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Original research

Antioxidant activity and mineral content of watermelon peel

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A B S T R A C T

Both synthetic and natural antioxidants are using in the food industries. The evaluation of the former showed its potential carcinogenic effects. So, different studies focused on natural materials as the rich source of antioxidant compounds. The aim of the present study was to evaluate some chemical properties of watermelon peel (WMP). So the minerals content, physicochemical parameters, antioxidant activity, total phenolic content, crude protein, fat, fiber, and ash determined in the WMP sample. The results showed that, watermelon peels contain protein (6.77 g/100g), fat (0.92 g/100g), ash (13.2 g/100g), fiber (24 g/100g), sodium (53.59 mg/100g), potassium (2074 mg/100g), calcium (468 mg/100g), copper (0.59 mg/100g), iron (12.08 mg/100g), magnesium (164.48 mg/100g), zinc (0.91 mg/100g) and phosphorus (107 mg/100g). It also indicates a significant free radical scavenging activity (IC_{50} of 147.30 mg/kg) and total phenolic content (2.47 g/100g). Finally, the WMP can consider as a good source of natural polyphenols, antioxidants and minerals.

Keywords: Watermelon peel, Atomic absorption spectroscopy, Physicochemical, Antioxidant activity, Mineral

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1. Introduction

By-products and wastes are produced during food processing and manufacturing. The main part of the food wastes contains valuable nutrients which possess economical value, human health benefits and reduced problem related to their disposal into the environment. Watermelon (*Citrullus Lanatus*) is a herbaceous plant of the cucurbit family with a short shrub, yellow flowers, juicy, and edible fruits. According to the previous studies, this fruit contains about 93% of water, 6% carbohydrates, 0.6% protein, 0.2% fat, vitamins, lycopene and minerals (0.2%) (Dube et al., 2020; Ghorbanalinejhad & Nobakht, 2017).

Watermelon is popular in tropical Asia, the US, India, China and Iran (Wang et al., 2016). Watermelon is also popular in Iran, which according to statistics were presented in 2016, produces more than 3 million tons per year (Ghorbanalinejhad & Nobakht, 2017).

Recently, special attention has been paid to food and agriculture wastes and by-products as a valuable source of polyphenols, natural antioxidants and dietary fiber. On the other hand, researchers are working on the improving the methods of recycling and reusing these wastes. In the food industries, such as the production of fruit juice, the fruit peels usually discarded as waste. The use of watermelon peel has been reporting as fertilizer or animal feed. In China, it is available in the powdered form and widely used as a traditional medicine to clear away heat and eliminate toxic substances of the body. Its pickled form also consumes in the southern US, Russia, Ukraine, Romania, and Bulgaria. The fermented and blended form of watermelon peel consumed as juice in Nigeria. Generally different applications of the watermelon peel in other countries show it has its own value and not just a waste (Ibrahim et al., 2017).

Antioxidants are added to the food in order to enhance the stability and shelf life of products due to its ability to prevent or inhibit the oxidation processes (Al-Sayed & Ahmed, 2013). The addition of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are restricted due to their potential carcinogenic potential and therefore, recently researchers are seeking to replace them with the natural alternatives (Jayaprakashaet al., 2003).

It was shown that the fruit peels contain bioactive compounds such as pectins, flavonoids, carotenoids and poly-methoxy flavones (Rafiq et al., 2018). Watermelon and its peels, as a natural alternative antioxidant (Al-Sayed & Ahmed, 2013), also contain other bioactive compounds such as citrulline (as a non-essential amino acid effective in the immune system and a key intermediate in the urea cycles) and lycopene (with a prominent role in the treatment of some cancer and cardiovascular diseases) (Naz et al., 2014).

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Hanan and Abdelrahman (2013) studied the antioxidant activity of the watermelon and Sharlyn melon peel, and showed their effects on the enhancement of the shelf-life of the cake produced by the substitution of wheat flour (at a 5% level) with watermelon peels.

In the present study, some physicochemical properties such as ash, protein, fat, fiber, phosphorus, total polyphenol, minerals and antioxidant value evaluated in WMP waste as low-cost agricultural waste material.

2. Material and Methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) purchased from Sigma–Aldrich (St. Louis, MO, USA). Folin-Ciocalteau phenol reagent and multielement standard solution for AA were supplied by Merck (Darmstadt, Germany). The double-distilled water purified using a Millipore system and used for the preparation of all standard and sample solutions. All other chemicals and solvents supplied by Merck or Sigma and were of analytical-reagent grade and used as received without further purification.

2.2. Apparatus

The concentration of metal elements determined with an Analytik Jena AG AAS ZEEnit 700P atomic absorption spectrometer (Jena, Germany) equipped with the flame and graphite furnace (GF) atomizers and Zeeman background correction mode. A Shimadzu UV-1700 Pharma spec. (Tokyo, Japan) was used for the determination of polyphenolic compounds, phosphorus and antioxidant activity (with a standard 10 mm path length cell). A muffle furnace (LMF4 from Carbolite, Bamford, Sheffield UK) employed for incineration of the samples. The protein percentage of the samples was determined by Gerhardt protein and nitrogen analysis system (Gerhardt, Germany).

2.3. Sample preparation

Watermelon samples were collected from the local farms in Torbat-Heydariyeh (Khorasan Razavi, Iran) in the summer of 2017. The edible portion was separated and the whole part of the white and green peel was dried in the shade. The WMP samples were powdered using a mechanical grinder and subsequently were sieved. The particles smaller than 0.3 mm were packaged and stored at 4°C until use.

2.4. Physicochemical characteristics

The AOAC recommended analysis methods were utilized for the determination of ash (942.05), crude protein (920.152) , crude fat (948.22) and fiber (2009.01) contents (AOAC, 2016). The ash was determined by the incineration of the sample (2 g) in a muffle furnace at 550 °C for 5 h. Crude protein (%total nitrogen × 6.25) (Morais et al., 2017) was determined by the Kjeldahl method using 2 g of the sample. The crude fat obtained by exhaustively extracting of each sample (2 g) in a Soxhlet apparatus using petroleum ether (boiling range 40-60°C).

2.5. Determination of crude fiber

Crude fiber is the loss on ignition of dried residue remaining after digestion of sample under specific conditions (Dube et al., 2020) with H_2SO_4 (1.25 %w/v) and NaOH (1.25 %w/v) solutions. Sample (5.0 g) was boiled in 150 mL of 1.25% H_2SO_4 solution for 30 min under reflux. The boiled sample was washed in several portions of hot water using a two-fold cloth to trap the particles. It was returned to the flask and boiled again in 150 mL of 1.25% NaOH for another 30 min under same condition. After washing in several portion of hot water the sample was allowed to drain dry before being transferred quantitatively to a weighed crucible where it was dried in the oven at 105°C to a constant weight. It was thereafter taken to a muffle furnace where it was burnt, only ash was left of it. The weight of the fiber was determined by difference and calculated as a percentage of the weight of sample analyzed (AOAC, 2016).

2.6. Determination of phosphorus

The Phosphorous determined as PO_4^{3-} by the spectrophotometric method (AOAC, 2016) using sodium phosphomolybdate in which the phosphorous present as the orthophosphate reacts with a sodium molybdate reagent to produce a yellow-orange complex. A mixture of WMP and Ca_2CO_3 was incinerated in a muffle furnace and the residue was transferred to a beaker with distilled water. An extra amount of hydrochloric acid was added and the beaker was heated to completely dry. Then nitric acid was added and subsequently heated. The solution was filtered after dilution. The final solution was mixed with molybdovanadate reagent solution and stored at 20°C for 20 minutes followed by measurement of the absorbance at 430 nm. The control sample also was prepared similarly, except that ethanol solution (70% v/v) used instead of the sample extract. A calibration curve prepared with phosphorus standard solutions. The presented results were an average value of three determinations and were expressed based on dry mass.

2.7. Measurement of the metal elements

Wet digestion of the WMP samples was performed with a combination of hydrochloric and nitric acids followed by AAS analysis of the metal elements. The initial mixture of the WMP (5.0 g) and HNO_3 (30 mL), was heated and gently boiled until the remaining of 3-6 mL of the solution. After the addition of concentrated HCl (25 mL), the heating continued and the solution volume was reduced to 10-15 mL. The residue was filtered through the blue band filter paper and after cooling, the digested sample was diluted to 50 mL with distilled water. The blank digestions were also carried out in the same way (Ainsworth & Gillespie, 2007; AOAC, 2016). Measurement conditions for flame and graphite furnace atomic absorption spectrometer and the instrumental parameters for graphite furnace are listed in Table 1 and Table 2, respectively.

2.8. Total phenolic compounds

The official spectrophotometric procedure with Folin-Ciocalteu reagent (diluted at a 1:100 ratio in distilled water) (Ainsworth & Gillespie, 2007) applied for the measurement of total polyphenolic compounds. Briefly, the homogenized powder of WMP (0.5 g) was extracted with 25 mL of aqueous-ethanol solution (70% v/v). The mixture completely was stirred for 1 h and the resultant suspension

centrifuged at 5000 rpm for 10 min. A part of the collected supernatant (200 µL) and gallic acid standard solution was mixed with the Folin-Ciocalteu reagent (1.5 mL). The test tubes were stirred for 5 min followed by the addition of 1.5 mL of Na_2CO_3 solution (6% w/v). The samples were stored in a dark place at room temperature for 60 min. A control sample also was prepared in the same way, except that ethanol 70% used instead of the sample extract. The absorption of the samples was read at 760 nm, a calibration curve constructed with standard gallic acid solutions, and total polyphenolic compounds were expressed as g of gallic acid equivalents per 100 g of dry matter.

Table 1. Measurement conditions for atomic absorption spectrometer.

Element	Atomizer	Gas	Flame	Lamp current (mA)	Wavelength (nm)	Calibration (µg/mL)	Modifier
Na	Flame	acetylene	oxidizing	5	330.2	0.2-1 50-300	Cs, 2000 µg/mL
K	Flame	acetylene	oxidizing	6	404.4	0.4-2 150-600	Cs, 2000 µg/mL
Ca	Flame	N_2O	oxidizing	10	239.9	1-5 150-600	K, 2000 µg/mL
Fe	Flame	acetylene	oxidizing	7	248.3	2-10	-
Mg	Flame	acetylene	oxidizing	3	202.6	0.2-0.6 5-20	La, 2000 µg/mL
Cu	Furnace	----	----	3	324.7	0.005-0.015	---
Zn	Furnace	----	----	5	213.9	0.005-0.020	---

Table 2. GFAAS conditions for the determination of Zn and Cu in WMP.

Step	Ramp temperature (°C)		Ramp time (°C/s)	Hold time (s)	Gas	Gas flow (mL/min)
	Cu	Zn				
preparation	50	50	1	2	-	-
injection	-	-	-	-	-	-
Drying	90	90	10	15	Ar	250
Drying	120	120	15	10	Ar	250
Ashing	800	400	10	5	Ar	250
Ashing	800	400	0	1	-	-
Atomization	2300	1800	0.8	1.1	-	-
Cleaning	2600	2100	1	2	Ar	250

Table 3. Physicochemical characteristics of WMP (n=3) (results ± SD).

Parameter	Protein (g/100g)	Fiber (g/100g)	Ash (g/100g)	Fat (g/100g)	IC50 (mg/kg)	Total phenolic compound (g/100g)
Amount	6.77 ± 0.47	24.00 ± 1.38	13.20 ± 0.42	0.92 ± 0.06	147.30	2.47

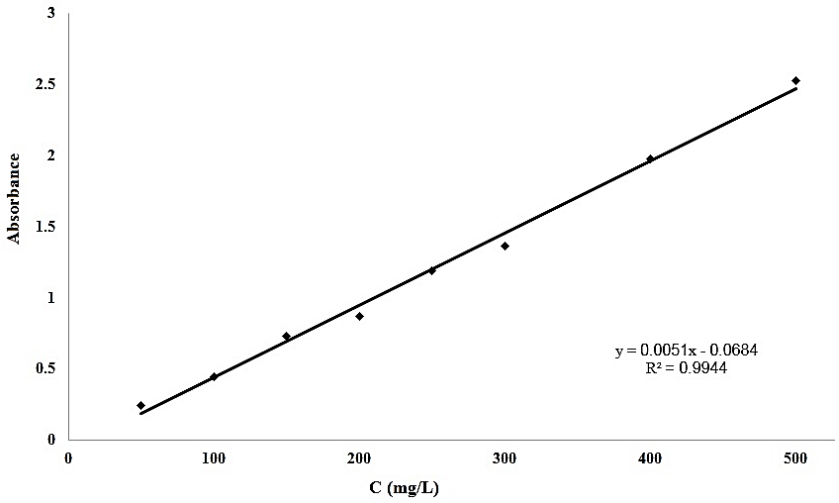

Fig. 1. Gallic acid calibration curve.

2.9. DPPH Free Radical Scavenging Activity (RSA)

The WMP samples (5 g) was dispersed in 25 mL of ethanol solution (70% v/v) and gently stirred for 24 h. The suspension centrifuged at 5000 rpm for 10 min and the collected supernatant was diluted in methanol, in the concentration range of 50-300 mg/L. After the addition of 2 mL of DPPH solution (0.004% v/v in methanol), the samples were stored in a dark place for 30 min at room temperature. The control sample also prepared in the same way, but methanol used instead of the sample solution. Subsequently, the optical density (OD) measured at 517 nm using a spectrophotometer and the following formula used for the calculation of RSA which expressed as the percentage inhibition.

$$\mathrm{DPPH}_{\text{scavenging activity}} = \left(\frac{\mathrm{A}_{\text{blank}} - \mathrm{A}_{\text{sample}}}{\mathrm{A}_{\text{blank}}}\right) \times 100 \qquad (1)$$

where A_{blank} and A_{sample} are the absorbance of the blank and sample solutions, respectively. Lower absorbance of the reaction mixture indicates higher free RSA.

3. Results and Discussion

3.1. Mineral composition of WMP

Dietary minerals are essential for the life that presented in the soil and water and absorbed by the plants or ingested by the animals. According to the human body demands the dietary minerals divided into the bulk and trace groups. The former was including calcium, magnesium, phosphorus, potassium, sodium, and sulfur, required in relatively large amounts. The second group needed only in very small quantities such as chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, selenium, and zinc (Lieberman & Bruning, 1997).

The mineral content of the WMP, as average values of four replicate measurements was determined. The obtained results of calcium, magnesium, potassium, sodium, copper, iron, zinc and phosphorus were 468.00 ± 0.12, 164.48 ± 0.20, 2074.00 ± 10.00, 53.59 ± 0.10, 0.59 ± 0.008, 12.08 ± 0.09, 0.91 ± 0.13 and 107.00 ± 0.17 mg/100g, respectively.

In the similar study by Morais et al. (2017) mineral content of WMP was investigated. These minerals were Ca, Mg, K, Na, Cu, Fe, Mn and Zn with mean value of 9770.9 ± 643.4, 260.4 ± 22.4, 3734.2 ± 208.9, 29.7 ± 2.3, 6.1 ± 0.3, 4.0 ± 1.0, 3.8 ±0.2 and 5.1 ± 2.9, respectively. Globally 200 to 300 different varieties of watermelon are accounted. Comparison between the results obtained in this study and in ours show that there is a difference between them. These differences may be due to the different varieties of watermelons that were analyzed.

3.2. Physicochemical properties of WMP

As presented in Table 3, WMP contained crude fat (0.92 ± 0.06 g/100 g), crude fiber (24.00 ± 1.38 g/100 g) and protein (6.77 ± 0.47g/100 g) in dry wt. The WMP sample also has an ash content of 13.2 ± 0.42 g/100 g dry wt. Morais et al. (2017) determined ash (10.2 ± 2.4 g/100 g), protein (10.2 ± 1.1 g/100 g), fat (1.8 ± 0.1 g/100 g) and fiber (39.1 ± 7.5 g/100 g) of WMP grown in Brazil. This study presents the result of the properties of ash, that is consistent with the results reported in the Morais et al. paper. Differences in the results of other physicochemical properties may be attributed to differences in the watermelon varieties studied. Based on the Iranian national standard, the ash content of maize, soybean oilcake and sunflower meal should be 1.5, 6 and 7%, respectively. The amount of crude fat, protein and fiber are comparable to the values reported by the Iranian national standard for the different variety of animal feeds. The crude fat is higher than those reported for maize, soybean oilcake and sunflower meal with the values of 3.5, 0.5, 2.0, and 2.5%, respectively (INSO 1445, 2018; Naz et al., 2014; Rafiq et al., 2018). Fat has the highest energy content in diets (2.25 times higher than carbohydrates), supplies the energy needed by an animal for normal body maintenance, and promotes the absorption of fat-soluble vitamins (Akinhanmi et al., 2008).

Protein also plays a part in the organoleptic properties of foods in addition to being a source of amino acid. Since the fruit are not potential source of protein, the fruit value of crude protein is low. The Protein content was lower than those reported for maize, soybean oilcake and sunflower meal with the values of 8, 42 and 34%, respectively (INSO 1445, 2018; Naz et al., 2014; Rafiq et al., 2018). The crude fiber content was higher than those reported for maize, soybean oilcake and sunflower meal with a value of 2.7, 3.37 and 14%, respectively (INSO 1445, 2018; Naz et al., 2014; Rafiq et al., 2018).

3.3. Total phenolic compounds

The polyphenols, as one of the plant constituents, have widely interested in food industries mainly due to antioxidant capacity and human health properties. The amount of total phenolic compounds in this research was 2473.45 mg gallic acid equivalent (GAE)/100 g. Gallic acid calibration curve was shown in Fig. 1. In the study Naguib and Tantawy (2019) was focused on the anticancer effect of some fruits peels aqueous extracts. Some important phytochemical, such asalkaloid, tannin, saponin, steroid, glycosidic cyanide, phytate, phenolic and flavonoidwere investigated. The level of phenol found in watermelon peel was 120.83 ± 0.038 µg/g dry wt (Naguib & Tantawy, 2019). In the study Rolim et al. (2018) determines total phenolic compound of melon peel and melon seed by HPLC. The polyphenol content of hydroethanolic (70:30 v/v) extract was found 203.1 ± 7.17 mg GAE/100 g dry extract. The difference between this results with our, is due to differences in the method of analysis and melon variety.

3.4. DPPH Free Radical Scavenging Activity (RSA)

The DPPH is a stable free radical, which loses its characteristic deep purple color by accepting hydrogen from a corresponding donor. The DPPH radical is commonly used as a substrate to estimate the activity of antioxidants. The results of DPPH usually expressed based on IC_{50} (the half half-maximal inhibitory concentration). In our study, the IC_{50} value under laboratory condition was equal to 147.3 mg/kg. Analyses of antioxidant properties were performed by Rolim et al. (2018) in melon seed and melon peel extract using various types of chain reactions: initiation (total antioxidant capacity, (TAC), reducing power), propagation (metal chelating), termination (sequestration of hydroxyl radicals, superoxide, DPPH), and oxygen reactive antioxidant capacity (ORAC) test. TAC as ascorbic acid equivalent, reducing power, DPPH activity scavenging and total ORAC value were 88.7 ± 2.99 mg/g, 17.1 ± 1.89 (%), 30.1 ± 0.16 (%) and 19.49 ± 0.97 (mmol trolox/100 g), respectively.

4. Conclusion

The present study investigates the minerals composition, physicochemical characteristic and antioxidant activity of the WMP extracts. The obtained results in this study confirm it is rich in bioactive components which have beneficial effects on human health. It should be stressed that the peels of different fruits can consider as a potential source of various antioxidant components, which are not exploited at the moment but could find practical application in many industrial branches. In many cases, the fruit peels are the waste products, hence their re-using as the antioxidant source, could bring measurable economical profits and contribute to the reduction of pollutions introduced by fruit and vegetable industries into the environment. It is necessary to consider both environmental (waste management, protection against pollution) and economic aspects (extraction profitability). Minerals are important in the diet because they serve as cofactors for many physiologic and metabolic functions and in their absence, clinical deficiencies may occur. Both Ca (468 mg/100 g) and K (2074 mg/100 g) detected at significant levels in WMP. The levels of Na (53.59 mg/100 g) and K suggested that the WMP might prove useful in lowering elevated blood pressure. A significant level of Fe (12.08 mg/100 g) was also present in the WMP. Fe, Cu, Zn, and Mg play an important role in biological systems.

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Conflict of interest

The authors declare that there is no conflict of interest.

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