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Full Length Research Paper

A study on gumbo seed grown in Congo Brazzaville for its food and industrial applications

J.M. Nzikou^{1*}, M. Mvoula-Tsieri¹, E. Matouba¹, J.M. Ouamba², C. Kapseu³, M. Parmentier⁴, S. Desobry⁴

¹ENSP – UMNG, Laboratoire de Physico-chimie et de Biotechnologie Alimentaires BP 69 Brazzaville – CONGO.

²Unité de Chimie du Végétal et de la Vie Faculté des Sciences – UMNG B.P. 69 Brazzaville – Congo.

³Département de Génie des Procédés et d'Ingénieries, ENSAI, BP 455 Ngaoundéré – Cameroun.

⁴ENSAIA – INPL, Laboratoire de Physico-chimie et Génie Alimentaires 2, avenue de la forêt de Haye, 54505 Vandoeuvre-lès-Nancy.

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Proximate composition, energy content and mineral concentrations of okra seeds grown in two localities of Congo Brazzaville were investigated. The paper also reports the physicochemical characteristics of the oil extracted from the seeds. Ash was highest (5.84±0. 2%) in Dolisie okra seeds (DOS) followed by Brazzaville okra seeds (BOS) with a value of 5.52±0.34%. Protein ranged from 25.48±0.57% in DOS to 23.73±0.35% in BOS. Crude fat content is of 29.31±0.83% for BOS and 25.71±0.44 % for DOS. Total carbohydrates were generally high in all the seeds and ranged from 31.84% in BOS to 231.27% in DOS. The seeds were found to be good sources of minerals. Phosphorus (1755.95–1464.87 mg/100 g), magnesium (3895.67-2743.5 mg/100 g) and potassium (124.59-116.05 mg/100 g), were highest in two okra seeds cultivars. The physical properties of the oil extracts showed the state to be liquid at room temperature and the colour to be golden-yellow, in general. Thermal analysis show that fatty acids melting point was lowest ranged between -25 °C and +6.55 °C. Gas liquid chromatography revealed that the major fatty acid was linoleic acid (34.89-44%), palmitic acid (25.2-28.3%) and oleic acid (21.9-24.08%). Abelomschus esculentus seeds oil (AESO) content long chain poly unsaturated acids as eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) and erucic acid (1.1-4.1%). It can be inferred that the oil seeds investigated are good sources of crude fat, crude protein, ash and minerals. The oil extracts exhibited good physicochemical properties and could be useful as edible oils and for industrial applications.

Key words: Nutritive values, unconventional oilseeds, *Abelomschus esculentus* seeds, erucic acid, essential fatty acid, activation energy.

INTRODUCTION

Gumbo or okra of scientific name Abelmoschus esculentus or Hibiscus esculentus is supposed to come from

*Corresponding author E-mail: nzikoumath@yahoo.com. Tel.: +242 537 42 79

Abbreviations: AESO, Abelmoschus Esculentus Seed Oil; BOS, Brazzaville Okra seed; DHA, Docosahexaenoic acid; DOS, Dolisie Okra seed; DPA, Docosapentaenoic acid; DSC, differential scanning calorimetry; EPA, Eicosapentaenoic acid; FAME, Fatty Acid Methyl Ester; FFA, Free Fatty Acid; IV, Iodine Value; L, Linoleic acid; Ln, Linolenic acid; MAG, Monoacylglycerol; O, Oleic acid; P, Palmitic acid; PUFA, Poly unsaturated fatty acid; PV, Peroxide Value (meq O₂.kg⁻¹); S, Stearic acid; SFA, Saturated fatty acid; SV, Saponification Value (mg KOH.g⁻¹); TAG, Triacylglycerol; and UFA, Unsaturated fatty acid.

Ethiopia; it was then propagated in North Africa, in the Mediterranean, in Arabia and India. The Egyptians were the first to plant it to the 12th before JC. *A. esculentus* is a tropical or subtropical plant which belongs to Malvaceae familiy. The world production of gumbo is estimated about 5 to 6 million tons by year (Siemonsma, 1982), which represents approximately 1.5% of vegetable production. In West Africa, gumbos occupy the second place of vegetable production after tomatoes. The chemical composition and nutritional or medicinal value of *A. esculentus* have been reported (Anon, 2003; Baytop, 1984; Aladesanwa, 2005; Benjamin, 1951; Çalışır, 2005; Siemonsma, 1982; Woolfe, 1977).

A. esculentus seed (AES) is a good source of oil and protein (Pavlos, 1975; Martin, 1982; Oyenuga, 1969). Studies has shown that A. esculentus seed oil (AESO) as a potential source of phospholipids and palmitic acid (Camciuc, 1997). Moisture transfer from okra has been described by applying the Fick's diffusion model, and the effective diffusivity was calculated. Effective diffusivity increased with increasing temperature. An Arrhenius relation with an activation energy value of 51.26 kJ.mol⁻¹ expressed the effect of temperature on the diffusivity (Doymaz, 2005).

Fat-free cookies have been prepared with okra gum or apple sauce, replacing margarine and egg yolk in high-fat cookies. Okra gum is an acceptable fat replacer in chocolate bar cookies (Romanchik-Cerpovicz, 2002). The *A. esculentus* seeds roasted are used as a good coffee substitute in Turkey (Çalışır, 2005).

The aim of this work was, first to contribute to the knowledge about the total carbohydrate, fatty acids (including seed oil), proteins content and mineral composition of okra seeds. Second, after displaying the nutritional profile of the plant, the data would aid knowledge of how important okra is nutritionally for the diet of people who consume it. This study will enable us to deduce the possible okra applications in food and cosmetic Industries.

MATERIALS AND METHODS

Seed material

A. esculentus seeds were obtained from Brazzaville and Dolisie markets. Then, they were further dryed in our Laboratory at about 40 °C and then crushed in Moulinex coffee blender (type Prep' line 850). Powdered A. esculentus seeds were kept at 5 °C in polyethylene bag before analysis.

Chemical analysis of powdered seeds

The recommended methods of the Association of Official Analytical Chemists (AOAC, 1984) were adopted to determine the levels of moisture, ash, crude protein and crude fat. Moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven, maintained at 105°C for 5.5 h. Ash was determined by the incineration of 1.0 g samples placed in a muffle furnace, maintained at 550 ℃ for 8 h. Crude protein (% total nitrogen x 6.25) was determined by the Kjeldahl method (Kjeldahl, 1883), using 1.0 g samples. Crude fat was obtained by exhaustively extracting 40.0 g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant (Dhellot, 2006). Total carbohydrate was obtained by difference; the energy content was calculated by multiplying the mean values of crude protein, crude fat and total carbohydrate by Atwater factors of 4, 9 and 4, respectively, taking the sum of the products and expressing the result in kilocalories per 100 g sample as reported by Onyeike et al. (1995). The minerals were determined by atomic absorption spectrophotometry. One gram samples, in triplicate, were dry ashed in a muffle furnace at 550°C for 8 h until a white residue of constant weight was obtained. The minerals were extracted from ash by adding 20.0 ml of 2.5% HCI, heated in a steam bath to reduce the volume to about 7.0 ml, and this was transferred quantitatively to a 50 ml volumetric flask. It was diluted

to volume (50 ml) with deionised water, stored in clean polyethylene bottles and mineral contents determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA). These bottles and flasks were rinsed in dilute hydrochloric acid (0.10 M HC1) to arrest microbial action which may affect the concentrations of the anions and cations in the samples. The instrument was calibrated with standard solutions.

Fatty acid composition analysis

The oils were converted to methyl esters using potassium hydroxide 2 N in methanol as described by Dhellot et al. (2006) and borontrifluoride-methanol by gas chromatography. Lipid sample (5-40 mg) was heated at 100°C with 1.5 mL hexane and 1.5 mL borontrifluoride-methanol (8% solution) under nitrogen atmosphere in a Teflon-lined screw capped vial (Linder, 2002). A Perichrom™ 2000 system gas chromatographs (Perichrom, Saulx les Chartreux, France), equipped with a flame-ionization detector, and was used for analyzing FAME. Chromatographic parameters were set as fellows: fused silica capillary column (30 m x 0.22 mm id. x 0.25 µm film thickness, BPX70 SGE Australia Pty. Ltd., analytical products), injector and detector temperatures 260 °C. The column temperature was programmed from 70 to 180 °C at 39.9 °C min⁻¹, and is kept at this temperature during 8 min. It then undergoes a second heating up to 220 °C (3 °C min⁻¹). Nitrogen was the carrier gas (1.1 bars). The Winilab software (Périchrom, Saulx-les-Chartreux) is used to integrate the chromatograms; the peak areas of triplicate injections were measured.oven temperature programming: held 5 min at 145 °C then ramped to 210 °C at 2 °C/min followed by a hold period of 10 min. Fatty acid were identified by comparison of their retention times with standard mixtures (PUFA1 from marine source and PUFA2 from animal source; Supelco, Bellfonte, P.A.).

Differential scanning calorimetry (DSC)

Rheological measurements

Rheological properties of oil were performed under steady shear and dynamic conditions using a thermostated Stresstech Rheologica® apparatus equipped with a UP30 stried plate-plate device (gap 0.5 mm). Experimental conditions were set as fellows: temperature gradient ranging from 45 to 5 °C at 1 °C/min, delay time 10 s; stress 50 Pa.

Unsaponifiables matters contents

5 g of oil are saponified with 30 ml 2 N potassium hydroxide in methanol. In yellowish solution obtained 50 ml of hexane are added, then 30 ml of distilled water. The organic phase is recovered and the aqueous phase is treated with 15 ml of hexane, with three recoveries. The organic fractions containing the unsaponifiables are

Parameter	BOS	DOS	Mean
Lipids (%)	29.31 ± 0.83	25.71± 0.44	27.51
Proteins (%)	23.73 ± 0.35	25.48 ± 0.57	24.60
R ₁	1.37 ± 0.2	1.13 ± 0.16	1.25
Ash (%)	5.52 ± 0.34	5.84 ± 0. 2	5.68
Moisture (%)	9.6 ± 1.5	11.7 ± 0.5	89.35
Total carbohydrate (%)	31.84	31.27	31.55
Calorific value (kJg ⁻¹)	2029	1916	1972
Mineral (mg/100g)			
Р	1464.87 ± 9.5	1755.95 ± 6.6	1610.41
Ca	79.58 ± 3.52	94.69 ± 2.15	87.13
Na	26.45 ± 0.64	79.12 ± 0.33	52.78
Mg	2743.50 ± 7.1	3895.67 ± 8.5	3319.58
K	124.59 ± 9.56	116.05 ± 1.63	120.32

Table 1. Abelomschus esculentus seed physico chemicals properties.

% Proteins = N_T x 6.25 ; R_1 = % lipids/% proteins ; Total carbohydrate = 100 – [%Lipids + % Proteins + % Ash + % Moisture]

collected, and then dried with Na_2SO_4 . Hexane is removed by Rotavapor apparatus, to recover the unsaponifiable part, which was then weighed.

Triacylglycerol (TAG) profile

Triacylglycerol (TAG) profile was obtained by High Performance Liquid Chromatography (HPLC). It is constituted by: HP 1050 pump (Hewlett Packard, Palo-Viola, CA, the United States); 20 µL Rheodyne loop injector valve model 7125 (Rheodyne, Cotati, CA, the United States), evaporative detector with diffusion of light Sedere Sedex 75 (Sedere, Alfortville, France). Column temperature was controlled using a furnace Croco-lash (Cluzeau, Sainte-Foy-lastat_cryostat julabo CPU F10, Touzart and Matignon, Ulis, France). Azur v2.0 Software (Datalys, Saint Martin d' Hérès, France) was used for data acquisition. TAG were separated at 20 °C using a Kromasil C18 250 X 4.6 mm (Thermo Quest, Ulis, France) column and was eluted from the column with a mixture of acetonitrile / dichloromethane (63:67) at the flow rate of 1 ml/min (Brody, 1994). Detector parameters were optimized and are as follows: $T = 37^{\circ}C$, P = 3.5 bars, Profit = 11, time constant = 1. Acetonitrile (Acros, New Jersey, the USA) and dichloromethane (Carlo Erba, Rodano, Italy) are HPLC quality. Oils were dissolved in MeCN/CH₂Cl₂ (50:50); the concentration and volume injected were adapted in such way that a peak taken in reference (PLL) has the same surface always appreciably.

RESULTS AND DISCUSSION

Chemical composition of *Abelmoshus esculentus* seed

Table 1 presents the average compositions of *A. esculentus* seed of the two studied cultivars. Okra seeds from Brazzaville and Dolisie cultivars contained 9.6 and 11.7% moisture, respectively. The ash, protein and fat contents in BOS and DOS were 5.52 and 5.84%; 23.73 and 25.48% and 29.31 and 25.71%, respectively. Accordingly, total carbohydrate content of okra seed ranged

from 31.84% for BOS to 31.27% for DOS. These results were in general higher than those reported by (Çalışır, 2005; Pavlos, 1975). Oyelade (2003) has shown that okra seeds cultivars could contain 22 to 44% of lipids and 40 to protein 50%.

Those differences may be attributed to the variability of the studied cultivars. The okra seeds also contained significant amount of important minerals (Table 1). The magnesium concentration (2743.50 - 3895.67 mg/100 g) was the highest, followed in descending order by phosphorus (1464.87 - 1755.95 mg/100 g), potassium (116.05 - 124.59 mg/100 g), calcium (79.58 - 94.69 mg/100 g) and sodium (26.45 - 79.12 mg/100 g). Potassium is an essential nutrient and has an important role in the synthesis of amino acids and proteins (Malik, 1982). Ca and Mg play a significant role in photosynthesis, carbohydrate metabolism, nucleic acids (Russel, 1973). Calcium assists in tech development (Brody, 1994). Magnesium is essential mineral for enzyme activity, like calcium and chloride; magnesium also plays a role in regulating the acid-alkaline balance in the body. High magnesium levels in drinking water have been linked to resistance to heart disease. Phosphorus is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body's acid-alkaline balance (Fallon, 2001).

Oils Chemical analysis

Presented in Table 2 is the result of chemical composition of oil extracted from two cultivars. *A. esculentus* seed oils are of pale yellow color. We did not observe remarkable differences for oils chemical indices; acidity is around 0.9% oleic acid, saponification value varies between 180.3 and 191.5 mg KOH/g, iodine value is 124.7 and peroxides value at 3 and 4 meq O₂/kg.

Table 2. Abelomschus esculentus seed oil physico-chemicals properties.

Parameter	BOS		DC	Mean	
Acidity	0.99 ± 0.1		0.85	0.92	
SV	180.3	3 ± 5.6	191.2 ± 7.5		185.9
IV	124.	7± 6.8	124.8	± 11.5	124.7
PV	4 ±	0.05	3 ±	0.2	3.5
η	37.5	± 0.31	41.5	± 0.3	39.50
Ea (KJ mol ⁻¹)	3.	.39	2.3	36	2.87
Unsaponifiables (%)	1.47	± 0.18	1.5 ±	0.12	1.49
Fatty acids	КОН	BF ₃	КОН	BF ₃	
16:0	26.85	25.75	28.29	25.2	26.52
18:0	2.79	1.74	3.2	2.47	2.55
SFA	29.97	27.83	31.87	27.99	29.42
16:1ω7	0.17	0.17	0.17	0.17	0.17
18:1ω9	23.5	23.94	24.08	21.9	23.36
20:1ω9	0.27	0.25	0.32	0.26	0.28
22:1ω9	1.64	1.11	4. 1	1.1	3.15
MUFA	25.58	25.47	28.67	23.43	25.79
18:2ω6	42.11	41.79	34.89	44	40.70
PUFA ω 6	42.11	41.79	34.89	44	40.70
18:3ω3	1.73	1.61	1.7	1.54	1.65
20:5ω3		0.29		0.25	0.27
22:5ω3		0.58	0.26	0.53	0.46
22:6ω3		1.14	1.28	1.01	1.14
PUFA ω3	1.84	3.62	3.24	3.33	3.01
Others	0.5	1.29	1.33	1.25	1.09
ω6/ω3	22.89	11.54	10.77	13.21	14.60
R ₂	2.32	2.55	2.10	2.53	2.37

Acidity in % oleic acid; SV: Saponification Value (mg KOH.g⁻¹); IV: Iodine Value; PV: Peroxide Value (meg O_2 .kg⁻¹); η : viscosity in m Pa .s; % Proteins = $N_T \times 6.25$; $R_2 = UFA/SFA$.

The nutritional value of fat depends on the quantity of free fatty acids (for example, acid butyric in butter) present. Oils whose free fatty acid content is below 3% can be used for cooking oils (Bassir, 1971; Onyeike, 2002). The low levels of % FFA in all the oils investigated indicate that the oils could probably be good edible oils that may be stored for a long time without spoilage via oxidative rancidity.

The high saponification values of the oils 180.3±5.6 mg KOH/g oil and 191.2±7.5 mg KOH/g oil for BOS and DOS, respectively, suggest that the oils could be good for soap making and in the manufacture of lather shaving creams (Eka, 1980; Hilditch, 1949).

lodine value is relatively high; gumbo oil is thus more unsaturated than nonconventional oils as *Dacryodes edulis* pulp (60-85) and *Canarium schwenfurthii* (71-95) (Omoti, 1987; Kapseu 1999). BOS and DOS oils have iodine values below 124.7 which place them in the non-drying group of oil. The oil could be utilized for cooking and may find application as a raw material in industries for the manufacture of vegetable oil-based ice-cream (Ibiyemi, 1992). Viscosity at 25 °C varies between 37.5

and 41.5 mPas. Unsaponifiables matters content is rather high (1.5%) thus guaranteeing the use of *A. esculentus* seeds oil in cosmetics industry.

Fatty acid composition

Fatty acid composition of the two studied seed oils is shown in Table 2. The most abundant fatty acids of okra seed oil were oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) which together composed about 90% of the total fatty acids. FAME (KOH) composition show that linoleic acid is the major component ranging from 42.11% for BOS oil to 34.89% for DOS oil, with palmitic (26.85-28.29%) and oleic (23.5-24.08%). Stearic (2.79-3.2%), erucic (1.64-4.1%) and linolenic acid (1.7%) are also present. Long chain poly unsaturated fatty acids such as docosapentaenoic (0.3%) and docosahexaenoic acids (1.28%) were detected in DOS oil.

 BF_3 method, FA composition gave the following results: linoleic acid (41.79 - 44%), palmitic acid (25.75 - 25.2%), oleic acid (23.94 - 21.9%), stearic acid (1.74-2.47%), linolenic acid (1.61 - 1.54%), erucic acid (1.1%) and PUFA

Table 3.	Linoleic	and	Linolenic	acid	content	of	usual	oils	and	fats	edibles	(from
CIQUAL.	databases	s. AFS	SSA report.	2003).							

Oil /Fat	Linoleic acid	Linolenic acid	18:2ω6/18:3ω3
Peanut oil	30.5	0	-
Rapeseed oil	21.2	9.6	2.21
Corn oil	55.9	0.9	62.11
Walnut oil	56.7	12.3	4.61
Grapeseed oil	67.3	0.3	224.33
Soybean oil	52.6	7.3	7.21
Sunflower oil	64.1	0.05	1282
Olive oil	12.9	0.85	15.18
Mixed oil	47	1.2	39.17
Abelomschus esculentus	40.70	1.65	24.66
Butter oil	1.16	0.46	2.53
Cream	0.52	0.12	4.33
African sheabutter	5.98	nd	-
Goose fat	12	nd	-
Palmkernel fat	2.7	nd	-
Cooking butter	12.4	1.24	10
Pig fat	8.1	nd	-

Table 4. Thermal analysis of *Abelomschus esculentus* seed oil.

Parameter	Brazzaville	Dolisie
Peak 1 (℃)	-24.7	-24.8
ΔH (J/g)	7.65	7.04
Peak 2 (℃)	-1.14	-1.98
ΔH (J/g)	6.87	12.35
Peak 3 (℃)	+3.13	+6.55
ΔH (J/g)	2.18	2.02

such as EPA (0.27%), DPA (0.55%), DHA (1.08%) are present. Linoleic acid, undoubtedly one of the most important polyunsaturated fatty acid in human food because of its prevention of distinct heart vascular diseases (Omode, 1995), is present in all the seed oils. Apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, linoleic acid also prevents high blood pressure. Also linoleic derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds (VIes, 1989). The presence of essential fatty acids and omega3 fatty acids in the seed oils make them nutritionally valuable. Linolenic acid content is on average of 1.6%. It can be used as frying fat. With $18:2\omega6/18:3\omega3$ ratio at 24.66 (Table 3) okra seed oil is more equilibrated than edible oils such as corn oil (62.11), grape seed oil (224.33) and sunflower oil (1282). All these give the okra seed oil dietetic potentialities. Erucic acid level is of interest. Application of erucic include lubricants, heattransfer fluids, surfactants, slip agents, emolients, cosmetics and coatings. It is also used in polyesters, plastics and nylons (Lühs, 1994).

Thermal analysis

The results of thermal analysis of oils are presented in Table 4. The obtained peaks were asymmetric and may indicate the presence of three components in oil extracted from the two cultivars. The first peak at low melting point appears at -24.8 °C with a fusion enthalpy (ΔH_f) of 7 J/g, corresponding to triglycerides formed by poly unsaturated fatty acids. The second melting point is at -1.1 °C (ΔH_f =6.87 J/g) for BOS cultivar and -2 °C (ΔH_f = 12.35 J/g) for DOS cultivar. This is a characteristic of mono unsaturated fatty acids. The last peak leaves to +3.1 °C for BOS cultivar and +6.55 °C for DOS cultivar, suggest the presence of mixed triglycerides groups with different melting points.

Viscosity

Viscosity at 25 °C varies between 37.5 and 41.5 mPas. In order to evaluate activation energies of various fatty acids classes contained in those oils, the influence of temperature on viscosity was studied. When the temperature increases, viscosity decreases exponentially (Igwe, 2004; Dzondo, 2005). For Dolisie, viscosity decreases from 44.63 to 38.16 mPas. For Brazzaville cultivar, it decreases from 41 to 32 mPas (Figure 1). The Arrhenius equation was used to determine activation energy starting from the viscosity results:

$$\eta = A e^{-Ea/RT}$$

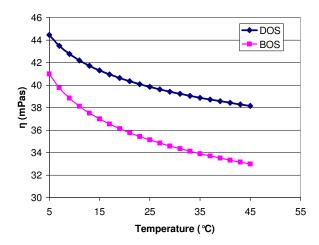


Figure 1. Effect of temperature on *Abelomschus esculentus* seed oil viscosity.

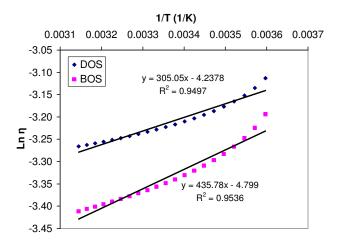


Figure 2. Arrhénius plot of *Abelomschus esculentus* seed oil.

A is the frequency factor called also exponential pre factor Energy. Ea = barrier to cross before the elementary flow can begin in kJ mol^{-1} , R = 8.31 $\text{Jmol}^{-1}\text{K}^{-1}$ (perfect gas constant) and T = absolute temperature (K). A plot of Ln η against 1/T (Figure 2) gave -Ea/RT which is the slope from which Ea was evaluated. Activation energies calculated were 2.36 kJ mol^{-1} for DOS cultivar and 3.39 kJ mol^{-1} for BOS cultivar. The higher the activation energy, the more stable the fatty acid is.

Triacylglycerol (TAG) profile

The chemical analysis of triacyl glycerols shows that *A. esculentus* seed oil contains 13 TAG of which 5 are in a majority representing 88% of total TAG. These include PLL (35-36.3%), POL (13.52-20.45%), PPL (12.99-15.22%), LLL (8.22-13.91%) and OLL (9.45-11.92%) (Table 5). TAG is the majority components of the food

Table 5. TAG content of *Abelomschus esculentus* seed oil.

TAG	Brazzaville	Dolisie
PLL	34.95	36.29
POL	20.45	13.52
PPL	15.22	12.99
LLL	8.22	13.91
OLL	9.45	11.92
OOL	3.30	2.95
P00	2.44	1.67
PPO	1.98	1.46
SLL	1.54	3.41
PSL	1.07	0.84
000	0.74	0.43
SOL	0.65	0.60

greasy substances (Carey, 1983). In rheological level, a fatty substance is liquid (oil) or solid (fat) at ambient temperature according to nature and the place occupied by fatty acid on the various positions of glycerol. In nutriational level, AG in central position on glycerol does not have the same one to become after pancreatic digestion (Small, 1991). Indeed, fatty acid located in external positions of the TAG is hydrolysed preferentially, and can be eliminated by the organization when they form with the intestinal calcium of insoluble salts. On the other hand, fatty acid located in central position of the molecule is preferentially and effectively absorbed in the form of β -mono acylglycérols (MAG) through the intestinal wall.

CONCLUSION

Considering A. esculentus seeds and its oil physicochemical properties, we can conclude that these oils could be used to meet part of nutritional requirements of animal or human foods. It could be considered as good sources of protein and minerals such as phophorus, magnesium, calcium and potassium. A. esculentus seeds oil have high contents of unsaturated fatty acids, it is more balanced that some edible oils such as grape seed oil, corn oil and sunflower oil. High unsaponifiable matters content guarantees the use the oils in cosmetics industry and we suggest the application of these oils for soap making and manufacture of lather shaving creams. Free fatty acids content and peroxides value were low indicating that the oils could qualify as good edible oils. However, the safety of okra seed oil must be tested before using it as an ingredient in food or cosmetic industries.

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