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Changes in pectic enzymes and cellulase activity during guava fruit ripening

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Abstract

Changes in activities of the cell wall degrading enzymes, pectinesterase (PE), polygalacturonase (PG) and cellulase, were studied during the ripening of white- and pink-fleshed guava fruit types. PE activity increased in both guava types up to the climacteric peak of respiration (flesh firmness of 1.21 kg/cm^2) and subsequently decreased. Activities of PG and cellulase increased progressively during the ripening of both guava fruit types with a high correlation between the increase in the activity of the two enzymes and the loss of fruit flesh firmness.

Keywords: Guava; Enzyme changes; Fruit ripening

1. Introduction

Guava (*Psidium guajava* L.) is a popular fruit crop in Sudan. It is grown almost in every state in the country. Although Sudan has great potential to produce high quality guavas and to export them to other countries, its marketability is still limited to local markets. This is due to the delicate nature of the fruit, poor handling practices, and inadequate transportation and storage facilities. Therefore, proper handling techniques and control of the ripening process are crucial for the development of a sound guava industry in Sudan.

Ripening of the guava fruit is characterized by softening of the flesh. Fruit softening is associated with cell wall disassembly (Seymour & Gross, 1996) and modifications to the pectin fraction are some of the most apparent changes that take place in the cell wall during ripening (Marin-Rodriguez, Orchard, & Seymour, 2002). The general observation is that softening is accompanied by solubilization of pectin, involving the action of enzymes pectinesterase (PE), polygalacturonase (PG) and pectate lyases (PL) (White, 2002). This notion was supported by reports of changes in cell wall pectic material in ripening mango (Roe & Bruemmer, 1981), tomato (Besford & Hobson, 1972) and pear (Ahmed & Labavitch, 1980a).

Studies on changes in the activities of the cell wall degrading enzymes in some Sudanese mango cultivars have been reported (Abu-Sarra & Abu-Goukh, 1992). However, data on cell wall degrading enzymes in guava fruit are limited.

This study was conducted to investigate changes in the activity of the cell wall degrading enzymes, PE, PG and cellulase during ripening of white and pink guava fruits.

2. Materials and methods

2.1. Experimental material

Mature green fruits of white- and pink-fleshed guava types were obtained from the University of Khartoum orchard at Shambat (Lat. 15°40′ N, long. 32°22′ E). Fruits were selected for uniformity of size, colour and freedom from blemishes. About 400 fruits, of each of the guava fruit types, were washed, dried, placed in carton boxes, and stored in the ripening room at 22±1 °C and 90–95% RH. Random samples of 16

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fruits from each type, were removed daily for respiration and flesh firmness determinations. Respiration rate was determined for each fruit of the sample separately using the total absorption method of Charlimers (1956). Flesh firmness was measured by a Magness and Taylor firmness tester (D. Ballauf Meg Co.) equipped with an 8 mm diameter plunger tip. Two readings were taken from the opposite sides of each fruit after the peel was removed. The fruits were labelled and stored at −12 °C and later sorted into seven groups (2.13, 1.82, 1.52, 1.21, 0.91, 0.61 and 0.30 kg/cm^2) according to their flesh firmness. Each group consisted of 10 fruits replicated five times.

2.2. Protein assay

The frozen fruits of the different groups were thawed separately for 90 min. Thirty grammes of pulp or peel from each group were homogenized in 100 ml of distilled water for one min in a Sanyo Solid State blender (model SM 228 P) and centrifuged at 10,000 rpm for 10 min in a Gallenkamp portable centrifuge (CF 400). The total protein was determined in pulp extract according to the protein-dye binding procedure described by Bradford (1976).

2.3. Enzyme assay

Guava fruits at designated stages of firmness were peeled, and 50 g of flesh were homogenized in two volumes of 100 mM sodium acetate buffer pH 6.0 containing 0.2% sodium dithionite ($Na_2S_2O_4$) and 1% polyvinyl pyrrolidone (PVP, MW 44,000) for 1 min using a Sanyo Solid State blender. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was stored at −12 °C (buffer extract).

The residue was suspended in two volumes of 1 M sodium acetate buffer, pH 6.0, containing 6% NaCl. The pH of the suspension was adjusted to 8.2 with 2 N NaOH. The sample was kept overnight at 4 °C with continuous stirring and then centrifuged. The supernatant was filtered twice using Whatman No. 1 filter paper. The filtrate (salt extract) was dialysed against distilled water for 48 h with four changes. All operations were carried out in an ice bath. This dialysed sample constituted the enzyme extract.

PE activity was determined according to the technique described by Nagel and Patterson (1967). The substrate used was a 1% (w/v) solution of pectin (Citrus; 150 grade; H.P. Bulmer Ltd., Hereford, England). The pH of the pectin solution was adjusted to 7.0 with 0.02 N NaOH. The reaction mixture contained 25 ml of the crude enzyme, 5 ml of 0.2 M sodium oxalate and 25 ml of substrate. The reaction mixture was incubated at 30 °C and continuously stirred by bubbling CO_2-free air through it. During the course of the reaction, the pH of the reaction mixture was maintained at 7.0 with 0.02 N NaOH. The amount of 0.02 N NaOH added was recorded every 15 min. Enzyme activity was expressed as milliequivalents of ester hydrolysed per minute per kilogramme of original fresh weight.

PG activity was determined by measuring the reducing groups released from polygalacturonic acid (Orange; Sigma Chem. Co). Reducing groups were measured according to the technique described by Nelson (1944) as modified by Somogyi (1952). The reaction mixture, containing 0.25 ml of crude enzyme, 0.25 ml of 100 mM sodium acetate buffer, pH 4.5, and 0.5 ml of 0.1% polygalacturonic acid solution, was incubated at 37 °C for 30, 60, 90, 120 and 150 min. At the end of each incubation period the amounts of reducing groups released were determined. A calibration curve was obtained using D-galacturonic acid as a standard. PG activity was expressed as μmol of galacturonosyl reducing groups liberated per minute per kilogramme original fresh weight.

Cellulase activity was determined by measuring the reducing groups released from carboxymethyl cellulose. The concentration of the reducing groups was determined, with D-glucose as a standard, as in the PG assay. The reaction mixture contained 0.25 ml of crude enzyme, 0.5 ml of 0.1% carboxymethyl cellulose, and 0.25 of 100 mM sodium acetate buffer, pH 5.0. Incubation was carried out at 37 °C for 2, 4, 6, and 12 h. Cellulase activity was determined as units, one unit being defined as the amount of the enzyme that catalysed the formation of one μmol reducing groups per hour per kilogramme of orginal fresh weight.

3. Results and discussion

3.1. Changes in fruit flesh firmness

Fruit flesh firmness of the two guava types studied showed a progressive decline during ripening. The decline in firmness observed was about eight-fold, from the hard mature green stage to the final soft ripe stage (Fig. 1). Most of this decline occurred during the first 10 days. A similar drop in guava fruit firmness was reported (Rodriguez, Agarwal, & Saha, 1971). Abu-Goukh and Abu-Sarra (1993) observed a rapid decrease in flesh firmness during ripening of three mango cultivars. A similar pattern of changes was reported for banana (Abu-Goukh, Ibrahim, & Yusuf, 1995), pear (Luton & Holland, 1986), apple, peach and apricot (Salunkhe & Wu, 1973) and date (Barrevelled, 1993).

3.2. Changes in respiration rate

The rate of CO_2 production, during ripening of both guava types exhibited a typical climacteric pattern of

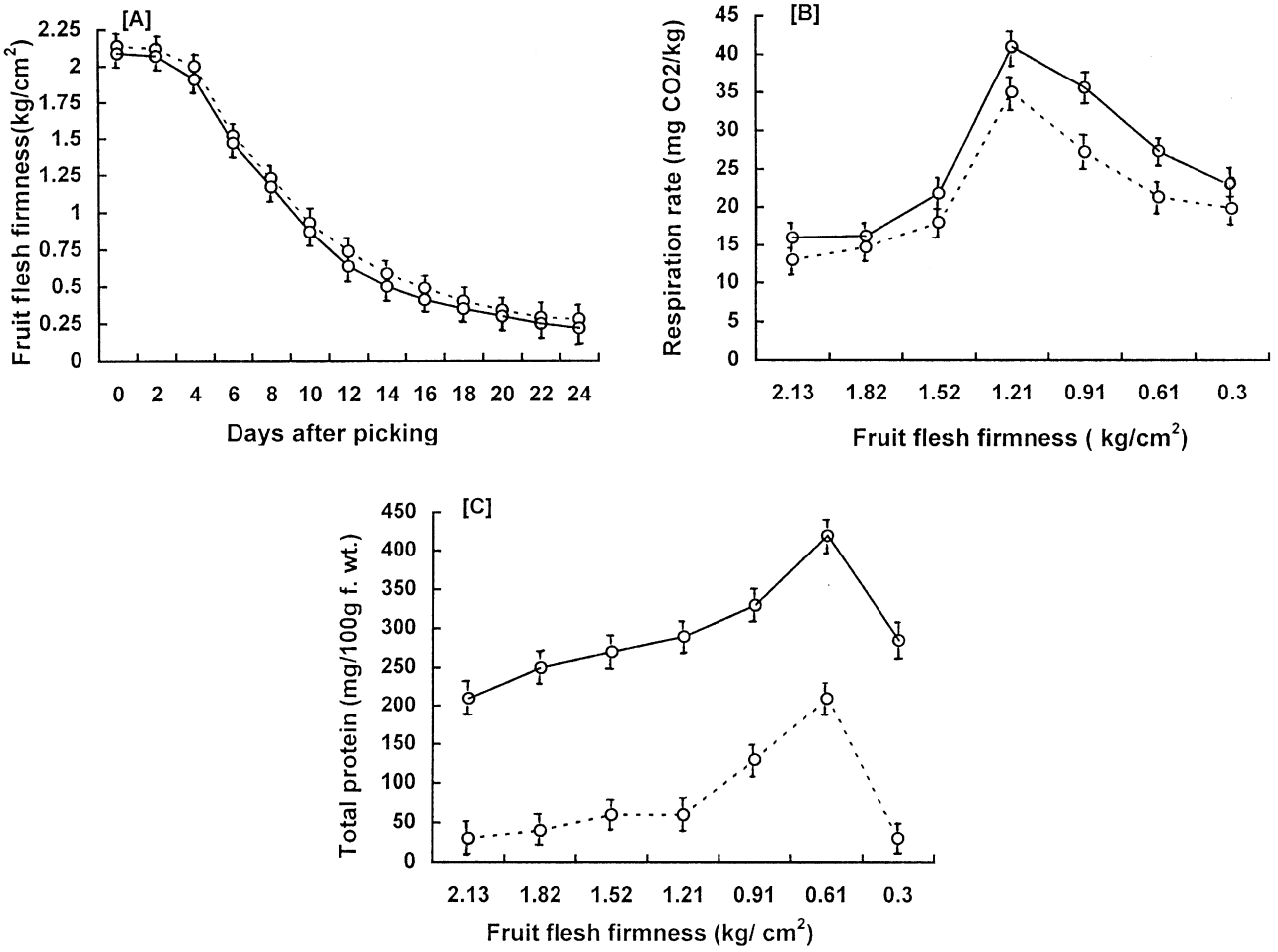

Fig. 1. Changes in fruit flesh firmness [A], respiration rate [B] and total protein content [C] during ripening of white (– – –) and pink (——) guava fruits [vertical bars represent SE].

respiration, with climacteric peak at 1.21 kg/cm^2 flesh firmness (Fig. 1). Respiration rate was significantly higher in the pink guavas than the white ones. A similar climacteric pattern was reported by Akamine and Goo (1979).

3.3. Changes in total protein

Total protein in pulp of the white and pink guava types increased systematically up to the full-ripe stage (fruit firmness 0.61 kg/cm^2) and then suddenly decreased (Fig. 1). Quantitative changes in soluble protein during fruit ripening have been repeatedly demonstrated (Mattoo & Modi, 1969). Abu-Goukh and Abu-Sarra (1993) reported that the total protein in pulp and peel of three mango cultivars increased up to the full-ripe stage and then decreased at the over-ripe stage. The increase in protein content during the climacteric phase coincided with increased activity of polygalacturonase and cellulase (Fig. 2). The decline in the protein at the over-ripe stage, was explained as breakdown of protein during senescence, which again supported the view that proteins in ripening fruits are mainly enzymes required for the ripening process (Frenkel, Klein, & Dilley, 1968).

3.4. Changes in enzyme activity

3.4.1. General

Enzyme activity was measured both in the buffer extract and the salt extract. The buffer extract contained large amounts of reducing sugars which resulted in high background readings in the reducing group assay. Although this problem can be lessened by dialysis of the extract, the time required was excessive. Activities in the buffer extract were extremely low compared with those of the corresponding salt extract. This is probably due to the fact that most cell wall enzymes are bound to the cell wall. To release them, either high salt concentration and/or high pH are necessary. Only the data of enzyme activities in the salt extract are presented.

Fruit softening during ripening is frequently attributed to the enzymatic degradation of cell wall materials (Ahmed & Labavitch, 1980b). The current theory is that PE removes the methyl groups of the galacturonic acid

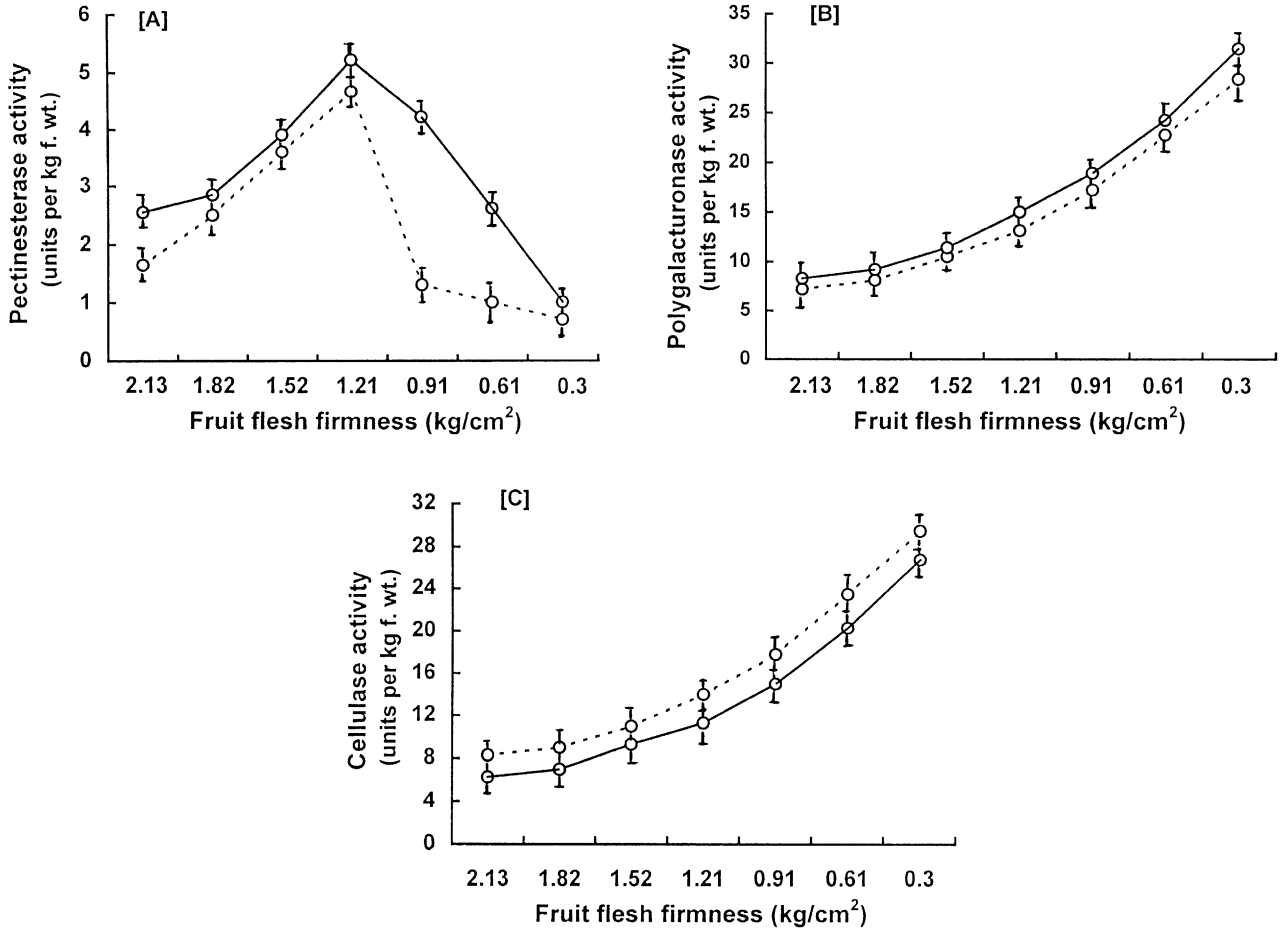

Fig. 2. Changes in pectinesterase (PE) [A], polygalacturonase (PG) [B] and cellulase [C] activities during ripening of white (– – –) and pink (——) guava fruits. [vertical bars represent SE].

polymers (Lee & MacMillan, 1970), which then enables PG to depolymerise the de-esterified polygalacturonide chain, and reduce its molecular weight (Benkova & Markovic, 1976). Cellulase cleaves the β1, 4 glucosidic bonds of cellulose (Babbitt, Powers, & Patterson, 1973). Recently, Marin-Rodriguez et al. (2002) reviewed the role of pectate lyases in fruit softening. Pectate lyases (PL) catalyse the Ca^{2+}-dependent cleavage of de-esterified pectin, which is a major component in the primary cell walls of many higher plants. PL activity has been obtained directly from banana pulp with a substantial increase in activity during ripening (Martin-Rodriguez, 2001). Fruits of tomato, strawberry and grape all express PL, where they may play a significant role in fruit softening (White, 2002). The exact sequence of events and the contribution of each of these enzymes to softening in fruits are still not clear.

3.4.2. Pectic enzymes

Fig. 2 shows the changes in pectinesterase (PE) activity during ripening of white and pink guava fruits. PE activity increased in both guava types up to the climacteric peak of respiration (flesh firmness of 1.21 kg/cm^2) (Fig. 1) and subsequently decreased. Abu-Sarra and Abu-Goukh (1992) reported that PE activity in " Abu-Samaka" mango increased up to shortly before the climacteric peak and then decreased. Ashraf, Khan, Ahmed, and Elahi (1981) found inconsistent patterns of PE changes during ripening of some mango cultivars. PE activity was found to increase during ripening of tomato (Buescher & Tigchellar, 1975), banana (Hultin & Lenine, 1965) and date (Al-Jasim & Al-Delaimy, 1972). PE activity was higher in the pink guava compared with the white ones (Fig. 2). This could be explained by differences in constituents of the white and pink guava types. White guava had the higher total soluble solids, total and reducing sugar, titratable acidity, phenolics and ascorbic acid contents (Bashir & Abu-Goukh, 2003).

Polygalacturonase (PG) activity progressively increased during fruit ripening in a similar manner in both guava types (Fig. 2). Very high correlation was observed between the increase in PG activity and loss in flesh firmness ($r^2 = 0.987$). Similar results were reported for mangoes (Abu-Sarra & Abu-Goukh, 1992; Roe & Burmmer, 1981), pears (Ahmed & Labavitch, 1980b),

peaches (Pressy, Hinton, & Avants, 1971), dates (Hasegawa, Macer, Kaszycki, & Crawford, 1969) and tomatoes (Hobson, 1962).

Pectic enzymes are apparently important in the softening of fruit and vegetable tissues during ripening (Ahmed & Labavitch, 1980b; Besford & Hobson, 1972). Due to the pronounced changes in cell wall components and in the degree of esterification of pectin, which are generally accepted to occur during fruit ripening, the softening process was thought to be a consequence of de-esterification of pectin, catalysed by PE, followed by pectin depolymerization, catalysed by PG (Ahmed & Labavitch, 1980b; Roe & Bruemmer, 1981). Consequently the slow rate of fruit softening observed by Abu-Sarra and Abu-Goukh (1992) in "Abu-Samaka" mango fruit in spite of its high PG activity, suggest a key role for PE in controlling the rate of fruit softening during fruit ripening.

Mattoo and Modi (1969) related the softening of chilling-injured mango to the increase in PE activity. PE activity was proposed for monitoring change in softening time in CA storage of avocado (Barmore & Rouse, 1976). The failure of the non-ripening mutant of tomato (*rin*) to ripen was also evaluated in terms of PE activity (Beuscher & Tigchelaar, 1975).

The ripe pericarp of tomatoes is rich in PG activity and it was long assumed to be the principal enzyme responsible for fruit softening. However, transgenic tomatoes, in which the accumulation of PG mRNA was suppressed, still softened normally (Smith, Watson et al., 1989). Also, in other fruits, such as strawberry and banana, PG activity is very low or absent, despite evidence for pectin solubilization and degradation (Huber, 1984; Smith, Seymour, Tucker, & Jeger, 1989). PL activity in banana pulp showed substantial increase in activity during ripening (Marin-Rodriguez, 2001). More recently, PL gene expression has been manipulated in transgenic strawberry fruits and suppression of the PL mRNA during ripening resulted in significantly firmer fruits (Jimenez-Bermudez et al., 2002). This suggests that PL could play an important role in fruit softening (Marin-Rodriguez, Orchard, & Seymour, 2002).

3.4.3. Cellulase

Cellulase activity increase was similar in both guava types during fruit ripening (Fig. 2). The correlation was very high between the increase in cellulase activity and flesh firmness ($r^2 = 0.991$).

The role of cellulase in fruit softening is uncertain. Cellulase activity increases during ripening of mango (Abu-Sarra & Abu-Goukh, 1992; Roe & Bruemmer, 1981), avocado (Pesis, Fuchs, & Zauberman, 1978), tomato (Hobson, 1968), date (Hasegawa & Smolenskey, 1971) and strawberry (Maurice & Palehett, 1976). No cellulase activity was detected during ripening of papaya fruit (Selvaraj, Pal, Subramanyam, & Lyer, 1982) or pear (Ahmed & Labavitch, 1980b). Tahir and Malik (1977) reported that, in "Pairi" mango, no appreciable change occurs in cellulose content during ripening. Therefore, in spite of the high correlation between the increase of cellulase activity and the loss of resistance to shearing force, no significant role in tissue softening can be ascribed to cellulase at the present time. In contrast, Roe and Bruemmer (1981), suggested that PG and cellulase are the degrading enzymes responsible for fruit softening during mango fruit ripening.

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