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Nutritional quality evaluation of four icebox cultivars of watermelon fruit during their development and ripening

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Abstract

Watermelon is a satiating fruit supplemented with health promoting components like sugars, antioxidants mainly lycopene, minerals etc. The biochemical composition, including antioxidants, and the specific activities of enzymes of watermelon fruit of four icebox cultivars were compared at their sequential stages of development and ripening and also an attempt has been made to determine their nutritional quality. The accumulation of sugars was found to be concomitant with the fruit development and ripening of all the presently studied icebox cultivars, but maximum accumulation of sugars occurred in 'Beauty' cultivar compared to that of other three cultivars. This phenomenon of sugar accumulation coincided with the increased activity of sucrose phosphate synthase in pre-ripened stage and decreased activities of invertases (acid, neutral) in the course of ripening of 'Beauty', but with their maximum activities in young fruit of 'Karina King'. Antioxidants such as lycopene, ascorbic acid, phenols, polyphenols, anthocyanin and flavanols were found in more quantity in the fruit of 'Beauty' followed by 'Suman 235' at their ripened stage than that of other cultivars of watermelon. Antioxidant enzymes, POD and SOD, displayed their significant activities during early stages of ripening in all the icebox cultivars. However, a strong positive correlation was observed between total polyphenols and lycopene with total antioxidant activity. The rate of mineral accumulation was higher in the early stages of fruit development in all the cultivars. Based on the patterns of accumulation of sugars, lycopene and other antioxidants and increased activities of enzymes in the fruit of Beauty', it may be concluded that the fruit of 'Beauty' has better nutritional quality when compared to all the other three cultivars of watermelon fruit.

Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), which belongs to the family *Cucurbitaceae*, is a warm-season crop and it is usually cultivated in the long warm growing seasons (Rosnah *et al*., 2010). Watermelon is of great economic importance with an estimated worldwide production of approximately 93.7 thousand million tons (Tarazona Diaz, 2011). Rosnah *et al.* (2010) stated that there are many watermelon cultivars that vary in shape, color of the rind and flesh. Almost 50 years ago, the first icebox watermelon variety was introduced in the U.S. but it is only recently these icebox watermelons have become commonly available in markets. Icebox watermelons are rapidly gaining in popularity due to their small size and also offer farmers a means of producing high quality watermelons locally. Dragovic-Uzelac (2007) stated that during fruit ripening several biochemical, physiological and structural modifications happen and melon fruit quality is a combination of such modifications that result in changes in color, texture, flavor, aroma (Chisari *et al.*, 2010) and also lead to the production of phenolic compounds and carotenoids.

The physical and chemical characterization of fruits and the quantification of their bioactive compounds are important for understanding their nutritional value and for increasing the quality and value of the final product. Fruits are a source of antioxidant compounds, such as phenolics, vitamins, carotenoids and minerals, which contribute to their chemo preventive effects (Rios de Souza *et al.*, 2012). Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules and thus prevent or repair the damage of the body cells that is caused by oxygen (Ismail *et al.*, 2010). Likewise, phenolic antioxidants inhibit free radical formation and cellular damage or cell death (Sun *et al*., 2002). Melons are rich in antioxidants like ascorbic acid, β-carotene, folic acid (Lester, 1997) and watermelon fruit is especially rich in lycopene. Lycopene is a carotenoid of great interest because of its antioxidant capacity in scavenging reactive oxygen species, which cause oxidative damage and loss of proper cell function (Tarazona Diaz, 2011). The analysis of antioxidants and antioxidant activity has been an important parameter for the nutritional quality of foods and its quantification gives the real

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evaluation of this nutritional value rather than the analysis of each single antioxidant compound (Ilahy *et al*., 2011).

The involvement of antioxidant enzymes in the regulation of free radical metabolism is well known, as superoxide dismutase (SOD; EC 1.15.1.1) enzyme catalyzes the dismutation of O_2^- in H_2O_2 while removal of H_2O_2 is done by the action of peroxidase (POD; EC 1.11.1.7) enzyme (Lacan and Baccou, 1998). Minerals are the essential regulators of physiological processes in humans and fruits contribute a major parts of them. In order to ensure the presence of minerals and trace elements in the diet at the required level, their amounts in plants need to be monitored (Konczak and Roulle, 2011).

Harvesting at the correct stage of maturity is essential to achieve optimum quality and also for maintenance of the quality after harvesting. The main objective of the present study was to compare the nutritional quality of four cultivars of watermelon by measuring different quality attributes at five different stages of development and ripening. The study was also focused on the elucidation of effect of various ripening stages on the nutritional composition of four cultivars of watermelon fruit. Besides, total antioxidant activity, phenolic compounds and ascorbic acid which attribute nutritional quality of fruit on the basis of their antioxidant properties were also measured.

Materials and Methods

Plant material

The fruit of four icebox cultivars of watermelon (cv F1 Arun, Beauty, Karina King and Suman 235) were collected at their sequential stages of development and ripening (viz: young, pre- mature, mature, pre-ripened and ripened) from different regions of Gujarat, and subjected for a comparative study of their nutritive value and antioxidant property.

Determination of phenolics and total antioxidant activity

The phenols (free, total) and polyphenols (free, total) were determined by the Folin-Ciocalteu (FCR) method, based on the procedures by Vinson *et al.* (2001). One gram of tissue was homogenized with 10 mL of 50% methanol for free phenols (FP), 50% methanol:HCL for total phenols (TP) and with 10 mL of 60% methanol for free polyphenols (FPP) while, 10 mL of 60% methanol:HCL for total polyphenols (TPP) and heated at 90ºC for 3 hours with vortexing every 30 min, then centrifuged at 5000 rpm for 5 min. A 0.4 ml of the aliquot was mixed with 0.5 ml of FCR (diluted 1:1 with distilled water) and after 3 min, 20% Na_2CO_3 was added. The solution was heated in a water bath for one min after which its absorption was measured at 750 nm (FP, TP) and 765 nm (FPP, TPP) respectively. The measurement was compared with a standard of Catechin and expressed as Catechin equivalent mg/g FW for FP and TP, while for FPP and TPP expressed as mg Gallic acid equivalent (GAE) mg/g FW.

Methanolic extracts (2 grams in 10 mL) were prepared for the determination of antioxidant activity. 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) and Narwal (2009) with some modifications. A 0.1 ml of aliquot was mixed with the 100µm 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 and expressed as total antioxidant capacity (TAC) in %.

Determination of total anthocyanins and flavanols

One gram of mesocarpic tissue was homogenized in 15 mL of 95% ethanol: 1.5 N HCL (85:15 v/v) and kept at 4ºC overnight and the samples were filtered, residues were washed to ensure the complete removal of pigments. The filtrates were completed to a total volume of 100 ml with the extractor solution and the absorbance was read at 535 nm and 374 nm to quantify anthocyanins and flavanols after 2 h at room temperature, following the method of Lees and Francis (1972).

Determination of lycopene and ascorbic acid

Lycopene content was determined following the procedure by Wang *et al.* (2005). 2 grams of the tissue was extracted in 20 mL of hexane:acetone (v/v) and the organic layer was collected until the solution turned colorless. The absorbance was measured at 502 nm for lycopene and expressed as µg/g FW. The quantitative analysis of ascorbic acid (AA) was performed as per the method of Roe (1964). A 2 g tissue was homogenized in 10 mL of 5% metaphosphoric acid and glacial acetic acid and centrifuged. 0.3 – 0.4 mL of aliquot was mixed with 1 mL of 2% 2, 4 Dinitrophenyl hydrazine (DNPH) and 2 drops of 10% thiourea, tubes were kept for incubation for 3 h at 37ºC. The reaction was terminated by 5 mL of 85% H_2SO_4 and absorbance was measured at 540 nm. The standard graph was prepared by using ascorbic acid and expressed as mg/g FW.

Antioxidant enzyme assay

POD in the extracts (one gram in 10 mL) were

assayed as per the method of Guilbalt (1976). The specific activity of the enzyme was expressed as 1 unit change in OD/min/mg protein. Superoxide dismutase (SOD) (EC 1.15.1.1) was assayed according to the method of Wang et al. (2004). 2 grams of mesocarpic tissue was extracted with 10 mL of extraction buffer (0.05 M sodium- phosphate buffer, pH 7.8 containing PVPP). The supernatant was taken for the assay of SOD and expressed the activity as the U/mg protein where one unit (U) inhibits the reduction of cytochrome C by 50% in a coupled system.

Determination of sugars and sugar metabolizing enzymes

The procedures adopted for the extraction and analyses of sugars were as those cited by Thimmaiah (1999). The fleshy portion of the fruit (one gram) was extracted with 10 mL of 80% ethanol, centrifuged and the supernatant was collected, evaporated on water bath and later dissolved the sugars. The quantitative analysis of reducing (RS) and non- reducing sugars (NRS) (Dinitrosalicyclic acid (DNS method) was performed as per the methods cited by Thimmaiah (1999). Results were expressed as mg/g FW and all the measurements were done in triplicate.

The method of Hubbard *et al.* (1989) was followed for the assay of sucrose phosphate synthase (SPS) (EC 2.4.1.14), sucrose synthase (SS) (EC 2.4.1.13) and invertases (EC 3.2.1.26) – acid and neutral. The frozen melon tissue (2-2.5 grams) was ground using a 10 mL buffer. 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 1.5 mL of 50 mM Mops-NaOH (pH 7.5), 0.2 mL of 15 mM $MgCl_2$, 0.2 mL of 5 mM fructose 6-P, 0.2 mL of 15 mM glucose 6-P, 0.1 mL of 10 mM UDPG, and 0.3 mL of 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.

To determine the acid invertase (AI) activity, reaction mixtures contained 1.5 mL of 100 mM citrate-phosphate buffer (pH 5.0), 0.2 mL of 120 mM sucrose, and 0.3 mL of the crude enzyme extract and for that of neutral invertase (NI) activity, the reaction mixtures contained 1.5 mL of 50 mM Mops-NaOH (pH 7.5), 0.2 mL of 90mM sucrose and 0.3 mL of 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.

Determination of minerals

The analysis of minerals was performed as per the methodology of Jackson (1973). One gram of dry material was further processed for the wet digestion by the diacid (1 $HCLO_4$: 3 HNO_3) mixture and allowed to stand overnight. The samples were heated on hot plate until solid particles nearly disappeared and heat until a clear colorless solution is obtained. Once digested, samples were further evaporated to near dryness. Once the reaction has subsided, samples were cooled and made upto 100 ml with milliQ water. The solution was allowed to stand overnight, filtered through a dry paper to remove silica without washing. The solution containing samples was retained and used for the analysis of minerals against the reagent blank by atomic absorption spectrophotometer.

Protein assay

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

Statistical evaluation

Data were represented as means ± standard deviation from three independent analyzes. One-way analysis of variance (ANOVA) was performed according to a factorial design on the basis of Complete Randomized Design (CRD), the stages of development and ripening and replicates as the main factor. 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

Phenolic compounds

Phenolic compounds which are widely distributed in plants have gained much attention due to their antioxidant activities and free radical-scavenging abilities, and they have potential beneficial implications for human health. Among the four cultivars of watermelon, the amount of FP was highest in the 'Suman 235' followed by 'F1 Arun' in the ripened stage. All other three cultivars had their high levels of FP in the early stages of development (Table 1). TP level was highest in 'Suman 235' as well as 'Beauty'. However, TP got accumulated maximum in the ripened stage in all the cultivars. Polyphenol

Table 1. Quantitative analysis of free (FP) and total (TP) phenols and free (FPP) and total polyphenols (FPP) of four icebox cultivars of watermelon fruit during their development and ripening (mg/g FW).

Cultivars	FP	TP	FPP	TPP
F1 Arun				
Young	0.364 ± 0.12^{a}	0.801 ± 0.01^{b}	0.261 ± 0.05^{c}	0.780 ± 0.10^{c}
Pre-mature	0.211 ± 0.026^{a}	0.016 ± 0.004^{a}	0.095 ± 0.005^{a}	0.280 ± 0.067^{a}
Mature	0.259 ± 0.03^{a}	1.30 ± 0.3^{c}	0.201 ± 0.02^{b}	0.357 ± 0.034^{ab}
Pre-ripened	0.832 ± 0.07^{b}	1.13 ± 0.08^{c}	0.132 ± 0.007^{a}	0.440 ± 0.0023^{b}
Ripened	0.910 ± 0.10^{b}	1.11 ± 0.04b^{c}	0.414 ± 0.03^{d}	0.818 ± 0.06^{c}
Beauty				
Young	0.844 ± 0.2^{b}	1.43 ± 0.2^{a}	0.738 ± 0.2^{b}	2.09 ± 0.18^{ab}
Pre-mature	0.713 ± 0.1^{b}	3.09 ± 0.1^{b}	0.418 ± 0.04^{ab}	2.57 ± 0.43^{bc}
Mature	0.052 ± 0.02^{a}	1.75 ± 0.3^{a}	0.342 ± 0.03^{a}	1.65 ± 0.16^{a}
Pre-ripened	0.137 ± 0.02^{a}	1.35 ± 0.1^{a}	0.237 ± 0.05^{a}	2.09 ± 0.53^{ab}
Ripened	0.054 ± 0.01^{ab}	2.95 ± 0.1^{b}	0.499 ± 0.3^{ab}	2.98 ± 0.27^{c}
Karina King				
Young	0.291 ± 0.02^{c}	0.026 ± 0.02^{a}	0.064 ± 0.04^{b}	0.121 ± 0.04^{a}
Pre-mature	0.242 ± 0.01^{b}	0.140 ± 0.03^{c}	0.057 ± 0.01^{b}	0.123 ± 0.02^{a}
Mature	0.179 ± 0.005^{a}	0.154 ± 0.006^{c}	0.020 ± 0.01^{a}	0.140 ± 0.008^{a}
Pre-ripened	0.195 ± 0.008^{a}	0.222 ± 0.02^{d}	0.144 ± 0.005^{c}	0.267 ± 0.023^{b}
Ripened	0.203 ± 0.008^{a}	0.096 ± 0.05^{b}	0.073 ± 0.03^{b}	0.268 ± 0.04^{b}
Suman 235				
Young	0.194 ± 0.01^{a}	1.20 ± 0.04^{ab}	0.128 ± 0.003^{a}	0.579 ± 0.1^{a}
Pre-mature	0.300 ± 0.03^{ab}	2.34 ± 0.09^{c}	0.224 ± 0.02^{b}	0.864 ± 0.1^{c}
Mature	0.347 ± 0.04^{b}	1.58 ± 0.3^{b}	0.551 ± 0.05^{d}	0.767 ± 0.1^{bc}
Pre-ripened	1.27 ± 0.1^{c}	1.04 ± 0.1^{a}	0.735 ± 0.0^{c}	1.41 ± 0.03^{d}
Ripened	3.18 ± 0.06^{d}	3.27 ± 0.3^{d}	0.261 ± 0.009^{c}	0.659 ± 0.06^{ab}

Values represented are Means ± SD; Values in columns with the same letter are not significantly different (P < 0.05), by Duncan's Multiple Range Test (DMRT).

Table 2. Quantitative analysis of total antioxidant activity (TAC) (%) and antioxidants (mg/g FW) of four icebox cultivars of watermelon fruit during their development and ripening

Cultivars	Lycopene (µg/g FW)	TAC	AA	Flavanols	Anthocyanins
F1 Arun					
Young	8.24 ± 0.03^{a}	48.80 ± 0.0009^{a}	0.713 ± 0.04^{b}	0.294 ± 0.03^{b}	3.30 ± 0.01^{b}
Pre-mature	8.63 ± 0.3^{a}	49.20 ± 0.004^{a}	0.859 ± 0.1^{c}	1.26 ± 0.03^{c}	6.20 ± 0.04^{c}
Mature	20.30 ± 1.0^{c}	53.10 ± 0.006^{b}	0.367 ± 0.07^{a}	0.044 ± 0.0^{a}	3.28 ± 0.1^{b}
Pre-ripened	23.06 ± 0.4^{d}	53.30 ± 0.006^{b}	0.759 ± 0.1^{d}	0.270 ± 0.01^{b}	2.02 ± 0.08^{b}
Ripened	30.73 ± 0.4^{e}	55.30 ± 0.04^{b}	0.917 ± 0.1^{d}	0.086 ± 0.02^{a}	1.46 ± 0.04^{a}
Beauty					
Young	4.69 ± 0.01^{a}	53.60 ± 0.003^{ab}	3.53 ± 0.5^{a}	0.745 ± 0.001^{e}	0.026 ± 0.008^{a}
Pre-mature	22.53 ± 0.1^{c}	49.80 ± 0.017^{a}	3.42 ± 0.2^{a}	0.302 ± 0.002^{c}	0.047 ± 0.003^{b}
Mature	21.21 ± 0.2^{b}	60.30 ± 0.003^{c}	7.91 ± 1.7^{c}	0.541 ± 0.001^{d}	0.063 ± 0.004^{b}
Pre-ripened	22.67 ± 0.7^{c}	55.30 ± 0.04^{b}	23.04 ± 2.2^{a}	0.230 ± 0.001^{b}	0.006 ± 0.004^{a}
Ripened	32.44 ± 1.1^{d}	54.10 ± 0.002^{ab}	6.20 ± 0.1^{b}	0.189 ± 0.004^{a}	0.098 ± 0.001^{c}
Karina King					
Young	5.88 ± 0.9^{b}	2.32 ± 0.008^{b}	0.366 ± 0.03^{a}	0.054 ± 0.02^{a}	0.039 ± 0.008^{a}
Pre-mature	0.998 ± 0.06^{a}	1.90 ± 0.007^{b}	0.611 ± 0.1^{c}	1.08 ± 0.1^{c}	0.292 ± 0.003^{c}
Mature	6.31 ± 0.1^{c}	0.290 ± 0.0006^{a}	0.866 ± 0.1^{c}	0.684 ± 0.2^{b}	0.076 ± 0.01^{a}
Pre-ripened	6.07 ± 0.4^{c}	0.110 ± 0.0007^{a}	1.12 ± 0.09^{d}	0.702 ± 0.1^{b}	0.187 ± 0.009^{b}
Ripened	11.82 ± 0.1^{d}	0.100 ± 0.004^{a}	1.07 ± 0.1^{d}	0.029 ± 0.01^{a}	0.010 ± 0.005^{a}
Suman 235					
Young	1.16 ± 0.006^{a}	19.88 ± 6.3^{c}	0.019 ± 0.004^{ab}	0.283 ± 0.006^{c}	0.141 ± 0.01^{b}
Pre-mature	1.80 ± 0.03^{b}	16.10 ± 5.8^{bc}	0.006 ± 0.001^{a}	0.232 ± 0.002^{b}	0.129 ± 0.008^{b}
Mature	1.03 ± 0.02^{a}	18.97 ± 8.3^{bc}	0.044 ± 0.002^{cd}	0.166 ± 0.002^{a}	0.078 ± 0.01^{a}
Pre-ripened	3.59 ± 0.01^{c}	11.50 ± 4.1^{a}	0.035 ± 0.02^{bc}	0.242 ± 0.003^{c}	0.145 ± 0.006^{b}
Ripened	1.77 ± 0.01^{b}	13.01 ± 3.9^{ab}	0.062 ± 0.01^{d}	0.268 ± 0.001^{d}	0.144 ± 0.001^{b}

Values represented are Means ± SD; Values in columns with the same letter are not significantly different (P < 0.05), by Duncan's Multiple Range Test (DMRT).

(FPP, TPP) concentration was highest in 'Beauty' and lowest in 'Karina King' cultivar (Table 1). The maximum accumulation of TPP was noticed in the ripe fruit of 'Beauty' and minimum in 'Karina King' (Table 1). Statistically a significant difference (P < 0.05) was observed in the accumulation of phenolic compounds in all the cultivars of icebox watermelon determined under the current study.

Several studies cited by Ismail *et al*. (2010), indicated that the phenolic content in plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In the studies by Kolayli *et al.* (2010) in different cultivars of standard, hybrid and grafted melons, parallel trend of results were observed. The varied trend of polyphenol content in ripened stage was probably due to the different degree of the biosynthetic pathways of these compounds affected during ripening and also may be due to genetic, environmental factors as well as postharvest processing conditions (Kolayli *et al*., 2010). Toor and Savage (2006) attributed that the reduced levels of phenols during advanced ripening stages were due to the breakdown of cellular structure, including vacuoles, where soluble phenolic compounds may accumulate during ripening.

Total antioxidant activity and other antioxidants

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. A significant level (P < 0.05) of antioxidant activity was exhibited by the mature fruit of 'Beauty in comparison to other cultivars of watermelon (Table 2). However, the main effect of ripening process on total antioxidant activity was observed in 'F1 Arun' cultivar. Our results regarding the antioxidant activity were in accordance with the studies on the antioxidants in the fruit like honeydew melon. According to Smirnoff (1995), during ripening, increased level of antioxidant activity might be a self-defensive response against the effects of oxidative stress. Lester (2008) advocated that as antioxidants can scavenge/neutralize AOS, the tissue exhibiting high antioxidant capacities would better resist oxidative stress than tissue with lower antioxidant potential.

Ascorbic acid is an active form of vitamin C and its amount varies in different species of fruits and vegetables. In all the investigated cultivars of watermelon, the content of AA expressed on a FW basis, markedly increased during fruit ripening. Table 2 shows the higher values of AA in the pre-ripened stage of 'Beauty' and lower in pre-mature stage of 'Suman 235'. Our results for AA are consistent with the observations of Tan *et al.* (2012) in different cultivars of pepper. Tan *et al*. (2012) in their studies demonstrated an increase in AA content during ripening and reasoned that it may be due to the active involvement of AA in removal of H_2O_2 in response to elevated oxidative stress. According to Lee and Kader (2000), the accumulation of AA during ripening depends on the type of fruit. Hodges and Lester (2006) in their studies in muskmelon stated that the phytonutrient, ascorbic acid accumulate differently in various cultivars in response to different growing locations and yearly conditions.

Anthocyanins and flavanols are important as they determine the color quality of fresh fruits and vegetables. In the presently analyzed cultivars of watermelon, the amount of total anthocyanins and

flavanols were statistically significant ($P < 0.05$) in the initial stages of development and ripening. The maximum amount of anthocyanins was found to occur in the pre-mature stage of 'F1 Arun' cultivar and decreased gradually till the ripened stage. A slightly higher rise in anthocyanin content was observed in the pre-ripened stage of 'Karina King' and ripened stage of 'Beauty' as indicated in Table 2.

As depicted in Table 2, flavanols accumulated maximum in pre-mature stage of 'F1 Arun' whereas in ripe fruit of 'Karina King' low values was noticed for flavanols. On the other hand, in other two cultivars 'Beauty' and 'Suman 235', accumulation of flavanols peaked in the young stage of watermelon and decreased in the subsequent stages of development. Lycopene is a carotenoid pigment responsible for the color of red fleshed watermelon. Significant accumulation of lycopene was observed in the ripened stage of 'Beauty' followed by 'F1 Arun'. However, insignificant accumulation of lycopene was noticed in the ripe fruit of 'Karina King' and pre-ripe fruit of 'Suman 235' respectively (Table 2). During the ripening of icebox cultivar of watermelon fruit, a massive deposition of lycopene occurred which in turn indicated that watermelon accumulates lycopene as its major fruit carotenoids. The carotenoid content in watermelon is also influenced by the color of the flesh (Tarazona Diaz, 2011). Dragovic Uzelac (2007) stated the reason for the accumulation of lycopene as, it may be due to enhanced carotenoid biosynthesis or by the catalytic activity of phytoene synthase of the first step of the formation of carotenoids. Perkins-Veazie *et al.* (2006) opined that a wide range in lycopene content exists among watermelon germplasm and that watermelon cultivars with very high lycopene contents are available. Carotenoid accumulation is a net result of biosynthesis, turnover and finally stable storage of the end products.

Correlation between total antioxidant activity and antioxidants

A correlation was drawn between antioxidant activity and various antioxidants in the four icebox cultivars of watermelon. Phenolic compounds are believed to account for a major portion of the antioxidant activity in many plants. However, from our findings, a weak correlation was observed between TP and total antioxidant activity in all the four cultivars of watermelon. There was a correlation with 0.152 (r^2) in 'F1 Arun' compared to other cultivars, (Table 3). A moderate correlation was obtained between TPP and DPPH values ($r^2 = 0.468$) in cultivar 'Beauty' compared to other cultivars as shown in Table 3. The linear correlation coefficient ($r^2 = 0.727$) value indicated a strong correlation between AA content and total antioxidant activity in fruit of 'Karina King' as presented in Table 3. However, as summarized in Table 3 in all the other cultivars, a low correlation was observed between AA and DPPH activity. In the fruit of 'F1 Arun', lycopene content correlated well ($r^2 = 0.987$) with the DPPH activity. The higher value for correlation indicated the elevated concentration of antioxidant compounds in watermelon fruit. Moderate level of correlation was observed in 'Suman 235', whereas 'Karina King' and 'Beauty displayed a low correlation between lycopene and antioxidant activity (Table 3). The phenolic compounds showed low level of correlation between DPPH activity in the present study which agreed with the studies by Demiray *et al.* (2009) and Sulaiman *et al.* (2011). The low correlation confirmed that other than phenolics, presence of non-phenolic antioxidants like ascorbic acid and lycopene might be accountable in enhancing the antioxidant activity (Sulaiman *et al.*, 2011).

Table 3. Linear correlation coefficient (r^2) between total antioxidant activity (DPPH value) and antioxidants of four icebox cultivars of watermelon fruit during their development and ripening

Cultivars	Linear correlation coefficient (r^2)			
	TP	TPP	AA	Lycopene
F1 Arun	0.152	0.041	0.0004	0.987
Beauty	0.105	0.468	0.091	0.001
Karina King	0.066	0.125	0.727	0.093
Suman 235	0.081	0.261	0.150	0.710

Table 4. Specific activities of peroxidase (POD), superoxide dismutase (SOD) (Units/mg protein) and acid (AI) and neutral invertase (NI) (µmol/h/mg protein) of four icebox cultivars of watermelon during their development and ripening

Cultivars	POD	SOD	AI	NI
F1 Arun				
Young	0.041 ± 0.02^{bc}	0.0009 ± 0.0003^{b}	0.004 ± 0.0008^{a}	0.001 ± 0.0006^{a}
Pre-mature	0.025 ± 0.01^{a}	0.002 ± 0.0003^{a}	0.006 ± 0.0004^{a}	0.004 ± 0.001^{a}
Mature	0.006 ± 0.001^{c}	0.005 ± 0.0004^{a}	0.001 ± 0.0005^{a}	0.009 ± 0.001^{a}
Pre-ripened	0.017 ± 0.001^{abc}	0.009 ± 0.003^{a}	0.004 ± 0.001^{a}	0.007 ± 0.002^{a}
Ripened	0.005 ± 0.001^{ab}	0.005 ± 0.0009^{a}	0.003 ± 0.0005^{a}	0.001 ± 0.0007^{a}
Beauty				
Young	0.094 ± 0.04^{b}	0.022 ± 0.007^{ab}	0.030 ± 0.002^{a}	0.011 ± 0.003^{a}
Pre-mature	0.005 ± 0.0009^{a}	0.005 ± 0.001^{a}	0.030 ± 0.002^{a}	0.003 ± 0.0002^{a}
Mature	0.163 ± 0.03^{c}	0.023 ± 0.02^{ab}	0.030 ± 0.002^{a}	0.026 ± 0.006^{a}
Pre-ripened	0.039 ± 0.005^{a}	0.028 ± 0.01^{b}	0.033 ± 0.002^{a}	0.053 ± 0.02^{b}
Ripened	0.019 ± 0.01^{a}	0.010 ± 0.006^{ab}	0.030 ± 0.002^{a}	0.005 ± 0.002^{a}
Karina King				
Young	0.281 ± 0.03^{bc}	0.474 ± 0.1^{b}	1.97 ± 0.1^{b}	0.069 ± 0.04^{a}
Pre-mature	0.100 ± 0.01^{a}	$0.020 \pm 0.007a$	0.079 ± 0.01^{a}	0.005 ± 0.001^{a}
Mature	0.338 ± 0.1^{c}	0.003 ± 0.001^{a}	0.051 ± 0.003^{a}	0.005 ± 0.001^{a}
Pre-ripened	0.245 ± 0.1^{abc}	0.0004 ± 0.0001^{a}	0.028 ± 0.005^{a}	0.008 ± 0.001^{a}
Ripened	0.164 ± 0.03^{ab}	0.0008 ± 0.0003^{a}	0.059 ± 0.004^{a}	0.008 ± 0.0006^{a}
Suman 235				
Young	0.0002 ± 0.00002^{c}	0.009 ± 0.001^{ab}	0.007 ± 0.003^{a}	0.009 ± 0.008^{a}
Pre-mature	0.0003 ± 0.00002^{ab}	0.016 ± 0.001^{ab}	0.0004 ± 0.0002^{a}	0.001 ± 0.0006^{a}
Mature	0.00005 ± 0.00^{a}	0.005 ± 0.003^{b}	0.0085 ± 0.008^{a}	0.002 ± 0.001^{a}
Pre-ripened	0.0002 ± 0.00001^{b}	0.011 ± 0.001^{c}	0.0009 ± 0.0005^{a}	0.003 ± 0.002^{a}
Ripened	0.0005 ± 0.00009^{ab}	0.012 ± 0.002^{a}	0.002 ± 0.001^{a}	0.002 ± 0.0001^{a}

Values represented are Means ± SD; Values in columns with the same letter are not significantly different ($P < 0.05$), by Duncan's Multiple Range Test (DMRT).

Antioxidant enzymes

Plants defend against the deleterious effects of free radicals by the action of antioxidant enzymes such as POD and SOD. The specific activities of POD

Table 5. Concentrations of Potassium (K), Sodium (Na), Iron (Fe), Copper (Cu), Zinc (Zn) and Manganese (Mn) of four icebox cultivars of watermelon during their development and ripening (mg/ Kg)

Cultivars	K	Na	Fe	Mn	Cu	Zn
F1 Arun						
Young	164.49	15.56	ND	0.247	0.186	0.924
Pre-mature	105.46	15.15	0.912	0.141	0.228	0.687
Mature	96.14	12.68	1.55	0.164	0.304	0.333
Pre-ripened	67.88	14.54	2.62	0.098	0.052	0.595
Ripened	48.58	11.04	ND	0.039	0.016	ND
Beauty						
Young	109.69	9.64	0.657	0.046	0.074	48.00
Pre-mature	39.66	9.42	0.673	0.021	0.020	1.31
Mature	72.74	32.39	0.686	0.038	0.053	9.10
Pre-ripened	9.74	1.31	0.238	0.008	0.008	0.545
Ripened	6.31	0.808	0.014	0.008	0.025	0.354
Karina King						
Young	31.52	40.82	0.590	0.075	0.039	
Pre-mature	51.80	101.65	0.600	0.109	0.059	
Mature	95.78	73.02	0.640	0.076	0.068	ND
Pre-ripened	43.60	59.20	0.430	0.041	0.070	
Ripened	22.26	45.69	0.260	0.059	0.018	
Suman 235						
Young	392.80	6.512	1.03	0.025	0.071	0.101
Pre-mature	430.65	8.07	0.656	0.036	0.031	0.130
Mature	28.15	8.58	1.05	0.054	0.099	0.178
Pre-ripened	12.25	10.75	0.827	0.098	0.126	0.289
Ripened	6.72	11.61	1.81	0.085	0.083	0.321

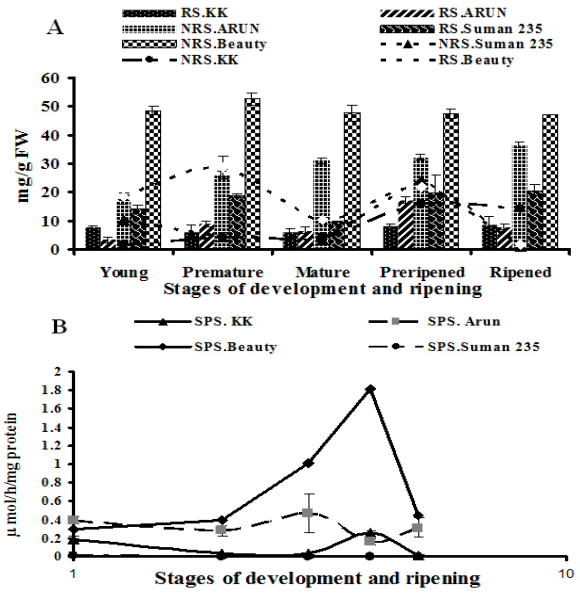

Figure 1. Quantitative analysis of reducing sugars (RS) and non-reducing sugars (NRS) (A) (mg/g FW) and Specific activity of sucrose phosphate synthase (SPS) (B) (µmol/hr/mg protein) of four icebox cultivars of watermelon fruit during their development and ripening

and SOD was significantly ($P < 0.05$) higher during the initial stages of development and ripening of the fruit of 'Karina King'. Table 4 indicated maximum POD activity in the mature stage of 'Karina King' followed by 'Beauty' cultivar. However, the pre-mature stage of 'F1 Arun' and ripened stage of 'Suman 235' influenced the POD activity. The lowest activity of SOD was obtained in the pre-ripened stage of 'Karina King' (Table 4). In a similar pattern, Chisari *et al.* (2010) observed in melons 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 Lamikanra 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. The high activity of the SOD in the mesocarpic tissue in the 'Karina King' watermelon cultivar is in agreement with the findings of Lester (2008) who advocated that the activity of the antioxidant enzymes in the inner mesocarp tissue may be associated with ripening that generates reactive oxygen species.

Sugars and their metabolizing enzymes

Sugar content is an important quality characteristic of the watermelon fruit. Among the four icebox cultivars studied, 'Beauty' displayed highest amount of reducing (28.67 mg/g FW) and non-reducing (52.71 mg/g FW) sugars. However, in all the cultivars comparable levels of sugars got accumulated in their ripened stage (Figure 1(A). The results of current investigation supports the findings of Lester (2008) in the honeydew melon who documented different profiles of sugars like sucrose, fructose and glucose as possible in the melon fruit and they derived from the sucrose hydrolysis, serve as a substrate for respiratory energy production to support growth and storage function in the hypodermal mesocarpic tissues. In a similar line of research in oriental melons by Shin *et al*. (2007) affirmed that at the postharvest maturity of oriental melons, the respiration was carried out by the normal process of TCA cycle. Previously various researchers (Hubbard *et al*., 1989; Seymour *et al*., 1993; Menon and Rao, 2012b) in their studies in melons reported that a substantial pool of hexose sugars is present throughout the development of muskmelon fruit, but sucrose was accumulated only in the final stages of ripening of the fruit as carbohydrates translocation occurred in melon fruits during ripening. The results of the present study regarding the accumulation of sugars in the icebox cultivars were consistent with that of the observations of Hubbard *et al.* (1989); Seymour *et al.* (1993); Menon and Rao (2012b) and Lester (2008).

The icebox cultivars accumulated high amount of sugars towards their ripening, but no significant difference ($P < 0.05$) was observed in the specific activity of SPS, a sugar synthesizing enzyme. As indicated in Figure 1(B), SPS exhibited maximum activity in 'Beauty' in its pre-ripened stage, while minimum of it was observed in 'Suman 235'. However, AI and NI exhibited no significant difference ($P <$

0.05) in their activities in the sugar cleaving process in the watermelon fruit under the current study. The maximum activities of AI (1.974 µmol/h/mg protein) and NI (0.0694 µmol/h/mg protein) was observed in the young stage of 'Karina King' and minimum in 'F1 Arun' as presented in Table 4. On the contrary, AI and NI enzymes displayed their maximum activities in the pre-ripened fruit of 'Beauty'. The increased activity of SPS suggests the possibility that sucrose may be synthesized from alternate substrates other than those derived from sucrose hydrolysis (Hubbard *et al.*, 1989). Immature fruit that lacked a detectable pool of sucrose had high invertase activity and low SPS activity. Lingle and Dunlap (1987) in their studies in muskmelon fruit emphasized that sugar composition, a very important aspect of fruit quality, that it may be influenced by environmental factors affecting the activity of these enzymes. Ultimately, melon fruit quality was determined based on the regulation of the sugar composition due to the changes in the relative activities of the sugar metabolizing enzymes.

The activities of acid and neutral invertases was high in the initial stages of watermelon fruit ripening in all the cultivars which are in agreement with the findings of studies by Menon and Rao (2012a) in watermelon and Lee *et al*. (1997) in muskmelon fruit. Results suggested that the ultimate source of sugar in watermelon fruit may be the photosynthate translocated from the mother plant. The deposition of that translocated sugar occurs within the fruit, and thus the sugar composition is determined by partitioning of sugar within the tissue, and the metabolism of sugar within each compartment. In the present study, an apparent relationship was maintained between the sugar composition of developing watermelon fruit and the relative activities of enzymes in sucrose metabolism.

Minerals

The concentration of mineral nutrients like Na, Fe and Zn on dry weight basis were distinguishably higher in the ripe fruit of 'Suman 235', whereas the other micro elements (Cu and Mn) showed their maximum concentrations in the pre-ripened stage of cultivar 'Suman 235'. In contrast to the presented data for 'Suman 235', other cultivars of icebox watermelon showed their higher mineral concentration (K, Na and Mn) in the early stages of development and ripening (Table 5).

Conclusion

The present study on four icebox cultivars of watermelon is a measure of their nutritional value in terms of pigments, sweetness and antioxidants. A significant level of accumulation of sugars, lycopene and other antioxidants like phenolics was observed in the fruit of 'Beauty' as compared to other cultivars which suggests that the cultivar 'Beauty' has better nutritional quality than that of other three cultivars analyzed under the current study. Hence the fruit of 'Beauty' can be considered as a supplement to make human diets more balanced as consumer quality acceptance is generally related to specific perceived quality attributes. Furthermore, the results of the present study could be used by farmers to determine the optimum stage of harvesting and by consumers to choose the cultivar with best nutritional quality.

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