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Pectinesterase extraction from Mexican lime (*Citrus aurantifolia* Swingle) and prickly pear (*Opuntia ficus indica* L.) peels

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Abstract

Extracts from Mexican lime (*Citrus aurantifolia* Swingle) and prickly pear (*Opuntia ficus indica* L.) peels were tested for their pectinesterase activity. A factorial design was applied in this study as a method for enzyme extraction in which the variables were the source of enzyme (prickly pear and Mexican lime peels) and the NaCl solution concentration (0–3.0 M). In all cases, enzyme extracts obtained using the same NaCl concentration from lime peel showed higher activity than extracts from prickly pear peel. NaCl concentration influenced the pectinesterase extraction process in both cases. Maximum enzyme activities were obtained with NaCl 0.5 M and 1.0 M for Mexican lime and prickly pear peels, respectively. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Pectinesterase (PE; pectinmethylesterase, pectin pectilhydrolase, EC 3.1.1.11) catalyses the hydrolysis of methoxyl groups of methylated galacturonic residues in pectin molecules (Sáez et al., 1983). This enzyme is present in many higher plants and can be found in different plant tissues, particularly those contained in fruits (Baron & Thibault, 1985).

Control of PE activity *in situ* is very important in the food industry because of its influence on the final product quality. For example, endogenous PE is activated *in situ* to control texture and firmness in processed fruits and vegetables (Pilnik & Voragen, 1991), and to produce low methoxyl pectins in citrus peels (Taylor, 1982). Contrary to this, native PE is inactivated to obtain cloudy citrus juices (Nath & Ranganna, 1977), high viscosity tomato juice and puree (Nath et al., 1983), and in those byproducts (citrus peels and apple pomace) to be utilised for the manufacture of high methoxyl pectins (May, 1990). Moreover, there are reports that exogenous plant PE is used *in vitro* to produce low methoxyl pectins (Komae & Misaki, 1989) and the vacuum-assisted infusion of fresh tissues (Baker & Wicker, 1996). Furthermore, endogenous and exogenous PE are important for analytical purposes (Lin et al., 1990; Horie & Rechnitz, 1995).

Extraction and quantification of endogenous PE is needed if one wishes to measure the effect of temperature on activation or inactivation processes so as to design proper blanching conditions, either to inactivate or to activate the enzyme. An enzyme extraction study is advisable to develop and optimise an extraction procedure for a given plant material rather than to adopt any procedure published in the literature (Fayyaz et al., 1993).

High PE activity can be detected in lime (Rouse & Atkins, 1954) and prickly pear (Joubert, 1993) peels. As limes and prickly pears are produced in Mexico, it was considered interesting to study the PE extraction process from these fruit peels which can be obtained as byproducts in local food factories. The results described here will be used for further studies on PE activation and inactivation processes.

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2. Materials and methods

2.1. Raw materials

Mature limes (*Citrus aurantifolia* Swingle) and prickly pears (*Opuntia fiucs indica* L.) were obtained in the local market (Saltillo, Coahuila). Limes were from Tecomán, Colima, and prickly pears from San Luis Potosí, México.

Both types of fruit were washed carefully with water. Limes were cut into halves and the juice was extracted. Seeds, juice sacs and rags were separated by hand and the remaining material composed of the flavedo and albedo tissues (peels) was used. Prickly pears were peeled by hand. Lime and prickly pear peels were frozen in liquid nitrogen and kept at −20°C until time of testing.

2.2. Extraction of PE from peels

Frozen lime and prickly pear peels were suspended in either water or NaCl solution (0.5–3.0 M). The ratio of peels to the extractant was 1:4 (w/v). Peel suspensions were homogenised quickly for 1 min in a blender (Osterizer). The homogenates were filtered with a filter paper and centrifuged at 500 ***g*** for 5 min. Ten millilitres of supernatant were adjusted to pH 7.0 with 1 M NaOH and immediately used to measure PE activity. All extraction steps were carried out at 4°C.

2.3. Determination of PE activity

PE activity was determined using the method proposed by Kertesz (1955). Concisely, the method involves the measurement of the releasing rate of carboxyl groups in a pectin solution (1% w/v), at 30°C, pH: 7.0. The substrate was prepared and stored according to the procedure described by Rouse and Atkins (1955). The reaction was started by the addition of 2 ml of extract to 20 ml of substrate and the pH of the reaction was maintained manually by the addition of a 0.01 M NaOH solution for 20 min. In all cases, initial NaCl concentration in the reaction mixture was adjusted to 0.3 M. The equivalent amount of NaOH solution used is proportional to the PE activity. One unit of PE activity was defined as the amount of enzyme able to release 1 μmol of carboxyl groups per minute under the above mentioned reaction conditions.

Table 1
The ANOVA of pectinesterase activity as function of peel type and extracting solution

SV	DF	SS	F
Origin (O)	1	8829.593	5401.163*
Concentration (C)	4	1604.271	981.351*
O × C	4	1222.434	747.777*
Error	20	1.635	
Total	29		

Effects are significant at $*\alpha = 0.05$.

2.4. Experimental design and statistical analysis

A factorial design with two independent variables (origin of the enzyme and type of extracting solution) was used to determine the influence of the variables on the extraction process. The response variable was PE activity (U/ml of extract). Treatments were carried out in triplicate. Results obtained with different treatments according to the experimental design used were analysed using computer software (SYSTAT programme).

3. Results and discussion

PE from lime and prickly pear peels was extracted with water and NaCl solutions of different concentrations (0.5–3.0 M). Highly significant differences were found ($P < 0.001$) in PE activity in the extracts, depending on the origin of peel used (Table 1). NaCl concentration in the extracting solution also showed a significant effect on the PE activity measured.

The effect of NaCl concentration of the extracting solution on PE activity from lime and prickly pear peels is shown in Fig. 1. PE activity in water extracts from both lime and prickly pear peels was low, showing similar values in both cases. The enzyme activity was increased substantially in lime peels when NaCl solutions were used as extractant. A major increase was observed in the

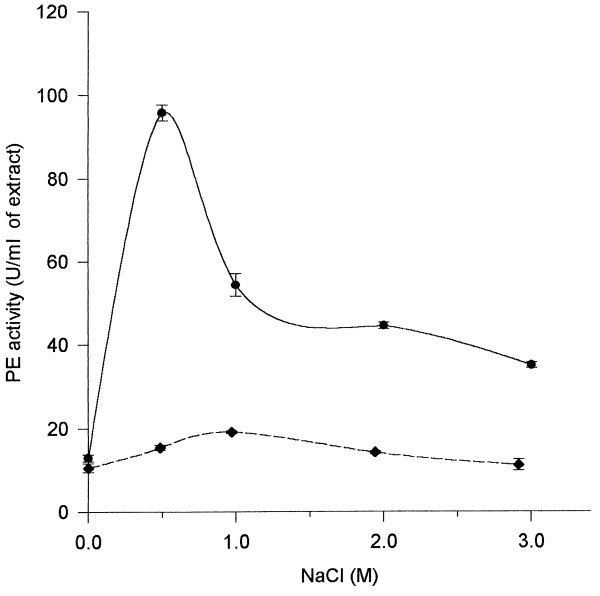

Fig. 1. Pectinesterase activity of extracts of prickly pear (–◆–) and Mexican lime (–●–) peels as a function of NaCl concentration in the extracting solution.

Table 2
Extraction conditions of some PE of plant origin

Origin	Factor	Level	Optimum	Experimental design	Reference
Mexican lime	NaCl	0–3.0 M	0.5 M	Factorial	This article.
Orange	NaCl	0–2.0 M	0.25 M	Monofactorial	MacDonell et al. (1945)
	pH	4.5–10.5	8		
Orange	NaCl	0.3–1.0 M	1 M	Factorial	Won and Walker (1995)
(thermostable PE)	pH	4.14–8.0	4.14		
	Time	—	5–10		
	Tempe	—	70°C		
	Enzyme*	—	0.2%		
	EGTA	—	5 mM		
Papaya	NaCl	0–2.5 M	2 M	Monofactorial	Fayyaz et al. (1993)
	pH	5–10	8		
	Time	0–8 h	5		
Persimmon	NaCl	0–4.8 M	1.6 M	Monofactorial	Awad (1985)
(astringent)	Trit	0–5%	1%		
	Albumin	0–5%	5%		
Persimmon	NaCl	0–3.2 M	0.2 M	Monofactorial	Awad (1985)
(nonastringent)	Triton	0–5%	L.E.		
	Albumin	0–5%	L.E.		
Prickly pear	NaCl	0–3.0 M	1.0 M	Factorial	This article.
Soursop fruit	NaCl	0.5–2.0 M	1.92 M	Fractional factorial and response surface methodolgy	Arbaisah et al. (1996)
	EDTA	0–0.01	L.E.		
	pH	4–9	8.4		
	Time	1–8 h	L.E.		
	PVP	0–2	L.E.		
Star fruit	NaCl	—	2.4 M	Response surface methodology	Hornung et al. (1996)
	pH	—	8.4		
	PVP	—	2%		
Tomato	NaCl	0.5–1.0%	0.5%	Monofactorial	Pirovani de Rodríguez and Di Pentima (1986)

*Cytolase 104.
EGTA: ethylene glycol-bis(β-aminoethylether) N,N,N′,N′-tetraacetic acid.
EDTA: ethylenediaminetetraacetic acid.
L.E.: low effect.
PVP: polyvinylpyrrolidone.

presence of 0.5 M NaCl and a smaller increase with 2.0–3.0 M NaCl. In comparison, a much smaller increase in PE activity was observed for the prickly pear extracted with 1.0 M NaCl. The activity of PE in lime peel was five times greater than that observed for the prickly pear.

PE is located in the cell walls of higher plants, and it is attached with different degrees of strength. The exact nature of these linkages is still unclear (Baron & Thibault, 1985; Fayyaz et al., 1993). Different authors have studied the desorption process of PE from persimmon [khaki, *Diospyros kaki*] (Awad, 1985), tomato (Pirovani de Rodríguez & Di Pentima, 1986), papaya (Fayyaz et al., 1993), soursop fruit [*Anona muricata* L.] (Arbaisah et al., 1996) and starfruit [*Averrhoa carambola* L.] (Hornung et al., 1996) and observed that it is closely related to the ionic strength of the extracting solution. Table 2 shows the reported results of PE extraction from different origins. As can be seen, a wide range of experimental conditions such as NaCl concentration, pH, extraction time, extraction temperature, addition of chelating agents, raw material, enzymes, etc., affects the PE extraction process.

The results show that the highest amounts of PE from lime and prickly pear peels can be obtained in a desorption process using an ionic strength of 0.5 and 1.0 M, respectively. The evaluation of the activation and inactivation processes of PE from these sources can be carried out appropriately under these extraction conditions. Nevertheless, a further optimisation of other process variables, such as extraction pH and time, is needed if the purpose of PE extraction is the industrial production of the enzyme. Results about these topics will be shown elsewhere.

Finally, it is remarkable that a local byproduct, such as lime peels, shows great potential as an exogenous source of PE for the food-processing industries.

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