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Characteristics of soursop natural puree and determination of optimum conditions for pasteurization

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The physico-chemical and microbiological characteristics of soursop natural puree were determined. The analysed parameters were: pH, titratable acidity, soluble solids, ascorbic acid, reducing sugars, viscosity, pectinesterase activity and cloud stability. A response surface methodology was used to establish the optimum conditions for pasteurization of soursop (*Annona muricata* L.) natural puree (2:1 w/v). In this study time-temperature combinations in the range of 15–120 sec and 50–90°C were the independent variables and their effects on enzyme inactivation and vitamin C retention were evaluated. The results implied an optimum pasteurization condition to be 69 sec and 78.8°C at pH 3.7. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Soursop (*Annona muricata* L.), a major source of vitamin C, can be processed to form natural puree. But it undergoes degradation during pasteurization and storage (Saguy *et al.*, 1978). The puree is produced from the white fleshy portion of the fruit and can be frozen (Magda, 1991; Sanchez Nieva *et al.*, 1970).

Pectinesterase (PE) is one of the most heat-resistant enzymes present in soursop fruit (Garces, 1969), and other tropical fruits (Fayyaz *et al.*, 1993; Fayyaz & Asbi, 1993; Fayyaz *et al.*, 1995) which could lead to gelation and precipitation of pectin in puree and juice with subsequent loss of cloud. Garces (1969) also reported that fresh soursop contains 0.36% pectin which could influence precipitation of pectin. Control of the pectinesterase activity is of prime importance in biotechnological processes which concern the production and storage of fruit juices and purees. To overcome quality defects, fruit products must be pasteurized at temperatures greater than that needed to control microbial growth and to inactivate the heat-stable pectinesterase.

There are examples of effects of pasteurization on soursop pulp and puree (Benero *et al.*, 1971; Sanchez Nieva *et al.*, 1970; Bueso, 1980) after addition of ascorbic acid and sugar for maximum nutrient retention. But optimization of pasteurization conditions by varying both time and temperature without any additives has not yet been studied. When many factors and interactions affect desired responses, response surface methodology (RSM) is an effective tool for optimizing the process (Hunter, 1959). RSM uses an experimental paradigm such as central composite design (CCD) to fit a model by the least squares technique. If the proposed model is adequate, as revealed by the diagnostic checking provided by an analysis of variance (ANOVA) and residual plots, contour plots can be usefully employed to study the response surface and to locate the optimum.

In the present study, response surface methodology was used to determine the processing conditions for maximum vitamin retention and enzyme inactivation in soursop natural puree. Those conditions will serve as a preliminary basis for conducting further studies on storage stability of soursop puree.

MATERIALS AND METHODS

Fruits

Soursop fruits (*Annona muricata* L.) of commercial variety were obtained from Dusun Labu Valley, Nilai, Negeri Sembilan, Malaysia at the mature green stage. The fruits, which had ripened earlier, were processed first and frozen at −20°C and other fruits were allowed to ripen at room temperature within the next 1–2 days.

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Preparation of puree

The fruits were washed, peeled, cored, deseeded and pulped by hand and macerated using a local made stone grinder (stone size 10 in). Water was added in the ratio of 2:1 (w/v) to facilitate the maceration process and it was repeated twice to achieve a smooth-textured puree. The physico-chemical and microbiological analysis of fresh puree was carried out in duplicate.

Experimental design

RSM was used as in the method of Arbaisah *et al.* (1996). Two responses were measured: enzyme inactivation (Y_1), defined as units of PE activity in 1 g puree and mg ascorbic acid retention (per 100 g) (Y_2). Two independent variables, temperature (X_1) and time (X_2) were used in this study. The other important variables were fixed: the total soluble solids content (8°Brix) and the original pH (3.7) of the puree. Each of the variables to be optimized was coded at 5 levels: −1.414 (lowest level), −1, 0 (medium level), 1 and 1.414 (highest level). The coded values of the independent variables were calculated for a rotatable design having the same predictive power in all directions from the centre point with the best precision (Cochran & Cox, 1957). Correspondences between these coded values and actual values are given in Table 1. The complete design consisted of 13 experimental points which included 5 replications of the centre point (Table 1). These replicates were used to allow for estimation of the 'pure error' sum of squares. Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors.

Statistical analysis

A software package (STATGRAPHICS, 1988) was used to fit the second-order models for analysis of variance and regression coefficient calculation. The regression coefficients were then used to make statistical calculations to generate response surface and contour plots to determine optimum conditions. The analysis yielded a polynomial equation of the form:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (1)$$

where,

Y = 'response' = pectinesterase activity (units) or ascorbic acid content (mg)

X_1 = temperature (°C)

X_2 = time (sec)

$b_0, b_1, b_2, b_{11}, b_{22}, b_{12}$ = constants.

Pasteurization

Two replicate samples of puree for each of the processing treatments were heated in screw-cap bottles in a constant temperature water bath with continuous shaking and continuous temperature monitoring was done using thermocouples. The thermal process treatment for pectinesterase inactivation and retention of ascorbic acid was performed in the temperature range of 50–90°C for 15–120 s.

Enzyme extraction

Extraction of pectinesterase enzyme was done according to Arbaisah *et al.* (1996). The soursop puree was blended at medium speed with extraction solution (1.92 M NaCl; pH 8.4) using a Waring blender (model 7011 S) for 1 min. The ratio of the fruit puree to the extractant was 1:3 (w/v) and the pH was maintained by addition of NaOH. The slurry was centrifuged at 15,000*g* for 30 min using a refrigerated centrifuge (Beckman, model J2-21 M/E) and the supernatant was collected for enzyme assay. All procedures were carried out at 4°C.

Enzyme assay

The pectinesterase activity was determined by the method of Kertesz (1955), as described by Korner *et al.*

Table 1. Level combinations for the two variables, temperature and time, used in the rotatable design for RSM

Pasteurization	Level of temperature		Level of time	
	coded variables	temp (°C)	coded variables	time (sec)
1	−1	55.85	−1	30.37
2	1	84.14	−1	30.37
3	−1	55.85	1	104.63
4	1	84.14	1	104.63
5	−1.414	50	0	67.50
6	1.414	90	0	67.50
7	0	70	−1.414	15
8	0	70	1.414	120
9	0	70	0	67.50
10	0	70	0	67.50
11	0	70	0	67.50
12	0	70	0	67.50
13	0	70	0	67.50

(1980). Briefly the method consists of a titrimetric measurement of the rate of carboxyl group liberation from one per cent pectin, 0.15 M NaCl solution at pH 7.0 and 30°C. The initial reaction velocity was measured by automatic titration of the liberated carboxyl groups with 0.02 M NaOH for 10 min in a TitraLab Autotitrator model VIT 90/ ABU 93/ SAM 90 (Radiometer, Copenhagen, Denmark). One unit of pectinesterase is defined as the activity corresponding to the release of one micro-mol of carboxyl group per minute.

Microbiology

Samples were serially diluted with sterile 0.1 per cent peptone and pour-plated in duplicate. Yeast and mould counts were conducted with rose-bengal chloramphenicol agar (DRBC agar base; Oxoid, England). Total plate counts and E. coli were conducted with plate count agar (Oxoid, England) and Eosin methylene blue agar levine (Gibco Diagnostics, Madison, Wisconsin, USA), respectively. Plates were incubated for 3 days (DRBC) and 48 h at 35°C (TPC & EMB) prior to counting.

Determination of total titratable acidity, pH and total soluble solids content

pH was determined with a glass-electrode pH meter (Hanna B 417); total titratable acidity was determined by AOAC (1980) method no. 22.061 and the results were expressed as percent citric acid. Total soluble solids content was determined using a hand refractometer (Otago) with a scale of 0–32°Brix.

Determination of cloud stability, viscosity and colour

Cloud stability measurement was carried out according to the method of Krop *et al.* (1974). The puree was diluted with water (1:3 w/v) to make juice. After filtration with muslin cloth 10 ml juice was centrifuged (Clements GS 15) for 10 min at 360*g*. The extinction of the supernatant was then measured at 660 nm in a 1 cm cuvette (Shimadzu UV-1201 UV-VIS Spectrophotometer) and this value is considered a measure for the cloudiness. The viscosity measurement was made using a Brookfield viscometer (model DV-II) using RV spindle no. 7 rotated at 50 rpm.

The colour of the soursop puree was measured with a Hunter automatic colour difference meter (Minolta CR-300, Japan). A white tile with the following parameters: $L = 97.95$; $a = -0.07$ and $b = 1.66$ was used as a reference plate.

Determination of ascorbic acid and sugars

Ascorbic acid was determined using the 2,6-dichlorophenol indophenol dye titration method (Ranganna, 1986). Sugars were analysed by high performance liquid chromatography (Hunt *et al.*, 1977). The standard solutions used were glucose, fructose and sucrose.

RESULTS AND DISCUSSION

The averages of the triplicate measurements of physical, chemical and microbiological analysis of soursop pulp and puree are shown in Tables 2 and 3. The values for pH, titratable acidity and ascorbic acid corresponded to the literature (Paull, 1982; Puziah & Salmah, 1994). Sugar and total soluble solids were slightly lower than that found by Chan & Lee (1975) and Puziah & Salmah (1994). In this study, pectinesterase in soursop pulp and puree showed slightly higher activity under the same conditions as used previously (Arbaisah *et al.*, 1996). This might be caused by differences in fruit variety, season and stages of fruit maturity.

During preparation of puree, contamination by microorganisms was inevitable. For fruit juices, the microbial population usually consists of yeasts, molds, lactic and acetic acid bacteria which are generally heat-sensitive. Other organisms usually are inhibited by the acid in fruit juices. Even intermediate acidities are suitable for the growth of only a few spore formers, such as *B. thermoacidurans* (*B. coagulans*) or *C. pasteurinum* (Banwart, 1981). The contamination may as many as 10^8 bacteria per g of which spores may be as many as 10^6 (Ranganna, 1986). By washing the fruits, contamination can be removed. In our study, yeast and mold, and total plate counts were 10×10 and 8.5×10^2, respectively. This observation for total plate count is supported by the findings of other workers (Li *et al.*,

Table 2. Physical and chemical characteristics of soursop pulp and puree

	Pulp	Puree
Titratable acidity (g/100g as citric acid)	1.02 ± 0.43	0.61 ± 0.02
Ascorbic acid(mg/100g)	20.9 ± 1.84	9.83 ± 0.26
PE activity (units/g)	32.1 ± 2.40	15.2 ± 0.15
Cloud stability (at 660 nm)	0.94 ± 0.06	0.65 ± 0.00
Total soluble solids (°Brix)	11.00 ± 0.4	8.00 ± 0.00
pH	3.70 ± 0.06	3.70 ± 0.04
Viscosity (cp)	25.60 ± 0.14	19.20 ± 0.16
Colour, *L*	65.89 ± 1.01	61.01 ± 0.35
a	−2.24 ± 0.10	−3.16 ± 0.00
b	6.04 ± 0.55	3.83 ± 0.10
Sugar (g/100g)		
Fructose	3.60 ± 0.27	3.09 ± 0.22
Glucose	2.97 ± 0.24	2.90 ± 0.29
Sucrose	1.02 ± 0.28	0.99 ± 0.05

Table 3. Microbiological analysis of fresh puree

Count	CFU/g
Yeast and Mold	10 × 10
Total count	85 × 10
E. Coli	Nil

Table 4. Pectinesterase activity (Y_1) and ascorbic acid content (Y_2) of pasteurized puree

Test	code		Responses	
	X_1	X_2	PE (units/g)	Ascorbic acid (mg/100g)
1	−1	−1	9.34	7.07
2	1	−1	0.21	2.04
3	−1	1	7.16	7.07
4	1	1	0.18	3.22
5	−1.414	0	13.9	4.78
6	1.414	0	0.00	1.27
7	0	−1.414	0.86	6.06
8	0	1.414	0.37	4.17
9	0	0	0.07	4.27
10	0	0	0.09	4.16
11	0	0	0.06	4.27
12	0	0	0.07	4.06
13	0	0	0.04	4.16

1989) who showed that population of total count in orange juice (pH 4.06) was 10×10^2. But the yeast and mold count in this study was higher than that in their observation. This may be due to some rotten fruits which permited yeast to proliferate in the acid environment of the fruit flesh (Hsu, 1975).

Table 4 presents results for pectinesterase inactivation and ascorbic acid retention of pasteurized puree. The relationship between these temperatures and times to the pectinesterase inactivation and ascorbic acid retention are represented by three dimensional surface plots (Fig. 1 a–b) and two dimensional contour plots (Fig. 2 a–b) generated by the model equation (Eq. 1) developed from the statistical analysis. From the plot the optimum condition was calculated by canonical analysis to find the stationary point and this gave a temperature of 78.8°C and time of approximately 69 seconds.

The models (Y_1) and (Y_2) are useful in indicating the direction in which to change the variables in order to maximize enzyme inactivation and retention of ascorbic acid in the puree.

The values of regression coefficient confirm that the most important factor affecting the pectinesterase inactivation was the temperature. Both its first order effect and second order effect were highly significant. A resultant polynomial for this variable is:

$$Y_1 = 0.0654 - 4.4678X_1 - 0.3683X_2 + 3.5424X_1^2 + 0.3842X_2^2$$

In the above model for Y_1, temperature had the largest coefficient ($b_1 = -4.4678$). The negative sign indicated that enzyme activity in the puree significantly decreased as temperature and time increased.

In comparison to this study, Garces (1969) had noted that in soursop (pulp diluted 1:1) pectinesterase can be inactivated at 85°C for 3 min. The optimum time of 69 s to inactivate pectinesterase used in this study was slightly lower than that found in aseptic processing of papaya puree (Chan & Cavaletto, 1982). In that study, the papaya puree of pH 3.9 was thermally processed at 93°C for 60 s to inactivate the pectinesterase enzyme. Further observations by Rothschild *et al.* (1975) that pasteurization temperatures necessary for commercially

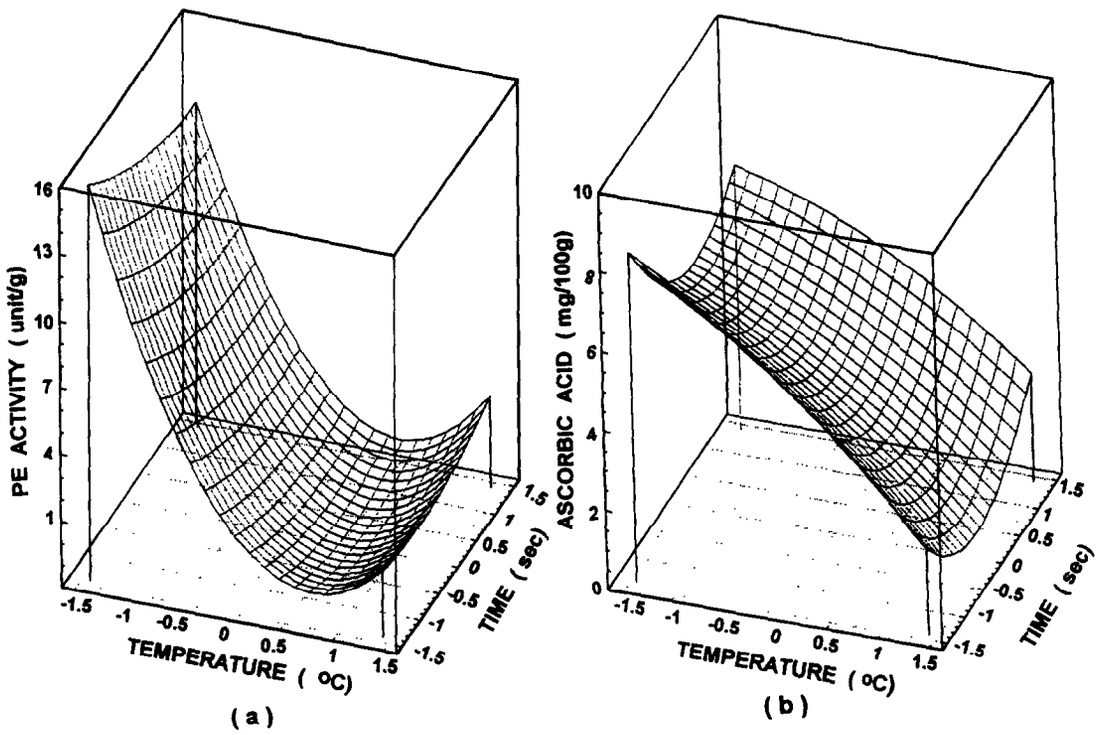

Fig. 1. Response surface plots for: a. Pectinesterase inactivation; b. Ascorbic acid degradation.

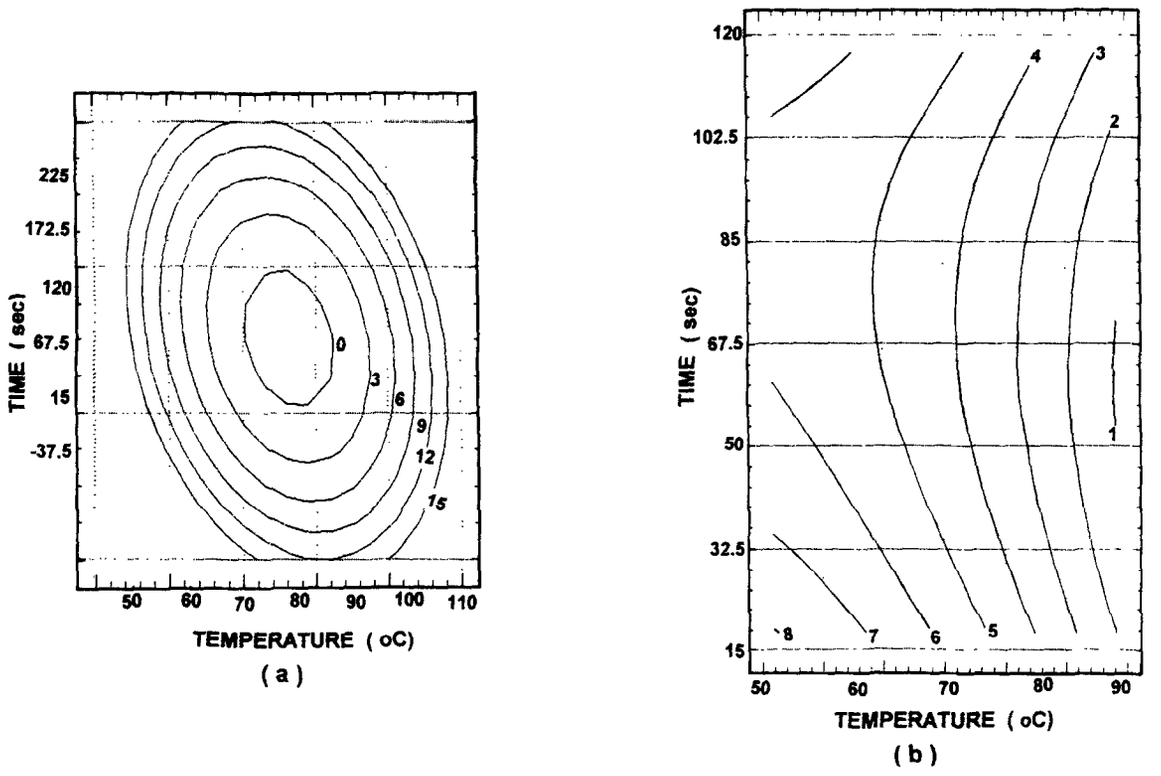

Fig. 2. Contour plots for: a. Pectinesterase inactivation; b. Ascorbic acid retention.

satisfactory juices and comminuted products decreased as the pH decreased are confirmed by our results. However in order to benefit from this effect, longer holding times are required for puree of low pH.

It is natural to expect some destruction of ascorbic acid when heating to higher temperatures. But in contrast to these studies Sanchez Nieva *et al.* (1970) found that heating has no appreciable effect on the ascorbic acid content of fresh pulp. The variance analysis of the ascorbic acid retention showed significant linear effects of temperature and quadratic effects of time and the polynomial was defined as:

$$Y_2 = 4.1820 - 1.7304\,X_1 - 0.1909\,X_2 - 0.3848\,X_1^2 + 0.6652\,X_2^2$$

Temperature (X_1) and time (X_2) had negative slopes ($b_1 = -1.7304$; $b_2 = -0.1901$) in the model for Y_2. These indicated that, when temperature and time increased, ascorbic acid decreased. The response surfaces of these variables are presented in Figs 1b and 2b. The estimated optimum was located outside the region covered by the experiment. In this study the ascorbic acid content at optimum PE inactivation was found to be 3.0 mg per 100 g pasteurized puree (Fig. 2b).

From a sensory panel evaluation it was found that the taste, smell and overall acceptability of pasteurized puree were not significantly different from those of the control. However, the colour and appearance of the pasteurized puree were improved.

CONCLUSION

This study shows that, at a constant pH of 3.7, a response surface methodology can be used to optimize the pasteurization conditions for soursop natural puree. These conditions are a temperature of 78.8°C and time 69 s.

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