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Fatty acid profile, tocopherols content and antioxidant activity of algerian pumpkin seeds oil (*Cucurbita pepo L*)

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Abstract. *Cucurbita Pepo* (Pumpkin) is a plant which is traditionally used to treat a wide variety of diseases, and through scientific investigation most of the properties have been validated. However, more scientific data is needed to support the various health claims. The aim of this study was to determine the fatty acids (FA) and Triacylglycerols (TAG) composition, tocopherols content and to evaluate the antioxidant activity of eight pumpkin seeds oil cultivated in Algeria. The results indicated that pumpkin seeds were rich in oil (15.8–33.5%) and the major unsaturated fatty acids were linoleic acid (42.1–48.5%) followed by oleic acid (18.4–39.6%), while the main saturated fatty acids were palmitic (13.91–20.00%).Unsaturated FA showed a preference for the internal position. Linoleic and oleic acids occurred predominantly in the sn-2 position as generally found in vegetable oils. The main TAGs were triunsaturated GU_3 and monosaturated GSU_2 as majorities compounds. The tocopherol content of the oils ranged from 7.7 to 31.9 mg/kg of oil for α-tocopherol, from 39.3 to 155.1 mg/kg for (β+γ)-tocopherol, and from 39.0 to 103.0 mg/kg for δ-tocopherol. The capacity that would be suitable for food and industrial applications, as well as high unsaturation FA and tocopherol content that could potentially improve the nutrition of human diets.The data of antioxidant power determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and phosphomolybdenum (PPM) complex methods show that the level of the antioxidant activity by two used assays was significantly compared to synthetic antioxidants. Also, it was demonstrated for the first time that the studied oils possessed a good antioxidant activity which may be associated with their alleged health benefits.

Keywords: Pumpkin seed oil, fatty acids (FA), Triacylglycerols (TAG), tocopherols, antioxidant activity

1. Introduction

The *Cucurbitacae* have been cultivated for long time not only for food but also for their medicinal properties. Particular medical properties have been attributed to each part of the fruit and the plant seeds [1].

Recently increased attention has been focused on the use of under-utilized agricultural products, as well as by products and wastes from food processing to produce food and feed [2]. Pumpkin seeds that remain in large quantities as waste product after the removal of the flesh could be used [3, 4].

Pumpkin seeds are consumed directly for human consumption as a snack food in many cultures throughout the world, and the seeds are especially popular in Arabian countries, after salting and roasting. The pumpkin seeds have been used as additives to some food dishes [3, 5]. Pumpkin seeds contain a strong therapeutic effect which can help eliminating intestinal parasites, clean blood vessels, normalize cholesterol level and stimulate kidney activity [6, 7]. Although the seeds of pumpkin are rich in oil and protein and the crop could potentially become another source of vegetable oil and protein, detailed studies on their composition and the properties of their oil are limited [3, 8].

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In Algeria, local pumpkin (*Cucurbita pepo*) has been grown widely in several major growing areas for many decades and can be considered one of the major vegetable crops. In the literature there are many reports on the composition and properties of pumpkin seeds especially *Cucurbita pepo* [15, 16, 30, 56], which concern mainly non-specific varieties. However, there are no studies on fatty acid composition, antioxidant properties and the health benefits of Algerian pumpkin oil seeds. Therefore, the aim of the present study was to investigate the triglyceride composition and the determination of antioxidant activity of the local pumpkin seed oil. The tocopherols content and profile were also determined. In addition, the physicochemical properties of oil and the fatty acids content were analyzed.

2. Experimental procedure

2.1. Chemicals

The chemicals used were of analytical reagent grade. stable 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Trolox, vitamine E, BHT, BHA, boron trifluoride (10% in methanol) and pancreatic lipase were brought from Sigma–Aldrich. Hexane, diethyl ether, formic acid, hydrochloric acid, calcium chloride, acetone and all the reagents were from Fisher Scientific.

2.2. Collection and preparation of samples

Seeds of local pumpkin were obtained from Sebgag (at 136 km at the north of Laghouat in the steppe region of Algeria). Pumpkins were collected between July and August 2008 and 2009. The ripe pumpkins were cut and then the seeds were separated. The separated seeds were air dried at room temperature. To simplify more, we have adopted the following abbreviations for the pumpkin seeds name: 81 : Sample N°1 collected in 2008; 82 : Sample N°2 collected in 2008; 83 : Sample N°3 collected in 2008; 84 : Sample N°4 collected in 2008; 91 : Sample N°1 collected in 2009; 92 : Sample N°2 collected in 2009; 93 : Sample N°3 collected in 2009; 94 : Sample N°4 collected in 2009.

2.3. Extraction of oil

The dried seeds were crushed using a domestic crusher and extracted with n-hexane using Soxhlet apparatus for 6 hours.The extract is dried by addition of a sufficient amount of anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The obtained oils were kept in amber glass bottles at 4°C.

2.4. Determination of the physicochemicals values of the oils

Acid value, saponification value, density and refractive index were determined according to the procedure described by the AOCS [9].

2.5. Purification of triacylglycerols (TAGs) by column chromatography

The TAGs were purified from the total lipid by column chromatography using silica gel (mesh 70–230). Activated silica gel (5 g per 1 g of the sample) was packed in dry n-hexane and total lipid was added to it. The column was washed with 80 ml of n-hexane to afford TAGs fractions. These fractions were further concentrated in vacuum and the purity was checked by TLC using an elution system of n-hexane as mobile phase. The purified TAGs showed only one spot indicating that these preparations contained no detectable lipid contaminants.

2.6. Pancreatic lipase hydrolysis

Lipase hydrolysis of the neutral TAGs was almost same as that described by [10]. To the Lipid sample (1 g in a screw-capped tube) were added ammonium chloride buffer (10 ml, 1.2 mol/L) at 8 pH, bile salt solution (2 ml, 25 %,

w/v) and calcium chloride solution (4 ml, 22%, w/v) were added. When the temperature of the melange is maintained at 37 °C, 100 mg of porcine pancreatic lipase were added and shaken vigorously on a magnetic stirrer. The reaction mixture was kept at 37 ± 0.5°C for 45 min. while shaking. The reaction is stopped by adding 1 ml of 6 M hydrochloric acid. Reaction mixture was immediately extracted with diethyl ether. Combined ethereal solution was washed with water and dried over anhydrous sodium sulphate. The dried samples were immediately fractionated by preparative thin layer chromatography (TLC) plates.

To isolate the 2-monoacylglycerols (2-MAGs), the lipolytic products (dissolved in 1 ml of chloroform) were applied on preparative plates in streaks. The chromatoplates were developed with a mixture of petroleum ether-diethyl ether-formic acid (60:40:1, v/v/v). Bands were detected under UV light. The components were separated out into four defined zones in the following order of increasing height travelled: MAGs, diacylglycerols (DAGs), free FAs and unreacted TAGs. The band corresponding to 2-MAGs (identified using standard sample run in the side of the plates) was scrapped off and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to dryness to obtain the purified 2-MAGs.

2.7. Gas chromatography analysis of TAGs and 2-MAGs

FAs methyl esters (FAME) of oil, TAGs and 2-MAGs were prepared by basic catalysed esterification using boron trifluoride-methanol method [11]. A Delsi gas chromatography, equipped with an FID detector and a Mega 10 column (25m×0.25 mm i.d, 0.25μm film thickness) was used to analyse the FAME(s). The GC conditions were as follows: initial oven temperature(150°C) heating rate 2°C/min, final temperature(200°C), injection port temperature (250°C), detector port temperature(250°C), hydrogen gas flow 30 mL/min, air flow 300 mL/min, and helium gas carrier flow 1 mL/min. The injection volume was 0.1μL. The fatty acids were identified by comparing their retention times with those of pure standards purchased from Sigma –Aldrich.

2.8. Tocopherols analysis

The quantitative and qualitative analysis of tocopherols were carried out by using an HPLC system composed of water 2690, quaternary pump; a thermostated column compartment, and a fluorescence detector (Excitation at 290 nm and emission at 330 nm), licsopher RP-18 column (250×4.6 mm, 5μm thickness, Merck) was used with a methanol: acetonitrile (70:30 v/v) mobile phase at an isocratic elution, flow rate with 1 mL/min. Quantification was carried out from a calibration based on the standards tocopherols.The isomer β-tocopherol was not resolved from γ-tocopherol by RP-chromatography. For this reason, we describe β-and γ-tocopherols together.

2.9. Measurement of antioxidant capacity

For the determination of the antioxidative capacity of our studied oils, two assays had been used for this purpose.Hydrogen and electron transfer from antioxidant analytes to DPPH· And Mo(VI) complex occur in the DPPH and phosphomolybdenum assay methods. The transfers occur at different redox potentials in the two assays and also depend on the structure of the antioxidant.

2.9.1. DPPH radical scavenging activity assay

Radical scavenging activity of pumpkin oil seeds against stable DPPH· (2, 2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically by the slightly modified method of Brand Williams [12], as described below. The solution of DPPH· in methanol (250μM) was prepared daily, before measurements. Various concentrations of 1 ml of sample solution diluted in 1-butanol were added to 1 ml of the DPPH· radical solution. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorption was measured at 517 nm. The antioxidant activity of the extract was expressed as an EC_{50} value defined as the concentration (in mg/ml) of the extract that inhibited the formation of DPPH radicals by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged. The DPPH radical scavenging activity obtained for each plant extract was compared with that of ascorbic acid, Trolox, BHA, BHTand α-tocopherol.

2.9.2. The phosphomolybdenum assay

The antioxidant activities of pumpkin oil seeds were evaluated according to the phosphomolybdenum method developed by Prieto et *al* [13]. Aliquots of 1 ml of sample solutions (in dimethyl sulfoxide) were combined in glass test tubes with 2 mL of reagent solutions (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were capped and incubated in a water bath at 70ºC for 90 min. Samples were cooled down at room temperature and the absorbance was measured at 695 nm. The total antioxidant activity of the extracts is reported as % reduction of Mo(VI) to Mo(V) and is calculated as the efficient concentration EC_{50} in mg/ml. The antioxidant capacity obtained for each oil extract was compared with that of ascorbic acid, Trolox, BHA, BHT and α-tocopherol.

2.10. Statistical Analysis

Results are presented as means ± SD from three replicates of each experiment. A*P* value <0.05 was used to denote significant differences among mean values. Statistical analysis of data was carried out by computer using MS-Excel. Strictly linear calibration curves were obtained for all of the methods using different calibration standards. Multiple comparison tests were used to analyze data.

2.11. Cluster analysis

Cluster analysis (CAH) was performed using hierarchical clustering (Ward's technique) with Euclidean distance measure. The calculus was performed using two sets of data. The first set of data which refer to the analysis of TAGs is composed of 13 different oils plants and 34 variables. Whereas, the second set of data which refer this time to the analysis of tocopherols (α-, (β+γ)- and *δ*-tocopherol) is composed of 12 oils of different plants and 4 variables.

Because we have similar results of TAGs and Total tocopherols content of all 8 samples, the TAGs and tocopherols data employed for the classifications (CAH) are taken as the means values.

3. Results and discussion

3.1. Physicochemical values of oils

The pumpkin seed oils were olive-green in colour, liquid at room temperature. The oil content of the eight samples of local *Cucurbita pepo* seeds were ranged from 15.8 to 33.5 % (Table 1). These values fell in the range reported for different species of *Cucurbita* (9.8–52.1%) and different varieties of *Cucurbita pepo* (31.2–51.0%) [14]. This oil content was much lower than that reported for the European varieties (60%) [15], Egyptian varieties (50.1–51.01%) [16] and also similar than that reported for the Eritrea ones (22–35%) [15]. However, it is higher than others such as lima beans (19.8%) and chickpeas (19%) [17]. This difference in the oil concentration can be explained by different extraction methods. Moreover, the oil content of the pumpkin seed collected in 2009 was higher than that collected in 2008. Also, in the present study, our oils content were found to exceed, or be comparable to, that of some common edible oils such as cottonseed (22–24%), sunflower (30–35%), soybean (18–22%), rapeseed (40–48%), and olive (12–50%) [18]. Variation in oil yield may be due to the differences in cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used. These findings showed that these seeds can be considered as a potential source of vegetable oil for domestic and industrial purposes.

Physicochemical values of the pumpkin seed oils are shown in Table 1. Physicals values of oils derive directly from their chemical structures and functional groups and greatly influence the functions of lipids in foods and the methods required for their manipulation and processing. They can also be used to assess the purity or quality of lipid material in reference to known standards or preferred characteristics [19].

Refractive index is used by most processors to measure the change in unsaturation as the fat or oil is hydrogenated. The refractive index of oils depends on their molecular weight, FA chain length, degree of unsaturation, and degree of conjugation [20]. The pumpkin seed oils showed a refractive index which ranged from 1.467 ± 0.0002 to 1.471 ± 0.0003, which were similar to those reported by A. Gohari Ardabili (2011) for *Cucurbita pepo* (1.4662) and

Table 1
Physicochemical properties and fatty acid composition of Pumpkin seed soil

	Pumpkins collected in 2008				Pumpkins collected in 2009			
	81	82	83	84	91	92	93	94
Oil %	15.8	26.7	27.2	22.5	27.1	29.8	28	33.5
AV (mg $_{(KOH)}$/g)	14.9 ± 2.3	16.8 ± 4.3	15.8 ± 8.5	17.2 ± 6.7	11.2 ± 2.2	15.1 ± 2.6	28.7 ± 3.9	23.4 ± 6.5
SV (mg $_{(KOH)}$/g)	195.0 ± 0.5	198.8 ± 3.7	198.6 ± 5.0	197.2 ± 1.4	197.5 ± 0.5	198.2 ± 1.1	197.7 ± 0.2	199.9 ± 0.1
$\eta^{27}{}_{D}$	1.468 ± 0.1	1.467 ± 0.2	1.468 ± 0.1	1.469 ± 0.2	1.469 ± 0.1	1.468 ± 0.5	1.468 ± 0.1	1.471 ± 0.3
d^{27}	0.846 ± 0.01	0.831 ± 0.04	0.882 ± 0.04	0.896 ± 0.06	0.875 ± 0.04	0.837 ± 0.03	0.879 ± 0.04	0.895 ± 0.08
U.M%(w/w)	6.54	5.50	4.15	2.33	3.18	3.40	2.96	4.72
C14 : 0	0.48	0.28	0.38	0.31	0.36	0.30	–	0.26
C16 : 0	20.0	16.2	18.8	16.2	15.3	13.9	18.0	15.8
C16 : 1	0.26	0.19	–	0.17	0.16	0.19	0.23	0.18
C17 : 0	0.29	0.18	–	0.22	0.21	0.23	0.24	0.11
C18 : 1	29.3	32.5	35.8	38.7	33.7	39.6	32.6	18.4
C18 : 2	46.5	48.5	42.4	42.1	47.7	43.9	46.8	44.0
C18 : 3	1.0	0.42	0.35	0.40	0.36	0.23	0.24	0.32
C20 : 0	1.5	1.1	0.99	0.89	1.04	0.65	0.87	0.72
C22 : 0	0.45	0.26	0.30	0.27	0.25	0.18	0.25	0.22
C23 : 0	0.06	–	–	–	–	–	–	–
C24 : 0	0.31	–	–	0.16	0.14	–	–	–
Total saturated	22.4	17.9	20.7	18.0	15.4	15.2	20.1	17.1
Total unsaturated	77.1	81.6	78.7	81.4	82	83.8	79.9	82.7
UFA/SFA	3.4	4.5	3.8	4.5	4.7	5.5	4.0	4.8

AV: Acid value; IV: Iodine value; SV: Saponification value; $\eta^{27}{}_{D}$: refractive index; d^{27}: density; U.M%: Unsaponifiable matter; UFA: Unsaturated fatty acids; SFA: Saturated fatty acids.

by Mohammed A. Alfawaz for *Cucurbita maxima* (1.4656).These were lower than the range reported for sunflower and olive oils; higher than that for palm, palm kernel and coconut oils; and within the range reported for canola, rapeseed and corn oils [19].

Generally, oils are lighter than water. Some however, are heavier than water, especially those which contain larger amounts of oxygenated constituents of the aromatic series. Densities of the oils were ranged between 0.831 ± 0.036 and 0.896 ± 0.060. These values were well comparable with the value obtained by Gohari et al. [19], for the Cucurbita pepo from Iran (0.9151 ± 0.0002). These values also are less than that reported for olive (0.910–0.920), canola (0.914–0.920) and some wild oilseed plants [21].

The unsaponifiable matter of oil is a small portion of oil which is extracted by organic solvent after the saponification of the oils with the alkali. It's a variety of nonglyceridic bioactive substances containing variable mixture of hydrocarbons, aldehydes, ketones, alcohols, sterols, pigments, and fat-soluble vitamins that may occur naturally or may be formed during processing or degradation of oils [22]. These minor substances of the oil contained in unsaponifiable matter have antioxidant and other health benefits in animals and in human subjects and useful in softening the skin [23]. Quantitatively, the value of unsaponifiable matter content of pumpkin seed collected in 2008 was higher than that collected in 2009.The content of unsaponifiable matters in the oils experimented was illustrated in Table 1. These values are higher than some Congo oil seed plants and cotton seed, [21] palm and sunflower seed oils [24], but it was in a close agreement with the 3–7% range reported by Anwar et al [25], for rice bran oil. Hence, more detailed examinations of the composition of the unsaponifiable fraction of this oil will be of special interest.

The Codex Alimentarius Commission expressed the permitted maximum acid values of 10 and 4 mg KOH/g oil for virgin palm and coconut oils, respectively [3]. Oil that is low in acidity is suitable for consumption. They must have acidity level less than 0.1 mg KOH/g [18]. All studied seeds had oils with high acidities, with acid number more

than 10 (Table 1). The current studied result is much higher than all of pumpkin seeds (*Cucurbita pepo* Subsp. pepo Var. Styriaka) grown in Ira) [19], pumpkin seed kernel oil [3] and some Congo oilseed plants [21]. This result explain that the studied oils could be become rancid. Therefore, considering that the oil studied was unrefined and its initial quality indicators were within the reported limits, the pumpkin seed oil can be regarded as edible oil with not good quality.

Saponification value is an indicator of the average molecular weight and hence chain length. It is inversely proportional to the molecular weight of the oil. The saponification values of oils obtained from the different seeds were ranged from 195.05 ± 0.50 to 199.86 ± 0.06 mg KOH/g Table 1.This result is fallen in the 174–197 range reported for the pumpkin seed oils [20]. This value indicated that the pumpkin seed oil had fatty acids with higher number of carbon atoms in comparison with coconut (248–265) and palm kernel (230–254) oils [20]. This result was in good agreement with the 185.5–195.3 range of Markovic and Bastic [26], however, it was lower than 200–218 range reported by Al-Khalifa [27], 206 of El-Adawy and Taha [16] and 201 of Tsaknis et al [28] and was higher than 132.3 reported by Younis et al. [15] for *Cucurbita* species. Furthermore, it fell in the range reported for palm, cotton and some medicinal plant seeds [3]. Moreover, many edible oils have saponification values between 195 and 200 [14]. All seeds studied oils have saponification values that are within this range. The oils having high saponification value (around 300) are useful for soap making, but no such oil was found in this study.

3.2. Fatty acid analysis

Data of FA composition of the pumpkin seed oils can be used to evaluate its stability and nutritional quality. A higher degree of oil unsaturation makes it more susceptible to oxidative deterioration. On the other hand, there are considerable data to recommend are duction in saturated and a moderate increase in monounsaturated and *n*-3 and *n*-6 polyunsaturated FAs in human nutrition in order to prevent coronary heart disease and other diseases [2]. The composition of FA varies depending on several factors including variety, growing area, climate and ripeness [29].

Gas chromatography (GC) analysis has shown that the dominant FA_S found in the pumpkin seed oils are: palmitic C16:0 (13.9–20.0%), oleic C18:1 (18.4–39.6%) and linoleic C18:2 (42.1–48.5%). The content of these three main FA_S ranges from 78.1 to 97.6% of the total FA composition of the oil. The FA composition of oil samples from the eight pumpkin studied seeds is given in Table 1.This FA profile is confirmed by several authors [15, 26, 56]. The studied pumpkin seed oils contained total percentage of the saturated FA_S which ranged from 15.3 to 22.4%, with the major one being palmitic acid (more than14%), while it was high inunsaturated FAs with a total content of (77.1–83.8%). This total content of the unsaturated FA_S was similar to that of the other studies on *Cucurbita pepo* and all pumpkin speciesin Cucurbitaceae [30, 56]. The main unsaturated FA_S were linoleic acid and oleic acid with different percentage.

In most other investigations on the FA composition of *Cucurbita pepo* [16], the percentage of linoleic acid (43.1–55.6%) and oleic acid (20.4–37.8%) were higher than obtained in the present study. Moreover, the composition of linoleic acid was found to be comparable to that found for the European variety, 36.6–60.8% [31]. But, compared to other seed oils, the amount of oleic and linoleic acids in our pumpkin seeds oils are relatively higher than for bitter melon seed oil (65.6%), kenaf seed oil (74.8%) and roselle seed oil (64.1%) [14]. This indicates that the *Cucurbita pepo L.* seed oil is a rich source of linoleic acid, and that the unsaturated nature of this oil qualifies it to be promising edible oil. Despite the high content of total unsaturated FA_S in the pumpkinseed oils, linolenic acid was very low (0.23–1.00%), which was in good agreement with all other similar studies. Also, the level of other FA_S in the pumpkin seed oils was very low, similar to the results reported in the literature [30].

Furthermore, for all oil samples of seeds, whenever the composition of linoleic acid C18:2 is high, the composition of oleic acid C18:1 is low. The composition of palmitic acid C16:0 remains constant. The trace amounts of the other higher FAs, e.g. linolenic and docanoic acids, found in the oil seeds collected in 2008 are also higher than those found in the oil of seeds collected in 2008 (Table 1). This conclusion is based on the assumption that experimental error encountered in the measurement of these trace amounts is negligible. These observations are also in accordance with the reported findings that due to the date of cultivation. Thus, the FA_S such as stearic acid C18:0 were not present in the studied seed oil samples. It is worth mentioning that a trace amount of nervonic acid C22:0 (Less than 0.45%) has been found in all the seed oil samples analysed in this study. Further, the ratios of UFA/SFA were ranged between 3.43 and 5.49. These ratios in the pumpkin oil seeds which collected in 2009 were higher than those collected in

2008. (5.64) and these values give the oils a good prevention of oxidation. Therefore, these ratios were higher than the Algerian Argan oil (2.1) [32] and for Algerian *Pistacia atlantica* fruits oil (2.7) [33], but were smaller than that for sorghum grains growing in Algeria (more than 5.7) [34]. Consequently, these values give the oils a good prevention of oxidation.

Early experiments showed that palmitic, myristic and lauric acids raised plasma cholesterol more than those with either shorter or longer chains [35]. Indeed the cholesterol-lowering effect was similar to that seen in oleic acid. However, saturated FA plays an important role in the structure of tissue [36]. The high level of linoleic and oleic acids confirm that the oil is liquid oil than solid oil; hence, it cannot easily solidify at ordinary temperature. It also implies that the consumption of this oil can prevent the risk of heart problems. The presence of high amounts of the essential linoleic acid suggests that the pumpkin seed oil is highly nutritious. As the pumpkin seed oil is rich in both oleic and linoleic acids, it may be used as edible cooking and salad oils or for margarine manufacture.

The structure of naturally occurring TAGs has been studied over a number of years with little agreement about the distribution of the FAs, except as to the prevalence of mixed acylglycerols. Several types of arrangement, for example, even random, partial random, and restricted random, have been proposed and defended. One reason that a number of theories developed has been the lack of suitable methods for the isolation and characterization of acylglycerols. In 1956, Mattson and Beck [37] demonstrated the specificity of pancreatic lipase for the cleavage of ester linkages at the 1 and 3 positions of TAGs and suggested that this offered a new tool for study of TAG structure, a tool with certain obvious advantages over previous means. The absorption of FA dependent not only on its chain length and degree of saturation, but also on its positional distribution in the glycerol backbone [38]. In the FA stereochemistry, the middle carbon is numbered sn-2. Gastric and pancreatic lipases hydrolyze FAs from the sn-1,3 positions of dietary TAGs to produce free FAs and 2-MAGs [39]. FA_S in the sn-2 position of dietary TAGs are preferentially absorbed through the intestinal wall, and those esterified to the sn-1,3 positions, especially long-chain unesterified saturated FA_S such as 16:0 are not well absorbed, in part because of melting points substantially above body temperatures and a strong tendency to form insoluble soaps with divalent cations, such as calcium and magnesium, and excreted in the feces which reduce their absorption by animals [40]. Recent studies demonstrated that changing the positional distribution (increasing proportion of the FA in the sn-2 position of TAG) would improve the fat absorption. In the present study, we have investigated whether the positional distribution of FA_S in pumpkin seed oilsTAGs.

The neutral TAGs were thus purified from the eight oils by column chromatography and tested using TLC plates which showed only one spot indicating that these preparations contained no detectable lipid contaminants. The results of separation of the seed oils show that the majority of the lipid molecular species is TAGs. The free FA_S and the partial glycerols are low in comparison, which is in agreement with the findings of other researchers [41]. TAGs were further esterified for GC. The GC data of TAGs (Table 2) reveal that all oils of pumpkin seeds contain a greater percentage of unsaturated acids (65.9–81.4%), with linoleic acid as the major unsaturated component followed by oleic acid with an amount which ranged between 17.1 and 38.6%. Whereas in all studied pumpkin sees oils, the presence of myristic acid C14:0 (Less than3%), though not in higher percentage, whereas it was completely absent in sample 82 and relatively higher in sample 83. The contents of palmitic and stearic acids were found to be range between 10.2–15.4 and 5.8–9.4%, respectively. Furthermore, some pumpkinseed oils also showed the presence of small amounts of lauric acid. However, our results (Table 2) did not show presence of traces of C20:0 and C22:1 as examined by Al-Khalifa [27]. However, the levels of C18:2 and other fatty acids varied among the samples referred. Comparing our pumpkin seed oils with others wholesome seed oils like sunflower, safflower, sesame and flax seed oils, it is evident that its amount of C16:0 and C18:0 is comparable with our pumpkin seed oils [37]. As well, the content of an essential FA (C18:2) in pumpkin seed oil is closely comparable to that of sunflower oil, however slightly lower than those of grape seed and safflower seed oils. These differences might be due to varying features such as harvesting time, seed drying conditions, seasonal variation and fruit seed maturity.

The FAs distribution in TAGs of the all pumpkin seed oils in the present analysis was in agreement to those reported in the literature for other *Cucurbitaceae* seed oils [42];generally containing low amounts of saturated FAs and high contents of poly unsaturated FAs, especially C18:2. It is now widely accepted that diet with low saturated FAs and high in polyunsaturated FAs is beneficial for health. The high proportion of PUFA and monounsaturated FAs (MUFA) and low amounts of saturated FAs (SFA) indicate the possible higher oxidation rate of *Cucurbitaceae* seed oils due to high degree of unsaturation. Although none of these oils are presently used on industrial scale, but some are used as cooking oil in several countries of Africa and the Middle East [27]. Furthermore, according to Smit et al. [43]

Table 2
Fatty acid composition of TAGs and the proportion of each FA in the 2-position

	TAGs %								2-MAG %							
	Pumpkins (2008)				Pumpkins (2009)				Pumpkins (2008)				Pumpkins (2009)			
	81	82	83	84	91	92	93	94	81	82	83	84	91	92	93	94
C12:0	–	–	2.7	–	0.3	–	–	6.3	0.2	0.6	0.5	–	–	–	–	1.1
C14:0	2.3	–	10.1	1.4	3.0	2.0	2.4	0.5	0.7	0.6	1.5	–	–	1.64	1.0	0.8
C16:0	12.4	14.4	15.4	10.2	10.6	13.4	13.6	12.5	6.2	3.0	2.3	2.0	4.0	4.77	1.5	13.7
C18:0	7.6	9.2	5.8	7.0	8.0	9.4	9.3	7.7	1.4	1.5	2.3	–	2.2	3.25	3.2	3.9
C18:1	27.0	38.6	16.8	25.6	29.8	32.1	19.3	17.1	38.4	39.5	29.9	28.9	37.3	29.1	17.7	20.3
C18:2	49.2	37.8	49.1	55.8	47.3	43.2	55.4	55.9	52.5	53.4	59.3	69.1	55.5	61.26	73.0	59.4
C18:3	–	–	–	–	–	–	–	–	0.51	0.2	1.6	–	–	–	1.2	–
C20:0	01.4	–	–	–	1.0	–	–	–	–	0.4	0.8	–	–	–	–	–
C20:1	–	–	–	–	–	–	–	–	–	1.0	–	–	1.0	–	–	0.7
C20:4	–	–	–	–	–	–	–	–	–	–	1.4	–	–	–	–	–
C23:0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24	–
C24:0	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–
TSFA	23.8	23.6	34.1	18.6	23.0	24.7	25.3	27.0	8.5	5.7	7.3	2.0	6.2	9.6	8.1	19.5
TUFA	76.2	76.4	65.9	81.4	77.0	75.3	74.7	73.0	91.5	94.3	92.9	98.0	93.8	90.4	91.9	80.5

TSFA: Total Saturated Fatty Acids; TUFA: Total Unsaturated Fatty Acids.

essential FAs are the compounds which cannot be endogenously synthesized and must be supplied through the diet because of their requirement for human body.

The normal TAGs separated from the oils were later hydrolyzed using Luddy et al [10] procedure to analyze the composition of 2-MAGs. The 2-MAGs were then separated from the lipolytic product by preparative silicate TLC. Pure 2-MAGs were immediately esterified and subjected to GC analysis. Table 2 shows the percentage composition of 2-MAGs. In all studied oils the unsaturated acids dominate on saturated FAs with linoleic acid (52.5–73.0%) as the major component at 2–position followed by oleic acid (17.8–39.5). The other FA_S present at 2-position were 16:0 (less than 14%) and 18:0 (less than 4%).Thus, there are traces of the others FA_S like C14:0. Therefore, in all pumpkin seeds oils samples the percentage of unsaturated acids were higher (more than 90%) except sample 94 which the unsaturated component dominates the 2-position with 80.5%. These results were in agreement with the work by Mattson and Volpenhein [44], which showed that oleic and linoleic acids are preferentially attached to the 2-position. This result is in concordance with the positional distribution theory suggested by Van Derwal [45], Coleman and Fulton [46], Gunstone [47] and Youngs [48], where the 2-MAG contains mostly unsaturated FA with 18 atoms of carbon. The remaining FA is randomly distributed among 1,3-positions of the TAG molecule. The composition in TAG components of the pumpkin oils was calculated from the FA found in the 1,3-positions and those determined in the 2-position for the oil, according to the distribution theories of Gunstone [47] and Coleman [46].The present data agree well with the "positional distribution theory" presented in graphs published by Gunstone [47]. The acylglycerol structure data also are in accordance with the correlation curves of Coleman [46]. The results are given in Table 3.Values less than 0.1% is not mentioned.

According to the Gunstone distribution theory, seven major TAGs were identified: LOL, LLL, OOL, LPL, OPL, LStL and OStL. The other TAGs are minors (less than 3%). The contents of these TAGs vary in a more or less significant way of a sample to another. Thus, in sample 84 the quantitative composition of LOL, LLL, LOO, LPL, OPL, LStL and OStL represents more than 83% of total TAGs, with prevalence of LOL (23%); whereas, lowest quantitative composition of these major TAGs was found in the sample 83 which represents 52.5% of total TAGs. In the other samples, the quantitative compositions of the seven main TAGs show similar content (average 70%) of total TAGs, with prevalence of LOL (10–23%). This method showed a good content for the eight preceding samples and these results confirm the resemblances observed in the compositions in FA_S. The components TAGs of the eight samples of *Cucurbita pepo* seed oils calculated by using Coleman distribution theory were mentioned in the Table 3.

Table 3
Triacylglycerols composition of total TAGs (%) in Pumpkin seed oils

	Coleman									Gunstone							
	Pumpkins (2008)				Pumpkins (2009)					Pumpkins (2008)				Pumpkins (2009)			
	81	82	83	84	91	92	93	94		81	82	83	84	91	92	93	94
ALL	1	–	–	–	0.74	–	–	–	A,L,L	1.1	–	–	–	0.78	–	–	–
AOL	0.76	–	–	–	0.5	–	–	–									
ALO	0.46	–	–	–	0.44	–	–	–	A,L,O	1.2	–	–	–	0.98	–	–	–
ALSt	0.24	–	–	–	0.18	–	–	–	A,L,St	0.31	–	–	–	0.23	–	–	–
AOO	0.34	3.5	–	–	0.3	7.3	–	–	A,O,O	0.34	–	–	–	0.31	–	–	–
AOSt	0.18	–	–	–	0.12	–	–	–	A,O,St	0.17	–	–	–	0.14	–	–	–
AOP	0.24	–	–	–	0.16	–	–	–	A,O,P	0.28	–	–	–	0.19	–	–	–
ALP	0.34	–	–	–	0.24	–	–	–									
LPA	0.12	–	–	–		–	–	–	L,P,A	0.51	–	–	–	0.31	–	–	–
LLL	11.9	4.8	11.5	16.6	10.3	7.1	15.8	17.4	L,L,L	11.1	5	9.9	16.7	9.9	7.5	15.7	15.7
LLO	10.6	12.2	5.4	16.3	12.4	14	13.6	10.1									
LOL	8.7	–	5.8	7	6.9	–	3.8	6	L,O,L	18.3	15.4	10.1	23	18.8	16.7	16.4	14.7
LLSt	5.4	4.2	3.9	7.1	5.2	5.1	8.4	6.2									
LStL	0.32	0.13	0.45	–	0.41	0.36	0.69	1.1	L,St,L	6.1	4.3	4.7	7.1	5.9	5.8	9.5	8.1
LLP	7.8	6.5	11.4	9.7	6.6	7.33	13.4	7.6									
LPL	1.4	0.26	0.45	0.48	0.75	0.5	0.33	4	L,L,P	10	6.8	12.5	10.3	7.8	8.3	13.9	13.1
LLM	1.5	–	7.5	1.4	2.2	0.9	2.1	0.22			–						
LML	0.16	–	0.29	–	–	0.16	0.21	0.23	L,M,L	1.8	–	8.3	1.4	2.3	1.2	2.4	0.52
LLLa	–	—	2	–	0.22	–	–	5.7									
LLaL								0.32	L,La,L	–	–	2.2	–	0.22	–	–	6.3
LOO	7.8	9	2.7	6.8	8.4	6.6	3.3	3.5									
OLO	2.4	7.8	0.63	4	3.7	6.9	2.9	1.5	O,L,O	10.1	15.8	3.5	10.6	11.8	12.4	5.7	4.6
LOSt	3.9	3.1	2	3	3.5	2.4	2	2.1									
LStO	0.28	0.34	0.2	–	0.5	0.7	0.6	0.66	L,St,O	6.8	8.9	3.2	6.6	7.5	8.6	6.7	4.9
StLO	2.4	5.3	0.92	3.5	3.1	5.1	3.6	1.8									
LOP	5.7	4.8	5.8	4	4.5	3.5	3.2	2.6									
LPO	1.2	0.68	0.2	0.48	0.9	1	0.28	2.3	L,P,O	11	14	8.6	9.5	9.8	12.3	9.7	8.1
PLO	3.5	8.2	2.7	4.7	4	6.2	5.8	2.22									
LOM	1.1	–	3.8	0.58	1.5	0.4	0.5				–						
MLO	0.70	–	1.8	0.68	1.3	0.8	0.9		M,L,O	2	–	5.7	1.3	2.8	1.8	1.7	0.32
LMO	0.14	–	0.14	–	–	0.3	0.18	0.14									
LOLa	–	–	1	–	0.14	–	–	1.6									
LaLO	–	–	0.48	–	0.12	–	–	1.7	La,L,O	—	–	1.5	–	0.28	–	-	4.1
LLaO	–	–		–	–	–	–	0.18									
LStSt	0.14	0.12	0.14	–	0.2	0.2	0.36	0.4	L,St,St	0.85	0.93	0.55	0.76	0.88	1.1	1.4	1
StLSt	0.60	0.9	0.33	0.77	0.66	0.9	1.1	0.55									
LStP	0.20	0.18	0.44	–	0.26	0.3	0.58	0.5									
PLSt	1.80	2.8	1.9	2.1	1.7	2.63	3.6	1.4	P,L,St	2.8	2.9	3	2.2	2.3	3.2	4.2	3.3
LPSt	0.64	0.22	0.14	0.2	0.38	0.4	0.18	1.4									
LStM		–	0.3	–			0.1										

Table 3
(*Continued)*

	Coleman									Gunstone							
	Pumpkins (2008)				Pumpkins (2009)					Pumpkins (2008)				Pumpkins (2009)			
	81	82	83	84	91	92	93	94		81	82	83	84	91	92	93	94
MLSt	0.34	–	1.3	0.3	0.56	0.3	0.56		M,L,St	0.51	–	2	0.3	0.68	0.48	0.74	0.13
LMSt		–	0.1	–	–	0.11	0.1										
LaStL	–	–		–		–	–	0.36									
LLaSt	–	–		–	–	–	–	0.12	L,La,St	–	–	0.53	–		–	–	1.3
LaLSt	–	–	0.34	–		–	–	1									
LPP	0.92	0.36	0.44	0.28	0.48	0.5	0.28	1.8									
PLP	1.3	2.2	2.9	1.4	1.1	1.9	2.8	0.83	P,L,P	2.2	2.3	4	1.6	1.5	2.3	3.1	2.7
LPM	0.18	–	0.3		0.16												
LMP	0.1	–	0.29	–	–	0.2	0.18	0.1	L,M,P	0.83	–	5.2	0.43	0.89	0.68	1.1	0.21
MLP	0.5	–	3.76	0.4	0.7	0.4	0.88										
LPLa	–	–		–		–	–	1.3									
LaLP	–	–	1	–		–	–	1.3	La,L,P	–	–	1.4	–		–	–	2.7
OOO	1.7	5.7	0.31	1.7	2.5	3.2	0.71	0.51	O,O,O	1.8	5.4	0.4	1.6	2.5	3.1	0.66	0.47
OOSt	1.8	3.9	0.46	1.4	2.1	2.4	0.88	0.62									
OStO	0.06	0.21		–	0.15	0.33	0.13	0.1	O,St,O	1.8	4.5	0.55	1.5	2.3	3.2	1.2	0.77
OOP	2.5	6.1	1.3	2	2.7	2.4	1.4	0.76									
OPO	0.28	0.43		0.12	0.27	0.5		0.34	O,P,O	3	7.1	1.5	2.2	3.1	4.6	1.7	1.2
OOM	0.5	–	0.88	0.28	0.88	0.42	0.22										
OMO		–		–	–	0.13			O,M,O	0.56	–	0.97	0.29	0.9	0.68	0.29	
OOLa	–	–	0.24	–		–	–	0.56	O,O,La	–	–	0.26	–		–	–	0.6
OStSt		0.14		–	0.12	0.2	0.16	0.12									
StOSt	0.44	0.67	0.17	0.32	0.44	0.45	0.27	0.19	St,O,St	0.46	0.96	0.19	0.35	0.56	0.84	0.51	0.32
OStP	0.1	0.22	0.1	–	0.16	0.3	0.24	0.14									
StOP	1.3	2.1	0.98	0.86	1.1	1.23	0.86	0.46	St,O,P	1.5	3	1	1	1.5	2.4	1.5	1
StPO	0.28	0.28		0.1	0.22	0.4		0.42									
OStLa	–	–		–		–	–	0.1	O,St,La	–	–	0.18	–		–	–	0.52
StOLa	–	–	0.18	–		–	–	0.34									
OPP	0.4	0.46	0.1	0.14	0.28	0.5	0.12	0.52	O,P,P	1.2	2.4	1.4	0.74	0.97	1.7	1.1	0.8
POP	0.93	1.6	1.4	0.59	0.71	0.9	0.69	0.29									
OPM		–			0.1												
POM	0.36	–	1.9	0.16	0.48	0.22	0.22		P,O,M	0.46	–	1.8	0.2	0.56	0.51	0.37	
OMP		–		–	–	0.2											
OPLa	–	–		–		–	–	0.38	O,P,La	–	–	0.48	–		–	–	0.84
POLa	–	–	0.5	–		–	–	0.42									
OMSt		–		–	–	0.14			O,M,St	0.28	–	0.67	0.14	0.42	0.35	0.26	0.28
StOM	0.26	–	0.64	0.12	0.38	0.16	0.14										
OLaSt	–	–		–	–	–	–	0.4									
StStP				–		0.14	0.16										
StPSt								0.13									
StPP	0.2	0.16		0.06	0.12	0.2		0.32									

Table 3
(*Continued*)

	Coleman									Gunstone							
	Pumpkins (2008)				Pumpkins (2009)					Pumpkins (2008)				Pumpkins (2009)			
	81	82	83	84	91	92	93	94		81	82	83	84	91	92	93	94
PStP			0.11	–		0.1	0.12										
StPLa	–	–		–		–	–	0.24									
PStM		–	0.14	–													
PPP	0.15	0.12	0.11	0.04	0.08	0.15		0.19									
PPM		–	0.14														
PPLa	–	–		–		–	–	0.28									
PLaL	–	–		–	–	–	–	0.14									
MLM		–	1.2		0.11				M,L,M		–	1.7		0.13			
MLLa	–	–	0.66	–		–	–		M,L,La	–	–	0.93	–		–	–	0.11
MOM		–	0.62						M,O,M		–	0.59					
MOLa	–	–	0.34	–		–	–		M,O,La	–	–	0.32	–		–	–	
LaLLa								0.47	La,L,La	–	–	0.12	–		–	–	0.68
LaOLa	–	–		–		–	–	0.16	La,O,La	–	–		–		–	–	0.21
LaPLa	–	–		–		–	–	0.11									
Others	1.44	0.32	2.78	0.34	1.58	0.84	1.37	1.06	Others	0.64	0.31	0.06	0.19	0.77	0.26	0.17	0.42

A : arachidic acid; L : linoleic acid; O : oleic acid; St : stearic acid; P : palmitic acid; M : myristic acid; La : lauric acid.

It appears that the TAGs containing oleic, linoleic and palmitic FA_S represent more than 50% of the total TAGs, because the content of these three acids are most important in the oil samples. Also, these oils have divergent quantitative compositions in TAGs distribution. Moreover, the individual content of the TAG varies from a sample to another. In addition, we observe that calculated values for TAGs containing two oleoyl and one linoleoyl chains (O, O, L) or one palmitoyl and two oleoyl chains (P,O,O)agree well with the corresponding values determined by Gunstone method. These results are in perfect agreement with those of the literature. We have observed that concerning the cucurbitaceous ones usually consumed in Congo, for species keep a similar profile in TAGs distribution with regard to our specie; it is about *Citrullus lanatus*, *Cucurbita pepo* and *Lagenaria siceraria* and *Cucurbita moschata* [49]. In conclusion, oleic and linoleic acids preferentially would thus be fixed in position 2 of glycerol and thus completely available for the organization. Such a possibility increases the nutritional interest of this botanical family.

In general, the physical properties of a fat are more easily interpreted using the classification of the TAGs in groups according to their unsaturation degree.Table 4 shows the TAG percentage in term of four main TAG categories: trisaturated (GS_3), disaturated (GS_2U), monosaturated (GSU_2), and triunsaturated (GU_3). For all oil samplesstudied,GS_2U, GSU_2 and GU_3 types were found, but no GS_3 was detected. The contribution of the GU_2S is also important when considering their sensorial properties at room temperature. As a consequence, the ratio between GS_2U and GU_2S amounts in oil samples is selectively associated with an increase in the technological functionality or improvement ability of the sensorial attributes of this raw-material. *Cucurbita pepo* seeds with lower GS_2U contents and higher GU_2S level scan lead to unsatisfactory crystallization characteristics [50]. However, the technical literature does not indicate the limits of these TAG classes suitable for adequate crystallization behaviour in different climate regions. According to our results, it's clear that tripalmitin (PPP) is the major component TAG among GS_3 deduced in the *Cucurbita pepo* seed oils. This value is agrees well with Kartha's restricted random distribution theory [32]; which shows that the amount of GS_3 must not exceed a value that permits its solubility in the substrate.

The values of disaturated TAG_s (GS_2U) ranged between 7.8 and 26.1%. These values, due to the fact that the oils had a reduced amount of saturated acids especially palmitic acid. Dipalmitolinolein (PPL) constitutes the principal TAG of the GSU_2 category. Concerning the di-insaturated TAGs category, the amount of the various TAGs types was clearly manifested by the fact of the FAs percentage. The palmitodilinolein (PLL) TAG is higher than the palmitidiolein (POO), because the content of linoleic acid is higher than that of oleic acid in the oil samples. As

Table 4
The four categories of total TAGs

		Coleman				Gunstone			
		GS_3	GS_2U	GSU_2	GU_3	GS_3	GS_2U	GSU_2	GU_3
Pumpkins (2008)	81	0.01	12.7	45.9	41.3	0.01	12.7	45.9	41.3
	82	0.04	12.5	45.7	41.7	0.03	12.5	45.7	41.7
	83	–	26.1	50.0	23.9	0.03	26.1	50.0	23.9
	84	0.04	7.80	40.2	52.0	0.04	7.8	40.2	52.0
Pumpkins (2009)	91	0	11.9	45.2	43.0	0	11.9	45.2	43.0
	92	0	13.7	46.7	39.6	0.01	13.7	46.6	39.6
	93	0	14.4	47.1	38.4	0.01	14.4	47.1	38.4
	94	0	16.4	48.2	35.4	0.02	16.2	48.3	35.5

GS_3: Trisaturated acylglycerols; GS_2U: Disaturated acylglycerols; GSU_2: Monosaturated acylglycerols; GU_3: Triunsaturated acylglycerols.

Table 5
Tocopherols composition of the *Pumpkin* seeds oil

	Pumpkins collected in 2008				Pumpkins collected in 2009			
	81	82	83	84	91	92	93	94
α-tocopherol (mg/kg of oil)	7.7	28.4	29.5	22.6	14.6	22.2	31.9	0.0
(β+γ)-tocopherol (mg/kg of oil)	155.1	61.1	126.7	39.3	75.5	90.1	46.4	68.0
δ-tocopherol (mg/kg of oil)	54.8	40.5	56.0	42.7	39.0	53.6	45.0	130.0
Total(mg/kg of oil)	217.6	130.0	221.2	104.7	129.1	166.0	123.4	170.9

well, the percentage of GSU_2in the all oils is the most important comparatively to the other categories. The total triunsaturated TAGs (GU_3) were ranged between 23.9 and 52.0%. The dilinoleiolein (OLL) TAGs type has the higher percentage in the all oils (10.2–23.0%), but the triolein TAGs (OOO) were the lowest with percentages varied between 0.4 and 5.4%.

3.3. Tocopherols analysis

The determination of tocopherol homologues in the pumpkin seed oils is important owing to their antioxidative effects and their positive nutritional influences in human metabolism as biological antioxidants [51]. Tocopherols have already been the subject of many studies describing their content in plant materials and some papers describe the effect of physical treatments like frying at 180°C and subjecting them to microwave heating but none on the effect of roasting [6].

The results were obtained from two repetitions. An average curve of peak areas relating to five selected concentrations was then plotted using a linear regression calculation. The coefficients of correlation R^2 were close to 1. As shown in Table 5, the pumpkin seed oils had a relatively high level of total tocopherols (104.7–221.2 mg/kg of oil), which would be expected to contribute good oxidative stability of the oil during storage and processing. The total tocopherols content in the studied pumpkin seed oil was relatively higher than that reported in the literature [6, 52]. Also, the seeds oil had total tocopherols content lower to other oleaginous seeds such as cotton (380–1200 mg/kg oil) and maize (330–3720 mg/kg oil). This content was higher than those of olive (114 mg/kg oil), Babassu (60–130 mg/kg oil) and coco (less than 50 mg/kg oil) [53]. While there were certain similarities in the levels of total tocopherols content, there was a difference in the separated individual tocopherolsamounts. (β+γ)-tocopherol levels were extremely high in all studied pumpkin seed oils (more than 40% of total tocopherols). δ-tocopherol was the second major component in all oil samples, accounting for 39.1–103.0 mg/kg of oil content. Sample number 94 was a rich source

of δ- tocopherol since its level (103.0 mg/kg of oil) was much higher than for other pumpkin seed oils and those of olive (0 mg/kg oil), maize (23 mg/kg oil) and Sunflower (0 mg/kg oil) [53]. The differences observed in the amounts of total tocopherol in the oils from different samples were perhaps due to the climate and date of collect. These results provide useful information for the industrial application of these seeds. High levels of δ- tocopherolin the oils, may contribute to the greater stability toward oxidation.

3.4. Antioxidant activity

The quantification of oxidative stress in populations appears to be a possible indicator for the magnitude of environmental risk factors. The interpretation of the results has been expressed by the EC_{50} parameter which is defined as the efficient concentration or the concentration of the substrate that causes 50 % loss of radical activity. The EC_{50} values were calculated by the linear regression method of plots of the percent of antiradical activity or reducing power against the concentration of the tested compounds. Trolox, α-tocopherol, BHA, BHT and ascorbic acid were used as water-soluble and lipid-soluble reference antioxidants in order to indicate the range of activity which is expressed by the different test systems (Table 6).

The model of scavenging stable radical DPPH is a widely used method to evaluate antioxidant capacities of natural products, and it has been used for olive oil and other vegetable oils as well as for individual antioxidant polyphenols [54, 55].

In the DPPH assay, all oil extracts scavenged $DPPH^{\bullet}$ radical. As shown in Table 6, the pumpkin seed oils were characterized by statistically significant differences in their antioxidant activity measured by the $DPPH^{\bullet}$ method. Hence, all the oil extracts showed similar DPPH radical scavenging activity. No significant difference of the antiradical power was observed between the oil extracts of pumpkin seeds which collected in 2008 and that collected in 2009. The highest antioxidant activity was displayed by the extract obtained from sample 94 pumpkin oils. Whereas, sample 91 possessed the lowest antiradical activity. Also, the Table 6 shows the comparison of the mean concentration for 50% free radical scavenging activity (EC_{50}) of pumpkin seed oils against 250 μM DPPH radical. The EC_{50} values of standards which ranged from 0.005 to 0.01 mg/ml were stronger than all tested oils.

Many researches could not find significant correlation between the total antioxidant content and antioxidant activity of the plant extracts. This may be because fruits and vegetables contain many different antioxidant components including carotenoids, vitamins, phenolic compounds, flavonoids, etc., which could affect the measurement of antioxidant activity. Moreover, in our study there was no significant correlation between antiradical activity and total tochopherol contents of the studied oils. But, the correlation coefficient between DPPH scavenging activity and vitamine E

Table 6
Antioxidant capacity of plant extracts as measured using the DPPH and phosphomolybdenum (PPM) assays

		DPPH assay EC_{50} (mg/ml)	PPM assay EC_{50} (mg/ml)
Pumpkins (2008)	81	23.8 ± 0.8	20.6 ± 0.9
	82	36.7 ± 1.8	24.4 ± 1.4
	83	25.1 ± 0.7	45.5 ± 3.1
	84	38.7 ± 0.2	18.8 ± 0.3
Pumpkins (2009)	91	41.7 ± 0.1	39.8 ± 3.9
	92	27.39 ± 5.04	41.5 ± 7.5
	93	22.0 ± 2.0	56.3 ± 0.2
	94	17.9 ± 3.0	31.1 ± 0.6
Vitamin C		0.009 ± 0.001	0.07 ± 0.001
Vitamin E		0.010 ± 0.002	0.08 ± 0.001
Trolox		0.005 ± 0.001	0.07 ± 0.001
BHT		0.006 ± 0.001	3.60 ± 0.002
BHA		0.006 ± 0.001	0.27 ± 0.005

contents had $R^2 = 0.2$. This result suggests that 21% of the antioxidant capacity of Algerian pumpkin seed oils results from the contribution of α-tocopherol compounds. Also, it can be concluded that antioxidant activity of oil extracts is not limited to tocopherols, but it's may also come from the presence of other antioxidant secondary metabolites. Moreover, as illustrated in Table 6, the best antioxidant activity was displayed by the extract oil obtained from sample number 94. This oil contained the highest amount of δ-tocopherol content. So, the good antiradical power of this oil extract may be due to the presence of the highest level of δ-tocopherol content.

The phosphomolybdenum method is routinely applied in laboratories to evaluate the total antioxidant capacity of plant extracts and a variety of grains and seeds [34, 56]. This method is based on the reduction of molybdenum by the antioxidants and the formation of a green (V) complex, which has absorption at 695 nm. The total antioxidant capacity observed in all oil extracts has EC_{50} values which ranged from18.8 ± 0.3 to 56.3 ± 0.2 mg/ml (Table 6). All extracts oils had smaller activity comparatively to standard antioxidants. The higher antioxidant capacity has been revealed for the sample 84. Its effect was almost less than five times as BHT. This antioxidant is currently used in therapy and food industry, but is accused of being dangerous to health and particularly BHT [57]. So, the results suggest that our oil extracts in particular sample 84 can replace synthetic antioxidants because they pose a good antioxidant activity to those observed for the antioxidant reference.Comparing these results with literature, similar values were reported for pumpkin seeds oil from Poland [4]. However, our pumpkin oil extracts have illustrated good antiradical activities comparatively to other plants oil such as Soybean and Sunflower [4].

Moreover, there was no relationship between total antioxidant and reducing activities (R^2 <0.40). Also, a bed correlation has been observed between the antioxidant activity and the level of α-tocopherol content ($R^2 \approx 0.50$). These results can be explained since the global antioxidant property of a plant extract is generally considered as the result of the combined activity of a wide range of compounds including, beside phenolics, peptides, organic acids and other components [58]. This is the reason why samples such as 82 and 93 with similar concentrations of total tocopherols, may vary in their antioxidant activities (PPM assay). It can be observed that the antioxidant activity of the oil extracts have no significant correlation with their antiradical activity measured by DPPH assay (R^2 <0.1). The discrepancy between results of DPPH and PPM assays may be related to the different mechanisms involved in each evaluation. In conclusion; our results suggest that the oil extracts of some pumpkin seeds investigated have radical-scavenging capacity. The re-introduction of the *Cucurbita pepo* seeds into the regular diet is acceptable and could be a relevant cultural practice in the control of diseases in which free radicals are involved.

Data on the antioxidant content pumpkin seed oil is very important for food scientists, doctors, industries and consumers, comparatively to the Mediterranean olive oil. Although, various methodologies have been developed for the quantitative assessment of different antioxidant compounds in edible oils. So, pumpkin seed oil could be an alternative to the expensive olive oil [59–61].

3.5. Cluster analysis

In order to highlight the correlation of TAGs and tocopherols contents of the pumpkin seeds (different cultivars in Algeria) with some common plants (Data provided by literature), we have chosen the method of cluster analysis, using the Ward's technique.

The result referring to the TAGs analysis showed the existence of two principal clusters (I and II) within the TAGs of the individuals of the investigated plants (Fig. 1). The group (I) cluster gathers the flowing plants: *Gossypum arboreum, Cucumis melo, Cucurbita maxima, Papaver somniferum, Heliantus annuus* and *Glycine maxima*. The second group (II) can be separated to two subgroups SG-1 and SG-2. The first subgroup (SG-1) is referred to the following plants: *Brassica napus* and *Olea europaea*. The second subgroup (SG-2) includes *Argania spinosa, Pistacia atlantica, Cucurbita pepo, Arachis hypogaea* and our studied *Cucurbita pepo L.* This results indicates that our seeds oil are dietary plants oils.

In the case of the tocopherols, the dendrogram of the Fig. 2 indicates the presence of two main groups I and II. The second group can divided in two subgroups SG-1 and SG-2. The first group is referred to the cluster of the following plants: *Arachis hypogaea, Gossypum arboretum, Heliantus Annuus* and *Olea europaea*. The second group comprise all the rest of the adopted plants (Pumpkin seeds individuals included). For the tocopherols cluster analysis, the Pumpkin individuals are dispatched between the two subgroups 1 and 2. The first subgroup is characterised by

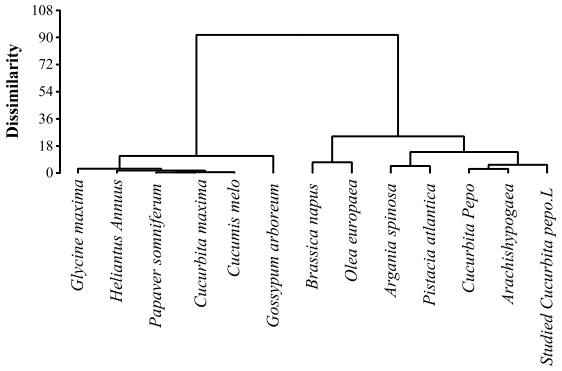

Fig. 1. Dendrogram obtained from the TAGs cluster analysis of studied Pumpkin seeds and 12 different plants oils.

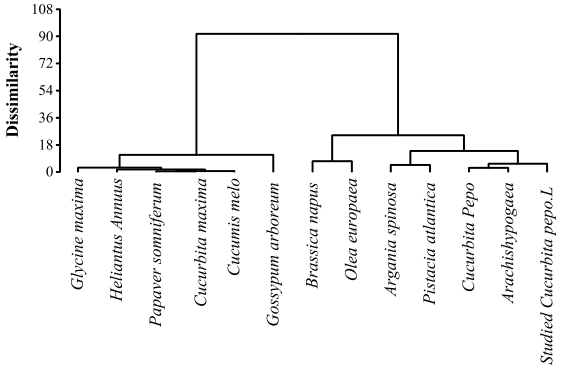

Fig. 2. Dendrogram obtained from the tocopherols cluster analysis of studied Pumpkin seeds and 11 different plants oils.

the highest contents of α-tocopherol. The differentiation of the SG-1 and SG-2 is based on the difference of the three tocopherols, i.e. SG-2 is characterised by higher contents of α-, and (β+γ)-tocopherol, but lower content of δ-tocopherol in comparison with SG-1.

4. Conclusion

In this study, we have investigated the oil content, FA, TAG and tocopherol composition and antioxidant activities of oils from 8 pumpkin samples, belonging to *Cucurbita pepo*, cultivated in Algeria. To date, nothing is known about the oil characteristics of any of the 8 samples selected in this study. Additionally, there are few studies on pumpkin seed oil from the many thousand cultivars of *Cucurbita pepo*. The results of the present study showed that Algerian pumpkin seed contained appreciable amounts of total tocopherols and crude fat. The oil, extracted from the pumpkin seeds, harvested from Sebgag, Laghouat, Algeria, was rich in linoleic acid (C18:2) as well as it exhibited low levels of saturated FAs, suggesting its potential food uses for health benefits. Furthermore, it was demonstrated for the first time that the studied oils possessed a good antioxidant activity which may be associated with their alleged health benefits. Also, knowledge gained from this study will help to determine the potential for seed oil from this pumpkin cultivar to be commercially exploited for industrial applications and incorporation into food formulations to improve human health. Additional research on the detailed physicochemical and bioactive properties of pumpkin seed oil is crucial to explore their commercial and functional foods applications.

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