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Ascorbic Acid, Lycopene and Antioxidant Activities of Red-fleshed and Yellow-fleshed Watermelons

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ABSTRACT

Ascorbic acid, lycopene and antioxidant activities of two varieties of seeded watermelon, red-fleshed and yellow-fleshed watermelon were analyzed in this study. The flesh of red-fleshed watermelon had higher ascorbic acid (86.32 mg/kg) and lycopene (9.50 mg/kg) contents compared to the ascorbic acid (52.05 mg/kg) and lycopene (0.04 mg/kg) contents of the flesh of yellow-fleshed watermelon. The whole fruit of red-fleshed watermelon had lycopene content of 2.60 mg/kg which was higher than that of whole fruit of yellow-fleshed watermelon (0.37 mg/kg) but, both had similar ascorbic acid contents. The anti-radical power and ferrous ion chelating activity values ranged from 0.0062-0.0090 and 2.94-27.90%, respectively.

Keywords: watermelon, antioxidant, vitamin C, lycopene.

INTRODUCTION

Watermelon belongs to the Cucurbitaceae family, which encompasses over 800 species of plants. They are collectively known as gourds or cucurbits which include watermelons, melons, cucumbers, pumpkins and so on. Watermelon is under the genera of *Citrullus*, which rank among the top ten in economic importance among vegetable crops globally [1]. According to Gusmini and Wehner [2], watermelon has been bred to improve yield, quality, and disease resistance, to diversify fruit and plant type (for an example, seeded versus seedless fruit) and to adapt to various production areas across the globe. Watermelon can have fruits with various sizes, shapes, rind patterns and flesh colors.

Different carotenoid patterns were found in red-fleshed watermelon and yellow-fleshed watermelon [3]. Yellow-fleshed watermelons contain many different carotenoids but they were all in low to trace amounts [4]. Red-fleshed watermelons contain high levels of lycopene and varying amounts of β –carotene [3]. Epidemiological studies have demonstrated that high consumption of fruits and vegetables containing lycopene is associated with reduced incidence of coronary heart disease and prostate cancer [5, 6]. The antioxidant components of red-fleshed watermelons were influenced by genotype and sampling area [7], fruit ripening stages [8] and different cultivars [9, 10, 11]. Limited similar study has been carried out on yellow-fleshed watermelon. In one study, Davis et al. [4] described a rapid and reliable light absorption method to assay total carotenoid content for canary yellow-fleshed watermelon that does not require organic solvents. The objective of this study was to determine the ascorbic acid content, lycopene content and the antioxidant activity of whole fruit and flesh of red-fleshed and yellow-fleshed watermelons.

MATERIALS AND METHODS

Chemicals and reagents
L-ascorbic acid, butylated hydroxytoluene (BHT) and 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical were purchased from Sigma-Aldrich (U.S.A.). Potato starch was purchased from Bendosen Laboratory Chemicals (Norway). Iron (II) sulphate 7-hydrate and ferrozine iron reagent were purchased from Acros Organics (Belgium). Ethanol, hexane, acetone and sulfuric acid were of analytical grade.

Plant material and sample preparation
Watermelons (seeded), red-fleshed and yellow-fleshed, were purchased from a local supermarket in Bandar Sunway, Selangor, Malaysia. A total of 3 fruits were purchased randomly for each type of watermelon at three different times. The red-fleshed watermelon was washed, drained and wiped-dry. It was cut into a few small portions and then blended to a paste-like state for approximately 2 minutes using a Waring blender. During the blending process, intermittent stops were required to minimize heating effect on the watermelons. The homogenized sample was centrifuged at 1000 g for 30 minutes and at 4°C before being filtered under suction. The sample was stored at -20°C until use within a week. These procedures were repeated with the skin of the watermelon, and the white part of the rind, excluded. Only the red-colored flesh was used. The same procedures were applied to the yellow-fleshed watermelons.

Determination of ascorbic acid
The ascorbic acid content in the sample was determined using the method of Suntornsuk et al. [12]. Starch indicator solution was prepared as follows. A gram of starch was weighed and a small amount of distilled water was added to it to form a paste. The paste was added to 200mL of boiling water while stirring and boiled for a few seconds. It was removed from heat immediately and allowed to cool.

Watermelon sample (25mL) was transferred into 250mL Erlenmeyer flask. Then, 25mL of 2N H_2SO_4 was added, followed by 50mL of distilled water and 3mL of starch indicator. The solution was mixed well prior to direct titration with 0.001N iodine. A blank titration was conducted prior to sample titration as well. One mL of iodine = 8.806mg ascorbic acid.

Determination of lycopene
Lycopene content was determined according to the method of Davis et al. [4] with some modifications. Approximately 0.6g of sample was weighed and added to 5mL of 0.05% (w/v) BHT in acetone, 5mL of 95% ethanol and 10mL of hexane. The homogenate was centrifuged at 400 g for 15 minutes at 4°C. After that, 3mL of distilled water was added. The vials were agitated for 5 minutes and left at room temperature to allow phase separation. The absorbance of upper hexane layer was measured in a 1cm-pathlength quartz cuvette at 503nm using a spectrophotometer. Hexane was used as blank. The lycopene content in the sample was estimated according to the equation:

$$\text{Lycopene (mg/kg tissue)} = \frac{A_{503} \times 31.2}{\text{mass of tissue (g)}}$$

where A_{503} is the absorbance of upper hexane layer

Determination of free radical scavenging activity
Free radical scavenging activity was determined according to the method of Suja et al. [13] with some modifications. Samples (1mL) each with different concentrations (30-400 mg/mL) were prepared and then added to 2mL DPPH solution (0.05M) in ethanol, respectively. The reduction of DPPH in the samples was measured at 517nm after 30 minutes against a blank assay (samples with similar concentrations were added to 2mL of ethanol, respectively). The percentage of remaining radical was calculated by dividing the absorbance of the sample with that of DPPH control and multiplied by 100. The amount of sample required to decrease the initial DPPH concentration by 50%, EC_{50}, was calculated graphically. The equation for anti-radical power is:

$$\text{Anti-radical power (ARP)} = 1 / EC_{50}$$

Determination of ferrous ion chelating activity
Ferrous ion chelating activity was determined according to the method of Lim et al. [14] with some modifications. $FeSO_4$ (2mM) and ferrozine (5mM) were prepared and diluted 20 times. One mL of samples, each with different concentrations (600-1500 mg/mL) was mixed with 1mL diluted $FeSO_4$ followed by 1mL of diluted ferrozine. All the

solutions were mixed well and allowed to stand for 10 minutes at room temperature. The absorbance of each sample was measured against blank (samples with similar concentrations were added with 2mL of distilled water, respectively) at 562nm. The chelating ability of the sample was calculated using the equation below:

$$\text{Chelating ability (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Statistical analysis

Data were interpreted by one-way analysis of variance (ANOVA) with Duncan's multiple-range test using SAS software package (SAS Institute Inc., Cary, NC, U.S.A.). Statistical significance was evaluated at $p < 0.05$ level.

RESULTS AND DISCUSSION

Ascorbic acid content

The ascorbic acid content of the flesh of red-fleshed watermelon (86.32 mg/kg) was the highest (Fig. 1). This value was lower than the ascorbic acid contents of red-ripe stage of red-fleshed watermelon cultivars (119.7-204.0 mg/kg) in the study of Tlili et al. [7] and six red-fleshed watermelon cultivars (98.0-261.8 mg/kg) in the study of Tlili et al. [8]. However, the ascorbic acid content of the red-fleshed watermelon in this study was higher than those reported by Isabelle et al. [15] (39.1 mg/kg), Leong and Shui [15] (37 mg/kg) and Opara [17] (~30 mg/kg). The ascorbic acid content of the flesh of yellow-fleshed watermelon in this study (Fig. 1) was significantly lower than that of the red-fleshed watermelon but was higher than that of the yellow-fleshed watermelon (55.2 mg/kg) in the study of Isabelle et al. [15]. Isabelle et al. [15] reported higher ascorbic acid content for yellow-fleshed watermelon as compared to that of red-fleshed watermelon. These differences are most likely due to the strong influence by genotype differences and external factors such as environmental conditions, maturity stage, harvest and post-harvest practices [7, 9, 10, 11].

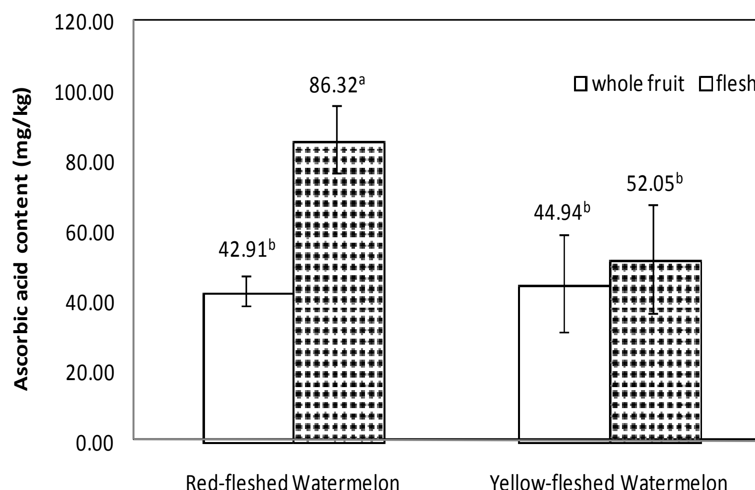

Fig. 1. Ascorbic acid content of red-fleshed and yellow-fleshed watermelons.

Results are expressed as means ± standard deviation.

*[*ab]Values with different superscript letters indicate significant difference at $p < 0.05$.*

Lycopene content

The lycopene content of the flesh of yellow-fleshed watermelon in this study was much lower than that of red-fleshed watermelon (Fig. 2). These results indicate that lycopene is present abundantly in the flesh of red-fleshed watermelon whereas yellow-fleshed watermelon lacks lycopene. This agrees with the study of Tadmor et al. [3] and Isabelle et al. [15] where no lycopene was found in the yellow-fleshed watermelons studied. According to Davis et al. [4], there is not one predominant carotenoid found in yellow-fleshed watermelon.

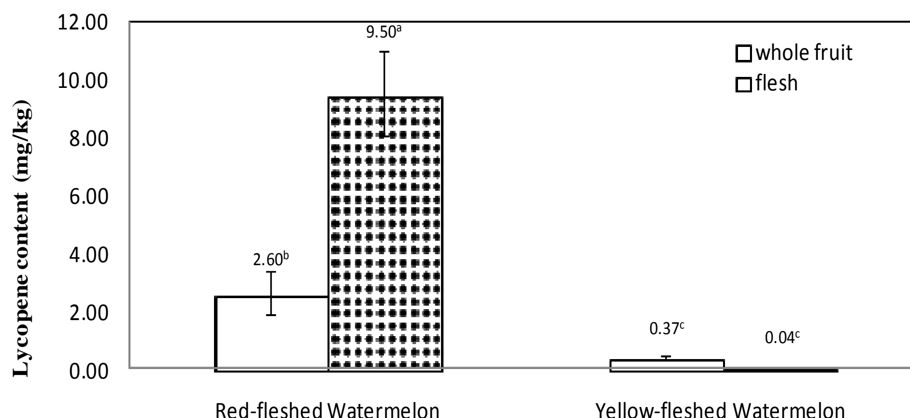

Fig. 2. Lycopene content of red-fleshed and yellow-fleshed watermelons.
Results are expressed as means ± standard deviation.
**abcValues with different superscript letters indicate significant difference at $p < 0.05$.*

The lycopene content of the whole fruit of red-fleshed watermelon was approximately four times higher than that of the flesh (Fig. 2). Since the same amount of watermelon (both for whole fruit and flesh) was used in the lycopene content analysis, this indicates that the flesh of red-fleshed watermelon contribute mostly to the high content of lycopene. As there was no significant difference in lycopene content between the whole fruit and flesh of yellow-fleshed watermelon, this indicates that there was no or trace amount of lycopene present in the skin of yellow-fleshed watermelon in this study.

The lycopene content of the flesh of red-fleshed watermelon in this study (Fig. 2) was lower than those reported by Isabelle et al. [15] (10.95 mg/kg), Tadmor et al. [3] (42.6 mg/kg), Tilli et al. [7] (42.7-102.4 mg/kg) and Tilli et al. [8] (44.5-64.5 mg/kg). This difference was due to red-fleshed watermelons varied in their lycopene content depending on genotype and environmental conditions [9]. Another study by Perkins-Veazie et al. [10] showed that different cultivars varied greatly in lycopene content, ranging from 33-100mg/kg in watermelon puree. Another study by Fish et al. [18] showed that lycopene content of red-fleshed watermelon puree was between 30-80 mg/kg fresh weight.

Free radical scavenging activity

DPPH radical was used in the evaluation of free radical scavenging activity of watermelons. There was no significant difference between the whole fruits of red-fleshed watermelon and yellow-fleshed watermelon in terms of the EC_{50} and ARP values (Table 1). However, the flesh of red-fleshed watermelon showed lower EC_{50} than that of yellow-fleshed watermelon. Thus, lower concentration of red-fleshed watermelon was required to decrease the initial DPPH solution by 50%. This corresponds to higher ARP value, which reflects higher efficiency of antioxidants in the fruits [19]. Higher ARP was observed in the flesh of red-fleshed watermelon compared to that of yellow-fleshed watermelon. This was most likely due to the higher contents of ascorbic acid and lycopene in the red-fleshed watermelon (Figs. 1 and 2). One ascorbic acid could reduce nearly two DPPH radicals [19]. Hence, the antioxidant capacity in the flesh of red-fleshed watermelon was more than that of yellow-fleshed watermelon.

Table 1: EC_{50} and ARP of red-fleshed and yellow-fleshed watermelons

Samples	EC_{50} (mg/mL)	ARP
Red-fleshed watermelon, whole fruit	149.8 ± 3.8^{ab}	0.0067 ± 0.0002^{ab}
Red-fleshed watermelon, flesh	112.2 ± 16.7^{b}	0.0090 ± 0.0013^{a}
Yellow-fleshed watermelon, whole fruit	121.8 ± 32.2^{ab}	0.0086 ± 0.0021^{ab}
Yellow-fleshed watermelon, flesh	165.1 ± 30.2^{a}	0.0062 ± 0.0010^{b}

Values are means ± standard deviation.
ab Different superscript letters within a column indicate significant difference at $p < 0.05$.

There was no significant difference in the EC_{50} and ARP values between the whole fruit and flesh of red-fleshed watermelon. These results were unexpected as the ascorbic acid and lycopene contents in the flesh were significantly higher (Figs. 1 and 2). This was most likely due to the presence of other antioxidants such as citrulline, phenolics and other forms of carotenoids that were not measured in this study. Citrulline, a non-protein amino has been detected in watermelon and the rind of watermelon was reported to contain more citrulline than the flesh [20, 21]. Citrulline is an efficient hydroxyl radical scavenger [22, 23]. As for yellow-fleshed watermelon, there was no

significant difference in EC_{50} and ARP values between the whole fruit and flesh. This was most likely due to the similar ascorbic acid and lycopene contents in the whole fruit and flesh of yellow-fleshed watermelon (Figs. 1 and 2). This indicates that yellow-fleshed watermelon probably lacks of citrulline or phenolics.

Ferrous ion chelating activity

Chelating power measures the effectiveness of compounds in extract to compete with ferrozine for ferrous ion. A high chelating power extract reduces the free ferrous ion concentration by forming a stable iron (II) chelate and thus decreasing the extent of Fenton reaction which is implicated in many diseases [24]. The chelating ability of the whole fruit of yellow-fleshed watermelon was higher than that of red-fleshed watermelon (Table 2). In this study, the ascorbic acid content in the whole fruit of yellow-fleshed watermelon was similar with that of red-fleshed watermelon (Fig. 1) whereas the lycopene content in the whole fruit of yellow-fleshed watermelon was significantly lower than that of red-fleshed watermelon (Fig. 2). This indicates that lycopene did not contribute much to the chelating ability of the whole fruit of yellow-fleshed watermelon. The higher chelating ability in the whole fruit of yellow-fleshed watermelon was most likely due to the presence of phenolics or other forms of carotenoids that were not measured in this study.

Table 2: Chelating ability of red-fleshed and yellow-fleshed watermelons

	Mean concentration (mg/mL)	Chelating ability (%)
Red-fleshed watermelon, whole fruit		
FIC 1	710.76	2.94 ± 0.27^{d}
FIC 2	1066.41	8.40 ± 1.58^{b}
FIC 3	1421.88	10.26 ± 2.07^{b}
Red-fleshed watermelon, flesh		
FIC 1	619.34	6.94 ± 1.15^{c}
FIC 2	928.85	8.21 ± 1.34^{b}
FIC 3	1238.68	8.42 ± 2.70^{b}
Yellow-fleshed watermelon, whole fruit		
FIC 1	646.18	20.79 ± 1.73^{a}
FIC 2	969.27	25.68 ± 7.76^{a}
FIC 3	1292.36	27.90 ± 8.82^{a}
Yellow-fleshed watermelon, flesh		
FIC 1	619.34	10.99 ± 1.25^{b}
FIC 2	928.85	13.71 ± 1.81^{b}
FIC 3	1238.68	14.73 ± 4.14^{b}

Values are means ± standard deviation.

^{abcd} Different superscript letters within a column indicate significant difference at $p < 0.05$.

There was no significant difference between the chelating ability of the flesh of yellow-fleshed watermelon and red-fleshed watermelon except for FIC 1 (Table 2) where the chelating ability of the flesh of yellow-fleshed watermelon for FIC 1 was higher than that of the red-fleshed watermelon. The ascorbic acid and lycopene contents of the flesh of yellow-fleshed watermelon were lower than those of red-fleshed watermelon (Figs. 1 and 2). There was also no significant difference between the chelating ability of the whole fruit and flesh of red-fleshed watermelon except for FIC 1 where the chelating ability of the flesh of red-fleshed watermelon in FIC 1 was higher than that of the whole fruit of red-fleshed watermelon (Table 2). The ascorbic acid and lycopene contents of the flesh of red-fleshed watermelon were higher than those of yellow-fleshed watermelon (Figs. 1 and 2). In addition, the chelating ability of the whole fruit of yellow-fleshed watermelon was higher than that of the flesh of yellow-fleshed watermelon. However, there was no significant difference in the ascorbic acid and lycopene contents between the whole fruit and flesh of yellow-fleshed watermelon. These indicate that ascorbic acid and lycopene did not contribute much to the ferrous ion chelating ability of the watermelons but was most likely influenced by the presence of phenolics or other forms of carotenoids that were not measured in this study. This is in accordance with the study of Choo and Yong [25] where ferrous ion chelating activity did not correlate with the ascorbic acid content.

CONCLUSION

The flesh of red-fleshed watermelon had higher ascorbic acid and lycopene contents compared to those of yellow-fleshed watermelon. The whole fruit of red-fleshed watermelon had higher lycopene content than that of yellow-fleshed watermelon but with similar ascorbic acid content. Both varieties of watermelon showed different antioxidant activity. Flesh of red-fleshed watermelon had the highest primary antioxidant property (free radical scavenging activity) whereas the whole fruit of yellow-fleshed watermelon had the highest secondary antioxidant property (ferrous ion chelating activity). This study also demonstrates that besides ascorbic acids and lycopene, other bioactive compounds contribute to the antioxidant activity of the two varieties of watermelon.

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