Essential oil composition and total phenolic compounds of naked and normal seed types of different accessions of *Cucurbita pepo* L. in Iran

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Abstract

Cucurbita pepo var. *styriaca* is known as a medicinal crop among other cucurbits and has been cultivated around many decades in some parts of Iran by local farmers. The present study was conducted on 8 accessions of C. *pepo* seed from various planting zones. All collected seeds were planted in Horticultural Science Research Field of University of Tehran in three replications with two observations in each replicate based on randomized complete block design. Seed characteristics and phytochemical composition were analyzed after fruit ripening. The result of variance analysis showed that all of the studied characteristics had significant differences among accessions. Gorgan accession (193 gr) and Mashhad accession (102 gr) had the highest and lowest seed weight, respectively which had seed coat in comparison with naked seed types. Among naked seeds, Shahroud accession revealed higher seed weight (148 g). Maximum oleic acid content, an unsaturated fatty acid (vital and important fatty acids), was for Esfahan accession as naked seed. The results showed that maximum total phenolic compound was related to Qom accession with 4 mg GAE/kg and Khomein accession as naked seed type had maximum antioxidant content. Although naked seed is known as medicinal cucurbits because of high amount of un-saturated fatty acid, this study demonstrated that other cucurbits like Chalous accession with normal seeds also had high amount of unsaturated fatty acid.

Key words: *Cucurbita pepo*; fatty acid; naked seed; phytochemical composition

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Introduction

Cucurbits are vegetable crops. Cucurbita genus belongs to *Cucurbitaceae* family and comprises of five domesticated species, *C. pepo* L. (Summer squash and Zucchini), *C. maxima* Duchesne (Pumpkin), *C. moschata* Duchesne (Butternut), *C. argyrosperma* Huber (*C. mixta* Pang.) (Cushaw), and *C. ficifolia* Bouché, all being native to America (Hadia et al., 2008). The plants of the family are collectively known as cucurbits that are distributed mainly in tropical and subtropical regions of the world (Kocyan *et al.*, 2007). The benefits of cucurbits fruits are very important in terms of human health, purification

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of blood, removal of constipation, and are good for digestion and supplying energy. Also seeds, flowers, and roots are consumed by humans. The secondary metabolite Cucurbitacin content of seed or fruit parts of some cucurbits are reported to possess purgatives, anthelmintic and anticancer properties (Bisognin, 2002). Pumpkin seeds have a high nutritional value: good source of potassium, phosphorus, and magnesium, and also containing high amounts of other trace minerals (calcium, sodium, manganese, iron, zinc, and copper) (Lazos, 1986).

Cucurbita pepo seeds normally have thick, leathery seed coat (hull) due to the strong lignification of some of its testa layers. The mutant styrian (naked) seed pumpkin (*Cucurbita pepo* L. convar. *Pepo* var. *styriaca* Greb. (Winkler, 2000)) Exposes a complete lack of lignification of the seed testa. This mutation appeared probably in the 1880's in the South-East of the then Austro-Hungarian-Monarchy (Zraidi et al. 2003).

According to FAO harvest area of cucurbit in Iran was 59774 ha, and the yield was around 9.6 ton/ha (FAO, 2011) and there is a great genetic diversity in the cultivated forms of the crop.

The four fatty acids present in significant quantities are palmitic, stearic, oleic, and linoleic acids (Stevenson et al., 2007). Nakic et al. (2006) evaluated the chemical characteristics of oils from naked and husk seeds of *Cucurbita pepo* L. and found some differences for fatty acid composition, tocopherol, sterol, and squalene content. Bandoniene et al. (2013) studied the distribution of trace elements in pumpkin seed and pumpkin seed oils in relation to the geographical origin of soils of several agricultural farms in Austria. Studies on different geographical origins showed variations of the elemental patterns that were significantly large, reproducible over the years and ripeness period while showing no significant influence of oil production procedure to allow a discrimination of geographical origin. A successful differentiation of oils from different regions in Austria, China and Russia classified with multivariate data analysis is demonstrated.

Various plants synthesize a plethora of different phenolic compounds, and the phenolic pattern can also be characteristic for the species. Different plant-derived classes of phenolic compounds have been isolated and characterized. They show manifold interesting properties including antioxidant, estrogenic or anti-estrogenic effects. In addition, amongst them are potential anticancer and cardio protective drugs, and some that possess antimicrobial and antiviral activities (Fruhwirth and Hermetter, 2007).

In the study by Applequist et al. (2006) great variation has been observed in fatty acid content of pumpkin seeds from *Cucurbita pepo* while other pumpkins or winter squash species, including *C. moschata*, *C. maxima*, and *C. argyrosperma* have been inadequately studied. Steavenson et al. (2007) reported that Oil content of 12 cultivars of pumpkin ranged from 10.9 to 30.9% and Total unsaturated fatty acid content ranged from 73.1 to 80.5%.

Considerable variation in total lipid content and composition within, as well as among, species was observed. Generally, *C. pepo* had the lowest proportion of saturated fatty acids, whereas *C. maxima* had the highest (Berenji and Papp, 2000; Gohari Ardabili et al., 2011). Hybridization in open-pollinated fruits affected seed fatty acid phenotype and open-pollinated fruits had higher total lipid content (Applequist et al., 2006). The seeds of *Cucurbita pepo* subsp. *pepo* var. *Styriaca* contain more than 80% unsaturated fatty acids with especially high amounts of polyunsaturated fatty acids (myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid) (Nawirska-Olszańska et al., 2013). The fatty acid pattern of Styrian pumpkin seed oil appears to be very similar to that of Styrian oil pumpkin seeds, thus it mainly consists of unsaturated fatty acids (Ielly et al., 2010).

Different kinds of cucurbits have been used for their seed as medicine or as nuts in nutrient regime in traditional celebrations. Also, cucurbit flesh has been used as fresh vegetable. The aim of the present study was to assess chemical composition of naked seed type in comparison with other true seed type to check the presence of phytochemicals in the seed extracts and introduce better accession.

Material and Method

Plant material

A collection comprising 8 accessions of cucurbit species which have been cultivated in Iran was chosen for this study. Accessions including 3 naked seed types were collected from west and north parts of the country (Table 1). All collected seeds were planted in three replications with two observations in each replicate in a randomized completely block design. Plants were irrigated, fertilized, and were treated against pests and pathogens according to standard agronomic methods. After fruits ripening, they were harvested and moved to laboratory for investigating their characteristics including fruit weight, fruit width, fruit length, seed width, seed cavity diameter, length, weight, and thickness, and analysis of phytochemical properties (fatty acid and total phenol content).

Fatty acid analysis

Dried samples of 8 accessions were extracted with chloroform: methanol (2:1, v/v) according to the method of Folch et al. (1957). Solid and non-lipid materials were removed before the solvent was evaporated under nitrogen gas. Fatty acid methyl ester was prepared by methylation of the total lipids as described by Joseph and Ackman (1992). Methyl esters were separated by gas chromatography (GC), under the following conditions. The detector temperature was 280° C, the injection port temperature was 260° C, and the column temperature was 180° C. Carrier gas (hydrogen) flow was 1 ml/min with a nitrogen flow of 30 ml/min. To identify each fatty acid, each retention time was compared with the standard reference.

Total phenolic compounds

Total phenolic content was determined by the Folin–Ciocalteau assay (Marinova et al., 2005) using Gallic acid as a calibration standard. A 0.5 ml extract was added along with 2.5 ml of Folin- Ciocalteu reagent followed by addition of 2 ml sodium carbonate (Na_2CO_3) (75 g/l). The sample was then incubated for 5 min at 50° C.

Table 1
Accessions used in this study, their species, and sites

No.	Species	Location
1	*C.pepo var. styriaca*	Khomein
2	*C.pepo* var. *styriaca*	Esfahan
3	*C.pepo* var. *styriaca*	Shahroud
4	*C.pepo*	Gorgan
5	*C.pepo*	W. Azerbaijan
6	*C.pepo*	Kerman
7	*C.pepo*	Qom
8	*C.pepo*	Mashhad

The absorbance was then measured at 760 nm using a spectrophotometer. The results were then expressed as mg Gallic acid equivalents per gram of extract (mg GAE/g) that was derived from a calibration curve.

Total Antioxidant compounds

The free radical scavenging activity of the samples was measured in accordance with the method of Brand-Williams et al. (1995) with modifications. The extracts were dissolved in 1.0 ml NaOH and the solutions were added to 1.0 ml DPPH. Solutions were kept at room temperature. The absorbance was measured at 515 nm utilizing UV-1601 Shimadzu spectrophotometer. The results were expressed as percentage of reduction of the initial DPPH. Absorption by test samples was calculated as follows:

DPPH scavenging effect (%) = $[(A0 - At) / A0] \times 100$

Where A0 is the absorbance of the control at t = 0 min, and At is the absorbance of the antioxidant at t = 15 min.

The IC50 is defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration by 50%. The IC50 of the samples was derived from the percentage of scavenging activity vs. concentration plot and was expressed as mg/ml.

Statistical analysis

Data was analyzed by using SPSS software. Duncan's multiple range tests were used to compare means for each trait and significance was accepted at p≤0.05 to identify the variability within populations.

Table 2
Variance analyze of important traits of 8 accessions in the study

source of variations	df	Mean Square												
		Palmetic	Stearic	Oleic	Linoleic	Phenol	Antioxidant	Seed cavity diameter	Seed weight	Seed Width	Seed Length	Fruit weight	Fruit length	Fruit width
accessions	7	5.95**	14.60**	506.40**	223.43**	0.01**	0.29**	18.73**	10365.73**	6.49**	128.29**	16.65**	211.43**	303.52**
block	2	0.001^{ns}	0.001^{ns}	0.0009^{ns}	0.003^{ns}	0.0004^{ns}	0.01^{ns}	2.16^{ns}	114.25^{ns}	5.96^{ns}	0.48^{ns}	3.14^{ns}	31.10^{ns}	17.98^{ns}
Error	16	0.002	0.002	0.00003	0.001	0.002	0.001	1.03	49.067	0.49	0.123	1.62	52.818	46.310
CV		0.38	0.73	0.01	0.05	5.31	9.87	3.09	5.85	0.45	0.09	28.69	20.72	12.94

Results

Morphological characteristics

The analysis of variance in data (Table 2) showed accessions relation with the studied traits. Accessions effects were found important and significant on palmitic, stearic, oleic and linoleic acids, phenol, antioxidant, seed weight, seed width, and seed length; however, there was no significant difference in the block effects on all of the studied traits.

Fruit parameters indicated more variety among different accessions. Fruit weight in Shahroud and Isfahan accessions was high among naked seed type. The highest seed cavity diameter (14.73 cm) and fruit width (62.53 cm) were observed in Gorgan accession while maximum fruit length (32 cm) was measured in Markazi accession from normal seed type. Coated seeds of Gorgan accession (193 g) and Mashhad accession (102 g) had highest and lowest seed weights, respectively (Table 3). Variation among naked seeds revealed that Shahroud accession had higher seed weight (148 g). The average length and width of seeds were observed about 13.89 mm and 7.13 mm, respectively.

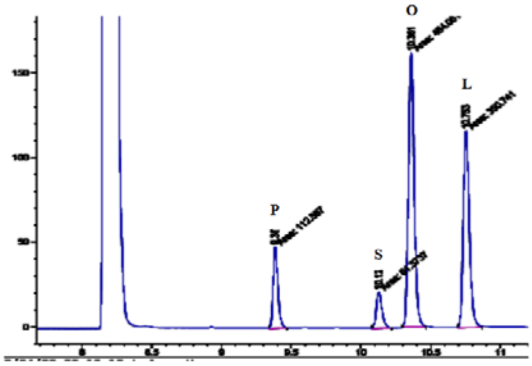

Fig. I. Gas chromatogram; Methyl Ester of fatty acid in Khomein accession; Linoleic acid: L, oleic acid: O, Stearic acid: S, Palmetic acid: P

Fatty Acid Compounds

The fatty acid contents were significantly different among the cucurbits seed samples. Results of the present study exhibited that in naked seed oil samples, oleic acid content was higher than other fatty acids but among normal seed types, unsaturated fatty acids was more.

The fatty acid profile of the cucurbit seed oils showed that maximum oleic acid content, a vital unsaturated fatty acid, was for Esfahan accession as naked seed. Linoleic acid content was higher in Kerman accession. Comparison of naked seed types revealed that Khomein had higher linoleic acid. However, naked seed accessions had high unsaturated oil as whole.

Study of the average fatty acid compounds showed that oleic acid (39.24%) was higher than linoleic (38.76%), palmitic (11.09%), and stearic (5.37%) acids. Stearic acid content was higher in Qom accession which also contained minimum palmitic acid (Table 3). On the other hand, Mashhad accessions were found to contain maximum amount of palmitic acid while at the same accessions had the lowest stearic acid. Considering the relation between

Table 3
Mean comparisons of important traits for 8 accessions in the study

Accessions	Palmetic	Stearic	Oleic	Linoleic	Phenol (mg GAE/kg oil)	Antioxidant (µg mL^{-1})	Fruit length (cm)	Fruit weight (kg)	Fruit width (cm)	Seed cavity diameter(cm)	Seed weight (gr)	Seed Wide (mm)	Seed length (mm)
Gorgan	11.36e	5.75 d	38.86 f	44.08b	0.36 c	0.94 c	31.23^{c}	2.06^{b}	62.53^{a}	14.73^{a}	193a	6.80f	3.16h
Esfahan	11.75 c	6.16 b	49.47 a	32.62 h	0.32 d	0.77 c	29.47^{e}	2.9^{a}	58.80^{b}	14.33^{b}	115e	7.25d	15.08 d
Shahroud	11.39 e	6.21 b	46.93 c	35.47f	0.33 d	1.23 b	29.20^{f}	2.97^{a}	55.85^{c}	12.65^{c}	148c	8.88a	17.05b
Markazi	11.66 d	5.78 c	48.06 b	34.51g	0.25 e	1.37 b	32^{a}	2.5^{b}	48.30^{f}	10^{f}	159b	7.28c	14.15f
Mashhad	13 a	0.13 f	5.21 h	39.99c	0.40 b	1.32 b	25.25^{g}	1.0^{d}	25.83^{m}	6.20^{h}	102h	6.18g	13.32g
Khomein	11.04 f	5.84 c	43.95 d	39.17e	0.31 d	1.69 a	30.10^{d}	1.89^{c}	51.25^{d}	11.20^{d}	130d	7.07e	18.53a
Kerman	11.88 b	5.3 e	37.84 g	44.98a	0.15 f	1.77 a	31.50^{b}	1.88^{c}	44.10^{n}	8.12^{g}	108g	6.03h	14.60e
Qom	10.70 g	6.38a	43.65 e	39.27d	0.44 a	0.87 c	25.25^{g}	1.5^{c}	49.15^{e}	10.85^{e}	110f	7.57b	15.26c

Similar letters in each column are not statistically different at 5% level of probability using Duncan test.

fatty acids, results showed that increase in stearic acid content coincided with the decrease in palmitic acid ad they seem to have antagonistic effect.

Total phenolic and antioxidant compounds

Various plants synthesize a plethora of different phenolic compounds and the phenolic pattern can also be characteristic for the species. The results showed that maximum total phenolic compound was related to Qom accession with 0.44 mg GAE/kg but three naked seed accessions showed no significant difference. Antioxidant contents of Khomein (naked seed) (1.69 µg ml^{-1}) and Kerman (normal seed) (1.77 µg ml^{-1}) were high but their total phenol contents were the lowest. This indicates that the presence and absences of seed coat was not effective in antioxidant capacity of the accessions.

Discussion

Our results about seed characteristics of *C. pepo* indicated more variation in them and this confirms the study reported by Wu et al (2011) on 89 accessions of *Cucurbita moschata* who found wide differences among seed length, width, thickness, and seeds weight of the accessions under study.

The thickness of the seed depends on its coat and embryo thickness (Gardingo et al., 2011). Milani et al. (2007) reported that seeds had lengths ranging from 12 to 14 mm; widths between 7 to 13 mm, and thicknesses ranging from 2 to 4 mm. Paksoy and Aydin (2004) found that *C. pepo* L. seed length, width, and thickness were 18.16, 9.80, and 2.67 mm, respectively. Selection based on seed characteristics would simply improve seed production and breeding program.

Cross between Styrian oil pumpkin with zucchini (Zraidi, 2005) revealed that pollination condition caused a coat on the seed surface by a thin lignified layer; therefore, culture condition affected generation, kind, and type of seeds. In Iran cucurbits are grown near each other and open pollination condition could affect different seed characteristics among them.

Environmental factors affect quality of seeds during seed development (Welbaum and Bradford, 1991). Different climatic conditions induce variation on traits on studied accessions.

Analysis of several Styrian pumpkin seed oils revealed that the content of polyunsaturated fatty acids (45.6 %) is considerably higher than the content of monounsaturated fatty acids (35.96 10%) or saturated fatty acids (18.56 20%) (Fruhwirth et al., 2003). Comparison of fatty acids contents of naked and normal seeds showed that the amount of oleic acid (unsaturated fatty acid) was more in Esfahan accession belonging to naked seed type while palmitic and stearic acids were higher in accessions with normal seed. The present study revealed that linoleic acid content was higher among fatty acids in normal seeds. In fact, all oil samples examined were rich in both linoleic and oleic acids. Naked seed oil samples in this study had higher oleic acid content compared with other fatty acids but the case in normal seeds was different and many samples of normal seeds had higher linoleic acid (Kerman, Markazi, and Gorgan accessions) and others had higher oleic acid content.

De Mello et al. (2001) reported that linoleic acid in melon was the principal fatty acid followed by oleic, palmitic, and stearic acids with concentrations approaching 51%, 31%, 8.5% and 6.1%, respectively. In contrast, the present study found that oleic acid content was higher than that of the linoleic acid which was then followed by palmitic and stearic acids in that order. Murkovic et al. (1996) reported that the relative amount of oleic acid is always negatively correlated with the relative amount of linoleic acid. This is due to the precursor-product relationship of these two fatty acids. Younis et al., (2000) studied properties of seed and variability in fatty acid composition of seed oil in African *Cucurbita pepo* L., and found that content of oleic and palmitic acids in *C. pepo* extract decreased as the average growing temperature decreased. This confirms that increased linoleic acid content is followed by decreased oleic acid content (Murkovic et al., 1996; Younis et al., 2000).

Wide variations were found in kind and amount of fatty acids and this may be due to various factors including the harvest time, level of maturity, seasonal variation, drying conditions, variety, source, presence of seed coat in some seeds, and storage conditions.

Balasundram et al. (2006) found no relation between antioxidant value and total phenolic compound whereas the present study suggests an indirect relation between antioxidant proportion and total phenol compound. Hence, increasing antioxidant content in accessions caused decrease in total phenol content. Fruhwirth and Hermetter (2007) reported that Styrian pumpkin seed oils showed considerable differences in their antioxidant capacities and their seed extracts possess considerable antioxidative capacities. They also suggested the antioxidant capacity of Styrian pumpkin seed oil as an important criterion for quality control with respect to the stability and shelf life of this oil. Lelley et al., (2010) reported a very high level of antioxidative capacity in the extracts with non-polar solvent of the pumpkin seed which is of high nutritional value.

Total phenolic content in this study ranged from 15 to 44 mg GAE/kg of oil while in the study by Andjelkovic et al. (2010) the total phenolic content (TPC) measured in the pumpkin seed oil samples ranged from 24.71 to 50.93 mg GAE/kg of oil.

Conclusion

Pumpkin seed have lots of valuable nutrients that are benefit for human health. In this study Esfahan and Shahroud accessions revealed higher content unsaturated fatty acid. Furthermore Shahroud accessions with high seed wide and weight could be favor naked seed type for commercial production to improve seed yield. Among normal types Kerman accessions with more content of linoleic acid and antioxidant capacity was as valuable accession. Evaluated *Cucurbita* genetic resources could improve crop production and suggested suitable accessions for breeding program.

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