

Nutritional quality of muskmelon fruit as revealed by its biochemical properties during different rates of ripening

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Abstract

Muskmelon (*Cucumis melo* L.) is one of the economically important fruit in the world and the important quality determining parameters of it are carbohydrates and antioxidants. In the present study antioxidants such as phenols, polyphenols, ascorbic acid, carotenoids, total antioxidant activity as well as sweetness related compounds like reducing, non-reducing and total sugars, starch and activities of various enzymes related to these biochemical components were evaluated in muskmelon at its sequential stages of development and ripening. Even though the amounts of sugars and phenolics increased with the progress of ripening, other antioxidants like ascorbic acid, carotenoids and level of total antioxidant activity however, decreased. The activities of antioxidant enzymes such as peroxidase and polyphenol oxidase gradually increased towards ripening with their maximum of it in the ripened and pre-ripened stage respectively, whereas catalase displayed its maximum activity in the premature stage. Sugar metabolizing enzyme, sucrose phosphate synthase exhibited positive correlation with the sucrose accumulation concomitant with the decreased activities of sucrose synthase, acid invertase and neutral invertase towards ripening of the muskmelon fruit. Ultimately the carbohydrates and bioactive compounds with their accumulation in the ripe muskmelon fruit suggests its use to the food scientists to maximize the nutritional value, thereby confirming muskmelon fruit as a nutritionally balanced source of dietary antioxidants.

Keywords

Bioactive compounds
enzymes
muskmelon
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sugars

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Introduction

Cucumis melo L. (Reticulatus group), commonly called as cantaloupe or muskmelon, is a member of the Cucurbitaceae family (Bailey, 1976). Consumer preference for this fruit is determined largely by its sweetness (i.e sugar content), flavor or aroma, texture and more recently as a rich source of phytonutrients (Lester, 2008). *Cucumis melo*, in addition to its superior consumer preference, is an extremely healthful food choice as they are rich in ascorbic acid, carotene, folic acid, and potassium as well as a number of other human health-bioactive compounds (Lester and Hodges, 2008). Hubbard *et al.* (1989) advocated that midway through their development, fruits of muskmelon undergo a metabolic transition marked by both physical and compositional changes such as netting of the exocarp, mesocarp softening, and the onset of sucrose accumulation. Prasanna *et al.* (2007) stated that 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.

Sugar content and composition are the major criteria used in judging the quality of the fruit of muskmelon. The composition of stored sugars in several plant species has been associated with key enzymes responsible for sucrose metabolism (Lingle and Dunlap, 1987). As per the studies in developing muskmelon fruit by Hubbard *et al.* (1989) sucrose phosphate synthase (SPS) (EC 2.4.1.14) and acid invertase (AI) (EC 3.2.1.26) are the determinants of sucrose accumulation and the role of sucrose synthase (SS) (EC 2.4.1.13) enzyme in sucrose accumulation remains obscure.

The biosynthesis and degradation of starch are said to be closely associated with fruit development and commercial quality formation (Zhang and Wang, 2001). Kanwal *et al.* (2004) correlated the amylase activity in fruits with the ripening process and rise in respiration. Further, Lajalo (2001) cited that the starch contents of fruits are degraded in a complex process, during ripening period, involving α and β -amylases (EC 3.2.1.1, EC 3.2.1.2). Plant cells possess enzymatic mechanisms that may delay the deleterious effects of free radicals with the action of antioxidant enzymes like superoxide dismutase (SOD) (EC 1.15.1.1) which catalyses the dismutation

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of O_2 in H_2O_2 , while removal of H_2O_2 is assumed by catalases (CAT) (EC 1.11.1.6) and/ or peroxidases (POD) (EC 1.11.1.7) (Lacan and Baccou, 1998).

However, a perusal of literature revealed that even though in the past, several researchers (e.g. Lingle and Dunlap, 1987; Hubbard *et al.*, 1989; Wang *et al.*, 1996; Gao *et al.*, 1999 and Lester, 2008) made attempts to study the development and ripening of the muskmelon fruit, these studies did not encompass the enzyme activity parameters. Moreover, complete profile of the quality traits, which are important for retail (e.g., sugar content, postharvest shelf life) and human nutrition (e.g., mineral, vitamin, and phytonutrient contents), of muskmelon has also least been investigated. Also little information is known regarding the correlation between the bioactive compounds and antioxidant activity in muskmelon fruit. Therefore, the present study has been undertaken with a view to fill the gap in the literature on the biochemical properties and various quality attributes in terms of their nutritional composition in the muskmelon fruit. It also provide base-line information regarding the biochemistry of the developing fruit and helps in the selection of the right harvesting maturity as well as in developing technologies for enhancing shelf life of muskmelon fruits, their transportation and storage conditions.

Materials and methods

The fruits of muskmelon (cv. Barvani.) were collected at their five sequential stages i.e. young, pre-mature, mature, pre-ripened and ripened from the river beds of Orsang River, Sankheda region of Gujarat, India. The selection of fruit for its sequential stages of development and ripening was made based on the morphological characteristics such as size, weight, color and softening of the rind rather than by days after anthesis.

Quantitative analysis of carbohydrates

One gram of the sample was weighed and extracted with 80% ethanol. The supernatant was collected and evaporated on water bath at 80°C for 5 min, added 10 ml of water and dissolved the sugars. The reducing and non-reducing sugars (by the DNS method), total sugars (by the phenol-sulphuric acid method) and the starch (by anthrone method) were estimated as per the methods described by Thimmaiah (1999).

Extraction and assay of amylases

Extraction of 1 g of fruit sample from the mesocarpic portion of the fruit was done with 5-10 volumes of ice-cold 10 mM calcium chloride solution

for 3 h at room temperature and the obtained extract was centrifuged at 4°C for 20 min. The supernatant was used as crude enzyme source. β -free amylase extraction was done in 66 mM phosphate buffer (pH 7.0) containing 0.5 M NaCl. The extract was centrifuged at 20,000 rpm for 15 min at 4°C and the supernatant was used for the assay. The assay of amylases was done by DNS method, as cited by Thimmaiah (1999).

Extraction and assay of sugar metabolizing enzymes

The method of Hubbard *et al.* (1989) was followed for the assay of SPS, SS and invertases - acid and neutral. The frozen melon tissue (4°C) was grounded in a chilled mortar using a 1:5 tissue-to-buffer ratio. Buffer contained 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.

Reaction mixtures were incubated at 25°C and terminated at 0 and 20 min with 30% KOH. 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 absorbance was measured at 620 nm. For SS assay, the reaction mixtures contained 10 mM fructose and did not contain fructose 6-P or glucose 6-P.

To determine the activity of AI, the reaction mixtures contained 100 mM citrate-phosphate buffer (pH 5.0), 120 mM sucrose and the crude enzyme extract and that of neutral invertase (NI) (EC 3.2.1.26) contained 50 mM Mops-NaOH (pH 7.5), 90 mM sucrose and enzyme extract. Reaction mixtures were incubated at 25°C and terminated at 0 and 30 min after initiation of the reaction with the enzyme extract.

Analysis of antioxidants

Extraction and analysis of phenolics and antioxidant activity

For free phenols, samples were extracted in 50% methanol/water and were vortexed for one minute and heated at 90°C for 3 h with vortexing every 30 min. After the samples were cooled, they were diluted with methanol and centrifuged for 5 min at 5000 rpm. Total phenols were extracted with 1.2 M HCl in 50% methanol/water and treated as above. For free polyphenols samples were extracted in 60% methanol and were vortexed for one minute and

heated at 90°C for 3 h with vortexing every 30 min. After the samples were cooled, they were diluted with methanol and centrifuged for 5 min at 5000 rpm. Total polyphenols were extracted with 1.2 M HCl in 60% methanol/water and treated as above. The phenolics were determined by the FCR method, based on the procedures by Vinson *et al.* (2001). The absorbance was measured at 750 nm for phenols (free, total) and 765 nm for polyphenols (free, total). The measurement was compared with a standard of catechin and expressed as catechin equivalent mg-1 fresh weight for phenols, while polyphenols were expressed as mg Gallic acid equivalent (GAE) mg-1 fresh weight. The antioxidant activity was evaluated by using the free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as per the method of Samee *et al.* (2006). A 0.1 ml of aliquot was mixed with the 100 µM of DPPH (dissolved in methanol), kept in dark for 30 min at room temperature and absorbance was measured at 517 nm against methanol as blank. The reading in triplicate was compared with the trolox standard and expressed as trolox equivalent antioxidant capacity (TEAC).

Extraction and analysis of carotenoids and ascorbic acid

The concentration of carotenoids was determined following the procedure by Wang *et al.* (2005). The tissue was extracted in a certain volume of hexane:acetone (v/v) and the organic layer was collected until the solution turned colorless. The absorbance was measured at 450 nm for carotenoids and expressed as mg g⁻¹ f. w. The quantitative analysis of ascorbic acid (AA) was performed as per the method of Roe (1964). A 2 g tissue was homogenized in 5% metaphosphoric acid and glacial acetic acid and centrifuged for 10 min at 5000 rpm. A known volume of aliquot was mixed with 2% 2, 4 dinitrophenyl hydrazine (DNPH) and 10% thiourea, tubes were kept for incubation for 3 h at 37°C. The reaction was terminated by 85% H₂SO₄ and absorbance was measured at 540 nm. The standard graph was prepared by using ascorbic acid and expressed as mg g⁻¹ f. w.

Extraction and assay of antioxidant enzymes

The tissue for the POD assay was homogenized in 0.1 M phosphate buffer (pH 7.2) and 1 mM polyvinyl pyrrolidone (PVP) and the supernatant was taken for the assay. POD was assayed as per the method of Guilbalt (1976). The specific activity of the enzyme was expressed as 1 unit change in OD min⁻¹.mg⁻¹ protein. CAT activity was assayed according to the method of Wang *et al.* (2004). The mesocarpic tissue was extracted with the extraction buffer (0.05 M

sodium-phosphate buffer, pH 7.8 containing polyvinyl pyrrolidone (PVPP). The supernatant was taken for the assay and expressed the activity as the U. mg⁻¹ protein. For the assay of polyphenol oxidase (PPO) (EC 1.14.18.1) enzyme, the tissue was homogenized in cold acetone and continuously stirred for 10 min. The homogenate was filtered, residue was collected and suspended in 0.1 M citrate phosphate buffer (pH 7.5) and kept overnight at 4°C. The enzymatic assay was performed according to method by Chisari *et al.* (2008) at 505 nm using 3, 4-dihydroxyphenylacetic acid (DOPAC) with 3 methyl-2-benzothiazolinone hydrazone (MBTH). The standard reaction mixture contained 40 mM DOPAC, 2% (w/v) MBTH, dimethylformamide (DMF), 50 mM sodium acetate buffer (pH 7.0) and the enzyme extract. Reaction was stopped at different times with 0.5 mL of 5% H₂SO₄. The blank was prepared by inverting the order between the enzymatic extract and H₂SO₄. 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 assay

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

Statistical Analysis

Data were represented as mean of triplicates. One way analysis of variance (ANOVA) was performed according to a factorial design on the basis of complete randomized design (CRD). 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 and its hydrolytic enzymes

Starch, is the main storage polysaccharide in many unripe fruits. An increase by 4 fold was observed in the starch content during the development of the muskmelon fruit from its young to premature stage with maximum of it in the premature stage (Figure 1 A). However the starch content decreased in a consistent manner with the advancement of ripening. As the accumulated starch provided the substrate for sugar synthesis during muskmelon ripening, the starch hydrolytic enzyme activities also were evaluated. From Figure 2, activity of α-amylase was high in the

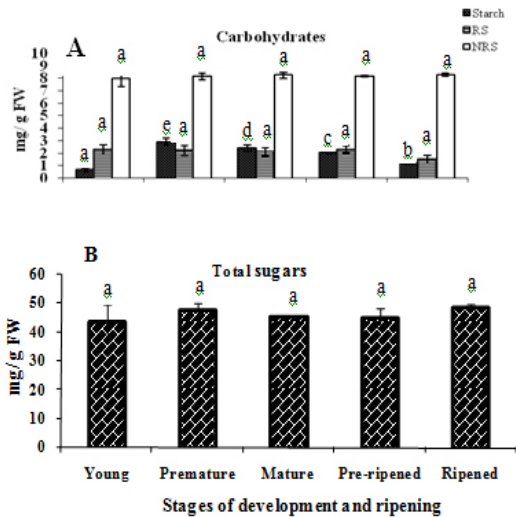


Figure 1. Quantitative analysis of starch (A), reducing sugars (RS) (A) and non-reducing sugars (NRS) (A) and total sugars (B) in muskmelon cultivars in its sequential stages of development and ripening (mg/g FW). Means with the same letter are not significantly different ($P < 0.05$), by DMRT

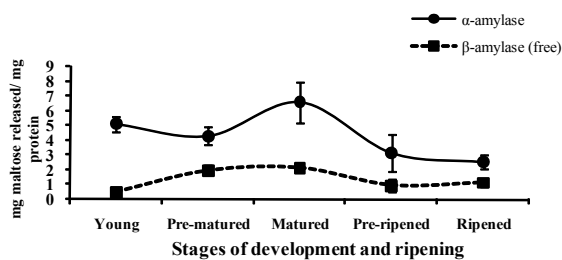


Figure 2. Specific activities of amylases (α , β -free) in muskmelon in its sequential stages of development and ripening (mg maltose released/mg protein). Means in the same column followed by the same letter are not significantly different ($P < 0.05$), by DMRT

pre-mature stage and achieved maximum activity in the mature stage of the fruit and declined thereafter. On the other hand, β -amylase (free) activity was barely detected during fruit development but with its maximum activity in the mature stage (Figure 2). From the results of the present study a progressive increase in the amylase activity was observed with the degradation of starch throughout the development and ripening of muskmelon fruit.

As the fruit attains maturity, the increase in amylase activity occurred together with the increase in various energy consuming processes such as respiration and ethylene production. Similar results were reported during the ripening process in mango fruit by Silva *et al.* (2008), where starch disappearance was concomitant to a five fold increase in the amount of sucrose, the most abundant sugar of the ripe fruits. The studies in avocado fruit related to the starch content and amylase activity by Pesis (1978) suggested that starch serves as a substrate for energy and is expected to find a higher hydrolysis rate in the pulp with higher energy consumption.

Sugars and its metabolizing enzymes

Sugars are the main quality determining parameters in the fruit. Quantitative analysis of sugars in muskmelon fruit showed inconsistency in reducing sugar content which was high initially and decreased subsequently, but increased in the pre-ripened stage (Figure 1 A). However, the amount of reducing sugars declined when the fruit attained fully ripe stage. During the entire length of fruit development and ripening of muskmelon, there was an increase in the quantity of the non-reducing sugars with the highest of it in the ripened stage (Figure 1 A). The concentration of total sugars was initially high in the pre-mature stage and decreased abruptly till the pre-ripened stage but statistically no significant difference was found. However, the maximum amount of total sugars was found to be accumulated in the ripened fruit of muskmelon (Figure 1 B). According to Beaulieu *et al.* (2003), reduction in the content of reducing sugars in the fully ripe stage of muskmelon fruit may be due to the accumulation of fructose and glucose in lesser quantities or they decrease or remain unchanged during ripening.

Lester (2008) in his studies in the mesocarp tissue of honeydew melon reported different profiles of sugars like sucrose, glucose and fructose. It was noted that sucrose hydrolyzed to fructose and glucose served as a substrate for respiratory energy production to support growth and storage function. This finding of Lester (2008) supports the work of Hubbard *et al.* (1989) who reported that a substantial pool of hexose sugars is present throughout the development of the fruit but sucrose accumulation was observed in the final stages of ripening of the fruit. The results of the present study regarding the accumulation of sucrose in muskmelon fruit are in agreement with that of Hubbard *et al.* (1989) and Lester (2008).

Studies by Moneruzzaman *et al.* (2008) are of the opinion that increase in the amount of non-reducing sugars from young to the full ripe stage of the muskmelon fruit might be due to the breakdown of starch concomitant with formation of non-reducing sugars. The gradual increase in the total sugar content found in the muskmelon fruit supports the observations of Tsuda *et al.* (1999). The probable reason for the increased total sugar content in ripened muskmelon fruit, as noted by Moneruzzaman *et al.* (2008), might be due to the conversion of starch to sugars.

SPS, involved in the sucrose synthesis process, activity increased with fruit development in a manner similar to the increase in sucrose concentration. It was noted that the significant ($P < 0.05$) activity of the enzyme was in the mature stage followed by the pre-

Table 1. 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 muskmelon

Stages	SPS ($\mu\text{mol}\cdot\text{hr}^{-1}\cdot\text{mg}^{-1}$ protein)	SS ($\mu\text{mol}\cdot\text{hr}^{-1}\cdot\text{mg}^{-1}$ protein)	AI ($\mu\text{mol}\cdot\text{hr}^{-1}\cdot\text{mg}^{-1}$ protein)	NI ($\mu\text{mol}\cdot\text{hr}^{-1}\cdot\text{mg}^{-1}$ protein)
Young	0.183 \pm 0.071 a	14.35 \pm 1.73 c	0.914 \pm 0.51a	1.196 \pm 0.43 c
Premature	0.044 \pm 0.018 a	3.54 \pm 0.46 a	0.068 \pm 0.04 a	0.100 \pm 0.004 a
Mature	1.29 \pm 0.76 b	12.89 \pm 3.09 c	1.87 \pm 1.35 ab	1.015 \pm 0.26 bc
Pre-ripened	0.504 \pm 0.16 a	4.43 \pm 2.9 ab	0.019 \pm 0.005 a	0.625 \pm 0.33 b
Ripened	0.414 \pm 0.017 a	7.68 \pm 1.43b	2.092 \pm 0.70 b	0.060 \pm 0.001 a

Means in the same column followed by the same letter are not significantly different ($P < 0.05$) by DMRT

ripened stage and decreased subsequently (Table 1). The high activity of SPS reaffirms its contribution in sucrose accumulation and also suggests that sucrose may be synthesized from alternate substrates other than those derived from sucrose hydrolysis (Hubbard *et al.*, 1989). The pattern of SPS activity suggested that photosynthate translocated from mother plant is the ultimate source of sugar in muskmelon fruit. The deposition of that translocated sugar within the fruit, and thus the sugar composition was determined by partitioning of sugar within the tissue, and the metabolism of sugar within each compartment (Lingle and Dunlap, 1987). SS, a sucrose cleaving enzyme showed inconsistent pattern in its activity during the different rates of ripening of the muskmelon fruit. SS activity was high in the green tissue of the fruit, decreased subsequently, but exhibited a remarkably high activity of 4 fold attaining the mature stage of the fruit. The activity of SS declined when the fruit attained ripened stage, indicating an accumulation of sucrose in the ripe and fruit of muskmelon (Table 1). Invertases (acid, neutral) which are also involved in the sucrose cleaving process were studied in different developmental stages of the fruit. Unlike SS, regarding the AI and NI, did not show any significant pattern of activities in sucrose cleaving mechanism. However the maximum activities of AI and NI were recorded in the ripe and mature fruit of muskmelon (Table 1). The activities of invertases in relation to ripening process showed negative correlation which retards further sucrose degradation. As stated by Lingle and Dunlap (1987), sugar composition, important fruit quality determining parameter, may be influenced by environmental factors affecting the activity of sugar metabolizing enzymes. Consequently the changes in the activities of these enzymes offered a testable hypothesis for the regulation of the sugar composition of muskmelon fruit and ultimately, fruit quality.

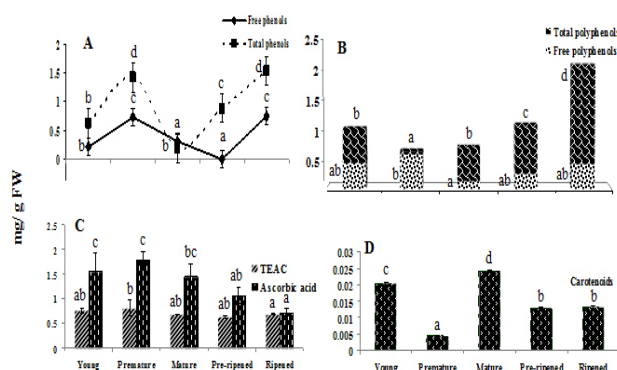


Figure 3. Quantitative analysis of phenols (A), polyphenols (B), Trolox equivalent antioxidant capacity (TEAC) (C), Ascorbic acid (C) and carotenoids (D) in muskmelon in its sequential stages of development and ripening (mg/ g FW). Means in the same column followed by the same letter are not significantly different ($P < 0.05$), by DMRT

Phenolics and total antioxidant activity

Phenolic compounds are widely distributed in plants which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health. The amount of phenolics was measured among which the content of free phenols was initially high and decreased thereafter. However the content of free phenols increased with the advancement of fully ripe stage (Figure 3 A). The amount of total phenols was high in the premature stage, decreased there after but a 2 fold increase was shown in their amount in the ripened stage of the fruit. Free polyphenols content showed inconsistency in its pattern in the muskmelon fruit. The quantity of total polyphenols progressively decreased up to mature stage and increased during the later stages of ripening of the muskmelon fruit (Figure 3 B). Evaluation of the antioxidant activity which is a nutritional quality assessing parameter and the scavenging activity of DPPH radicals is generally used as a basic screening method for testing the antiradical activity of a large variety of compounds. Total antioxidant activity evaluated, was high in the early stages with its maximum in the pre-mature stage. With the onset of ripening, no significant changes was observed in the total antioxidant activity and decreased attaining the ripened stage of the muskmelon fruit (Figure 3 C).

The varied pattern of polyphenol content in ripened stage was probably due to the different extent by which the biosynthetic pathways of these compounds are affected during ripening. Several studies cited by Ismail *et al.* (2010) indicating that the phenolic content exhibited antioxidant and redox properties which allowed them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Toor and Savage (2006) attributed that the breakdown of

Table 2. Specific activities of antioxidant enzymes- peroxidase (POD), polyphenol oxidase (PPO) and catalase (CAT) during development and ripening of muskmelon

Stages	POD (Units. mg ⁻¹ protein)	PPO (Units. mg ⁻¹ protein)	CAT (Units. mg ⁻¹ protein)
Young	0.004 ± 0.0029 ab	0.00893 ± 0.0037a	0.00071 ± 0.0004a
Pre-mature	0.011 ± 0.0056 b	0.0185 ± 0.0124ab	0.0040 ± 0.00095a
Mature	0.0019 ± 0.0009 ab	0.00262 ± 0.0005a	0.00103 ± 0.00099a
Pre-ripened	0.0019 ± 0.00064 a	0.0340 ± 0.0094c	0.00018 ± 0.00015a
Ripened	0.0124 ± 0.00061 ab	0.0041 ± 0.0012a	0.0017 ± 0.00095a

Means in the same column followed by the same letter are not significantly different ($P < 0.05$), by DMRT

cellular structure including vacuoles occurred where soluble phenolic compounds may accumulate, during the advanced ripening stage of fruits, which ultimately leads to the reduction in the content of phenols. The results obtained from the present study regarding the antioxidant activity were in accordance with the studies on the antioxidants obtained in the fruits like honeydew melon and persimmon. Lester (2008) advocated that the tissue exhibiting high antioxidant capacities would better resist oxidative stress as the active oxygen species (AOS) are neutralized by antioxidants, when compared to the tissue with lower antioxidant potential.

Ascorbic acid and carotenoids

Ascorbic acid is an active form of vitamin C and its content varied in different species of fruits and vegetables. Young fruit of muskmelon had more ascorbic acid content than the fully ripened fruit (Figure 3 C). The results showed that ascorbic acid was dependent on the maturity stage. Markus *et al.* (1999) attributed this increase of ascorbic acid with the onset of ripening, and later decreased with advanced ripening, to the antioxidant role of ascorbic acid, which increases with the increased respiration rate in climacteric fruits. Hodges and Lester (2006) in their studies in muskmelon reported that the phytonutrient, ascorbic acid accumulate differently in various cultivars grown in different locations and conditions. Generally, when fruits become over ripe, vitamin C content declines, concurrently with the degradation of fruit tissues (Kalt 2005).

The concentration of carotenoids increased in a consistent pattern up to the mature stage with the maximum in same stage. The amount of carotenoids declined progressively in the subsequent stages of ripening with minimum of it in the pre-ripened stage (Figure 3 D). Similar studies in watermelon fruit by Tlili *et al.* (2011) reported the accumulation of lycopene in chromoplast which is a carotenoid, and

predicted that the progressive activation of molecular mechanisms involved in carotenogenesis regulation may be the reason for this. Bramley (2002) also observed in his studies in tomato that the molecular mechanisms may include regulation at transcriptional or post transcriptional level, metabolite flux into the carotenoids pathway and carotenoids sequestration. Perkins-Veazie *et al.* (2006) opined that carotenoid accumulation is a net result of biosynthesis, turnover and finally stable storage of the end products.

Antioxidant enzymes

Plants defend against the deleterious effects of free radicals by the action of antioxidant enzymes such as POD, PPO and CAT. POD activity was initially high and declined gradually until the pre-ripened stage. However a sudden increase in the POD activity by 6 fold was observed in the muskmelon fruit attaining the ripened stage (Table 2). A similar trend was observed in melons and Chisari *et al.* (2010) stated that POD activity could contribute to determine the firmness of outer tissues, together with the processes involved in early stages of ripening. The previous studies in melons by Laminkara and Watson (2001) indicated that POD activity is not necessarily related to the total phenol content of fruits and vegetables. Enzymatic antioxidants function to scavenge the reactive oxygen species and resist oxidative stress. PPO displayed inconsistency in its activity as it showed increase by 2 fold with development of the fruit from the young to the pre-mature stage. However the activity of the enzyme declined suddenly in the mature stage but exhibited of a significant level ($P < 0.05$) in its activity by 13 fold in the pre-ripened stage and decreased with the onset of fully ripening process (Table 2).

The trend of activity of PPO, during ripening of melon, when at earlier stages corresponded an accelerated metabolism related with higher rates of ethylene production than at more advanced maturity stage (Chisari *et al.*, 2010). CAT enzyme evaluated showed increased pattern of activity in the early stages of ripening with maximum in the premature stage (Table 2). Furthermore the activity declined consistently with the onset of advanced stage of ripening but exhibited an increase in the fully ripe stage of the muskmelon fruit. Earlier reports in cantaloupe melons (Ben-Amor *et al.*, 1999) also exhibited similar trend of CAT activity in the fruit. H_2O_2 is capable of rapid diffusion across the cell membranes and in the ripened fruit its level rises which triggers the catalase in the detoxification of H_2O_2 (Abbasi *et al.*, 2010). Masia (1998) advocated that there is a strong correlation between the surge of cellular oxidants at the onset of ripening and

increase in antioxidant enzymes including CAT. Thus the increased activities of these enzymes suggested the continuous production of reactive oxygen species with a parallel increase in their detoxifying/scavenging enzymes.

Conclusion

The important fruit quality determining parameter, carbohydrates, based on the data obtained indicated that sweetening of muskmelon fruit seemed to be as a result of sucrose, which is the predominant sugar as the sucrose accumulation was highly correlated with the starch degradation. In this regard the mobilization of the starch in the mother plant could be from the concerted action of activities of amylases. The sugar metabolizing enzymes also positively correlated with the accumulation of sucrose in the muskmelon fruit and therefore the overall carbohydrates and enzymatic profiles suggest a common feature for non photosynthesizing starch-accumulating organs. The study of bioactive compounds and antioxidant activity is of great relevance both to human health and commercial purposes as it provides valuable information about their synthesis thereby evaluating the best harvest period to reach highest antioxidant potential. Ultimately the accumulation of carbohydrates and bioactive compounds in the ripe muskmelon fruit suggests its use to the food scientists to maximize the nutritional value, thereby confirming muskmelon fruit as a nutritionally balanced source of dietary antioxidants and also essential in revealing the biosynthetic pathways of these compounds in muskmelon.

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References

Abbasi, N. A., Singh, Z. and Khan, A. S. 2010. Dynamics of antioxidant levels and activities of reactive oxygen-scavenging enzymes in 'Pink lady' apple fruit during maturation and ripening. *Pakistan Journal of Botany* 42: 2605-2620.

Bailey, L. H. and Bailey, E. Z. 1976. *Hortus Third*. Macmillan Pub. Co. New York.

Beaulieu, J. C., Lea, J. M. and Eggleston, G. 2003. Sugar and organic acid variations in commercial cantaloupes and their inbred parents. *Journal of the American*

Society for Horticultural Science 128: 531-536.

Ben-Amor, B., Latche, A., Bouzayen, M., Pech, J. C. and Romojaro, F. 1999. Inhibition of ethylene biosynthesis by antisense ACC oxidase RNA prevents chilling injury in Charentais cantaloupe melons. *Plant Cell Environment* 22: 1579-1586.

Bliss, C. I. 1967. *Statistics in Biology, statistical methods for research in the natural sciences*. vol.1 McGrawHill Book Co., N.Y., USA, 558p.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Bramley, P. M. 2002. Regulation of carotenoid formation during tomato fruit ripening and development. *Journal of Experimental Botany* 53: 2107-2113.

Chisari, M., Barbagallo, R. N. and Spagna, G. 2008. Characterization and role of polyphenol oxidase and peroxidase in browning of fresh-cut melon. *Journal of Agricultural and Food Chemistry* 56: 132-138.

Chisari, M., Barbagallo, R. N., Spagna, G. and Artes, F. 2010. Distribution of degradative enzymatic activities in the mesocarp of two melon groups. *International Journal of Food Science and Technology* 45: 1016-1023.

Gao, Z., Petreikov, M., Zamski, E. and Schaffer, A. A. 1999. Carbohydrate metabolism during early development of sweet melon (*Cucumis melo*). *Physiologia Plantarum* 106: 1-8.

Guiltbalt, G. G. 1976. *Handbook of enzymatic methods of analysis*, pp. 147, Marcel Dekker Inc., New York.

Hodges, D. M. and Lester, G. E. 2006. Comparisons between orange- and green-fleshed non-netted and orange-fleshed netted muskmelons: Antioxidant changes following different harvest and storage periods. *Journal of the American Society for Horticultural Science* 131: 110-117.

Hubbard, N. L., Huber, S. C. and Pharr, D. M. 1989. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. *Plant Physiology* 91: 1527-1534.

Ismail, H. I., Chan, K.W., Mariod, A. A. and Ismail, M. 2010. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. *Food Chemistry* 119: 643-647.

Kalt, W. 2005. Effects of production and processing factors on major fruit and vegetable antioxidants. *Journal of Food Science* 70: 11-19.

Kanwal, B., Anjumzia, M., Rahman, Y. K. and Sheikh, M. A. 2004. Purification and characterization of α -amylase from apple (*Malus pumila*). *International Journal of Agriculture and Biology* 6: 233-236.

Lacan, D. and Baccou, J. C. 1998. High levels of antioxidant enzymes correlate with the delayed senescence in non netted muskmelon fruits. *Planta* 204: 377-382.

Lajalo, F. M. 2001. Partial characterization and relation to main changes in carbohydrate composition during ripening. *BioScience, Biotechnology, and Biochemistry* 65: 2174-2180.

- Laminkara, O. and Watson, M. A. 2001. Effects of ascorbic acid on peroxidase and polyphenol oxidase activities in fresh-cut cantaloupe melon. *Journal of Food Science* 66: 1283-1286.
- Lester, G. E. 2008. Antioxidant, sugar, mineral, and phytonutrient concentrations across edible fruit tissues of orange-fleshed Honeydew melon (*Cucumis melo* L.). *Journal of Agricultural and Food Chemistry* 56: 3694-3698.
- Lester, G. E. and Hodges, D. M. 2008. Antioxidants associated with fruit senescence and human health: Novel orange fleshed non-netted honey dew melon genotype comparisons following different seasonal productions and cold storage durations. *Postharvest Biology and Technology* 48: 347-354.
- Lingle, S. E. and Dunlap, J. R. 1987. Sucrose metabolism in netted muskmelon fruit during development. *Plant Physiology* 84: 386-389.
- Markus, F., Daood, H. G., Kapitany, J. and Biancs, P. A. 1999. Change in the carotenoid and antioxidant content of spice red pepper (paprika) as a function of ripening and some technological factors. *Journal of Agricultural and Food Chemistry* 47: 100-107.
- Masia, A. 1998. Superoxide dismutase and catalase activities in apple fruit during ripening and post-harvest and with special reference to ethylene. *Physiologia Plantarum* 104: 668-672.
- Moneruzzaman, K. M., Hossain, A. B. M. S., Sani, W. and Saifuddin, M. 2008. Effect of stages of maturity and ripening conditions on the biochemical characteristics of tomato. *American Journal of Biochemistry and Biotechnology* 4: 336-344.
- Perkins-Veazie, P., Collins, J. K., Davis, A. R. and Roberts, W. 2006. Carotenoid content of 50 watermelon cultivars. *Journal of Agricultural and Food Chemistry* 54: 2593-2597.
- Pesis, E., Fuschs, Y. and Zauberman, G. 1978. Starch content and amylase activity in avocado fruit pulp. *Journal of the American Society for Horticultural Science* 103: 673-676.
- Prasanna, V., Prabha, T. N. and Tharanthan, R. N. 2007. Fruit ripening phenomena- An overview. *Critical Reviews in Food Science and Nutrition* 47: 1-19.
- Roe, J. H. 1964. Chemical determination of ascorbic, dehydroascorbic acid and diketogluconic acids. In *Met Biochem Anal I* (Ed Gluk D) p.113-139. New York: Interscience.
- Samee, W., Engkalohakul, M., Nebbua, N., Direkrojanavuti, P., Sornchaithawatwong, C. and Kamkaen, N. 2006. Correlation analysis between total acid, total phenolic and ascorbic acid contents in fruit extracts and their antioxidant activities. *Thai Pharmaceutical and Health Science Journal* 1: 196-203.
- Silva, A. P. F. B., Nascimento, J. R. O. D., Lajolo, F. M. and Cordenunsi, B. R. 2008. Starch mobilization and sucrose accumulation in the pulp of Keitt mangoes during postharvest ripening. *Journal of Food Biochemistry* 32: 384-395.
- Thimmaiah, S. K. 1999. Standard methods of biochemical analysis. Kalyani Publishers, New Delhi, India.
- Tlili, I., Hdidier, C., Lenucci, M. S., Ilahy, R., Jebari, H. and Dalessandro, G. 2011. Bioactive compounds and antioxidant activities during fruit ripening of watermelon cultivars. *Journal of Food Composition and Analysis* 24: 923-928.
- Toor, R. K. and Savage, G. P. 2006. Changes in the major antioxidant components during postharvest storage. *Food Chemistry* 99: 724-727.
- Tsuda, T., Chachin, K. and Ueda, Y. 1999. Studies on keeping capacity of imported carabo mango fruit from the Philippines. *Journal of the Japanese Society for Horticultural Science* 69: 669-674.
- Vinson, J. A., Su, X., Zubik, L. and Bose, P. 2001. Phenol antioxidant quantity and quality in food: fruits. *Journal of Agricultural and Food Chemistry* 49: 5315-5321.
- Wang, Y., Wyllie, S. G. and David, N. L. 1996. Chemical changes during fruit the development and ripening of the fruit of *Cucumis melo* (cv Makdimon). *Journal of Agricultural and Food Chemistry* 44: 210-216.
- Wang, Y.S., Tian, S. P., Xu, Y., Qin, G.Z. and Yao, H. J. 2004. Changes in the activities of pro and anti-oxidant enzymes in peach fruit inoculated with *Cryptococcus laurentii* or *Penicillium expansum* at 0 or 20°C. *Postharvest Biology and Technology* 34: 21-28.
- Wang, Z. F., Ying, T. J., Bao, B.L. and Huang, X. D. 2005. Characteristics of fruit ripening in tomato mutant epi. *Journal of Zhejiang University Science* 6B: 502-507.
- Zhang, D. and Wang, Y. 2001. β -amylase in developing apple fruits: activities, amounts and subcellular localization. *Science in China* 45: 429-440.