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Full Length Research Paper

Phenolic compounds, protein, lipid content and fatty acids compositions of cactus seeds

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Opuntia ficus indica **seeds harvested in three different years were investigated for total phenols, protein, total lipid content and fatty acids composition. Seeds were found to be rich in phenolic compounds (268.4 mg/100 g). Seeds contained also 6.0% of protein and 5.5% of oil. Unsaturated fatty acids accounted for the majority of the fatty acids (83.2%). Linoleic acid with 56.6% was the main fatty acid followed by oleic acid (20.1%). Significant differences were observed between total phenols, protein and total lipid content of seeds collected in three different harvested years, whereas no differences in fatty acid (FA) compositions were detected. The use of this plant species as a source of seed oil for dietary, industrial and pharmaceutical applications is discussed.**

Key words: Cactus (*Opuntia ficus indica*), seeds, phenolic compounds, storage protein, oil, fatty acids.

INTRODUCTION

In recent years consumers have tended to attach greater importance to the quality rather than the quantity of foodstuffs. Moreover, the promotion of products with certain specific characteristics can be of considerable benefit (Romero et al., 2003). Only a small portion of plant material is utilized directly for human consumption. Plant seeds are important sources of phytochemicals for nutritional, industrial, and pharmaceutical applications (Tlili et al., 2009). Moreover, the continued increase in world population and the ever-increasing demand for oils necessitates the need to investigate new sources of oils (Omode et al., 1995). For these reasons, new plant sources of phytochemicals, especially from underexploited seeds, have been investigated (Tlili et al., 2009).

Recently, there has been an increase in the number investigations into the possible use of vegetable oils as a of source of oleochemicals to supplement petrochemicals (Falade et al., 2008). Indeed, because the apparent relationship between the amounts or types of fatty acids consumed and the incidence of some disease has been noted, knowledge of the composition of edible fats has been considered to be essential (Kinsella, 1986). Several studies have shown the dietary importance of fatty acid composition of lipids. Recently, it was proven by clinical evidence that PUFAs are able to alleviate symptoms of certain diseases such as coronary heart disease, stroke and rheumatoid arthritis (Calder, 2008). Because of the noticeable importance of PUFAs in human health and nutrition, different means are used to increase the human consumption of PUFAs from different food sources such as direct intake as food additives and nutraceuticals as well as indirect consumption through the enrichment of PUFAs in important species in aquaculture (Lewis et al., 1999).

Accordingly, consumption of proteins, as a general class of macronutrients, is not normally associated with adverse effects. Absence or inadequacy of protein diet causes malnutrition, which is the most acute problem of the developing countries (Iqbal et al., 2006). A time has come to assess and explore the best possible measure to combat such an important issue (Gupta et al., 2010). Less reliance upon animal protein and increased

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consumption of plant protein by humans has been proposed as a partial solution (Anonymous, 1974). For these reasons, proteins from seeds of non-conventional plants, which are found in abundance, should be explored (Tlili et al., 2011).

Epidemiological studies show that many phytonutrients of fruits and vegetables may be beneficial in protecting the human body against damage by reactive oxygen and nitrogen species. Thus, it is considered important to increase the antioxidant intake in the human diet and one way of achieving this is by enriching food with antioxidants. Many authors reported a high values of natural antioxidants from many different seeds such as, sunflower (Kubicka et al., 1999), mango (Puravankara et al., 2000), grape (Jayaprakasha et al., 2001), canola (Naczk et al., 1998), and sesame (Shahidi et al., 1997). Phenols are a major group of antioxidant phytochemicals with interesting properties (Meot-Duros and Magné, 2009; Tlili et al., 2010).

Some authors suggested that the phytochemical content of some plants is influenced by numerous pre-harvest factors, including genotype, rootstock, climatic conditions, agronomic practices and harvesting time, but also by post-harvest factors, including storage conditions and processing procedures (Cevallos-Casals et al., 2006; Lee and Kader, 2000).

The aim of this work is to study the phenolic compounds, protein, lipid content and fatty acid composition of seeds of cactus (*Opuntia ficus indica*) and whether these compounds are affected or not when seeds were harvested in different years. Indeed, cactus belongs to the family Cactaceae. It is known as a multi-purpose plant since it can be used as natural wind break barrier, soil stabilizer; it can be cultured as crop for the production of fruits, vegetables and forage for human and animal; it can be used as raw-industrial material to produce several sub-products such as wine, candies, jellies and flour.

MATERIALS AND METHODS

Chemicals

All solvents used in the experiments were purchased from Fluka (Ridel-de Haën, Switzerland).

Plant material

Fruits of cactus were harvested in August 2007, 2008 and 2009 from Chebika in Kairouan (Tunisia). The fruit were hand-picked and then we separated the seeds for the analysis. All the analyses were performed in triplicate.

Phenolic compounds content

Samples (100 mg) were ground in a mortar and repeatedly extracted with 100% methanol (4.5 ml) until extracts were colorless. For the determination of total phenolic compounds an aliquot of the methanolic extract (0.1 ml) was mixed with 0.2 ml of Folin-Ciocalteu reagent, 2 ml of water, and 1 ml of 15% sodium carbonate, and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Rutin was used as the standard for the calibration curve, and the total phenolics were expressed as milligrams of rutin equivalents per gram of sample.

Protein contents

The protein content of the samples was determined according to Bradford (1976) method, using a Uvikon 930 spectrophotometer. Briefly, samples (0.1 g) were vigorously homogenized at 4°C in buffer containing 50 mM Tris–HCl, 200 mM NaCl, pH 8.5, and centrifuged (10,000 g for 20 min). To 50 µl of protein extract we added 50 µl of distilled water and 2 ml of Bradford reagent. The absorbance was measured at 595 nm. BSA (Sigma Aldrich) was used as the standard for the calibration curve (0 to 150 µg).

Oil content

The oil content was determined according to ISO method 659:1998 (ISO, 1999). About 5 g of the seeds was ground in a mortar and extracted with petroleum ether in a Soxhlet apparatus for 6 h. The solvent was concentrated on a rotary evaporator under reduced pressure at 60°C. The oil was dried by a stream of nitrogen and stored at -20°C until use.

Determination of fatty acid composition

Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography on a Hewlett Packard Model 5890 gas chromatograph (Palo Alto, CA, USA) using a CPSIL-88 column (100 m 9 0.25 mm i.d. film thickness 0.20 lm; Varian, Les Ulis, France) equipped with a flame ionization detector. Hydrogen was used as a carrier gas (inlet pressure, 210 kPa). The oven temperature was held at 60°C for 5 min, increased to 165°C at 15°C /min and held for 1 min, and then to 225°C at 2°C /min and finally held at 225°C for 17 min. The injector and the detector were maintained at 250 and 280°C, respectively. FAMEs were identified by comparison with commercial and synthetic standards. The data were processed using the EZChrom Elite software (Agilent Technologies, Massy, France).

Statistical analyses

All samples were analyzed in triplicate. Statistical analyses were performed using XLSTAT 2009. Data were expressed as mean ± SE using ANOVA. Differences at $p<0.05$ were considered statistically significant by Duncan's new multiple range test. The relationship between parameter was described as Pearson correlation matrix and r^2 coefficient. Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Phenolic compounds content

To our knowledge little study was carried out on total phenols in seeds of cactus. Results in (Table 1) show that seeds of cactus are rich in phenolic compounds. The content of total phenols (mg Rutin equivalent /100 g dry weight) is ca. 268 mg RE /100 g DW. Statistical analyses show significant differences between the total phenols

Table 1. Total phenols, protein and oil content of cactus seeds (*O. ficus-indica)*.

	2007	2008	2009	Mean
Weight of 100 seeds (g)	0.89 ± 0.09	1.42 ± 0.41	1.17 ± 0.09	1.16 ± 0.18
Total phenols (mg/100 g)	222.24 ± 0.075	254.79 ± 0.32	328.46 ± 0.04	268.45 ± 54.42
Protein (%)	3.45 ± 0.04	6.61 ± 0.15	8.10 ± 0.07	6.05 ± 0.23
Oil (%)	4.40 ± 1.05	6.85 ± 1.55	5.41 ± 0.86	5.55 ± 1.23

content of the three years which is in agreement with other authors (Romero et al., 2003). The values are ca. 222, ca. 254 and ca. 328 mg RE /100 g DW. Indeed, the biosynthesis of secondary metabolites, although controlled genetically, is affected strongly by environmental influences and climatic conditions (Yanivie and Palevitch, 1982).

Phenolic compounds are receiving increasing attention because of their health promoting effects, attributed to their antioxidant activity. Indeed, these compounds may have beneficial effects on human health (Meot-Duros and Magné, 2009; Tlili et al., 2010).

The high level of phenolic compounds makes seeds of *O. ficus indica* an excellent natural source of these compounds with a possible dietary, industrial and pharmaceutic utility.

Storage protein content

The approximate contents of the seed protein of O. ficus indica harvested in different years are shown in Table 1. The mean value (ca. 6%) show that cactus seeds contained a considerable level of proteins, but less than the protein content of cereals (ca. 10%) as suggested by Gueguen and Lemarie (1996) and more less than the legume seeds (ca. 25%) as suggested by Dulau and Thebaudin (1998). Indeed, Saénz (1995) suggested that cactus seeds are deficient in proteins.

Statistical analyses show significant differences between the total protein content between the three years. The values are ca. 3, 6 and 8%. Indeed, the storage content of plants varies with soil and climatic conditions (Breene et al., 2007). Due to the fact that the safety of proteins introduced into crops through genetic modification for humans or animals may not be known (Delaney et al., 2008). As cactus is widely spread in the five continents through the world, these proteins could be used as healthy ingredients in nutraceutical foods and can constitute a new food source for different population sectors.

Oil content and fatty acids composition

The oil level presented in Table 1 show that cactus seeds contained about 5.5%. The values are different between the three years and they are 4.4, 6.85 and 5.41% for 2007, 2008 and 2009, respectively. These differences might be due to climatic conditions as reported by other authors (Harwood, 1984; Breene et al., 2007) who suggested that environmental factors, such as light, temperature, and water stress, affect lipid levels.

Although the values seems low compared to other plants such as cotton seeds (Cherry and Leffler, 1984) pitaya seeds (Ariffin et al., 2009), or caper seeds (Tlili et al., 2009), but it encourage the use of this plant as a source of oil due the increase of the demand of new oils. The fatty acids were selected to examine any changes in oil composition as a function of year harvest. The total fatty acid composition is a parameter of quality of oils. Indeed, recently consumers have tended to attach more importance to the quality rather than the quantity of foodstuffs. Figure 1 show a typical chromatogram of fatty acid profile detected in cactus seeds. Fourteen fatty acids were identified (Table 2).

Extracted oils were mainly unsaturated (ca. 83%). This is in agreement with Ramadan and Morsel, (2003). The monounsaturated fatty acids (MUFAs) were ca. 26% and the polyunsaturated fatty acids (PUFAs) were ca. 57%, this is due to the fact that the major fatty acid was the linoleic fatty acid (ca. 56%). This gives an important value to this oil. Other parameters related with the nutritional aspects are the unsaturated/saturated ratio, which for cactus seed oil shows a mean value of 4.95 because of the high linoleic and oleic content.

Indeed, it was proven by clinical evidence that PUFAs are able to alleviate symptoms of certain diseases such as coronary heart disease, stroke and rheumatoid arthritis (Calder, 2008). Moreover, linoleic acid (as ω-6 fatty acid) can be transformed by the organism to a series of long-chain fatty acids, precursors of eicosanoids, a family of compounds with 20 carbons, including leucotriens and prostaglandins which have an important role at the vessel level and for blood coagulation. Also, it has beneficial properties for skin, and for this purpose it is used by the cosmetics products industry (Letawe et al., 1998; Darmstadt et al., 2002). Linolenic acid (as ω-3 fatty acid) was also detected in appreciable level (ca. 0.5%). Indeed, this compound has importance in the secondary prevention of coronary heart disease and in the prevention of cancer (Simopoulos, 1999). These results bring attention to the possible use of cactus seed oil as a natural source of PUFAs for nutritional, industrial or pharmaceutical purpose. Indeed, different means are

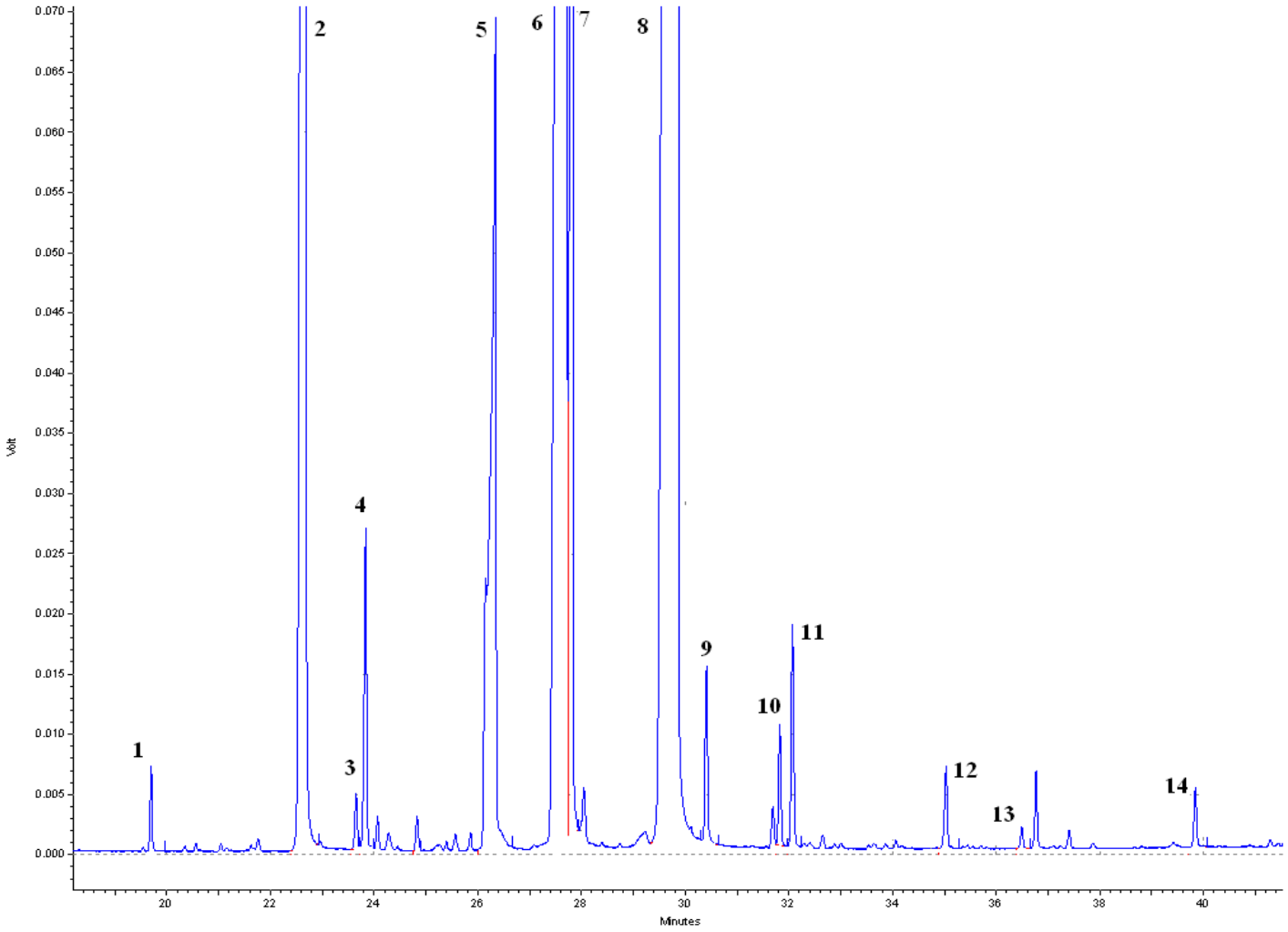

Figure 1. Typical chromatogram of fatty acids of seed oil from *O. ficus-indica*. Peaks: 1: 14:0; 2: 16:0; 3: 16:1n-9; 4: 16:1n-7; 5: 18:0; 6: 18:1n-9; 7: 18:1n-7; 8: 18:2n-6; 9: 20:0; 10: 20:1n-9; 11: 18:3n-3; 12: 22:0; 13: 22:1n-9; 14: 24:0.

used to increase, directly or indirectly, the human consumption of PUFAs (Lewis et al., 1999). Nutrition societies suggest a balanced ratio of n-6/n-3 may be necessary for optimal health, normal development and prevention of chronic disease (Simopoulos, 2002). Thus, the high ratios of this fatty acids found in our study suggest that limitations on the use of seed oil as a food ingredient should be noticed and other ingredients which could balance the ratio should be used at the same time.

Our results show that seeds of cactus are also rich with monounsaturated fatty acid (ca. 26%), and the major monounsaturated fatty acid is oleic acid. It is known that monounsaturated fatty acid has a fundamental role in many diseases such as cardiovascular prevention (Carrero et al., 2007). Results show also that the ration 18:1(n-7)/18:1(n-9) was ¼. This is in agreement with other plants (Hu et al., 1994). Moreover, Kleiman and Payne-Whal (1984) reported that many common vegetable oils contain small amounts of C18:1n-7 (1 to 15%). Cactus seed oil contain about 0.39% of very long-chain saturated fatty acids (VLCSFA) fatty acids with chain length exceeding 20 carbons, which are important constituents and intermediates of the cuticular surface layers of plant tissues (Gurr et al., 2002).

There were no significant changes in the oil composition between the different year yields. This is in agreement with other authors Romero et al., (2003). Moreover, the absence of difference between the different years gives great importance because of the effects on nutritional properties and oxidative stability of cactus seed oil.

Conclusions

This study demonstrated that cactus seeds could be considered as a source for natural phenolic antioxidants (ca. 268 mg/100 g). Proteins (ca. 6%) and lipid (ca. 5.5%)

Table 2. Fatty acids composition of cactus seeds (*O. ficus-indica)*.

Name	Code	R.T.	2007	2008	2009	Mean
14:0	1	19.696	0.15 ± 0.005	0.13 ± 0.005	0.15 ± 0.001	0.14 ± 0.01
16:0	2	22.641	12.63 ± 0.07	12.34 ± 0.04	11.76 ± 0.006	12.24 ± 0.44
16:1n-9	3	23.644	0.13 ± 0.001	0.11 ± 0.001	0.11 ± 0.001	0.12 ± 0.01
16:1n-7	4	23.829	0.67 ± 0.001	0.64 ± 0.001	0.64 ± 0.001	0.65 ± 0.01
18:0	5	26.262	3.52 ± 0.02	3.52 ± 0.03	4.05 ± 0.11	3.69 ± 0.3
18:1n-9	6	27.622	19.71 ± 0.06	19.18 ± 0.08	21.69 ± 0.16	20.19 ± 1.32
18:1n-7	7	27.756	5.15 ± 0.03	4.86 ± 0.06	4.47 ± 0.23	4.83 ± 0.34
18:2n-6	8	29.786	56.49 ± 0.07	57.87 ± 0.5	55.55 ± 0.02	56.63 ± 1.16
20:0	9	30.390	0.37 ± 0.05	0.33 ± 0.001	0.37 ± 0.006	0.36 ± 0.02
20:1n-9	10	31.809	0.24 ± 0.001	0.23 ± 0.001	0.25 ± 0.001	0.24 ± 0.01
18:3n-3	11	32.053	0.53 ± 0.001	0.44 ± 0.001	0.49 ± 0.001	0.47 ± 0.04
22:0	12	35.009	0.21 ± 0.001	0.19 ± 0.006	0.19 ± 0.005	0.19 ± 0.01
22:1n-9	13	36.482	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001	0.05 ± 0.001
24:0	14	39.828	0.16 ± 0.001	0.13 ± 0.001	0.14 ± 0.006	0.14 ± 0.01
∑ SFA			17.04 ± 0.09	16.63 ± 0.07	16.66 ± 0.12	16.78 ± 0.23
∑ MUFAs			25.95 ± 0.03	25.07 ± 0.03	27.21 ± 0.08	26.08 ± 1.07
∑ PUFAs			57.02 ± 0.07	58.31 ± 0.05	56.04 ± 0.02	57.12 ± 1.14
∑UFA			82.97 ± 0.09	83.38 ± 0.07	83.26 ± 0.11	83.20 ± 0.21
18:1n-7/18:1n-9			1/4	1/4	1/4	1/4
UFA/SFA			4.86	5.01	4.99	4.95
VLCSFA (>20c)			0.42	0.37	0.38	0.39

present, are relatively high. Results showed also that cactus seed oil is rich source of UFA (ca. 83%) for human health. Linoleic was the major fatty acid followed by oleic acid. The results also clearly indicate that there were no differences in FA compositions between the three collected years, while a notable difference was obtained for phenoilcs, proteins and lipids content. These results may offer a scientific basis for use of the seeds and oils both in human nutrition and some industrial and pharmaceutical products. Much research work will need to be conducted on the seed oil of this plant in order to determine the specific functions of cactus seeds.

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