

Some Physico-Chemical Properties of Edible and Forage Watermelon Seeds

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ABSTRACT: The crude oil, crude protein, crude ash, crude fiber, total phenol and antioxidant activity values, peroxide values, specific gravity, the refractive index and acid value of *Citrullus lanatus* and their oils were determined. Fatty acid composition of seeds belong to both watermelon were determined by Gas Chromatography (GC). These oils are important sources of essential fatty acid, linoleic acid (63.19% to 72.03%). Oleic acid contents of seeds ranged between 17.55% (Forage watermelon kernel) to 24.65% (watermelon kernel). Cd, Cr, Mn contents of watermelon kernel were found between 0.02 to 0.09 mg/kg, 0.37 to 1.46 mg/kg and 6.08 to 11.31 mg/kg, respectively. Ca, K, Mg, Na, P and S were found as major elements in seed samples. Total phenol contents of watermelon seeds ranged between 0.13 mg GAE /100 mg to 0.30 mg GAE/100 mg. Antioxidant activity of *Citrullus lanatus* (Thunb.) seeds (5.06 and 13.90%) were found higher than those of normal seeds (1.31 and 4.42%). Peroxide values of watermelon oils ranged between 7.6 meqO₂/kg to 11.7 meqO₂/kg.

KEY WORDS: Watermelon, Total phenol, Antioxidant activity, Fatty acid.

INTRODUCTION

Cucurbits are among the economically most important vegetable crops worldwide and are grown in both temperate and tropical regions (Pitrat *et al.*, 1999; Paris, 2011; Bisognin, 2002; Sanjur *et al.* 2002) [1-4]. Watermelon is an important horticultural crop, mostly known for its sweet and juicy fruit, grown in warm climates all over the world (Robinson & Decker-Walters, 1997; Jeffrey, 2001; Munisse *et al.*, 2011) [5-7]. *C.lanatus* is an annual species

containing cultivated semidomesticales and wild forms, widely distributed in tropical and subtropical areas (Jeffrey, 2001) [8]. Watermelon is mostly cultivated as an under sown intercrop together with cereals or root crops (Malanyaire, 1998; Ikeorgu, 1991) [9,10] in the same way as other cucurbits (Ndoro *et al.* 2007) [11]. Watermelon seed as a food source is reported (Loukou *et al.*, 2007) [12]. From a nutritional point of view, the red and sweet watermelon

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flesh is an important source of carotenoids, including lycopene and beta-carotene, a precursor of vitamin A (Seliawan et al. 2001; Edwards et al. 2003) [11,29]. Limited studies were carried out on *Citrullus lanatus* *vercitroides* and *Citrullus lanatus* (Thunb.) *Matsumura & Nakai* var. *Citroides* (Balley) Mansf (Forage watermelon).

The aim of this study was to determine some physico-chemical properties, fatty acid composition, total phenol and antioxidant activity of watermelon (*Citrullus lanatus* *vercitroides* and *Citrullus lanatus* (Thunb.) *Matsumura & Nakai* var. *Citroides* (Balley) Mansf (Forage watermelon)).

EXPERIMENTAL SECTION

Material

Watermelon seeds (*Citrullus lanatus* *vercitroides* and Forage watermelon (*Citrullus lanatus* (Thunb.) *Matsumura & Nakai* var. *citroides* (Balley) Mansf) were obtained from local markets in Konya provinces in Turkey. The seeds were cleaned and washed of any adhering residue and dried at 45 °C in a drying oven for 24h. Dried seeds were ground in a mill and screened through a mesh of 0.5 mm in diameter. The whole ground seeds were added into coloured bottle and kept in a refrigerator (+4 °C) before use.

Methods

The moisture content of pulp and pits were established separately by drying a sample (about 5 g) in a drying oven at 100±5 °C during 24 h. Crude fat, crude fiber, crude protein, crude ash, and peroxide values, specific gravity, the refractive index and acid values of samples were determined according to the Association of Official Analytical Chemists (AOAC, 2000) [4]. For oil analysis, each sample was homogenized and subjected to extraction for 6 h with petroleum ether (boiling range 30-60 °C) in a Soxhlet apparatus. The extracted oil was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure in a rotary evaporator. Oil percentages were determined by weight difference. For peroxide value (meq/kg), a known weight of watermelon seed oil was dissolved in a mixture of acetic acid / chloroform (3:2 v/v), and a saturated solution of KI (1 mL) was then added. The liberated iodine was titrated with sodium thiosulphate solution in the presence of starch as indicator. Ash was determined in a muffle furnace at 900 °C for 8 h (AOAC. 1990). [3]

Protein content was determined by the Dumas Nitrogen Analyzer (DNA) (Velp NDA 701- Italy). Protein was calculated using the general factor (6.25).

Working conditions of DNA:

O₂ flow rate: 400 mL/min

He flow rate: 195 mL/min

Combustion reactor temperature 1030 °C

Reduction reactor temperature 650 °C

Pressure: 881.0 mbar

Total phenol

Total phenolic contents of seeds were estimated using Folin Ciocalteu (FC) reagent as described by Yoo et al., 2004 [14] with some modifications. About a 0.5 mL aliquot of the aqueous date extract was mixed with 2.5 mL of 1:10 Folin Ciocalteu reagent and 1.5 mL of 20 % Na₂CO₃. Absorbance was measured at spectrophotometer (517 nm) (Shimadzu, Japan) after 30 min standing at room temperature. Gallic acid was used as a standard and the total phenolics were expressed as mg/g Gallic Acid Equivalents (GAE).

Radical Scavenging Assay

The free radical scavenging activity of the seed extracts was determined by using 1,1-diphenyl-2-picrylhydrazyl or DPPH (Lee et al., 1998) [15].

Determination of fatty acids

Fatty acid composition for seed oils were determined using a modified fatty acid methyl ester method as described by Hıřıl, 1998 [12]. The oil was extracted three times for 2 g air-dried seed sample by homogenization with petroleum ether. The oil samples (50-100 mg) was converted to its Fatty Acid Methyl Esters (FAME). The methyl esters of the fatty acids (1 µL) were analysed in a gas chromatography (Shimadzu GC-2010) equipped with a Flame Ionization Detector (FID), a fused silica capillary column (60 m x 0.25 mm i.d.; film thickness 0.20 mikrometere). It was operated under the following conditions: oven temperature program. 90 °C for 7 min. Raised to 240 °C at a rate 5 °C/min and than kept at 240 °C for 15 min); injector and detector temperatures, 260 and 260 °C; respectively, carrier gas. nitrogen at flow rate of 1.51 mL/min; split ratio. 1/50 µL/min.

A Standard fatty acid methyl ester mixture (Sigma Chemical Co.) was used to identify sample peaks.

Table 1: Physico-chemical properties of *Citrullus* seeds.

Properties	<i>Citrullus lanatus</i> var <i>citroides</i>		<i>Citrullus lanatus</i> (Thunb.) Matsumura&Nakai var. <i>citroides</i> (Balley) Mansf	
	Kernel	Hulled kernel	Kernel	Hulled kernel
Moisture (%)	4.56±0.13	5.71±0.73	3.61±0.18	4.55±0.27
Crude protein (%)	3.98±1.13	16.06±1.27	32.71±2.32	18.13±0.87
Crude oil (%)	52.34±1.47	26.83±1.38	43.32±2.17	23.31±1.08
Crude ash (%)	3.73±0.67	2.31±0.33	3.76±0.89	2.59±0.13
Crude fiber (%)	5.99±0.89	45.72±3.71	3.04±0.32	44.70±3.71
Total phenol (mgGAE/g)	0.13±0.07	0.28±0.03	0.24±0.03	0.30±0.08
Antioxidant activity (%)	1.31±0.11	4.42±0.73	5.06±0.68	13.90±1.2

Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times (AOAC 1990) [3]. Quantitative analyses of the fatty acids were performed using the heptadecanoic acid methyl ester as internal Standard. The results are mean values of three replicates.

Determination of element contents

Collected watermelon seed samples were dried at 70 °C in a drying cabinet with air-circulation until they reached constant weight. Later, about 0.5 g dried and ground sample was digested by using 5mL of 65% HNO₃ and 2 mL of 35% H₂O₂ in a closed microwave system (Cem-MARS Xpress) at 200 °C. The volumes of the digested samples were completed to 20 mL with ultra-deionized water and mineral concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP AES; Varian-Vista, Australia). Distilled deionized water and ultrahigh-purity commercial acids were used to prepare all reagents, standards, and samples. After digestion treatment, samples were filtrated through whatman No. 42. The filtrates were collected in 50 mL flasks and analysed by ICP-AES. The element contents of the seed samples were quantified against standard solutions of known concentrations which were analysed concurrently (Skujins, 1998) [30].

Working conditions of ICP-AES:

Instrument: ICP-AES (Varian-Vista)
 RF Power: 0.7-1.5 kW (1.2-1.3 kW for Axial)
 Plasma gas flow rate (Ar) : 10.5-15 L/min. (radial) 15" (axial)
 Auxilary gas flow rate (Ar): 1.5 L/min
 Viewing height: 5-12 mm

Copy and reading time :1-5 s (max.60 s)

Copy time: 3 s (max. 100 s)

Statistical analyses

Results of the research were analysed for statistical significance by analysis of variance (Püskülcü & İkiç, 1989) [29]. This research was performed by three duplicates with a replicate.

RESULTS AND DISCUSSION

The crude oil, crude protein, crude ash, crude fiber, total phenol and antioxidant activity values of Forage watermelon are shown in Table 1. From the experimental results, it is possible to state that these seeds which are used contained important quantities of these natural compounds. The protein contents of Forage watermelon kernel is more than results of other seed and with hull seeds. These values are high than those of sesame seed, caper, cactus, carob seeds, almond and cereals that contain between 5% and 25% protein (Özcan, 1995; Özcan, 2010; Tlili et al. 2011) [23,31]. The oil content of watermelon kernel (52.34%) was found high compared with content of Forage watermelon kernel (43.32%). The crude ash contents of water melon seeds changed between 2.31% to 3.76%. In addition, fiber contents ranged between 3.04% to 45.72%. While the fiber contents of hulled seeds were found at the high levels (44.7 % to 45.72%), fiber contents of corresponding kernels were found as 3.04% and 5.99%.

It is concluded also that watermelon kernel seeds show the high content of lipid (52.34%), followed by Forage watermelon kernel (43.32%). The protein contents were 29.23%, 36.0%, 29.55% and 24.79% for *C.lanatus*,

Table 2: Fatty acid composition of *Citrullus* seed oils (%).

Properties	<i>Citrullus lanatus</i> var. <i>citroides</i>		<i>Citrullus lanatus</i> (Thunb.) Matsumura&Nakai var. <i>Citroides</i> (Balley) Mansf	
	Kernel	Hulled kernel	Kernel	Hulled kernel
Stearic	9.41	9.16	9.65	9.73
Oleic	24.65	23.15	17.55	17.81
Linoleic	63.19	63.59	72.03	71.76
Alpha-linoleic	0.58	0.84	0.21	0.06
Linolenic	0.31	-	0.28	0.10
Total	98.14	96.74	99.72	99.46

C.mannii, *C.melo* and *A.hypogaea*, respectively. The highest estimates of fat content was observed with *C.lanatus* (56.67%) followed in decreased order *C.melo* (42.67%) (Loukou et al., 2007) [17].

Fatty acid composition of seeds belong to both watermelon are presented in Table 2. These oils are important source of essential fatty acid, linoleic acid (63.19% to 72.03%). Also, oleic acid contents of seeds ranged between 17.55% (Forage watermelon kernel) to 24.65% (watermelon kernel). This gives a nutritional and medicinal importance for these plant seeds. Indeed, linoleic acid has a beneficial role for the human body (Letawe et al., 1998) [16]. The major saturated fatty acid is the stearic acid (9.16% to 9.73%). Results show that all studied seeds are rich with unsaturated fatty acids (more than 75%). Moreover the monounsaturated fatty acid has an important physiological role (Colquhaun et al. 1996) [10]. The fatty acid content of the *Citrillus lanatus* seed oil showed that 71.9% is unsaturated (Oluba et al. 2008) [22]. These results indicate that both *C.lanatus* seed oils could be a good source of table oil. It high content of linoleic acid is of particular interest especially in the fight against atherosclerosis (Oluba et al., 2008) [22].

The element contents of seeds are given in Table 3. Element contents of samples were found to be different depending on the hulled and nonhull seeds. Cd, Cr, Mn and Ni contents were found low and in a similar range for all samples. Cu and Fe contents of watermelon seed samples were found between 9.11 mg/kg (watermelon hulled seed) to 19.07 mg/kg (Forage watermelon kernel) and 90.58 mg/kg (watermelon kernel) to 230.09 mg/kg (Forage watermelon kernel). In addition, Zn levels were determined between 33.69 mg/kg (watermelon hulled seed) to 77.58 mg/kg (Forage watermelon kernel).

Mn contents of Forage watermelon hulled seed and watermelon hulled seed seeds were established lower compared to Forage watermelon kernel and watermelon kernel seeds. Ca, K, Mg, Na, P and S were found as major elements in seed samples. Magnesium, phosphorus, sulfur and zinc contents of Forage watermelon hulled seed and watermelon hulled seed samples were found low according to Forage watermelon kernel and watermelon kernel samples. In addition, Ca contents of Forage watermelon kernel (1166.63 mg/kg) and Forage watermelon hulled seed (1362.72 mg/kg) were determined higher than those of watermelon kernel (465.07 mg/kg) to watermelon hulled seed (824.72 mg/kg). While potassium contents of samples changed between 6660.77 mg/kg (watermelon hulled seed) to 8271.24 mg/kg (Forage watermelon hulled seed), calcium contents ranged between 5075.75 mg/kg (watermelon hulled seed) to 8396.93 mg/kg (watermelon kernel). As a result, Ca, K, Mn and P contents of seeds were found at high levels. These samples are a source of elements for animal feeding. Ziyada & Elhussien, 2008 [35] reported that *Citrullus lanatus* oil contained 0.65 ppm Fe, 0.21 ppm Cu, 0.41 ppm Zn, 0.01 ppm Ni, 9.00 ppm Ca, 0.16 ppm Mn, 3.05 ppm Co and 7.00 ppm Mg. Also, the same researchers determined 9.97% palmitic, 7.78% stearic, 14.69% oleic and 67.56% linoleic acids in *C.lanatus* oil.

Present results are also similar to that of previous study on *Citrullus* and *Cucurbita pepo* seed oil which was found to contain mostly palmitic, stearic, oleic and linoleic acids, with linoleic acid as the most abundant (Murkovic et al., 1996; Ziyada & Elhussien, 2008; Oluba et al. 2008) [20,22,35]. Total phenol contents of watermelon seeds ranged between 0.13 mgGAE /g to 0.30 mgGAE/g. The highest total phenol was established in

Table 3: Physical and chemical properties of citrullus kernel oils.

Properties	<i>Citrullus lanatus</i> var. <i>citroides</i>		<i>Citrullus lanatus</i> (Thunb.) Matsumura & Nakai var. <i>Citroides</i> (Balley) Mansf	
	Kernel	Hulled kernel	Kernel	Hulled kernel
Peroxide value (meq O ₂ /kg)	7.6±0.18	9.3±0.13	9.8±0.008	11.7±0.32
Acid value (%)	2.7±0.21	3.2±0.1	1.9±0.3	2.8±0.3
Refractice index	1.453±0.009	1.467±0.011	1.450±0.012	1.463±0.013
Specific gravity	0.927±0.007	0.938±0.013	0.929±0.017	0.942±0.009

Table 4: Element contents of Citrullus kernels (mg/kg).

Properties	Elements													
	Ca 317.93 (wavelength)	B 249.77	Cd 214.43	Cr 267.71	Cu 327.39	Fe 238.20	K 766.49	Mg 279.55	Mn 257.61	Na 589.59	Ni 231.60	P 213.61	S 181.97	Zn 213.85
Forage watermelon kernel	1166.63	12.22	0.03	0.57	19.07	230.09	7794.64	3787.64	10.90	218.36	3.01	7545.04	2661.94	77.58
Forage watermelon hulled seed	1362.72	11.94	0.09	1.07	16.40	190.41	8271.24	3593.76	6.15	201.57	3.54	5985.15	2256.36	62.73
Watermelon kernel	465.07	13.60	0.02	0.37	13.27	90.58	7793.59	3916.89	11.31	74.61	5.96	8396.93	2871.26	48.07
Watermelon hulled seed	824.72	12.62	0.03	1.46	9.11	150.05	6660.77	3277.27	6.08	337.78	6.82	5075.75	1920.60	33.69

Forage watermelon kernel. In addition, antioxidant activity of seeds were found between 1.13% to 13.90%. Antioxidant activity of green seeds (5.06 and 13.90%) were found higher than those of normal seeds (1.31 and 4.42%).

Peroxide values of watermelon oils ranged between 7.6 meq O₂/kg to 11.7 meq O₂/kg. Samples have an acid values between 1.9 mg KOH/g to 3.2 mg KOH/g. In addition, while refractive index changed between 1.450 to 1.467, specific gravity of oils 0.927 to 0.942. peroxide values are higher than the values recorded for *Bauchinia racemora* (4.9 meq/kg) seed (Amoo & Moza, 1999) [6] and *Citrullus lanatus* (Egusi Melon) seed oil 8.3 meq/kg) (Oluba et al., 2008). [22] Peroxide value depends on a number of factors such as the state of oxidation, the method of exytaction used and the type of fatty acids present in the oil. Also, the high peroxide values may be due to causing lipid oxidation resulting from the absorption of oxygen, which increases the formation of peroxides (Cheftel & Cheftel, 1992; Oluba et al., 2008). [8,22] The result of the refractive index, 1.450 to 1.467 are in close agreement with the value of 1.46 obtained for *B.sapida* (Akintayo et al., 2002) [5] and *C.lanatus* oils (1.45) (Oluba et al., 2008) [22]. The acid values of samples (1.9 to 3.2 mg KOH/g) are relatively low compared to that reported for fluted pumpkin (3.5 mg KOH/g) (Christian,

2006) [9]. The low acid value of the oil indicates that it is good as edible oil.

CONCLUSIONS

Edible and forage watermelon seeds are among the economic cally most important vegetable erops. Seed oil contained important essential fatty acids such as linoleic acid. In addition, water melon seeds contnined major and minor elements.

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