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Research Article
Chemical Composition, Flavonoids and β-sitosterol Contents of Pulp and Rind of Watermelon (*Citrullus lanatus*) Fruit

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Abstract

Background: Recently, there is increasing demand for consumption of natural and healthy foods. Fruits (and watermelon among them) are believed to contain variety of dietary phytochemicals that may have beneficial effect on human health. **Objective:** The aim of this study was to prepare three samples from watermelon fruit (of Saudi origin) and to determine proximate composition, mineral, vitamin, flavonoids and beta sitosterol contents of the three samples. **Methodology:** Three samples were prepared from interior pulp region, exterior pulp region and from the rind of watermelon fruit. Proximate composition was determined by conventional official methods, analytical determination of minerals was performed by ICP-MS (inductively coupled plasma-mass spectrophotometer), vitamins and flavonoids were determined by High Performance Liquid Chromatography (HPLC) and β-sitosterol by Gas Chromatography (GC) techniques. Statistical analysis was performed by using SPSS software. **Results:** Moisture (ranged from 85-95%) was the predominant component in the samples. Carbohydrates ranged from 62.00-87.14% in dry weight basis, while fat content was low in the three watermelon samples. Potassium was the predominant mineral and ranged from 100.50-489.24 mg/100 g, vitamin C ranged between 2.50-8.30 mg/100 g, vitamin B6 0.060-0.150 mg/100 g and vitamin E 0.01-0.04 mg/100 g in the three watermelon samples. β-carotene concentration was high in the interior pulp (0.610 mg/100 g) compared to that in the rind (0.120 mg/100 g). Rutin was the only flavonoid detected in watermelon fruit, rutin concentration in the interior pulp sample was 1.66 mg/100 g. β-sitosterol is a phytosterol found in all parts of watermelon fruit, ranging from 0.140-0.627 mg/100 g. **Conclusion:** The results of this study may highlight the potential of watermelon fruit as a source of nutrients and anti-oxidants that may be considered for human nutrition and health.

Key words: *Citrullus lanatus*, chemical composition, β-carotene, flavonoids, phytosterols, anti-oxidants

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Competing Interest: The authors has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Citrullus lanatus (watermelon) family Cucurbitaceae[1] is one of the largest fruit crop in the world, in 2013 watermelon global production approximated 109 million tons[2]. Watermelon fruit is composed of flesh (68%), seeds (2%) and rind approximately 30% of the total mass of the fruit[3]. The sweet interior flesh of watermelon fruit is usually deep red or pink and in sometimes orange, yellow or may be green if not ripe[4]. Basic macronutrients of watermelon fruit include carbohydrates, protein, dietary fiber and fat and their percentages in wet weight basis were 7.50, 0.61, 0.40 and 0.15%, respectively[5]. Also, watermelon fruit is considered as a rich source of vitamins A, B, C and E and minerals K, mg, Ca and Fe and antioxidants e.g., phenolics and carotenoids[6]. Further, Tlili *et al.*[7] described watermelon fruit as one with high natural antioxidant capacity. Flavonoids have recently found more attention because of their suggested roles against free radicals. Kaempferol is one of the bioflavonoids and found in plants and plant-derived foods[8]. Quercetin is a type of bioflavonoid antioxidant abundant in the human diet e.g., onions are considered as a rich source of querectin[9]. Another flavonoid is rutin which has a wide range of pharmacological properties (e.g., antioxidative activity) that had been evidenced in human medicine and nutrition[10]. β-sitosterol is a predominant phytosterol used to treat heart diseases, hypercholesterolemia and prevention of some other diseases[11]. Beneficial effects of watermelon fruit may be due to its content of bioactive compounds that have antioxidant or anti inflammatory properties[12]. Example of such bioactive compounds in watermelon fruit is citrulline (an amino acid) that may influence atherosslerosis[13]. The aim of the present study was to prepare three samples from different parts of watermelon fruit (of Saudi origin) and to determine proximate, mineral, vitamin, flavonoids and sitosterol compositions of watermelon fruit samples.

MATERIALS AND METHODS

Materials: Ripe fruits of watermelon (*Citrullus lanatus*) were collected from local farms in Riyadh region, Saudi Arabia. All chemicals and reagents used in this study were of analytical grade.

Methods

Preparation of watermelon samples: Three watermelon samples were extracted from three different parts of the fruit; sample Watermelon Interior Pulp (WMIP) from the interior flesh or pulp, sample WMIP from the exterior flesh or pulp and sample Watermelon Rind (WMR) prepared from the rind or peel of watermelon fruit. Each sample was collected from 10 fruits, mixed together, then cut into small pieces and used fresh for analysis or dried at 50°C for 48 h using air oven, the dried samples were ground to a fine powder and stored at 4°C for further analysis.

Proximate composition: Moisture, protein (N×6.25), fat, crude fiber and ash contents of watermelon samples were determined by the standard methods of association of official analytical chemists[14].

Mineral determination: Watermelon samples were prepared for mineral analysis using wet digestion method[14]. The analytical determination of the minerals (K, Mg, Mn and Fe) was carried out by ICP-MS (Inductively coupled plasma-mass spectrophotometer) ELAN 9000 (Perkin Elmer Sciex Instruments, Concord, Ontario, Canada). The operation conditions and the calibration of the instrument were followed as found in the instructions of the instrumental (IPC-MS) manual.

Vitamin determination: The analytical procedures described by Sami *et al.*[15] for vitamins determination were followed with modifications. The HPLC-grade solvents were used and water used for HPLC was prepared with millipore simplicity instrument. All vitamins standards were of chromatography grade and were purchased from Sigma chemical company USA, the running conditions were as follows:

- **Mobile phase: For vitamin E:** 92% methanol+8% distilled water, vitamins B, B_6 and C: 30% methanol+70% phosphate butter. β-carotene: 40% acetonitrile+10% ethanol+50% distilled water
- **Column: Vitamin E and β-carotene:** Symmetry C_{18} 75 mm×4.6 mm×3.5 µm column operated at 30°C. vitamin E, B, B_6: EC 250/4 Nucleodur 100-5 C18ec column operated at 30°C
- **Detector:** For all vitamins: waters UV/visible 2489-285 nm detector was used
- **Sample size:** Volume of 20.0 µL was used for all vitamins

Flavonoids determination: Quantitative Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method with UV detection developed by Olszewska[16] for the quantification of flavonoids was followed for determination of kaempferol, quercetin and rutin in watermelon samples with some modifications:

- **Mobile phase:** 50% methanol+49% distilled water+1% acetic acid

- **Column:** EC 150/3 nucleodur polar Tec 5 µm operated at 30°C
- **Detector:** Waters UV/visible 3489-365 nm
- **Flow rate:** 1.0 mL min^{-1}, samples size: 20.0 µL
- **Standard:** Rutin standard 2.40 ppm, quercetin standard 2.25 ppm and kaempferol standard 1.10 ppm

Sitosterol determination: For determination of b-sitosterol from watermelon samples the official method[14] No 994.10 was followed using Gas Chromatography (GC) and the running conditions as follows:

- **Mobile phase:** Helium
- **Column:** Restek Rxi 5SII MS 30 m×0.25 mm×0.25 µm, operated at 300°C
- **Detector:** Shimadzu GC 2010 plus with FID and detector gases: Zero air and hydrogen
- **Sample size:** 2.0 µL
- **Standard:** Beta-sitosterol standard 173.0 ppm

Statistical analysis: Analyses were carried out in triplicate and data were expressed as Mean±Standard Deviation. Duncan's multiple range test (using SPSS Software 17.0) was performed to determine differences ($p \leq 0.05$) between means.

RESULTS AND DISCUSSION

Proximate composition of watermelon: Values of moisture, protein, fat, fiber, ash and carbohydrates for watermelon fruit fleshy parts (WMIP, WMEP) and the rind (WMR) are presented in Table 1. Moisture is the predominant component in the three samples in wet weight basis, ranging from 85.98-95.00%. While carbohydrates content for the three samples is high ranged from 62.00-87.14% in dry weight basis. Approximately 82% of carbohydrates in the flesh of mature watermelon fruit are sugars, fructose, glucose and sucrose are the main sugars present[5]. Fat contents for the three watermelon samples (Table 2) were the lowest compared to other components and ranged between 0.64 and 1.09% in dry weight basis. Proximate composition of the flesh or pulp of watermelon fruit was reported as 95.51, 0.55, 0.43 and 6.8% for moisture, protein, fat and carbohydrates, respectively[17]. While Al-Sayed and Ahmed[18] reported the composition of watermelon rind in dry weight basis for ash, fat, protein and carbohydrates as 13.09, 2.44, 11.17 and 56.00%, respectively. The fiber content of the rind of watermelon fruit (sample WMR) was calculated in dry weight basis to be 18.54% and this value is high than the fiber content in sample WMIP and WMEP. Consumption of fiber foods has been most extensively studied regarding health benefits[19].

Mineral composition of watermelon samples: The concentrations of the minerals (K, Mg, Mn and Fe) in the three watermelon samples are shown in Table 2. Potassium concentration in flesh of watermelon fruits (samples WMIP and WMEP) and in the rind (WMR) is remarkably high compared to the other minerals. Leterme *et al.*[20] stated that k presented an average of 32% of the total mineral content of samples of tropical fruits and unconventional foods investigated. The mineral composition of sample WMIP (Table 2) is very comparable to that of watermelon flesh where K, Mg, Mn and Fe concentrations were reported as 112, 10.0, 0.04 and 0.24 mg/100 g, respectively[5]. The rind (sample WMR) contained higher levels of the minerals investigated in this study as compared to sample WMIP and WMEP (Table 2). Huang *et al.*[21] reported levels of K, Mg, Mn and Fe in the rind of watermelon fruit as 107.48, 3.50, 0.19 and 0.35 mg/100 g, respectively and in the flesh as 20.85, 1.88,

Table 1: Proximate composition (%) of watermelon fruit samples in wet weight basis

Watermelon samples	Moisture	Protein	Fat	Fiber	Ash	Carbohydrates**
WMIP	90.82±0.235^{b}*	0.60±0.020^{b}	0.10±0.006^{b}	0.20±0.035^{c}	0.28±0.030^{c}	8.00
		(6.54)***	(1.09)	(2.18)	(3.05)	(87.14)
WMEP	95.00±0.120^{a}	0.12±0.010^{c}	0.05±0.050^{b}	0.60±0.040^{b}	1.13±0.055^{b}	3.10±0.115
		(2.40)	(1.00)	(12.0)	(22.60)	(62.00)
WMR	85.98±0.105^{c}	0.94±0.006^{a}	0.09±0.010^{a}	2.60±0.070^{a}	1.32±0.030^{a}	9.07±0.155
		(6.92)	(0.64)	(18.54)	(9.42)	(64.48)

WMIP: Watermelon interior pulp, WMEP: Watermelon exterior pulp, WMR: Watermelon rind or peel, *Mean±Standard Deviation (N = 3). Means with different letters in the same column are significantly different ($p \leq 0.05$). **Carbohydrates calculated by difference, ***Protein, fat, fiber, ash and carbohydrates values between brackets are calculated in dry-weight basis

Table 2: Mineral composition of watermelon fruit samples (mg/100 g dry samples)

Watermelon samples	Potassium (K)	Magnesium (Mg)	Manganese (Mn)	Iron (Fe)
WMIP	100.50±1.955^{*c}	12.18±0.286^{c}	0.039±0.0251^{b}	0.188±0.0115^{b}
WMEP	489.24±9.715^{a}	14.65±0.595^{b}	0.043±0.0058^{b}	0.092±0.0070^{c}
WMR	447.33±2.205^{b}	34.90±1.043^{a}	0.240±0.0250^{a}	0.611±0.0125^{a}

*Means±Standard Deviation (N = 3). Means with different letters in the same column are significantly different ($p \leq 0.05$)

Table 3: Vitamins content of watermelon fruit samples (mg/100 g fresh weight)

Watermelon samples	Vitamin C	Vitamin B_6	Vitamin B1	β-carotene	Vitamin E
WMIP	8.30±0.2520*[a]	0.070±0.100[b]	**	0.610±0.0300[a]	0.040±0.0000[a]
WMEP	2.50±0.0100[c]	0.060±0.0100[b]	-	-	0.010±0.0000[c]
WMR	5.35±0.058[b]	0.150±0.0300[a]	-	0.120±0.000[b]	0.020±0.0000[b]

*Means±Standard Deviation (N = 3). Means with different letters in the same column are significantly different ($p \leq 0.05$). **Not detected

Table 4: Flavonoids and β-sitosterol contents of watermelon fruit (mg/100 g fresh weight)

Watermelon samples	Kaempferol	Quercetin	Rutin	β-sitosterol
WMIP	**	-	1.660±0.0700*	0.300±0.0300[b]
WMEP	-	-	-	0.627±0.0152[a]
WMR	-	-	-	0.140±0.0200[c]

*Means±Standard Deviation (N = 3). Means with different letters in the same column are significantly different ($p \leq 0.05$). **Not detected

0.05 and 0.29 mg/100 g, respectively. They concluded that rind of watermelon fruit contains higher levels of the minerals investigated compared to the flesh of watermelon fruit.

Vitamin content of watermelon samples: Vitamin C, B_6, E and β-carotene were determined in the three watermelon samples (Table 3). Vitamin C concentration ranged between 2.50 and 8.30 mg/100 g in watermelon fruit pulp (samples WMIP and WMEP), while in the rind (WMR) vitamin C concentration is 5.35 mg/100 g fresh weight. These results are a little lower than that reported by Johnson *et al.*[22] for vitamin C in watermelon pulp and rind. They found vitamin C concentration to be 9.39 and 7.63 mg/100 g fresh weight in watermelon pulp and rind, respectively. Vitamin B_6 concentration in sample WMR (Table 3) is higher than vitamin B_6 concentration in sample WMIP and WMEP and the flesh or pulp in this study contains comparable amount of vitamin B_6 as reported by ESHA[5] for the flesh of watermelon fruit (0.06 mg/100 g fresh weight). Vitamin E levels for the three samples (WMIP, WMEP, WMR) are lower than that reported for watermelon fruit by Chun *et al.*[23]. They found vitamin E concentration in watermelon (expressed as total tocopherol and tocotrienol) to be 0.06 mg/100 g edible weight. The provitamin A (β-carotene) concentration of the interior pulp (WMIP) is much higher than that of the rind (WMR) as shown in Table 3 and values of β-carotene in this study are much higher than the values of β-carotene as reported by Johnson *et al.*[22] for fresh watermelon pulp and fresh watermelon rind (15 and 76.9 mg/100 g, respectively). The antioxidant properties of watermelon fruit are evidenced by the presence of vitamin C, E, lycopene and β-carotene which help to neutralize free radicals and prevent oxidative stress and its related diseases[4].

Flavonoids and sterols content in watermelon fruit: Three flavonoids were determined in watermelon fruit in this study (kaempferol, quercetin and rutin) as shown in Table 4. Only rutin was detected in WMIP or interior pulp sample. The value of rutin in WMIP sample was found to be 1.66 mg/100 g fresh weight. The values of rutin in dry fruits of apple and sweet cherry were 170 and 180 mg/100 g, respectively as reported by Atanassova and Bagdassarian[24]. Rutin is a flavonol glycoside and *in vitro* and *in vivo* studies showed the antioxidant and anti-inflammatory properties of rutin and its role in the metabolism of glucose and lipids[25].

β-sitosterol content in WMEP sample is higher than that in WMIP or WMR samples (Table 4). Values of β-sitosterol of watermelon sample in the present study are lower than β-sitosterol levels in watermelon, banana, orange and avocado fruits reported by Duester[26] as 1, 11, 17 and 76 mg/100 g edible weight, respectively. β-sitosterol and other phytosterols function as anticholesterolemic agent, lowering cholesterol in the serum by mechanism involves inhibition of cholesterol absorption in the intestines and decreasing of cholesterol synthesis in the liver[27] .

CONCLUSION

Flesh or pulp and rind are the parts of watermelon fruit that analyzed for macro- and micronutrients, flavonoids and β-sitosterol in this study. The results showed that watermelon fruit contains high percentages of moisture and carbohydrates and low percentage of fat. Potassium (and some other minerals) and vitamins C, E and B_6 found in variable amounts in watermelon samples. β-carotene found in high amount in the interior flesh or pulp. Rutin (a flavonoid and antioxidant) detected only in the interior pulp sample. While β-sitosterol (phytosterol) found in all parts of watermelon fruit. Nutrients and antioxidants investigated in the present study may make watermelon fruit a reliable food source with regard to human nutrition and health.

SIGNIFICANCE STATEMENTS

This study discovers the potential of Saudi cultivar watermelon fruit as a source of phytochemicals that can be beneficial for human nutrition. This study will help the searcher to uncover the critical areas of food applications that can use watermelon fruit chemical constituents. Thus, new information about watermelon fruit macro and micronutrients can be obtained and used in a practical way.

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