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RESEARCH NOTE

Pectinesterase extraction from papaya

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Pectinesterase (EC.3.1.1.11) was extracted from papaya fruit (*Carica papaya* L.) var. *Exotica*. Incubation time, pH and NaCl concentration influenced the extraction process of pectinesterase from papaya fruit. A maximum activity of 6·98 units/min ml was obtained with 2 M NaCl solution, pH 8, and at 5 h incubation time.

INTRODUCTION

The occurrence of the enzyme pectinesterase (EC.3.1.1.11) in many higher plants has been summarised by Voragen and Pilnik (1989). Native pectinesterase (PE) can be used for protecting and improving the texture and firmness of several processed fruits and vegetables as well as in the extraction and clarification of fruit juices (Voragen & Pilnik, 1989).

The properties of PE from plant sources have been reported by many investigators. Therefore it is of interest to purify PE from the papaya fruit to study its physicochemical characteristics. The first step in enzyme purification is to isolate the enzyme, and for this reason an effective method of extracting PE from papaya is necessary before its purification and characterisation. In this note a study is described to see the effect of NaCl concentration (extraction liquid), pH and incubation time on the extractability of PE and to compare the results with some other procedures from the literature. Further studies on purification and characterisation will be published elsewhere.

MATERIALS AND METHODS

Papaya fruits (*Carica papaya* L.) var. *Exotica*, were supplied by the Malaysian Agricultural Research and Development Institute (MARDI), Serdang. The fruits were thoroughly washed with water, peeled, seeds removed and then homogenised in a Waring blender. The homogenised pulp was instantly frozen at −80°C and stored at −20°C till further use.

PE extraction at varying NaCl concentration

After thawing at 4°C, PE was extracted from the pulp with pure water and NaCl solution of various concentrations (0·5–2·5 M) in a Waring blender for 3 min. The ratio of pulp to extracting solution was 1:2. The samples were incubated in a cold room for 4 h and the pH was maintained at 7·0 during the incubation time by adding either 2 M NaOH or 2 M HCl. The homogenate was centrifuged at 15 000*g* for 30 min and the supernatant was dialysed for 24 h against 1% NaCl solution and centrifuged at 15 000*g* for 30 min. The supernatant was used as a crude extract of the enzyme for the PE activity.

Enzyme extraction at different pH levels

After thawing at 4°C, 250 g pulp was homogenised with 500 ml of 2 M NaCl solution for 3 min. The homogenate was divided into six equal portions and the pH was adjusted from 5 to 10 by adding 2 M NaOH or 2 M HCl. Samples were then incubated in a cold room for 4 h and the pH was maintained at the desired levels by 2 M NaOH or 2 M HCl. The homogenate was centrifuged at 15 000 *g* for 30 min. The supernatant was assayed for PE activity. All the procedures were carried out at 4°C.

Enzyme extraction at different incubation times

After thawing at 4°C, 100 g pulp was homogenised with 200 ml 2M NaCl, pH 8·0, for 3 min and was incubated in a cold room for 8 h. During the incubation time, the pH of the homogenate was maintained at 8·0 by adding either 2 M NaOH or 2 M HCl. Samples were taken out after 1 h intervals and were filtered through No. 54 Whatman filter paper in a Buchner funnel with a slight vacuum. The filtrate was used for PE activity. All the procedures were carried out at 4°C.

Enzyme assay

PE activity was determined by the method described by Korner *et al.* (1980). Briefly, the method consisted 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 initial reaction velocity was measured by automatic titration of the liberated carboxyl groups with 0·02 M sodium hydroxide, in a TitraLab Autotitrator model VIT 90/ABU 93/SAM 90 (Radiometer, Copenhagen, Denmark). One PE unit is defined as the activity corresponding to the release of 1 μmol of carboxyl group per minute.

Enzyme extraction according to literature

The extraction procedure that was developed in this study was compared with those adopted by different authors (Lourenco & Catutani, 1984; Augustin *et al.*, 1985; Ghazali & Leong, 1987) for extraction of different enzymes from different fruits. In all of these experiments the same amount of pulp was used.

RESULTS AND DISCUSSION

Different authors have used different levels of NaCl concentration, different pH levels and different incubation times for the extraction of PE from various fruits and vegetables (Chang *et al.*, 1965; Al-Delaimy & Ali, 1969; Versteeg *et al*, 1978; Korner *et al*, 1980; Lourenco & Catutani, 1984; Fayyaz & Asbi, 1989; Komae *et al.*, 1990). Our findings (Fig. 1) also verified that different NaCl concentrations, pH levels and incubation times affected the extractability of PE from papaya fruits. Raising the concentration of NaCl initially increased the PE activity but at higher concentrations the enzyme activity decreased (Fig. 1A) Maximum enzyme extraction was obtained at 2 M NaCl concentration.

The result of this experiment concurred with other observations reported in the literature (Pozsar-Hajnal & Polacsek-Racz, 1975) according to which pectolytic enzyme may be more successfully extracted with NaCl solution than with water. Our results are also in agreement with the findings of Marfo and Oke (1989) who showed that increasing concentration of NaCl initially increased protein extractability from flamboyant seeds, but at higher salt concentration extractability decreased. Our results showed that NaCl concentration had a strong influence on the release of the enzyme which was probably bound strongly to the cell wall components of the papaya fruit. It is possible that a certain concentration of NaCl was necessary to extract the enzyme by way of osmosis from the cells (Polacsek-Racz & Pozsar-Hajnal, 1976) and/or it could be that increasing NaCl concentration increased protein solubility, a phenomenon called 'salting-in' (Price & Stevens, 1982). However, higher concentrations of NaCl decreased protein solubility by 'salting-out' (Price & Stevens, 1982) and this caused inactivation of the enzyme.

In the next experiment 2 M NaCl solution was used as an extraction medium but the pH levels were varied. Figure 1B shows the effect of pH on the PE extraction from papaya fruit. It was found that as the pH increased the extractability of enzyme also increased. The optimum pH for extraction was found to be pH 8·0. There was only a slight increase from pH 8·0 to 10·0. It was found that, beyond pH 10, gel-like material began to form in the suspension. These results are in agreement with the findings of Nilo Rivas *et al.* (1981) and Marfo and Oke (1989), who found that in alkaline conditions there was an increase in extractability of protein from sesame and flamboyant seeds. Marfo and Oke (1989) found that solubility of flamboyant seed proteins increased sharply from pH 5·0 to 8·0 and gradually from pH 8·0 to pH 11 but at pH 10 the solution became very slimy and difficult to centrifuge. However, it has been reported that PE became inactivated when allowed to stand in solutions having pH below 3 or above 8 (Kertesz, 1955). According to our results, there was only a slight difference in the extractability of PE at pH 8 and 10. For these reasons pH 8·0 was selected for the next step to see the effect of incubation time on PE extraction.

Figure 1C shows the effect of incubation time on the PE extraction from papaya fruits. Maximum enzyme extraction was obtained at 5 h incubation time. Beyond 5 h, extractability decreased as shown by the decrease in enzyme activity. The reason for this could be the

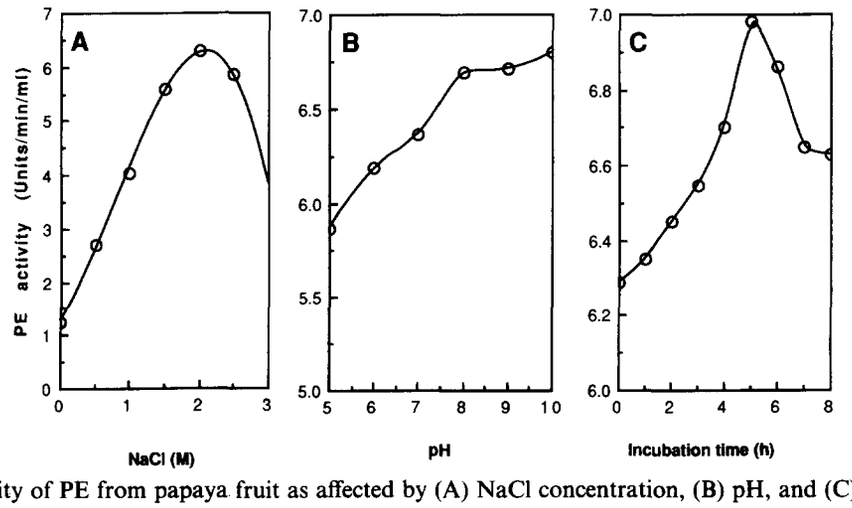

Fig. 1. Extractability of PE from papaya fruit as affected by (A) NaCl concentration, (B) pH, and (C) incubation time.

Table 1. Comparison of different procedures for extraction of pectinesterase from papaya

Extraction procedure	PE activity (Units/min/100 g fruit)
Lourenco and Catutani, 1984	876
Augustin *et al.*, 1985	1 129
Ghazali and Leong, 1987	1 475
This study	1 902

effect of prolonged exposure of the enzyme at very high NaCl concentration. From the extraction procedure developed in this study, maximum enzyme activity of 6·98 units/min ml was obtained by extraction with 2 M NaCl, at pH 8·0 and at 5 h incubation time.

A comparative study was carried out for extraction of papaya PE by using procedures developed for papaya (Lourenco & Catutani, 1984), guava (Augustin *et al*, 1985), and star fruit (Ghazali & Leong, 1987). The results obtained are shown in Table 1, from which it can be seen that the extraction procedure developed in this study gave the maximum extraction value for papaya PE.

This work shows that for an enzyme extraction study it is advisable to develop and optimise an extraction procedure for a given fruit rather than to adopt any procedure published in the literature.

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