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Physico-chemical changes during growth and development of papaya fruit. II: Chemical changes

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ABSTRACT

Chemical changes during growth and development of female and hermaphrodite fruits of 'Ekostika I', 'Ekostika II' and 'Baladi' papaya fruits were studied. Respiration curves exhibited a typical climacteric pattern. Respiration rate progressively declined to a minimum value at physiological maturity (120 DAA) and then increased in a climacteric pattern with peak of respiration at 140 DAA. Total soluble solid (TSS) and total sugars slightly increased up to physiological maturity and then sharply increased during the ripening phase (125-145 DAA). Reducing sugars, titratable acidity and total protein slightly decreased till physiological maturity and then increased, reaching a peak, which coincided with the climacteric peak of respiration, and subsequently decreased. Phenolic compounds progressively decreased and ascorbic acid content steadily increased. Papaya fruits should be harvested shortly (5-10 days) after physiological maturity, where the fruit attains maximum size and weight, still firm, climacteric rise phase has just started, TSS and sugars start to shoot up and phenolic compounds and acidity are reasonably low.

Keywords: Papaya fruit, chemical changes, growth and development.

INTRODUCTION

Papaya (*Carica papaya* L.) is an important fruit crop throughout the tropical and sub-tropical countries (Salunkhe and Desai, 1984). The ripe fruit is consumed fresh for dessert and in fruit salad or processed. It is highly accepted worldwide and the demand for fresh papaya fruit is increasing for its high content of vitamin C and provitamin A, which has a protective effect against cancer, and its low-calorie status that is recommended for low hypocaloric diets (Lobo and Cano, 1998). In addition papaya fruit is a good source of papain and chymopapain. Both are digestive proteolytic enzymes that digest protein and used as meat tenderizer, as digestive medicine, in pharmaceutical, brewing and tanning industries, and in manufacture of chewing gum (Nakasone and Paul, 1998).

In Sudan, papaya fruit is grown in the southern and central states. Its popularity among consumers is increasing, for its nutritional and medicinal value. Although Sudan has great potential to produce high quality papaya fruits for local and export markets, its marketability is still limited to local markets. This is due to the delicate nature of the fruit, limited know-how, poor handling practices and inadequate transportation and storage facilities.

Harvesting papayas at proper level of maturity is essential for good quality produce. Over-maturity or under-maturity affects the quality adversely. Papaya fruits are generally picked when they exhibit a slight overall loss of green color, with some hint of yellow color at the blossom end. For long distance shipments the fruits are harvested at color-break stage (Kader *et al*, 2002). It is difficult to distinguish immature-green from mature-green fruits. Immature-green fruits will not ripen after long-distance refrigerated transport. Minimum soluble solids content of 11.5% is required by Hawaiian grade standards (Kader *et al*, 2002). Many physical and chemical changes undergone by the developing fruit have been used as means of assigning the optimal picking dates for immediate consumptions or storage. None of these parameters are reliable individually for determining harvest maturity. It usually requires a combination of chemical and physical parameters, coupled with considerable experience (Salunkhe and Desai, 1984).

In a previous study (Shattir and Abu-Goukh, 2010), the physical parameters were evaluated. They found that the fruits of the three cultivars reached physiological maturity after 120 days from anthesis (DAA) and the ripening phase was 125-145 DAA. This study was conducted to evaluate the chemical changes during growth and development of papaya fruit to provide base-line information regarding the biochemistry of the developing fruit and to assist in determining harvest maturity of papaya fruit.

MATERIALS AND METHODS

Experimental site and setup: The experiment was conducted at the Demonstration Farm of Faculty of Agriculture, University of Sennar (44° 12´N, 38° 8´E), which lies in a semi arid zone with heavy clay soils. Two introduced papaya cultivars 'Ekostika I' and 'Ekostika II' released by Malaysian Agricultural Research Development Institute (MARDI) and a local 'Baladi' cultivar were used in the experiment. The papaya trees were raised as described earlier (Shattir and Abu-Goukh, 2010).

Experimental material: Twenty trees from each cultivar were selected randomly (10 female and 10 hermaphrodite) from each replicate. The fruits were tagged at time of anthesis. Chemical changes were determined on 20 fruits picked randomly at the designated stage in both fruit types of the three cultivars. These parameters were determined every 15 days after anthesis (DAA) up to the physiological maturity and then at 5-day intervals till the full-ripe stage. The experiment was arranged in a randomized block design with four replications.

Parameters studied: Respiration rate was determined in a flowing system by total absorption method and expressed in mg CO2 per kg-hr (Mohamed-Nour and Abu-Goukh, 2010). Total soluble solids (TSS) were measured directly from the fruit juice using a Kruss hand refractometer (model HRN-32).Two readings were taken from opposite sides of each fruit and the mean values were calculated and corrected according to the refractometer chart. Thirty grams of pulp of each fruit types of each cultivar were minced and homogenized in 100 ml of distilled water for one min in an electric blender and centrifuged at 10,000 rpm for 10 min in a Gallenkamp portable centrifuge (CF-400). The volume of the supernatant, which constituted the pulp extract, was determined. Total sugars were determined in pulp extracts according to the anthrone method of Yemm and Willis (1954). Reducing sugars were measured according to the technique described by Somogyi (1952). Total and reducing sugars were expressed in grams per100 g fresh weight. The protein dye-binding procedure of Bradford (1976) was used for total protein determination. Titratable acidity was measured according to the method described by Ranganna (1979) and was expressed as percent citric acid. Total phenolic compounds were determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Ascorbic acid content was measured using the 2, 6-dichlorophenol-indophenol titration method of Ruck (1963) and was expressed in mg per100g fresh weight

Statistical analysis: Analysis of variance (Gomez and Gomez, 1984), followed by Fisher's protected LSD test with a significant level of $P \leq 0.05$ were performed on the data.

RESULTS AND DISCUSSION

Changes in respiration rate: The respiration curves of both fruit types of the three papaya cultivars exhibited a typical climacteric pattern (Fig. 1). Respiration rate progressively declined from 15 DAA to a minimum value at physiological maturity (120 DAA) and then increased in a climacteric pattern with peak of respiration at 140 DAA (Fig. 1). Similar pattern was reported in papaya (Selvaraj *et al.,* 1982), mango (Abu-Goukh *et al*., 2005; Lakshaminarayana *et al.,* 1970) and banana (Munasque and Mendoza, 1991). Respiration rate was lowest in 'Baladi' papayas, compared to 'Ekostika I' and 'Ekostika II' at all stages of growth and development (Fig. 1). The differences were minor between 'Ekostika I' and 'Ekostika II' and between the female and hermaphrodite fruits within cultivars. Respiration rate is an excellent indicator for metabolic activity of the tissues, and thus is a useful guide for the potential storage life of fresh fruits and vegetables (Wills *et al.,* 1998). Respiration rate is inversely proportional to shelf-life of the product, the lower rate the longer shelf-life (Day, 1993). Fruits of 'Baladi' cultivar with the lowest respiration rate (Fig. 1) and the highest flesh firmness (Shattir and Abu-Goukh, 2010) at all stages of development, could have a longer shelf-life, compared to the other two introduced cultivars.

Changes in total soluble solids: Total soluble solids (TSS) slightly increased in both fruit types of the three cultivars up to physiological maturity (120 DAA) and then sharply increased during the ripening phase. That increased was about 2.2- folds in both fruit types of the three cultivars (Fig. 2). Similar trends were reported during growth and development in mango (Abu-Goukh *et al*., 2005) and guava (Stivastava and Narasimham,1967) and during ripening in banana (Osman and Abu-Goukh, 2008), mango (Abu-Goukh and Abu-Sarra, 1993), guava, (Bashir and Abu-Goukh, 2003) and tomato (Ahmed and Abu-Goukh, 2003). TSS were significantly lower in the local 'Baladi' cultivar, compared to the introduced ones, which had almost similar amounts of TSS (Fig. 2).

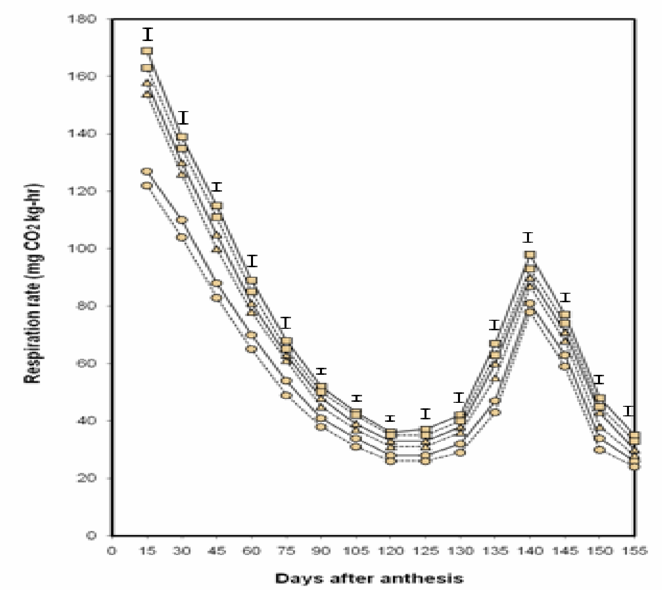

Fig. 1: Changes in respiration rate during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (o), 'Ekostika I' (∆) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

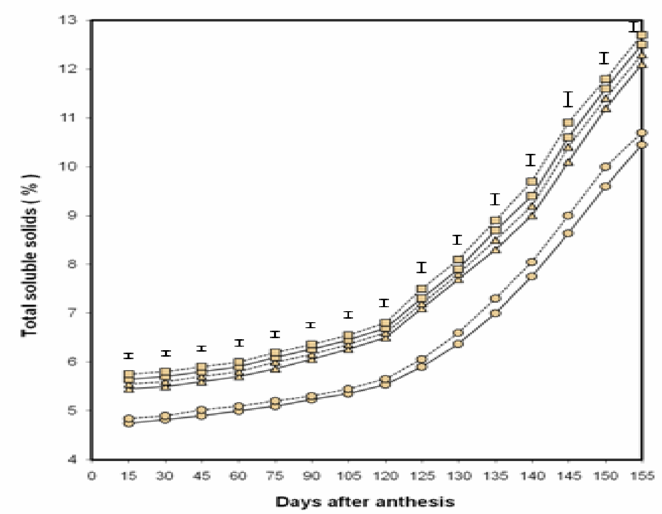

Fig 2: Changes in total soluble solids (TSS) during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (o), 'Ekostika I' (∆) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

Changes in total sugars: Changes in total sugars during growth and development of both fruit types of the three papaya cultivars were similar to those of TSS. Total sugars slightly increased up to physiological maturity (120 DAA) and then sharply increased afterwards (Fig. 3). This is in agreement with earlier reports that sugars do not begin to accumulate in papaya fruit until 100 days from anthesis or during the last 28-42 days of fruit development (Nakasone and Paull, 1998). Selvaraj *et al.* (1982) studied compositional changes during growth and development of four papaya cultivars, reported an increase in dry matter content from about 7% at 15 DAA to 13% at harvest. During that period, there was a steady decline in alcohol-insoluble solids, starches and several minerals, and an increase in total sugars. Similar pattern was found in mango (Abu-Goukh *et al.,* 2005), banana (Munasque and Mendoza, 1991) and guava (Stivastava and Narasimham, 1967). The remarkable increase in total sugars observed during the ripening phase may be attributed to the increase in starch hydrolysis or sugars conversion. Chan and Kwok (1975) attributed the increase in total sugars to the action of papaya-invertase enzyme (β fructo-furanoside enzyme) that catalyzes the conversion of sucrose. Sucrose metabolism during growth and ripening of papaya fruit were studied by Zhou and Paull (2001). They reported that sucrose phosphate synthase (SPS) activity remained low throughout fruit development. The activity of sucrose synthase (SS) was high 14 DAA and decreased to less than one-fourth within 56 DAA, then remained constant afterwards. Acid invertase (AI) activity was low in the young fruits and began to increase 90 DAA and reached a peak more than 10-folds higher, 125 DAA, as sugar accumulated in the flesh. These results suggested that SS and AI are the two major enzymes that may determine papaya fruit sink strength in the early and late developmental phases, respectively. The activity of AI paralleled sugar accumulation and may involve in phloem sugar unloading.

Changes in reducing sugars: Reducing sugars slightly decreased till physiological maturity (120 AA) in both fruit types of the three cultivars, then sharply increased, reaching a peak at 140 DAA, which coincided with the climacteric peak of respiration, and subsequently decreased thereafter (Fig. 4). That increase was 3.1-, 3.2- and 3.3-folds in the female fruits and 3.0-, 3.2- and 3.3-folds in the hermaphrodite fruits of 'Baladi', 'Ekostika I' and Ekostika II' cultivars, respectively. Similar pattern of reducing sugars changes was reported in mango (Abu-Goukh and Abu-Sarra, 1993; Abu-Goukh *et al.,* 2005) and guava (Bashir and Abu-Goukh, 2003). Climacteric fruits, in particular, may show considerable changes in sugar content during fruit ripening (Whiting, 1970). During ripening, starch and sucrose are converted to glucose, which is the main substrate utilized in respiration (Wills *et al*., 1998).

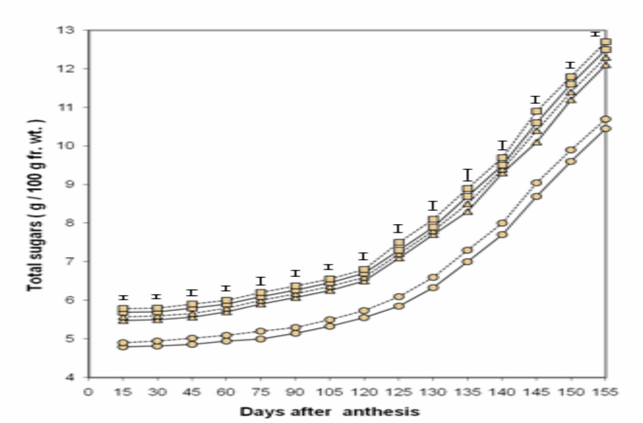

Fig. 3: Changes in total sugars during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (ο), 'Ekostika I' (Δ) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

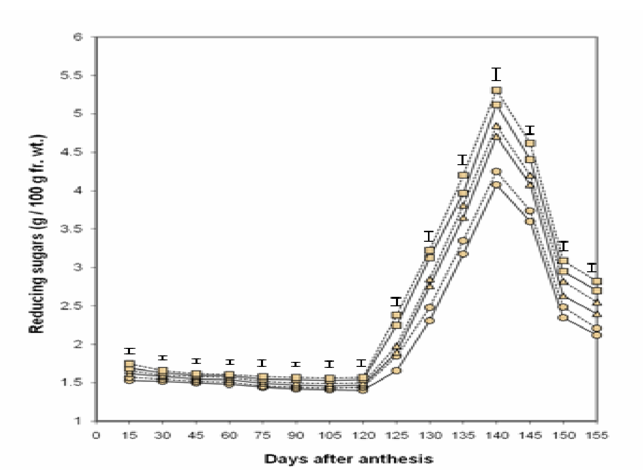

Fig. 4: Changes in reducing sugars during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (ο), 'Ekostika I' (Δ) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

Changes in total protein: Total protein gradually decreased up to physiological maturity (120 DAA) then sharply increased to reach a peak, which coincided with the climacteric peak of respiration at 140 DAA and finally dropped (Fig. 5). That increase in total protein during the climacteric rise was about 3-folds in both fruit types of the three cultivars. Similar results were reported during growth and development of mango fruits (Abu-Goukh *et al*., 2005; Lakshaminarayana *et al*., 1970). Quantitative changes in soluble protein during fruit ripening have been repeatedly demonstrated (Mattoo and Modi, 1969). Abu-Goukh and Abu-Sarra (1993) reported that 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. Similar results were reported in guava (Bashir and Abu-Goukh, 2003) and tomato (Ali and Abu-Goukh, 2005). During the climacteric phase of respiration, there is a decrease in free amino acids, which reflects an increase in protein synthesis, while during senescence, the level of free amino acids increases reflecting, breakdown of enzymes and decrease in metabolic activity (Wills *et al*., 1998). Proteins and free amino acids are minor constituents of fruits and not known to have a role in determining the eating quality. Proteins in fruits are mostly functional, such as enzymes, rather than acting as a storage pool, as in grains and nuts. Changes in their content indicate variation in metabolic activities during development of the fruit (Wills *et al*., 1998). The observation that the amino acid methionine may be possibly act as an immediate precursor of ethylene in fruits (Yung *et al*., 1982), signifies the importance of amino acids metabolism in fruit ripening. The increase in protein content during the climacteric phase coincided with increased activity of polygalacturenase and cellulase enzymes in mango (Abu-Sarra and Abu-Goukh, 1992), guava (Abu-Goukh and Bashir, 2003) and tomato (Ali and Abu-Goukh, 2005). Chen and Paull (2005) reported that the endoxylanase (EC 3.2.18) and A32.5-kDa xylanase (Cpa EXY1) activities increased progressively during ripening and softening of papaya fruits. The decline in the protein at the over-ripe stage was explained as breakdown of protein during senescence, which again supports the view that proteins in ripening fruits are mainly enzymes required for the ripening process (Abu-Goukh and Bashir, 2003; Ali and Abu-Goukh, 2005; Frenkel *et al*., 1968).

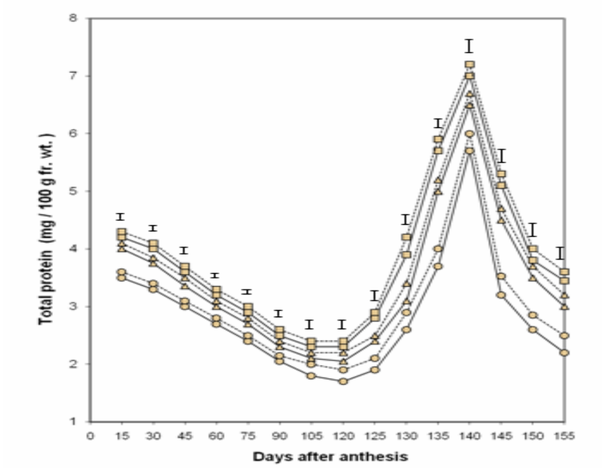

Fig. 5: Changes in total protein during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (o), 'Ekostika I' (Δ) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

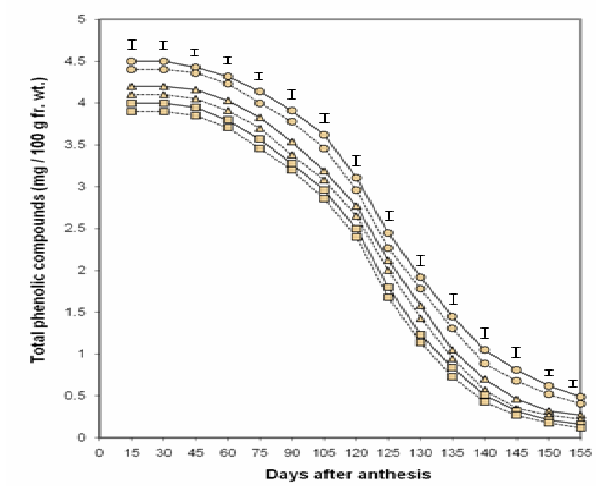

Fig. 6: Changes in total phenolic compounds during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (o), 'Ekostika I' (Δ) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

Changes in total phenolic compounds: Phenolic compounds progressively decreased throughout growth and development in both fruit types of the three papaya cultivars (Fig. 6). At the final ripening period (155 DAA), total phenolics drop was 9.2-, 15.5- and 25.0-folds in the female fruits and 10.7-, 18.6-,and 27.8- folds in the hermaphrodite fruits of 'Baladi', 'Ekostika I' and 'Ekostika II' cultivars, respectively. Similar results were reported during growth and development in mango (Abu–Goukh *et al*., 2005, Lakshaminarayana *et al.,* 1970), guava Mowlah and Itoo,1982) and date (Al-Ogaidi and Mutlak, 1986). The decrease in phenolic compounds with fruit ripening was also reported in banana (Ibrahim *et al.,* 1994), mango (Abu-Goukh and Abu-Sarra, 1993) and guava (Bashir and Abu-Goukh, 2003). Phenolic compounds have been repeatedly demonstrated to play a vital role in plant resistance and protect fruits and vegetables against insect pests (Abu-Goukh *et al.,* 2003) and diseases (Raa, 1968). Abu-Goukh *et al.* (2003) found a negative correlation between phenolic compounds and insect infestation during storage of dry dates. A key role was proposed for phenolics in resistance of dates to insect infestation during storage (Abu-Goukh *et al.*, 2003; Al-Ogaidi and Mutlak, 1986). Phenolics were reported to be higher in peel than pulp of mango (Abu-Goukh and Abu-Sarra, 1993) and guava (Bashir and Abu-Goukh, 2003).

Changes in titratable acidity: Titratable acidity steadily decreased during growth and development of both fruit types of the three cultivars, reached a minimum value at physiological maturity (120 DAA) then increased systematically to reach a peak, which coincided with the climacteric peak of respiration (140 DAA) and then declined sharply thereafter (Fig. 7). Abu-Goukh *et al.* (2005) found that titratable acidity slightly increased in pulp and peel of three mango cultivars up to 20 days before physiological maturity and progressively decreased afterwards. During fruit ripening, titratable acidity was reported to increase up to the climacteric peak and declined afterwards in papaya (Selvaraj *et al.,* 1982), mango (Abu-Goukh and Abu-Sarra, 1993), banana (Munasque and Mendoza, 1991) and guava (Bashir and Abu-Goukh, 2003). These results support the view that acids can be used as substrates for respiration when sugars have been consumed or participated in the synthesis of phenolic compounds, lipids and volatile aromas and provide in addition, a series of metabolites which are used in many processes that reflect dominance of sweet flavor in papaya fruit (Ulrich, 1970).

Changes in ascorbic acid content: Ascorbic acid content progressively increased during growth and development of both fruit types of the three cultivars (Fig. 8). The increase from 15 DAA to 155 DAA was 2.3- folds in 'Baladi', 2.2- folds in 'Ekostika I' and 'Ekostika II' in both fruit types. This is in agreement with the findings of Arrolia *et al.* (1975) who found that ascorbic acid gradually increased during growth and development of papaya fruit and reaching the maximum value at ripeness. The increasing trend of ascorbic acid during growth and development of papaya fruits is an exception to what is generally demonstrated in many fruits. In guava fruits, ascorbic acid content increased gradually during growth and development, reached a maximum values at mature-green stage and then decreased rapidly as fruits ripened (Agnihortri *et al.,* 1962). Ahmed and Abu-Goukh (2003) found an increase in ascorbic acid content in tomatoes up to the table-ripe stage and slight decreased afterwards during ripening. Ascorbic acid was demonstrated to decrease during ripening in mango (Mohamed and Abu-Goukh, 2003) and guava (Bashir and Abu-Goukh, 2003). Both types of 'Ekostika I' and 'Ekostika II' cultivars had higher ascorbic acid content throughout growth and development, compared to 'Baladi' cultivar (Fig. 8).

CONCLUSION

Papaya fruits should be harvested shortly (5-10 days) after physiological maturity, where the fruit attains maximum size and weight, still firm, climacteric rise phase has just started, TSS and sugars start to shoot up and phenolic compounds and acidity are reasonably low.

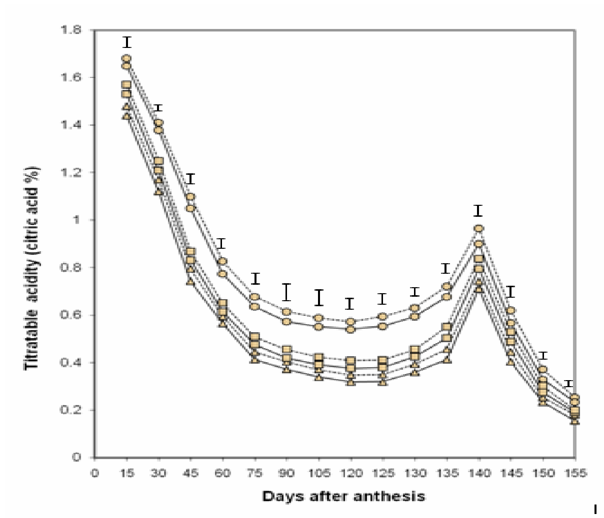

Fig. 7: Changes in titratable acidity during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (o), 'Ekostika I' (Δ) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

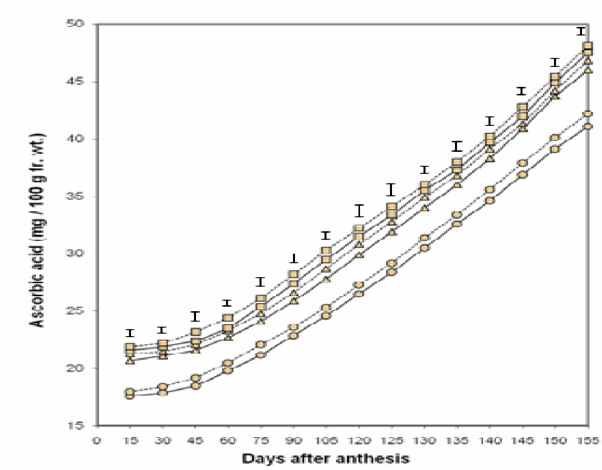

Fig. 8: Changes in ascorbic acid content during growth and development of female (_____) and hermaphrodite (……..) fruits of 'Baladi' (o), 'Ekostika I' (Δ) and 'Ekostika II' (□) papaya cultivars. Vertical bars represent LSD (5%).

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