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Activity of pectinmethylesterase, pectin content and vitamin C in acerola fruit at various stages of fruit development

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Abstract

In order to know which stage of fruit development is better for acerola industrialization, we studied the PME specific activity, pectin content and vitamin C at various stages of development. The acerola fruits were classified according to colour and weight in five stages: immature green (2.62–3.21 g), green (4.04–4.83 g), mature green/yellow (5.03–5.88 g), pale-red (6.16–6.77 g) and ripe mature (6.92–8.37 g). The results showed that the highest content of pectin and vitamin C occurred at the immature green stage, $4.51 \pm 0.1\%$ yield, 2424 mg/100 g of pulp and decreased as fruit ripened, $2.99 \pm 0.03\%$ yield, 957 ± 0.0 mg/100 g of pulp, respectively. However, at the same stages, the values of PME specific activity were lowest, 0.61 ± 0.01 and 0.55 ± 0.0 units g^{-1}/g of pulp, respectively. The highest value of PME activity was 2.08 ± 0.01 units g^{-1}/g of pulp in the green stage.

Keywords: Pectin methylesterase; Pectin; Vitamin C; Acerola; Fruit development

1. Introduction

The interest of consumers and producers of acerola (*Malpighia glabra* L., *Malpighia punicifolia* L or *Malpighia emarginata* DC) and its derived products has increased in Brazil and all over the world (Cooper, 1971; Facciola, 1990; Morton, 1987). Acerola fruit is rich in vitamin C as well as carotene, thiamin, riboflavin, niacin, proteins, and mineral salts, mainly iron, calcium and phosphorus. Acerola has been used as a remedy against flus and colds, pulmonary disturbance, liver ailments and irregularities of the gall bladder. Used in heavy dose, it has beneficial effects on viral hepatitis, varicella as well as poliomyelitis. Acerola is rich in antioxidant activity, which may be due in part to its high vitamin C content. Its main use is the production of juices, concentrates pulp and the production of vitamin C (Gonzaga, Amaral, & Saueressig, 1996). Acerola has also shown active anti-fungal properties (Caceres, 1993).

The enzyme pectin methylesterase (PME, EC: 3.1.1.11) is present in most plant tissues and is likely to play an important role in plant metabolism. During fruit ripening PME removes methyl groups from the cell wall pectic constituents which can then be depolymerized by polygalacturonase, decreasing the intercellular adhesivity and tissue rigidity. PME could also be involved in the elongation process of primary walls during cell growth, and this enzyme is found in many plant pathogens (Alonso, Rodriquez, & Canet, 1995). PME activity has been reported to control apoplastic pH and, in turn, the cell expansion process (Bordenave & Goldberg, 1994; Moustacas, Nari, Borel, Noat, & Ricard, 1991; Nari, Noat, Diamantidis, Woudstra, & Ricard, 1986; Ricard & Noat, 1986).

The action of some enzymes, such as pectin methylesterase, polygalacturonase and peroxidase, will have a pronounced effect on the observed quality of fresh and processed products (Tijskens, Rodis, Hertog, Proxenia, & Dijk, 1999). The aim of blanching fruits and vegetables prior to sterilization is, among others, the activation and/or inactivation of enzymes present in the plant tissue (Pilnik & Voragen, 1991; Tijskens et al., 1999). PME enzymes are the principal agents responsible for fruit juice clarification (Lozano et al., 1988). Thus, PME control is very important in the preservation of the cloud of citrus juice.

There are industrial processes where clarification of fruit juices is or is not required. For example, in citrus

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juice, the PME enzyme is usually inactived by thermal treatment to prevent the clarification process (Balaban, Arreola, Marshall, Peplow, Wei, & Cornel, 1991; Körner, Zimmerman, & Berk, 1980).

PME removes methoxyl groups from methylated pectin substances (Whitaker, 1984) and releases pectic acid as a product of enzymatic catalysis. These pectic acids can react with calcium ions present in the medium, to form insoluble calcium pectate complexes, inducing loss of cloud in citrus juice. So, due to the importance of PME in the quality of fruit juice, we studied its activity at various stages of acerola development.

Pectin is a colloidal material, capable of forming gels; this ability is utilized in the food industry in the preparation of jams, jellies, confectionery and low fat dairy products (Bardof & Dedersen, 1990; Nighojkar, Srivastava, & Kumar, 1995). These authors also used it as a component in pharmaceutical preparations, such as antidiarrheal, detoxicant formulations. Pectin was also shown to affect glucose metabolism by lowering the glucose response curve (Simpson, Egyankor, & Martin, 1984; Voragen, Rodis, Hertog, Proxenia, & Dijk, 1995). On the other hand, the sources which may be used for the commercial manufacture are quite limited. Two main natural sources of commercial pectin are apple and citrus fruit rinds (Kravtchenko, Voragen, & Pilnik, 1992). So, we developed this study to verify if acerola could be used an alternative source of commercial pectin.

Because acerola has a high vitamin C (ascorbic acid, AA) content, it may also be used for the production of vitamin C concentrates for pharmaceutical purposes or enrichment of fast foods (Byrne, 1993). Vitamin C is an indispensable component in diets and its constant use is recognized to prevent many pathologies (Genaro & Bertolo, 1990). Vitamin C may also be used in soft drinks as an oxidant for flavour ingredients, in meat and meat-containing products for curing and picking, in flour to improve baking quality, in beer as a stabilizer, in fats and oils as an antioxidant, and in a wide variety of foods for vitamin C enrichment (Byrne, 1993). This study evaluates the stages of maturation that present the highest vitamin C content.

This is achieved by determining the PME specific activity, pectin content and vitamin C in acerola fruit at various stages of fruit development.

2. Materials and methods

2.1. Preparation of pulp

Acerola fruits were collected at different points of a single plant to guarantee a homogeneous sample. The fruits were separated according to colour, diameter, weight and height, and classified in five stages of the development, based on colour and weight (Table 1). Twenty fruits were collected at each of the stages. Each sample was obtained by the mixture of fruits at the same stage of development collected from various trees. The weights of each of the fruits were obtained and the mean values ± standard deviations are reported in Table 1. The pulp was obtained by passing the fruits through a depulper.

2.2. Determination of pectin

A rapid and quantitative method (Shelukhina & Fedichkina, 1994) was employed. This method is based on the titration of de-esterified pectins after acid precipitation.

2.3. Extraction of the PME

The enzyme was extracted in borate–acetate buffer 50 mM, pH 8.3; with 0.60 mol/l NaCl solution at 4°C. The ratio of acerola material to extractant was 1:3 (w/v). The homogenate was squeezed through two layers of gauze and the extract was centrifuged for 10 min at 10,000×***g*** to remove the solid particles (Körner et al., 1980). The precipitate was discarded. The supernatant was brought to 70% saturation by addition of solid ammonium sulfate and centrifuged at 10,000×***g*** for 10 min, after standing for 1 h. The precipitate was re-suspended in borate–acetate buffer in the ratio of 1:3 (w/v).

2.4. Determination of PME activity

PME activity was measured titrimetrically, estimating free carboxyl groups formed in pectin as a result of enzyme action. The amount of 0.1 M NaOH required to maintain the median reaction at pH 8.0 (27 ± 2°C) was measured by the method described by Kertsz (1955). The enzyme substrate was a 0.25% citrus pectin solution containing 0.15 M NaCl. One unit of PME was defined as the amount of enzyme which released 1 μmole of carboxyl groups min^{-1}. PME activity was calculated by the following formula (Balaban et al., 1991):

$$\text{PME (units/ml)} = \frac{(\text{ml NaOH})\ (\text{Molarity of NaOH})\ (1000)}{(\text{time})\ (\text{ml sample})}$$

Table 1
Classification of fruits at five stages of development according to colour and weight

Stage of fruit development	Colour	Range of weight (g)
1	Immature green	2.62–3.21
2	Green	4.04–4.83
3	Mature green/yellow	5.03–5.88
4	Pale red	6.16–6.77
5	Ripe mature	6.92–8.37

Table 2
Relationship of the mean values±standard deviations of ascorbic acid content, pectin content, total protein content and PME specific activity at different stages of acerola development[a]

Stage of fruit development	Ascorbic acid (mg/100 g of pulp)	Pectin (% yield)	Protein (mg/ml)	PME specific activity (units g^{-1}/g of pulp)
1	2424±0.0a	4.51±0.1a	2.15±0.0a	0.61±0.01d
2	1814±0.09b	3.63±0.03b	2.03±0.0a	2.08±0.01a
3	1458±0.0c	3.00±0.03c	2.13±0.0a	1.22±0.01b
4	1454±0.0c	2.67±0.03d	2.31±0.0b	0.79±0.03c
5	957±0.0d	2.99±0.03c	3.20±0.0c	0.55±0.0d

[a] Mean values with the same letters in the same column are not significantly different ($P > 0.01$) by Tukey test.

The specific activity per gram of pulp was calculated by the following formula:

$$\text{Specific activity (units } g^{-1}\text{/g of pulp)} = \frac{\text{units}}{(\text{g of total protein}) \times (\text{g of pulp})}$$

2.5. Protein determination

Protein concentration was determined according to the method of Hartree (1972), using bovine serum albumin (Sigma Chemical Co.) as standard.

2.6. Vitamin C determinations

The content of vitamin C was determined by the 2,6-dichloroindophenol titrimetic method (JAOAC, 1984).

2.7. Statistical analysis

A minimum of three replications of the experiment were performed. The values were analyzed by the test of Tukey to verify if they were significantly different.

3. Results and discussion

The analyses of pectin content and vitamin C content at various stages of the fruit development (Table 2, Figs. 1 and 2, respectively) showed that peak values occurred at the immature green stage and decreased on ripening. The values of vitamin C content were in agreement with those determined by Itoo, Aiba, and Ishihata (1990), which showed that the highest values occurred in pale green fruit.

In relation of the total protein content, the results showed no significant variations at the four initial stages of fruit development (Table 2 and Fig. 3). There was a rise of the total protein content at the ripe mature stage.

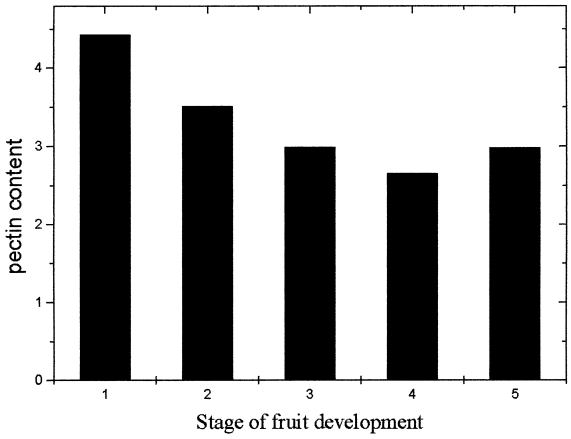

Fig. 1. Pectin content (% yield) at various stages of acerola fruit development (stages classification: 1, immature green; 2, green; 3, mature green; 4, pale red; 5, ripe mature).

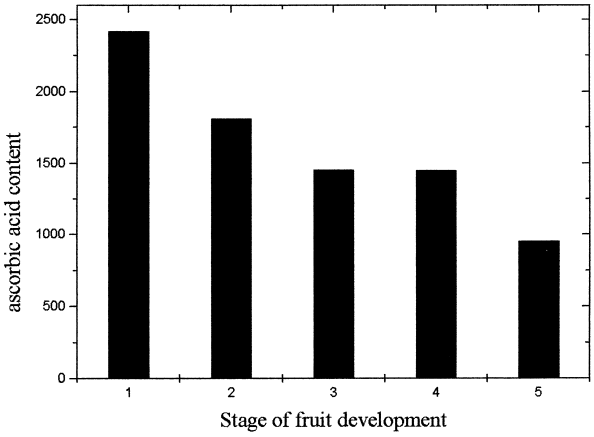

Fig. 2. Ascorbic acid content (vitamin C, mg/100 g of pulp) at various stages of acerola fruit development (stages classification: 1, immature green; 2, green; 3, mature green; 4, pale red; 5 ripe mature).

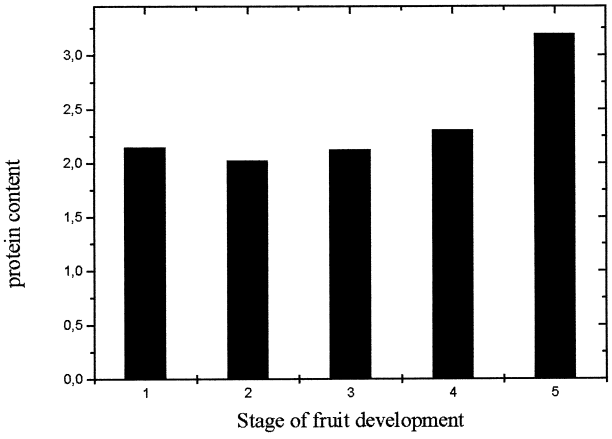

Fig. 3. Total protein content (mg/ml) at various stages of acerola fruit development (stages classification: 1, immature green; 2, green; 3, mature green; 4, pale red; 5, ripe mature).

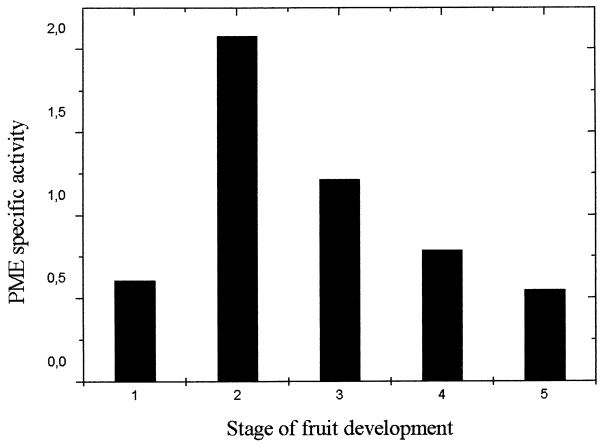

Fig. 4. Specific activity of PME (units g^{-1}/g of pulp) at various stages of acerola fruit development (stages classification: 1, immature green; 2, green; 3, mature green; 4, pale red; 5, ripe mature).

Comparing these values with PME specific activity variations, from immature green to ripe mature stage, suggests that the increase of total protein is probably not due to increase in biosynthesis of PME enzyme molecules (Table 2).

The highest value of PME specific activity occurred at the green stage and decreased on ripening (Fig. 4). These results agree with those reported by Abu-Sarra and Abu-Goukh (1992), Awad and Young (1980), and Nagel and Paterson (1967), that demonstrate highest activity at the immature stages of pear, avocado and mango, respectively. However, PME activity was found to increase during softening of cherry (Barret & Gonzalez, 1994), tomatoes (Pressey & Woods, 1992), African mango (Aina & Oladunjoye, 1993) and pear (Bartley, Knee, & Casimir, 1982). In durian aril, the PME activity was higher at the more ripe stage than the less ripe and the activity increased only slightly during softening (Ketsa & Daengkanit, 1999). Our results show that, since the ripe mature stage has a low PME activity, it is a better stage to use in acerola juice industrialization (Fig. 4). On the other hand, it is necessary to know the stability of the PME of acerola juice because of its implication in the juice cloud.

It has been stated that, at the unripe stage, the pectin occurs together with cellulose to form the so called protopectin, as an insoluble form. In this way, the pectin is protected from degradation by such agents as pectolytic enzymes, alkalis and acids which may be present in the plant tissue. However, on fruit ripening, the protopectin is broken and the cellulose withdraws its protection from the pectin. The latter then becomes susceptible to the degradative substances named above (Joslyn, 1970; Simpson et al., 1984).

The values obtained in this study show that, during the development (green stage) there is a decrease in the content of pectin and increase in the values of PME specific activity (Table 2). With a decrease of the PME specific activity, the pectin content increased slightly (ripe mature stage). These results agree with the hypothesis of the Laats, Grosdenis, Recourt, Voragen and Wichers (1997), that PME is able to catalyze the de-esterification of pectic compounds in the plant cell wall. After this, the demethylated pectin could be enzymatically hydrolyzed by polygalacturonase (PG; endoPG, EC: 3.21.15; exoPG, EC: 3.2.1.67), resulting in a decrease in degree of polymerization of the pectin chains and the loss of firmness of the tissue.

Thus, our studies demonstrate that acerola (*Malpighia glabra* L.) at immature green and green stages can be useful as a pectin source for the confectionary industries and for enrichment of food as dietary fibre.

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