INTER-VARIETAL VARIATION IN THE COMPOSITION OF OKRA (*HIBISCUS ESCULENTUS* L.) SEED OIL

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Abstract

The seeds from two varieties namely Sabz Pari and Punjab-8 of Okra (*Hibiscus esculentus*), grown under similar environment, exhibited oil content 11.72 and 13.42%, respectively. Protein, fiber, moisture and ash contents were found to be 20.00, 29.60, 7.26 and 5.18 and 23.68, 27.41, 8.35 and 6.23, respectively. The physicochemical characteristics of the extracted oils were as follows: iodine value, 111.6-114.9; refractive index (40°C), 1.4620-1.4640; density (24°C), 0.904-0.908 gcm⁻³, saponification value, 180.3-185.8 (mg of KOH g⁻¹of oil); unsaponifiable matter, 0.61-0.65%; color (1-in. cell), 3.40-7.00 R + 34.00-70.00 Y; acid value, 3.49-4.67 (mg of KOH g⁻¹ of oil); peroxide value, 7.29-8.47 meq kg⁻¹. Tocopherols (α , γ and δ) contents of the oils accounted for 653.0-696.5, 2.13-3.33 and 1.01-1.11 mg kg⁻¹, respectively. The major fatty acids of the tested oils were: linoleic acid (29.90-31.70%), palmitic acid (29.50-31.20%), oleic acid (26.69-28.19%) and stearic acid (4.90-6.10%). A small amount of cylcopropaneoctanoic acid with contribution up to 2.0% was also established. Most of the studied parameters of Sabz Pari and Punjab-8 *H.esculentus* seed oils were quite comparable with those of typical *Hibiscus* seed oils reported in the literature.

Introduction

Okra [*Hibiscus esculentus* L. (syn. *Abelmoschus esculentus* L. Moench)] is one of the most widely known and utilized species of the family Malvaceae (Bayer & Kubitzki, 2003; Naveed *et al.*, 2009). Okra, a tropical to subtropical flowering plant, 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, commonly known as "lady finger", is primarily a vegetable crop grown for its immature pods that can be consumed as a fried or boiled vegetable or may be added to salads, soups and stews (Crossley & Hilditch, 1951; Kashif *et al.*, 2008). The crop grows well in hot weather, especially in the regions with warm nights (>20 °C) (Ndunguru & Rajabu, 2004). In Pakistan, okra (locally known as "*bhindi*") is represented by one species: *H. esculentus.* It is widely grown in the plain areas of the country, particularly in the provinces of Punjab and Sindh, and is valued as a source of good income for the growers. Total area under okra cultivation in Pakistan is estimated to be 2.21×10^5 hectares yielding about 2.86×10^6 tons of green pods (Kashif *et al.*, 2008).

The seeds from fully mature and ripened okra pods are sometimes used for chicken feed. These have been used on a small scale for the production of oil (Oyelade *et al.*, 2003) and some time consumed after roasting as a coffee substitute (Crossley & Hilditch, 1951). The seeds, exhibiting antispasmodic and sedative effects, have also been in use as an aid to digestion and as nervine (Crossley & Hilditch, 1951).

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Some previous studies revealed the potential of okra seed as a source of oil and protein for both the temperate and tropical regions (Crossley & Hilditch, 1951; Rubatzky & Yamaguchi, 1997; Oyelade *et al.*, 2003). According to Andras *et al.*, (2005), oil concentration of okra seeds from Greece was found to be 15.9 to 20.7%, depending on the extraction method. The oil was found to contain a high level of linoleic acid (up to 47.4%) and tocopherols isomers. Savello *et al.*, (1980) also reported that okra seed oil is a rich source of linoleic acid, a polyunsaturated fatty acid essential for human nutrition.

As the demand for vegetable oils is rapidly increasing due to the growing human population and the expanding oleo-chemicals industry, the exploration of some nonconventional and newer resources of vegetable oils is of much concern. Okra, which is currently grown mainly as a vegetable crop, has potential for cultivation as an oilseed crop because its mature pods contain high quantity of seeds containing considerable amount of oil which could be characterized and utilized for commercial purposes. As far as we know, no comprehensive characterization and comparison of the oils produced from seeds of two varieties of okra (Sabz Pari and Punjab-8), commonly grown in Pakistan, have yet been reported. Therefore, the present research work was undertaken with the key objective to appraise and compare the detailed physico-chemical characteristics of seed oils of two varieties of okra cultivated under local environment.

Materials and Methods

Materials and reagents: The seeds of two locally grown varieties of okra (*H. esculentus*) namely Sabz Pari and Punjab-8 were obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Sabz Pari is a locally developed cultivar of okra whereas the origin of Punjab-8 is India. Both of the okra varieties were harvested under similar environmental conditions (same place and same time) at the experimental farms of the AAARI, Faisalabad, Pakistan in 2008. All reagents (analytical and HPLC) used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Standards of tocopherols [DL- α -tocopherol, (+)- δ -tocopherol, (+)- γ -tocopherol], and fatty acid methyl esters were obtained from Sigma Chemical Co. (St. Louis, MO).

Oil extraction: The crushed seeds (approximately 400 g) of okra were placed in a Soxhlet apparatus and then extracted with *n*-hexane for 6 h on a water bath. Excess solvent was removed under vacuum in a rotary evaporator (Eyela, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan) at 45°C. The resulting oil was stored at 4°C until further analyzed.

Analysis of oilseed residues: The remaining oilseed meal after oil extraction was analyzed for protein, fiber, and ash contents. Protein content (N×6.25) was determined following the AOAC method 954.01 (AOAC, 1990). Fiber content was determined according to the ISO method 5498 (ISO, 1981). Measurement of ash content was followed by the ISO method 749 (ISO, 1997).

Analysis of extracted oils

Physical and chemical parameters of oils: Determinations of density, refractive index, iodine value, peroxide value, acidity, saponification value and unsaponifiable matter of the extracted oil were carried out following AOCS official methods Cc 10a-25, Cc 7-25, Cd 1-25, Cd 8-53, F 9a-44, Cd 3-25 and Ca 61-40, respectively (AOCS, 1997).

CHARACTERIZATION OF OKRA SEED OIL

Tocopherol content: Tocopherols were analyzed by D-2500 Hitachi HPLC in normal phase mode (Hitachi, Tokyo, Japan) following a method of Current Protocols in Food Analytical Chemistry (Wrolstad, 2003). A 20- μ L sample was injected into a Supelcosil LC-Si column (250 × 4.6 mm, Supelco Inc., Supelco Park, Bellefonte, USA). A mobile phase of ethyl acetate/acetic acid/hexane (1:1:198, v/v/v) was used at the flow of 1.5 mL min⁻¹. The detector monitored UV absorbance at 295 nm. Tocopherols were identified by comparing their retention times with those of pure standards (Sigma Chemical Co.) and quantification was based on an external standard calibration for each isomer separately.

GC/MS fatty acid composition: Okra seed oils were transmethylated into fatty acid methyl esters (FAMEs) following a standard IUPAC method 2.301 (IUPAC, 1987). FAMEs were analyzed by gas chromatography/mass spectrometry (GC/MS), using Agilent-Technologies (Little Falls, CA, USA) 6890N Network GC system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto injector. The separation was performed on Agilent Technologies capillary column HP-5MS (30 m × 0.25 mm; film thickness 0.25 μ m). A sample volume of 1.0 μ L was injected into the column with split ratio 100:1. The carrier gas used was Helium at a flow rate of 1.2 mL min⁻¹. The column temperature was programmed from 150°C to 250°C at a linear ramp rate of 4°C min⁻¹, while the initial and final hold up time was 1 and 5 min, respectively. An electron ionization mode, with ionization energy of 70 eV, was used for GC/MS detection. Injector and MS transfer line temperature were set at 250°C and 260°C, respectively. The scanning mass range was selected from 30-550 *m/z* (mass-to-charge ratio). The unknown FAMEs were identified on the basis of matching of their relative

The unknown FAMEs were identified on the basis of matching of their relative retention times with those of standards of FAMEs (Sigma Chemical Co., St Louis, MO, USA). FAMEs were further identified and authenticated using their MS spectra compared to those from the NIST mass spectral library of the GC/MS system.

Statistical analysis: Three different seed samples of each of the okra varieties (Sabz Pari and Punjab-8) were assayed and analyzed individually in triplicate. Data is reported as mean $(n = 1 \times 3 \times 3) \pm SD$. For all investigated parameters, the analysis of variance (ANOVA) was used to determine significant differences between groups, considering a level of significance of less than 5% (*p*<0.05). A statistical software STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) was used.

Results and Discussion

The data from the analysis of *H. esculentus* seeds and oils of the two varieties, together with literature values of oils of identical *Hibiscus* species have been summarized in Tables 1-4. As far as the oil yield of *H. esculentus* seeds is concerned, there was a significant (p<0.05) variation of oil content between Sabz Pari and Punjab-8 varieties of seeds (Table 1). The oil concentration was high (13.42%) in the seed samples of the Indian-based Punjab-8 variety, whereas, the locally developed Pakistani variety (Sabz Pari) had lower oil content i.e., 11.72%. The variation observed in the *H. esculentus* seeds of the investigated varieties with regard to oil content might have been due to different genetic makeup of the varieties. Similarly, variation of seed oil content (11.72 to 13.42%) of the investigated *H. esculentus* seeds of Sabz Pari and Punjab-8 varieties

grown in Pakistan was considerably higher than that reported (8.21%) for *H. esculentus* seeds from Turkey (Calisir *et al.*, 2005), however the present values were lower than that reported for *H. esculentus* seeds (16.31%) from Greece (Andras *et al.*, 2005).

The analysis of oilseed residue revealed a significant (p<0.05) variation in seed protein contents of the Sabz Pari (20.0%) and Punjab-8 (23.6%) varieties of *H. esculentus*. The protein content (20.00-23.68%) in the present analysis of *H. esculentus* oilseeds were higher than those (19.10%) reported earlier (Calisir *et al.*, 2005). The present analysis showed the okra seed meal to be a good source of protein, with potential utilization as an ingredient in animal feed.

The contents of fiber (27.4-29.6%) and moisture (7.2-8.3%) for the seeds of the investigated *H.esculentus* varieties were not significantly (p>0.05) varied with each other. The ash content (5.2-6.2%) varied significantly (p<0.05) between the varieties investigated. Overall, the values of protein, fiber and ash contents in Sabz Pari *H. esculentus* seed were higher than those reported for Turkish *H. esculentus* seed (Calisir *et al.*, 2005).

Physical and chemical parameters of the investigated *H. esculentus* seed oils are presented in Table 2. No significant (p>0.05) differences were observed in the physical and chemical characteristics of the seed oils of Sabz Pari and Punjab-8 varieties of *H. esculentus*, except for color and peroxide value (PV). The iodine value (IV) (111.6 g of iodine/ 100 g of oil) of the *H.esculentus* seed oil of Sabz Pari was somewhat lower than that of Punjab-8 variety (114.9 g of iodine/100 g of oil). The present iodine values of the *H. esculentus* seed oils were lower than those reported for okra seed oil from Los Baños (120 g of iodine/100 g of oil) (Pham *et al.*, 2002).

The saponification values (180–185 mg of KOH g⁻¹of oil) were higher than those (171) for *H. esculentus* seed oils from Los Baños (Pham *et al.*, 2002). Unsaponifiable matter (0.61-0.65%) of the seed oils tested was lower than that (0.81%) of *H. esculentus* seed oils reported in the literature (Pham *et al.*, 2002). The information on the data for refractive index at 40°C (1.4620-1.4640), density at 25°C (0.904 and 0.908 g cm⁻³) and acid value (mg of KOH g⁻¹of oil) 3.49-4.67% for *H. esculentus* seed oils of the Sabz Pari and Punjab-8 varieties are reported for the first time. The intensity of color (3.40-7.00 R + 34.00-70.00 Y), which is mainly due to the extraction of pigments from seeds, of the investigated *H.esculentus* oils was higher than that of another non-conventional vegetable oil i.e., *Moringa oleifera* oil (Anwar *et al.*, 2005). The peroxide value (7.29-8.47 m.eq kg⁻¹ of oil), which measures hydroperoxide products of the oils (McGinely, 1991), of the investigated *H. esculentus* seed oils was slightly higher than that reported (6.7) for *Hibiscus* oil previously (Pham *et al.*, 2002).

Table 3 shows the contents of different tocopherols in the tested *H.esculentus* seed oils. The levels of α -, γ -, and δ -tocopherols in the non-degummed *H.esculentus* seed oil of Sabz Pari variety were 696, 3 and 1 mg kg⁻¹, respectively, whereas those in the Punjab-8 variety were 653, 2 and 1 mg kg⁻¹, respectively. The content of γ -tocopherol of the *H.esculentus* seed oil of Sabz Pari variety was significantly (p<0.05) higher than the Punjab-8 variety. However, no significant (p>0.05) variation was observed in the contents of α -, and δ -tocopherols between the oils from the two varieties. The present content of α -tocopherol in the seed oil was slightly lower but the concentration of γ tocopherol was almost comparable to that reported for *H. esculentus* seed oil from Greece (Andras *et al.*, 2005).

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Constituents	Sabz Pari	Punjab-8	P Value	Calisir et al., (2005)
Oil content (%)	11.72 ± 0.22	13.42 ± 0.19	0.000	8.21
Moisture (%)	7.26 ± 0.63	8.35 ± 0.48	0.076	6.35
Fiber (%)	29.60 ± 1.19	27.41 ± 1.25	0.093	26.34
Ash (%)	5.18 ± 0.16	6.23 ± 0.20	0.002	4.63
Protein (%)	20.00 ± 0.75	23.68 ± 0.61	0.003	19.10
*Values are mean \pm SE	, for three seed sample	s of each Okra variet	v. analyzed indi	ividually in triplicate.

Table 1. Proximate analysis of two varieties of Okra seeds*.

Tabla 2	Dhysico chomical	charactoristics	of two variatios	of Okro cood oils*	

Physico-chemical characteristic	Sabz Pari	Punjab-8	P Value	Pham <i>et al.</i> , (2002)
Iodine value (g of I/100 g of oil)	111.6 ± 3.20	114.9 ± 2.50	0.190	120.51
Refractive index (40°C)	1.4620 ± 0.004	1.4640 ± 0.003	0.961	NR
Density (g/cm ³) 25°C	0.908 ± 0.02	0.904 ± 0.03	0.978	0.95
Saponification value(mg of KOH g ⁻¹ of oil)	180.3 ± 2.53	185.8 ± 3.80	0.105	170.82
Unsaponifiable mater (%)	0.66 ± 0.05	0.61 ± 0.04	0.248	0.82
Color (1" cell) (red Unit)	3.40 ± 0.12	7.00 ± 0.09	0.000	NR
Yellow unit	34.00 ± 2.70	70.00 ± 3.81	0.000	NR
Acid value (mg of KOH g ⁻¹ of oil)	3.49 ± 0.09	4.67 ± 0.08	0.061	NR
Peroxide value (m.eq kg-1 of oil)	7.29 ± 0.33	8.47 ± 0.47	0.024	6.71

 *Values are mean \pm SD for three Okra seed oils of each variety, analyzed individually in triplicate. NR = not reported

Tocopherols (mg kg ⁻¹)	Sabz Pari	Punjab-8	P Value	Calisir <i>et al.</i> , (2005)
α-tocopherol	696.46 ± 17.80	653.04 ± 30.01	0.097	780
γ-tocopherol	3.33 ± 0.15	2.13 ± 0.20	0.001	2.3
δ-tocopherol	1.01 ± 0.10	1.11 ± 0.05	0.196	NR
Total tocopherols	700.80	656.08		782.30

Table 3. Tocopherols contents of two varieties of Okra seed oils*.

*Values are mean ± SD for three Okra seed oils of each variety, analyzed individually in triplicate. NR = not reported

The content (653-696 mg kg⁻¹) of the major tocopherol component, α -tocopherol, of the investigated *H. esculentus* seed oils, was higher than that of soybean (99.5 mg kg⁻¹), maize (282 mg kg⁻¹), cotton seed (338 mg kg⁻¹) and palm (89 mg kg⁻¹) oils but comparable to that of sunflower oil (670 mg kg⁻¹) (Rossell,1991). The concentration of both of γ -, and δ -tocopherol in the tested *H. esculentus* seed oils was quite lower than those reported for commonly available vegetable oils such as cottonseed, groundnut, palm, sunflower and olive oils (Rossell, 1991). Tocopherols in vegetable oils are believed to protect inheriting polyunsaturated fatty acids from oxidation. α -Tocopherol has stronger vitamin E potency, whereas the δ -tocopherol possesses greater antioxidant activity than either γ -, β - or α -tocopherols (Ozcan *et al.*, 2005).

	RT ^b	Sabz Pari	Punjab-8	- Ala	Literature	ature
			20	LABING	Andras et al., (2005)	Pham et al., (2002)
Myristic acid	16.73	0.19 ± 0.04	0.22 ± 0.04	0.840	NR	0.29
Palmitic acid	20.90	31.20 ± 0.65	29.50 ± 0.70	0.915	32.50	32.23
almitoleic acid	20.35	0.30 ± 0.05	0.31 ± 0.06	0.631	NR	0.308
Margaric acid	22.72	0.20 ± 0.07	0.17 ± 0.06	0.781	NR	NR
Stearic acid	24.57	4.90 ± 0.15	6.10 ± 0.10	0.000	2.80	3.94
Oleic acid	24.20	28.19 ± 0.50	26.69 ± 0.79	0.006	16.1	30.13
inoleic acid	24.08	29.90 ± 0.80	31.70 ± 0.89	0.289	47.4	30.05
inolenic acid		QN	QN		1.2	0.38
Arachidic acid	28.87	0.87 ± 0.06	1.02 ± 0.05	0.003	NR	0.36
Behenic acid	36.16	0.30 ± 0.05	0.56 ± 0.09	0.003	NR	0.81
Erucic acid		ND	QN		NR	0.27
2-octyl-cyclopropaneoctanoic acid	26.13	2.00 ± 0.10	1.89 ± 0.10	0.288	NR	NR
2-hexyl-cyclopropaneoctanoic acid	22.23	0.20 ± 0.05	0.27 ± 0.03	0.633	NR	NR
Otherse	I	1.70 ± 0.10	1.25 ± 0.12	0.018	NR	NR
FA		37.66	37.57		35.3	37,64
MUFA		28.49	27.00		16.1	30.44
PUFA		29.90	31.70		48.4	30.43

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SFA saturated fatty acids, MUFA mono unsaturated fatty acids, PUFA poly unsaturated fatty acids

"Not identified fatty acid as group

ND = not detected NR = not reported

^bRetention times in minutes

The total tocopherol contents in the tested okra seed oil of Sabz Pari variety (700.8 mg kg⁻¹) and Punjab-8 variety (656.1 mg kg⁻¹), noted to be appreciably higher than those reported for groundnut (407.4 mg kg⁻¹) and palm (107 mg kg⁻¹) oils, were found to be lower than those of soybean (1549 mg kg⁻¹), maize (1423 mg kg⁻¹), cottonseed (787.2 mg kg⁻¹), canola (766 mg kg⁻¹) and sunflower (709 mg kg⁻¹) oils (Rossell, 1991).

Table 4 depicts the fatty acid composition (FAC) of the seed oils of Sabz Pari and Puniab-8 varieties of *H. esculentus* as analyzed by GC/MS (Fig. 1. typical GC/MS chromatogram). The seed oils of the investigated varieties mainly consisted of linoleic acid (29.90-31.70%) followed by palmitic, oleic and stearic acids with amounts of 29.50-31.20, 26.69-28.19 and 4.90-6.10%, respectively. A small quantity, ranging from 0.99-1.26%, of minor fatty acids including C14:0, C16:1, C17:0, C22:0 was detected. Also, the presence of cyclopropanoid fatty acids: 2-octyl- cyclopropaneoctanoic acid (1.89-2.00%) and 2-hexyl-cyclopropaneoctanoic acid (0.20-0.27%) was established. The cyclic fatty acids, dihydrosterculic acid (cyclopropanoid acid) and sterculic acid (cyclopropenoid acid), are often present in bacterial cell membranes and also occur in some seed oils of the family Malvaceae, Bombaceae and Tiliaceae (Bao et al., 2003; Knothe, 2006). Among the cyclopropene fatty acids (CPE-FAs) and cyclopropane fatty acids (CPA-FAs)-containing oilseeds, cottonseed and lychee (Litchi sinensis) seed are the most prevalent (Bao et al., 2003; Knothe, 2006). Cottonseed oil contains ~1% CPE-FAs and lychee seed oil has about 41% CPA-FAs (dihydrosterculic acid). The biological role of CPE-FAs and CPA-FAs fatty acids in plants is still indecisive; however, these might function as antifungal agents (Bao et al., 2003). The feeding of oilseed meals containing such fatty acids to animals is linked with different physiological disorders such as pinktinted albumen in eggs, reduced egg production and delayed of sexual maturity in hens and an increase in the amount of hard fats in dairy cows (Bao et al., 2003). Although CPE-FAs and CPA-FAs may have anti-nutritional attributes in animal diets, the chemical reactivity of these acids can potentially be utilized for various oleo-chemical applications (Bao et al., 2003).

The contents of saturated fatty acids (SFA), mainly comprising palmitic and stearic acids, in Sabz Pari and Punjab-8 *Hibiscus* seed oils, 37.66 and 37.57%, respectively did not vary considerably between the varieties tested. These amounts of SFA were comparable with those reported for *H. esculentus* seed oil from Los Baños (35.3%) (Pham *et al.*, 2002) and Greece (37.6%) (Andras *et al.*, 2005). The present level of SFA in *H. esculentus* seed oils, noted to be higher than cottonseed oil (*ca.* 27%), was quite lower than palm oil (*ca.* 50%). The contents of monounsaturated FA (MUFA), which mainly contained oleic acid, were noted to be higher in the Sabz Pari variety (28.49%) and lower in the Punjab-8 variety (27.00%) of *H. esculentus* seed oils.

The level of MUFA (27.00-28.49%) in the present analysis of *H. esculentus* seed oils was higher than those investigated for okra seed oils (16.1%) from Greece (Andras *et al.*, 2005). However, it was considerably lower than those reported for *H. esculentus* seed oil (30.4%) from Los Baños (Pham *et al.*, 2002). The contribution of polyunsaturated fatty acid (PUFA) in the Punjab-8 variety at 31.70%, was slightly higher than that in Sabz Pari variety (29.90%). The present levels of PUFA were comparable to those reported for okra seed oil (30.4%) from Los Baños (Pham *et al.*, 2002) but lower than those from Greece (48.4%) (Andras *et al.*, 2005). The significant (p<0.05) differences between *H. esculentus* seed oils of Sabz Pari and Punjab-8 varieties were observed for oleic, stearic, arachidic and behenic acids while, the other fatty acids showed non-significant (p>0.05) variation between the varieties. Some reports in the literature showed that *H. esculentus* seed oil

contained a variety of FA, with palmitic (16:0), oleic (18:1) and linoleic (18:2) acids predominating (Pham *et al.*, 2002; Andras *et al.*, 2005).



Fig. 1. Gas chromatography/mass spectrometry chromatogram of typical *H.esculentus* seed oil (variety Punjab-8).

Regardless of the fact that Pakistan is mainly an agricultural country, it is unable to produce vegetable oils sufficient for the domestic requirements of about 3.0 million metric tons (MMT) per annum. The annual seed oil production from indigenous resources is estimated to be 0.8 MMT (Anwar et al., 2008) which necessitates the need to explore some newer and non-conventional oilseed sources. As the climate in most parts of Pakistan is warmer, the soil is fertile, so Okra (*H.esculentus*) appears to be a potentially valuable crop, which can yield useful oil. The results of our study demonstrated that the oils derived from the tested varieties of Okra seed from Pakistan, are a good source of an essential FA (C18:2) and valuable tocopherols suggesting their utilization for human consumption. The tested oils might also be useful for preparation of Various commodities of oleo-chemical interest due to presence of a reasonable proportion of SFA, MUFA and PUFA along with

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small amount of CPA-FAs of industrial interest. Evaluation of nutritional and antinutritional factors, if any, of Okra seed oils is further recommended. **References**

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