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Partial purification, heat stability and kinetic characterization of the pectinmethylesterase from Brazilian guava, Paluma cultivars

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Abstract

Pectinmethylesterase (PME) was extracted from guava fruit (*Psidium guajava* L.), cultivar Paluma, by 70% ammonium sulphate saturation and partially purified by gel filtration on Sephadex G100. Gel filtration showed PME isoenzymes with different values of molecular mass. Two samples were examined: concPME (70% saturation by ammonium sulphate) and Iso4 PME (one of the isoforms from gel filtration with the greatest specific activity). Optimum pH of the enzyme (for both samples) was 8.5 and optimum temperature ranged from 75 and 85 °C. The optimum sodium chloride concentration was 0.15 M. The K_M and V_{max} ranged from 0.32 to 0.23 mg ml^{-1} and 244 to 53.2 μmol/min, respectively, for concPME and Iso4PME. The activation energies (E_a) were 64.5 and 103 kJ/mol, respectively, for concPME and Iso4PME. Guava PME, cv Paluma, is a very thermostable enzyme, showing great heat stability at all temperatures studied.

Keywords: Pectinmethylesterase; Isoenzymes; Heat stability; Guava fruit

1. Introduction

Brazil with its 2.2 millions hectares of fruit cultivars, is one of the biggest fruit producer of the world, producing more than 30 millions tons annually, which is approximately 10% of the total world fruit production (Musser, 1995). The culture of guava is one of the principle activities of the horticulture of the State of São Paulo, and the production of the guava for export is of great economic importance (Martin & Kato, 1988; Pereira & Martinez, 1986).

The Paluma guava cultivar was obtained from the Ruby-supreme guava cultivar (Pereira & Martinez, 1986). It is very productive; it has late production, with large fruits (average weight 160 g), of oblong form, with pulp stable in transport and conservation, low number and medium size of seeds and pleasant flavour and odour (Pereira & Martinez, 1986). Guava fruit is rich in antioxidant activity, maybe due to its high vitamin C content (the concentration is ten times higher than in orange) (Ito, Yamaguchi, Ohata, & Ishihata, 1980), as well as sugar, vitamins A and B, pectic substances (pectin), proteins and mineral salts, mainly iron, calcium and phosphorus. It is consumed fresh or made into juice, nectar, puree, jam or jelly. Guava has been used as an additive in other juices and purees, thus increasing the content of vitamin C (Martin & Kato, 1988; Medina, 1988). Due to its good pectin content, it has viscosity properties useful for obtaining purees. Guava puree is reprocessed into nectars, juice drink blends, jams, jellies

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and syrups that give it great industrial application (Chem Chin et al., 1984, Ito et al., 1980; Kashyap, Vohra, Chopra, & Tewari, 2001; Seymour, Taylor, & Tucker, 1993).

The enzyme pectinmethylesterase (PME; EC: 3.1.1.11), also known as pectin esterase, catalyses hydrolysis of the methoxyl group of pectin, forming pectic acid as a product of the reaction. This enzyme acts preferentially on a methyl ester group of a galacturonate unit next to a non-esterified galacturonate unit. The decrease in the degree of pectin methoxylation, may in its turn, trigger different processes related to texture and firmness (Tijskens, Rodis, Hertog, Proxenia, & Van Dijk, 1999). Such catalytic action makes PME one of the most important enzyme in the industrialization and preservation of fruits, juices or other industrial products that involve the presence or absence of intact pectin (Alonso, Howell, & Canet, 1997; Giovane, Quagliolo, Servillo, & Balestrieri, 1990; Javeri & Wicker, 1991; Lin, Liu, Chen, & Wang, 1989; Nighojkar, Srivastava, & Kumar, 1995). PME commonly occurs in various parts of higher plants, including fruits, and it has been found in pathogenic fungi and bacteria (Arbaisah, Asbi, Junainah, & Jamilah, 1996; Giovane et al., 1994). Studies of PME in fruits show that it normally exists as two or more isoforms, each comprising a simple polypeptide chain with molecular mass ranging from 10 to 60 kDa (Alonso et al., 1997; Giovane et al., 1990; Markovic & Joernvall, 1992).

Previous research on pectinmethylesterase carried out in our laboratory, with samples provided by food industries of the Araraquara region, has shown residual enzyme activity at different steps of the industrial processing of guava fruit. The samples analysed included fresh fruit upto the production of guava sweet, and also stored guava puree (no published result). Those studies suggested that residual catalytic PME action is probably responsible for the necessity of adding commercial pectin to stored guava pulp during industrial processing, causing increase of the cost of the final product. The purpose of this work was to study the guava PME enzyme, by kinetic characterization, in order to check the presence of isoenzymes and to determine PME heat stability, which is important for the food industry of the Araraquara and Monte Alto regions, State of São Paulo, Brasil.

2. Materials and methods

2.1. Fruit sampling and preparation of the pulp

Guava fruits, Paluma cultivars, were collected from several points of the boxes containing fruits awaiting industrial processing. To assure a representative sample, the use of this variety was suggested by Indústria e Comércio de Conservas Alimentícias PREDILETA-Ltda., de São Lourenço do Turvo, Matão-SP, Brasil, since it is the one most used (90% of the total of the processed fruits) in the industrial processing of guava products (sweet, gels, purees and others). The fruits were kept frozen (below 4 °C).

2.2. Extraction of the PME

The pulp was obtained by passing the fruits through a despulper.

Unless otherwise indicated, all steps were performed at 4 °C. The pulp was homogenized in a blender with borate–acetate (extractor buffer), 50 mM, pH 8.3, containing 0.20 M NaCl. The ratio of the pulp mass (g) to buffer solution (ml) was 1:3.The mixture was centrifuged at 15,000*g* for 10 min, and the supernatant was then squeezed through cheese cloth to remove solid particles (Korner, Zimmermann, & Berk, 1980). Then, filtrate supernatant was brought to 70% saturation by addition of solid ammonium sulphate, and was centrifuged at 15,000*g* for 10 min after standing for 2 h. The pellet was suspended in cold borate–acetate buffer in a ratio of 1:1 (w/v). This fraction was named *concentrated PME (concPME)*.

2.3. Partial purification of concPME

Two millilitres of the concentrated PME sample (2.25 mg protein/ml) were loaded in a Sephadex G100 column (from Sigma Chemical Co), column size 48×1.2 cm, previously equilibrated with borate–acetate buffer, pH 8.3, with 0.15 M NaCl. Elution was performed at a flow rate of 0.25 ml/min, and fractions of 2 ml were collected. All fractions with absorbance at 280 nm were assayed for PME activity, pooled and stored at −15 °C. This pooled enzyme sample was named Iso4 PME.

2.4. PME activity assay and protein determination

The PME activity was determined by measuring the amount of free carboxyl groups formed as a result of enzyme action on pectin (as substrate) by the titrimetric method of Kertesz (1955). The standard reaction mixture consisted of 0.125% citrus pectin solution (substrate, 29.5 ml), 0.15 M NaCl and enzyme solution (0.5 ml). After the pH was adjusted to 8.0 with 0.01 M NaOH or 0.01 M HCl, this reaction mixture was incubated at 50 °C and titrated with 0.1 M NaOH. Units of PME (UA) and specific activity were calculated according to Assis, Lima, and Oliveira (2000).

2.5. Protein determination

Protein concentration was determined in all enzyme extracts from the fruits by Hartree (1972) method, using