

Nutraceutical value and quality attributes of icebox watermelon fruit as influenced by ripening

Soumya, V. Menon and *Ramana Rao, T. V.

Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat – 388120, India

Article history

Received: 20 April 2017
Received in revised form:
6 June 2017
Accepted: 8 June 2017

Abstract

Watermelon is a quintessential summer fruit prolific in sugars and antioxidants, mainly lycopene which boosts the health esteem incredibly by promotion of heart health and cancer prevention. The results indicated that ripe fruit of 'Simran' cultivar was a good source of total sugars (65.89 mg/g FW), antioxidants like ascorbic acid (11.38 mg/g FW), carotenoids (26.15 µg/g FW) and also a rich source of minerals such as sodium (16.23 mg/kg) and zinc (7.43 mg/kg). Besides that sugar accumulation was concomitant with the increased activities of sugar metabolizing enzymes like sucrose phosphate synthase and sucrose synthase. During ripening, a remarkable increase in the activities of β-galactosidase and cellulase were marked, whereas the polygalacturonase activity was found to be low and it remained fairly constant in later stages of ripening. Furthermore, the activities of superoxide dismutase and catalase also reached their peak level in the ripe fruit of 'Simran'. This study revealed the nutraceutical value of the icebox watermelon 'Simran' based on the significant accumulation of antioxidants, phenolics, minerals and higher antioxidant capacity. Moreover, it gives an insight into the further characterization of antioxidants to increase the productivity of melons with improved quality traits which are highly appreciated by consumers and producers and also to exploit them as nutritional supplements.

Keywords

Antioxidant activity
Icebox watermelon
Nutritional quality
Phenolics
Ripening
Sugars
Sucrose phosphate synthase

© All Rights Reserved

Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) belongs to the gourd family called Cucurbitaceae and the genus *Citrullus*. Watermelon is a healthful and popular fruit for humans. The major composition of watermelon is 91.45 g water; the remaining of it constitutes 7.55 g of carbohydrates, 0.40 g fiber, 8.1 mg ascorbic acid and 7-112 mg of various minerals in 100 g of the fruit (USDA, 2011). Watermelon fruit contains zero cholesterol that helps to remove/lower cholesterol level and also has strong diuretic tendencies that reduce the formation of kidney stones (Inuwa *et al.*, 2011). During ripening, watermelon fruit produces low amount of ethylene and is therefore considered as a non-climacteric fruit (Salman-Minkov and Trebitsch, 2008). Lycopene, a major carotenoid pigment and antioxidant, is abundant in red fleshed watermelon (Perkins-Veazie *et al.*, 2006). Icebox watermelon, varying in shape, color and size was first introduced in U.S. almost 50 years ago. This type of watermelon became popular recently and is commonly available in grocery stores, markets etc. due to their small size and convenient accommodation in refrigerators and also ideal for small families (Miles *et al.*, 2004).

According to Akin *et al.* (2008), sugars, organic acids, phenolics and carotenoids are naturally predominant in fruits and vegetables and these components play a major role in maintaining the fruit quality and determining their nutritive value. Some other components (phytochemicals) of fruits and vegetables are strong antioxidants and modify the metabolic activation and detoxification/disposition of carcinogens and they may even influence processes that may change the course of the tumor cell. Antioxidant capacity of fruits may be associated with several parameters, including the ripening stages and matrix of the plant product. During the fruit ripening process, phenolic compounds occur and they contribute to the sensory nutritive quality of fruit, particularly aroma, taste, flavor and color as they exhibit antioxidant property (Tan *et al.*, 2012). Likewise, polyphenols, which have health promoting properties, prevent degenerative diseases as well as microbial spoilage of food products (Gordon *et al.*, 2012).

One of the most important quality attributes of fruit is sweetness which is due to the predominance of sucrose in melons during their final stage of development (Yamaguchi *et al.*, 1977; Hubbard *et al.*, 1989; Burger and Schaffer, 2007). In the later

*Corresponding author.
Email: tadapanenirao@yahoo.com

stages of development and ripening, fruit sucrose accumulation commences with an increased sucrose phosphate synthase (SPS; EC 2.4.1.14) and decreased activities of invertases like acid invertase (AI; EC 3.2.1.26) (Lester, 2008).

A wide spectrum of nutritional quality evaluation which has been carried out previously in the ripe melon fruit showed the abundance of sugars, phenolics, ascorbic acid, minerals, total antioxidant activity and sugar related enzymes such as sucrose phosphate synthase, sucrose synthase (SS; EC 2.4.1.13), antioxidant enzymes like peroxidase (POD; EC 1.11.1.7), polyphenol oxidase (PPO; EC 1.14.18.1), catalase (CAT; EC 1.11.1.6) and also softening enzymes such as polygalacturonase (PG; EC 3.2.1.15) and β -galactosidase (β -Gal; EC 3.2.1.23) (Hubbard *et al.*, 1989; Lester, 2008; Yativ *et al.*, 2010; Menon and Rao, 2012a; Menon and Rao, 2012b; Menon and Rao, 2013).

However, our knowledge on the ripening behaviour and nutritional importance of icebox watermelon is limited and fragmented, and consumers are still unaware of the health promoting property of icebox cultivar of watermelon. It is envisaged that the results of the present study would be helpful for harvesting at appropriate stage of maturity so as to take the full advantage of its optimum nutritional quality.

Materials and Methods

Plant material

The fruits of watermelon (cv. Simran) were collected at its five sequential stages of development and ripening, viz: young, pre-mature, mature, pre-ripened and ripened from the fields of Dehgam, Gujarat.

Total sugars

The extraction of total sugars (TS) and its quantification was performed by phenol-sulphuric acid method (Thimmaiah, 1999). Briefly, the melon tissue was hydrolyzed with 2.5 N HCl for 3 h in a boiling water bath. After hydrolysis, the extract was centrifuged and collected the supernatant. The reaction mixture contained 0.02 ml of aliquot, 1 ml of 1% phenol, and 5 ml of 96% sulphuric acid (H_2SO_4). The mixture was left for 3 min at room temperature and later, the tubes were placed in a water bath at 25-30°C for 20 min. After the incubation, the color developed was measured at 490 nm and the values were expressed as mg/g Fresh weight (FW).

Sugar metabolizing enzymes

The extraction and assay of sugar metabolizing enzymes were conducted as per the methodology described by Hubbard *et al.* (1989). Briefly, for each stage, melon tissue (2.5 gram) was extracted in a 10 ml of buffer containing 50 mM 3-(N-morpholino) propane sulfonic acid – sodium hydroxide (MOPS-NaOH) (pH 7.5), 5 mM magnesium chloride ($MgCl_2$), 1 mM Ethylene diamine tetra acetic acid (EDTA), 2.5 mM dithiothreitol (DTT), 0.05% (v/v) Triton X-100, and 0.5 mg/ml bovine serum albumin (BSA). Samples were centrifuged at 10,000 g for 10 min and the supernatants were collected. For the assay of SPS activity, the reaction mixture contained the crude enzyme extract, 15 mM $MgCl_2$, and buffer containing 50 mM Mops-NaOH (pH 7.5), 5 mM $MgCl_2$, 10 mM uridine diphosphate glucose (UDPG), 15 mM glucose 6-P and 5 mM fructose 6-P. This 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 water bath at 40°C for 20 min, and measured the absorbance at 620 nm. The procedure for the assay of SS enzyme was identical to that of SPS except that the reaction mixtures contained only 50 mM MOPS-NaOH, 10 mM fructose and the enzyme extract.

Phenols and polyphenols

Phenolics were analyzed spectrophotometrically using the Folin-Ciocalteu method (Vinson *et al.*, 2001). Free phenols (FP) and total phenols (TP) were extracted by using 10 mL of 50% methanol/water, 1.2 M HCl in 50% methanol/water (v/v) respectively and were vortexed for one minute and heated at 90°C for 3 h with vortexing at an interval of 30 min. One gram of sample was homogenized in 10 ml of 60% methanol for free polyphenols (FPP), and it was vortexed for one minute and treated as above. Total polyphenols (TPP) (one gram tissue) were extracted with 10 ml of 1.2 M hydrochloric acid (HCl) in 60% methanol/water (v/v) and treated as above. Samples for phenolics were cooled, diluted with methanol and centrifuged at 5000 rpm for 10 min. Phenols (FP, TP) were measured at 750 nm, while polyphenols (FPP, TPP) were measured at 765 nm. Values obtained were expressed as catechin equivalent mg/g FW for phenols, while that of polyphenols were expressed as mg Gallic acid equivalent (GAE)/g FW against the external calibration.

Total antioxidant activity and ascorbic acid

The antioxidant activity was evaluated by using the free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) (Narwal, 2009) on triplicate samples of the

homogenate. In this assay, 100 μ L of aliquot of the extract and 2.9 mL of 100 μ M of DPPH (dissolved in methanol) were mixed. The absorbance was set at 517 nm and it was recorded after 30 min incubation in dark at room temperature, where methanol served as a blank. The total antioxidant capacity (TAC) was expressed in % activity. The quantitative analysis of ascorbic acid (AA) was determined by following the method by Roe (1964).

Carotenoids

Carotenoid extraction and estimation was carried out as per the methodology described by Wang *et al.* (2005). The mesocarpic tissue (2.5 g) was extracted in 40 mL of 60%:40% of the organic solvent, hexane:acetone (v/v). The organic layer formed was collected initially and extraction was repeated until the solution turned colorless and the absorbance was measured at 450 nm.

POD, CAT, SOD

One gram of mesocarpic melon tissue was homogenized for the POD assay in 10 mL of 0.1 M phosphate buffer (pH 7.2) and 1 mM polyvinyl pyrrolidone and the supernatant was taken for the assay. The specific activity of the enzyme was expressed as 1 unit change in OD/min/mg protein (Guiltbalt, 1976). CAT and superoxide dismutase (SOD; EC 1.15.1.1) were 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 polypyrrolidone. The supernatant was taken for the assay and expressed the activity as Units/ mg protein.

β -Gal

The β -Gal activity was assayed according to the method of Nakamura *et al.* (2003). One gram of the tissue was homogenized with 10 mL of 0.01 M sodium-phosphate buffer (pH 7.2) that contained 50 mM sodium chloride (NaCl). The homogenate was centrifuged at 4°C for 40 min and the supernatant was used as enzyme source. The assay mixture, which consisted of 50 mM sodium acetate containing 0.2 mg/ml of BSA (pH 4.0) and 10 mM of p-nitrophenyl β -D galactopyranoside (PNPG), was incubated at 30°C for 5 min. Next, 0.4 mL of the enzyme was added and incubated for 5 min at the same temperature. The reaction was quenched by the addition of 0.5 M sodium carbonate and the p-nitrophenol formed was determined at 405 nm. The specific activity is expressed in p-nitrophenol released/ mg protein.

PG and cellulase (Cx)

Three grams of melon tissue was homogenized with 10 mL of 0.02 M phosphate buffer (pH 7.0), 20 mM cysteine-HCL, 20 mM EDTA and 0.05% Triton X-100. The homogenate was centrifuged and the supernatant was assayed for the enzyme activity of PG and Cx (Srivastava and Dwivedi, 2000). The enzyme activity was expressed as 1 unit = PG activity produced per mg reducing group/hr/mg protein. The specific activity of Cx was expressed as mg glucose released/h/mg protein. The protein content in all the enzymes was also assayed (Bradford, 1976).

Minerals

One gram of dry material was further processed for the wet digestion by the diacid perchloric acid-nitric acid (1 HClO₄: 3 HNO₃) mixture and allowed to stand overnight. The digested samples were further heated on hot plate until solid particles nearly disappeared. The process of heating was continued until a clear colorless solution is obtained. Once the digestion process is over, samples were further evaporated to near dryness. After this, samples were cooled and made upto 100 ml with milli Q water and the solution was then allowed to stand overnight. This solution was filtered through a dry paper and further analyzed for the minerals such as potassium (K), sodium (Na), iron (Fe) and zinc (Zn) by atomic absorption spectrophotometer (AAS) following the methodology by Jackson (1973).

Statistical analysis

Statistical analysis has been performed using the software IRISTAT (v. 3.1, IRRI, Manila, Philippines) and the comparison of averages of each developmental stage was based on the analysis of variance (One-way ANOVA) according to Duncan's multiple range test (DMRT) at a significance level of $P < 0.05$. The results were represented as average \pm standard deviation of the replications per developmental stage (Bliss, 1967).

Results and Discussion

Total sugars

It is very important to know the biochemical composition based nutritional value of the specific cultivar of watermelon so as to meet the demand of the consumer and to accept it as a nutritionally rich fruit. Therefore, the biochemical composition of watermelon during its sequential stages of development and ripening is presented in this study. Sweetness is one of the major attributes contributing to the internal quality of the fruit and cultivars with

higher amount of sugars are promoted in the market. A significant difference ($P < 0.05$) in the amount of TS was observed throughout the development and ripening of 'Simran' as presented in Figure 1 (A). Green fruits contained lesser amount of TS, whereas with the onset of maturity, the TS content increased and a higher accumulation of it was noticed in the red ripe watermelon fruit. Pareek *et al.* (2014) reported rapid sugar accumulation during maturation and sucrose being a major sugar accumulates faster and in a higher level during the ripening process. The results of the present study are also in close agreement with TS levels reported previously for muskmelon (*Cucumis melo* L.) (Tsuda *et al.*, 1999; Menon and Rao, 2012b). Sweetness is quantified by the sweetness index which is based on the TS accumulation in fruits. Interestingly, the TS content of ripe strawberries varied between 26 and 94 mg/g FW which indicated the sweetness index of these cultivars of strawberry (Basson *et al.*, 2010). This data supported the current findings for TS which had its highest level (65.89 mg/g FW) in 'Simran' cultivar indicating its rich sweetness index.

Sugar metabolizing enzymes

Sugar composition in fruits is related to the activities of their metabolizing enzymes. As illustrated in Figure 1 (B), the highest level (0.517 $\mu\text{mol/h/mg}$ protein) of SPS activity occurred in the pre-mature stage. With the onset of maturity, the activity of SPS abruptly declined, while its activity increased slightly to 0.417 $\mu\text{mol/h/mg}$ protein during watermelon ripening. In comparison with the SPS enzyme, the activity of SS was found to be less in the initial stages of development. However, during maturation and ripening, SS enzyme displayed a significant level ($P < 0.05$) of activity. As shown in Figure 1 (B), the activity of SS increased about twice during the ripening of 'Simran'. This result for SS activity was consistent with those reported by McCollum *et al.* (1988) and Schaffer *et al.* (1987) in muskmelon and papaya (*Carica papaya* L.) (Gomez *et al.*, 2001) respectively, but different from those observed in various types of melons by some of the researchers (Hubbard *et al.*, 1989; Menon and Rao, 2012a; Menon and Rao, 2012 b). Hayata *et al.* (1999) observed varying levels of SS activity at an early stage of muskmelon development. Based on the studies in parthenocarpic melons, it was reported that increased activity of SS exhibited a positive relation with sucrose accumulation in the later stages of development (Hayata *et al.*, 2001).

Phenolic compounds

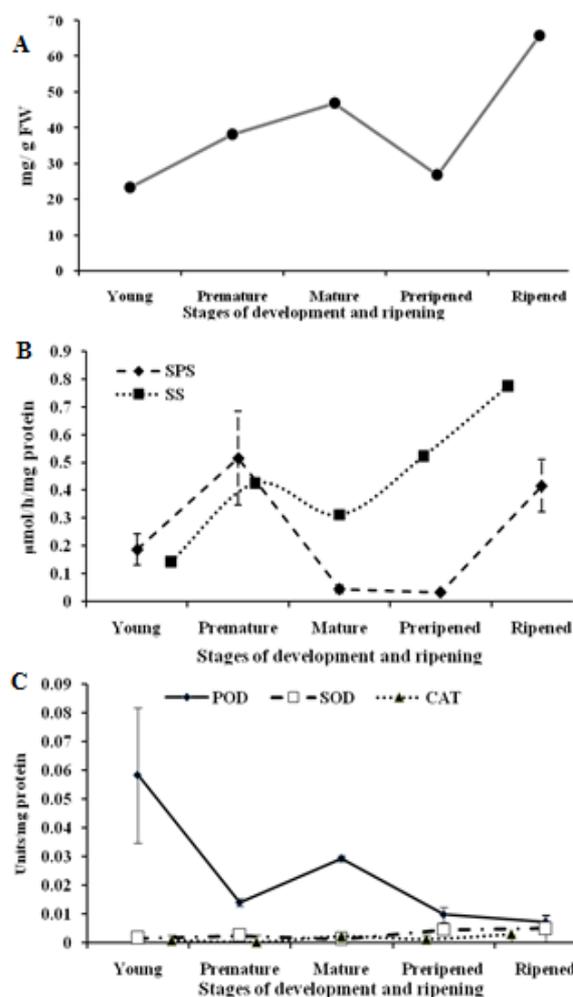


Figure 1. Changes in (A) Quantity of total sugars (TS) (mg/g FW), specific activities of (B) sucrose phosphate synthase (SPS) and sucrose synthase (SS) ($\mu\text{mol/h/mg}$ protein), (C) peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) (Units/mg protein) of 'Simran' at its five sequential stages of development and ripening.

From the nutritional point of view, melons are one of the fruits with highest phenolics than the other consumed fruits. The accumulation of FP was significant ($P < 0.05$) with its maximum value (0.589 mg/g FW) in the pre-mature stage of 'Simran'. Maturation did not modify the FP content in this particular cultivar as the mature fruit exhibited lower values than that of the pre-mature fruit. However, a slight rise from 0.395 to 0.528 mg/g FW in the levels of FP was observed as the fruit continued its growth towards ripening, as depicted in Table 1. Similarly, TP achieved a transient increase in its amount in the pre-mature stage with a precipitous decrease with the onset of maturity. During the remainder of ripening period, a slight increment in the levels of TP was noticed as shown in Table 1. The pattern of accumulation of phenols (FP, TP) and polyphenols (FPP, TPP) during the development and ripening

Table 1. Changes in the free phenol (FP), total phenol (TP), free polyphenol (FPP) and total polyphenol (TPP) content of 'Simran' at its five sequential stages of development and ripening (mg/g FW).

Stages	FP	TP	FPP	TPP
Young	0.369 ± 0.040 ^a	0.647 ± 0.09 ^a	0.671 ± 0.06 ^a	2.64 ± 0.07 ^b
Pre-mature	0.589 ± 0.009 ^b	1.400 ± 0.01 ^c	0.927 ± 0.04 ^b	3.18 ± 0.10 ^c
Mature	0.405 ± 0.020 ^a	0.966 ± 0.07 ^b	0.848 ± 0.17 ^b	2.40 ± 0.17 ^a
Pre-ripened	0.395 ± 0.030 ^a	0.888 ± 0.04 ^b	0.760 ± 0.03 ^b	2.20 ± 0.14 ^a
Ripened	0.528 ± 0.130 ^b	1.34 ± 0.04 ^c	0.923 ± 0.06 ^b	2.80 ± 0.10 ^b

Values represent the average of three replicates ± standard deviation. Different letters in same column indicate that means are significantly different ($P < 0.05$) by Duncan Multiple Range Test (DMRT).

of 'Simran' was similar, although their levels were quantitatively different. These results support the findings of Soumya and Rao (2014) who conducted a comparative study in four icebox cultivars of watermelon.

The results presented in Table 1 showed that the concentrations of polyphenols had increased remarkably as the fruit continued its growth from the young to the pre-mature stage. The decrease in the level of polyphenols coincided with the maturation process for the presently analyzed cultivar of watermelon fruit. In contrast, a progressive increment in the level of polyphenols was noticed in the ripened stage as presented in Table 1. These results for phenolics are in accordance with the previous reports on plum indicating that the process of ripening had no significant influence on the TP content (Usenik *et al.*, 2013). Moreover, evidences from the previous studies shows that in fruits and vegetables, genetic control is the primary factor which determines the phenolics, and the variations would depend upon the ripening stages at the time of harvesting, environmental factors and analytical methodology (Ilahy *et al.*, 2011).

TAC, AA and carotenoids

In the current investigation, the level of TAC was analyzed based on the measurement of scavenging ability of antioxidants towards the stable DPPH• radical and it was evaluated during the sequential stages of development and ripening, and the values are presented in Table 2. Young fruit contained higher levels of total antioxidant activity, but it got declined abruptly in the further stages of fruit development. However a significant and remarkable TAC level was observed in the pre-ripe fruit of 'Simran' which diminished eventually. Menon and Rao (2012b) noticed a similar trend for TAC in muskmelon fruit during its development and ripening. A comparative study of fermented and nonfermented watermelon

rind was performed by Erukainure *et al.* (2011) and they summarized from their studies that the unfermented rind possessed highest antioxidant capacity. Giovanelli *et al.* (1999) concluded from their studies that during post harvest storage, TAC tend to increase and this phenomenon was related to the metabolic processes at the time of ripening and also the production of lipophilic antioxidants such as phenolics, carotenoids and lycopene.

The levels of AA had increased slightly in the early stages of development of 'Simran', but they began to decrease by four fold with the onset of maturity. As presented in Table 2, the process of ripening caused to accumulate AA abundantly. In a study carried out by Matsufuji *et al.* (2007) on different colored peppers, it was documented that the AA got accumulated at higher levels in red colored pepper than that of the yellow one. Likewise, Marti *et al.* (2011) also reported varying levels of AA during maturation and ripening of pepper. This behaviour may be due to the oxidizing property of AA during the early stages of development. Moreover depending upon the cultivar, environmental and cultural conditions of fruits, the levels of AA may vary (Lee and Kader, 2000; Marti *et al.*, 2011).

Fruit ripening is characterized by the conversion of chloroplasts to chromoplasts, which involves the carotenoid accumulation (Cooper, 2004). Interestingly, a higher accumulation of carotenoids was observed in the pre-mature stage of 'Simran'. The concentration of carotenoids decreased as the fruit proceeded in its development from the pre-mature to the mature stage and these results were consistent with the previous studies on carotenoids in fruits (Karvouni *et al.*, 1995; Li *et al.*, 2006). Moreover, carotenoids got accumulated in watermelon in a lower level during the remainder of the ripening process (Table 2).

Table 2. Changes in the carotenoids and ascorbic acid (AA) content and the level of total antioxidant activity (TAC) of 'Simran' at its five sequential stages of development and ripening.

Stages	Carotenoids ($\mu\text{g/g FW}$)	AA (mg/g FW)	TAC (%)
Young	23.94 \pm 0.78 ^a	2.08 \pm 0.17 ^{ab}	18.51 \pm 0.55 ^c
Pre-mature	34.34 \pm 0.81 ^d	3.43 \pm 0.61 ^b	0.449 \pm 0.058 ^a
Mature	30.91 \pm 0.15 ^c	0.87 \pm 0.19 ^a	25.34 \pm 0.88 ^d
Pre-ripened	23.68 \pm 0.05 ^a	2.61 \pm 0.44 ^b	32.20 \pm 0.96 ^e
Ripened	26.15 \pm 0.03 ^b	11.38 \pm 0.25 ^c	12.92 \pm 0.53 ^b

Values represent the average of three replicates \pm standard deviation. Different letters in same column indicate that means are significantly different ($P < 0.05$) by Duncan Multiple Range Test (DMRT).

Antioxidant enzymes

It was clear from Figure 1 (C) that the activity level of POD was found to be high in the early stages of fruit development. However, the activity level for POD declined in the subsequent developmental stages of 'Simran'. The activity of POD increased significantly ($P < 0.05$) during the maturation of fruit and declined strongly by 3 fold towards the end of ripening. Our data concur with those prior findings in muskmelon (Chisari *et al.*, 2010) and watermelon (Menon and Rao, 2012a) fruit that the POD activity was found to be significant in the early stages of fruit development. CAT is one of the most important antioxidant enzymes in plant cells having the ability to scavenge the free radicals and its increased activity has been observed during fruit development (Huang *et al.*, 2007). The specific activity of CAT enzyme of watermelon during its development is illustrated in Figure 1 (C). The activity level for CAT in the young fruit was 0.0009 units/mg protein which reached to 0.0003 units/mg protein in the pre-mature fruit. A transient increase in the activity was observed subsequently, but with its remarkable activity (0.003 units/mg protein) in the ripe fruit of watermelon. Similar to the results of the present study, other authors also reported increased level of CAT activity indicating its role during the fruit development in active oxygen detoxification as well as defence (Srivastava and Dwivedi, 2000; Qusti *et al.*, 2010).

During the early stages of fruit development, a lower level of SOD activity was observed. Figure 1 (C) indicates a significant level of SOD enzyme as the fruit proceeds towards ripening. In a similar manner, higher activity of SOD was also noticed in casaba melon which indicated that various defence mechanisms in plants were triggered due to the higher SOD levels (Lester *et al.*, 2009). Previous

observations in melons also suggest that higher activity of SOD may be related to the lower softening rate and thereby enhance the shelf life of the fruit (Lacan and Baccou, 1998).

Softening enzymes

A significant level ($P < 0.05$) of β -Gal enzyme activity was noticed in the pre-mature fruit, but with a transient decrease of it during maturation. Ripening significantly affected the β -Gal activity in the red fleshed watermelon fruit, as displayed in Table 3. The findings of the present study are in agreement with the previous works performed on pepper (Tan *et al.*, 2012), watermelon (Menon and Rao, 2012a) and tomato (Smith and Gross, 2000). Based on the studies on pepper, Ogasawara *et al.* (2007) stated that wall softening coincides with the loss of considerable amount of galactose residues from the cell wall. The results of the present study emphasize further on the implication of β -Gal enzyme in the rapid softening process in the fruit.

A gradual and consistent increase in the activity of PG was observed throughout the developmental process of 'Simran'. The maximum and significant level of activity of PG was observed in the mature fruit, but with a diminution in its activity in the subsequent stages of ripening (Table 3). In a similar line, our previous work in muskmelon fruit showed highest PG activity during the maturation process (Menon and Rao, 2014). The general behaviour of Cx in fruits is its increase in the initial stages of development as well as at later stages of ripening. In a parallel manner, Cx activity levels was 0.068 in the young stage, but declined abruptly to 0.006 in the current study as the fruit reached pre-mature stage, the values expressed as mg glucose released/h/mg protein. A significant ($P < 0.05$) level of Cx activity was however noticed in the fully ripe fruit indicating a positive relation with

Table 3. Specific activities of β -galactosidase (β -gal), polygalacturonase (PG) and cellulase (Cx) of 'Simran' at its five sequential stages of development and ripening.

Stages	β -Gal (μ mol pnp released/mg protein)	PG (mg galacturonic acid released/h/mg protein)	Cx (mg glucose released/h /mg protein)
Young	0.256 \pm 0.02 ^b	0.270 \pm 0.05 ^a	0.068 \pm 0.011 ^{ab}
Pre-mature	0.379 \pm 0.015 ^c	0.627 \pm 0.11 ^b	0.006 \pm 0.001 ^{ab}
Mature	0.210 \pm 0.011 ^a	1.222 \pm 0.23 ^c	0.022 \pm 0.005 ^a
Pre-ripened	0.273 \pm 0.018 ^b	0.886 \pm 0.29 ^b	0.085 \pm 0.050 ^b
Ripened	0.392 \pm 0.010 ^c	0.608 \pm 0.01 ^b	0.039 \pm 0.003 ^{ab}

Values represent the average of three replicates \pm standard deviation. Different letters in same column indicate that means are significantly different ($P < 0.05$) by Duncan Multiple Range Test (DMRT).

Table 4. Mineral concentration of macro elements such as potassium (K), sodium (Na) and micro elements, iron (Fe) and zinc (Zn) of 'Simran' at its five sequential stages of development and ripening (mg/kg).

Stages	K	Na	Fe	Zn
Young	30.78 \pm 0.010 ^d	19.37 \pm 0.018 ^a	6.61 \pm 0.007 ^d	0.473 \pm 0.007 ^a
Pre-mature	36.75 \pm 0.009 ^a	19.02 \pm 0.009 ^d	9.47 \pm 0.002 ^a	0.879 \pm 0.002 ^b
Mature	28.98 \pm 0.027 ^c	11.74 \pm 0.006 ^a	4.06 \pm 0.001 ^a	2.43 \pm 0.004 ^c
Pre-ripened	18.14 \pm 0.005 ^b	11.99 \pm 0.012 ^b	5.01 \pm 0.003 ^b	2.70 \pm 0.010 ^d
Ripened	7.24 \pm 0.007 ^a	16.23 \pm 0.007 ^c	5.90 \pm 0.006 ^c	7.43 \pm 0.003 ^a

Values represent the average of three replicates \pm standard deviation. Different letters in same column indicate that means are significantly different ($P < 0.05$) by Duncan Multiple Range Test (DMRT).

the wall softening process, as depicted in Table 3. A similar report for Cx was shown in studies by Liu *et al.* 2008. Furthermore, Liu *et al.* (2008) concluded from their research that accelerated activity of Cx is an indication of relaxation and extension of cell wall and might stimulate the synthesis of new substances.

Minerals

The concentration of mineral nutrients such as K, Na, Fe and Zn on a dry weight basis was measured during the development and ripening of 'Simran' and it showed a progressive decrease in the concentration of K and Na during the ripening process, as displayed in Table 4. In 'Simran', interestingly significant accumulation of Na and K was observed in the young and pre-mature stages respectively. A comparative study of outer subpeel mesocarp and inner mesocarp of melons showed a similar pattern to that of the current study (Lester, 2008). Moreover, Lester (2008) stated that sub peel mesocarp, being a metabolically active tissue, requires maximum mineral supply for cellular membrane integrity enhancing the postharvest life.

As it could be seen from Table 4, the concentration

of micronutrient, Fe was 9.47 mg/kg in the pre-mature fruit, but exhibited a transient decrease in its value to 4.06 mg/kg with the onset of maturity. However, with the progression of ripening these values for Fe increased slightly to 5.90 mg/kg. In comparison to the accumulation pattern of K, Na and Fe in the fruit, a different pattern was observed for Zn accumulation. A consistent trend in the concentration of Zn was noticed all throughout the development and ripening of 'Simran'. A significant ($P < 0.05$) accumulation of Zn was found in the ripe watermelon fruit. Similar trend of mineral accumulation was noticed in another icebox cultivar 'Suman 235' in our previous studies (Soumya and Rao, 2014). Besides, results of the present study also supports the views of Julian-Loeza *et al.* (2011) that Zn is essential for the metabolism of carbohydrates, proteins and lipids.

Conclusion

The results of the present study indicate that 'Simran' is a rich source of antioxidants besides possessing strong antioxidant activity in scavenging

the reactive oxygen species. The data presented in the current research also provides information related to higher accumulation of macro and micro minerals, sugars and activities of various enzymes during ripening process. Thus these results are helpful to gain a better insight of the health-related compounds of icebox watermelon, 'Simran' during its development and ripening. These aspects are necessary for the consumer that demands the prevention of health problems through nutrition and certain fruit quality traits including fruit size, internal quality like flesh color etc. Moreover, these fundamental findings of the present research are an important factor to choose varieties with a high antioxidant potential as a nutraceutical source. This will help for further studies on all aspects of these nutraceuticals before advocating them as a boon to human health. In future, research on health benefits of icebox watermelon fruit from a therapeutic and breeding perspective needs to be focused on key areas such as identification of molecular, genetic mechanisms that regulate the synthesis of these nutraceuticals. This will further help to develop cultivars rich in a variety of antioxidants and other metabolites which ensure the essential nutrients in human diet as a functional food.

Acknowledgment

The authors are grateful to University Grants Commission (UGC), New Delhi for financial support under the scheme of its Major Research Project; F. No. 36-204/2008 (SR) dated 26th March 2009.

References

- Akin, E. F., Karabulut, I. and Topcu, A. 2008. Some compositional properties of main Malatya apricot (*Prunus armeniaca* L.) varieties. *Food Chemistry* 107: 939-948.
- Basson, C.E., Groenewald, J.H., Kossmann, J., Cronje, C. and Bauer, R. 2010. Sugar and acid related quality attributes and enzyme activities in strawberry fruits: Invertase is the main sucrose hydrolyzing enzyme. *Food Chemistry* 121: 1156-1162.
- Bliss, C. I. 1967. *Statistics in Biology, Statistical methods for research in the natural Sciences*. 1st ed. USA: McGrawHill Book Co.
- 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.
- Burger, Y. and Schaffer, A. A. 2007. The contribution of sucrose metabolism enzymes to sucrose accumulation in *Cucumis*. *Journal of American Society for Horticultural Science* 132: 704-712.
- 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.
- Cooper, D. A. 2004. Carotenoids in health and disease: Recent scientific evaluations, research recommendations and the consumer. *Journal of Nutrition* 134: 221S-224S.
- Erukainure, O.L., Oke, O.V., Adenekan, S.O. and Ajiboye, J.A. 2011. Antioxidant activities, total phenolics and flavanoid level of watermelon rinds subjected to *Saccharomyces cerevisiae* solid media fermentation. *Fermentation Technology Bioengineering* 2: 11-16.
- Giovanelli, G., Lavelli, V., Peri, C. and Nobili, S. 1999. Variation in antioxidant components of tomato during vine and post-harvest ripening. *Journal of the Science of Food and Agriculture* 79: 1583-1588.
- Gomez, M., Lajalo, E. and Cordenunsi, B. 2001. Evolution of soluble sugars during ripening of papaya fruit and its relation to sweet taste. *Journal of Food Science* 67: 442-447.
- Gordon, A., Friedrich, M., Martins, Da Matta., Moura, C. F. H. and Marx, F. 2012. Changes in phenolics composition, ascorbic acid and antioxidant capacity in cashew apple (*Anacardium occidentale* L.) during ripening. *Fruits* 67: 267-276.
- Guiltbalt, G. G. 1976. *Handbook of enzymatic methods of analysis*. 1st ed. New York: Marcel Dekker Inc.
- Hayata, Y., Li, X-X., and Osajima, Y. 1999. Effects of CPPU application on the sucrose metabolizing enzymes activities of melon (*Cucumis melo* L.) in the first growth stage (in Japanese). *Proceedings in 34th congress by society of chemical regulation in plants*, 152-153.
- Hayata Y, Li X-X. and Osajima Y. 2001. Sucrose accumulation and related metabolizing enzyme activities in seeded and induced parthenocarpic muskmelon. *Journal of American Society for Horticultural Science* 126: 676-680.
- Huang, R.R., Xia, L., Hu, L. and Wang, M. 2007. Antioxidant activity and oxygen-scavenging system in orange pulp during fruit ripening and maturation. *Scientia Horticulturae* 113: 166-172.
- 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.
- Ilahy, R., Hdider, C., Lenucci, M.S., Tlili, I. and Dalessandro, G. 2011. Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. *Journal of Food Composition and Analysis* 24: 588-595.
- Inuwa, H.M., Aina, V.O., Gabi, B., Imola, I. and Veronica, T. 2011. Determination of differences in nutrient composition of *Citrullus vulgaris* (watermelon) fruit after plucking. *British Journal of Dairy Science* 2: 27-30.
- Jackson, M. L. 1973. *Soil and chemical analysis*. 1st ed. New Delhi: Prentice Hall of India Pvt. Ltd.

- Julian-Loeza, A. P., Santos-Sanchez, N. F., Valadez-Blanco, R., Sanchez-Guzman, B. S. and Salas-Coronado, R. 2011. Chemical composition, color, and antioxidant activity of three varieties of *Annona diversifolia* safford fruits. *Industrial Crop Products* 34: 1262-1268.
- Karvouni, Z., John, I., Taylor, J. E., Watson, C. F. and Turner, A. J. and Grierson, D. 1995. Isolation and characterization of a melon cDNA clone encoding phytoene synthase. *Plant Molecular Biology* 27: 1153-1162.
- Lacan, D. and Baccou, J. C. 1998. High levels of antioxidant enzymes correlate with delayed senescence in non-netted muskmelon fruits. *Planta* 204: 377-382.
- Lee, S. K. and Kader, A. A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology* 20: 207-220.
- Lester, G. 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., Jifon, J. L. and Crosby, K. M. 2009. Superoxide dismutase activity in mesocarp tissue from divergent *Cucumis melo* L. genotypes. *Plant Foods for Human Nutrition* 64: 205-211.
- Li, Z., Lihu, Y., Yang, Y. and Li, A. 2006. Transgenic approach to improve quality traits of melon fruit. *Scientia Horticulturae* 108: 268-277.
- Liu, X., Ming-An, L., Guo-Tao, D., Shan-Bo, C. and Ya-Jun, R. 2008. Changes in the activity of PG, PE, Cx and LOX in pulp during fruit growth and development of two different ripening-season pear cultivars. *American-Eurasian Journal of Agricultural Environmental Science* 3: 445-450.
- Marti, M. C., Camejo, D., Vallejo, F., Romojaro, F., Bacarizo, S., Palma, J. M. Sevilla, F. and Jimenez, A. 2011. Influence of fruit ripening stage and harvest period on the antioxidant content of sweet pepper cultivars. *Plant Foods for Human Nutrition* 66: 416-423.
- Matsufuji, H., Ishikawa, K., Nunomura, O., Chino, M. and Takeda, M. 2007. Antioxidant content of different colored sweet peppers, white, green, yellow, orange and red (*Capsicum annum* L.). *International Journal of Food Science and Technology* 42: 1482-1488.
- McCollum, T. G., Huber, D. J. and Cantliffe, D. J. 1988. Soluble sugar accumulation and activity of related enzymes during muskmelon fruit development. *Journal of American Society for Horticultural Science* 113: 399-403.
- Menon, S. V. and Rao, T. V. R. 2012a. Enzyme activities during the development and ripening of watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) fruit. *International Journal of Plant Developmental Biology* 6: 21-26.
- Menon, S. V. and Rao, T. V. R. 2012b. Nutritional quality of muskmelon fruit as revealed by its biochemical properties during different rates of ripening. *International Food Research Journal* 19: 1621-1628.
- Menon, S. V. and Rao, T. V. R. 2013. Nutritional quality evaluation of nonnetted muskmelon fruit during its development and ripening. *Nutrition and Food Science* 43: 398-406.
- Menon, S. V. and Rao, T. V. R. 2014. Health promoting components and related enzyme activities of muskmelon fruit during its development and ripening. *Journal of Food Biochemistry* 38: 415-423.
- Miles, C., Kolker, K., Becker, G., Garth, L., Reed, J. and Smith T. 2004. Icebox watermelon variety trial. Washington State University.
- Nakamura, A., Moaeda, H., Mizuno, M. and Koshi, N. 2003. β -galactosidase and its significance in ripening of "Saijyo" Japanese persimmon fruit. *Bioscience Biotechnology and Biochemistry* 67: 68-76.
- Narwal, S. S. 2009. *Plant Biochemistry*. 1st ed. USA: Houston Studium Press.
- Ogasawara, S., Abe, K. and Nakajima, T. 2007. Pepper β -galactosidase 1 (PBG1) plays a significant role in fruit ripening in bell pepper (*Capsicum annum*). *Bioscience Biotechnology and Biochemistry* 71: 309-322.
- Pareek, S., Benkeblia, N., Janick, J., Cao, S. and Yahia, E. M. 2014. Postharvest physiology and technology of loquat (*Eriobotrya japonica* Lindl.) fruit. *Journal of Science of Food and Agriculture* 94: 1495-1504.
- Perkins-Veazie, P., Collins, J., Davi, A. R. and Roberts, W. 2006. Carotenoid content of 50 watermelon cultivars. *Journal of Agricultural Food Chemistry* 54: 2593-2597.
- Qusti, S. Y., Abo-Khatwa, A. N. and Bin-Lahwa, M. A. 2010. Free radical scavenging enzymes of fruit plant species cited in the holly Quran. *World Applied Science Journal* 9: 338-344.
- Roe, J. H. 1964. Chemical determination of ascorbic, dehydroascorbic acid and diketogluconic acids. In Gluk, D. (Ed). *Methods of Biochemical Analysis*, p. 113-139. New York: Inter Science.
- Salman-Minkov, A. and Trebitsch, T. 2008. Characterization of watermelon fruit development, Cucurbitaceae, In Pitrat, M. (Ed). *Proceedings of IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae*, p- 333. France: Avignon.
- Schaffer, A. A., Aloni, B. and Fogelman, E. 1987. Sucrose metabolism and accumulation in developing fruit of *Cucumis*. *Phytochemistry* 26: 1883-1887.
- Smith, D. L. and Gross, K. C. 2000. A family of at least seven β -galactosidase genes is expressed during tomato fruit development. *Plant Physiology* 123: 1173-1184.
- Soumya, V. and Rao, T. V. R. 2014. Nutritional quality evaluation of four icebox cultivars of watermelon fruit during their development and ripening. *International Food Research Journal* 21: 631-639.
- Srivastava, M. K. and Dwivedi, U. N. 2000. Delayed ripening of banana fruit by salicylic acid. *Plant Science* 158: 87-96.
- Tan, C. K., Ali, Z. M. and Zainal, Z. 2012. Changes in ethylene production, carbohydase activity and

- antioxidant status in pepper fruits during ripening. *Scientia Horticulturae* 142: 23-31.
- Thimmaiah, S. K. 1999. Standard methods of Biochemical analysis. 1st ed. India: Kalyani Publishers.
- Tsuda, T., Chachin, K. and Ueda, Y. 1999. Studies on keeping capacity of imported carabo mango fruit from the Philippines. *Journal of Japan Society of Horticultural Science* 69: 669- 674.
- United States Department of Agriculture (USDA). National nutrient database for standard reference, May 2011. Basic report of watermelon raw. Retrieved on July 2013 from NDB Website <http://ndb.nal.usda.gov/shared/PDF/foodreports.pdf>
- Usenik, V., Stampar, F. and Kastelec, D. 2013. Phytochemicals in fruits of two *Prunus domestica* L. plum cultivars during ripening. *Journal of the Science of Food and Agriculture* 93: 681-692.
- Vinson, J. A., Su, X., Zubik, L. and Bose, P. 2001. Phenol antioxidant quantity and quality in food: fruits. *Journal of Agricultural Food Chemistry* 49: 5315-5321.
- 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 of Science* 6B: 502-507.
- Yamaguchi, M., Hughes, D. L., Yabumoto, K. and Jennings, W. C. 1977. Quality of cantaloupes: variability and attributes. *Scientia Horticulturae* 6: 59-70.
- Yativ, M., Harary, I. and Wolf, S. 2010. Sucrose accumulation in watermelon fruits: Genetic variation and biochemical analysis. *Journal of Plant Physiology* 167: 589-596.