Volatile constituents and polyphenol composition of *Opuntia ficus-indica* (L.) Mill from Morocco

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Abstract

The aim of this study is the extraction and the identification of volatile organic compounds (VOCs) and total phenolic compounds of three *Opuntia ficus-indica* (L.) Mill., species from Morocco, namely *Dellahia, Aissa* and *Shoul*. The VOCs were extracted with Solid-Phase Microextraction (SPME) associated to gas chromatography-mass spectrometry (GC-MS) analysis. The antioxidant compounds in extracts were determined by liquid- Chromatography-Mass Spectrometry (LC-MS). The study allowed the identification of forty-six compounds for the VOCs. The most abundant compounds in the three varieties (*Dellahia, Aissa* and *Shoul*) were 2-hexanal and n-hexanol with a percentage of 10.6%, 10.9% and 44.0% for the first compound and 10.3 %, 59.0%, 18.7% for the second, successively. The extraction of phenolic compounds permitted the identification and quantification of 15 fractions of flavonoids and phenolic acids. Caffeic acid was the most abundant phenolic acid with 16.0 and 10.8 mg/100g of plant material respectively for *Dellahia* and *Aissa*. For flavoinoids composition, isorhamnetin was the major compound. It accounted respectively for 40.5% and 43.2% for *Dellahia* and *Aissa*. We can conclude that cactus pear fruits are a good source of natural antioxidants and the major compounds responsible of flavor in the studied varieties are 2-hexanal and n-hexanol.

Keywords: *Opuntia ficus indica*, SPME, LC- MS, GC-MS, volatile organic compounds, antioxidant compounds.

Résumé

Le but de cette étude était l'extraction de composés organiques volatils (COVs) et les composés phénoliques totaux des variétés de figue barbarie «*Opuntia ficus indica* (L.) Mill» les plus répandues au Maroc à savoir *Dellahia, Aissa* et *Shoul*. Les COVs ont été extraits par microextraction sur phase solide (SPME) couplée à la chromatographie en phase gazeuse et à la spectrométrie de masse (GC-MS). Les composés phénoliques dans les extraits ont été étudiés par chromatographie en phase liquide couplée à la spectrométrie de masse (LC-MS). L'étude a permis l'identification de quarante-six composés pour les COVs. Les composés les plus abondants dans les trois variétés étudiées (*Dellahia, Aissa* et *Shoul*) sont le n-hexanol et le 2-hexanal avec respectivement un pourcentage de 10,6%; 10,9% et 44,0% pour le premier composé et 10,3%; 59%; 18,7% pour le deuxième. L'analyse des composés phénoliques a permis l'identification et la quantification de 15 fractions de flavonoïdes et acides phénoliques. L'acide caféique est l'acide phénolique le plus abondant avec 16,0 et 10,8 mg/100 g de matériel végétal respectivement pour *Dellahia* et *Aïssa*. Pour les flavonoïdes; isorhamnetin était le principal composé, il représentait respectivement 40,5% et 43,2% pour *Dellahia* et *Aïssa*. Nous pouvons conclure que les fruits de figues de barbarie constituent une bonne source naturelle d'antioxydants naturels et par conséquent leurs bienfaits sur la santé sont inestimables.

Mots-clés: *Opuntia Ficus Indica*, SPME, LC- MS, GC-MS, composés organiques volatils, composés phénoliques

INTRODUCTION

The *Opuntia* cactus is a xerophyte of about 200 to 300 species and grows mainly in arid and semiarid zones. Due to their remarkable genetic variability, *Opuntia* plants show a high ecological adaptivity and can therefore be encountered in places of virtually all climatic conditions (Moßhammer et *al.,* 2006). The Opuntia *ficus-indica* L. is the most cultivated species of the genera. Several authors confirm that the cactus is native to Mexico, which is the main world producer. The species is also widespread in Mediterranean area, South Africa and central-South America (Inglese et *al*., 2002).

The fruit is a many-seeded berry with a thick peel, enclosing a delicately flavoured seedy pulp. The flavours of selected cactus pear fruit varieties resemble that of strawberry, watermelon, honeydew melon, fig, banana or citrus (Savio, 1987). Cactus pear fruits are a source of nutrients and vitamins (Sawaya et *al*., 1983; Teles et *al*.,1984) and are eaten fresh, dried or preserved in jams, syrups or processed into candy-like products (Hoffman, 1980).

Scientific studies have indicated that several parts of *O. ficus-indica* have diuretic and antigotous effects (Galati et *al*., 2002**)**.

The cladodes are utilized to reduce serum cholesterol level and blood pressure, for treatment of ulcers, rheumatic pain, wounds, fatigue, capillary fragility, and liver conditions (Agozzino et *al*., 2005). In addition, a recent study showed the potential antigenotoxic activities of cactus cladodes against the mycotoxin zearalenone, a potent estrogenic metabolite (Zorgui et *al*., 2009).

The seeds contained in the pulp produce oil that is composed mainly of fatty acids, sterols and vitamins (Ghazi et *al*., 2013).

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Both cladodes and fruits contain a wide range of secondary metabolites presenting health-promoting activities (Munoz-de-Chavez et *al*., 1995). Among the minor compounds of *O. ficus-indica*, the most important are polyphenols (Khatabi et *al*., 2011**)** that may act as antioxidants against oxidative damage.

Phenolic compounds are strongly correlated to the antioxidant activity (Cotelle, 2001**)** and several reports showed a linear correlation between antioxidant activity and polyphenol content (Elhadi Yahia et *al*., 2011; Khatabi et *al*., 2011; Kuti, 2004), therefore the knowledge of the polyphenolic composition in the food is of great interest.

The aroma profile in plant is influenced by environment, variety and other factors such as growing geographical location or time of collection (Arena et *al*., 2001).

In the last years, the analysis of volatile organic compounds (VOCs) is becoming of great interest; it is well known that flavor of food is related to the volatile compounds perception by the nose. In addition, plant VOCs play a key role in the growing of the plants by interactions with other plants or they can be produced as a response against some pathogen or insect pests (Loi et *al*., 2008).

For the relevant assessment of *O. ficus-indica* quality, main compounds responsible for the aroma must be known and an appropriate analytical method is needed. Analysis of food aroma could be approached by different techniques (solvent extraction, distillation, solid-phase microextraction (SPME), headspace techniques, adsorption traps) each of them having its inherent problems and possible bias (Belitz et *al*., 2004; Hofmann et *al*., 2005; Kaiser, 2006; Stephan et *al*., 2000). However, SPME coupled with gas chromatography-mass spectrometry (GC-MS) and comparison of spectra and retention indices of reference compounds, stays the best technique for the analysis of the volatile compounds (Molyneux, 2007; Zhou et *al*., 1999).

To our knowledge, this study is the first conducted to analyze volatile organic compounds in Moroccan prickly pear.

Therefore, the objectives of this study are the identification of the chemical composition of volatile fraction and quantification of polyphenols of three cultivars of *O. ficus-indica* from Morocco, namely *Dellahia*, a white cultivar, *Aissa*, a red cultivar and *Shoul*, a yellow cultivar.

MATERIAL AND METHODS

Plant material

Ripe fruits of three important cultivars of Morocco; *Aissa* from Sidi Ifni (South of Morocco), *Dellahia* from El-Hoceima (North of Morocco) and *Shoul* (in the central region) were collected during the month of September.

Harvesting of fruits from wild plant was done by done by simple random sampling; the spines were removed manually using brushes. They were immediately transported and stored in a freezer.

Extraction and identification of volatile organic compounds

SPME is based on the adsorption of volatile compounds present in the head space of food on a thin fiber covering the removable internal needle of a microsyringe. The adsorbed vapors are then desorbed by direct introduction of the microsyringe into the injector of a gas chromatograph (Arena et *al*., 2001).

SPME analysis: A 100 µm PDMS/DVB/CAR coated fiber was conditioned prior to use according to manufacturer instructions. The conditioned fiber was injected through the septum, and suspended in the headspace of a 40 mL glass vial containing 5 g of homogenized whole fruit (pulp, seeds and skin), to which was added 5mL of saturated aqueous NaCl solution. The fiber was exposed for extracting volatiles for 60 min at 40 °C under stirring. The fiber was then retracted and removed from the vial, and placed immediately into the injector of the GC. Thermal desorption was performed into the injector at a temperature of 250 °C for 5 min in splitless injection mode.

An aliquot of 2 µL was injected into the GC–MS equipment and analyzed under the same conditions mentioned before to determine the retention time of each n-alkane necessary for the Kovats indexes calculations (Elhadi Yahia and Mondragon-Jacobo, 2011).

The GC-MS analysis was carried out using a Hewlett Packard 5890 GC, equipped with a Hewlett Packard 5971 MS system (Palo Alto, USA) operating in the EI mode at 70 eV, using the column HP5.

Identification of the individual components was performed by comparison with the co-injected pure compounds or by matching the MS fragmentation patterns and retention indices with the built in libraries or literature data and commercial mass spectral libraries (NIST/EPA/NIH 1999).

The formula used to obtain Kovats indexes was:

$$I = 100n + 100z (\log t RA - \log t Rn) / (\log t RN - \log t Rn)$$

Where I is the Kovats index, A is the unknown compound, n is the number of carbon atoms in the smaller n-alkane, N is the number of carbon atoms in the larger n-alkane, z is the difference in the carbon atoms in the smaller and larger n-alkanes, and tR is the retention time.

Extraction and determination of phenolic compounds by LC-MS

The phenolic fraction of each *O. ficus-indica* (pulp) sample was extracted with a solution composed of ethanol:water 70:30 (v/v); using a ratio of sample to solvent 1:25 w/v at room temperature overnight. The resulting extract was then filtered through paper filter and the solvent was evaporated using a rotary evaporator under vacuum at 40°C.

LC-MS analysis conditions: Full scan ESI-MS and collision induced dissociation (CID) ESI-MS/MS analyses of samples were performed on an ABSciex API2000 spectrometer. The analytical parameters were optimized (declustering potential -22 eV, focusing potential -316 eV, and entrance potential -6 eV) by infusing a standard solution of chlorogenic acid (1 mg/ ml in methanol 50%) into the source at a flow rate of 10 mL/min. Data were acquired in the negative ion MS and MS/MS modes.

RESULTS AND DISCUSSION

Identification of volatile organic compounds

The analysis of VOCs of Moroccan cactus fruits using SPME- GC-MS technique resulted in the identification of forty-six compounds (Table 1). The identified molecules represented 84.8% of the whole volatiles for *Dellahia*, 92.2 % for *Aissa* and 92.1 % for *Shoul* varieties. These compounds were tentatively identified by comparison of retention index and MS fragmentation pattern both with pure standard coinjection and, when pure standard was not available, by comparison with built in libraries and literature data.

According to the literature, sixteen volatile compounds were found in Italian fruits of *O. ficus*-indica (Arena et *al*., 2001) and 61 in Mexican fruits of *O. ficus-indica* (Robert and Takahashi, 1978), representing successively 100% and 95% of whole volatiles in fruits.

The comparison between the chemical compositions of the VOCs using SPME- GC-MS of the three cultivars showed that *Shoul* cactus differs from *Dellahia* and *Aissa*. Indeed, the aroma profile of *Shoul* cultivar differed both qualitatively and quantitatively with respect to *Dellahia* and *Aissa* cactus.

Many terpenes derivatives have been found in the analyzed samples like camphene. Results showed that it represented 5.3% and 0.6% of the aroma profile of *Dellahia* and *Aissa*, respectively, while it was not detected in *Shoul.*

The most abundant compound in *Aissa* cultivars was (2E)-nonenal with a percentage of 16.7, while it was absent in Shoul variety and it exists in limited quantity in *Dellahia* with a 4.8%.

The n-hexanol represented also an important percentage in different cultivars analyzed with respectively 10.3 %, 18.7 % and 5.9 % for *Dellahia*, *Shoul* and *Aissa*. According to studies conducted by Arena et *al.,* (2001), this compound exists in larger amount in Italian fruit (26.4%) (Table 2).

One of the aldehyde responsible for the aroma profile of citrus was Decanal [a], this compound exists in trace amounts in fruits of *Opuntia ficus indica* with 0.2%, 0.6% and 0.3% for successively *Dellahia*, *Aissa* and *Shoul*, whereas, it was not detected in Italian fruit (Arena et *al*., 2001).

For nonanal, it was more present in *Aissa* (2.8%) followed by *Dellahia* (1.4%) and *Shoul* (1.1%), while it was not detected in Italian fruit (Arena et *al*., 2001).

E-2 hexen-1-ol was the major compound in Italian fruit (56.8%) (Arena et *al*., 2001), however, this compound was detected in its stereoisomers form (2 hexen-1-ol Z) in Moroccan fruit in small amounts (4.3%).

According to the literature, our results were in agreement with those reported by Robert and Takahashi (1978). These researchers found that the alcohols were the major class of compound represented with trans-2-Hexen-1-ol and n-hexanol even though numerous esters and carbonyl compounds are also present at low concentrations. However, Ammar et *al.,* (2012) analyzed two species of prickly pear flowers *Opuntia ficus indica* (L.) Mill. and *Opuntia stricta* (Haw.) and concluded that carboxylic acid was the main compound (28-97%), followed by terpenes (0.2-57%), esters (0.2-27%) and alcohols (<1.8%).

Another study was conducted by Bergaoui et *al.,* (2007) on cladodes, flowers and fruits of *Opuntia lindheimeri var. linguiformis* L. Benson from Tunisia. The results showed that the saturated fatty acid were the most important compounds in the three organs represented by tetradecanoic acid (3.2-13.6%) and hexadecanoic acid (8.5-17.3%). The authors have identified also butyl tetradecanoate (8.1-21.5%) and (E)-3-butyldiene phthalide (6.9-15.8%).

Determination of phenolic compounds

In order to investigate the occurrence of phenolic compounds, the qualitative analysis of *Opuntia ficus-indica* was performed by LC-MS allowing to identify 15 compounds belonging to two families as shown in Figure 1 and 2: flavonoids (kaempferol, isorhamnetin, quercetin, rutin) and phenolic acids (quinic acid, caffeic acid, acetylcaffeic acid, caffeic acid derivative, chlorogenic acid, caffeoylquinic acid, dicaffeoylquinic acid, rosmarinic acid, p-coumaric acid, ferulic acid and syringic acid).

The amount of the identified compounds was determined in different cactus pear cultivars using a rapid and sensitive LC-MS/MS (MRM) method.

According to figure 1, the overall polyphenolic content in *Dellahia* was higher than that of *Aissa*.

In terms of phenolic compounds identified, the most abundant was caffeic acid with respectively 16.0 and 10.8 mg/100g of plant material for *Dellahia* and *Aissa*.

In a study performed by Albano et *al.,* (2015) on Italian prickly pear fruit, they found that the total phenol content was 89.2 mg GAE (Gallic Acid Equivalents)/100 g of fresh weight in purple fruits and 69.8 mg GAE /100 g of fresh weight in orange fruit. These values were similar to those found in prickly pear cultivars from Saudi Arabia, California, and Tunisia (Abdel-Hameed et *al*., 2014; Stintzing et *al*.,2005; Yeddes et *al*., 2013) and lower than that reported by El Mostapha et *al.,* (2014). Fernandez-Lopez et *al*., (2010) reported a much higher phenolic content but it referred to a whole (skin and pulp) red-skinned fruit.

The total phenols of some prickly pear analyzed are similar to clarified apple or white grape juices (Mullen et *al*., 2007). Other juices extracted from cranberries, pomegranate, tropical fruits, purple grape, and apple contained more than three times the total phenolics determined in the set of prickly pear juices analyzed (Butera et *al*., 2002).

In terms of flavonoid composition, isorhamnetin derivatives was the major component, it accounted for 40.5 % (0.8 mg/100g of plant material), quercetin derivatives for 33.1%, kaempferol derivatives for 17.3 % and rutin derivatives accounted for 8.9% of total flavonoids in *Dellahia*.

For *Aissa* cultivar, isorhamnetin derivatives accounted for 43.2 % (0.4 mg/100 g of plant material), quercetin derivatives for 21.6%, rutin derivatives for 18.5% and kaempferol derivatives accounted for 16.4 % of total flavonoids. These values were lower than those reported by El Mostapha et *al.,* (2014) (4.94; 9.00 and 0.78 mg/100 g of plant material in of isorhamnetin, quercetin and kaempferol, successively).

Kuti (2004) analyzed different types of prickly pears and concluded that purple skinned fruits contained the highest amounts of flavonoids.

Almost half of flavonoid compounds is composed of isorhamnetin for both studied varieties with 40.5 % and 43.2%, for *Dellahia* and *Aissa*, successively. These values are higher than those found by Kuti (2004) in American prickly pear fruit: the isorhamnetin was about 34.6 % for green skinned and 2.0 %, for purple skinned, while it was not detectable in red and yellow skinned. However, the total flavonoids were composed of about 96.8% quercetin (90.5 ± 11.5 mg/g fresh weight) in purple-skinned, 93.1% quercetin (51.0 ± 4.6 mg/g fresh weight) in red-skinned, and 100% quercetin (9.8 ± 3.0 mg/g fresh weight) in yellow-skinned cactus pear fruits, respectively.

It appears that *Opuntia ficus indica* contains flavonoids common to other fruits and vegetables (Peterson and Dwyer, 1998). However, the flavonoid types and contents, as in other fruits and vegetables, vary with the cultivars (Bilyk and Sapers, 1986).

One of the more interesting findings in this study regarding the phytochemical content of Moroccan prickly pear is that they had relatively high content of phenolic acids (e.g. caffeic acid) and flavonols (e.g. isorhamnetin).

The isorhamnetin and caffeic acid which showed the highest contents in this study have a lot of health benefits; the first compound may reduce the risk of cancer, improve heart health and ease diabetes complications [b], and the second is able to protect cells against damage caused by free radicals (Chung et *al*., 2006).

Ndhlala et *al.,* (2007) analyzed the total phenolics and flavonoids of ethanol extracts of prickly pears from Zimbabwe belonging to *Opuntia megacantha* and found that the pulp contained approximately 180 µg/g GAE (gallic acid equivalents) and 10 µg/g cathecin. However, Chang et *al*., (2008) reported that they found 915 µg/g GAE and 292 µg quercetin/g in the *Opuntia dillenii* fruits extracts from Taiwan.

CONCLUSION

The chemical composition of Moroccan prickly pear was determined for the first time by SPME coupled with GC-MS. Major compounds responsible of flavor in the variety studied of *Opuntia Ficus Indica* are 2-Hexanal and n-hexanol.

For phenolic compounds, this investigation showed the potential value of cactus pear fruits as a good source of natural antioxidants and that consumption of cactus pear

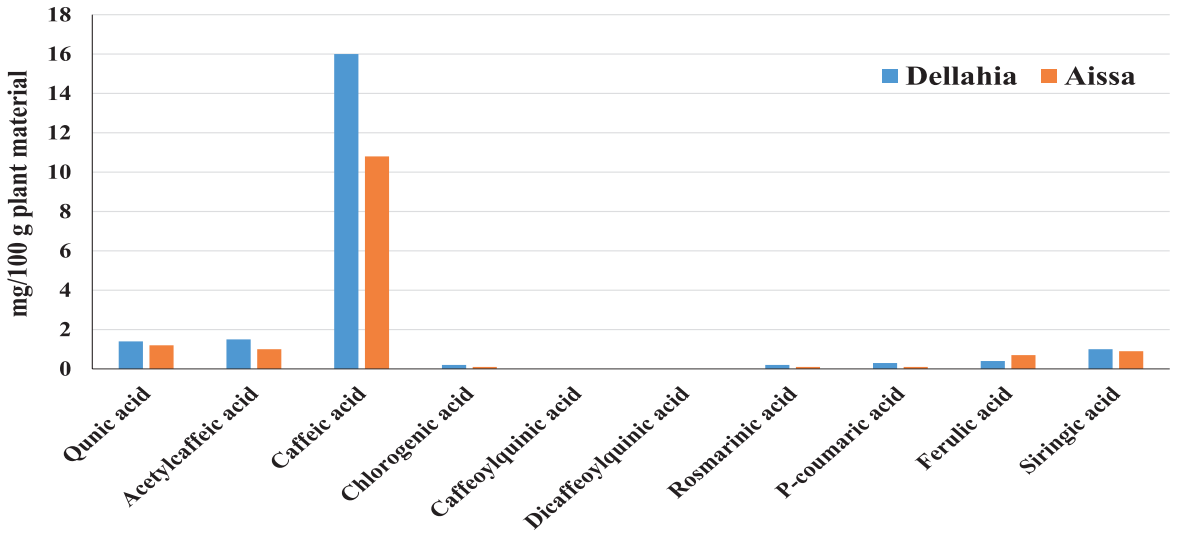

Figure 1: Composition of phenolic compounds for Moroccan prickly pear

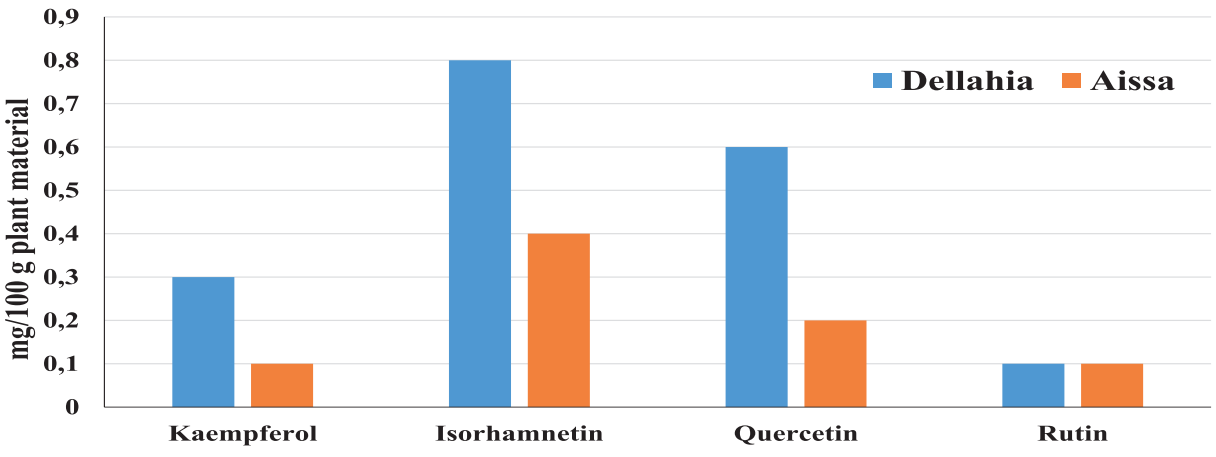

Figure 2: Composition of flavonoid compounds for Moroccan prickly pear

fruit may contribute substantial amounts of antioxidants to the diet. Based on the available data in this study and the phytochemical contents of cactus pear fruits, there is a high likelihood that cactus pear fruits may provide the types of nutritional and health benefit associated with consumption of fruits and vegetables in general.

Table 1: Percentage of VOCs of Moroccan prickly pear

Name	R.I	% *Dellahia*	% *Aissa*	% *Shoul*
Hexanal	800	0.7	2.3	2.3
2-hexanal	848	10.6	10.9	44
(Z)-2-hexen-1-ol	863	10.8	3.6	nd
n-hexanol	865	**10.3**	5.91	18.7
3,5 hexadien-1-ol Z	887	trace	trace	nd
styrene	891	0.3	trace	nd
2,4- hexadienal (E,E)	912	0.3	trace	nd
β pinene	934	0.2	trace	nd
camphene	948	**5.3**	**0.6**	**nd**
4-hexen-3-one 5 methyl	955	0.2	0.2	0.9
2,3 pentadiene	965	trace	trace	nd
1-heptanol	969	0.1	trace	nd
1-octen 3-ol	979	0.4	trace	nd
3-octanone,2-methyl	983	0.1	trace	nd
2-decanynoic acid	986	0.2	trace	nd
Furan,2-pentyl	992	1.8	1.4	0.8
Octanal	1003	trace	0.8	1.5
α phllandrene	1006	0.1	trace	nd
(E) 3-hexenyl acetate	1013	0.6	3.5	2.4
3-hexenil catetate	1016	0.4	5.7	11.1
ρ –cymene	1025	1.4	0.5	1.2
Limonene	1029	3.9	0.8	2.3
1,8-cineole	1032	0.6	0.4	nd
βocimène	1049	0.6	0.6	nd
2-octenal E	1058	0.3	0.9	nd
3-carene	1060	0.8	trace	nd
γ terpinene	1069	0.5	trace	1.5
1-octanol	1071	1	0.8	0.2
Methyl benzoate	1096	0.2	0.3	nd
Linalool	1100	3.8	3	0.1
Nonanal	1104	**1.4**	**2.8**	**1.1**
Methyl octanoate	1125	trace	0.1	0.9
2,6 nona dienal, E,Z	1154	2.3	14.4	nd
(2E)-nonenal	1160	**4.8**	**16.7**	**nd**
Trans cis-2,6nonadien-1-ol	1167	1.9	3.7	nd
2-nonen-1-ol E	1169	3.9	7.5	trace
Nonen-1-ol,E	1171	2.7	trace	nd
Decanal 4-E	1194	trace	0.4	0.5
Decanal	1206	**0.2**	**0.6**	**0.3**
5-hexen-3yn-2ol,2-methyl	1239	trace	trace	nd
4-hexen-1-ol,2,ethynyl-2,5 dimethyl	1256	0.3	trace	nd
Methyl-4-decenoate	1310	7.2	1.8	1.5
Methyl decanoate	1325	1.5	0.4	nd
Ethyl 4 decenoate	1381	0.8	trace	nd
α farnesene	1510	1	trace	nd
Total identified	-	**84.8**	**92.2**	**92.1**

Table 2: Comparison of VOCs of Moroccan and Italian Cactus prickly pear cultivars

Origin	% n-hexanol	% Nonanal	% Decanal	References
Italian fruit	26.4	Not detected	Not detected	Arena et *al.,* (2001)
Dellahia	10.3	1.4	0.2	This study
Aissa	5.9	2.8	0.6	This study
Shoul	18.7	1.1	0.3	This study

REFERENCES

Abdel-Hameed E.-S.S., Nagaty M.A., Salman M.S., Bazaid S.A. (2014). Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (*Opuntia ficus-indica* Mill.) growing in Taif, KSA. *Food Chem*. 160: 31–38.

Agozzino P., Avellone G., Caraulo L., Ferrugia M., Flizzola F. (2005). Volatile profile of sicilian prickly pear (*Opuntia ficus-indica*) by SPME-GC/MS analysis. *Italian Journal of Food Science* 17: 341–348.

Albano C., Negro C.,Tommasi N., Gerardi C., Mita G., Miceli A., De Bellis L., Blando F. (2015). Betalains, Phenols and antioxidant capacity in cactus pear [*Opuntia ficus-indica* (L.) Mill.] fruits from Apulia (South Italy) genotypes. *Antioxidants* 4: 269-280

Ammar I., Ennouri M., Khemakhem B., Yangui T., Hamadi A.(2012). Variation in chemical composition and biological activities of two species of *Opuntia* flowers at four stages of flowering. *Industrial Crops and Products* 37: 34–40.

Arena E., Campisi S., Fallico B., Lanza M.C., Maccarone E. (2001). Aroma value of volatile compounds of prickly pear (*Opuntia ficus indica* (L.) Mill. *Cactaceae*). *Italian Journal of Food Science* 13: 311-319

Belitz H.-D., Grosch W., Schieberle P. (2004). Aroma compounds. In *Food chemistr*y (3rd ed., pp. 342–408). Berlin: Springer.

Bergaoui A., Boughalleb N., Ben Jannet H., Harzallah-Shiric F., El Mahjoub M, Mighri Z.(2007). Chemical composition and antifungal activity of volatiles from three *Opuntia* species growing in Tunisia. *Pakistan Journal of Biological Sciences* 10: 485-2489.

Bilyk A., Sapers G. M. (1986). Varietal differences in the quercetin, kaempferol, and myricetin contents of highbush blueberry, cranberry, and thornless blackberry fruits. *Journal of Agricultural & Food Chemistry* 34: 585–588.

Butera D., Tesoriere L., Di Gaudio F., Bongiorno A., Allegra M., Pintaudi A.M., Kohen R., Livrea M.A. (2002). Antioxidant activities of Sicilian prickly pears (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. *J Agric Food Chem.* 50: 6895–6901

Chang S. F., Hsieh C.L., Yen G.C. (2008). The protective effect of *Opuntia dillenii* Haw fruit against low-density lipoprotein peroxidation and its active compounds. *Food Chem.* 106: 569–575.

Chavez-Santoscoy R.A., Gutierrez-Uribe J.A., Serna-Saldívar S.O. (2009). Phenolic composition, antioxidant capacity and *in vitro* cancer cell cytotoxicity of nine prickly pear (*Opuntia spp.)* Juices. *Plant Foods Hum Nutr*. 64 :146–152.

Chung M. J., Walker P. A, Hogstrand C. (2006). Dietary phenolic antioxidants, caffeic acid and Trolox, protect rainbow trout gill cells from nitric oxide-induced apoptosis. *Aquatic Toxicology* 80: 321-328.

Cotelle N. (2001). Role of flavonoids in oxidative stress. *Current Topics in Medicinal Chemistry*, 1: 569–590.

Elhadi Yahia M., Mondragon-Jacobo C. (2011). Nutritional components and anti-oxydant capacity of ten cultivars and lines of cactus pear fruit (*opuntia spp*.), *Food Research International* 44: 2311–2318.

El-Mostafa K., El Kharrassi Y., Badreddine A., Andreoletti P., Vamecq J., El Kebbaj M.S., Latruffe N., Lizard G., Nasser B., Cherkaoui-Malki M. (2014). Nopal cactus (*Opuntia ficus-indica*) as a source of bioactive compounds for nutrition, health and disease. *Molecules* 19: 14879- 14900.

Fernandez-Lopez J.A., Almela, L., Obón J.M., Castellar M.R. (2010). Determination of antioxidant constituents in cactus pear fruits. *Plant Foods Hum. Nutr*. 65: 253–259.

Galati E. M., Monforte M. T., Tripodo M. M., d'Aquino A., Mondell, M. R. (2001). Antiulcer activity of *Opuntia ficus-indica* (L.) Mill. (*Cactaceae*): Ultra-structural study. *Journal of Ethnopharmacology* 76: 1–9

Galati E. M., Tripodo M. M., Trovato A., Miceli N., Monforte M. T. (2002). Biological effect of *Opuntia ficus indica* (L.) Mill. (Cactaceae) waste matter. *Journal of Ethnopharmacology* 79: 17–21.

Ghazi Z., Ramdani M., Fauconnier M. L., El Mahi B., Cheikh R. (2013). Fatty acid Sterols and vitamin E composition of seed oil of *Opuntia ficus indica* and *opuntia Dillenii* from Morocco. *Journal of Materials and Environmental Science* 4-6: 967-972.

Hoffman W. (1980). The many uses of prickly pears (*Opuntia Mill*.) in Peru and Mexico. *Plant Resources & Development* 12: 58–68

Hofmann T., Rothe M., Schieberle P. (2005). State-of-the-art in flavour chemistry and biology. In *Proceedings of the 7th wartburg symposium*. Eisenach: Deutsche Forschungsanstalt für Lebensmittelchemie.

Inglese P., Basile F., Schirra M. (2002). Cactus pear fruit production In P. S. Nobel (Ed.). *Cacti biology and uses* 163-183.

Kaiser R. (2006). Introduction to the world of natural scents. In *Meaningful scents around the world* (pp. 1–14). Zürich: Verlag Helvetica Chimica Acta.

Khatabi O., Hanine H., Elothmani D., Hasib A. (2011). Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly fruits (*Opuntia ficus indica*). *Arabian Journal of Chemistry*.

Kuti J.O. (2004). Antioxidant compounds from four *Opuntia* cactus pear varieties. *Food Chemistry* 85: 527-533.

Liu J., Tang X., Zhang Y., Zhao W. (2012). Determination of the Volatile Composition in Brown Millet, Milled Millet and Millet Bran by Gas Chromatography/ Mass Spectrometry. *Molecules* 17: 2271- 2282.

Loi R.X., Solar M.C., Weidenhamer J.D.(2008). *Journal of Chemical Ecology* 34,70-75.

Loro J.F., Del Rio I., Pérez-Santana L. (1999). Preliminary studies of analgesic and anti-inflammatory properties of *Opuntia dillenii* aqueous extract. *J. Ethnopharmacol.* 67: 213-218

Moßhammer M.R., Stintzing F.C., Carle R. (2006). Cactus Pear Fruits (Opuntia spp.): A Review of Processing Technologies and Current Uses. *Journal of the Professional Association for Cactus Development* 8:1-25.

Molyneux R. J., chieberle P. (2007). Compound identification: A journal of agricultural and food chemistry perspective. *Journal of Agricultural and Food Chemistry* 55: 4625–4629.

Mullen W., Marks S.C., Crozier A. (2007). Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. *J Agric Food Chem.* 55:3148–3157.

Munoz-de-Chavez M., Chavez A., Valles V., Roldan J.A. (1995). Plants in human nutrition. *World Review of Nutrition and Dietetics* 77, 109–134.

Ndhlala A. R., Kasiyamhuru A., Mupure C., Chitindingu K., Benhura M., Muchuweti M. (2007). Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. *Food Chem*.103: 82–87.

Nicolosi A., Smundo C., Campisi S., Cataldi Lupo C., Fallico B., Maccarone E. (1995). Microestrazione in fas solida di component volatile dell aroma. Atti Del 2 congresso Nazionale di Chimica degli alimenti, 757. LA Grafica Ed. Messina.

Park E. H., Kahng J. H., Lee S. H., Shin K. H. (2001). An anti-inflammatory principle from cactus. *Fitoterapia,* 72: 288–290.

Peterson J., Dwyer, J. (1998). Taxonomic classification helps identify flavonoid-containing foods on a semiquantitative food frequency questionnaire. *Journal of American Dietetic Association* 98 :677–682, 685.

Robert A. F, Takahashi J.M. (1978). Volatile Constituents of Prickly Pear (*Opuntia ficus indica* Mill., de Castilla Variety). *J. Agric. Food Chem*., 26: 835-837.

Rychlik M., Schieberle P., Grosch W. (1998). Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants. *Lichtenbergstraße*, Germany.

Savio Y. (1987). Prickly pear cactus. *Cactus and Succulent Journal (USA)*, 59: 113–117.

Sawaya W. N., Khatchadourin H., Safi W., Al-Muhammed H. M. (1983). Chemical characterization of prickly pear pulp, *Opuntia ficus-indica* and manufacturing of prickly pear jam. *Journal of Food Technology*, 18:183–193.

Sjövall O., Lapveteläinen A., Johansson A., Kallio H. (1997). Analysis of volatiles formed during oxidation of extruded oats. *J. Agric. Food Chem*. 45: 4452-4455.

Stephan A., Bücking M., Steinhart H. (2000). Novel analytical tools for food flavours – review. *Food Research International* 33: 199–209.

Stintzing F.C., Herbach K.M., Mosshammer M.R., Carle R., Yi W.,Sellappan S., Akoh C., Bunch R., Felker P. (2005). Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. *J. Agric. Food Chem*. 53: 442–451.

Teles F., Stull J., Brown W., Whitting F. (1984). Amino and organic acids of prickly pear cactus (*Opuntia ficus-indica L.*). *Journal of the Science of Food and Agriculture* 35: 421–425.

Yang X., Pepparad T. (1994).Solid phase microextraction for flavor analysis. *Journal of Agricultural and Food Chemistry* 42,1925.

Yeddes N., Cherif J.K., Guyot S., Sotin H., Ayadi M.T. (2013). Comparative study of antioxidant power, polyphenols, flavonoids and betacyanins of the peel and pulp of three Tunisian *Opuntia* forms. *Antioxidants* 2: 37–51.

Zhou M., Robards K., Glennie-Holmes M., Helliwell, S. (1999). Analysis of volatile compounds and their contribution to flavor in cereals. *Journal of Agricultural and Food Chemistry* 47: 3941–3953.

Zorgui L., Imen A.-B., Yosra A., Hassen B., Wafa H. (2009). The antigenotoxic,activities of cactus (*Opuntia ficus-indica*) cladodes against the mycotoxin zearalenone in Balb/c mice: prevention of micronuclei, chromosome aberrations and DNA fragmentation. *Food and Chemical Toxicology* 47: 662–667.

[a]URL: https://en.wikipedia.org/wiki/Decanal. Accessed; 23.12.2014.

[b]URL:http://www.phytochemicals.info/phytochemicals/isorhamnetin.php. Accessed; 23.12.2014.