

Fruit Characteristics and Chemical Proprieties of Juice and Seeds of Three *Opuntia ficus indica* Cultivars

صفات الفاكهة والخصائص الكيميائية لعصير و بذور ثلاثة أنواع صبار

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Abstract: Morphological parameter and biochemical proprieties of three Tunisian *Opuntia ficus indica* cultivars (Thorny, Gialla and Rossa) were investigated. Large variations were observed between the mean values of the different cactus cultivars. The source of this variation is probably the genetic without excluding the geographic effects. Gialla and Rossa seems more preferment especially for the consumers. Moreover, seeds of cactus are rich in oil, phytosterols and proteins. The data obtained in this study will be important as an indication of the potentially nutraceutical, economic and industrial utility of seeds of cactus fruit as a new source of oils and proteins. **Keywords:** Cactus (*Opuntia ficus-indica*); Fruit; Juice; Acidity; Sugar; Seeds; Lipids; Unsaponifiables; Sterols; Proteins.

المستخلص: يهدف هذا البحث إلى دراسة بعض الخصائص المورفولوجية والبيوكيميائية لثلاث أصناف من الصبار. أظهرت النتائج تباينا كبيرا بين الأنواع الثلاثة. هذا التباين ربما مرده إلى الاختلاف الوراثي من غير أن نهمل العامل الجغرافي. بينت النتائج أيضا أن بذور نبتة الصبار غنية أيضا بالزيوت، الفيتوستيرول و البروتينات. إن البيانات التي تم الحصول عليها في هذه الدراسة تعتبر مؤشرا مهما لبذور الصبار كمصدر جديد من الزيوت والبروتينات. **كلمات مدخلية:** الصبار، فاكهة، عصير، حامضية، سكريات، بذور، دهنيات، غير قابل للتصبن، زيوت، بروتينات.

INTRODUCTION

Cactus belongs to the family *Cactaceae*. The family *Cactaceae* contains about 130 genera and the prickly pear cactus (*Opuntia ficus-indica*) belongs to the genus *Opuntia* (Russell and Felker, 1987). It is widely spread in the five continents throughout the world. Species of *Opuntia* (cactus pear) grow in all parts of the American continent,

from Canada to Patagonia and in the course of time have been cultivated in different areas of Europe, particularly in the Mediterranean countries, as well as in Africa and Australia (Le Houérou, 1996, Lahsasni *et al.*, 2004). It is known as a multi-purpose plant since it can be used as natural wind break barrier, soil stabilizer, re-vegetation resource to control water and wind erosion in eroded soils (Nobel, 1994). Ben Salem

et al. (1996) also noted the increased importance of cactus as livestock feed in arid and semi-arid zones due to its drought resistance, high biomass yield, high palatability and tolerance to salinity.

Succulent pads of cactus serve as source of water for livestock in dry areas. Various species of *Opuntia* provide an important source of fodder especially in arid and semi-arid environments (Gebremariam et al., 2006). It can be cultured as crop for the production of fruits, vegetables and forage for animal feed or used as raw-industrial material to produce several sub-products such as wine, candies, jellies, flour (Hegwood, 1990; Flores-Valdéz, 1995). The fruits of the plant are also useful to humans (Snyman, 2004). Moreover, cactus is known in the traditional medicine for its hypoglycemic and hypolipidemic actions (Fernandez et al., 1992). Cactus fruits are eaten fresh, dried or preserved in jams, syrups or processed into candy-like products. Cactus pear 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). The nutritional importance of cactus pear fruit is mainly due to the content antioxidants, fibres and free amino acids (Stintzing et al., 2001; Bensadón et al., 2010). Moreover, Piga et al. (2003) suggested that the minimal processing did not decrease the main nutritional and health-promoting properties of cactus fruit during storage at 4°C for up to 9 days. In some countries cactus pear juice is consumed at home, in vegetarian restaurants or in local health-food stores.

The aim of this work is to study some characteristics (pH, acidity, °Brix and reducing sugar) of juice and storage content (lipid and protein) of three Tunisian *Opuntia ficus indica* cultivars. The importance of these results is an indication of the nutraceutical and economic utility of Tunisian cactus.

MATERIALS AND METHODS

Plant Material

Fruits of three *Opuntia* species were harvested from Sbiba (Kasserine) in August 2008 and from Soliman (Nabeul) in November 2008. The fruit were hand-picked; seeds were separated

and the juice frozen and stored at -80 °C. Analyses were performed in triplicate.

Fruit

The following parameters were measured: fresh weight of fruit, fruit length, fruit diameter, fruit colour, seed number and total weight of seeds per fruit.

Juice

In this study we have crushed the fruit without peel. The juice, free of seeds, was filtered to remove cell debris. We evaluated the physico-chemical properties of juice by measuring the pH, the acidity and the total sugars. The freshly collected juice was pressed out with a manual piston press and the total soluble solids were measured by means of a hand-held refractometer (Brix 0~23 %) and expressed in Brix degrees (°Bx). Acidity was determined by titration with 0.1 N NaOH and expressed as percent of citric acid; phenophtalein was used as the indicator. The pH was measured with a Fisher accumet pH meter. Reducing sugars, as percent inverted sugar, were determined by the Miller (1959) method.

Seeds

Oil Content

The oil content was determined according to ISO (1999) method 659:1998. About 25 g of the seeds were ground in a mortar until dough and extracted with petroleum ether in a Soxhlet apparatus for 6 h. The solvent was concentrated using a rotary evaporator, under reduced pressure at 45 °C. The oil was dried by using a stream of nitrogen and stored at -20 °C until use.

Unsaponifiable Fraction

To separate the unsaponifiable fraction, oil from seeds was treated with ethanolic potassium hydroxide solution (2N) to transform the fatty acyl esters into potassium salts that are soluble in water. Extracted lipids were treated with 50 mL of 2M KOH-ethanol solution, and the mixture was refluxed, with constant stirring, for 1 h. Then, 50 mL of water were added. The unsaponifiable fraction was extracted with 3 x 40 mL of diethyl ether. The organic extract was separated and washed with 3 x 40 mL of distilled water and then dried over anhydrous sodium sulfate, filtered, and

concentrated using a rotary evaporator.

Separation of the Sterolic Fractions by Thin-Layer Chromatography (TLC)

The unsaponifiable matter (5% in chloroform) was separated on TLC plates (20 × 20 cm) coated with KOH–methanol (2 N) impregnated silica gel (0.25 mm), previously activated by heating at 100 °C for 1 h. The unsaponifiable fraction (250 µL) was spotted on the plates. Elution was performed using hexane/diethyl ether 65:35 (v/v) as the mobile phase. The plates were then sprayed with a 0.2% solution of 2',7'-dichlorofluorescein in ethanol, and the sterol pink bands appeared under UV light. Sterol bands were scraped off separately and dissolved into warm chloroform (5 mL). The obtained solutions were dried over anhydrous sodium sulfate and filtered through Whatman filter paper. The chloroform was evaporated by nitrogen stream, and sterolic fractions were dried in an oven at 103 °C.

GC-FID Analysis

Sterol contents were determined according to Stiti *et al.* (2007). Sterolic fractions were acetylated with acetic anhydride/pyridine (2:1 by volume). Acetate derivatives were then purified and analyzed by GC using a Varian GC, model 8300, equipped with a flame ionization detector (FID) and a DB-1 capillary column (25 m × 0.32 mm i.d., 0.25 µm thickness, J&W scientific), with H₂ as the carrier gas (2 mL/min). The temperature program included a fast rise from 60 to 230 °C (30 °C min⁻¹), a slow rise from 230 to 280 °C (2 °C min⁻¹), and a plateau at 280 °C for 10 min. Free cholesterol was used as an internal standard, indeed many authors reported that this compounds was undetected in cactus seed oil cactus seed oil (Ramadan and Morsel, 2003; Salvo *et al.*, 2002). Sterols were identified by gas-chromatography mass-spectrometry (GCMS) analyses performed on an Agilent gas chromatograph equipped with an on-column injector and a DB-5 (J&W Scientific) capillary column (30 m × 0.25 mm, i.d.) and interfaced to a 5973 MSD using electron impact at 70 eV. Spectra were compared to those of authentic samples or our own reference sterols or to literature data.

Storage Protein Contents

Protein contents were determined according to the AOAC (1984) official method using a micro-Kjeldhal apparatus. Each sample (100 mg) was digested for about an hour with 100 mg of digestion mixture (8g K₂SO₄ + 20g CaSO₄ + 2g selenium) and 6 mL of concentrated H₂SO₄. When digestion was completed, the solution became clear. The solution was then made up to 30.0 mL in a volumetric flask with distilled water. For the nitrogen determination, 10 mL of 2% boric acid solution was first taken in a beaker with a few drops of methyl red as indicator. Then 10 mL of the digested mixture, 30 mL of 40% NaOH solution, and 10 mL of distilled water were transferred to the distillation chamber. Ammonia was liberated, and it combined with NaOH to form NH₄OH, which was then received into the boric acid solution to form ammonium borate (pink colour to yellow). Distillate (ammonium borate) was then titrated with 0.1 N H₂SO₄. The volume of acid that had been added at the point when the colour of the distillate changed from yellow to pink was recorded. Protein was calculated according to the following formula: %protein = %N × 6.25.

Statistical Analysis

The results were analysed by ANOVA and means separated by a Duncan Test when the samples were significantly different ($p \leq 0.05$).

RESULTS AND DISCUSSION

Fruit Characteristics

Results show that the colour is characteristic of the cultivar. Indeed, colour plays an important role in consumer selection of food. Moreover, natural colorants from plant sources are receiving recently growing interest from both food producer and consumers due to the fact that these fruit or vegetable colouring extracts may contain additional ingredients of nutritional value.

The thorny cultivar has a yellow-orange colour. Gialla is greenish while the Rossa is pink. Indeed, red and yellow cactus fruits are important edible sources containing betalains which demonstrate an important antioxidant property (Stintzing *et al.*, 2003). Moreover, Moßhammer *et al.* (2005) suggested that cactus fruits are a

suitable source to provide customised hues for colouring foodstuffs.

Results show also that the thorny cultivar, presents the smallest dimensions. The average weight of the fruit is 74.25 g; length and diameter are 6.67 cm and 3.94 cm, respectively (Table 1). The highest mass is detected with Rossa and Gialla (140.72 g and 132.09 g, respectively). Using the statistical analysis a significant difference was detected. The fruit of Gialla and Rossa, although larger, contains 234 seeds/fruit whereas the thorny cultivar contains 265 seeds/fruit. Cultivar Gialla and Rossa are two late cultivars. Indeed, removing the spring flush of flowers and cladodes induces reflowering in cactus pear (Barbera *et al.*, 1991). Removal time determines the extent of reflowering, and the emergence of new flower buds decreases if the removal of the spring flush is delayed until the late stages of bloom (Barbera *et al.*, 1991). Then the fruits produced are larger and mature in autumn. Indeed, fruit mass or size is very important since consumers associate it with better value for money (deWit *et al.*, 2010). These results show the importance of the cultivars Gialla and Rossa intended for export. They have the larger size and the lower number of seeds. Indeed, Karababa *et al.* (2004) and Bekir (2006) suggested that size and weight of cactus pear fruit is influenced by locality, season and environment. Whereas, Felker *et al.* (2002) reported that fruit size is not determined by environmental or edaphic factors, but genetic factors. The current results support the effect of variety on fruit size.

Juice Characteristics

The volume of juice per fruit, the pH and the acidity of the three cultivars are presented in table 2. The high volume of juice are obtained with Rossa (55 mL), followed by Gialla (44mL) and thorny cultivar (24mL). Statistical analysis shows a significant difference between cultivars. Different studies were done in order to reduce the pH value of the juice (Espinosa *et al.*, 1973). In our study, the pH is 5.77, 5.81 and 5.96 for the cultivar Rossa, thorny and Gialla, respectively. No significant differences were observed between the pH values. These values are in agreement with those reported by other authors (Stintzing *et al.*, 2003). The thorny cultivar show the high acidic juice (21.06 mmol l⁻¹) while the two late cultivars have the lower acidity (14 and 9 mmol l⁻¹, for Rossa and Gialla, respectively). Cassano *et al.* (2010) reported that juice from *Opuntia* collected from Sicily is characterised by a high pH value (5.5–5.7) and a very low acidity (0.03% in citric acid).

Sugar content is an important criterion of fruit quality for consumers who prefer sweet fruit (Inglese *et al.*, 1995). The °Bx for Gialla is 12.53 ± 0.01 and is higher than from the other cultivars (11.93 and 11.53, for thorny and Rossa, respectively). In addition, statistical analysis shows the absence of significant differences between the values (Table 2). These values are in agreement with the results published by other authors (Stintzing *et al.*, 2003) who reported that °Bx for Gialla and Rossa from Italy are 12.3 and 13.6, respectively. Moreover, Turker *et al.*

Table 1. Morphological parameter of *Opuntia ficus-indica* fruit.

	Fresh weight (g)	Length (cm)	Diameter (cm)	Seeds / fruit	Seeds weight/fruit (g)	Fruit colour
Thorny	74.25 ± 14.95	6.67 ± 0.65	3.94 ± 0.44	265 ± 68.55	8.84 ± 3.31	Yellow-Orange
Gialla	132.09 ± 21.55	8.73 ± 1.02	4.99 ± 0.51	234 ± 68.69	6.79 ± 2.31	Greeny
Rossa	140.72 ± 25.56	9.57 ± 0.91	4.96 ± 0.51	236 ± 53.42	6.86 ± 0.54	Pink

Table 2. Juice characteristics of *Opuntia ficus-indica* cultivars.

	volume of juice/fruit (ml)	pH	Acidity (mmol l ⁻¹)	°Brix	Reducing sugar
Thorny	24.68 ± 6.71	5.81	21.06 ± 2.57	11.93 ± 0.01	1.26±0.08
Gialla	44.77 ± 10.11	5.96	9.86 ± 2.20	12.53± 0.01	3.27±0.20
Rossa	55.43 ± 11.92	5.77	14 ± 0.8	11.53 ± 0.01	4.39±0.38

(2001) reported that °Bx from Turkish cultivar is between 12.2 and 15.8. Indeed, total soluble sugars is highly influenced by crop management and environment since cactus pear fruit grown in dry areas are sweeter than those grown in humid areas (Modragon-Jacobo, 2001) or being irrigated (Inglese *et al.*, 1995). Reducing sugar is between 1.26 and 4.39 g/fruit. Gialla and Rossa are always more rich in reducing sugar than thorny cultivar. Piga *et al.* (2003) suggested that reducing sugar in cultivar from Italy is from 10 to 15%. Whereas, our results show that values are between 1.7 and 3.11%. Indeed, the fruit of opuntia is considered a very sweet fruit and this is due to the presence of reducing sugars in the pulp, especially glucose and fructose (Russell and Felker, 1987; Stintzing *et al.*, 2003).

Seed Oil

Table 3 show the total seed oil content of the three opuntia cultivar. The values are from 6.93 ± 0.57 % (thorny) to 7.76 ± 1.12 % (Rossa). These results are in agreement with those reported by Coskuner and Tekin (2003) who reported an oil content of 6.91 %. Whereas, Ennouri *et al.* (2005) reported that oil content of cactus seeds is between 10.90 % and 11.05 % for cultivar from Sfax (Tunisia). Moreover, Salvo *et al.* (2002) reported that oil content from Italian cultivar is about 9.14 %. Ramadan and Morsel (2003) reported that oil content from German cactus is about 9.9%. These differences may be related to geographic or genetic conditions since the oil content of crops varies with the crop cultivars, soil and climatic conditions of an area (Breene *et al.*, 2007). Ramadan and Morsel (2003) reported that cactus oil contained mainly unsaturated fatty acids (ca. 76%) and an appreciable level of fat-soluble vitamin.

Table 3. Seed oil, unsaponifiables and protein content of *Opuntia ficus-indica* seeds

	Lipid	Unsaponifiables	Protein
Thorny	6.93 ± 0.57	1.36 ± 0.04	9.65
Gialla	7.22 ± 1.35	1.87 ± 0.28	12.78
Rossa	7.76 ± 1.12	2.39 ± 0.02	11.06

Unsaponifiable content are between 1.36% (thorny) and 2.39% (Rossa). These values are in agreement with those reported by other authors (Ramadan and Morsel 2003) for German cultivar (2.01%), whereas the values for Italian cultivar

are about 3.33% (Salvo *et al.* 2002). These values are in the higher range of those found for sunflower (Karleskind and Wolff 1996) and also for some underexploited seed oil like caper seeds (Tlili *et al.*, 2009). These results are useful for the consumer and also for the producer of oils. Indeed, the unsaponifiable fraction of vegetable oils has applications in cosmetics and pharmacology due to its biological properties.

Sterols comprise a major portion of the unsaponifiable matter of most vegetable oils (Kiritsakis and Christie, 2000). Figure 1 show a typical chromatogram corresponding to the GC analysis of sterol fraction. Eleven sterols were identified.

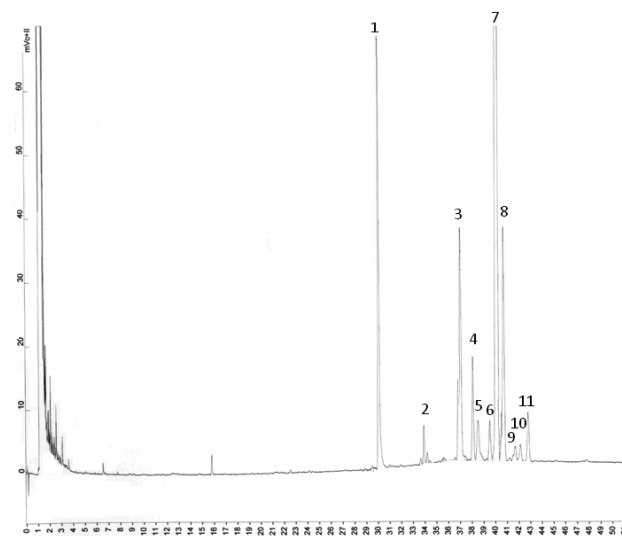


Fig. 1. Typical chromatogram of sterols from unsaponifiable matter extracted from *Opuntia ficus indica* seeds.

Peaks : 1 Cholesterol (internal standard), 2 24, Methylenecholesterol, 3 Campesterol, 4 Campestanol, 5 Stigmasterol, 6 Clesosterol, 7 β - Sitosterol, 8 Δ^5 Avenasterol, 9 $\Delta^{5,24}$ Stigmastadienol, 10 Δ Stigmastenol, 11 Δ^7 Avenasterol.

The sterol composition is summarized in Table 4. β -sitosterol was the most abundant sterol as it occurs for most of the plant species (Grunwald, 1975). The percentages are ca. 72% (thorny), ca. 70% (Rossa) and 69% (Gialla) with an average of 70.74%. Indeed, β -sitosterol is the major sterol in olive oil with 80% (Sanchez *et al.*, 2004) in coffee beans with 50% (Carrera *et al.*, 1998) or in caper seeds with 57% (Tlili *et al.*, 2010). Δ^5 avenasterol and campesterol are present with ca. 9% and ca. 7%, respectively. The percentages of campestanol, clerosterol,

Table 4. Sterol composition of *Opuntia ficus-indica* seed oil

Samples	Thorny	Rossa	Gialla	Mean
Sterols	%	%	%	%
24, Methylcholesterol	0.37	0.82	1.06	0.73
Campesterol	6.12	7.8	9.5	7.81
Campestanol	2.47	3.67	3.55	3.21
Stigmasterol	0.18	1.64	2.35	1.37
Clerosterol	3.46	2.81	1.46	2.55
β -sitosterol	72.46	70.77	69.06	70.74
Sitostanol	Tr	Tr	0.34	0.34
Δ^5 Avenasterol	10.86	9.45	8.78	9.66
$\Delta^{5,24}$ Stigmastadienol	1.17	0.79	0.89	0.93
Δ^7 Stigmastenol	0.58	0.58	0.85	0.65
Δ^7 Avenasterol	2.33	1.67	2.16	2.03
Total	100	100	100	100

Δ^7 avenasterol and stigmasterol are between 3.21% and 1.37%. While the percentages of 24, methylcholesterol, sitostanol, $\Delta^{5,24}$ stigmastadienol and Δ^7 stigmastenol are less than 1%. The sitostanol was quantified only in Gialla (0.34%), whereas in the other samples it was detected in trace quantities.

These compounds are known to have a wide range of beneficial biological activities and physical properties. Recently it was reported that phytosterols/phytosterols may regulate cholesterol metabolism (Yang *et al.*, 2006; Calpe-Berdiel *et al.*, 2009). Phytosterols plays an important role in treatment of breast cancer (Awad *et al.*, 2008). Savage *et al.* (1997) reported that Δ^5 avenasterol can act as an antioxidant. Campesterol can regulate lipoprotein metabolism in the intestine and regulate biliary secretion (Ho and Pal, 2005).

These results present a suggestion of the possible dietary and pharmaceutical utility of cactus seed oil as a good source of phytosterols.

Storage Protein Content

Legume seeds with about 25% of protein are the most important source of these compounds for human and animal nutrition, whereas protein content of cereals is between 10 % and 15 % (Gueguen and Lemarie, 1996). In Table 4, our results show that protein content is ranged between 9.65 % (thorny) and 12.78% (Gialla). These differences may be related to geographic or genetic conditions since the

storage content of plants varies with the crop cultivars, soil and climatic conditions (Breene *et al.*, 2007). These values are in agreement with results published by El-Kossori *et al.* (1998), but higher than those reported by Sáenz-Hernández (1995) who suggested that cactus is deficient in protein (ca. 3%). These results confirm the nutritional value of opuntia seeds. Indeed, protein-calorie malnutrition (PCM) is a major nutritional syndrome affecting more than 170 million preschool children and nursing mothers in developing Afro-Asian countries (Iqbal *et al.*, 2006). For these reasons, proteins from non-exploited seeds of plants should be explored.

CONCLUSIONS

Results show significant differences between the cultivars. The thorny cultivar showed the small fruit (ca. 74 g). Whereas, the Gialla and Rossa fruit are ca. 132g and ca. 140g, respectively. Gialla and Rossa fruit contains 235 seeds/fruit while the thorny cultivar contains 265 seeds/fruit. The pH, the acidity and the sugar content of the juice were also studied. The important volumes of juice (44.77 ml and 55.43 ml) were obtained with Gialla and Rossa cultivars respectively. The thorny cultivar had the high acidity (ca. 21 mmol l⁻¹) compared to Gialla and Rossa (9 and 14 mmol l⁻¹). Total sugars of the three cultivars are quite similar (ca. 12 °Brix) and the pH is between 5.77 and 5.96. Oil seeds values was about 7 %. The unsaponifiable fractions are

2.39%, 1.87% and 1.36% for Rossa, Gialla and thorny cultivars, respectively. β -sitosterol was the major sterol (70%) followed by Δ^5 avenasterol and campesterol. The storage protein content is arranged between ca. 9% and ca. 13%. These results show the nutraceutical and industrial importance of cactus fruit/seeds.

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