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Determination of optimum conditions for pectinesterase extraction from soursop fruit (*Anona muricata*) using response surface methodology

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Optimum conditions for the extraction of pectinesterase from soursop (*Anona muricata*) have been established. A fractional factorial design and response surface methodology was applied in this study, as a means of improving the method for developing an enzyme extraction procedure. Among the variables tested, NaCl and pH showed greater significant effects, while PVP, EDTA and incubation time seemed to have a lowering effect on the efficiency of pectinesterase extraction from soursop. The maximum enzyme extraction was obtained by using 1.92 M NaCl solution at pH 8.4

INTRODUCTION

Pectinesterase (E.C.3.1.1.11) occurs commonly in various parts of higher plants including the fruit. It belongs to the subdivision of enzymes which hydrolyse carboxylic acid esters. Pectinesterase catalyses the release of methyl ester group from C6 of methylated galacturonate polymers (pectin) (Versteeg, 1979).

The significance of pectinesterase in fruit processing industry has been well studied especially in citrus fruit (Pilnik & Voragen, 1991). In fresh or under pasteurised citrus juices pectinesterase de-esterifies the pectin, producing methanol and low methoxyl pectin. Subsequently precipitation of low methoxyl pectin by calcium ions results in cloud loss, a serious quality defect because of the appearance, reduction of taste and aroma components and the increased sensitivity to oxidation (Joslyn & Pilnik, 1961).

Previous research on pectinesterases carried out in our laboratory has shown that different sources yielded different forms and characteristics of pectinesterases (Fayyaz & Asbi, 1993). Methods of extraction also vary with the variety since different results may be obtained with different solvents and conditions of extraction medium. The purpose of this research was to establish the optimum conditions for extracting pectinesterase enzyme from soursop (*Anona muricata*) fruit pulp using a fractional factorial design and response surface methodology as a preliminary basis for conducting further studies in the purification of soursop pectinesterase.

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MATERIALS AND METHODS

Citrus pectin and polyvinylpyrrolidone (PVP) were obtained from Sigma Chemical Co., St Louis, Illinois, USA, and sodium chloride and ethylenediaminetetra-acetic acid (EDTA) were purchased from BDH Chemicals, Poole, UK. Soursop fruits were provided by Labu Valley, Nilai, Negeri Sembilan, Malaysia. Ripe soursops were peeled, seeds removed and the pulp obtained were homogenized with an extraction medium using a Waring blender (model CB6) before being frozen at −60°C. The samples were kept at −20°C until further use.

Extraction procedure

The frozen soursop pulp was blended at medium speed with extraction solution using a Waring blender (model 7011S) for 1 min. The ratio of the pulp to the extractant was 1:3 and the pH was maintained by addition of NaOH. The slurry was centrifuged at 15000 *g* for 30 min using a Beckman refrigerated centrifuge (model J-21M/E) and the supernatant was collected for enzyme assay. All procedures were carried out at 4°C.

Table 1. Responses of five variables in a fractional factorial design in determining the significant effects of extraction conditions of pectinesterase from soursop

Test	NaCl	EDTA	pH	Time	PVP	Response
1	0.5	0.00	4.0	8.0	2.0	2.4
2	2.0	0.00	4.0	1.0	0.0	3.2
3	0.5	0.01	4.0	1.0	2.0	3.3
4	2.0	0.01	4.0	8.0	0.0	3.9
5	0.5	0.00	9.0	8.0	0.0	5.3
6	2.0	0.00	9.0	1.0	2.0	6.3
7	0.5	0.01	9.0	1.0	0.0	4.6
8	2.0	0.01	9.0	8.0	2.0	4.9

The effects determined were:
NaCl = 0.6700
EDTA = −0.1100
pH = 2.1150
Time = −0.2150
PVP = −0.0100.

Experimental design

A fractional factorial design was adopted which permits modelling of interactions among factors using an efficient number of combinations rather than the full set (Cox & Cochran, 1957). In accordance with this experimental design, eight extraction procedures were conducted. The eight data points obtained were then analysed statistically to determine which two variables had significant effects. In the next following step, using a central composite design consisting of two variables (NaCl and pH), extraction procedures for response surface methodology were evaluated. It consisted of five levels with 13 experimental points and five replications of the centre points. The five levels for each of the two variables were coded as −1.414, −1, 0, +1, +1.414 and an analysis of variance was computed (Cox & Cochran, 1957).

The optimum conditions of extraction were plotted as a peak on the response surface which was generated by a statistical computer program (STSC, 1986). The analysis yielded a second degree polynomial equation of the form:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_{12} + b_{22}x_{22} + b_{12}x_1x_2 \quad (1)$$

where y = response = pectinesterase activity (units); x_1 = NaCl concentration in M; x_2 = pH; b_0, b_1, b_2, b_{11}, b_{22}, b_{12} = constants.

Enzyme assay

The pectinesterase activity was determined by the method of Kertesz (1955) as described by Korner *et al.* (1980). Briefly the method consists of a titrimetric measurement of the rate of carboxyl group liberation from a 1% pectin, 0.15 M NaCl solution at pH 7.0 and 30°C. The released carboxyl groups were titrated with 0.02 N NaOH for 10 min using a Titralab Autotitrator model VIT 90/ABU 93/SAM 90 (Radiometer, Copenhagen, Denmark). One unit of pectinesterase was defined as 1 μmol of carboxyl group produced per min.

RESULTS AND DISCUSSION

The mean responses for all treatments are shown in Table 1. Among the variables tested to determine their effects on the extraction of pectinesterase from soursop pulp, pH and NaCl concentration showed somewhat significant positive effects. pH had a high significant effect (2.12) while the significant effect for NaCl was much lower (0.67). These results showed that pH had greater significant effect than NaCl concentration of the extraction medium on the release of the enzyme from the cell walls of soursop fruit.

Previous studies had noted that pectinesterase which is situated in the free space between the cell walls and the main part is bound by ionic interaction. It can be liberated from the cell wall fraction from orange by raising the pH above 7.0 and increasing the ionic strength (McDonell *et al.*, 1945; Jansen *et al.*, 1960). According to Awad (1985), pectinesterase extraction was more efficient when NaCl was added during extraction of pectinesterase from persimmon fruit.

PVP is often used in the extraction of enzymes from plant materials in order to prevent inhibition by polyphenols. However, in this study (Table 1) adding PVP had negative effects on the ability of pectinesterase to be extracted from soursop pulp. Awad & Young (1980) also found that PVP supressed rather than stimulated pectin methylesterase in extraction of this enzyme from avacado fruit. Hence it would appear that soursop fruit did not contain phenolic compounds which interfere with pectinesterase activity. Adding EDTA to the extraction fluid also seemed to have a lowering effect on the efficiency of extraction of pectinesterase from soursop (Table 1). Awad (1985) also reported that pectin methylesterase from persimmon fruit displayed less sensitivity towards EDTA. He noted that addition of EDTA to the extraction buffer used was unnecessary. From Table 1, it can be shown that the length of time to solubize pectinesterase did not seem to be critical, i.e. the incubation time had very little effect (−0.22) on the ability of pectinesterase to be extracted from soursop. From this part of the study, variables that showed low

Table 2. Coefficients and analysis of variance of the second degree polynomial equation

Test	Code	Code	Responses
1	−1	−1	2.3
2	+1	+1	1.4
3	−1	−1	5.9
4	+1	+1	3.5
5	−1.414	0	2.0
6	+1.414	0	2.8
7	0	−1.414	2.4
8	0	+1.414	5.3
9	0	0	4.5
10	0	0	4.5
11	0	0	4.6
12	0	0	4.7
13	0	0	4.8

where:

Code	NaCl (M)	pH
−1.414	0.50	4.0
−1	1.02	5.5
0	2.25	6.7
+1	3.48	8.0
+1.414	4.00	9.0

giving
$b_0 = 4.63$
$b_1 = -0.27$
$b_2 = 1.23$
$b_{11} = -1.08$
$b_{22} = -0.35$
$b_{12} = -0.38$

	d.f.	ss	ms
First-order terms	2	12.77	16.39
Second-order terms	3	9.05	3.02
Lack of fit	3	2.94	0.98
Experimental error	4	0.09	0.02
Total	120	24.860	4.8

effects were therefore ignored and these were PVP, EDTA and incubation time.

Equation (1) was fitted to the experimental data (Table 2). From the analysis of the coefficients, pH and NaCl concentration of extraction medium appeared to influence the response. The relationships between these two variables of the pectinesterase extraction are represented by the three dimensional surface plot (Fig. 1). The response increased and decreased within the experimental extraction procedure. This indicated the existence of an optimum level within the range of pH and NaCl concentration tested. From the plot (Fig. 1), the optimum condition was calculated by canonical anaylsis to find the stationary point and this gave pH of approximately 8.4 and the concentration of NaCl as 1.92.

The optimum concentration of NaCl used in this study was similar to that found by us in extracting pectinesterase from papaya fruits (Fayyaz *et al.*, 1993). In that study we found that there was only a slight increase in the extractability of pectinesterase from

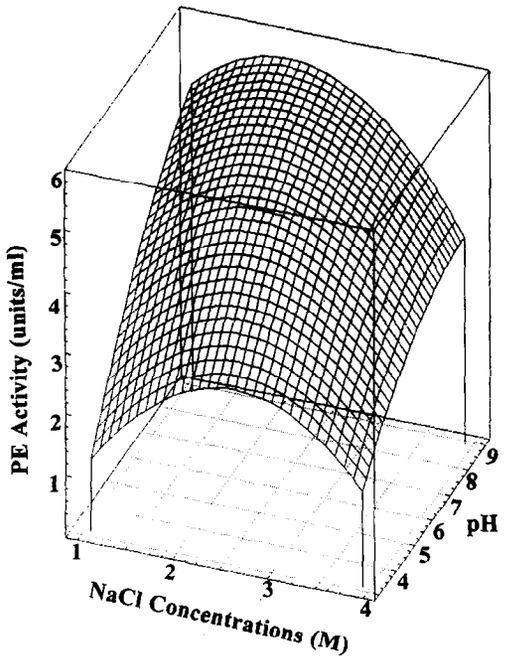

Fig. 1. A response surface plot for the effect of NaCl concentration and pH on pectinesterase extraction from soursop fruit.

papaya fruit using 2 M NaCl solution at pH 8–10. Marfo & Oke (1989) observed that beyond pH 10 a gel-like material began to form in the suspension and the solution became very slimy and difficult to be centrifuged. Whitaker also mentioned that for plant enzymes, the extraction fluid usually contained sufficient buffer and salt to maintain the pH near 7.5 and at ionic strengths of 0.1–0.5. The results in this study are in agreement with previous studies (McDonnell *et al.*, 1945; Jansen *et al.*, 1960) which indicated that pectinesterase can be liberated from the cell wall fraction of orange by raising the pH above 7.0 and increasing the ionic strength.

CONCLUSION

This study shows that a fractional factorial design and response surface methodology can be used in determining the optimum conditions for extraction of pectinesterase from soursop fruit. These conditions are pH of 8.4 and 1.92 M NaCl.

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