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PHYSICO-CHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF PRICKLY PEAR SEED OIL AND ANTIOXIDANT ACTIVITY OF ITS UNSAPONIFIABLE MATTER

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ABSTRACT

The chemical composition of prickly pear seeds *(Opuntia ficus-indica* L.*)* was determined. The results ascertained that prickly pear seeds have high contents of crude fibers, crude proteins and oil. The seeds were investigated as a source of oil which constituted 12.28% of the whole seed. The obtained data proved that the physico-chemical properties of the prickly pear seed oil (PPSO) such as specific gravity, refractive index, color, stability, acid value, iodine value and saponification value compared well with those properties of other commonly edible oils (*e.g.* soybean and corn oil). The results purported that (PPSO) has a high degree of total unsaturation, 84.86%. Linoleic acid was found to be the dominant one which constituted 63.04%, followed by oleic acid, 19.42% and palmitic acid, 10.7 %. Meanwhile, linolenic acid was found in a very low level. The unsaponifiable matter (USM) of prickly pear seed oil was extracted and analyzed using GC/MS. The results revealed that β- sitosterol was the major component of the sterolic fraction (64.8%) followed by campesterol (13.8%), meanwhile, 24- methyl cycloarthenol was found to be the main component of the alcoholic fraction (48.8%), followed by cycloarthenol (35.9%). Also, the unsaponifiable matter of PPSO was examined for its antioxidant activity by using sunflower oil. The obtained results proved that the (USM) exhibited antioxidant activity, specially, at 1200 ppm, where the antioxidant activity resembled that of 200 ppm of BHA (synthetic antioxidant). Furthermore, the antioxidant activity of (USM) at 1500 ppm was superior to that of BHA. Therefore, prickly pear seed oil could be used as edible oil, besides, its unsaponifiable matter which exhibited a potent antioxidant activity to suppress lipid oxidation.

Key words:*antioxidant activity, fatty acids, prickly pear,seed composition, prickly pear seed oil, unsaponifiable matter.*

1. INTRODUCTION

Prickly pear, a member of the Cactaceae Family and a native to arid and semi arid regions, is widely distributed in many parts of the world such as Africa, Australia and Mediterranean basin. It contains high content of some chemical constituents which give added value to its fruit on a nutritional and technological functionality basis (Piga, 2004).

The potential supply of the fruit by-products may be enormous. Millions of pounds of fruit seeds are discarded yearly, resulting in disposal problems and proper utilization of these waste products could lead to important new sources of oil which could be used in functional foods (Ramadan and Morsel, 2003a). The seeds of the prickly pear fruit constitute about 10-15% of the pulp. These seeds contain about 12% oil, with a linoleic acid of 50.9%. Thereon, PPSO could be extracted and used as an alimentary oil (Lopez and Burgos, 1973). Chiefly, the biological studies on rats purported that PPSO exhibited a significant decrease in serum glucose, total cholesterol and LDL-cholesterol in the treated rats (Ennouri, *et al.*, 2005b). Also, the findings of Tan and Che Man (2000) purported that the lipid pattern of prickly pear seed was comparable with that of sunflower and grape seed oils.

Another point of view is the unsaponifiable matter of PPSO where sterols comprise the bulk of the unsaponifiable matter in many oils. They are of interest due to their antioxidant activity and impact on health. Recently, sterols have been added to vegetable oils as an example of a

successful functional food. This type of product is now available and lowers blood LDL-cholesterol by around 10-15% as apart of a healthy diet (Ntanios, 2001).

Concerning the unsaponifiable matter presented in PPSO, Krifa *et al.* (1993) affirmed that the USM of PPSO constitutes high content of sterols, mainly, β- sitosterol (67%) and campesterol(13%). Likewise, Ramadan and Morsel (2003b) and Kuti (2004) pronounced that PPSO contained γ- tocopherol and β- carotene. These compounds exhibited antioxidant activity, thus, it could be used as natural antioxidants instead of synthetic ones such as BHT and BHA, which have been suspected of being responsible for liver damage and carcinogenesis (Jassen, and Vries 1997).

The present investigation was planned to extract the oil of prickly pear seeds and determine the physico-chemical properties and fatty acid composition. Also, to separate the unsaponifiable matter of prickly pear seed oil and determine their composition using GC/MS, then, examine their antioxidative activity on refined sunflower oil.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Fruit samples

Mature prickly pear fruits, *Opuntia ficus-indica* were purchased from El-Oboor Market, Cairo. The fruits were sorted, washed, dried and hand picked, separated into peel and pulp. The pulp was squeezed for the separation of the seeds.

2.1.2. Sunflower oil and BHA

Refined sunflower oil was obtained from Cairo for Oils and Soaps Co., Cairo, Egypt.
Butylated hydroxyl anisole (BHA) was purchased from Sigma Chemical Co., (London, Ltd. Poole).

2.2. METHODS

2.2.1. Fruit physical measuremeats

Whole fruits, pulp, peel and seed weights were determined and the percentage of each parameter was calculated according to the method of Duru and Turker (2005).

2.2.2. Preparation of prickly pear seeds

The seeds were washed with distilled water several times, air- dried at ambient temperature and then milled by hummer mill.

2.2.3. Chemical composition of prickly pear seeds

Moisture, total ash and crude fiber contents were determined according to AOAC method (AOAC, 1995). The oil yield was determined from seed powder of 5g (AOAC, 1995). Total nitrogen was determined by the Kjeldahl procedure and crude protein was calculated as N X 6.25. Total carbohydrates were calculated by difference.

2.2.4. Extraction of prickly pear seed oil

The seed powder oil was extracted with hexane in a soxhlet extractor for 9 hr according to the method reported by Ennouri *et al.*(2005a). The organic phase was then removed using a rotary evaporator under reduced pressure. The oil was flushed with a stream of nitrogen and stored at -20° C in sealed tubes prior to analyses.

2.2.5. Extraction of unsaponifiable matter

The unsaponifiable matter was extracted according to the method of (AOCS, 1993), and then stored at -20° C in sealed glass tubes prior to analyses and using.

2.2.6. Physico-chemical analysis of prickly pearseed oil

Specific gravity, refractive index, color (using Lovibond Tintometer), acid value, iodine number, peroxide value, saponification number and unsaponifiable matter of (PPSO) were determined according to the standard methods of AOCS (1993). Stability of oil was determined at 75° C as the method described by Banias *et al.*, (1992).

2.2.7. Determination of fatty acid composition

The fatty acid composition of PPSO was analyzed by GC/MS after transesterification. Fatty acid methyl esters were prepared in the presence of 2N potassium hydroxide in methanol and analyzed on a Hewlett-Packard model 5890 series gas chromatograph equipped with a flame ionization detector and a capillary column : carbowax 20 M (0.3 mm internal diameter, 50 m length and 0.3 μm film thickness). The operational conditions were: injector temperature 220°C, detector temperature 275°C, column temperature 50°C for 5 min then raised again to 240°C at rate of ci. Carrier gas was nitrogen at a flow of 1.2 ml/min as mentioned by Ennouri *et al.*, (2005a).

2.2.8. Determination of unsaponifiable matter composition

The unsaponifiable matter was sailinized prior to chromatographic analysis. The unsaponifiable matters were derivatized with the addition of pyridine (0.5 ml), hexamethyl disilosane (100 μl) and trimethyl chlorosilane (40 μl). The sterols and triterpene alcohol were then centrifugated and the upper layer was analyzed using a Vrian CP- 3800 gas chromatography equipped with a flame ionization detector and a capillar column, ZEBRN ZB- 5; Phenomenex (0.25 mm internal diameter, 30 m length and 0.25 μm film thickness) was coupled to a mass spectrometer (Saturn 2000 GC MS/MS). The oven temperature was fixed at

150°C for 5 min and then raised to 250°C at 15°C/min and held for 20 min. Carrier gas was helium at a flow of 1 ml/min. Quantification of ompounds was carried out using the internal standard method (Bereau *et al.*, 2003).

2.2.9. Evaluation of antioxidant effectiveness

The antioxidant activity of USM extracted from PPSO was measured. The oven test at 75° C was used as described by Economou, *et al.* (1991). Twenty- five grams of refined fresh sunflower oil was placed in 100 ml open-mouthed beakers and used as control and another sample of oil with BHA (200 ppm) for comparison. Another samples of oil (25 g each) with different concentrations of USM (200, 400, 600, 800, 1000, 1200 and 1500 ppm). The samples were covered with watch glasses and placed at 75° C±1 till rancidity took place. Peroxide value was determined according to the standard methods of AOCS (1993).

The induction periods (the time needed for the peroxide value to become 20) were determined by plotting the peroxide values of samples *vs.* time as described by Banias *et al.*, (1992).

2.2.10. Statistical analysis

The collected results of the induction periods of sunflower oil as affected by USM addition were subjected to statistical analysis using one way analysis of variance according to the method of Snedecor and Cochran (1980). The LSD was calculated at 0.05% probability.

3. RESULTS AND DISCUSSION

The results manifested that the fruits of Prickly pear contained 51% peels, 41% pulp and 8% seeds. These results are in agreement with those obtained by Piga (2004) and Duru and Turker (2005).

3.1. Chemical composition of prickly pear seeds

The results of chemical composition of PPS are presented in Table (1). Oil extracted from PPS constituted 12.28% of the whole seeds. This result is in the same line with that obtained by Ramadan and Morsel (2003b) and Ennouri *et al.*,(2005a). Also, it could be observed that the seeds contained 15.22% moisture, 7.42% crude protein, 47.91% crude fibers, 2.32% total ash and 30.07% total carbohydrates.

The results of total ash and other extract were higher than that reported by Coskuner and Tekin (2003), while, crude fibers and total carbohydrates contents were lower than that obtained by the same authors. The observed differences could be probably due to the origin of the fruits. Meanwhile, these values are in accordance with the values found by Ennouri *et al.*, (2005a).

3.2. Physico-chemical properties of PPSO

Table (1): Chemical composition of prickly pear seeds (dry wt. basis).

[a] Constituents	g/100g
Crude proteins	7.42
Crude fibers	47.91
Total ash	2.32
Ether extract	12.28
Total carbohydrates	30.07
Moisture	15.22

[a] Means of three determinations.

The PPSO had a light yellow color. The physico-chemical properties of PPSO are displayed in Table (2). From the results, it could be observed that low acid and peroxide values indicated that no hydrolytic and oxidative rancidity occurred during extraction of oil. Like most vegetable oils, PPSO had a relatively high iodine value (116), which reflects a high degree of unsaturation. Also, due to the iodine value, PPSO oil could be classified as a semi-drying oil. This value is higher than that obtained by Ennouri *et al.*, (2005a) (116 *vs.* 101.5). PPSO also exhibited higher stability compared with that of sunflower oil (Table, 5), meanwhile, the average value of unsaponifiable matter was high as compared to other vegetable oils (3.42%). This value is higher than that reported by Sawaya and Khan (1982) who purported that they were 1.96%. Also, the result recorded for saponification number was higher than that value found by Ennouri *et al.*,(2005a) (190 *vs.* 169). The observed difference is possibly due to both of variety and origin of the fruits. Generally, all characteristics of PPSO are in closed levels with those of other common vegetables oils.

Table (2): Physico-chemical properties of prickly pear seed oil.

[a] Properties	
Specific gravity (25° C)	0.904
Refractive index (25° C)	1.478
Color (Lovibond Tintometer)	0.7 red, 20.0 yellow
Stability of oil (at 75° C)	3.9 hr
Acid value as oleic	0.94
Iodine number (Wijs)	116
Peroxide value (meq O_2/kg oil)	2.2
Saponification number	190
Unsaponifiable matter (%)[b]	3.42

[a] Results are mean values of three determinations.

[b] Result is avalue of one determination.

3.3. Fatty acid composition of PPSO

This study was conducted on fatty acid composition of PPSO; results are listed in Table

(3). It could be noticed that, linoleic was found to be the dominant fatty acid (63.04%). This result agreed well with that obtained by Sawaya and Khan, 1982 and Barbagallo and Spagna, (1999). Meanwhile, Ennouri *et al.* (2005a) reported a higher linoleic acid content than the obtained result (73.3% *vs.* 63.04%). The recorded difference is possibly due to the degree of maturity of the fruits as reported by Coskuner and Tekin (2003). The second one was oleic acid, 19.42%, followed by palmitic acid, 10.7% and stearic acid, 4.32%. The oil contained 15.14% saturated, 21.54% mono-unsaturated and 63.32% poly unsaturated fatty acids. These results are approximately in agreement with those obtained by Krifa *et al.* (1993), who purported that these values comprised 18.0, 25.0 and 57.0%, respectively. Also, it could be noticed that both total saturation and unsaturation ratio of PPSO are close to that of soybean oil. Meanwhile, linoleic acid content and total unsaturation of PPSO are nearer to that of corn oil (Table 3). In general, the higher level of unsaturation and particularly high level of linoleic in conjunction with low level of linolenic acid, which affects adversely the stability of the oil, indicated that PPSO might be an excellent potential source of edible oil for human and /or animal consumption as reported by Sawaya and Khan (1982) and Ennouri *et al.*, (2005b).

sterolic and alcoholic fractions. Concerning the sterolic fraction, the data revealed that five components, representing 94.9% of the total sterols were identified. Also, it could be noticed the preponderance of β –sitosterol (64.8%, of the total sterols), followed by campesterol (13.8%), stigmasterol (6.1%), lanosterol (5.8%) and Δ -5 avenasterol (4.4%). These results are lower than those obtained by Ramadan and Morsel (2003a) who reported that β –sitosterol comprised 72%, followed by campesterol (18%) while stigmasterol and lanosterol constituted 3%. With reference to the alcoholic fraction, data indicated that the main components are 24- methyl cycloarthenol (48.8%), followed by cycloarthenol (35.9%) and β –amyrin (6.4%). These values were higher than those in the work of Salvo *et al.*, 2004, whereas, the values were 34.2, 29.3 and 2.2%, respectively. These differences may be attributed to the variety fruits, besides, the conditions of determination. From the above mentioned results, it could be noticed the importance of unsaponifiable matter of PPSO, especially, the higher content of sterols, which have antioxidant activity and impact on health (Karifa *et al.*, 1993, Ntanios, 2001 and Ramadan and Morsel, 2003 a&b).

3.5. Antioxidant activity of unsaponifiable matter

Initially, the addition of USM at various concentrations did not affect either the color or the flavor of sunflower oil. The data of the oxidative

Table (3): Fatty acid composition of prickly pear seed oil compared with some edible oils.

Fatty acid	Prickly pear seed oil	Soybean* oil	Corn oil*	Sunflower* oil	Cotton seed oil
Myristic $C_{14:0}$	0.12	0.2	-[b]	-	0.7
Palmitic $C_{16:0}$	10.7	10.7	11.0	6.0	22.7
Palmitoleic $C_{16:1}$	2.12	0.3	-	0.1	0.6
Stearic $C_{18:0}$	4.32	3.9	1.7	4.0	2.3
Oleic $C_{18:1}$	19.42	22.8	25.8	16.5	17.3
Linoleic $C_{18:2}$	63.04	50.8	59.8	72.4	55.8
Linolenic $C_{18:3}$	0.28	6.8	1.1	0.5	-
Other	-	4.5	0.6	0.5	0.6
Total saturation	15.14	14.8	12.7	10.0	25.7
Total unsaturation	84.86	80.7	86.7	89.5	73.7
Mono unsaturation	21.54	23.1	25.8	16.6	17.9
Poly unsaturation	63.32	57.6	60.9	72.9	55.8
[c] U/S ratio	5.60	5.45	6.83	8.95	2.86

*Results of U.S. corn oil reported by Strecker *et al.* (1990).
*Results of soybean, sunflower and cotton seed oils were reported by Hui (1996).
[b] Not detected.
[c] U / S ratio = ($C_{16:1}$ + $C_{18:1}$ + $C_{18:2}$+ $C_{18:3}$/ $C_{14:0}$ + $C_{16:0}$ + $C_{18:0}$).

3.4. Composition of unsaponifiable matter

The composition of unsaponifiable matter of PPSO using GC/MS is listed in Table 4. It could be observed that two fractions were identified;

stability of sunflower oil as affected by using different concentrations of USM, are displayed in

Table (4): Composition of unsaponifiable matters of prickly pear seeds oil.

Component	Relative percent
Sterolic fraction	
β - Sitosterol	64.8
Campesterol	13.8
Stigmasterol	6.1
Lanosterol	5.8
Δ -5 Avenasterol	4.4
Total identified	94.9
Total un-identified	5.1
Alcoholic fraction	
24- Methyl cycloarthenol	48.8
Cycloarthenol	35.9
β -Amyrin	6.4
Total identified	91.1
Total un-identified	8.9

Table (5). It could be observed that USM at 400 ppm had a little effect on the induction period (3.8 days). A positive correlation between USM concentration and its antioxidative activities was noticed. At the concentration 1200 ppm, USM showed a similar induction period as BHT at 200 ppm where the induction period recorded 11.6 and 11.5 days, respectively.

Furthermore, USM exhibited higher antioxidant activity at 1500 ppm compared to BHA as shown in Table 5. With reference to the antioxidant activity of USM, it could be ascribed

Table (5): Effect of unsaponifiable matters of PPSO at various concentrations on induction period of sunflower oil

Unsaponifiable matter concentration (ppm)	Induction period (day) **	R.I.P#
None	*3.2 ^{g} ± 0.10	1.0
200	3.8 ^{f} ± 0.27	1.2
400	3.8 ^{f} ± 0.35	1.2
600	5.8 ^{e} ± 0.20	1.8
800	7.5 ^{d} ± 0.27	2.3
1000	10.8 ^{c} ± 0.27	3.4
1200	11.6 ^{b} ± 0.35	3.6
1500	13.5 ^{a} ± 0.31	4.2
BHA (200 ppm)	11.5 ^{b} ± 0.3	3.6

* The initial peroxide value of the oil samples was 1.2 meq O_2/ kg oil.
** Induction period, days needed for peroxide value to become 20 at 75° C ± 1.
# R.I.P (Relative induction period):indction period for control= 1.
Results are means of three determinations ± standard deviations.
- Means which are not significantly different are followed by the same letter.

to the presence of sterol fraction as reported by (Ntanios, 2001 Ramadan and Morsel (2003)$_a$ and Salvo *et al.*, (2004), where β- sitosterol accounted to 64.8% of the total sterol content in PPSO (Table, 4).

Based on the abovementioned results, prickly pear seed oil singled out to be a good potential source of alimentary oil, besides, the activity of unsaponifiable matter against oxidation as a natural antioxidant, which could prolong the shelf life of edible fats and oils.

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الخصائص الطبيعية و الكيميائية و تركيب الأحماض الدهنية لزيت بذور التين الشوكي و النشاط المضاد للاكسدة للمواد غير القابلة للتصبن

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ملخص

تهدف الدراسة إلي الاستفادة من مخلفات التين الشوكي خاصة البذور للحصول علي زيت يمكن استخدامه في الأغراض الغذائية. و قد تم تقدير التركيب الكيميائي لبذور التين الشوكي حيث أظهرت النتائج ارتفاع محتواها من الألياف و البروتين الخام و الزيت. كما تم استخلاص زيت بذور التين الشوكي و الذي يمثل ١٢,٢٨ % من وزن البذور. تم تقدير الخصائص الطبيعية و الكيميائية له الوزن النوعي، معامل الانكسار، اللون، ثبات الزيت، الرقم اليودي، الرقم الحمضي ورقم التصبن. لوحظ تقارب خصائص زيت بذور التين الشوكي مع مثيلاتها في بعض الزيوت الغذائية الشائعة مثل زيت فول الصويا و زيت الذرة. و من ناحية أخري لوحظ ارتفاع درجة عدم التشبع للزيت (٨٤,٨٦%). و يعتبر حمض اللينوليك الحمض الدهني السائد في زيت بذور التين الشوكي و يمثل ٦٣,٠٤%، يليه حمض الاوليك (١٩,٤٢%)، ثم حمض البالمتيك(١٠,٧%). بينما أوضحت النتائج انخفاض محتوي الزيت من حمض اللينولينيك.

تم استخلاص المواد غير القابلة للتصبن و تقدير تركيبها باستخدام جهاز التحليل الكروماتوجرافي الغازي وتحليل طيف الكتلة. أظهرت النتائج أن مركب البيتا- سيتوستيرول يمثل المركب الرئيسي (٦٤,٨ %) بالنسبة لإجمالي الجزء الاستيرولي ، يليه مركب الكامبستيرول (١٣,٨%)، بينما يمثل مركب ٢٤- ميثيل سيكلو أرثينول المركب الرئيسي (٤٨,٨%) بالنسبة لإجمالي الجزء الكحولي، يليه مركب سيكلو أرثينول(٣٥,٩%).

تم اختبار قدرة المواد غير القابلة للتصبن المستخلصة من زيت بذور التين الشوكي كمضادات أكسدة طبيعية لتقليل معدل أكسدة زيت دوار الشمس من خلال تتبع رقم البيروكسيد. و قد سجلت المواد غير القابلة للتصبن نشاطا مضادا للأكسدة خاصة عند استخدامها بتركيز ١٢٠٠ جزء في المليون حيث أعطت نفس النتائج المتحصل عليها باستخدام BHA بتركيز ٢٠٠ جزء في المليون، بينما استخدام تلك المواد بتركيز ١٥٠٠ جزء في المليون أعطي تأثيرا مضادا للأكسدة يفوق ذلك المتحصل عليه باستخدام BHA.

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