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Antioxidant Indices of Watermelon Juice and Lycopene Extract

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Abstract: In the current project, indigenously grown promising watermelon variety (Sugar baby) was characterized for its antioxidant potential. The watermelon juice and lycopene extract were quantified by HPLC that depicted 4.53±0.05 and 6.27±0.06 mg/100mL of lycopene, respectively. Furthermore, the watermelon juice and lycopene extract showed Total Phenolic Contents (TPC), beta-carotene assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) as 23.63±1.09 and 97.15±5.01 mg/100g GAE, 49±3.10 and 73±3.20%, 29.11±1.91 and 57±3.22% and 21.67±1.21 and 37.60±1.12 mM FRAP/g, respectively. Consequently, the watermelon proved as a good source of antioxidant with special reference to lycopene.

Key words: Watermelon, lycopene, antioxidant, DPPH, beta-carotene assay, FRAP

INTRODUCTION

Phytonutrients are plant derived materials performing key role in maintaining human health, especially in disease prevention. In recent era, phytochemicals based nutraceuticals particularly from fruits and vegetables are becoming popular due to consumer awareness regarding their health enhancing potential. Epidemiological studies have correlated the consumption of these constituents with declining the incidence of several physiological threats (Engelhard *et al*., 2006). These bioactive ingredients are diverse in nature like phenolics and carotenoid showing considerable antioxidative activity (Shahidi, 2009).

Watermelon (*Citrullus lanatus*) botanically considered as fruit belonging to the family *Cucurbitaceae* (Edwards *et al*., 2003) is originated from Kalahari desert of Africa but nowadays cultivated abundantly in tropical regions of the world. Presently, China is the leading producer followed by Turkey, United States, Iran and Republic of Korea (Zohary and Hopf, 2000; Lucier and Lin, 2001). It has great economic importance with 29.6 million tons estimated production worldwide. Pakistan holds promising position in watermelon production, nevertheless, selected verities are cultivated commercially (Quek *et al*., 2007; FAO, 2010). The watermelon fruit has deep green or yellow colored smooth thick exterior rind with gray or light green vertical stripes.

Inside the fruit is pink, red or even yellow in color with small black seeds embedded in the middle third of the flesh. Generally, watermelon flesh is the main consumable portion, however, outer rind is also used in some parts of the world (Levi *et al*., 2001; Wehner *et al*., 2001; Oms-Oliu *et al*., 2009). Watermelon contributes a plethora of nutritional agents as antioxidants (lycopene, beta-carotene etc.) and some specific amino acids (arginine, citrulline etc.). Fresh watermelon consumption is considered a healthy addition to diet owing to the presence of lycopene. Majority of the watermelon is being sold on the basis of sweetness but presently, ruby color imparted by lycopene is also a convincing factor (Kamil *et al*., 2011). Considering the nutritional profile, consumption of 100 g of watermelon provides 30 Kcal to the body. It contains almost 92% water and 7.55% of carbohydrates, out of which 6.20% are sugars and 0.40% dietary fiber. Vitamins like thiamine, riboflavin, niacin and folate are also present. Additionally, it is a good source of potassium and also contains magnesium, calcium, phosphorus and iron (Quek *et al*., 2007).

The lycopene activity in the body depends on its molecular and physicochemical properties and site of action within the cells. It acts as singlet oxygen and free radical quencher. Moreover, conjugated double bonds play a vital role in energy transfer. Lycopene shows scavenging ability for singlet oxygen due to excited energy state related to conjugated double bonds. Therefore, lycopene scavenging rate is reported to be higher than beta-carotene and α-tocopherol. Being a reactive carotenoid, lycopene follows three possible routes to interact as adduct formation, transferring electron to free radical and by allelic hydrogen abstraction. Lycopene mode of action depends on the position within the cell as it lies parallel underneath the surface of cell membrane. Moreover, it significantly inhibits free radicals invasion at membranes surface and serves as primary defense system. Lycopene combinations with other antioxidants have proven synergistic behavior to scavenge reactive oxygen species. Its synergistic role with vitamins E and C and

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other carotenoids has affirmative impact on human health (Huang *et al*., 2007; Skibsted, 2012).

Lycopene owns unique chemical properties due to 11-conjugated linearly arranged double bonds that enable its absorption in the body tissues. Nevertheless, lycopene lacks pro-vitamin A activity due to deficient in beta-ionone ring. Research investigations explicated that free radical quenching ability of lycopene is more than twice of beta-carotene and ten times of α-tocopherol. It is an efficient antioxidant and has ability of trapping free hydroxyl radicals. Furthermore, lycopene is a good source of electron transference with second order reaction activity (Basuny *et al*., 2009). Considering the facts, present research project was planned to characterize locally grown watermelon (Sugar baby) with special reference to lycopene, a potent antioxidant.

MATERIALS AND METHODS

The current research was carried out in the Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad.

Procurement of raw material: Watermelon (Sugar baby) was procured from fruit market considering the quality traits like uniformity in size, color, shape and abrasion free followed by grading and washing. Refrigerated conditions were ensured during storage to aid in further analysis and potential application.

Characterization of watermelon: Watermelon flesh was separated from the rind followed by blending and subjected to various analysis including physicochemical profiling, mineral assay and juice extraction.

Physicochemical analysis: Watermelon flesh was analyzed for moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract by using triplicate samples.

Moisture contents: Moisture content of watermelon were measured by drying weighed fruit flesh sample in Air Forced Draft Oven (Model: DO-1-30/02, PCSIR, Pakistan) at 105±5°C till constant weight according to AACC (2000) Method No. 44-15A.

Crude protein: Estimation of crude protein was done via Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) following the guidelines of AACC (2000) Method No. 46-30.

Crude fat: Crude fat was determined through Soxtec System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) as described in AACC (2000) Method No. 30-25.

Crude fiber: For the measurement of crude fiber, fat free sample was subjected to digestion for 30 min with 1.25% H_2SO_4 followed by digestion with NaOH 1.25% solution while using Labconco Fibertech (Labconco Corporation Kansas, USA) by adopting the protocol of AACC (2000) Method No. 32-10.

Ash: Ash contents in dry sample were determined after charring followed by direct incineration at 550°C in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) till grayish white residue following the procedure of AACC (2000) Method No. 08-01.

Nitrogen Free Extract (NFE): The Nitrogen Free Extract (NFE) was determined by following the expression:

NFE (%) = 100 - (CP + Crude fat + Crude Fiber + Ash) %

Where CP = Crude protein

Mineral assay: Watermelon was subjected to mineral profile considering the instructions of AOAC (2006). Purposely, Na and K were determined through Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge), whilst Ca, Fe and Mn by Atomic Absorption Spectrophotometer (Varian AA20, Australia).

Juice extraction: Watermelon flesh was subjected to juice extraction followed by sieving. Extracted juice was subjected to antioxidant assays as described below.

***In vitro* studies**

Lycopene extraction and quantification: Lycopene was extracted using organic solvents i.e., hexane, acetone and petroleum ether in ratio of 2:1:1, respectively and 0.05% Butylated Hydroxytoluene (BHT) as extracting medium to attain maximum recovery (Perkins-Veazie *et al*., 2001). Extracted lycopene was quantified through HPLC following the procedure of Charoensiri *et al*. (2009).

Free radical scavenging ability: Free radical scavenging activity of watermelon juice and extracted lycopene was determined by DPPH method (Muller *et al*., 2011).

Antioxidant activity: Extracted juice and lycopene was subjected to antioxidant activity assay based on coupled oxidation of beta-carotene and linoleic acid through spectrophotometer (470nm) by following the method of Taga *et al*. (1984).

Total phenolics: Estimation of Total Phenolic Contents (TPC) of juice and extracted lycopene was carried out using Folin-Ciocalteu method as described by Singleton *et al*. (1999).

Ferric Reducing Antioxidant Power (FRAP): FRAP was conducted by following the procedure of Muller *et al.* (2011).

Statistical analysis: The data obtained for each parameter was subjected to statistical analysis in order to determine the level of significance (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Characterization of watermelon: Characterization of experimental material is an obligatory step for the assessment of component of interest. Physicochemical assay along with sensory profiling are the decisive factors for designer food development that ultimately support the dietary efficiency in animal feeding investigation. With intent, watermelon was probed for its proximate profiling i.e., moisture, crude protein, crude fat, crude fiber, ash and Nitrogen Free Extract (NFE). Alongside, mineral quantification and antioxidant indices of watermelon are discussed in the upcoming section for meticulousness regarding the nutritional profile of watermelon.

Compositional profiling: The watermelon compositional analysis elucidated that moisture, crude protein, crude fat, crude fiber, ash and Nitrogen Free Extract (NFE) were 92.02±1.65, 0.49±0.02, 0.11±0.001, 0.32±0.06, 0.27±0.03 and 6.79±0.25%, respectively (Table 1).

The present results of proximate assay are in corroboration with the findings of Inuwa *et al.* (2011), estimated moisture, crude protein, crude fat, crude fiber and ash contents as 93.40 to 94.60, 0.50 to 0.60, 0.10 to 0.15, 0.30 to 0.40 and 0.50 to 0.55%, respectively. According to them, the variations in watermelon composition were due to varietal differences. The current outcomes are also in accordance with the values reported by United States Department of Agriculture (USDA, 2010) as 91.45 g moisture in 100 g sample of watermelon. The remaining traits like protein, fat, ash and dietary fiber were 0.61, 0.15, 0.25 and 0.40 g/100g, respectively. The findings of instant investigation for moisture content are in accordance with the study of Arocho *et al.* (2012), narrated variations for moisture contents in two different harvest seasons of watermelon. They noticed the moisture content 90.99±0.19 and 90.16±0.26% for September 2009 and June 2010 harvesting period, respectively. They concluded that the differences in moisture content are also dependent on harvesting time. Previously, Yau *et al.* (2010) delineated moisture content as an associated factor with the watermelon flesh crispness. According to their findings, moisture content varied from 91.80 to 94.10%. Similarly, Shofian *et al.* (2011) explicated 92.47±0.12% of moisture in red watermelon.

In the present exploration, the watermelon minerals like potassium (K), calcium (Ca), sodium (Na), iron (Fe) and zinc (Zn) were recorded as 126±2.36, 5.60±0.21, 0.81±0.03, 0.26±0.01 and 0.031±0.001 mg/100g, respectively. Earlier, USDA (2010) published the values for potassium, calcium, sodium, iron and zinc as 112, 7, 1,0.24 and 0.10, respectively. In an investigation, Inuwa *et al.* (2011) recorded variations in watermelon iron contents from 0.18 to 0.33 mg/100g. Moreover, Proietti *et al.* (2008) reported 154 mg/100g of potassium in watermelon sample. It has been observed that potassium ranged from 107 to 114 mg/100g, whilst, Na, Ca and Mn 0.70, 6.40 and 0.027 mg/100g, respectively (Colla *et al.*, 2006). The recorded deviations in proximate composition and mineral contents of current study are might be due to the changes in agronomic practices, geographical conditions, ripening stage and harvesting season.

In the present exploration, watermelon juice and lycopene extract were quantified through High Performance Liquid Chromatography (HPLC). The recorded values for watermelon juice and lycopene extract were 4.53±0.05 and 6.27±0.06 mg/100mL of lycopene, respectively. Likewise, the observed values for Total Phenolic Contents (TPC), beta-carotene assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) for watermelon juice and lycopene extract were 23.63±1.09 and 97.15±5.01 mg/100g GAE, 49±3.10 and 73±3.20%, 29.11±1.91 and 57±3.22% and 21.67±1.21 and 37.60±1.12 mM FRAP/g, respectively (Table 2).

The findings are in agreement with Barba *et al.* (2006) who computed lycopene through HPLC in various fruits and vegetables i.e., watermelon, tomato, medlar, persimmon, pepper and carrot. They expounded that watermelon and tomato had higher lycopene contents. The recorded value for watermelon lycopene extract

Table 1: Compositional profiling of watermelon

Proximate assay (%)	
Moisture	92.02±1.65
Crude protein	0.49±0.02
Crude fat	0.11±0.001
Crude fiber	0.32±0.06
Ash	0.27±0.03
Nitrogen Free Extract (NFE)	6.79±0.25
Minerals (mg/100g)	
Potassium (K)	126±2.36
Calcium (Ca)	5.60±0.21
Sodium (Na)	0.81±0.03
Iron (Fe)	0.26±0.01
Zinc (Zn)	0.031±0.001

Table 2: Antioxidant indices of watermelon juice and lycopene extract

Parameters	Watermelon juice	Lycopene extract
Lycopene (mg/100mL)	4.53±0.05	6.27±0.06
TPC (mg/100g GAE)	23.63±1.09	97.15±5.01
Antioxidant activity beta-carotene (%)	49±3.10	73±3.20
DPPH (%)	29.11±1.91	57±3.22
FRAP (mM FRAP/g)	21.67±1.21	37.60±1.12

using hexane: Ethanol: Methanol (2:1:1) as solvents were 6.50±0.1 to 7.30±1.0 mg/100g. The current results are in harmony with the outcomes of Charoensiri *et al.* (2009), probed 37 fresh fruits obtained from different areas of Bangkok for their lycopene contents. They identified red watermelon as one of the richest sources of lycopene 6.69 mg/100g moreover, papaya and guava fruits are also good sources.

Earlier, Perkins-Veaize *et al.* (2006) accessed 50 different watermelon cultivars for lycopene quantification and narrated wide variations ranging from 3.52±2.30 to 11.20±12.90 mg/100g. They further suggested 150 g serving size of watermelon for reasonable intake of lycopene. Similarly, Katherine *et al.* (2008) extracted 3.70 mg/100g of lycopene at 60°C and reported a decline with gradual rise in extraction temperature upto 75°C. Earlier, Fish and Davis (2003) observed the effect of freezing conditions on lycopene content of watermelon. They measured lycopene in 11 individual watermelons and reported 4.29 to 7.82 mg/100g of lycopene with an average of 5.36 mg/100g. They deduced that initial freeze-thaw at -20°C reduced lycopene from 4 to 6%. However, storage at -20°C decreased lycopene by 30 to 40% whilst at -80°C a reduction upto 5 to 10% was recorded during one year.

The instant results for lycopene in watermelon juice are slightly lower than the findings of Oms-Oliu *et al.* (2009), observed 6.20 mg/100mL. They attributed watermelon juice as one of the excellent sources of lycopene. In a study, Liu *et al.* (2012) assessed the effect of mild heat treatment on overall quality parameters of watermelon juice and found 6.25 mg/100mL of lycopene. According to their observations, quality attributes like lycopene content, pH and color remained stable throughout the study. In a parallel research, Zhang *et al.* (2011) examined watermelon juice for lycopene, color, degree of browning and viscosity after mild heat treatment. They elucidated non-significant effect of slight heat processing on lycopene content.

The current results for total phenolics in watermelon sample are in harmony with Reddy *et al.* (2010), they computed 26±2.50 mg/100g GAE of TPC. Recently, Naveen *et al.* (2012) probed various fruits of Indian market for their beta-carotene assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) values. The recorded beta-carotene assay and DPPH values for watermelon were 63 and 39%, respectively. Conclusively, watermelon showed remarkable free radical scavenging and antioxidant activity owing to potent antioxidant i.e., lycopene.

The present results for total phenolic contents are in accordance with the outcomes of Shofian *et al.* (2011). They stated total phenolic contents 29.32 mg/100g for fresh watermelon that was comparatively higher than muskmelon. Earlier, Melo *et al.* (2008) recorded 25% DPPH scavenging activity of Portuguese watermelon aqueous extract.

The existing results for lycopene and Ferric Reducing Antioxidant Power (FRAP) are in conformity with the investigations of Tlili *et al.* (2011), they assessed various watermelon varieties for these traits. Lycopene contents for Giza, Dumara, P403, P503 and Aramis were 9.69, 4.27, 4.54, 10.24 and 7.10 mg/100g, respectively. Besides, FRAP values ranged from 22.90 to 37.40 mM FRAP/g strengthened the present results. Additionally, Guo *et al.* (2003) recorded FRAP value 16 mM FRAP/g for watermelon pulp of Jingxin variety.

Findings of Fu *et al.* (2011) reported similar trend for TPC comparable with that of present research. They delineated total phenolic contents of three different watermelon varieties i.e., Jintong, red pulp and yellow pulp by 23.15±1.47, 24.66±1.04 and 18.62±1.32 mg/100g GAE, respectively. One of their peers, Tlili *et al.* (2011) observed total phenolic contents of watermelon cultivars at various ripening stages and stated that Giza, Dumara, P403 and P405 had 26.02±0.6, 24.64±0.5, 20.08±0.6 and 18.34±0.5 mg/100g GAE of TPC, respectively.

One of the researchers groups, Muller *et al.* (2011) explicated the mechanism of ferric reducing antioxidant power of lycopene. They found that only lycopene was an effective ferric reducing compound among various carotenoids. The ferric reducing power is influenced by conjugated double bonds of the compound. The acyclic intermediate colorless plant carotenoids like phytoene, phytofluene and neurosporene did not show substantial activity to reduce ferric ions owing to low conjugated double bond numbers with 3, 5 and 9, respectively. However, acyclic carotenoid lycopene with 11 conjugated double bonds encompasses ability to overlap the chromophore thus displayed high ferric reducing power. The findings of current investigation are in conformity with their results regarding ferric reducing power of lycopene. The lycopene extract exhibited relatively higher FRAP value due to high concentration of lycopene as compared to watermelon juice.

Earlier, Takeoka *et al.* (2001) assessed relative high concentration of lycopene and its subsequent antioxidant activity in tomato paste as compared to juice. They noticed 25.20 to 64.80 and 5.20 to 18.20% antioxidant activity of tomato paste and juice, respectively. They explained that heat treatment concentrated lycopene thus showed relative high antioxidant activity. This phenomenon strengthened the instant results for concentrated lycopene extract that exhibited higher antioxidant activity than that of watermelon juice. Alongside, in a parallel study, Dewanto *et al.* (2002) reported 27.93 to 62.09% of total antioxidant activity as a function of lycopene concentration at various temperatures.

The present results are also supported by the research work of Egydio *et al.* (2010) for DPPH radical scavenging ability i.e., 18.90±0.7 to 49.7±1.5%. The variations in

DPPH activity of lycopene containing juice depends on the purity of the sample. In current case, the lycopene extract had more purity than watermelon juice thus exhibited higher DPPH radical scavenging ability.

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