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Enzyme Activities during the Development and Ripening of Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) Fruit

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ABSTRACT

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai; Cucurbitaceae), is an excellent source of sugars and antioxidants, mainly lycopene, which makes it a nutritionally good fruit. The present study was carried out with the purpose of evaluating the activities of various enzymes in relation to the biochemical processes occurring at five sequential stages of watermelon fruit: young, pre-mature, mature, pre-ripened and ripened. Maximum activity (0.010 mg maltose released/mg protein) of β-amylase (free), one of the starch hydrolyzing enzymes, was found in the pre-mature stage, while β-amylase (bound) exhibited highest activity (0.063 mg maltose released/mg protein) during the pre-ripened stage. Sugar metabolizing enzymes in the relevant metabolic pathways of sugars were also quantified. Sucrose phosphate synthase and sucrose synthase exhibited maximum activities in the young and pre-mature stages, respectively. These activities were significantly higher ($P < 0.05$) than in other development and ripening stages. The sucrose-cleaving enzyme, acid invertase, showed remarkably high activity (0.014 µmol/h/mg protein) in the ripe stage, as did neutral invertase in the mature stage (0.029 µmol/h/mg protein). β-Galactosidase activity (0.035 µmol pnp/mg protein) was maximum in the mature stage, while activities of polygalacturonase (0.025 mg glucose released/mg protein) and cellulase (0.01 mg glucose released/mg protein) were maximum in the young stage. The specific activity of peroxidase was significantly high (0.0034 units/mg protein) in the pre-mature stage whereas polyphenol oxidase exhibited inconsistent activity, but with a maximum in the ripened stage (3.49 units/mg protein). The overall results indicate a positive relation between enzymes (sucrose phosphate synthase, sucrose synthase, β-galactosidase, amylases and polyphenol oxidase) and the development and ripening of watermelon fruit.

Keywords: antioxidant enzymes, softening, sucrose phosphate synthase, sucrose metabolism

INTRODUCTION

Citrullus lanatus, commonly known as watermelon, belongs to the Cucurbitaceae family which consists of about 120 genera and more than 800 species (Jeffrey 1990). Among the 25 species in the genus *Cucurbita*, four species of *Citrullus* are recognized and one of which is widely grown for its edible fruit (USDA 1997). Though watermelon was first cultivated in Africa its cultivation flourished in the Mediterranean regions for centuries before it was introduced into Asia, including India (Pratt 1970). In India, which is the third largest producer of watermelon in the world, the plains of Uttar Pradesh, Gujarat, Maharashtra and Andhra Pradesh are the major states that cultivate watermelon (Singh *et al*. 2009). The Food and Agricultural Organization statistics (FAOSTAT) report published in 2009 indicates watermelon production in India to be 0.3% of the total watermelon production in the world (3,282,35 tonnes).

C. lanatus, an annual species which exists as cultivated, semi-domesticated or wild forms (Munisse *et al*. 2011), is grown worldwide usually in regions with a long warm growing season (Yau *et al*. 2010). Watermelons vary in shape from globular to oblong; the rind color of these fruits also varies from light to dark green and may be solid, striped or marbled. Flesh color varies from yellow, orange, pink or red in most of the commercial varieties. These fruits contain 91.45 g water, 7.55 g carbohydrates, 0.4 g total dietary fiber and 0.6 g protein in a 100 g of edible flesh (USDA 2006). The principal attributes of eating quality in watermelon fruit are good flesh color, flesh crispness, and sweetness (Pratt 1970). The red flesh watermelon is an important source of carotenoids, including lycopene, β-carotene and also citrulline (Munisse *et al*. 2011).

According to Prasanna *et al*. (2007), fruit ripening is "a highly coordinated, genetically programmed and irreversible phenomenon involving a series of physiological, biochemical and organoleptic changes that leads to the development of a soft and edible fruit with desirable quality attributes". Payasi *et al.* (2009) opined that softening is a developmentally programmed ripening process, associated with biochemical changes involving hydrolytic enzymes such as polygalacturonase, pectin methyl esterase, pectate lyase, rhamnogalacturonase, cellulase and β-galactosidase. Softening of melon fruit during ripening involves modifications of cell walls but the mechanisms and enzymes involved are not well characterized (Ranwala *et al*. 1992a).

Sugar accumulation, a key process in determining fruit quality, is controlled by both translocation of sugars and their metabolism in developing fruits (Yativ *et al*. 2010). Liu *et al*. (2010) conducted an in-depth study on the mechanism of grafting effects on muskmelon carbohydrate metabolism. They found that in the latter growth stages of the fruit sucrose accumulation commences with an increased activity of sucrose phosphate synthase (SPS; EC 2.4.1.14) and decreased activity of acid invertase (AI; EC 3.2.1.26). Studies involving watermelon by various researchers in the past have been restricted to understanding the ripening process with emphasis on sugar metabolism (Hubbard *et al*. 1989; Gao *et al*. 1999; Stepansky *et al*. 1999) and on the activities of various enzymes such as β-galactosidase (β-Gal; EC 3.2.1.23) (Ranwala *et al*. 1992a), peroxidase (POD; EC 1.11.1.7), polyphenol oxidase (PPO; EC 1.14.18.1) (Lamikanra and Watson 2001), polygalacturonase (PG; EC 3.2.1.15) and α-amylase (EC 3.2.1.3) (Matsui and Yoshida 1992). However, information regarding the activities of en-

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zymes involved in the physiological processes of watermelon during successive stages of development and ripening is limited.

According to Prasanna *et al*. (2007), amylases are involved in starch degradation where α-amylases produce a mixture of glucose and maltose, whereas β-amylases (EC 3.2.1.3) catalyze the production of maltose. The actions of POD and PPO primarily affect the ability of fresh and processed fruits and vegetables to retain their characteristic flavor and color (Lamikanra and Watson 2001).

In the present study, we have investigated the changes in the activities of enzymes in watermelon fruit to provide an insight into the physiological changes associated with different stages of watermelon fruit development and also to open avenues for future interrogation into the molecular mechanism of watermelon fruit ripening process.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used were uridine 5-diphosphoglucose (UDPG) disodium salt and 3,4-dihyrdoxyphenylacetic acid (DOPAC) from Sigma-Aldrich (St. Louis, MI, USA) and Alfa Aezar (MA, USA) respectively. 3-(*N*-morpholino) propane sulphonic acid (MOPS), dithiothreitol (DTT), 3-methyl 2-benzothiazolinone hydrazone (MBTH), *p*-nitrophenyl β-galactopyranoside (PNPG), *O*-dianisidine, glucose 6-P and polyvinylpyrrolidone (PVP H-30) were from Hi-Media (Mumbai, India). All other chemicals and reagents used for the present study were of analytical grade.

Plant material

The fruit of watermelon (cv. 'GEN 108') representing five sequential developmental stages, viz.: young, pre-mature, mature, pre-ripened and ripened stages were collected from watermelon plants grown in research plots maintained in the vicinity of Bhanuben Ratilal Doshi School of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India.

Experimental design and sampling methods

Three watermelon fruit samples were harvested for each developmental stage and, after recording physical parameters such as weight, length and diameter, the mesocarpic portion of fresh cut fruit was subjected to assay the enzyme activities.

Extraction and assay of enzymes

1. Amylases

One gram of the fruit sample was weighed and extracted in 5-10 parts of ice-cold 10 mM calcium chloride solution for 3 h at room temperature and the obtained extract was centrifuged twice at 8000 × *g* at 4°C for 20 min. The supernatant was used as enzyme source for amylases. β-amylase (free) extraction was done in 66 mM phosphate buffer (pH 7.0) containing 0.5 M NaCl, centrifuged and the pellet was re-suspended in the phosphate buffer containing 0.5% β-mercaptoethanol which was used as a source of β-amylase (bound). The reaction mixture for the amylase assay contained 1 ml starch and 1 ml enzyme which was incubated at 27°C for 15 min. The reaction was stopped by adding 2 ml of dinitrosalicyclic acid (DNS), and then heated in a boiling water bath for 5 min to activate the enzyme followed by the addition of 1 ml of 40% Rochelle salt to stop the reaction. The final volume in each sample was made up to 10 ml with distilled water and the absorbance was read at 560 nm (Thimmaiah 1999).

2. Sucrose phosphate synthase, sucrose synthase, acid invertase, neutral invertase

The method of Hubbard *et al.* (1989) was followed for the assay of SPS, sucrose synthase (SS; EC 2.4.1.13) and invertases (EC 3.2.1.26) – acid and neutral. The frozen middle mesocarpic melon tissue was extracted using a 1:5 tissue-to-buffer ratio with the buffer containing 50 mM Mops-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 0.05% (v/v) Triton X-100, and 0.5 mg/ml BSA. Homogenates were centrifuged at 10,000 g for 30 s. Reaction mixtures for the assay of SPS activity contained 50 mM Mops-NaOH (pH 7.5), 15 mM $MgCl_2$, 5 mM fructose 6-P, 15 mM glucose 6-P, 10 mM UDPG, and the crude enzyme extract. The mixture was boiled for 10 min to destroy any unreacted fructose or fructose 6-P. After cooling, 1 mL of a mixture of 0.14% anthrone in 13.8 M H_2SO_4 was added and incubated in a 40°C water bath for 20 min, and measured at 620 nm. For the SS assay, the reaction mixtures contained only 10 mM fructose. To determine the AI activity, reaction mixtures contained 100 mM citrate-phosphate buffer (pH 5.0), 120 mM sucrose and the crude enzyme extract. For the activity of neutral invertase (NI) the reaction mixtures contained 50 mM Mops-NaOH (pH 7.5), 90 mM sucrose and the enzyme extract. Reaction mixtures were incubated at 25°C and terminated by placing tubes in boiling water at 0 and 30 min after initiation with enzyme extract.

3. Polygalacturonase

The mesocarpic tissue was homogenized in 0.017 M Tris-HCl buffer (pH 10) containing 5 mM 2-mercaptoethanol and then centrifuged for 15min. The pellet was collected and incubated in 5 ml of extraction buffer (1.7 M NaCl, 50 mM sodium citrate and 15 mM EDTA. pH 5.5). 1% PG and 2 M NH_4Cl were incubated at 37°C. The reaction was initiated by the addition of enzyme extract and incubated further for another 30 min. The reaction was terminated by 5% TCA and centrifuged. After incubation, the reaction mixture was made up to a total volume of 3 ml, followed by addition of 3ml DNS. The mixture was then heated in a boiling water bath for 5 min, warmed, and after the development of red colour, 1 ml of 40% Rochelle salt was added. The amount of reducing sugars formed was measured at 510 nm (Thimmaiah 1999).

4. β-Galactosidase

β-Gal was assayed following the method of Nakamura *et al*. (2003). First the mesocarpic tissue was homogenized in 10 mM sodium-phosphate buffer (pH 7.2) containing 50 mM NaCl. The homogenate was centrifuged for 40 min at 4°C and the supernatant was used later as the enzyme source. The assay mixture consisted of 50 mM sodium acetate containing 0.2 mg/ml of BSA at pH 4.0 and 10 mM of PNPG. The reaction mixture was incubated at 30°C for 5 min before the addition of the enzyme. The enzyme activity was assayed for 10 min and the reaction was stopped by adding 0.5 M sodium carbonate and the resulting *p*-nitrophenol formed was determined at 405 nm (SL207 MINI SPEC, ELICO, India).

5. Cellulase

The mesocarpic tissue was homogenized in 100 mM sodium acetate buffer (pH 6.0) containing 0.2% sodium dithionite and 1% PVP and centrifuged for 20 min. The residue was suspended in 2 volumes of 1 M sodium acetate buffer (pH 6.0) containing 6% NaCl, incubated overnight at 4°C, and then centrifuged. The supernatant was used for the cellulase (EC 3.2.1.4) enzyme assay. A reaction mixture of 1% of carboxymethyl cellulose and 100 mM sodium acetate buffer (pH 5.0) was incubated at 37°C. The reaction was initiated by the addition of 1ml of the enzyme and the aliquots of 0.5 ml were withdrawn at intervals of 2, 4, 6 and 12 h. The reducing sugars formed were determined as described above, following the method cited by Thimmaiah (1999).

6. Peroxidase

The mesocarpic tissue was homogenized in ice-cold 0.1 M phosphate buffer, pH (6.0), strained through two folds of muslin cloth, and centrifuged for 20 min at 4°C. The assay was performed by following the method used by Patel and Rao (2009). The reaction mixture contained 0.01 M *O*-dianisidine, 20 mM H_2O_2, 0.1 M phosphate buffer, pH (6.0) and 2.4 ml distilled water. The mixture was incubated at 30°C and the reaction was started by the addition of the enzyme. After 5 min the reaction was stopped by the addition of 2 N H_2SO_4 and the absorbance was measured at 430 nm.

The enzyme activity was expressed in units, where one unit is the increase in OD by 1 assuming standard conditions.

7. Polyphenol oxidase

The mesocarpic tissue was homogenized in cold acetone and continuously stirred for 10 min. The homogenate was filtered through Whatman No. 42, residue was collected and suspended in 0.1 M citrate phosphate buffer (pH 7.5) and kept overnight at 4°C, before refiltering. The enzymatic assay was performed according to method by Chisari *et al*. (2008) at 505 nm using the standard reaction mixture containing 40 mM DOPAC, 2% (w/v) MBTH, dimethylformamide (DMF), 50 mM sodium acetate buffer (pH 7.0) and the enzyme extract. The reaction was stopped at different times by adding 0.5 mL of 5% H_2SO_4. The blank was prepared by inverting the order between the enzymatic extract and H_2SO_4. One unit of PPO activity was defined as the amount of enzyme that produces 1 µmol of MBTH-DOPAC per minute at 25°C.

Protein content

Protein content in the enzyme was determined by following the method of Bradford (1976) with bovine serum albumin (BSA) as a standard.

Statistical analysis

Data was represented as means ± standard deviation from three biological independent analyses. One-way analysis of variance (ANOVA) was performed according to a factorial design using a Complete Randomized Design (CRD), with the stages of development and ripening and replicates as the main factors. Duncan's Multiple Range Test (DMRT) was employed to determine the statistical significance ($P < 0.05$) of the differences among the mean values. Significant differences were indicated by different letters in the table. The statistical analysis of the data was performed using the IRRISTAT software (Bliss 1967).

RESULTS AND DISCUSSION

Starch hydrolyzing enzymes

An altered specific activity of amylase during the course of watermelon fruit development was observed. As shown in the **Table 1**, amylase activity increased gradually with its lowest value (0.0019 mg maltose released/mg protein) in the pre-mature stage and maximum activity (0.049 mg maltose released/mg protein) in the ripened stage. However, intermediate phases between well-defined developmental stages showed about 9% reduction before the surge (**Table 1**). **Fig. 1** shows the specific activity of both free and bound form of β-amylase enzyme. During fruit ripening process, the specific activity of β-amylase (free) which occurs considerably at high level in pre-mature stage is significant at $P < 0.05$, but with minimal changes of it during ripening (**Fig. 1**). However, β-amylase (bound), showed an increase in its activity up to the pre-ripened stage (0.063 mg maltose released/mg protein), following which there was a significant decrease in the activity of this enzyme upon ripening (**Fig. 1**). Amylase enzyme hydrolyzes starch to yield monomeric carbohydrates in tomato (*Lycopersicon esculentum* L.) (Alam *et al*. 2006). The minor changes in activity of amylases during ripening may suggest the presence of different isoforms, with ability to degrade specifically insoluble polysaccharides or soluble carbohydrates depending on the time of their expression (Kossman and Lloyd 2000). According to Ahmed (2009), the amylase activity in muskmelon (*Cucumis melo* L.) decreased with the advancement of ripening as the starch was absent or only minor starch reserves was present for conversion to sugars. Similarly, Lima *et al*. (2001) reported that in mango (*Mangifera indica* L.), only traces of the starch can be detected and amylase activity also reduced substantially when the fruit is fully ripened. Amylases in fruits are involved in the transformation of starch to low molecular weight sugars which in turn may be used during respiration and other energy consuming processes. Thus the present data regarding the activity of amylases indicate that the increase in β-amylase activity was closely related to the breakdown of starch during the development and ripening of watermelon fruit.

Table 1 Specific activity of amylases during development and ripening of watermelon.

Stages	Amylase [a]
Young	0.022 ± 0.004 a
Premature	0.0019 ± 0.0015 a
Mature	0.209 ± 0.030 a
Pre-ripened	0.017 ± 0.012 a
Ripened	0.049 ± 0.025 a

[a] mg maltose released/mg protein

Means followed with the same letter within each column are not significantly different, by Duncan's multiple range test (DMRT). $P < 0.05$

* Values represent mean ± Standard Deviation (SD)

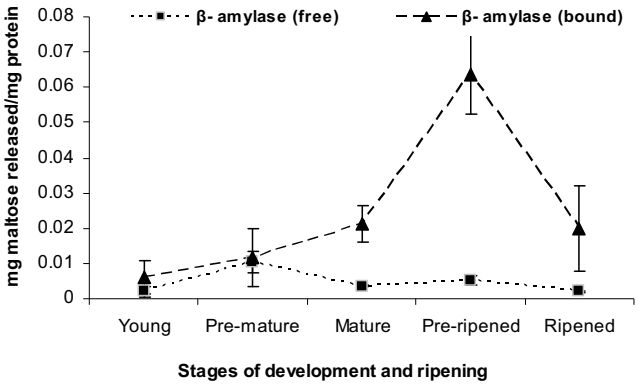

Fig. 1 Specific activities of β-amylases (free, bound) (mg maltose released/mg protein). Values represent mean ± standard deviation (SD).

Sugar metabolizing enzymes

As the watermelon fruit continued its growth, the specific activity of SPS increased significantly in the pre-mature stage ($P < 0.05$). However, upon ripening, the SPS activity declined to 0.0088 µmol/h/mg protein. A similar trend was observed for the activity of SS which displayed maximum in the pre-mature stage (0.343 µmol/h/mg protein) and decreased in the subsequent stages of development and ripening (**Table 2**). The data for activities of sucrose cleaving enzymes (AI and NI) showed that even though the specific activity of AI increases in the young fruit, it subsequently declined. However when fully ripe, the activity of AI increased by 4 fold (0.014 µmol/h/mg protein), while NI showed remarkably higher activity in the mature stage (0.029 µmol/h/mg protein), but decreased in later stages (**Table 2**). Likewise, Ranwala *et al*. (1992b) and Poovaiah and Veluthambi (1985) reported that soluble AI activity increases as fruit ripens and can be correlated with an increase in hexose sugars in muskmelon and strawberry (*Fragaria* x *virginiana* Duch.) respectively. In the early stages of watermelon fruit development, the activities of AI and NI enzymes are found to be low, while that of SS was high.

The SPS implicated in sucrose synthesis exhibited low activity towards ripening. The probable reason for this outcome is evident from published work in cherry laurel (*Prunus laurocerasus* L.), where this decrease was attributed to the enzymatic hydrolysis of sucrose to glucose and finally fructose that gets translocated to the fleshy portion of the fruit (Var and Ayaz 2004). SS involved in the sucrose cleavage process showed increasing activity in the early stages of fruit development, while activities of AI and NI were low. This supports the findings of Liu *et al*. (2010) who suggested that SS activity is high in the early stages of muskmelon so as to make up for the deficiency of invertase activities to hydrolyze sucrose.

Information regarding the relative activity of the sugar

Table 2 Specific activities of sugar metabolizing enzymes- sucrose phosphate synthase (SPS), sucrose synthase (SS), acid invertase (AI) and neutral invertase (NI) during the development and ripening of watermelon.

Stages	SPS [a]	SS [a]	AI [a]	NI [a]
Young	0.015 ± 0.0005 c	0.084 ± 0.064 a	0. 0.0042 ± 0.0001 a	0.0052 ± 0.0006 a
Pre-mature	0.014 ± 0.0013 c	0.343 ± 0.08 b	0. 0.0045 ± 0.00021 a	0.0015 ± 0.0002 a
Mature	0.011 ± 0.0013 b	0.309 ± 0.173 ab	0.0038 ± 0.00043 a	0.029 ± 0.009 b
Pre-ripened	0.010 ± 0.0044 b	0.208 ± 0.084 ab	0.0055 ± 0.00045 a	0.004 ± 0.00045 a
Ripened	0.0088 ± 0.01 a	0.214 ± 0.181 ab	0.014 ± 0.0165 b	0.0097 ± 0.0037 a

[a] µmol/h/mg protein
Means followed with the same letter within each column are not significantly different, by Duncan's multiple range test (DMRT). $P < 0.05$
* Values represent mean ± Standard Deviation (SD)

cleaving enzymes during the early stages of development in muskmelon may shed light on physiological and developmental roles of these enzymes (Gao *et al*. 1999). During the sucrose accumulation phase, when SPS activity is high and AI is low, sucrose accumulation may occur via a dual mechanism, i.e. intact storage of the translocated sucrose, without hydrolysis and re-synthesis of sucrose via SPS (Hubbard *et al.* 1989). Furthermore, Hubbard *et al*. (1989) reported higher activity of SPS in muskmelon and opined that sucrose may be synthesized from alternate substrates other than those derived from sucrose hydrolysis. The enzyme activity data alone cannot determine the most likely metabolic pathways but the distinct patterns of activity presented may suggest preferred pathways at different developmental stages of the fruit (Gao *et al*. 1999). Sucrose was reported as the predominant sugar in ripe watermelon fruit (Elmstrom and Davis 1981a; Pardo *et al*. 1996) and the relative concentration of sucrose is influenced by cultivar and stage of maturity (Elmstrom and Davis 1981b; Chisholm and Picha 1986). According to Fukuoka *et al*. (2009) fruit temperatures in watermelon have a major role in sugar accumulation during the latter half of the fruit developmental period. Lingle and Dunlap (1987) stated that the activities of the sugar metabolizing enzymes in muskmelon may be affected by environmental factors. In view of these observations and findings in muskmelon and watermelon, a positive association can be made between the activities of sucrose metabolic enzymes and sucrose accumulation which ultimately determines the fruit quality of watermelon.

Softening enzymes

PG, one of the major enzymes involved in the softening process and cell wall modification, showed fluctuation in its activity throughout the development and ripening of watermelon fruit. PG activity was statistically significant at $P < 0.05$ in the young stage (**Fig. 2**). Wallner and Walker (1975) stated that PG is absent in green fruits of tomato but show a dramatic increase in its activity towards ripening. This increase is assumed to be functionally significant in terms of cell wall degradation. However, our findings are in contradiction with a previously published report on muskmelon fruit in which the authors have attributed the softening process to be PG dependent (McCollum *et al*. 1989; Matsui and Yoshida 1992). According to Hobson (1962), an increase in the activity of PG has long been associated with fruit ripening, although the amount detected varies between species. Research by Gross (1991) in tomato and Gray *et al*. (1992) indicated that even though PG is important for degradation of pectins, it is not the sole determinant of tissue softening during ripening. This conclusion is supported by the findings of Figueroa *et al*. (2010). Studies in Chilean strawberry (*Fragaria chiloensis*) by Figueroa *et al*. (2010) confirmed that PG activity was more related to pectin solubilization rather than depolymerization.

As the watermelon fruit continues its growth, in the mature stage β-Gal showed highest level of activity, which is significant at $P < 0.05$, but decreased eventually (**Fig. 3**). In muskmelon fruit, Ranwala *et al*. (1992a) reported involvement of β-Gal in the modification of cell wall components during the ripening process. Studies of β-Gal in apples by Bartley (1974) demonstrated that increased activity of the enzyme during ripening could hydrolyze galactan and therefore β-Gal may be responsible for the low content of the cell wall. Wallner and Walker (1975) in their observations on glycosidases in tomato fruit revealed that though high β-Gal activity was found during ripening, there was no release of galactose either from isolated cell walls or a galactose-rich polysaccharide. Studies by Ali *et al*. (2004) revealed higher β-Gal activity in mature tropical fruits which indicates that it contributes in cell wall modifications and softening.

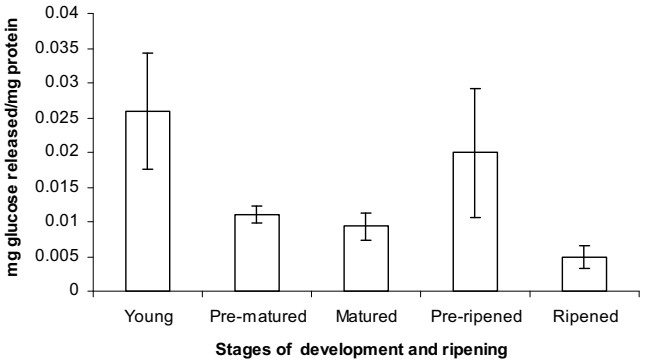

Fig. 2 Specific activity of polygalacturonase (PG) (mg glucose released/mg protein). Values represent mean ± standard deviation (SD).

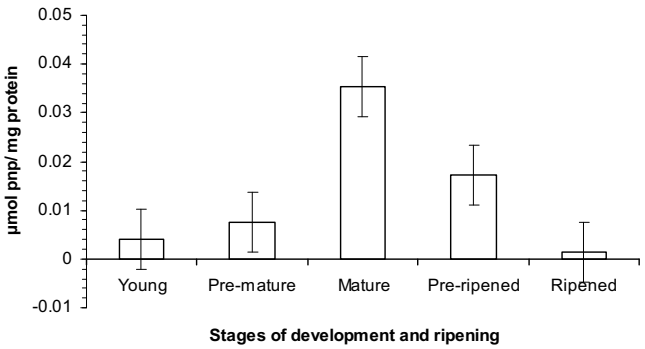

Fig. 3 Specific activity of β-galactosidase (β-Gal) (µmol pnp/mg protein). Values represent mean ± standard deviation (SD).

Cellulase exhibited maximum activity (0.010 mg glucose released/h/mg protein) in the young stage but decreased thereafter until the ripened stage (**Fig. 4**). A study by Colinas-Leon and Young (1981) showed that cellulose is lost from the cell wall only after the initiation of solubilization of other cell wall components (like uronic acids and reducing sugars), which indicates that cellulase acts similar to hemicellulase that hydrolyzes other polysaccharides. However, Awad and Young (1979) reported that cellulases may be synthesized or activated in the early stages of ripening. Hobson (1968) concluded from his studies that cellulase plays an insignificant role in fruit softening. Similarly, Ali *et al*. (2005) also reported cellulase activity in tomato fruit, but they did not attribute any significant role of it in the tissue softening. The loss of fruit firmness was associated with the increasing activity of β-Gal and thus

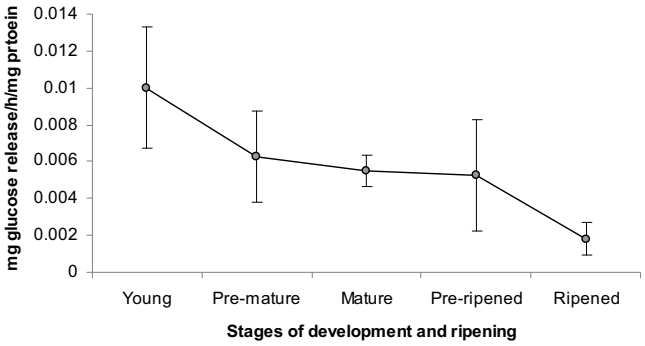

Fig. 4 Specific activity of cellulase (mg glucose released/mg protein). Values represent mean ± standard deviation (SD).

Table 3 Specific activities of antioxidant enzymes - peroxidase (POD) and polyphenol oxidase (PPO).

Stages	POD[a]	PPO[a]
Young	0.00086 ± 0.0001 a	2.58 ± 0.174 b
Pre-mature	0.00294 ± 0.002 a	1.86 ± 0.119 a
Mature	0.00103 ± 0.0009 a	2.069 ± 0.265 ab
Pre-ripened	0.00024 ± 0.000073 a	1.715 ± 0.089 a
Ripened	0.00343 ± 0.0026 a	3.49 ± 0.706 c

[a] units/mg protein

Means followed with the same letter within each column are not significantly different, by Duncan's multiple range test (DMRT). $P < 0.05$

* Values represent mean ± Standard Deviation (SD)

these enzymes play a subsequent role in softening process of watermelon fruit.

Therefore, the results related to the activity of softening enzymes of this study support the earlier findings on cell wall modification and softening process.

Antioxidant enzymes

POD, one of the antioxidant enzymes, displayed an increase in its activity during the early stages (**Table 3**) of the watermelon fruit development and maximum activity in the ripened stage. However no significant difference in the activity of POD was observed during the development and ripening of the watermelon fruit. A similar trend in POD activity was observed in sunberry (*Physalis minima* L.) fruit (Patel *et al*. 2011) and muskmelon (Chisari *et al*. 2010). Lamikanra and Watson (2001) suggested that relatively high POD activity levels could substantially limit the commercial shelf-life of cantaloupe melon. Furthermore, the possible limiting of the substrate H_2O_2 and high oxygen atmosphere application to prevent oxidative stress leads to decreased POD activity (Oms-Oliu *et al*. 2008). In contrast, PPO showed an inconsistent pattern in its activity throughout the growth and development of the watermelon fruit. The activity of PPO was initially high in the young stage, declined thereafter, but exhibited statistically significant ($P < 0.05$) increased activity by two fold in the fully ripened stage (**Table 3**). According to Lamikanra and Watson (2001) POD activity is not necessarily related to the total phenol content of fruits and vegetables and only a relatively small part of food phenolics can serve as substrates for PPO. The activities of PPO and POD were found to be higher in the beginning of the melon development and during the final process of its ripening. These increased activities of PPO and POD may be due to higher metabolic activity during the developmental stages of melon fruit. However Chisari *et al*. (2008) noted the synergistic effect of PPO-POD in muskmelon through the generation of H_2O_2 in PPO-catalyzed reactions and also the action of POD of semi-quinonic intermediates of PPO-catalyzed reactions as oxidizing substrates.

CONCLUSION

The data obtained from the present study for the sugar metabolizing enzymes provides an important foundation for future investigations of carbohydrate metabolism which determines to a large extent crop quality and yield in the melon species. The results of softening process suggests that the β-Gal enzyme has a positive relationship, while PG and cellulase activities were negatively correlated with the softening process, an indication of better postharvest storage life. Thus the changes in the relative activity of enzymes analyzed under the present study should help in improvement of the shelf life and quality of watermelon fruit. Further, a comprehensive study combining ultrastructural and biochemical aspects of watermelon fruit would provide an insight into the relationships between its structural and metabolic aspects of enzyme activities.

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