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Metabolic behavior and quality changes of yellow-fleshed watermelon at different stages of ripening

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ABSTRACT
Watermelon is a fruit known to be rich in health promoting phytonutrients and antioxidants. The present study was aimed at evaluating the nutritional potential of yellow-fleshed watermelon (*Citrullus lanatus* (Thunb.) Matsum. &Nakai), a less studied fruit, based on its composition and biochemical activities during development and ripening. Total sugars (TS), antioxidants (phenols, polyphenols, ascorbic acid, flavanols) and antioxidant capacity, sugar related enzymes (sucrose phosphate synthase, sucrose synthase), antioxidant enzymes (peroxidase, polyphenol oxidase) and softening enzymes (polygalacturonase, cellulase, β-Galactosidase) were determined at five sequential stages of development and ripening of the fruit. The amount of TS increased considerably during the fruit development and a significant accumulation of it occurred in the ripe fruit. Significant accumulation of phenols, polyphenols,flavanols and ascorbic acid was also observed in fully ripened fruit. Sucrose phosphate synthase displayed a sharp increase in its activity in the pre-ripened stage, but eventually it declined. Besides this, a positive relation with the activity of sugar metabolizing enzyme and sugar accumulation was observed. With the progression of ripening, the concentrations of sodium, zinc, copper showed an increase, while potassium, iron and manganese exhibited a reverse trend. The increased levels of phytochemicals and sugars confirmed that yellow-fleshed watermelon cultivars are equivalent to the red-fleshed watermelon cultivars with respect to its nutritional quality and improves the potential for its increased commercialization.

KEYWORDS: antioxidant activity, ascorbic acid, phenols, ripening, sugars,sucrose phosphate synthase

I. INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. &Nakai) is an important horticultural crop cultivated around the world (Perkins-Veazie et al. 2006) and it accounts for about 2% of the world's total vegetable production (FAO 1994). Watermelon accumulates lycopene as its major mesocarp carotenoids, which have recently aroused interest as health promoting phytochemicals. In general, carotenoids being antioxidants and precursors of vitamin A, exhibit many health-promoting activities, including lowering blood pressure and preventing heart disease (Lewinsohn et al. 2005). Flesh color is an important quality attribute that determines attractiveness and is indicative of health promoting benefits of watermelon (Bang et al. 2010). A comparable range of fruit colors is available in watermelon such as white, yellow (pale, canary, salmon), orange and red (King et al. 2009). Carotenoids are mostly responsible for the different flesh color in watermelon and neoxanthin is the predominant carotenoid in yellow-fleshed watermelon (Zhao et al. 2013).

Fruit ripening involves physiological, biochemical, and structural changes such as cell wall hydrolysis, pigment synthesis and degradation, carbohydrate metabolism, and generation of secondary metabolic compounds which influence appearance, texture, flavor and aroma and the nutritional quality of fruit and ripening is a genetically programmed process (Li et al. 2006; Mworia et al. 2012). Sweetness in melon is characterized by metabolic transition during its development that may result in highest accumulation of the disaccharide sucrose (Dai et al. 2011). Glucose and fructose accumulate in the initial stages of fruit development, whereas sucrose accumulates during ripening and after harvest in watermelon fruit. Sucrose content is a predominant factor determining sweetness of watermelon (Ikeshita et al. 2010). The accumulation of sucrose seems to be related to sucrose-phosphate synthase (SPS, EC 2.4.1.14) and sucrose synthase (SS, EC 2.4.1.13) and acid invertase (AI, EC 3.2.1.26) activities (Dai et al. 2011).

Nutritional status is an important trait determined by the abundance of minerals and antioxidant-related phytochemicals that influence

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the quality and postharvest behavior of fruits. Antioxidants are a group of compounds capable of delaying or inhibiting the oxidation of other molecules thus preventing the generation of free radicals. Therefore, their analysis is necessary as they are considered as important nutritional factors. Furthermore, quantification of antioxidants and antioxidant activity portrays the nutritional value evaluation more than just the analysis of single components (Menon and Rao, 2014a).

An elaborate and highly redundant plant reactive oxygen species (ROS) network, composed of non-enzymatic antioxidants and antioxidant enzymes plays a major role in preserving the levels of ROS under tight control. Non enzymatic antioxidants like phenols, ascorbate, glutathione and antioxidant enzymes such as catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7), superoxide dismutase (SOD, EC 1.15.1.1) and polyphenol oxidase (PPO, EC 1.14.18.1) have been viewed as a synergistic antioxidant defensive system, whose combined purpose is to protect cells from active oxygen damage (Agarwal and Pandey 2004).

Ripening is associated with textural changes, which occur as a result of primary wall disassembly. During ripening process in fruits, softening is associated with the alterations in cell wall and middle lamella structure, whereby the pectin wall is increasingly depolymerized (Li et al. 2006). Cell wall degrading enzymes such as polygalacturonase (PG, EC 3.2.1.15), β-galactosidase (β-Gal, EC 3.2.1.23), pectin methyl esterase (PME, EC 3.1.1.11) and cellulase (Cx, EC 3.2.1.4) are the enzymes involving in softening of fruits.

Literature is replete with information on the biochemical composition and enzymatic activities in red-fleshed watermelon (Lewinhson et al. 2005; Ikeshita et al. 2010; Yativ et al. 2010; Menon and Rao 2012a). However, to the best of our knowledge, no comprehensive study the nutritional quality of yellow watermelon has been carried out yet. Consumers normally prefer red-fleshed watermelon. Awareness about the health promoting effects of yellow-fleshed watermelon is very scanty among the consumers as well as producers. Therefore, the present study was envisaged to portray a comprehensive view of the bioactive compounds and the enzymes which are involved in improving the nutritional quality of yellow watermelon during different stages of its development and ripening.

II. MATERIALS AND METHODS

Plant Material

The fruits of yellow-fleshed watermelon (**cv.158**) were collected from the fields of Dehgam region of Gujarat, India at five stages of their development *viz.* young, pre-mature, mature, pre-ripened and ripened

Determination of total sugars and their related enzymes

Total sugars (TS) content was analyzed by following the phenol-sulphuric acid method as cited by Thimmaiah (1999). The mesocarpic tissue of fresh watermelon (1 gm) was extracted in 10 mL of 80% hot ethanol. Supernatant was collected and evaporated on a water bath at 80ºC for 5 min, added 10 ml of water and dissolved the sugars. This extract was used for estimation of TS. The reaction mixture contained a known aliquot of the extract, 1 ml of 1% phenol, and 5 ml of 96% H_2SO_4. After 10 min tubes were placed in a water bath at 25-30ºC for 20 min. The color formed was measured using spectrophotometer (MINI-SPEC 400) at 490 nm.

The methodology described by Hubbard et al. (1989) was followed for assay of sugar metabolizing enzymes. The frozen melon tissue was homogenized in a 1:5 tissue-to-buffer ratio. Extraction 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, 0.5 mg/ml of BSA. The homogenate was centrifuged at 15000 rpm for 15 min at 4ºC and the supernatant was used for the enzyme assay. Reaction mixtures for the assay of SPS activity contained 1.5 mL of 50 mM MOPS-NaOH (pH 7.5), 20 µL of 15 mM $MgCl_2$, 10 µL of 5 mM fructose 6-P, 10 µL 15 mM glucose 6-P, 1 µL of 10 mM uridine diphosphate glucose (UDPG), and 50 µL of crude enzyme extract . Reaction mixture was incubated at 25°C and the reaction was terminated at 0 and 20 min with the addition of 70 µL 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 the absorbance was measured at 620 nm. For the assay of SS enzyme, the reaction mixtures contained 10 mM fructose and all the other components, but did not contain fructose 6-P or glucose 6-P.

Estimation of phenolics, flavanols and ascorbic acid

Total phenolics and polyphenolics in the extracts were determined spectrophotometrically according to the protocol described by Vinson et al. (2001). Total phenols (TP) were extracted from the mesocarpic tissue (1 gm) in a mixture of 1.2 M HCl

in 10 mL of 50% 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. Total polyphenols (TPP) were extracted with 1.2 M HCl in 10 mL of 60% methanol and treated as above. The absorbance was measured at 750 nm for total phenols and 765 nm for total polyphenols. TP content was calculated from the calibration curve using catechol as a standard and TPP was calibrated from standard curve prepared with gallic acid.Results were expressed as catechin equivalent in milligram per gram fresh weight (mg/g (FW)) for TP and as gallic acid equivalents (mg GAE/g FW) for TPP, respectively.

For analysis of flavonoids, one gram of mesocarpic tissue was homogenized in 20 mL of 95% ethanol: 1.5 N HCL (85:15 v/v) and kept at 4ºC overnight as per the methodology of Lees and Francis (1972) and the samples were filtered and residues were washed to ensure complete removal of pigments. The filtrates were pooled and made up to a total volume of 100 ml with the same solution. The absorbance was determined at 374 nm after keeping for 2 h at room temperature.

Ascorbic acid was determined using dinitro phenylhydrazine (DNPH) according to the method described by Roe (1964). Sample of watermelon fruit (1 gm) was homogenized in 10mL of 5% metaphosphoric acid: glacial acetic acid mixture and then centrifuged for 10 min at 5000 rpm. The reaction mixtures containing 1 mL of 2% DNPH, a few drops of 10% thiourea and 0.2 mL of homogenate were incubated for 3 h at 37ºC. After the incubation period, reaction was terminated by the addition of 85% H_2SO_4, measured the absorbance at 540 nm and expressed the value as mg/g (FW).

Antioxidant activity

The antioxidant activity was evaluated by using the free radical 2, 2-diphenyl-1picrylhydrazyl (DPPH) as per the methods of Samee et al. (2006) and Narwal (2009). A 0.1 ml of aliquot was mixed with the 100 µM of DPPH (dissolved in methanol), kept in the dark for 30 min at room temperature and the absorbance was measured at 517 nm against methanol as blank. The total antioxidant capacity (TAC) was expressed in % activity.

Assay of antioxidant enzymes

One gram of tissue was homogenized in 10 mL of 0.1 M phosphate buffer (pH 7.2) containing 1 mM polyvinyl pyrrolidone (PVP), centrifuged and the supernatant was taken for assay of POD. The specific activity of the enzyme was expressed as 1 unit of enzyme considered as OD_{460}/min/mg protein, as per the method by Guilbalt (1976). For the assay of PPO enzyme activity, one gram of tissue was homogenized in 15 mL of cold acetone and continuously stirred for 10 min. The homogenate was filtered using a cheese cloth, the residue was collected and suspended in 10 mL of 0.1 M citrate phosphate buffer (pH 7.5) and kept overnight at 4°C. The enzyme assay was performed using 3,4-dihydroxyphenylacetic acid (DOPAC) with 3-methyl-2-benzothiazolinone hydrazone (MBTH) as substrates and measuring the absorbance at 505 nm (Chisari et al. 2008).

Assay of softening enzymes

β- Galactosidase

The mesocarpic tissue (2 gms) was homogenized in 15 mL of 10 mM sodium-phosphate buffer (pH 7.2) containing 50 mM NaCl. The homogenate was centrifuged at 14, 000 rpm for 40 min at 4°C and the supernatant was collected and used as the enzyme source. The assay mixture consisted of 1 mL of 50 mM sodium acetate containing 0.2 mg/ml of BSA at pH 4.0 and 0.1 mL of 10 mM of PNPG. The reaction mixture was incubated at 30°C for 5 min before the addition of the enzyme. After the addition of 0.1 mL of enzyme, the mixture was incubated for 10 min and the reaction was terminated by the addition of 0.5 mL of 0.5 M sodium carbonate and the *p*-nitrophenol formed was determined by measuring the absorbance at 405 nm and expressed one unit of enzyme activity as µmol *pnp* released/mg protein (Nakamura et al. 2003).

Assay of polygalacturonase and cellulase

Softening enzymes, PG and CMC-Cx activities were determined as per the methodology of Srivastava and Dwivedi (2000). The melon tissue (2 gms) was homogenized in a mortar using a 1:5 tissue-to-buffer ratio at (4ºC). The extraction mixture contained 10 mL of 0.02 M phosphate buffer (pH 7.0) containing 20 mM cysteine-HCL, 20 mM EDTA and 0.05% Triton X-100. The homogenate was centrifuged and the supernatant was assayed for enzyme activities of PG and CMC-Cx. The reaction mixture for Cx assay contained 1.5 mL of 100 mM acetate buffer (pH 5.0) and 0.2 mL of the enzyme extract, which was incubated at 37ºC for 16 h. After the incubation period, carboxymethyl cellulose (CMC) was added to the control tubes and the color developed was analyzed by DNS method. The absorbance was measured at 545 nm and expressed the specific activity of cellulase as one unit of activity as mg glucose released/h/ mg protein

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The reaction mixture for the assay of PG activity contained 1.5 mL of 200 mM sodium acetate buffer (pH 4.5), 0.5 mL of 1% PG (polygalacturonic acid) (pH 4.5) and 0.2 mL of enzyme extract and made up to a total volume of 3 ml with distilled water. The mixture was incubated for 1 h at 37ºC and the reducing sugar formed was determined by DNS method. In control tubes, substrate was added after the incubation period. The reducing groups formed were estimated against D-galacturonic acid as a standard and the absorbance was measured at 540 nm. 1 unit of PG activity = 1 mg reducing group formed/h/mg protein.

Mineral Analysis

Analysis of minerals was performed as per the methodology of Jackson (1973).One gram of dry material was digested by the acid mixture (1 $HCLO_4$: 3 HNO_3) keeping on a hot plate overnight until a clear colorless solution was obtained. The digested samples were evaporated to near dryness. Samples were then cooled and made up to 100 ml with de-ionized water. The samples which were cooled and pooled with water were allowed to stand overnight, filtered through a dry paper to remove if any residues of silica are present. The solution containing samples was retained and used for analysis of minerals against the reagent blank by atomic absorption spectrophotometer (AAS).

Protein assay

Protein content in the crude enzyme samples was determined according to Bradford (1976) using 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 data was performed using the IRRISTAT software (Bliss 1967).

III. RESULTS

Sugars and its related enzymes

Sugar content is one of the major attributes that determine the commercial maturity and fruit quality of watermelon. The amount of TS varied significantly between the developmental stages as given in Fig. 1 where, the values were higher in the young stage, but decreased in the subsequent stages. However, the TS got accumulated in its maximum (Fig. 1) as the progressed towards ripening. We found that the activity of SPS, sucrose synthesizing enzyme, was inconsistent during the development and ripening of watermelon. The activity of SPS was found to be high in the young stage, decreased subsequently, but a sudden rise (13%) in it was noted in mature stage (Table 1). A significant level of SPS activity ($P < 0.05$) was noted in the ripened stage of the yellow watermelon fruit. With the onset of maturation, a progressive increase in the SS activity was observed. The activity of SS declined abruptly in the pre-ripened stage, but increased sharply being the highest of its level during the ripening (Table 1).

Antioxidants and antioxidant enzymes

Phenolic compounds are a class of antioxidants, which act as free radical terminators and also play a role in the defense mechanism of plants. In the present study, the concentrations of FP, TP and FPP and TPP were determined in yellow-fleshed watermelon fruits during its development and ripening. A gradual increase in the levels of FP and TP was noticed, (Fig. 2). During maturation, however, there was a decline in the amount of TP. A sharp and consistent rise in the levels of TPP was noticed until the maturation of the fruit, but showed a subsequent decline in its amount as indicated in Fig. 2. A substantial amount of FPP and TPP was found in the ripe fruit, which was statistically significant ($P< 0.05$).

From the nutritional point of view, other antioxidants such as flavanols were also analyzed during the development and ripening of watermelon. A steady increase in flavanol level appeared to be present during the development and ripening of yellow watermelon as shown in Table 2. A significant ($P < 0.05$) level of flavanol was noticed in the ripe fruit of watermelon. Fruits and vegetables are the most important dietary source of ascorbic acid; it is an important nutritional and health related attribute of plant foods. In the early stages of fruit development, the amount of AA was higher, but dropped in its level during maturation. A considerable increase in the concentration of AA, however, was noticed in the ripened stage (Table 2).

The antioxidant activity is an important parameter for assessing the nutritional value of a fruit. A high level of total antioxidant activity was noticed in young fruit which declined in the subsequent stages of development (Table 2). However, a slight increase in antioxidant activity was noticed in the ripe fruit.

Higher level of POD activity was observed in the young stage of watermelon fruit. The intermediate phases between the well-defined stages showed reduction before the surge. However, the highest and significant ($P < 0.05$) activity level of POD was noticed in the ripe fruit (Table 3). PPO activity was inconsistent during the development and ripening. Initially, the activity was high, but declined with the onset of maturity. At the pre-ripened stage, a rapid increase in the activity of PPO by 10-fold was observed, but the activity declined eventually (Table 3).

Softening enzymes

β-Gal activity increased appreciably throughout the development and ripening of watermelon fruit. A significantly high activity ($P< 0.05$) of β-Gal was evident in the ripe fruit. β-Gal activity showed a positive relation with the tissue softening process. PG enzyme exhibited a consistent pattern of activity from the young to mature stages, but a decline in its activity by two-fold was noticed in the pre-ripened stage. A marked increase in the activity of PG by 3.4-fold was observed in the ripe stage thereby influencing the process of softening (Fig. 3). In contrast, the activity of Cx was fluctuating throughout the development and ripening of the fruit. A significantly high level ($P < 0.05$) of activity of Cx was however, found in the mature stage, but a decrease by 15-fold was noticed in the subsequent stages till the end of ripening.

Minerals

A gradual and significant increase was observed in the content of Na during the fruit development. A significant accumulation of Na was found to be in the ripe fruit of yellow watermelon. However, an opposite trend was observed for Zn where highest concentration of it appeared during maturation and lowest level during ripening. The accumulation of Mn and Cu, which are essential for human health, was found to be maximum in the premature and mature fruit, respectively. The concentrations of both these minerals declined significantly as the fruit progressed to full ripening stage as presented in Table 4.

IV. DISCUSSION

Total sugars got accumulated in the early stages of fruit development, decreased with maturation, but its maximum level was found in the ripe yellow watermelon fruit. This result is in conformity with the previous reports by Liu et al. (2013) on sweet and non-sweet watermelon fruits. They have reported that sucrose level was highest in the ripened stage in non-sweet watermelons, whereas in sweet type, it was high in early maturity stages. Yativ et al. (2010) believed that phloem unloading and sugar metabolism are the factors for higher TS levels with the commencement of ripening, and also it may be due to the transformation and spatial compartmentalization of sugars (McCollum 1987). Therefore, TS levels in watermelon are determined by the sucrose accumulation as it indicates the sweetness of the fruit.

Sucrose metabolizing enzyme activities are relevant and predominant in sucrose accumulation phenomenon in yellow watermelon fruit during ripening and followed the trend, which was noticed in our earlier studies on red-fleshed watermelon (Menon and Rao 2012a) and muskmelon (Menon and Rao 2012b) fruit. Lingle and Dunlap (1987) in their studies in muskmelon fruit emphasized that sugar composition, a very important aspect of fruit quality, may be influenced by environmental factors affecting the activity of these enzymes.

Phenolic compounds are important as they participate in the defense mechanism, contribute to some organoleptic and quality properties in food and are highly beneficial for health due to their antioxidant property. Accumulation of high levels of TP and TPP in the ripe fruits of yellow watermelon was observed in the present study. The main factors influencing the presence, quantity and quality of phenols in plant foods are genetic factors, environmental conditions, degree of ripeness, variety, etc. (Melo et al., 2006; Miletic et al. (2012). On the other hand, Toor and Savage (2006) reported that during the advanced ripening stage of fruits, the breakdown of cellular structure including vacuoles occur where soluble phenolic compounds may accumulate which ultimately lead to the reduction in the content of phenols.

In the present study, a conspicuous accumulation of AA was observed in ripe fruit of yellow-fleshed watermelon. The results are in agreement with the findings of Lee and Kader (2000), Gordon et al. (2012), and Siddhique et al. (2013). According to Lee and Kader (2000), accumulation of AA during ripening depends on the type of fruit. Gordon et al. (2012) determined the AA levels during ripening of cashew apple and stated that its formation seemed to depend, in particular, on the species. Higher AA content was noticed by Lester (2008) in the mesocarpic tissue of ripe honeydew melon. He also observed a similar phenomenon in orange-fleshed melon and concluded that the increased level of AA might be concomitant with the higher antioxidant capacity requirement in the mesocarp which is the physiologically active region of the fruit. Zhao et al.

(2011) recorded high amount of AA in grafted muskmelon fruit during its ripening and suggested that endogenous AA in fruits played a major role in maintaining its quality and shelf life.

As far as we know very little information is available on the activity of antioxidant enzymes (POD and PPO) in yellow-fleshed watermelon fruit. In the present study, POD exhibited higher activities in the early stages of fruit development and also in the final stage of ripening. This is in agreement with our earlier findings on red-fleshed watermelon (Menon and Rao, 2012a) and that of Chisari et al. (2010) on muskmelon. Further, Chisari et al. (2010) have pointed out that POD might promote the firmness of outer tissues, together with the processes involved at earlier ripening stages. Lamikanra and Watson (2001) have put forth a hypothesis that the activity of POD is not necessarily related to the total phenol content of fruits and vegetables and only a relatively small part of food phenolics can serve as substrates for PPO, which seems to be more plausible. According to Chisari et al., (2009) the increased activity of PPO in the initial stages of development could be related to accelerated metabolism of fruits. In the present case also, increased activities of PPO and POD may be due to the higher metabolic activity during the developmental stages of the yellow-fleshedmelon fruit.

It was found that ripening process did not significantly influence the total antioxidant activity in yellow watermelon. Ilahy et al. (2011) have shown high antioxidant activity during ripening in tomato containing high lycopene content. The highest antioxidant activity was also noticed in rockmelon extracts, which was related to the high levels of phenolics present in the extracts (Norrizah et al. 2012). Isbilir et al. (2012) stated that the differences in the antioxidant activity which occurs in ripening stages may possibly be due to the change in the amount of phytochemicals at different ripening stages and could be correlated with the phenolic compounds present in the fruit extracts. This aspect could be considered as an indication of the superior antioxidant potential of yellow watermelon which suits the consumer requirement of nutritive and health promoting foods.

The results of activity of β-Gal enzyme demonstrated its positive relation with softening process of yellow watermelon, which is in accordance with the earlier reports by Ranwala et al. (1992), Menon and Rao (2014) in melons and Konozy et al. (2012) in tomato. Ranwala et al. (1992) reported high activity of β-Gal towards ripening of melons and indicated its function in cell wall modification. Progressive increase in PG activity was reported during ripening of 'Galia' and 'Piel de sapo' melons by Chisari et al. (2009). During melon ripening, a shift to a lower molecular-mass distribution of hemicellulose polymers occurs and that leads to substantial solubilization and break down of pectins, particularly the water-soluble pectins (Chisari et al. 2009).

A considerable variation in the concentration of minerals was noticed during the development and ripening of yellow watermelon. Data obtained for the mineral distribution are in accordance with the findings of Mahmood et al. (2012) in various berries in which they observed higher levels of minerals in unripe fruits as compared to the ripe ones. According to Lester (2008), the distribution pattern of minerals across various fruit tissues was consistent with their physiological functions and tissue requirements on their dry weight basis. The level of minerals in fruit depends on various factors such as uptake, transloction, remobilization, distribution, competition between organs, mobility and interaction of nutrients. Based on these factors these minerals vary in their distribution pattern in fruits (Paul et al. 2012).

V. CONCLUSION

The current investigation provides novel information on the nutritional potential of yellow-fleshed watermelon fruit based on the findings on the accumulation of nutraceutical components such as sugars, phenolics, ascorbic acid, and minerals, particularly, Na during fruit development and ripening, which will enhance the current understanding of the importance of this fruit. It is also evident from this study that the yellow-fleshed watermelon has high nutritional potential and health relevance when compared to that of red fleshed watermelon. The harvesting of fruit in its optimal maturity stage is necessary for obtaining a better quality produce. The present study clearly indicates the most suitable stage for harvesting, which will improve the commercialization of the fruit. Moreover, the outcome of this study provides additional and informative data for targeted patient specific management of nutritional needs by consumption of yellow and for exploiting it to prevent chronic diseases. However, further research is needed to address the health enhancing potential of yellow watermelon fruit.

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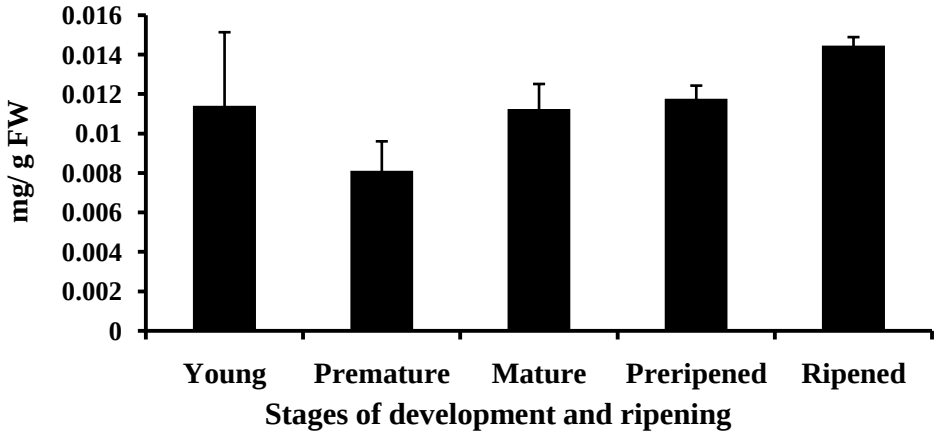

Figure 1: Changes in total sugars (TS) during the development and ripening of yellow watermelon (mg/gFW)

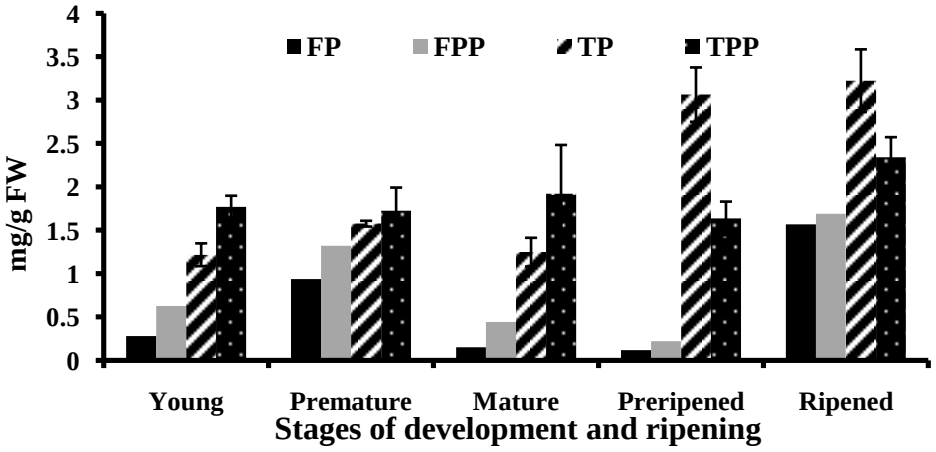

Figure 2: Changes in phenolics like free (FP), total phenols (TP), free polyphenols (FPP) and total polyphenols (TPP)) during the development and ripening of yellow watermelon (mg/gFW)

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Stages	SPS	SS
Young	0.320 ± 0.28^{a}	0.222 ± 0.11^{a}
Pre-mature	0.054 ± 0.004^{a}	0.170 ± 0.017^{a}
Mature	0.064 ± 0.009^{a}	0.608 ± 0.18^{a}
Pre-ripened	0.567 ± 0.23^{a}	0.095 ± 0.005^{a}
Ripened	1.83 ± 0.75^{b}	7.12 ± 4.9^{b}

Table 1: Specific activities of sucrose phosphate synthase (SPS), sucrose synthase (SS) during the development and ripening of yellow watermelon (µmol/h/mg protein).

Stages	AA	TAA	Flavanol
Young	3.26 ± 0.25^{a}	56.78 ± 13.87^{b}	0.064 ± 0.006^{a}
Pre-mature	8.24 ± 0.81^{c}	44.05 ± 2.05^{a}	0.141 ± 0.002^{b}
Mature	3.27 ± 0.57^{a}	46.85 ± 2.74^{ab}	0.162 ± 0.008^{c}
Pre-ripened	5.92 ± 0.20^{b}	36.74 ± 2.06^{a}	0.253 ± 0.008^{d}
Ripened	10.83 ± 0.25^{d}	39.81 ± 2.11^{a}	0.286 ± 0.005^{e}

Table 2: Changes in the content of ascorbic acid (AA), flavanols, (mg/g FW) and the level of total antioxidant activity (TAA) (%) during the development and ripening of yellow watermelon

Stages	POD	PPO
Young	10.08 ± 2.29^{b}	0.0038 ± 0.0033^{ab}
Pre-mature	5.99 ± 0.22^{a}	0.0094 ± 0.0024^{ab}
Mature	7.92 ± 0.62^{ab}	0.0061 ± 0.004^{ab}
Pre-ripened	7.92 ± 0.75^{ab}	0.0626 ± 0.05^{b}
Ripened	8.073 ± 0.74^{ab}	0.0002 ± 0.0001^{a}

Table 3: Specific activities of peroxidase (POD), polyphenols oxidase (PPO) during the development and ripening of yellow watermelon (Units/mg protein).

Stages	Na	Zn	Cu	Mn
Young	10.44 ± 0.006^{b}	0.180 ± 0.001^{b}	0.052 ± 0.0002^{a}	0.059 ± 0.0002^{d}
Pre-mature	10.73 ± 0.012^{d}	0.218 ± 0.001^{d}	0.092 ± 0.0002^{c}	0.083 ± 0.0003^{e}
Mature	9.14 ± 0.003^{a}	0.264 ± 0.001^{e}	0.144 ± 0.0001^{e}	0.056 ± 0.0004^{c}
Pre-ripened	11.06 ± 0.001^{e}	0.210 ± 0.001^{c}	0.072 ± 0.0001^{b}	0.050 ± 0.0001^{a}
Ripened	10.59 ± 0.007^{c}	0.170 ± 0.001^{a}	0.104 ± 0.0001^{d}	0.054 ± 0.0003^{b}

Table 4. Changes in the concentration of sodium (Na), zinc (Zn), copper (Cu) and manganese (Mn) during the development and ripening of yellow watermelon (mg/kg).

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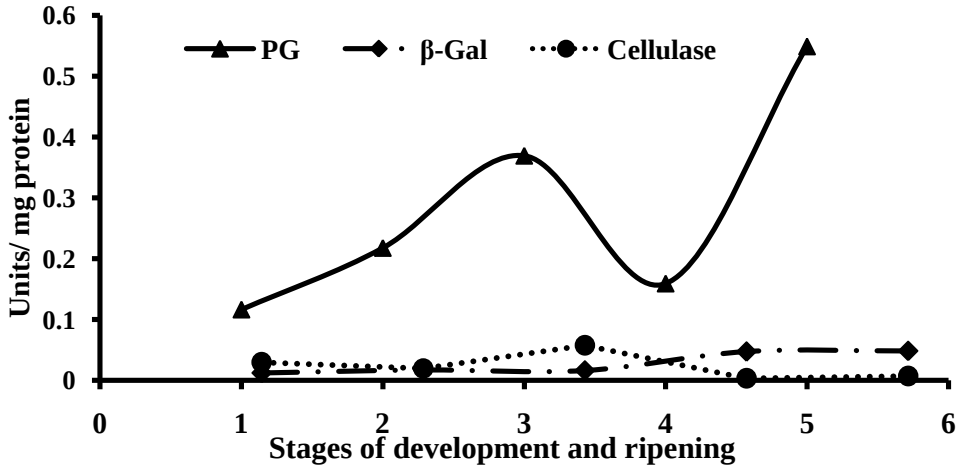

Figure 3: Specific activities of polygalacturonase (PG), β-Galactosidase (β-Gal) and cellulase (Cx) during the development and ripening of yellow watermelon (Units/mg protein).

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