}+1.18E\text{-}004\ X_1^{2\ ***}+1.81E\text{-}003\ X_2^{2\ *} \quad (5)$$

where X_1 = enzyme concentration (ppm) and X_2 = incubation time (min).
*Significant at 0.05 level, **Significant at 0.01 level, ***Significant at 0.001 level.

It was seen on equation 5 that enzyme concentration and incubation time showed negative linear effects on ascorbic acid, which was significant at $p<0.001$ and $p<0.01$, respectively. Whilst, enzyme concentration and incubation time had positive quadratic terms at $p<0.001$ and $p<0.05$, respectively. There was no interaction effect between the variables. The response surface plot shown in Fig.1d demonstrates that the ascorbic acid was mostly influenced by enzyme concentration. A rapid increase of ascorbic acids related to increasing enzyme concentration. The application of enzyme concentration (> 850 ppm) and incubation time (> 70 min) produced the juice with the highest ascorbic acid. This was attributed to the pectinase hydrolysis on the guava pomace and ascorbic acids were released into the juice (Kuar *et al.*, 2009).

Optimization and validation: The optimum condition for the production of guava juice containing maximum yield, TSS and ascorbic acid was determined by the numerical optimization with chosen each variable and

response, as shown in Table 4. The analysis predicted that the conditions which contained 869.36 ppm enzyme concentration and 71.27 min incubation time would produce the maximum values for guava puree viscosity = 160.07 cps, yield = 85.10%, TSS = 5.60°Brix and ascorbic acid = 54.27 mg/100 ml.

The suitability of the model equations was performed using the recommended optimal conditions. The experimental results showed that the guava juice (n = 3) contained 159.35 cps puree viscosity, 83.74% yield, 5.48 °Brix TSS and 53.86 mg/100 ml ascorbic acid, which was close to the predicted values, indicating that each model was quite accurate in prediction.

Table 1. Independent variables and their coded and actual values used for analysis

Independent variables	Unit	Symbol	Coded levels				
			-1.41	-1	0	1	1.41
Enzyme concentration	ppm	X_1	500	558.56	700	841.44	900
Incubation time	min	X_2	30	38.78	60	81.22	90

Table 2. The central composite rotatable design for guava juice production with enzyme treatment and experimental data of responses

Experimental number	Independent variables		Physicochemical properties						
	Enzyme concentration (X_1)	Incubation time (X_2)	Puree viscosity (cps)	pH	Titratable acidity (%)	Clarity (Abs_{660})	Yield (%)	TSS (°Brix)	Ascorbic acid (mg/100ml)
No enzymat treatment			232.17	4.13	0.60	1.823	68.54	4.70	45.83
1	-1	-1	192.33	3.71	0.65	1.716	75.35	4.90	46.65
2	-1	+1	183.86	3.72	0.62	1.748	78.65	5.15	48.35
3	+1	-1	174.65	3.72	0.67	1.748	81.35	5.22	52.05
4	+1	+1	161.35	3.71	0.63	1.718	84.35	5.50	53.36
5	-1.41	0	181.67	3.73	0.60	1.638	75.23	5.22	47.75
6	+1.41	0	160.07	3.70	0.64	1.779	85.14	5.65	55.36
7	0	-1.41	197.67	3.70	0.62	1.739	78.70	4.75	45.95
8	0	+1.41	172.25	3.72	0.62	1.739	84.36	5.30	50.95
9	0	0	187.73	3.72	0.64	1.726	82.85	5.05	46.35
10	0	0	189.60	3.71	0.62	1.732	83.62	5.12	47.76
11	0	0	185.55	3.71	0.61	1.739	82.72	5.18	47.22
12	0	0	187.53	3.72	0.65	1.733	83.03	5.15	46.59
13	0	0	186.53	3.71	0.62	1.738	81.38	5.12	46.45

Table 3. Regression coefficients for physicochemical properties of guava puree and juice

Response[1)]	F-value	P-value	R^2	Adj- R^2	Pred- R^2	Adeq-precision	Coefficient variation	Lack-of-fit (Prob > F)
Y_1	46.28	<0.0001	0.9706	0.9497	0.8236	21.145	1.41	0.0692
Y_2	2.79	0.1090	0.3581	0.2297	-0.2802	4.821	0.21	0.1900
Y_3	2.72	0.1142	0.3521	0.2225	-0.1717	4.826	2.68	0.4880
Y_4	3.56	0.0679	0.4161	0.2993	-0.2749	5.556	1.54	0.0014
Y_5	41.26	<0.0001	0.9672	0.9437	0.8327	18.943	0.97	0.2146
Y_6	42.08	<0.0001	0.9678	0.9448	0.8531	23.709	1.04	0.3286
Y_7	43.35	<0.0001	0.9687	0.9464	0.8482	19.257	1.46	0.2647

[1)] Y_1: guava puree viscosity, Y_2 : pH, Y_3 : titratable acidity, Y_4 : clarity, Y_5 : yield, Y_6 : TSS and Y_7 : ascorbic acid.

Table 4. Criteria and output for numerical optimization of guava juice production

Criteria	Goal	Limit	Output
Enzyme concentration (ppm)	In the range	500-900	869.36
Incubation time (min)	In the range	30-90	71.27
Puree viscosity (cps)	In the range	160.07-197.67	160.07
Yield (%)	Maximize	75.23-85.14	85.10
TSS (°Brix)	In the range	4.75-5.65	5.60
Ascorbic acid (mg/ 100 ml)	Maximize	45.95-55.36	54.27
Desirability			0.939

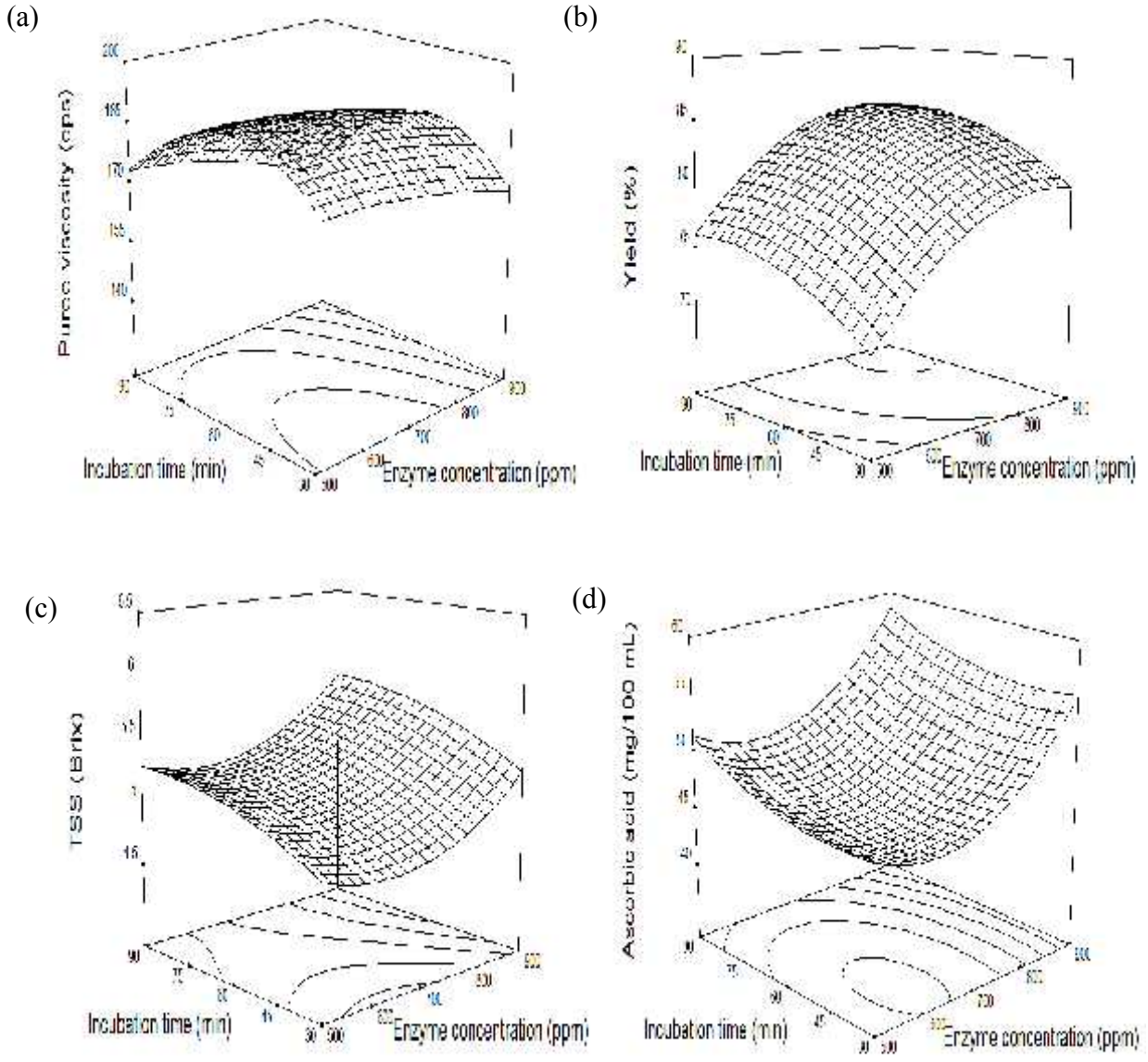

Fig. 1. Response surface plots of physicochemical properties of guava puree and juice:
(a) puree viscosity, (b) yield, (c) TSS and (d) ascorbic acid

Conclusion: By using pectinase, the guava mash showed a decrease in viscosity and the juice was high in yield, TSS and ascorbic acid as compared to that without enzyme treatment. The CCRD study indicated that the increment of enzyme concentration and incubation time significantly related to high values for yield, TSS and ascorbic acid of guava juice. The enzyme concentration was the greatest effect to lower the viscosity of guava puree, resulting in a better filtration process. The optimized enzyme treatment by adding 869.36 ppm pectinase in guava mash and incubated for 71.27 min was recommended. Under this condition, the predicted values were 85.10% yield, 5.60 °Brix TSS and 54.27 mg/100 ml ascorbic acid. This result obtained would be beneficial for juice industry to increase the yield and ascorbic acid of the product.

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