

## Chemical Composition of Okra Seeds and Some Physico-Chemical Characteristics of Extracted Oil

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### ABSTRACT

Okra (*Abelmoschus esculentus* L.) seeds were analyzed and characterized in terms of physical properties, chemical composition, mineral content and some oil characteristics. Seeds were found to be a good source of crude oil and protein being 15.20% and 24.32% on dry weight basis, respectively. The seeds possessed high content of crude fiber and could be considered as a good source of K, Na, Mg and Ca. The extracted oil was slightly high in saponification value and relatively low in iodine value, so it could be classified as a semi – dry oil. In addition, the crude oil showed low peroxide and acid values indicating its high stability against deterioration. Total lipid fractions of okra seed oil consist mainly of nine classes in which triacylglycerols being the major class. Palmitic, oleic and linoleic acids were the major fatty acids and constituted about 90.71% of the total fatty acids.

**Keywords:** Okra seeds, physical properties, chemical composition, oil characteristics.

### INTRODUCTION

The okra plant (*Abelmoschus esculentus* L. Moench) is one of the most widely known and utilized species of the family Malvaceae (Calisir *et al.*, 2005). This plant is an economically important vegetable crop grown in tropical and subtropical parts of the world. It is widely distributed from Africa to Asia, in Southern European, the Mediterranean and all of the America (Oyelade *et al.*, 2003, Andras *et al.*, 2005). Okra is known by many local names in different parts of the world. It is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spain, guibeiro in Portuguese, bhindi in India and Pakistan, banya in Turkey and bamyah in Egypt (Adams, 1975, Camciuc *et al.*, 1998, Anwar *et al.*, 2010). Okra is cultivated for its fibrous green fruits or pods containing round seeds. The fruits are harvested when immature and eaten as a vegetable. Its water extract contains thick slimy polysaccharides (mucilage) and is used to thicken soups and stews (El-Mahdy & El-Sebaiy, 1984, 1988, Sengkhamparn *et al.*, 2009).

In Syria, Egypt, Greece, Iraq, Jordan, Lebanon, Turkey and Yemen, and other parts of the Eastern Mediterranean including Cyprus, okra is widely used in a thick stew made with vegetables and meat. In Indian and Pakistani cooking it is sautéed or added to gravy-based preparations and is

very popular in North India and Pakistan. In Western parts of India, okra is one of the most popular vegetables and is often cooked in daily meals. Generally, okra is stir-fried with spices and some sugar. In Caribbean islands, okra is cooked and eaten as soup, often with fish. In Haiti, it is cooked with rice and maize; It is also used as a sauce for meat. It became a popular vegetable in Japanese cuisine toward the end of the 20<sup>th</sup> century, served with soy sauce and *Katsuobushi* or as *tempura*. It is used as a thickening agent in gumbo, served in the Southern United States. The immature pods may also be pickled, chilled, frozen, dried and canned for later use (Calisir *et al.*, 2005, Amin *et al.*, 2007). Okra is said to be very useful against genito-urinary disorders, spermatorrhoea and chronic dysentery. Its medical value has been reported in caring ulcers and relief from hemorrhoids (Adams, 1975).

The seeds can be ground and sifted to yield a high-protein, high-oil product of considerable nutritional value. The meal can be used as partial substitute for wheat flour in conventional oven-baked products.

The oil appears to be as good as cottonseed oil for food purposes, and contains considerably less gossypol and cyclopropenoid fatty acids (Martin & Ruberte, 1979, Martin *et al.*, 1979, Savello *et al.*, 1980, Telek & Martin, 1981).

It has been reported that the seeds of mature

okra pods sometimes used for poultry and animal feed and also consumed after roasting as a coffee substitute. Also okra seeds have been used on a small scale for oil production (Crossley & Hilditch, 1951, Savello *et al.*, 1980, Rubatzky & Yamaguchi, 1997, Oyelade *et al.*, 2003, Andras *et al.*, 2005). In addition, Anwar *et al.* (2010) found that okra seed oil is an acceptable feedstock for biodiesel production.

In Egypt, usually three harvesting are taken from okra plant, while the third one is left due to uneconomic impact. So, huge amounts of okra seeds are wasted every year.

Little attention has been paid with regard to Egyptian okra seeds as unexploited source of oil and protein. El-Katib (1947) used okra flour as a supplement to corn flour to improved dough quality. Abdalla & Melton (1991) studied the chemical composition of two popular okra varieties (Balady & Tourky) in Egypt.

In the present study, whole dry okra seeds of one popular Egyptian variety (*Eskandarani*) were used to evaluate their physical properties and chemical composition including proximate composition, total gossypol, cyclopropenoid fatty acids, total phenolic compounds, total tocopherols and antioxidant activity. Moreover, some physico-chemical characteristics of the crude oil extracted from the seeds were also evaluated. Such knowledge will be of great interest in evaluating the nutritional value of okra seeds and its extracted oil.

## MATERIALS AND METHODS

### Materials:

The famous Egyptian variety (*Eskandarani*) of okra (*Abelmoschus esculentus* L.) seeds was obtained from the Department of Vegetable Crops, Faculty of Agriculture, Alexandria University, Alexandria, Egypt (Season, 2012). The foreign matters and infested seeds were removed manually. Cleaned okra seeds were milled using Kenwood mixer (Model BL 350, PK 100/ AD, England). The milled flour was sieved through a 40 mesh sieve and the powder was packed in Kilner jars and kept at 4°C until analysis.

### Methods:

#### Physical methods

Physical properties of okra seeds including weight of seeds, 100 seed mass, seed dimensions

(length, width and thickness), bulk density, hull and kernel percentages were determined according to the methods reported by Calisir *et al.* (2005).

#### Chemical methods

Proximate analysis of okra seed flour including moisture, crude protein ( $N \times 6.25$ ), crude ether extract, crude fiber and total ash was carried out according to the AOAC (2003) procedures unless otherwise stated. Nitrogen-free extract was calculated by difference. Total gossypol was determined by the AOCS (1997). Total tocopherols were determined colourimetrically using a rapid method described by Tsen (1961). Phenolic substances as mg gallic acid equivalent (GAE/100g) was assayed colourimetrically according to the method of Hagerman & Butler (1978). Cyclopropenoid fatty acids were measured by the modified method of Bailey *et al.* (1966). Antioxidant activity was measured by the N,N-dimethyl-P-phenylenediamine dihydrochloride (DMPD) according to Fogliano *et al.* (1999).

Minerals (Fe, Cu, Mg, Ca, Mn, Zn, Cd and Pb) were measured as described in the AOAC (2003) using Perkin Elmer Atomic Absorption Spectrophotometer (Model 2380). On the other hand, Na and K were determined using flame photometer (Model PE P7, England).

#### Physico-chemical characteristics of okra seed oil:

A portion of the okra seed powder was subjected to extraction with chloroform: methanol (2:1v/v) according to the procedure of Folch *et al.* (1957). Solvent was removed in a rotary evaporator at 40°C and the oil was flushed with nitrogen gas and stored in a sealed glass at -18°C until analysis.

Refractive index (RI) at 25°C, specific gravity (sp. Gr) at 25°C, iodine value (IV), saponification value (SV), peroxide value (PV as  $mEqO_2/kg$  oil), unsaponifiable matter (%) and colour (in. cell) of okra seed oil using a Lovibond tintometer (Model E, England) were determined as described in AOCS (1997). Total lipid extract was fractionated into different classes using the TLC technique according to the method of Mangold & Malins (1960) on glass plates (20 × 20 cm) precoated with 0.25 mm silica gel G-60. The developing solvent system used was petroleum ether: diethyl ether: glacial acetic acid (70 : 30 : 2 v/v/v). After running, the plate was air dried and the separated spots were

visualized by iodine vapour. Lipid classes were identified by their  $R_f$  values according to Rahma & Abd El-Aal (1988).

Fat sample was extracted using petroleum ether and then the methyl ester was prepared using methanol-  $H_2SO_4$  acid. Gas chromatographic analysis was carried out using ACME model 6100 GC (Young LIN Instrument Co., Korea) fitted with a split/ splitless injector and FID detector. Nitrogen was used as the carrier gas with a flow rate of 0.5 ml/ min. The components were separated on a 30-m Sp- 2380 fused – silica capillary column with a 0.25 – mm i.d. and 0.2 -  $\mu$ m film thickness (Supelco Bellefonte, PA). The detector temperature was set at 260°C. The injector temperature was set at 220°C and in split mode (Split ratio 80 : 1). The column was initially maintained at 140°C for 5 min, and the temperature was subsequently increased to 240°C at a rate of 4°C/ min, (Total program time was 30 min). Standard fatty acid methyl esters were used for identification.

#### Statistical analysis:

Mean values and the standard deviations were calculated using the method described by Steel & Torrie (1980).

## RESULTS AND DISCUSSION

#### Physical properties of okra seeds:

As shown in Table (1), weight of seed (g), seed index (g/ 100 seeds) and bulk density (g/  $cm^3$ ) were 0.06, 5.67 and 0.55, respectively. Comparing with some oil seeds, it has been reported that both seed index and bulk density of okra seeds were lower than those of sunflower (Abd El-Aal, 1976), safflower (Asker *et al.*, 1994), water melon and pumpkin seeds (Datta & Lal, 1977, Abd El-Aal & Rahma, 1988). The data showed that okra seed dimensions (length, width and thickness) were 5.35, 4.88 and 4.24 mm, respectively. The percentages of hulls and kernels of okra seeds were 41.53 and 58.47%, respectively. It has been reported that the percentages of kernels of water melon, grape, sunflower, cantaloupe and safflower seeds were 52.3, 40, 44 – 57, 31.19 and 52 – 72%, respectively (Abd El-Aal & Rahma, 1988, Kamel *et al.*, 1985, Abd El Aal 1976, Abdel-Nabey & Attia, 1998 and Asker *et al.*, 1994). On the other hand, the results of the physical properties obtained here agree well with those reported by Martin & Rhodes (1983), Oyelade *et*

*al.* (2003) and Calisir *et al.* (2005).

**Table 1: Physical properties of okra seeds**

Property	Value*
Weight of seed (g)	0.06 ± 0.001
Seed index (g/ 100 seeds)	5.67 ± 0.106
Bulk density (g/ $cm^3$ )	0.55 ± 0.168
Seed dimensions:	
Length (mm)	5.35 ± 0.007
Width (mm)	4.88 ± 0.004
Thickness (mm)	4.24 ± 0.003
Hulls %	41.53 ± 1.378
Kernels %	58.47 ± 1.36

\* Mean of three determinations ± S.D.

#### Chemical composition of okra seed flour:

Data in Table (2) show the proximate chemical composition of okra seed flour. It can be noted that the flour had high concentration of crude protein and crude ether extract being 24.32 and 15.20%, respectively. On the other hand, crude fiber and nitrogen free extract were 25.55 and 30.93%, respectively. The high content of crude fiber is mainly due to the higher value of hulls as mentioned in Table (1). Thus, removal of hulls would reduce the fiber content and thus concentrate both oil and protein and also improve the efficiency of oil extraction. The data in Table (2) reveal also that okra seed flour contained 4.00% total ash. The results of the proximate chemical composition of okra seed flour in the present study are in accordance with those reported by (Karakoltsidis & Constantinides, 1975, Savello *et al.*, 1980, Martin & Rhodes, 1983, Aminigo & Akingbala, 2004, Calisir *et al.*, 2005, Jarret *et al.*, 2011, Anwar *et al.*, 2011, Soares *et al.*, 2012).

Okra seed flour contained lower amounts of total gossypol as well as cyclopropenoid fatty acids being 0.003 and 1.05%, respectively. Gossypol, a toxic phenolic compound present in cottonseed was not found in significant amounts in okra seeds (Table 2). It has been reported that much higher levels of 0.015 – 0.020% of free gossypol can cause toxicity symptoms and death to animals (Berardi & Goldblatt, 1980). Cyclopropenoid fatty acid content in the common okra varieties is negatively correlated with protein content and to a lesser extent with kernel fraction and seed weight (Martin & Rhodes, 1983). They reported that cycloprope-

noid fatty acid content varied between 0.26% and 5.59%. The results obtained in the present study are in accordance with those reported by Al-Wandawi (1983), Abdalla & Melton (1991), Andras *et al.* (2005) and Anwar *et al.* (2011).

The results in Table (2) show that okra seed flour contained considerable amounts of phenolic compounds and low level of total tocopherols being 25 mg GAE/ 100 g and 0.07%, respectively. These results are in good agreement with those reported by Adetuyi & Komolafe (2011). It has been reported that okra seeds contain different type of phenolics. These compounds appear to have anti-cancer and antioxidant effects in human body (Rao, 1985, Arapitsas, 2008). With regard to tocopherol content, Karakoltsidis & Constantinides (1975) mentioned that tocopherol content in okra seeds was 30.40 mg/ 100 g. On the other hand, Abdalla & Melton (1991) found that the total tocopherol content of two Egyptian okra seed varieties varied from 0.042 to 0.048%. The results obtained here are in accordance with those reported by Calisir *et al.* (2005) and Anwar *et al.* (2011). Since the tocopherols are antioxidants and  $\gamma$ -tocopherol is about three times as active as  $\alpha$ -tocopherol, it might be expected that okra seeds having the highest content of  $\gamma$ -tocopherol (Anwar *et al.*, 2011) and would be very stable.

**Table 2: Chemical composition of okra seed flour**

Component	Value*
Moisture (%)	10.16 $\pm$ 0.075
Crude Protein (%)	24.32 $\pm$ 0.454
Crude ether extract (%)	15.20 $\pm$ 0.176
Total ash (%)	4.00 $\pm$ 0.112
Crude fiber %	25.55 $\pm$ 0.211
N-free extract** (%)	30.93 $\pm$ 0.321
Total gossypol (%)	0.003 $\pm$ 0.001
Cyclopropenoid fatty acids (%)	1.05 $\pm$ 0.003
Phenolic Substances (mg GAE/ 100g)***	25.00 $\pm$ 0.003
Total tocopherols (%)	0.07 $\pm$ 0.001
Antioxidant activity (%)	49.00 $\pm$ 1.00

\* Mean of three determinations  $\pm$  S.D. (On dry weight basis)

\*\* By difference

\*\*\* Gallic acid equivalent

Table (2) shows that okra seed flour had a relatively high percentage of antioxidant activity be-

ing 49%. These results confirmed the possibility of using okra seed flour as an antioxidant source. These results agree well with those of Adalakun *et al.* (2009) who found that the antioxidant activity of okra seed flour was 48.34%. On the other hand, Adetuyi & Komalafe (2011) found that the addition of okra seed flour to plantain flour increased the antioxidant activity of the resultant fortified plantain flour.

### Mineral contents of okra seed flour:

Elemental compositions of okra seed flour are shown in Table (3). The elements of K, Na, Mg and Ca were the major elemental constituents, while Fe, Mn, Zn and Cu were present in the range of 11.71 to 16.50 mg/ 100g of okra seed flour. Other elements such as Cd and Pb are found in the range of 0.11 to 1.48 mg/ 100g, respectively. These results are similar to those reported by Savello *et al.* (1980), Al-Wandawi (1983) and Rao (1985).

**Table 3: Mineral contents of okra seed flour**

Element	mg/ 100g*
K	1590.06 $\pm$ 7.400
Mg	820.79 $\pm$ 3.265
Na	508.64 $\pm$ 2.260
Ca	202.31 $\pm$ 1.213
Zn	16.07 $\pm$ 0.242
Fe	16.00 $\pm$ 0.187
Cu	16.50 $\pm$ 0.096
Mn	11.71 $\pm$ 0.065
Cd	0.11 $\pm$ 0.006
Pb	1.48 $\pm$ 0.008

\* Mean of three determinations  $\pm$  S.D. (On dry weight basis)

It has been reported by Adalakun *et al.* (2010) and Adalakun *et al.* (2012) that soaking of okra seeds in water reduced all investigated minerals which are time dependant. Blanching process reduced all minerals except Mg. On the other hand, malting process reduced P, K, Mg and Fe, while increase in Ca, Na, Zn and Mn were observed. Roasting of okra seeds increased all the minerals except P and Mg.

### Physico-chemical characteristics of okra seed oil

The physico-chemical characteristics of the oil extracted from okra seeds are presented in Table

(4). The crude oil had a light yellow colour and acceptable odour. It was liquid at room temperature and had low free fatty acids (1.07 as % oleic acid) and unsaponifiable matter (0.635%). Values of refractive index and specific gravity are within the range for most crude vegetable oils (Swern, 1979). The oil was slightly high in saponification value (188.62) and relatively low in iodine value (103.25). Thus, okra seed oil could be classified as a semi-dry oil such as cottonseed, sunflower, and corn oils. The crude oil showed low peroxide and acid values being 0.984 m Eq O<sub>2</sub>/ kg oil and 2.13 indicating its high stability to deterioration. The intensity of the colour of crude okra seed oil (35.0 Y, 3.6 R and 0.2 B), which was mainly due to the extraction of pigments from the seeds, however it was quite similar to the other crude vegetable oils (Pham *et al.*, 2002, Andras *et al.*, 2005, Anwar *et al.*, 2005, Anwar *et al.*, 2011).

**Table 4: Physico-chemical properties of okra seed oil**

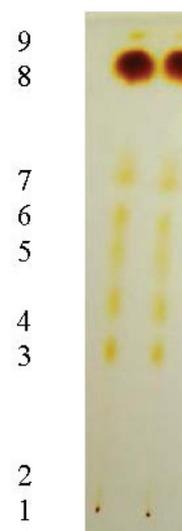
Property	Value*
Refractive index (25/ 25°C)	1.4635 ± 0.004
Specific gravity (25°C)	0.9035 ± 0.03
Free fatty acids (as % oleic acid)	1.07 ± 0.12
Acid value	2.13 ± 0.15
Peroxide value (m EqO <sub>2</sub> / kg oil)	0.984 ± 0.23
Iodine value	103.25 ± 1.85
Saponification value	188.62 ± 2.16
Unsaponifiable matter (%)	0.635 ± 0.04
Lovibond colour ( in. cell)	
Yellow	35.0 ± 0.0
Red	3.6 ± 0.01
Blue	0.2 ± 0.01

\* Mean of three determinations ± S.D.

### Lipid classes of okra seed oil

The results of the fractionation of the total lipid classes of okra seed oil are shown in Fig. (1). The total lipids of okra seed oil consisted mainly of 8 fractions of acylglycerols and non acylglycerol components in addition to the polar lipid class located on the base line. Triacylglycerols class was found to be the major fraction of okra seed oil. The other classes can be arranged, based on their R<sub>F</sub>, as follows: monoacylglycerols, 1, 2 and 2, 3 diacylglycerols, sterols, 1, 3 diacylglycerols, unknown, free fatty acids, triacylglycerols, hydrocarbons and

sterolesters based on the front line. These results are in accordance with those reported by Sengupta *et al.* (1974) and Abdalla & Melton (1991). The same trend was found in some unexploited sources of crude oil such as safflower seed oil (Asker *et al.*, 1994), grape seed oil (Abou Rayan *et al.*, 1998), cantaloupe seed oil (Abdel-Nabey & Attia, 1998), date pits oil (Abdel-Nabey, 1999), prickly pear oil (Abdel-Nabey, 2001) and flaxseed oil (Abdel-Nabey, *et al.*, 2013).



**Fig. 1: Thin layer chromatogram of total lipids of okra seed oil**

Coating material: Silica gel G Merk type 60

Solvent system: Petroleum ether: diethyl ether: glacial acetic acid (70 : 30 : 2 v/v/v)

Detection: Exposure to iodine vapour

- |                                  |                                  |
|----------------------------------|----------------------------------|
| 1- Polar lipids                  | 6- Unknown                       |
| 2- Monoacylglycerols             | 7- Free fatty acids              |
| 3- 1, 2 and 2, 3 diacylglycerols | 8- Triacylglycerols              |
| 4- Sterols                       | 9- Hydrocarbons and sterolesters |
| 5- 1, 3 diacylglycerols          |                                  |

### Fatty acid composition of okra seed oil

Fatty acid composition of okra seed oil is presented in Table (5). The unsaturated fatty acids represented 67.71% of the total fatty acids. The main unsaturated fatty acids were linoleic (37.6%) followed by oleic acid (23.37%) with trace amounts of palmitoleic, linolenic, C20:1 and C22:1. On the other hand, the saturated fatty acids represented 31.24% of the total fatty acids. The saturated fatty acids were found to be composed mainly of palmitic acid (29.74%) followed by stearic acid (5.29%) and trace amounts of C12: 0, C14: 0, C17: 0 and C22: 0.

**Table 5: Fatty acid composition of okra seed oil**

Fatty acid	%*
C12 : 0	0.44 ± 0.02
C14 : 0	0.26 ± 0.01
C16 : 0	29.74 ± 0.23
C16 : 1	0.34 ± 0.01
C17 : 0	0.33 ± 0.02
C18 : 0	5.29 ± 0.09
C18 : 1	23.37 ± 0.22
C18 : 2	37.60 ± 0.52
C18 : 3n3	0.40 ± 0.03
C20 : 1	0.57 ± 0.01
C22 : 0	0.47 ± 0.02
C22 : 1	0.14 ± 0.01
Other	1.05 ± 0.02
TSFA (S)**	31.24 ± 0.42
TUFA (U)***	67.71 ± 0.56
U/ S ratio	2.17 ± 0.24

\* Mean of three determinations ± S.D.

\*\* Total saturated fatty acids

\*\*\* Total unsaturated fatty acids

Some reports in the literature showed that okra seed oil contained a variety of fatty acids with linoleic, palmitic, oleic and stearic acids predominating (Pham *et al.*, 2002, Andras *et al.*, 2005 & Anwar *et al.*, 2011). With regard to the fatty acid composition, okra seed oil was similar to corn oil and cotton seed oil, while corn oil had a lower percentage of stearic acid, and cotton seed oil had higher percentage of palmitic acid. The presence of high content of unsaturated fatty acids especially linoleic acid (an essential fatty acid), proved to be a highly nutritious oil and can be a good substitute for sunflower and corn oils for use in diets intended to reduce high level of blood cholesterol.

As shown in Table (5), the ratio of unsaturated to saturated fatty acids was 2.17 : 1. This ratio agreed well with that reported by (Karakoltsidis & Constantinides, 1975, Savello *et al.*, 1980, Al-Wandawi, 1983, Rao, 1985, Abdalla & Melton, 1991, Anwar *et al.*, 2005, Jarret *et al.*, 2011, Soares *et al.*, 2012).

As a conclusion on the basis of the oil composition and its characteristics, okra seed oil can be used as a good source for essential fatty acid (C18 : 2 ω6), besides okra seed can be considered as a good source of protein especially in the de-

hulled and defatted form. Therefore, further study is required to investigate amino acid composition, digestibility, functional properties and the application of the dehulled and defatted okra seed meal in some food products.

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## التركيب الكيماوى لبذور الباميا وبعض الخواص الطبيعية والكيماوية للزيت المستخلص

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تم دراسة بعض الصفات الطبيعية والتركيب الكيماوى ومحتوى المعادن وخواص وتركيب زيت بذور الباميا. ولقد وجد أن بذور الباميا تعتبر مصدراً جيداً لكل من البروتين والزيت حيث أحتوت البذور على ١٥,٢٠٪ زيت خام، ٢٤,٣٢٪ من البروتين على أساس الوزن الجاف. كذلك أحتوت البذور على نسبة عالية من الألياف الخام ويمكن اعتبارها مصدراً لبعض العناصر المعدنية مثل البوتاسيوم، الصوديوم، الماغنسيوم والكالسيوم. أثبتت الدراسة أن زيت بذور الباميا له رقم تصبن عال نسبياً ورقم يودى منخفض نسبياً ويمكن تصنيفه على أنه زيت نصف جاف. كذلك أظهرت الدراسة أن محتوى الزيت الخام منخفض من البيروكسيدات والأحماض الدهنية الحرة مما يدل على ثباته العالى ضد التدهور. أحتوت لبييدات بذور الباميا على تسعة أقسام ومثل فيها ثلاثى أسايل الجليسرولات المكون الرئيسى. أظهرت الدراسة أن حمض البالمتيك بالإضافة إلى حمض الأوليك، اللينوليك كانت الأحماض السائدة فى زيت بذور الباميا وتمثل حوالى ٩٠,٧١٪ من الأحماض الدهنية الكلية.

