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Comparison on Optimization of Star Fruit Juice Using RSM between Two Malaysian Star Fruit Varieties (B11 and B10)

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ABSTRACT

In Malaysia, two star fruit varieties, B11 and B10, are planted for commercial purposes. These types of star fruits are suitable for making juices. However, the fresh star fruit juice is cloudy, viscous and green in colour, necessitating the use of enzymes to clarify the juice. Thus, the aim of this study was to establish the optimum conditions for enzymatic treatment of star fruit (B11) juice using response surface methodology (RSM) and to compare the optimum conditions of this variety (B11) with the B10. Star fruit juice (B11) was treated with pectinase enzyme at different enzyme concentrations, incubation times, and temperatures. The effect of this enzymatic treatment was analyzed based on turbidity, clarity, and viscosity. The regression models describing the changes of turbidity, clarity and viscosity were established with the coefficient of determination, R^2, which were greater than 0.8. The optimum operating conditions for clarifying star fruit juice (B11) was found to be at 0.01% enzyme concentration at 30 min of incubation time and 30°C of temperature using response surface methodology. The method of treatments for B10 was similar to that of B11. The two varieties of star fruit (B11 and B10) showed different optimum conditions on enzyme concentration and incubation time, however there was no difference in terms of incubation temperature at optimum conditions.

Keywords: B10, B11, enzymatic clarification, response surface methodology, star fruit juice, clarity, turbidity, viscosity

INTRODUCTION

Nowadays, tropical fruit juices have become popular, and the positive trend is more people like to drink juices especially in the morning replacing the traditional caffeinated drinks. This positive trend is mainly due to the wholesomeness that the fruit juices offer in terms of nutritional benefits, all enriched with vitamin, fibres or other ingredients. Fruit juices have potentially open up new market opportunities tailoring fruit products to consumer demands.

One of the most popular tropical fruit juices is star fruit or Carambola. Star fruit is sweet and slightly acidic, succulent and juicy with attractive flesh and distinctive flavour. Star fruit can be found in different shapes ranging from oblong to ellipsoid. The fruit when cross-sectionally cut, produces beautiful star shapes, from which the name 'starfruit' is derived. The skin is translucent, smooth and waxy with yellowish green colour skin when ripe. The flavour is variable and ranges from light sour to sweet. These fruits are relatively inexpensive as well as extremely rich in vitamins C, low fat and cholesterol free.

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Star fruit has been planted commercially in some countries such as Malaysia, China, India and Taiwan. Star fruit has approximately seventeen different cultivars (varieties), and each has its own origin, flavour and production levels (Vaillant *et al.*, 1999). In Malaysia, varieties B10 and B11 are planted for commercial purposes. Star fruit is easy to process and transform to juices, and the juice could be used in tropical drinks and smoothies. Recently, many countries have been involved in big scale star fruit juice processing especially manufacturers in Malaysia, Hawaii and China. Star fruit juices are usually cloudy, contain colloidal suspension, and green in colour, and this is associated with the quality of the juice which may influence consumer's acceptability. A clear juice is usually more acceptable by the consumer and tends to be easily marketed.

The cloudiness of the juices is mainly due to the presence of pectin. Pectin can be associated with plant polymers and the cell debris which are fibrous-like molecular structure. The cloudiness could be removed by enzymatic depectinization. Several studies have been reported on depectinization using enzymatic treatment such as pectinases that could effectively clarify the fruit juices (Alvarez *et al.*, 1998; Ceci and Lozano, 1998; Isabella *et al.*, 1995; Lee *et al.*, 2006; Rai *et al.*, 2004; Sin *et al.*, 2006; Yusof and Ibrahim, 1994). The pectinase hydrolyzes pectin and causes pectin-protein complexes to flocculate. The resulting juice from this pectinase treatment contains much lower amount of pectin and reduces its viscosity, which is advantageous in facilitating the subsequent filtration processes.

The enzymatic hydrolysis of pectin depends on several physicochemical factors such as incubation time, incubation temperature and enzyme concentration (Isabella *et al.*, 1995; Lee *et al.*, 2006; Rai *et al.*, 2004; Sin *et al.*, 2006; Sreenath and Santhanam, 1992). Enzymes are expensive biocatalysts and clearly, juice manufacturers want to minimize their operating costs by using enzymes at optimum conditions. It is desirable to obtain the optimum conditions which will maximize the effectiveness of juice clarification.

Optimization of different parameters that influence the depectinization rate was the main reason for this work. Response Surface Methodology (RSM) is an effective method to carry out optimization studies. RSM is a statistical tool that uses quantitative data from appropriate experimental design to determine and simultaneously solve multivariate equations (Giovanni, 1983). RSM can reduce the number of experimental trials needed to evaluate multiple parameters and their interactions, thus, less time consuming compared to other approaches. RSM has been widely applied in optimization processes in food industries (Lee *et al.*, 2006; Sin *et al.*, 2006; Wong *et al.*, 2003; Yusof *et al.*, 1988).

Liew Abdullah *et al.* (2007) and Liew Abdullah (2007) studied the optimization conditions for clarification of B10 star fruit juice using a commercial enzyme. The objectives of this work were to establish the optimum process conditions (incubation time, temperature and enzyme concentration) for enzymatic clarification of star fruit juice using Response Surface Methodology for B11 and compare that to a previous study with variety B10 (Liew Abdullah *et al.*, 2007; Liew Abdullah, 2007).

MATERIALS AND METHODS

Fruit

Fresh star fruits (*Carambola averrhoa* L.) of variety B11 were purchased from a local market in Serdang, Malaysia. Colour index 3 (25% to 75% yellow) was chosen for the ripeness of the fruit for this study (Colour index according to the FAMA[1] Standards grade for starfruit).

Enzyme

Pectinex Ultra SP-L from *Aspergillus niger* obtained from Novozymes Switzerland AG, Dittengen, Switzerland, was used for enzymatic treatment of star fruit juice and stored at 4°C. The activity of pectinex ultra SP-L enzyme was 26,000 PG per ml.

Juice Extraction Process

Star fruits were washed, peeled, deseeded and the star fruits were blended using a food blender (Panasonic, Malaysia) for 2 – 3 min until a homogenous solution was obtained. The juice was filtered out using a cheese cloth to separate the pulp from the juice. *Fig. 1* shows the extraction steps and subsequent clarification using enzymatic treatment of star fruit juice.

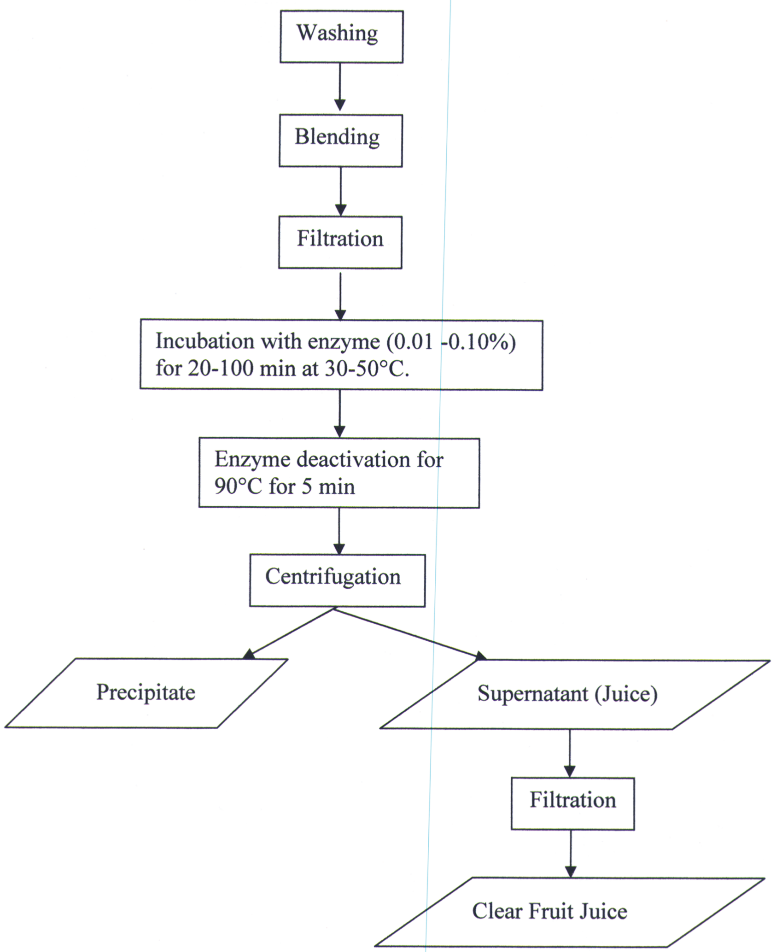

Fig. 1: Steps for extraction and subsequent clarification by enzymatic treatment of star fruit juice

[1]FAMA is Malaysia Federal Agriculture Marketing Authority.

Enzyme Treatment

The juice was strained using a muslin cloth. For each experiment, about 150 ml juice was treated with different enzyme conditions as shown in Table 1. The independent variables for enzymatic treatments were incubation time, X_1 (30 – 100), incubation temperature, X_2 (30 – 50) and concentration of enzyme used, X_3 (0.01 – 0.1). The temperature of enzymatic treatment was adjusted to the desired level using a water bath (Model 903, Protech Electronic, Malaysia, ± 0.5ºC). At the end of enzymatic treatment, the enzyme in the sample was inactivated by heating the suspension at 90ºC for 5 minutes in a water bath. The treated star fruit juices were centrifuged at 3000g for 10 min (Avanti J-25, Beckman Coulter, USA) and the supernatant was collected. Then, the juice was filtered through a filter paper (Whatman No.1, Whatman International Ltd., England) using Eyela vacuum aspirator. The filtrate was collected for further analysis.

TABLE 1
Effect of incubation time, incubation temperature and enzyme concentration on three responses

	Independent variables			Dependent variables		
	Incubation time (min)	Incubation temperature (ºC)	Enzyme Concentration (%)	Turbidity (NTU)	Clarity (abs)	Viscosity (cps)
Trial	$X_1(x_1)$	$X_2(x_2)$	$X_3(x_3)$	y_1	y_2	y_3
1	65(0)	40(0)	0.01(-1)	56.5	0.059	1.4
2	65(0)	40(0)	0.1(+1)	11.9	0.025	1.4
3	65(0)	30(-1)	0.055(0)	14.8	0.039	1.4
4	65(0)	50(+1)	0.055(0)	15.0	0.020	1.4
5	30(-1)	40(0)	0.055(0)	19.4	0.030	1.3
6	100(+1)	40(0)	0.055(0)	15.3	0.020	1.3
7	100(+1)	50(+1)	0.1(+1)	23.0	0.027	1.4
8	100(+1)	50(+1)	0.01(-1)	24.3	0.058	1.2
9	100(+1)	30(-1)	0.1(+1)	24.0	0.031	1.2
10	100(+1)	30(-1)	0.01(-1)	22.0	0.056	1.4
11	30(-1)	50(+1)	0.1(+1)	21.5	0.031	1.3
12	30(-1)	50(+1)	0.01(-1)	12.7	0.043	1.3
13	30(-1)	30(-1)	0.1(-1)	16.5	0.060	1.3
14	30(-1)	30(-1)	0.01(-1)	32.2	0.019	1.3
15	65(0)	40(0)	0.055(0)	32.2	0.019	1.4
16	65(0)	40(0)	0.055(0)	33.9	0.021	1.4
17	65(0)	40(0)	0.055(0)	34.1	0.021	1.3
18	65(0)	40(0)	0.055(0)	34.3	0.020	1.4
19	65(0)	40(0)	0.055(0)	34.7	0.020	1.3

Turbidity Analysis

Turbidity was determined using a portable Turbidimeter (Model 2100P, Hach Company, Loveland, Colorado,USA) and the results were reported as Nephelometric Turbidity Units (NTU).

Clarity Analysis

Clarity was determined by measuring the absorbance at 660nm using a UV-VIS spectrophotometer (Model UV-1201, Shimadzu Corporation, Japan). Distilled water was used as a reference.

Viscosity Analysis

The viscosity of the juice was determined using a Brookfield viscometer (Model LVDV-II+, Brookfield Engineering Laboratory, Inc., Middleboro, USA) at 100 rpm with spindle SC4-18 at room temperature ± 27ºC.

Experimental Design

Response Surface Methodology (RSM) was used in this study to determine the optimum conditions for the enzymatic clarification of star fruit juice. The experimental design and statistical analysis were performed using ECHIP Software Version 6 (Echip Inc., Hockessin, Delaware, USA).

The experiments were based on a central composite rotational design (Cochran and Cox, 1957) with a quadratic model in order to study the combined effect of the three independent variables (incubation time, temperature and enzyme concentration). These three independent variables were represented as X_1, X_2 and X_3, respectively. Each independent variable had 3 levels which were -1, 0, and +1. Based on Baumann (1981), these three chosen variables were responsible for the mechanism of enzyme activity in the juice. A total of 19 combinations including five replicates of the center point were carried out in random order according to a central composite design configuration for the three chosen variables. The experimental design in the coded (x) and actual (X) levels of variables is shown in Table 1. The dependent variables (y) measured were turbidity (y_1), clarity (y_2), and viscosity (y_3) of the star fruit juice. These dependent variables were expressed individually as a function of the independent variables known as response function. The variance for each factor assessed was partitioned into linear, quadratic and interactive components and were represented using the following second order polynomial function.

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (1)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1, b_2 and b_3 (linear coefficient), b_{11}, b_{22} and b_{33} (quadratic coefficient), and b_{12}, b_{13} and b_{23} (interactive coefficient). The significance of all terms in the polynomial functions were assessed statistically using F-value at a probability (p) of 0.001, 0.01 or 0.05. The regression coefficients were then used to generate contour maps from the regression models. The three-dimensional plots were generated by keeping one variable constant at the center point and varying the other variables within the experimental range.

Method for B10

The treatment and experimental methods for B10 (Liew Abdullah *et al.*, 2007; Liew Abdullah, 2007) was similar to that for B11.

RESULTS AND DISCUSSION

Statistical Analysis

The experimental values for all responses (independent variables) under different conditions are presented in Table 2 (B11). The independent variables and dependent variables (responses) were fitted to the second order polynomial function and examined for the goodness of fit. The R^2 or coefficient of determination which is defined as the ratio of explained variation to the total variation is a measure of the degree of fit (Haber and Runyon, 1977). The R^2 values for turbidity, viscosity and clarity were 0.996, 0.829 and 0.964, respectively. The closer the value of R^2 approaches unity, the better the empirical model fits the actual data. The smaller the value of R^2, the less relevant was the dependent variables in the model in explaining the variation of behavior (Little and Hills, 1978; Mendenhall, 1975). The result for this study was above 0.8. The values of R^2 for turbidity, viscosity and clarity for B10 were slightly less than B11 which were 0.880, 0.779 and 0.864 respectively (Liew Abdullah *et al.*, 2007).

Turbidity

Turbidity is considered as 'muddy' for a juice, thus, indicating the turbidity should be minimal for marketing purposes. Therefore, clear and sparkling star fruit is required. The response surfaces for turbidity can be visualized in *Fig. 2a*, which shows the contour map for the effect of the independent variables on turbidity of B11.

TABLE 2
Regression coefficient, R^2, values for four dependent variables for enzymatic clarification of star fruit juice

Regression coefficient	Turbidity (NTU)	Viscosity (cps)	Clarity (abs)
b_0	16.4495	1.37773	0.0231278
b_1	-393.333 ***	-1.24444 ***	-0.416 ***
b_2	-0.171429 ***	-0.000857143 *	-0.000159143 **
b_3	-0.084	-0.000699999	-0.000315
b_{12}	6809.21 ***	-9.82561	6.12957 **
b_{22}	0.0086438 ***	-2.44056e-005	2.49989e-006
b_{32}	-0.0071134	0.000451031	3.06237e-005
b_{12}	2.46825 ***	-0.0079365	0.00254762 *
b_{13}	5.52778 **	0.0388889	0.000305556
b_{23}	-0.00703571 **	5.71429e-005	-4.89286e-006
R^2	0.996	0.829	0.964
p	0.000 ***	0.0138 *	0.000 ***

Subscripts: 1 = enzyme concentration; 2 = incubation time; 3 = temperature.
* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

By referring to Table 2, the turbidity (B11) was significantly affected by incubation time and enzyme concentration for both the linear and quadratic cases. Both independent variables showed a negative effect on the linear terms and a positive effect on the quadratic terms. It was a significant interaction effect between enzyme concentration and incubation time with a positive effect on turbidity. This means that the action of the enzyme was dependent on the incubation time during the enzymatic treatment of the star fruit juice.

The turbidity of the fruit juice is mainly caused by the polysaccharides in the juice such as pectin (Grassin and Fauquembergue, 1999). Thus, increase in enzyme concentration and incubation time might decrease the turbidity of the juice. *Fig. 2a* shows the effect of enzymatic treatment at a fixed temperature (40ºC). The turbidity of the juice decreased drastically when enzyme concentration increased. These results strongly agree with those reported by Alvarez *et al.* (1998); as the enzymatic treatment process took place, the amount of pectin in the juice decreases and hence, reducing the turbidity of the juice. Similar trends were observed by Liew Abdullah *et al.* (2007) for variety B10 juice, at a fixed temperature (30ºC), turbidity decreased with an increase in enzyme concentration (*Fig. 2b*).

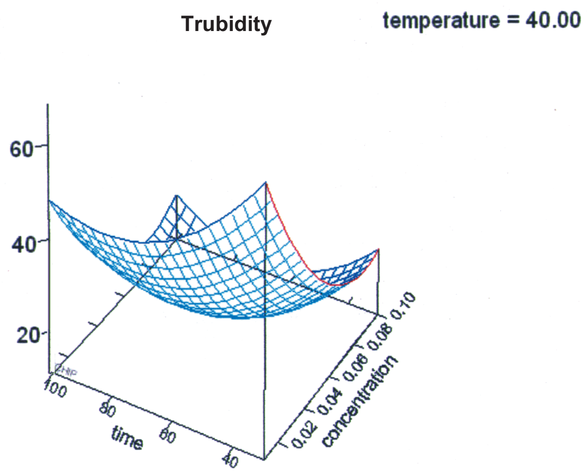

Fig. 2a: Response surface for turbidity of star fruit juice (B11) as a function of time and enzyme concentration (at 40ºC)

Clarity

Clarity is another important index of clarified juice (Sin *et al.*, 2006). Clarified juice is a natural juice that is pulpless and clear in appearance. It is observed from Table 2 that clarity mainly depends on the enzyme concentration as its quadratic effect was positive and significant at $p<0.001$. The incubation time also significantly affects the clarity in the linear case. There are also significant interaction effects between incubation time and enzyme concentration at $p< 0.05$ with a positive effect, which indicates that the incubation time was dependent on enzyme concentration.

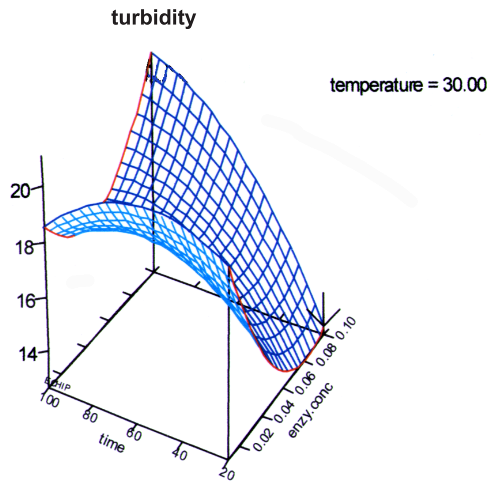

Fig. 2b: Response surface for turbidity of star fruit juice (B10) as a function of time and enzyme concentration (at 30ºC) (Adapted from Liew Abdullah et al., 2007)

Fig. 3a shows a 3D plot for juice clarity with enzyme concentration and incubation time at a fixed temperature of 40ºC for variety B11. It was evident that the absorbance value decreased with an increase in enzyme concentration. Low absorbance values indicate a clearer juice is being produced. It was also observed that the absorbance values decreased with increased the incubation time. The time required to obtain a clear juice is inversely proportional to the concentration of enzyme used at constant temperature (Kilara, 1982). *Fig. 3b* (Liew Abdullah *et al.*, 2007) shows the plot for variety B10 juice clarity. From the figure, it can also be observed that the absorbance value decreased with increasing enzyme concentration and incubation time at fixed temperature.

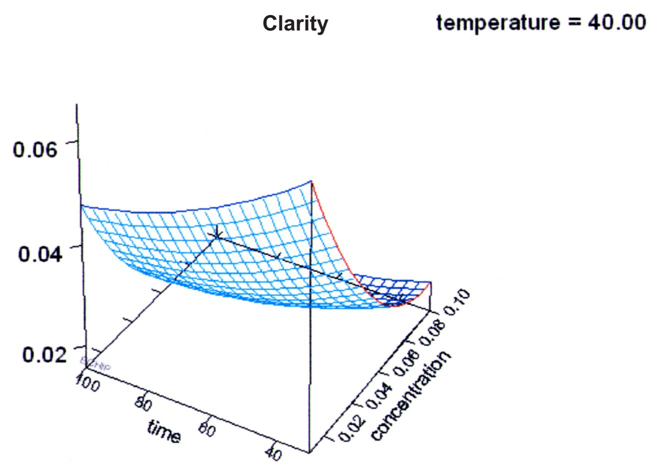

Fig. 3a: Response surface for clarity of star fruit juice (B11) as a function of time and enzyme concentration (at 40ºC)

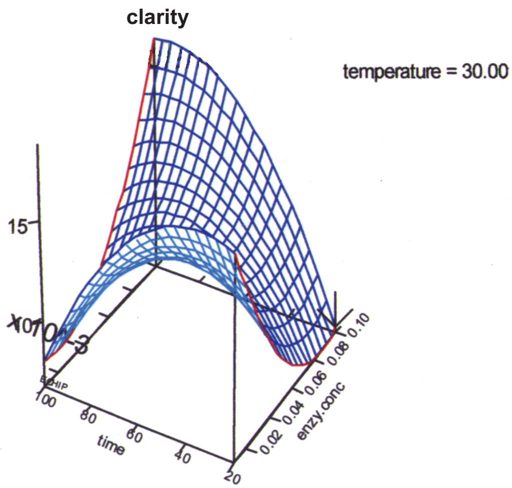

Fig. 3b: Response surface for clarity of star fruit juice (B10) as a function of time and enzyme concentration (at 30°C) (Adapted from Liew Abdullah et al., 2007)

Viscosity

The viscosity of the star fruit juice decreased as the enzyme concentration increasing as shown in *Fig. 4a* for variety B11. Enzyme concentration had a negative effect on viscosity for the linear case, showing a highly significant level at $p<0.001$. Upon enzymatic treatment, the degradation of pectin leads to a reduction of water holding capacity. Free water was released to the juice and its viscosity was reduced.

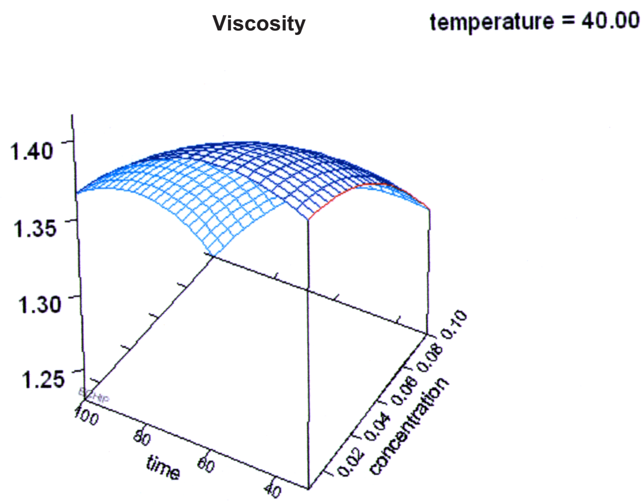

Fig. 4a: Response surface for viscosity of star fruit juice (B11) as a function of time and enzyme concentration (at 40°C)

Fruit juice with high viscosity may lead to a few problems during the filtration process (Vaillant *et al.*, 1999). To get a better filtration performance, it is recommended that fruit juices be enzymatically treated before filtration for the purpose of hydrolyzing soluble polysaccharides responsible for its high viscosity (Cheryan and Alvarez, 1995). Urlaub (1996) reported that the viscosity of fruit juice could be reduced via enzymatic hydrolysis of pectin. Thus, juice with lower viscosity is preferable in the enzymatic clarification (Sin *et al.*, 2006). *Fig. 4b* (Liew Abdullah *et al.*, 2007) shows the plot for viscosity for variety B10. It can be observed that the viscosity was significantly reduced at a higher enzyme concentration.

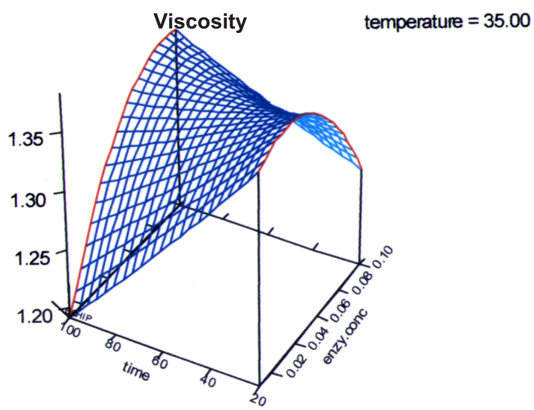

Fig. 4b: Response surface for viscosity of star fruit juice (B10) as a function of time and enzyme concentration (at 35°C) (Adapted from Liew Abdullah et al., 2007)

Optimization

During juice clarification, the cost of pre-treatment using enzyme is very important. Therefore, the best combination of process variables for response functions (turbidity, clarity, viscosity) need to be determined by taking into account the cost of enzyme used. The optimum conditions for the clarification of star fruit juice using enzymatic treatment were determined by superimposing the contour plots of all responses. The condition would be considered optimum if the turbidity, absorbance value, and viscosity were at a minimum. The criteria applied for graphical optimization were: (a) minimum turbidity, (b) minimum absorbance value, and (c) minimum viscosity. The computer generated plots for turbidity, clarity and viscosity for the variety B11 (*Figs. 2a–4a*), and with the criteria being set previously, produced an optimum region in the superimposed plot as shown in *Fig. 5a.* These criteria were selected as they are important parameters of the physical characteristics of the clarified juice. *Figs. 2a–4a* show the optimum conditions for each response, while *Fig. 5a* (variety B11) shows the optimum combined conditions that was found to be at 0.01% enzyme concentration at 30°C for 30 min. *Fig. 5b* from the work of Liew Abdullah *et al.* (2007) shows the superimposed plot for the optimum conditions for each response for variety B10. Liew Abdullah *et al.* (2007) concluded that the optimum combined conditions for B10 was 0.1% enzyme concentration at 30°C for 20 min. Table 3 shows the differences in characteristics between B10 and B11 in terms of turbidity, clarity, viscosity and optimum conditions for enzymatic treatment.

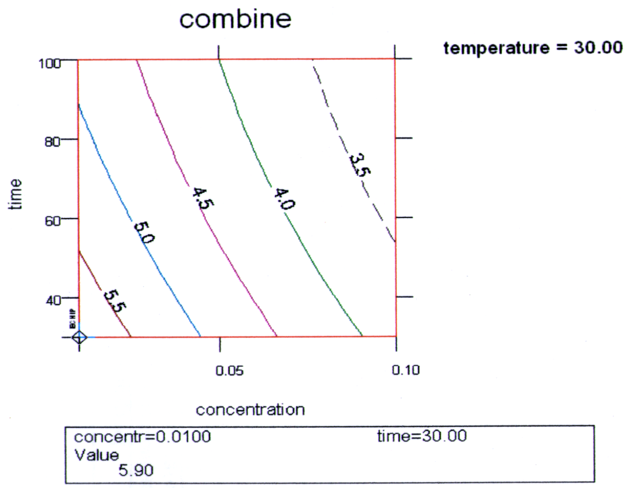

Fig. 5a: Contour plots for optimum combined condition (B11) as a function of enzyme concentration and incubation time at 30°C

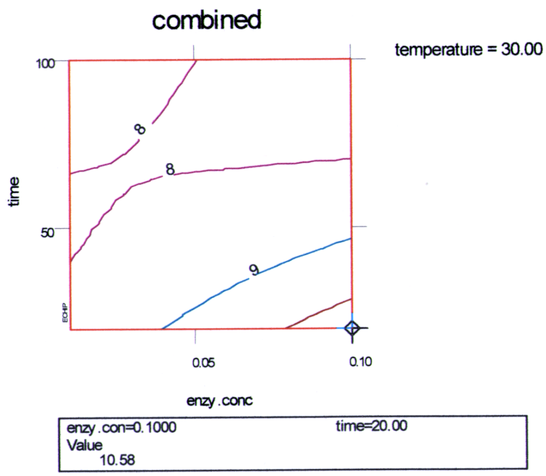

Fig. 5b: Contour plots for optimum combined condition (B10) as a function of enzyme concentration and incubation time at 30°C (Adapted from Liew Abdullah et al., 2007)

TABLE 3

Comparison on characteristics between B10 and B11 varieties

Characteristics	B10	B11
R^2 (Turbidity)	0.880	0.996
R^2 (Viscosity)	0.779	0.829
R^2 (Clarity)	0.864	0.964
Optimum incubation temperature (°C)	30	30
Optimum incubation time (min)	20	30
Optimum enzyme concentration (%)	0.10	0.01

CONCLUSIONS

Different conditions for enzymatic treatment affect the turbidity, clarity and viscosity of the star fruit juice. Therefore, statistical analysis using RSM could be used to establish the optimum process variables for enzymatic clarification of star fruit juice. By using response surface and contour plots, the optimum set of operating variables could be obtained graphically in order to achieve the desired pre-treatment levels for the star fruit juice for clarification processes. It is recommended that the enzymatic treatment clarification conditions for variety B11 of the star fruit juice was 0.01% enzyme concentration at temperature 30ºC and 30 min incubation time. These values are different from the optimum conditions for B10 variety which was 0.1% enzyme concentration at 30ºC for 20 min. It can be concluded that different varieties of star fruit need different optimum conditions of enzymatic treatment for the clarification of the juice. It may be due to the structural difference between these two varieties and also their properties and composition which influence the susceptibility of the fruit to enzyme.

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