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Optimization of Pectinase Enzyme Liquefaction of Banana 'Gros Michel' for Banana Syrup Production

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ABSTARCT

The pectinase enzyme liquefaction of banana 'Gros Micheal' pulp was optimized by response surface methodology (RSM). The effect of pectinase enzyme concentrations (0-0.2%) and incubation times (60-300 min) on juice yield (%), total soluble solids (TSS), recovery soluble solids (RSS), clarity ($\%T_{670}$) and browning index (A_{420}) of juice were studied using central composite design of experiments. Results showed that pectinase enzyme concentration played an important role that significantly ($p \leq 0.001$) influenced most of dependent variables of banana juice. The coefficient of determination (R^2) of yield (%), recovery soluble solids (RSS), clarity ($\%T_{670}$) and browning index (A_{420}) were 0.907, 0.924, 0.804 and 0.793, respectively. The optimum condition for enzymatic extraction was 0.15% of pectinase enzyme incubated for 120 min at 50°C. The yield was ≥ 62%, RSS ≥14, $\%T_{670} \geq 96\%$ and $A_{420} \leq 0.1$. The physicochemical compositions of syrup were mostly three times higher than juice owing to the concentration that increased from 24 to 74° Brix except for pH, clarity ($\%T_{670}$), CIE L* which were not changed.

Key words: banana, syrup, enzymatic optimization, pectinase enzyme, response surface methodology

INTRODUCTION

Increase awareness in health issue leads to increase in consumption of natural products. Epidemiological studies have consistently shown that high fruit and vegetable consumption is associated with a reduced risk of several chronic diseases such as coronary heart disease, cardiovascular disease, cancer, aging, atherosclerosis neurodegenerative disease (such as Parkinson and Alzheimer), and inflammation (Dillard and German, 2000). The use of natural sweeteners rather than sucrose is an interesting area for the food industry. The high fructose corn syrup as sweetener has increased in using since recent years. Other raw materials like sugarcane, sugar beet and corn as sources of syrup with high fructose content are important at present. Recently, grapes have been investigated as another potential source of syrup. Meta *et al*. (2005) studied the possibility of commercial black walnut syrup (*Juglans nigra*) to compete with maple syrup. The pure walnut syrup could be commercially potential as a replacement for pure maple syrup. Saenz *et al*. (1998) studied the use of cactus pear (*Opuntia ficus* indica L.) to obtain a new liquid sweetener.

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The sensory evaluation revealed the relative sweetness of cactus pear syrup was similar to glucose syrup but lower than fructose syrup. The three native sun-dried date (*Phoenix dactylifera* L.) varieties, syrup and by-products were examined for proximate composition, dietary fiber, total phenolics and total antioxidant activity. Carbohydrate was predominant component in all date varieties. Seed and press cake were found to be good sources of dietary fiber and total phenolics (Al-Farsi *et al*., 2007).

Thailand has been exporting banana for many years. The number of export banana increased gradually from 2,700 ton/year in 2002 to 8,500 ton/year in 2004. A considerable number of bananas are rejected because they do not meet quality standard. The Hom Thong banana (*Musa* AAA group 'Gros Michel') were rejected from the three banana export stations more than 200 ton/year (Tadakittisarn, 2004). While some fruits could be sold at low prices in the local market, most are considerable as waste and as presenting significant environmental problems. Since banana has high sugar content and been recognized for its desirable flavor, banana syrup can then be an alternative for utilization of the disqualified banana. Banana syrup is the nutrition sweetener since banana is a good source of carbohydrate, dietary fiber, vitamins and minerals especially potassium (Bates *et al*., 2001). The banana syrup is industrially performed in order to reduce storage packaging, handling and shipping costs.

The most efficient extraction of banana juice is one of the most important steps for banana syrup production. However, some tropical fruits including banana are usually too pulpy and pectinacious to yield juices. One of the most effective methods is the enzymatic liquefaction technique. The commercial pectinolytic enzymes are used as processing aids for pectin degradation which settled down organic particles in suspension. The use of pectinolytic enzymes not only resulted in higher yield of juice but also preserves the nutrients, original color and flavor (Jareel *et al*.,1978). Shahadan and Abdullah (1995) used 0.04% pectinase enzyme (Pectinex Ultra SP-L, Novozymes A/S, Denmark) at 30° C with pH 3.4 for banana juice extraction. Koffi *et al*. (1991) used the mixture of pectinase, cellulase and hemicellulase enzymes which were effective in viscosity reduction and filterability improvement in the preparation of clarify banana juice. Viquez *et al*. (1981) studied the production of clarifiy banana juice using six commercial pectinolytic enzymes to disintegrate other fruit pulps, reduce viscosity and clarified juices. The enzymatic process should be optimized with temperature, time and enzyme concentration to maximize the yield and quality of banana juice.

One of the most effective tools for optimizing the process when many factors and interactions affect the desired response is response surface methodology (RSM). The basic theoretical and fundamental aspects of RSM have been reviewed (Hu, 1999). RSM reduces the number of experimental trials made to evaluate multiple parameters and their interactions. RSM has been widely applied for optimizing processes in the food industry (Shahadan and Abdullah, 1995; Rai *et al*., 2004; Lee *et al*., 2005). It usually uses an experimental design such as a central composite design (CCD) to fit a first- or second-order polynomial by least significant technique. An equation is used to describe how the test variables affect the response, determine the interrelationship among the test variables, and describe the combined effect of all the test variables in the response. The contour plots can be usefully employed to study the response surfaces and locate the optimum.

This study aimed (1) to study the effect of enzyme concentration and incubation time on yield, TSS, RSS, clarity and browning index and (2) to optimize the enzymatic liquefaction of banana puree by response surface methodology. The physicochemical properties of banana juice

and syrup obtained were also determined.

MATERIALS AND METHODS

Materials

Disqualified banana (*Musa accuminata* AAA group 'Gros Michel') were collected from Thungkawat Gardening Group, Chumphon province, Thailand. Commercial pectinolytic enzyme (Pectinex Ultra SP-L, Novozymes A/S, Denmark) was used. This enzyme was prepared from *Aspergillus aculentus* and used in food industry for fruit juice processing to reduce viscosity. It contained different pectinolytic and cellulolytic enzymes [endo-polygalactulonase (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].

Methods

Sample preparation

The bananas were ripen for 5-7 days at 25 ± 2°C and 90-95% RH until they reached stage 7 according to standard peel color index (CSIRO, 1972). Consequently, all ripen bananas were steam blanched for 16 min before they were peeled off. The pulps were blended and the puree obtained was then frozen at -20°C until further study.

Enzymatic liquefaction

The liquefaction was performed according to the method described by Kashyap *et al*. (2001) with modification as shown in Figure 1. Banana puree (500g) was thawed overnight at 4°C, thoroughly mixed with enzyme and subjected to the treatments given in Table 1. The samples were kept stirring under certain temperature (50 ±1°C) controlled by water bath (Viquez *et al*., 1981). At the end of the treatment, the enzyme in sample was inactivated by heating at 100°C for 15 min in water bath. The enzyme treated pulp was centrifuged at 8,000 rpm for 20 min and the supernatant was filtered through the vacuum filtration to obtain clear juice.

Dependent parameters estimation

The juice yield was estimated as percentage of the juice obtained based on the initial pulp. The total soluble solid (TSS) content was determined using hand refractometer (Atago, Japan). Recovery of soluble solid (RSS) was computed (Al-Hooti *et al*., 2002) as followed:

$$RSS = \frac{\text{weight of extract x TSS of extract}}{\text{weight of banana pulp}}$$

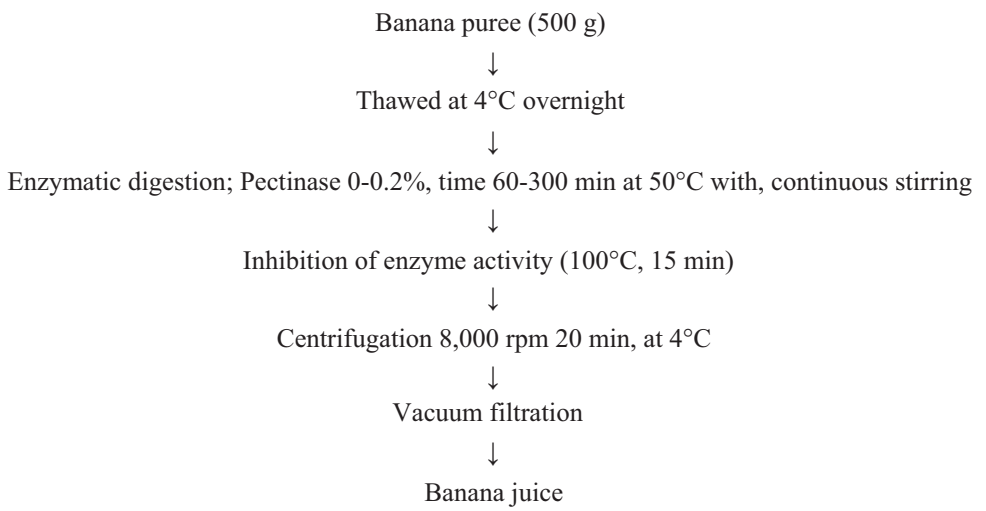

Figure 1 The process of banana liquefaction.

Clarity and browning index of juice were determined by measuring %transmittance at 670 nm and absorbance at 420 nm, respectively, using spectrophotometer (Jasco, V-530, Japan) (Johnson *et al*., 1995; Youn *et al*., 2004).

Experimental design

In the investigation, two variables (five levels of each variable) and second–order central composite experimental design (Hu, 1999) was employed. The independent variables were enzyme concentration (x1) 0-0.2%v/w, reaction time (x2) 60-300 min each at five levels: -α, -1, 0, 1, +α. The experimental design in coded (x) and actual (X) levels on variable were shown in Table 1.

Statistical analyses

A second-order polynomial equation was used to fit the experimental data given in Table 1. The model proposed for the response (Y) was

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_2 x_2 + e \quad (1)$$

where Y was the predicted response for yield, TSS, RSS, clarity ($\%T_{670}$) and browning index (A_{420}), b_0 (constant term) b_1 and b_2 (linear effects) b_{11} and b_{22} (quadratic effects) and b_{12} (interaction effects) and ε (random error). Response surfaces and contour plots were developed using the fitted quadratic polynomials equation obtained from regression analysis.

Banana syrup preparation

The clear banana juice obtained from the selected optimized enzymatic liquefaction process was concentrated using rotary vacuum evaporator at 40°C until the 74±1° Brix TSS was obtained. Then banana syrup and banana juice were physicochemically characterized.

Physicochemical determination

The physicochemical properties of banana juice and banana syrup were determined. The pH of sample was measured using pH meter (Cyberscan 500, Singapore). Titratable acidity was determined by diluting 10 ml of juice or 10 g of syrup with 40 and 75 ml water, respectively, followed by titration against 0.1 N sodium hydroxide until pH 8.3 was obtained. Moisture content was determined by Electronic moisture analyzer (Boeco, SM001, Germany) at 95°C. Viscosity was measured using viscometer (Brookfield, DV20, USA) with small sample adapter and needle no.S27. Clarity and browning index of juice were determined by measuring %transmittance and absorbance at 670 and 420

Table 1 Matrix of experimental central composite design (CCD) for enzymatic liquefaction of banana puree.

Treatment	Pectinase concentration (%)	Incubation time (min)
	X1 (x1)	X2 (x2)
1	0.05 (-1)	120 (-1)
2	0.05 (-1)	240 (1)
3	0.15 (1)	120 (-1)
4	0.15 (1)	240 (1)
5	0 (-α)	180 (1)
6	0.2 (+α)	180 (1)
7	0.10 (0)	60 (-α)
8	0.10 (0)	300 (+α)
9	0.10 (0)	180 (0)
10	0.10 (0)	180 (0)
11	0.10 (0)	180 (0)
12	0.10 (0)	180 (0)

nm, respectively using spectrophotometer (Jasco, V-530, Japan). The commission internationale de L' Eclairage (CIE) system reference measures the lightness, redness and greenness (L* a* b* value) measured using tintometer (Lovibond, PFX195, UK). Reducing sugar was analyzed by Somogyi-Nelson's solution (Zhu *et al*., 1992). Total phenolic content was colorimetrically measured with Folin-Cioculteu reagent (Singleton and Rossi, 1965) whereas sugar content was determined by high performance liquid chromatography (HPLC) (Shimadzu, LC10A, Japan). The starch was determined by iodine solution (Carrin *et al*., 2004). Pectin was analyzed in form of galacturonic acid using m-hydroxydiphenal method (Kintner and van Buren, 1982). Polysaccharides and total sugar were determined by the phenol-sulfuric acid method (Saha and Brewer, 1994) and protein was measured by Lowry's method (Lowry *et al*., 1951).

RESULTS AND DISCUSSION

Statistical analysis

The experimental value and analysis of variance for five response variables; yield, TSS, RSS, clarity and browning index under different treatment conditions are presented in Tables 2 and 3, respectively. It showed that the response surface model developed for all response variables were adequate. The determination coefficient (R^2) values for all response variables were higher than 0.75 except TSS (0.54), indicating that the regression model explained the reaction well.

Effects of parameters

The different parameters (yield, TSS, RSS, clarity and browning index) of enzyme extracted juice compared with the untreated juice were shown in Table 4. It was clear that the quality of juice from enzyme extraction process was improved.

Banana juice yield extracted by the enzymatic liquefaction varying from 59.44 to 65.29% were greater than the control (without enzymatic treatment; 43.20%) (Table 4). The regression model (Table 3) showed that the yield was significantly affected by linear and quadratic of enzyme concentration. The linear effect ($p \leq 0.001$) was positive; whereas the quadratic effect ($p \leq 0.001$) was negative thus resulting in a curvilinear increase in yield for all incubation times. The incubation time had a very minor

Table 2 Treatment schedule for two-factor CCD and the response in terms of yield, TSS, RSS, clarity (%T670) and browning index (A420).

Treatment*	Yield (%)	TSS (°Brix)	RSS (°Brix)	Clarity(% T670)	Browning index(A420)
1	60.84	23.80	14.11	97.52	0.099
2	61.25	24.00	14.21	96.41	0.111
3	66.61	23.20	15.45	98.05	0.091
4	65.87	23.20	15.28	99.41	0.073
5	43.20	23.20	10.02	62.26	0.723
6	64.00	23.20	14.85	98.01	0.094
7	59.44	23.20	13.79	97.00	0.096
8	65.01	23.20	15.08	97.74	0.103
9	64.81	23.20	15.04	96.04	0.099
10	65.29	24.00	15.67	97.83	0.106
11	64.50	23.80	15.35	98.26	0.091
12	63.74	23.80	15.17	98.03	0.091

Note : Treatment numbers represented enzymetic liquefaction of banana puree shown in Table 1

influence on yield. The yield was linearly related to incubation time, therefore providing a linear increase in yield with incubation time for all enzyme concentrations (Figure 2a). These results agreed with Rastogi and Rashami (1999) that juice yield was found to be a function of linear and quadratic effects of enzyme concentration and Shahaden and Abdullah (1995) that enzyme concentration more affected banana juice yield than other factors (pH and temperature). Degradation of pectin by pectinase enzyme led to a reduction of water holding capacity, and therefore, free water released to the system caused reduction of viscosity and increasing in yield (Kashyap *et al*., 2001; Lee *et al*., 2006).

RSS was the calculated parameter from yield and TSS, although R^2 value of TSS was less than 0.75, TSS was increased with enzymatic treatment (Table 4). The R^2 of RSS value in the regression model (Table 3) was the highest of all responses (0.924), indicating that the regression model of RSS could explain the reaction well. The RSS value was significantly affected by first ($p \leq 0.001$) and second orders ($p \leq 0.001$) of enzyme concentration. The incubation time had a very minor influence on RSS value. It might also be observed from Figure 2b that RSS increased with increasing enzyme concentration until its maximum and then slightly decreased. It was found that all first order parameters had positive effects on the RSS, and at the same time the second order parameters had negative effects in both enzyme concentration (x_1) and incubation time (x_2). Furthermore, results of RSS showed similar trends as yield.

The clarity was an important index of clarified juice (Sin *et al*., 2006). It was observed from Table 3 that the clarity increased (linear positive effect) and decreased (quadratic negative effect) with the increasing of enzyme concentration. Increase in enzyme concentration may increase the rate of clarification by exposing

Table 3 The regression coefficients, R^2 and *p* of 2 variables for enzymatic liquefaction.

Regression coefficient	Yield (%)	RSS(∝Brix)	Clarity(% T_{670})	Browning index(A_{420})
b_0	37.723***	7.971***	68.676***	0.597***
b_1	306.28***	77.525***	478.633***	-8.393***
b_2	0.074	0.025	0.002	0.00001076
b_{11}	-1098.1***	-287.1**	-1768**	31.097**
b_{22}	0	-0.0000605	0	0
R^2	0.906	0.924	0.804	0.793
p	0.001	0.001	0.003	0.004

Note : ** Significant at 0.01 level
*** Significant at 0.001 level

Table 4 Yield, TSS, RSS, clarity and browning index of untreated and enzyme-treated juices.

Parameter	Untreated banana Juice	Enzyme-treated banana Juice
Yield (%)43.20	59.44-65.29	
TSS (°Brix)	23.20	23.20- 24.00
RSS (°Brix)	10.02	13.79-15.67
Clarity (%T_{670})	62.26	96.04-99.41
Browning index (A_{420})	0.723	0.073-0.111

Note : Subscripts: 1 was enzyme concentration, 2 was incubation time
*** Significant at 0.001 level, ** Significant at 0.01 level, * Significant at 0.05 level
b_0 was constant term, $b_1$and b_2 was linear effect, $b_{11}$and b_{22} was quadratic effect and b_{12} was interaction effect

part of the positively charged protein beneath, thus reducing electrostatic repulsion between cloud particles which caused these particles to aggregate into larger particles and eventually settled out (Shahaden and Abdullah, 1995; Sin *et al*., 2006).

The appearance characteristic, for example color was the first judgment of a clarified juice quality. A dark product would mean that the product was deteriorating and it was usually less appealing to the consumers (Abdullah *et al.,* 2007). Color of banana juice extracted by the enzymatic liquefaction varied from 0.073-0.111 of A_{420} which less than control (without enzymatic treatment; 0.723 of A_{420}) (Table 4). It was observed from Table 3 that browning index decreased (linear negative effect) and increased (quadratic positive effect) with the pectinase enzyme concentration ($p \leq 0.001$). It was clear from Figure 2d that the browning index decreased with the increasing of enzyme concentration. Increase in enzyme concentration may increase the rate of juice liquefaction and clarification. This could be due to that increased agglomeration of floc as more pectin was degraded by higher enzyme concentration. As time increased, the fine particles in juice may also slowly settle down (Sin *et al*., 2006). Therefore the absorbance at 420 nm was reduced until the extraction in steady state that no more juice was extracted then the A_{420} was increased because of that some light absorbed

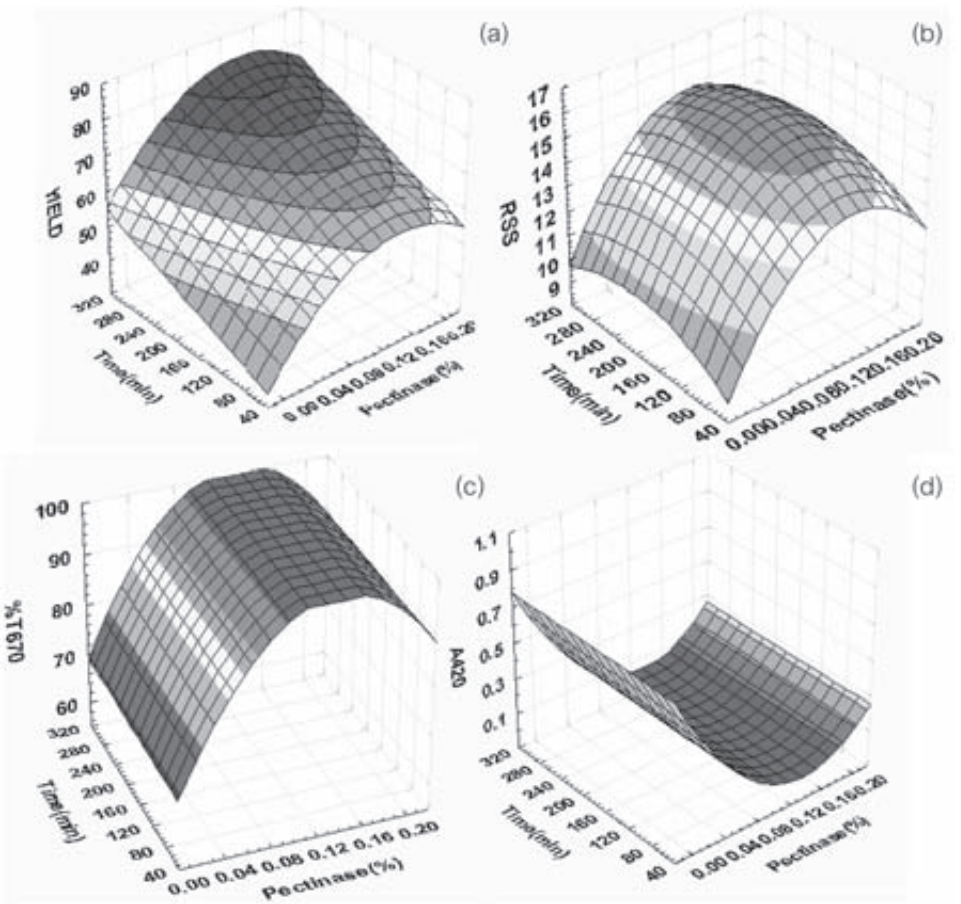

Figure 2 Response surfaces showing the effect of pectinase concentration and incubation time on (a) yield (b) RSS (c) clarity ($\%T_{670}$) and (d) browning index (A_{420}).

particles were extracted. The result of browning index was inversed with the clarity (Figure 2c and 2d).

Optimization of enzyme liquefaction

The optimum extraction condition was determined by superimposing the contour plots of the significant responses. The final product would be considered optimum with high yield and less time of processing. Emphasis was placed on RSS and clarity since they were important indexes of physical characteristics of all responses. RSS could be represented better than yield and the clarity and browning index were in the same trend. The criteria produced an optimum region in the superimposed plot if RSS was ≥15 and clarity (T_{670}) ≥ 97% (Figure 3). The optimum combined condition was found to be at 0.09-0.18% of pectinase enzyme concentration incubated for 100-300 min. From this combined area, specific 6 points were selected among enzyme concentrations (0.10 - 0.17%) and times (100 - 200 min) for banana puree liquefaction. The result showed that the treatment with higher enzyme concentration (>0.15%, 120 min) gave high clarity and less browning index significantly different when compared with the lower enzyme concentration although the RSS were not different. Thus, the condition at 0.15% enzyme concentration incubated for 120 min at 50° C was chosen for banana juice extraction.

Physicochemical composition of banana juice and banana syrup

The banana juice was prepared using the optimized condition (0.15% enzyme and 120 min at 50°C) and concentrated to obtain banana syrup. The physicochemical properties were determined and compared with the banana juice (Table 5). The physicochemical compositions of syrup were usually three times higher than those of juice owing to the concentration of syrup that higher than juice for three times (from 24 to 74° Brix) except for pH, clarity (%T_{670}), CIE L* which were not changed.

The soluble solid of banana juice (24 ° Brix) was less than that of Kayinja banana (34.9 ° Brix) (Kyamuhangire *et al.*, 2002) but pH was higher (4.98 and 4.31, respectively). Sucrose glucose and fructose contents were 0.13, 12.25 and 13.03%, respectively which were different from the ones obtained in this study (12.14, 6 and 5 %,

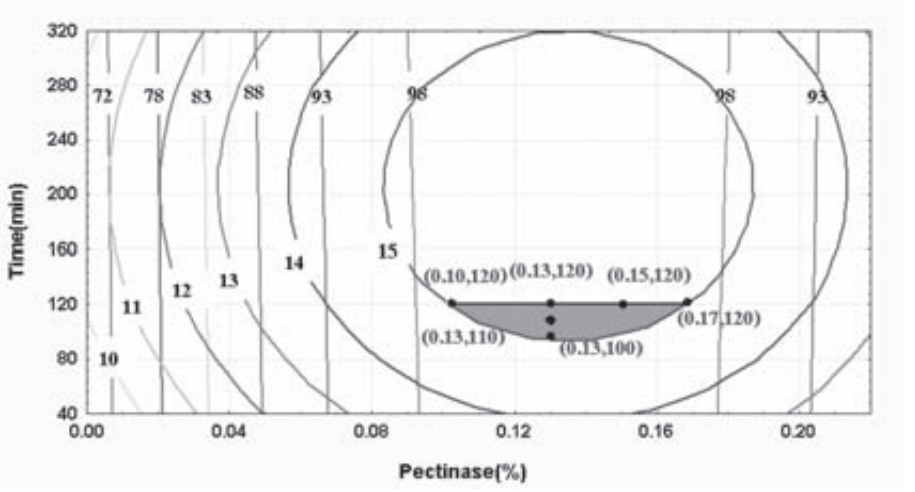

Figure 3 The 6 selected points within the superimposed contour plot of optimized area for banana liquefaction among varied enzyme concentrations (0.10% -0.17%) and times (100-120 min).

respectively). The physical properties of juice including clarity ($\%T_{670}$) and color CIE L* a* b* were 97.8, 97.04, -1.448 and 5.239, respectively, which were more clear and lighter than Barangan banana juice; 94.7, 33.92,-0.73 and 2.33, respectively (Shahadan and Abdullah, 1995).

The banana syrup was such a new product, its pH value (5.0) was between those of cactus pear syrup (4.31) (Saenz *et al.,* 1998) and date syrup (5.32-7.56) (Al-Farsi, 2003). Glucose and fructose contents were 17 and 14.8%, respectively, which were less than those of date syrup (26.6 and 34.5%, respectively) although 36% sucrose were detected in banana syrup. The content of total phenolics in banana syrup was 0.377% higher than date syrup (0.096-0.162%) (Al-Farsi *et al*., 2006). Recognition of antioxidant activities of many polyphenols had realized thinking toward the health benefits provided by many of these compounds (Bravo *et al.,* 1998). Banana syrup also contained more dietary fiber (1.727 %) than date syrup (0.01%) (Al-Farsi *et al*., 2007).

CONCLUSION

The disqualified export banana could be developed as value added banana syrup. The response surface methodology appeared to be valuable tool for optimizing enzyme concentration and incubation time for enzymatic liquefaction of banana puree. The optimized enzymatic process with 0.15% pectinase enzyme and extraction for 2 hours at 50°C yielded ≥ 62% juice, RSS ≥14, %T670 ≥ 96% and A420 ≤ 0.1. The physicochemical properties of banana juice from optimized condition and syrup obtained were also determined. After being concentrated, the

Table 5 The characteristics of optimized banana juice and banana syrup.

Characteristic	Banana juice	Banana syrup
TSS (° Brix)	24.0 ± 0	74 ± 0
pH	4.980 ± 0.00	4.995 ± 0.01
Acidity (% malic acid/100g)	0.2272 ± 0.01	0.704 ± 0.04
Moisture (%)	77.163 ± 0.15	29.96 ± 0.30
Viscosity (cP)	0.33 ± 0.11	807.50 ±157.33
Clarity ($\%T_{670}$)	97.81 ± 0.15	98.22 ± 0.40
Color A_{420}	0.080 ± 0.001	0.365 ± 0.01
CIE L*	97.043 ± 0.04	95.133 ± 0.13
a*	-1.448 ± 0.12	-4.906 ± 0.65
b*	5.239 ± 0.09	20.085 ± 0.42
ΔE*	19.107 ± 0.10	4.965 ± 0.17
Starch (g %)	0.262 ± 0.001	1.887 ± 0.11
Total sugar(g %)	32.88 ± 2.23	55.049 ± 2.14
Reducing sugar (g %)	8.206 ± 0.041	33.895 ± 3.87
Sucrose (g %)	12.137 ± 1.21	35.992 ± 0.78
Glucose (g %)	6.010 ± 0.70	16.947 ± 0.63
Fructose (g %)	4.968 ± 0.48	14.759 ± 0.81
Polysaccharide (g %)	0.62 ± 0.016	2.523 ± 0.47
Protein (mg %)	6.69 ± 1.80	31.823 ± 0.76
Pectin (g %)	0.456 ± 0.10	1.727 ± 0.03
Polyphenol (g %)	0.116 ± 0.001	0.377± 0.01

composition of banana syrup was not changed; the nutrients and original color were still preserved. Results from this study might serve as a guideline for a large-scale liquefaction of banana juice from banana discarded from the export operation. This banana juice could be used to produce banana syrup.

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