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Optimization of enzymatic clarification of sapodilla juice using response surface methodology

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Abstract

Response surface methodology is a statistical procedure frequently used for optimization studies. It uses quantitative data from an appropriate experimental design to determine and simultaneously solve multivariate problems. In this study, a two-factor central composite design was used to establish the optimum conditions for enzymatic clarification of sapodilla juice. Sapodilla juice was treated with pectinase enzyme at different incubation times (30–120 min), temperature (30–50 °C) and enzyme concentration (0.03–0.10%). These three factors were used as independent variables whose effects on turbidity, clarity, viscosity and colour (L values) were evaluated. Significant regression models describing the changes of turbidity, clarity, viscosity and colour (L values) with respect to the independent variables were established, with the coefficient of determination, R^2, greater than 0.8. The results indicated that enzyme concentration was the most important factor affecting the characteristics of the juice as it exerted a significant influence on all the dependent variables. The recommended enzyme clarification condition was 0.1% enzyme concentration at 40 °C for 120 min.

Keywords: Sapodilla juice; Enzymatic clarification; Response surface methodology

1. Introduction

A large variety of new clarified fruit juice products have recently appeared in the local market. However there are no clarified tropical fruit juices. Therefore sapodilla with its unique flavour and aroma may be able to diversify the market, thus providing a totally new experience for the consumer. Sapodilla is a fleshy berry, generally globose, conical or oval with one or more seeds. The fruit generally weight about 75–200 g, ranging from 5 to 9 cm in diameter. The fruit has a thin rusty brown scurfy skin and a yellowish brown or red pulp with a pleasant, mild aroma and an excellent taste (Mickelbart, 1996; Thompson, 2003). It is normally eaten fresh, but sometimes it is served as candy, dehydrated slices, jelly and juices.

For clarified products, clarity and homogeneity are two important characteristics, which are achieved by the complete removal of all suspended solids. The suspended solids are mainly polysaccharides (pectin, cellulose, hemicellulose, lignin and starch), proteins, tannin, metals and microorganisms (Vaillant, Millan, Dornier, Decloux, & Reynes, 2001). Crude sapodilla juice obtained after hot water extraction is turbid, yellowish brown in colour, very viscous and tends to settle during storage, necessitating the use of enzymes to clarify the juice.

In order to degrade pectins and polysaccharides, an enzymatic treatment of the crude juice is usually carried

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out using pectinases (Grassin & Fauquembergue, 1996; Kashyap, Vohra, Chopra, & Tewari, 2001; Vaillant et al., 1999; Viquez, Laetreto, & Cooke, 1981). Pectinases hydrolyze pectin and cause pectin–protein complexes to flocculate. The resulting juice has a much lower amount of pectin and also a lower viscosity, which is advantageous for the filtration process (Alvarez, Alvarez, Riera, & Coca, 1998; Isabella, Geraldo, & Raimundo, 1995; Koffi, Sims, & Bates, 1991).

Enzymatic hydrolysis of pectic substances depends on several physicochemical factors such as incubation time, enzyme concentration and incubation temperature (Baumann, 1981; Lanzarini & Pifferi, 1989; Rai, Majumdar, DasGupta, & De, 2004). The present work involves optimization of different parameters affecting the enzymatic clarification process. The general practice for determining the optimal operating conditions is by varying one parameter and keeping the others at a constant level. The major disadvantage of this single variable optimization is that it does not include interactive effects among the variables, and therefore, it does not depict the net effects of various parameters on the reaction. In order to overcome this problem, optimization studies have been carried out using response surface methodology (RSM). The basic theoretical and fundamental aspects of RSM have been reviewed (Cochran & Cox, 1957; Giovanni, 1983; Henika, 1982; Myers & Montgomery, 2002; Thompson, 1982). RSM reduces the number of experimental trials needed to evaluate multiple parameters and their interactions; therefore, it is less laborious and time-consuming than other approaches. RSM has been widely applied for optimizing processes in the food industry (Batistuti, Barros, & Areas, 1991; Frank, 2001; Luciane, Hilary, Aparecida, & De Silva, 2001; Mudahar, Toledo, & Jen, 1990; Pietrasik & Li-Chan, 2002; Shieh, Koehler, & Akoh, 1996; Yusof, Talib, Mohamed, & Bakar, 1988).

The objective of the present study was to establish the optimum conditions (incubation time, temperature and enzyme concentration) for enzymatic clarification of sapodilla juices using RSM.

2. Materials and methods

2.1. Materials

2.1.1. Enzyme source

Pectinex 3X L from *Aspergillus niger* was obtained from Novo Nordisk Ferment Ltd., Dittingen, Switzerland and stored at 4 °C. The activity of pectinase enzyme is 5000 IU per ml. The commercial enzyme contained mainly pectin transeliminase, polygalacturonase and pectinesterase. Other side activities include small amounts of hemicellulase and cellulase (Novo Nordisk Ferment, 2001).

2.1.2. Fruits

Sapodilla fruits (*Achras sapota*) were obtained from Teluk Intan, Perak, Malaysia at commercial maturity (16–18° Brix). The fruits were first allowed to ripen for 2–3 days (19–22° Brix) at room temperature and then frozen at −18 °C before processing.

2.2. Juice extraction

Fruits were peeled, deseeded and blended using a Waring blender (National, model Kenwood chef, Malaysia) for 2–3 min until a homogenous fruit pulp was obtained. Hot water extraction was used to facilitate the maceration process and to help extract more juice from the pulp. Water was added in a ratio of 1:1 (weight/volume). Juice extraction was carried out at 60 °C for 120 min. The pH and total soluble solids of the juice obtained were 4.7 and 10.2° Brix, respectively.

2.3. Enzyme treatment

The juice was strained through a cheese cloth. For each experiment, about 200 ml juice was subjected to different enzyme treatment conditions as shown in Table 1. The incubation temperature was controlled using a water bath (Model 903, Protech Electronic, Malaysia). The pH of the juice was maintained between 4.6 and 4.8. At the end of enzyme incubation, the enzyme was inactivated by heating the suspension at 90 °C for 5 min. The treated juices were centrifuged at 3000*g* for 10 min (Avanti J-25, Beckman Coulter, USA) and the supernatant was collected. The juice was filtered through a Whatman no. 1 filter paper using Eyela vacuum aspirator (Model A-3s, Tokyo Rikakikai Co., Ltd., Japan) at 25 mm Hg. The juice obtained from each sample was then bottled and pasteurized at 90 °C for 10 min.

2.4. Physical and chemical analysis

2.4.1. Turbidity

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

2.4.2. Clarity

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

Table 1
Effects of incubation time, temperature and enzyme concentration on four dependent variables

Independent variables							Dependent variables			
	Coded variables			Uncoded variables						
Treatment	X_1	X_2	X_3	Time (min)	Temp. (°C)	Enzyme conc. (%)	Turbidity (NTU)	Clarity (abs)	Viscosity (cps)	L value
15	0	0	0	75	40	0.065	16.6	0.0260	1.39	54.55
1	−1	0	0	30	40	0.065	34.4	0.0316	1.40	52.15
9	1	−1	1	120	30	0.1	22.3	0.0295	1.25	53.77
15	0	0	0	75	40	0.065	16.2	0.0210	1.35	54.01
10	−1	−1	1	30	30	0.1	19.0	0.0213	1.39	53.17
7	1	1	1	120	50	0.1	18.8	0.0140	1.34	54.89
15	0	0	0	75	40	0.065	17.0	0.0290	1.40	54.21
3	0	−1	0	75	30	0.065	18.1	0.0300	1.42	54.01
8	−1	1	1	30	50	0.1	29.9	0.0270	1.34	54.00
15	0	0	0	75	40	0.065	15.0	0.0210	1.38	54.42
12	−1	1	−1	30	50	0.03	65.3	0.0658	1.40	49.36
5	0	0	−1	75	40	0.03	49.7	0.0511	1.45	51.73
15	0	0	0	75	40	0.065	14.6	0.0250	1.40	54.38
11	1	1	−1	120	50	0.03	43.2	0.0511	1.39	51.88
13	1	−1	−1	120	30	0.03	52.1	0.0528	1.43	51.59
2	1	0	0	120	40	0.065	21.1	0.0176	1.28	54.11
6	0	0	1	75	40	0.1	12.2	0.0168	1.25	54.25
4	0	1	0	75	50	0.065	14.8	0.0192	1.41	54.10
14	−1	−1	−1	30	30	0.03	68.9	0.0750	1.46	48.10

2.4.3. Viscosity

Viscosity was measured using a Brookfield viscometer (Model LVDV-II+, Brookfield Engineering Laboratory, Inc., Middleboro, USA) at 100 rpm with spindle SC4-18.

2.4.4. Colour measurement

The colour of the clarified juice was measured using a Hunter Laboratory Calorimeter (model SN 7877, Ultrascan, Hunter Associates Laboratory, Inc., Virginia), where $+L$' represents lightness and $-L$' represents darkness.

2.5. Experimental design

RSM was used to determine the optimum conditions for the enzymatic clarification of sapodilla juice. The experimental design and statistical analysis were performed using ECHIP Software Version 6 (Echip Inc., Hockessin, Delaware, USA).

Central composite design (CCD) was employed to study the combined effect of three independent variables—incubation time, temperature and enzyme concentration—coded as X_1, X_2 and X_3, respectively. The minimum and maximum values for incubation time were set at 30 and 120 min, incubation temperature at 30 °C and 50 °C, while 0.03% and 0.1% for the effect of enzyme concentration. According to Baumann (1981), these are the key factors that influence the mechanism of enzyme activity in the juice. The complete designs consisted of 19 combinations (including five replicates of the center point) and were carried out in random order (Table 1). Variance for each factor assessed was partitioned into linear, quadratic and interactive components were represented as shown below:

$$Y = \boldsymbol{b}_0 + \sum_{i=1}^{j} \boldsymbol{b}_i \boldsymbol{x}_j + \sum_{i=1}^{j} \boldsymbol{b}_{ij} \boldsymbol{x}_i^2 + \sum \sum_{i \neq j=1}^{j} \boldsymbol{b}_{ij} \boldsymbol{x}_i \boldsymbol{x}_j, \quad (1)$$

where, $\boldsymbol{b}_0$ is the constant, $\boldsymbol{b}_i$ the linear coefficient, $\boldsymbol{b}_{ii}$ the quadratic coefficient and $\boldsymbol{b}_{ij}$ the cross product coefficient. $\boldsymbol{x}_i$ and $\boldsymbol{x}_j$ are the levels of the independent variable. The three-dimensional plots were drawn by keeping one variable constant at the center point and varying the other two variables within the experimental range. The combinations of the three independent variables together with the responses are shown in Table 1. The responses measured were turbidity, clarity, viscosity and colour (L value).

3. Results and discussion

Coefficient of determination, R^2, is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit (Haber & Runyon, 1977). It is also the proportion of the variability in the response variables, which is accounted for by the regression analysis (Mclaren et al., 1977). When R^2 approaches unity, the better the empirical model fits the actual data. The smaller the value of R^2, the less relevance the dependent variables in the model have in explaining the behavior variation.

Table 2
Regression coefficients and R^2 value for four dependent variables for enzymatic clarified sapodilla juices

Regression coefficient	Turbidity (NTU)	Clarity (abs)	Viscosity (cps)	L value
b_0	16.449484	0.023128	1.377732	54.223608
b_1	−0.133333*	−0.000124**	−0.000667***	0.021022*
b_2	−0.084000	−0.000315	−0.000700	0.035900***
b_3	−505.714303*	−0.534857*	−1.600000*	49.771423*
b_1^2	0.005229*	0.000002	−0.000015	−0.000484**
b_2^2	−0.007113	0.000031	0.000451	−0.000556
b_3^2	11256.049479*	10.132549**	−16.242336	−914.790594*
b_{12}	−0.005472**	−0.000004	0.000044	−0.000189
b_{13}	2.468254*	0.002548***	−0.007937	−0.358730**
b_{23}	7.107141**	0.000393	0.050000	0.142856
R^2	0.996	0.964	0.829	0.978
p	0.0000	0.0000	0.0138	0.0000

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

Analysis of variance for the four response variables (Table 2) indicated that the response surface models developed for all the response variables were adequate. The R^2 values for these response variables were higher than 0.8, indicating that the regression models explained the reaction well.

3.1. Turbidity

Turbidity in fruit juices can be a positive or a negative attribute depending on the expectation of the consumers (Hutchings, 1999). In the case of orange and tomato juices, the juices are usually cloudy and have colloidal suspensions. However, this cloud is desirable and acceptable by the consumers. For clarified fruit juices, a juice that has an unstable cloud or whose turbidity is considered "muddy" is unacceptable to be marketed as clear juices (Floribeth, Celsa, & Cooke, 1981).

Time and enzyme concentration significantly ($p < 0.001$) affected the turbidity in both linear and quadratic manners. Both independent variables showed a negative effect on linear terms but showed a positive effect on its quadratic terms. The interaction effect between incubation time and temperature was negative; whereas it showed a positive interaction effect between incubation temperature and enzyme concentration ($p < 0.01$). The interaction between incubation time and enzyme concentration was also significant ($p < 0.001$), and its effect was positive on turbidity meaning that the action of enzyme was dependent on the incubation time during enzyme treatment (Fig. 1).

According to Grassin and Fauquembergue (1999), pectin was the main cause of turbidity. Application of enzyme will reduce the turbidity by degrading the pectin content in the juice. It is evident from Fig. 1 that at a fixed temperature, the turbidity decreased with increase in enzyme concentration. However, turbidity decreased

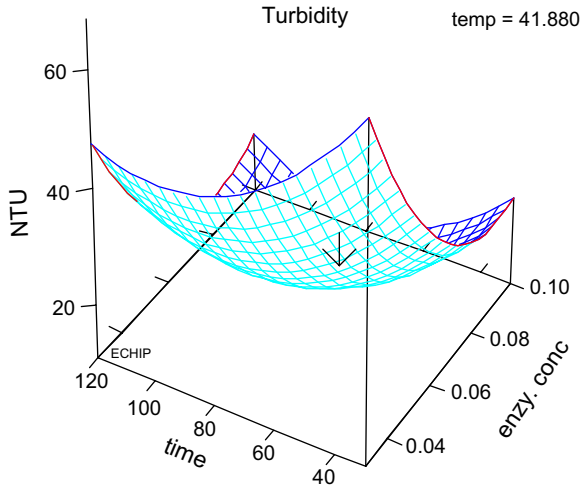

Fig. 1. Three-dimensional plots for turbidity as a function of enzyme concentration and incubation time at 41.88 °C.

with time up to 90 min and increased thereafter. This phenomenon may be due to the formation of haze particles that consisted of protein–carbohydrates or protein–tannin complex as time increased.

3.2. Clarity

Upon enzyme treatment, pectolytic enzymes break down the pectin molecules, which facilitate the formation of pectin–protein flocs, leaving a clear supernatant and significantly removing the colloidal aspect of the juices (Alvarez et al., 1998; Yusof & Nurzarina, 1994). Therefore, clarity is an important index of clarified juice.

From Table 2, it may be observed that clarity depended on the enzyme concentration as its linear effect was negative at $p < 0.001$ and its quadratic effect was positive at $p < 0.01$. It showed only a significant interaction effect between time and enzyme concentration

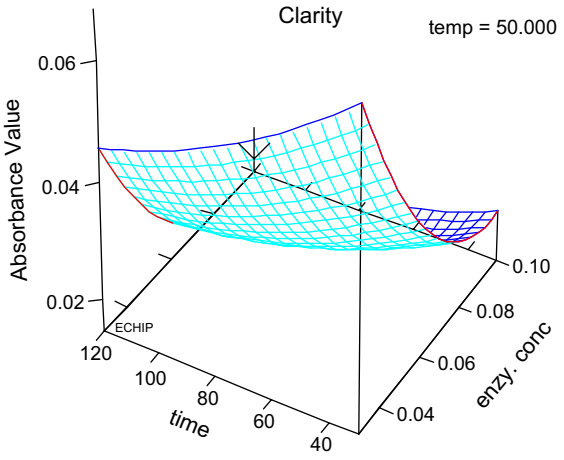

Fig. 2. Three-dimensional plots for clarity as a function of enzyme concentration and incubation time at 50 °C.

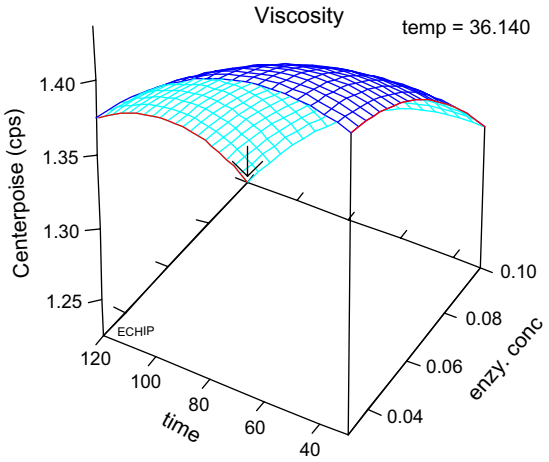

Fig. 3. Three-dimensional plots for viscosity as a function of enzyme concentration and incubation time at 36.14 °C.

at $p < 0.05$ with a positive effect, therefore the incubation time was dependent on enzyme concentration. Fig. 2 shows the clarity with enzyme concentration and incubation time at a fixed temperature. It was clear from the figure that absorbance value decreased with the increase in enzyme concentration. Clarity showed the lowest absorbance value at highest enzyme concentration. Lower absorbance values indicated a clearer juice. Increase in enzyme concentration may increase the rate of clarification by exposing part of the positively charged protein beneath, thus reducing electrostatic repulsion between cloud particles which cause these particles to aggregate to larger particles and eventually settle out. It may also be observed from the figure that the absorbance values decreased with increasing incubation time at fixed temperature. As shown in Table 2, the linear negative effect of time at $p < 0.01$ level was more dominant.

3.3. Viscosity

Enzyme concentration had a negative effect on viscosity at linear terms, showing a highly significant level of $p < 0.001$. Viscosity was significantly reduced with higher enzyme concentration as observed in Fig. 3. The pectinaceous substances possess a high water holding capacity and developed a cohesive network structure. Degradation of pectin by enzyme led to a reduction of water holding capacity and therefore, free water was released to the system to further reduce the viscosity. Incubation time also affected the viscosity at linear terms with a negative effect but to a lesser extent ($p < 0.05$). Incubation time showed a maximum viscosity at 90 min but reduced as the incubation time increased (Fig. 3).

Some authors (Alvarez et al., 1998; Kashyap et al., 2001; Vaillant et al., 2001) showed that fruit juices with high viscosity may lead to problems during the filtration process. The formation of a highly swollen fouling layer on the membrane surface will greatly reduce the performance. To enhance filtration performance, fruit juices are usually treated before filtration with enzyme preparation aimed at hydrolyzing mainly soluble polysaccharides responsible for high viscosity (Cheryan & Alvarez, 1995). The decreased in the viscosity of fruit juices due to enzymatic hydrolysis of pectin has been reported by Urlaub (1996). Therefore the juice with lower viscosity is preferable in the enzymatic clarification of juice.

3.4. Colour

The L value is a measure of lightness and so this should be as high as possible for clarified juices. Colour is an important sensory attribute (Brimelow & Groesbeck, 1993). A dark product is usually less appealing to the consumers as it may indicate deterioration. The CIE system of colour measurement transforms the reflection or transmission spectrum of the object into three-dimensional space using the spectral power distribution of the illuminant and the colour matching functions of the standard observer (MacDougall, 1993). L value generally showed similar trends as turbidity where incubation time and enzyme concentration were the most influential independent variables, as reflected in the significant linear and quadratic terms ($p < 0.01$). Incubation time and enzyme concentration have a positive linear effect and negative quadratic effect. The other factor that also contributes to L value was temperature. Temperature showed a significant positive effect ($p < 0.05$) at linear terms. In addition, the interaction effect of time and enzyme concentration was significant at $p < 0.01$ with negative effect. L value with enzyme concentration and time at fixed temperature is presented in Fig. 4. A parabolic curve was obtained for the L value

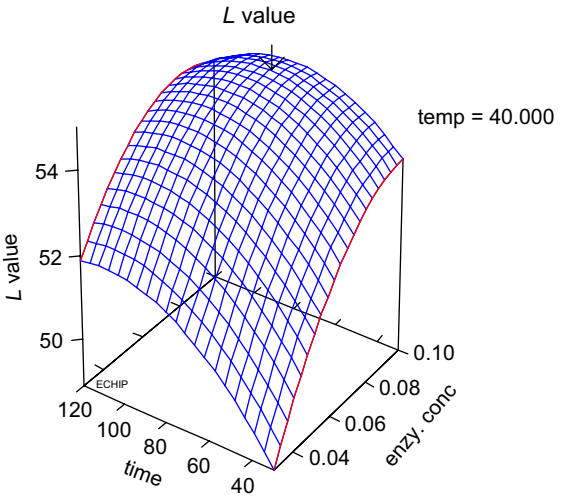

Fig. 4. Three-dimensional plots for L value as a function of enzyme concentration and incubation time at 40 °C.

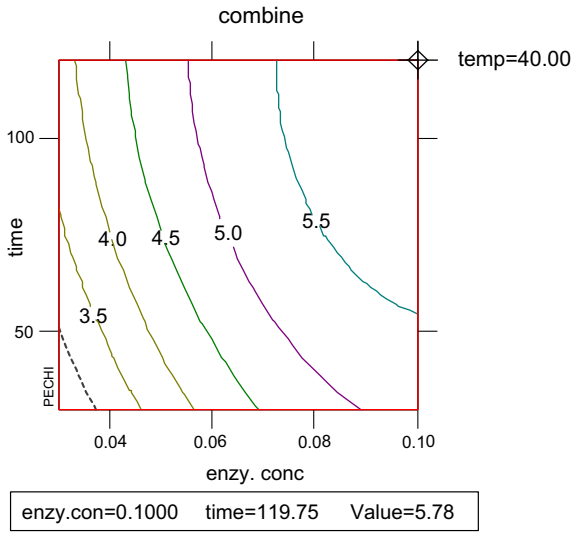

Fig. 5. Contour plots for optimum combine condition as a function of enzyme concentration and incubation time at 40 °C.

in relation to the enzyme concentration and incubation time. This may be due to increased agglomeration of floc as more pectin was degraded by higher enzyme concentration. As time increased, the fine particles in the juice may also slowly settle down. Therefore, it is clear that increasing enzyme concentration and incubation time increased the L value.

In general, enzyme concentration was the most important factor influencing the enzyme clarification. Its effect on all the dependent variables was significant ($p < 0.001$) at linear regression. On the other hand, its quadratic regression showed significance ($p < 0.01$) for L value, clarity and turbidity. Temperature was found to be the least important since the model did not show any significant effect on both linear and quadratic regression. However, there was a significant effect ($p < 0.05$) on L value at linear regression. According to Kilara (1982), temperature may aid in the rate of enzymatic clarification process as the temperature is below denaturation temperature (40–60 °C). Therefore, moderate temperature could and should be used during enzymatic clarification of sapodilla juice.

3.5. Optimization

The optimum clarification condition was then determined by superimposing the contour plots of all the responses. The final product would be considered optimum if the turbidity, absorbance value and viscosity were as low as possible while the L value was as high as possible. Therefore, the criteria applied for graphical optimization were as follows: (a) minimum turbidity (b) minimum absorbance value (c) minimum viscosity (d) maximum L value. The computer generated plots for turbidity, clarity, viscosity and L value (Figs. 1–4); and the criteria outlined above produced an optimum region in the superimposed plot (Fig. 5). Emphasis was placed on turbidity, clarity, viscosity and L value since they are important indexes of the physical characteristics of the clarified sapodilla juice. Figs. 1–4 also showed the optimum conditions for each respective response. Therefore, by overlaying all the responses, the optimum combined condition was found to be at 0.1% enzyme concentration at 40 °C for 120 min (Fig. 5).

4. Conclusion

Statistical analysis using RSM appeared to be a valuable tool for optimizing the effects of incubation time, temperature and enzyme concentration on enzymatic clarification of sapodilla juice. The response surface and graphical optimization methods lead to a better understanding for optimizing the clarification process. The recommended enzyme clarification condition was 0.1% enzyme concentration at 40 °C for 120 min.

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References

Alvarez, S., Alvarez, R., Riera, F. A., & Coca, J. (1998). Influence of depectinization on apple juice ultrafiltration. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 138*, 377–382.

Batistuti, J. P., Barros, R. M. C., & Areas, J. A. G. (1991). Optimization of extrusion cooking process for chickpea

(*Cicer arietinum*, L.) defatted sour by response surface methodology. *Journal of Food Science, 56*, 1695–1698.

Baumann, J. W. (1981). Application of enzymes in fruit juice technology. In G. G. Birch, N. Blakebrough, & K. J. Parker (Eds.), *Enzymes and food processing* (pp. 129–147). London: Applied Science Publication.

Brimelow, C. J. B., & Groesbeck, C. A. (1993). Colour measurement of foods by colour reflectance instrumentation. In E. Kress-Rogers (Ed.), *Instrumentation and sensors for the food industry* (pp. 63–96). Oxford: Butterworth Heinemann.

Cheryan, M., & Alvarez, J. R. (1995). Food and beverage industry application. In R. D. Noble & S. A. Stern (Eds.), *Membrane separation technology principles and applications* (pp. 443–465). London: Elsevier.

Cochran, W. G., & Cox, G. M. (1957). Some methods for the study of response surfaces. In *Experimental designs* (2nd ed.). New York: John Wiley and Sons Inc., pp. 335–375.

Echip software—version 6.0 windows (1993). Wheeler, B. Reference Manual. Echip Inc.: Hockessin, Delaware, USA.

Floribeth, V., Celsa, L., & Cooke, R. D. (1981). A study of the production of clarified banana juice using pectinolytic enzymes. *Food Technology, 16*, 115–125.

Frank, R. (2001). Blending response surface methodology and principal components analysis to match a target product. *Food Quality and Preference, 12*, 457–465.

Giovanni, M. (1983). Response surface methodology and product optimization. *Food Technology, 37*, 41–45.

Grassin, C., & Fauquembergue, P. (1999). Enzymes, fruit juice processing. In M. C. Flickinger & S. W. Drew (Eds.), *Encyclopedia of bioprocess technology, fermentation, biocatalysis, bioseparation* (pp. 1030–1061). New York: John Wiley and Sons, Inc.

Grassin, C., & Fauquembergue, P. (1996). Application of pectinases in beverages. In J. Visser & A. G. J. Voragen (Eds.), *Pectins and pectinases* (pp. 453–462). Elsevier Science B.V.

Haber, A., & Runyon, R. (1977). *General statistics* (3rd ed.). Reading, MA: Addison-Wesley Publishing Company.

Henika, R. G. (1982). Use of response surface methodology in sensory evaluation. *Food Technology, 36*, 96–101.

Hutchings, J. B. (1999). *Food colour and appearance* (2nd ed.). An Aspen Publication (pp. 19–21).

Isabella, M. B., Geraldo, A. M., & Raimundo, W. F. (1995). Physical–chemical changes during extraction and clarification of guava juice. *Food Chemistry, 54*(4), 383–386.

Kashyap, D. R., Vohra, P. K., Chopra, S., & Tewari, R. (2001). Applications of pectinases in the commercial sector: A review. *Bioresource Technology, 77*, 215–227.

Kilara, A. (1982). Enzymes and their uses in the processed apple industry: A review. *Process Biochemistry, 23*, 35–41.

Koffi, E. K., Sims, C. A., & Bates, R. P. (1991). Viscosity reduction and prevention of browning in the preparation of clarified banana juice. *Journal of Food Quality, 14*, 209–218.

Lanzarini, G., & Pifferi, P. G. (1989). Enzymes in the fruit juice industry. In C. Cantavelli & G. Lanzarini (Eds.), *Biotechnology applications in beverage production* (pp. 189–222). New York: Elsevier Science Publishers Ltd.

Luciane, C. M., Hilary, C. M., Aparecida, M., & De Silva, A. P. (2001). Optimization of the roasting of robusta coffee (*C. Canephora conillon*) using acceptability tests and RSM. *Food Quality and Preference, 12*, 153–162.

MacDougall, D. B. (1993). Principles of colour measurement for food. In E. Kress-Rogers (Ed.), *Instrumentation and sensors for the food industry* (pp. 39–62). Butterworth Heinemann.

Mclaren, C. G., Bartolome, V. I., Carrasco, M. C., Quintana, L. C., Ferino, M. I. B., Mojica, J. Z., et al. (1977). *Experimental design and data analysis for agricultural research, Vol. 1*. Los Banos, Laguna: International Rice Research Institute.

Mickelbart, M. V. (1996). Sapodilla: A potential crop for subtropical climates. In J. Janick (Ed.), *Progress in new crops* (pp. 439–446). Alexandria, VA: ASHS Press.

Mudahar, G. S., Toledo, R. T., & Jen, J. J. (1990). A response surface methodology approach to optimize potato dehydration process. *Journal of Food Processing and Preservation, 14*, 93–106.

Myers, R., & Montgomery, D. C. (2002). *Response surface methodology*. New York, USA: Wiley.

Novo Nordisk Ferment, Ltd., Dittingen, Switzerland (2001). Fruit and Vegetable Product Sheet.

Pietrasik, Z., & Li-Chan, E. C. Y. (2002). Response surface methodology study on the effects of salt, microbial transglutaminase and heating temperature on pork batter gel properties. *Food Research International, 35*, 387–396.

Rai, P., Majumdar, G. C., DasGupta, S., & De, S. (2004). Optimizing pectinase usage in pretreatment of mosambi juice for clarification by response surface methodology. *Journal of Food Engineering, 64*, 397–403.

Shieh, C. J., Koehler, P. E., & Akoh, C. C. (1996). Optimization of sucrose polyester synthesis using response surface methodology. *Journal of Food Science, 61*, 97–100.

Thompson, A. K. (2003). Fruit and vegetables: Harvesting, handling and storage. In A. K. Thompson (Ed.), *Chapter 12: Postharvest technology of fruits and vegetables* (2nd ed.). UK: Blackwell Publishing Limited.

Thompson, D. R. (1982). Response surface experimentation. *Journal of Food Processing and Preservation, 6*, 155–188.

Urlaub, R. (1996). Advantages of enzymatic apple mash treatment and pomace liquefaction. *Fruit Processing, 6*(10), 399–406.

Vaillant, F., Millan, A., Dornier, M., Decloux, M., & Reynes, M. (2001). Strategy for economical optimization of the clarification of pulpy fruit juices using crossflow microfiltration. *Food Engineering, 48*, 83–90.

Vaillant, F., Millan, P., O'Brien, G., Dornier, M., Decloux, M., & Reynes, M. (1999). Crossflow microfiltration of passion fruit juice after partial enzymatic liquefaction. *Journal of Food Engineering, 42*, 215–224.

Viquez, F., Laetreto, C., & Cooke, R. D. (1981). A study of the production of clarified banana juice using pectinolytic enzymes. *Journal of Food Technology, 16*, 115–125.

Yusof, S., & Nurzarina, I. (1994). Quality of soursop juice after pectinase enzyme treatment. *Food Chemistry, 51*, 83–88.

Yusof, S., Talib, Z., Mohamed, S., & Bakar, A. (1988). Use of response surface methodology in the development of guava concentrate. *Journal of Science and Food Agricultural, 43*, 173–186.