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Extraction, thermal stability and kinetic behavior of pectinmethylesterase from hawthorn (*Crataegus pubescens*) fruit

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Abstract

Pectinmethylesterase (PME) extracted from hawthorn (*Crataegus pubescens*) fruit was evaluated for its thermal stability and kinetic behavior. The enzyme extraction process was established after studying different NaCl concentrations (0.5–3.0 moles/L). A maximum PME extraction of 51.61 units/mg protein was obtained using 2 moles/L NaCl. Kinetic parameters (K_m and V_{max}) were determined using a commercial citrus pectin and *C. pubescens* pectin as substrates. The effects of NaCl concentration, pH and temperature on PME activity were investigated. PME showed higher affinity for *C. pubescens* pectin (K_m and V_{max} of 2.84 mg/mL protein, and 64.10 units/mg protein, respectively) than for citrus pectin. *C. pubescens* PME extract showed maximum activity at 0.4 moles/L NaCl, pH 7.5, and 55 °C. The E_a and Q_{10} for thermal activation were 36.27 kJ/mol and 2.01 (20–30 °C), respectively. About 50% of the activity still remained after heating for 25 min at 60 °C, and it was completely inactivated by incubation at 80 °C for 10 min. The Q_{10} and E_a values for thermal inactivation reaction were 20.06 (70–80 °C) and 146.16 kJ/mol, respectively. These results provide useful information about the factors that affect the activity of *C. pubescens* PME, and might be used as a starting point for texture control during post-harvest handling and processing of this fruit.

Keywords: Pectinmethylesterase; *Crataegus pubescens*; Thermal stability; Kinetic behavior; Hawthorn pectin extraction

1. Introduction

Crataegus pubescens (H.B.K.) Steud., known as *Crataegus stipulosa (Kunth)* Steud., *Crataegus succulenta and Crataegus mexicana*, is a member of the Rosaceae family, and is one of the approximately 200 species of hawthorns genus *Crataegus*, which has its origin in China and México (Borys, 1996; Hobb & Foster, 1990). It is cultivated in Asia, Australia (Phipps, 1983), Canada (Dickinson, 1985), México, Central America and Ecuador (Hobb & Foster, 1990). Some of the largest plantations of *C. pubescens* flourish in México, with an important annual fruit production (24,000 T) during the fall (Secretaria de Agricultura Ganadería, Desarrollo Rural, and Pesca y Alimentación (SAGARPA), 2004). The species *C. pubescens* produces the largest fruits between the genus with specimens up to 44.42 mm in diameter. These fruits are like small apples, with a thin skin covering a fleshy pulp. They are green and hard in the unripe stage, and during ripening they become sweet, soft, flesh with a skin color ranging from yellow through red to dark purple (Herrera-Guadarrama, 1990). Ripe *C. pubescens* fruits are edible and are eaten fresh. They are rich in vitamin C, carotene and mineral salts, mainly calcium, phosphorus, iron and also have a high content of pectin (Higareda, Salazar-Montoya, & Ramos-Ramírez, 1995; Morales, Babinsky, Bourges, & Camacho, 1999). Many food products, like concentrated pulp, jam, jellies, and marmalades can be processed from this fruit, making it of economical importance; however, the full potential of this fruit has not been exploited yet (Borys, 1996). This is probably due to the short shelf-life of the fruits (4–8 days) for excessive

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softening after harvesting, and the lack of studies about the control of pulp texture during processing (Vivar, 2000).

Textural changes in fruits have been attributed to pectin degradation caused by pectic enzymes (Whitaker, 1984). Softening of fruits is brought about by the removal of the methyl ester groups from pectin (Rexová-Benková & Markovic, 1976) in the cell wall by pectinmethylesterase (PME, EC 3.1.1.11), allowing the action of polygalacturonase (EC 3.2.1.15) over the resulting polymer producing a reduction in the intercellular adhesiveness and tissue rigidity (Huber, 1983; Takuo, Sakamoto, & Hallaert, 1993). Thus, control of native PME activity in fruits for the maintenance and improvement of the texture characteristics of fruits and vegetables (Fischer & Bennet, 1991; Rombouts & Pilnik, 1978) is of critical importance in food industry. Of course, the activity and properties of plant PME depend on the source, environmental and physico-chemical conditions such as pH and temperature. Considering that there are no data about the properties of PME from *C. pubescens*, the aim of the present work was to evaluate the thermal stability, and the activity and kinetic behavior from a PME extract from this hawthorn fruit.

2. Materials and methods

Fruits of *C. pubescens* harvested in the mature stage of development from a wild orchard in the State of Michoacan, Mexico, during the last week of November, were used for this work.

2.1. Proximal chemical analysis of C. pubescens fruit

C. pubescens fruits without seeds were used as PME source and its proximal chemical analysis was performed according to AOAC 925.10, 923.03, 920.87, and 920.39 methods (Association of Official Analytical Chemists (AOAC), 1995). Samples were analysed by triplicate for moisture, ash, protein, and fat content. Carbohydrates content were estimated by difference.

2.2. Extraction and chemical analysis of pectin from C. pubescens fruits

Pectin from *C. pubescens* fruits was extracted from the alcohol insoluble solids (AIS) prepared by mixing *C. pubescens* pulp with ethanol (1:2 w/v). The extraction was performed with distilled water at pH 5, 80 °C and 30 min. After centrifugation at 9860*g* for 15 min, the water-soluble pectin was precipitated with ethanol and dried at room temperature. The galacturonic acid content and esterification degree (DE) in pectin was estimated using *m*-hydroxidiphenyl-sulphuric acid method (Blumenkrantz & Asboe-Hansen, 1973) and the titration method of Schultz (1965), respectively.

2.3. Pectinmethylesterase extraction from C. pubescens fruit

C. pubescens fruits were pre-treated by quick-freezing under liquid nitrogen and storage at −70 °C until extraction. Frozen fruits were ground in a mortar to a fine powder at −30 °C, and the seeds were separated and discarded. All further processes were carried out at 4 °C. The fruit powder was treated with antioxidant agents in order to prevent any loss of PME activity by phenolic inhibition during extraction (Anderson, 1968). The powder was homogenised in 500 mg/L sodium *meta*-bisulfite (Baker) solution (1:8 w/v) using a mixer (Thermolyne Maxi-Mixer, USA) for 5 min. The slurry was centrifuged at 8900 *g* for 15 min, and the pellet was resuspended in 500 mg/L (1:6 w/v) of the same solution and recentrifuged. The pellet was resuspended in a mixture of sodium *meta*-bisulfite containing 10 mg/mL polyvinylpolypyrrolidone (Sigma) in proportion 1:4 w/v followed by centrifugation. Supernatant was discarded and the pellet was resuspended in two parts of NaCl (Baker) solution (0.5–3 moles/L) to establish the concentration for maximum PME extraction. The pH of the mixture was adjusted to 7.5, magnetic-stirred for 3 h, and the pH maintained by addition of NaOH 0.25 moles/L. The slurry was centrifuged at 8900*g* for 10 min. The clear supernatant was collected and named *C. pubescens* PME crude extract. The *C. pubescens* PME extraction was estimated as PME units/mg protein from two independent lots at each NaCl concentration in the extracting solution.

2.4. Determination of pectinmethylesterase activity

PME activity was evaluated following the method described by Kertesz (1955), and measuring the liberation rate of carboxyl groups from 10 g/L (w/v) standard citrus pectin (DE 74.2%, Sigma) in 0.1 moles/L NaCl, pH 7.5, and 30 °C. The reaction was started by addition of 1 ml of the enzymatic extract (protein content of 150 μg/mL), to 9 ml of the pectin solution. The pH of the reaction mixture was kept by addition of 0.4 g/L NaOH using a Titralab Autotitrator (TIM900 Radiometer, Copenhagen). One unit of PME activity was defined as 1 μequivalent of carboxyl groups released per minute, under the above-mentioned reaction conditions.

2.5. Determination of protein

Protein in the PME extracts was assayed colorimetrically according to the method of Bradford (1976). The bovine serum albumin protein (Sigma) was used as standard protein.

2.6. Determination of the kinetic parameters of C. pubescens PME

The kinetic parameters of PME were estimated using citrus pectin (Sigma) as standard substrate and the pectin