DOI: http://dx.doi.org/10.1590/1678-457X.6579

Influence of harvest year in the physicochemical properties and antioxidant activity of flaxseed hull oils from Tunisia

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Abstract

The objective of this research was to determine the effect of five years on physico-chemical characteristics and antioxidant activity of flaxseed hull oils. Oil and protein on flaxseed hull varied between 20.15 and 22.56 and 17.45 and 19.14, respectively. The albumin fraction dominated the seed hull protein composition (44 - 47%). Flaxseed hull oils showed significantly high unsaturated to saturated fatty acid ratios (6.61 - 8.36) and calculated oxidizability (Cox) values (10.88 - 12.30). The physiochemical parameters determined included saponification value (SV) (174 -182 mg KOH/g), unsaponifiable matter (UM) (1.32 - 1.64%), peroxide value (PV) (1.70 - 2.20 mequiv/kg), Oxidation value (OV) (4.40 - 5.90) and oil stability (1.10 - 1.42 h). The highest content of total phenolic acids and total tocopherols were recorded on hull sample collected in 2008. Flavanoid content ranged from 11 to 19 mg/100 g oil. Antioxidant activity differed significantly; the greatest inhibition (61.38%) was for the hull sample collected in 2008.

Keywords: flaxseed hull oil; antioxidant activity; chemical composition.

Practical Application: Practical application for the study entitled "Influence of harvest year in the physicochemical properties and antioxidant activity of flaxseed hull oils from Tunisia" regard the possibility of knowing the chemical composition of flaxseed hull produced in Tunisia from autochthonous variety. It is very important to understand if and how harvest year influence the physicochemical characteristics of flaxseed hull. Valuable information will be provided for flaxseed hull breeders and growers in developing and producing functional food resources and products.

1 Introduction

Flaxseed hulls, a low-valued co-product represents a potential source of value-added healthy products. The hull, including the seed coat and endosperm, constitutes 36% of the total weight of hand-dissected flaxseed or 22% of the seed when obtained mechanically (Oomah & Mazza, 1998). Flaxseed hulls correspond roughly to 27% of the total weight of the seed (Herchi et al., 2014a). The hull consists of an outer, true hull, which is tough and fibrous, with no oil and protein, and an inner soft hull containing some oil and protein. With the success of a patented dehulling process (Cui & Han, 2006); the whole flaxseed could be efficiently separated into a kernel (63%) fraction and a hull (37%) fraction in large scale (Cui & Han, 2006). Oil content of flaxseed hulls varies from 26% to 30% depending on processing conditions representing approximately 18% of the total seed oil (Oomah, 2003). This oil from hulls obtained by dry abrasive dehulling contained significantly higher levels of palmitic acid and lowest level of stearic and oleic acids compared to those from the whole seed (Oomah & Mazza, 1998). Since the hulls are a low value product obtained from the seed dehulling, the recovery of important compounds, such as oil, tocopherols and total phenolics present on flaxseed hulls could contribute for the advance of this by-product utilization.

Flaxseed hulls are a rich source of soluble and insoluble (dietary) fiber, and contain a very high concentration of the lignan, secoisolariciresinol diglycoside (SDG), which exhibits high antioxidant activity (Renouard et al., 2010). Mostly present on flaxseed hull, phenolic compounds are natural antioxidants, which are believed to have protective effects against degenerative diseases in humans, such as cardiovascular diseases, cancer and diabetes mellitus (Renouard et al., 2010). Investigating the composition, physicochemical and antioxidant properties of flaxseed hulls will assist in exploring its potential for the food industry health and safety. Although flaxseed hull composition has been studied during maturation (Herchi et al., 2014a), However, no information is available concerning the effect of harvest year on the chemical composition and antioxidant activity of flaxseed hull oils.

Therefore, the main objective of this study was to determine for the first time the physicochemical characteristics of flaxseed hull oils and antioxidant activity depending harvest year, to increase its value and contribution to the development of new omega-3 products for the functional foods and nutraceutical applications.

Received 25 Nov., 2014
Accepted 04 Feb., 2015
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2 Materials and methods

2.1 Chemicals and reagents

All solvents and standards used in the experiments were purchased from Fisher Scientific Company (Ottawa, Ontario, Canada).

2.2 Plant material

Flaxseed hull (N105) was obtained from Institut National Recherche Agronomie Tunis (INRAT), Tunisia. The dehulled seed and hulls were separated by air aspiration.

2.3 Proximate composition

The dry matters contents of flaxseed hull were determined by drying in an oven at 105 °C during 24 h to constant weight (Association of Official Analytical Chemists, 1990). The crude protein contents were calculated from nitrogen contents (N x 6.25) obtained using the Kjeldahl method by (Association of Official Analytical Chemists, 1990). The crude fat contents were determined by continuous extraction in a Soxhlet apparatus for 5 h using petroleum ether as solvent (Association of Official Analytical Chemists, 1990). The total ash contents were determined by incinerating flaxseed hull (2 g) in a furnace at 550 °C for 6 h, then weighing the residue after cooling to room temperature in a desiccator (Association of Official Analytical Chemists, 1990). The carbohydrate contents were determined by difference that is by deducting the mean values of other parameters that were determined from 100. Vitamin C (Ascorbic acid) content was determined by application of the 2.6 dichloroindophenol volumetric method (Association of Official Analytical Chemists, 1990). The sample calorific value was estimated (in Kcal) by multiplying the percentage crude protein, crude lipid and carbohydrate by the recommended factor (4, 9 and 4.125 respectively) used in vegetable analysis (Hunt et al, 1987). Digestible crude protein was estimated as follows: Digestible crude protein (g) = (protein (g) × 0.96 – 4.21) (Barrett and Larkin, 1977).

2.4 Protein fractionation

Protein fractions were isolated by sequentially extracting flaxseed hull with different solvents (the hull to solvent ratio was 1:10, w/v, in each case) according to the modified Osborne scheme, as described by Chavan et al (2001): Samples (1 g hull) were dispersed in 10 ml distilled water by stirring with a magnetic stirrer and extracted over 45 min periods at room temperature (25 °C). The suspension was then centrifuged at 5,000 × g for 15 min, and the resultant supernatant was filtered. The residues were re-extracted twice more with the same solvent and the recovered filtrates were combined and designated the "water-soluble fraction". The residue was then extracted successively with 0.5 M NaCl solution (pH 7.0), 70% (v/v) ethanol at 65 °C in a shaking water bath, and 0.1 M sodium hydroxide in order to separate the total hull proteins into albumin, globulin, prolamin and glutelin fractions, respectively. Filtrates containing the desired protein fractions were dialyzed against distilled water for 48 h at 4 °C and separately lyophilized. The protein content of each fraction was determined by the micro-Kjeldahl procedure (Association of Official Analytical Chemists, 1984).

2.5 Gas chromatography–flame ionization detection (GC-FID)

The quantification of fatty acids methyl esters was performed using a gas chromatography – flame ionization detection (GC–FID) apparatus. Fatty acid methyl esters were prepared by simultaneous extraction and methylation following the procedure described by Metcalfe et al. (1966) modified by Lechvallier (1966). Methyl esters were analyzed by GC, using an HP 4890 gas chromatograph equipped with a FID detector on a capillary column coated with Supelco wax TM 10 (30 m long × 0.25 mm i.d., and 0.2 µm film thickness). Helium was used as the carrier gas at a flow rate of 1ml/ min. Temperatures of the column, detector, and injector were 200, 250, and 230 °C, respectively. The identification of the peaks was achieved by retention times by means of comparing them with standards analyzed under the same conditions. The area under each peak was measured and the percentage expressed in regard to the total area. To evaluate the efficiency of the desaturation pathway during maturation process (Mondal et al., 2010) the desaturation ratios from oleic to linoleic (ODR, oleic desaturation ratio) and from linoleic to linolenic acid (LDR, linoleic desaturation ratio) were calculated as follows (Equations 1 and 2):

$$ODR = [\%\ C18{:}\ 2 + \%\ C18{:}\ 3\ /\ \%\ C18{:}\ 1 + \%\ C18{:}\ 2 + \%\ C18{:}\ 3] \times 100 \quad (1)$$

$$LDR = [\%\ C18{:}\ 3\ /\ \%\ C18{:}\ 2 + \%\ C18{:}\ 3] \times 100 \quad (2)$$

The magnitude of desaturation ratios represents the amount of substrate which is successfully desaturated from C18:1 to C18:2 and C18:3, thus providing a proportional measure of the desaturating enzymes activities during seed maturation. The Cox value of the oils was calculated based on the percentage of unsaturated C18 fatty acids, applying the formula proposed by Fatemi & Hammond (1980) (Equation 3):

$$Cox\ value = [1(18{:}1\%) + 10.3\ (18{:}\ 2\%) + 21.6\ (18{:}3\%)]\ /\ 100 \quad (3)$$

2.6 Physicochemical characteristics

Determination of Density, Saponification value (SV), Acid value (AV), Free fatty acids (FFA), Iodine value (IV), *p*-anisidine value (*p*-AV), Peroxide value (PV), UV absorption characteristics (K_{232} and K_{270}), and unsaponifiable matter (UM) of the extracted oil was carried out by standard IUPAC methods for the analysis of fats and oils (Dieffenbacher & Pocklington, 1987). Oxidation value (OV) was calculated from Holm's equation, OV = *p*-AV + 2 (PV), while theoretical flavor scores (F) were obtained from equation F = 7.7 – 0.35 (OV) (List et al., 1974). Oxidative stability was evaluated by the Rancimat method (Gutierrez, 1989). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 743 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 3 g warmed to 100°C and air flow of 10 l/h.

2.7 Total chlorophyll and carotenoids

A 1.5 g sample of flaxseed hull oil was fully dissolved in 5 mL cyclohexane. Chlorophyll and carotenoid were determined colorimetrically following the method of Minguez-Mosquera et al (1991). The maximum absorption at 670 nm is related to

the chlorophyll fraction and at 470 nm is related to carotenoid fraction. The values of the coefficients of specific extinction applied were $E_0 = 613$ for the pheophytin as a major component in the chlorophyll fraction and $E_0 = 2{,}000$ for lutein as a major component in the carotenoid fraction. Thus the pigment contents were calculated as follows (Equations 4 and 5):

$$\text{Chlorophyll (mg/kg)} = (A_{670} \times 10^6) / (613 \times 100 \times d) \quad (4)$$

$$\text{Carotenoid (mg/kg)} = (A_{470} \times 10^6) / (2,000 \times 100 \times d) \quad (5)$$

Where A is the absorbance and d is the spectrophotometer cell thickness (1 cm). The data reported is based on oil weight (mg/ g flaxseed hull oil).

2.8 Polyphenols contents

Extraction and determination of total phenolic acids and flavanoids contents were carried out according to the method of Gutfinger (1981).

2.9 Total Sterols (TS) Content

The TS content was quantified according to the Lieberman- Burchard color reaction (Sabir et al,. 2003). Lieberman–Burchard reagent (sulfuric acid and acetic anhydride) reacts with sterols to produce a characteristic green color whose absorbance is determined by spectrophotometry at 640 nm.

2.10 Total Tocopherols (TT) Content

The TT content was determined according to the colorimetric method described by Wong et al. (1988).

2.11 Determination of antioxidant activity

The hull oil obtained was subjected to screening for its possible antioxidant activity. The oil was assessed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay. All the data were the averages of triplicate determinations of three tests. The DPPH free radical-scavenging activity of oil was measured using the method described by Gorinstein et al. (2004). A 0.1 mM solution of DPPH in methanol was prepared. An aliquot of 0.2 mL of sample was added to 2.8 mL of this solution and kept in the dark for 30 min. The absorbance was immediately measured at 517 nm. The ability to scavenge the DPPH radical was calculated with the following Equation 6:

$$\text{Inhibition percentage} = (I\,\%) = [(A_0 - A_1) / A_0] \times 100 \quad (6)$$

Where A_0 is the absorbance of the control, A_1 is the absorbance in the presence of sample.

2.12 Statistical analysis

Statistical analysis was performed by using the Proc ANOVA in SAS (software version 8). All analyses were replicated three times for each sample.

3 Results and discussions

3.1 Effect of harvest year on the proximate composition and protein fractionation

The proximate composition of flaxseed hull over five years, 2005 - 2009 are shown in Table 1. As can be seen from the table, the proximate composition was significantly year harvest depending. Moisture content ranged from 5.85% to 7.87%. The oil content was found in the range of (20.15% - 22.56%). There was a significant difference ($p \leq 0.05$) in the oil content of flaxseed hull between the years 2005 and 2007. Oil is synthesized from carbohydrates either from current photosynthesis or from the remobilization of storage carbohydrates. Therefore, oil accumulation greatly depends on the carbon economy and photosynthesis ratio of the crop during seed filling (Aguirrezábal et al., 2009). The highest protein content (19.14%) was obtained in the year 2006, while the lowest protein content (17.45%) was obtained in the year 2005. Riedell et al. (2009) reported that variations in seed protein concentration induced by weather, water and nitrogen availability, response to temperature, solar radiation, CO_2, regional conditions, and growing techniques are much larger than variations due to genotype. Yalcin et al. (2011) investigated the protein and oil content of flaxseed grown in 2008 and 2009. They reported that the oil and protein content of flaxseed samples were affected by the harvesting year. There is no study in the literature about the effect of cultivation year on flaxseed hull composition. The total carbohydrate of flaxseed hull was not influenced by the variance in harvesting years, because a significant difference was not observed among total carbohydrate values over the five years ($p \leq 0.05$). Harvest year had no significant effect ($p \leq 0.05$) on

Table 1. Proximate composition of flaxseed hull depending harvest year.

Years	2005	2006	2007	2008	2009
Moisture (%)	6.79±0.32^a	6.28±0.20^a	5.85±0.57^b	6.12±0.42^b	7.87±0.35^a
Oils (%)	20.15±0.46^a	21.70±0.22^a	22.56±0.14^b	21.45±0.38^a	20.36±0.26^a
Protein (%)	17.45±0.20^a	19.14±0.24^b	18.42±0.38^b	18.32±0.44^a	17.50±0.31^a
Ash (%)	4.10±0.10^a	5.23±0.19^a	5.11±0.15^a	4.60±0.12^a	5.73±0.23^a
Total carbohydrate (%)	51.51±0.41^a	47.65±0.43^a	48.06±0.26^a	49.51±0.21^a	48.54±0.36^a
Ascorbic acid (Vitamin C)	1.27±0.05^a	1.44±0.17^a	1.71±0.12^a	1.30±0.10^a	1.82±0.14^a
Digestible protein (%)	12.54±0.12^a	14.16±0.11^b	13.47±0.16^b	13.38±0.10^b	12.59±0.12^a
Energy (Kcal/ 100g)	464±0.42^a	469±1.16^a	475±1.12^b	471±0.87^b	454±0.92^a
Energy (Kj/ 100g)	1943±1.56^a	1964±3.72^a	1989±3.68^b	1972±2.70^b	1901±3.18^a

Values given are the means of three replicates ± standard deviation; Means with different letters (a–c) within a row are significantly different at ($p \leq 0.05$).

ascorbic acid content (Vitamin C). Digestible protein content increased from 2005 to 2006, and then it decreased. The highest content was in 2006. Osborn solubility based protein fractionation data (Table 2) indicated that albumin was the major fraction (44 - 47%) followed by globulins (39 - 41%) and with a low content of prolamins (7 - 10%) and glutelins (4 - 6%). Osborn proteins were significantly ($p \leq 0.05$) affected by the harvesting year. Solar radiation reaching the earth surface changes from year to year (Tulukcu et al., 2009). This change in solar radiation may cause an alteration in the photosynthesis ratio of agricultural crops and the composition of oilseeds. Other factors such as rainfall, differentiation in cultivation area, vegetation phase, countries and geographical zones all have different impacts on plant production and composition (Tulukcu et al., 2009).

3.2 Effect of harvest year on physicochemical characteristics

Tables 3 and 4 lists the fatty acid composition and different physicochemical characteristics of flaxseed hull oils at different harvest year. There is not any study in the literature on the effect of cultivation year on the fatty acid compositions of flaxseed hull and physicochemical properties. α-Linolenic acid is the main fatty acid (6.94 - 7.40%), and palmitic acid (3.53 -5.71%) is the main saturated fatty acid in all flaxseed hull samples. The levels of fatty acids in flaxseed hull were the same in all samples. There was no significant difference ($p \leq 0.05$) in the palmitic acid and the stearic acid (C18:0) amounts of flaxseed hull between 2005 and 2009 harvesting years. However, there was significant difference ($p \leq 0.05$) in oleic acid between the years 2006 and 2009. There was also significant difference ($p \leq 0.05$) in linoleic acid and linolenic acid between the years 2005 and 2009. The highest level of C18:3 in flaxseed hull sample obtained from the 2005 year growing season suggest Δ15 desaturase activity. The level of linoleic acid (18:2) varied throughout the harvest year. This fact may be justified by the effect of oleic acid desaturase (Δ12-desaturase) which catalyzes the conversion of oleic acid to linoleic acid. According to these results, we can say that the fatty acid composition of flaxseed hull was affected by the harvesting year. Yalcin et al. (2011) investigated the fatty acid composition of flaxseed grown in the 2008 and 2009 harvesting years. They reported that while the amount of palmitic, stearic and oleic acids decreased, the amount of linolenic acid increased in the 2009 harvesting year. However, linoleic acid was not affected by harvesting year. The predominance of linolenic and oleic acids, certainly adds an extra dimension to the nutritional value of flaxseed hull oil. Linoleic and linolenic acids are the most important essential FAs required for growth, physiological functions and maintenance (Pugalenthi et al., 2004; Galvão et al., 2014). The ratio of MUFA to PUFA (0.35 - 0.49) is an indicator of oxidative stability of the oil. SFA and UFA play important role in degree of saturation and trans-esterification of oil. Present investigation showed a lower percentage of SFA (10.47 - 13.02) and a higher percentage of UFA (86.08 - 87.73) and is suitable for trans-esterification. It can be observed that flaxseed hull oils are distinguished from each other by small difference in UFA/SFA ratio and Cox value. The ratio of omega 3: omega 6 was found in the range of (2.45 - 3.06). These results suggested that flaxseed hull oils were of good nutritional quality according to the Japanese recommendations (Davis & Kris-Etherton, 2003). The FA composition of the oils corroborates measurements of the physicochemical characteristics of the oils (Tables 3 and 4). The saponification value varied significantly among the oils and was greater in flaxseed hull oil obtained in 2008. The saponification value is an indicator of the average molecular weight and, hence, chain length. It is inversely proportional to the molecular weight of the lipid. All the oil samples had low values compared to common vegetable oils with average SV range of 175–250 (Farhoosh et al., 2008). The range of AVs (1.42 - 1.85 mg KOH/g) resemble closely to that reported earlier for flaxseed oil (0.50 - 2.50 mg KOH/g) (Choo et al., 2007) but lower than AVs reported for Phaseolus vulgaris (11.0 - 19.2 mg KOH/g) seed oil (Mabaleha & Yeboah, 2004). The measurement of acid value encompasses acids formed by oxidation and those formed by hydrolysis. The IV, which is considered as a measure of the oil unsaturation, was found in the range of (170 - 177 g I_2/100 g). The differences in the IVs between different flaxseed hulls oils were due to their different fatty acid compositions. All the respective values were similar to those reported previously (Herchi et al., 2014a). The Unsaponifiable matter (USM) content of flaxseed hull oils ranged from 1.32 to 1.78% and there was no statistically significant difference among them. The USM content is extensively used as an index of the quality of refined oil or as a control index of the refining process (Farhoosh et al., 2008).

The data of Table 4 show the quality indices of flaxseed hull oil depending harvest year. FFA and PV are used as the most important indicator for seed oil quality. A low FFA value indicates that the stability of the oil extracted in 2008 was higher than those other oils**.** The lowest PV was (1.70 mequiv/kg) found in the oil extracted in 2008. The *p*-AV values of 1.00 - 1.70 anisidine units suggest the presence of low amounts of secondary oxidation products in the test oil samples. The OV (4.40 -5.90) indicates that low oxidative activity might be due to either lipoxygenase or autoxidation. Harvest year had significant effect ($p \leq 0.05$) with respect to FFA, PV, *p*-AV and OV for all samples. Table 4 shows theoretical flavor scores (F) of the oil from all flaxseed hull samples. Although the equation was developed for soybean

Table 2. Protein fractionation yields depending harvest year.

Years	2005	2006	2007	2008	2009
Osborn protein fraction					
Albumin	45±1.26[a]	47±1.70[b]	44±1.23[a]	47±1.59[b]	45±1.19[a]
Globulin	41±0.77[a]	40±0.89[a]	39±0.66[b]	41±0.31[a]	40±0.49[a]
Prolamin	4±0.14[a]	4±0.10[a]	6±0.18[b]	4±0.10[a]	5±0.12[a]
Glutelin	9±0.22[a]	8±0.19[a]	10±0.26[b]	7±0.29[a]	9±0.24[a]

Values given are the means of three replicates ± standard deviation; Means with different letters (a–c) within a row are significantly different at ($p \leq 0.05$).

Table 3. Fatty acids composition (% GC area, mean of three measurements) and physicochemical properties depending harvest year.

Years	2005	2006	2007	2008	2009
Saturated fatty acids (SFA)					
C16:0 (palmitic acid)	7.38±0.08[a]	7.25±0.12[a]	6.94±0.06[a]	7.40±0.10[a]	7.31±0.11[a]
C18:0 (stearic acid)	4.12±0.05[a]	4.36±0.06[a]	3.53±0.05[a]	3.58±0.07[a]	5.71±0.07[a]
Total	11.50±0.13[a]	11.61±0.18[a]	10.47±0.11[a]	10.98±0.17[a]	13.02±0.18[a]
Monounsaturated fatty acid (MUFA)					
C18:1 (oleic acid)	23.10±0.18[a]	22.89±0.15[a]	24.78±0.22[a]	23.11±0.20[a]	28.24±0.24[b]
Total	23.10±0.18[a]	22.89±0.15[a]	24.78±0.22[a]	23.11±0.20[a]	28.24±0.24[b]
Polyunsaturated fatty acids (PUFA)					
C18:2 (linoleic acid) ω6	15.84±0.18[a]	17.31±0.20[b]	16.21±0.18[a]	16.71±0.22[b]	16.77±0.26[b]
C18:3 (linolenic acid)ω3	48.52±0.51[a]	47.03±0.34[b]	46.64±0.62[b]	47.91±0.39[a]	41.07±0.42c
Total	64.36±0.69[a]	64.34±0.54[a]	62.85±0.80[b]	64.62±0.61[a]	57.84±0.68[b]
EFA	64.36±0.69[a]	64.34±0.54[a]	62.85±0.80[b]	64.62±0.61[a]	57.84±0.68[b]
UFA	87.46±0.87[a]	87.23±0.69[a]	87.63±1.02[a]	87.73±0.81[a]	86.08±0.92[a]
ω3/ ω6 ratio	3.06±0.18[a]	2.71±0.14[a]	2.87±0.20[a]	2.86±0.16[a]	2.45±0.12[b]
Linolenic /Oleic ratio	2.10±0.20[a]	2.05±0.12[a]	1.88±0.22[b]	2.07±0.14[a]	1.45±0.17[b]
PUFA/ SFA	5.59±0.24[a]	5.54±0.18[a]	6.00±0.16[a]	5.88±0.26[a]	4.44±0.14[b]
UFA/SFA	7.60±0.31[a]	7.51±0.28[a]	8.36±0.32[b]	8.00±0.38[b]	6.61±0.22[a]
MUFA/PUFA	0.35±0.04[a]	0.35±0.04[a]	0.39±0.05[a]	0.35±0.03[a]	0.49±0.06[b]
Cox value	12.28±0.14[a]	12.17±0.12[a]	12.00±0.10[a]	12.30±0.15[a]	10.88±0.08[b]
ODR	73±0.74[a]	73±0.70[a]	71±0.43[a]	73±0.56[a]	67±0.37[b]
LDR	75±0.58[a]	73±0.66[a]	74±0.55[a]	74±0.50[a]	71±0.40[a]
Physicochemical properties					
Relative density at 25 ºC/g cm-3	0.941±0.02[a]	0.938±0.01[a]	0.940±0.01[a]	0.935±0.02[b]	0.937±0.01[b]
Saponification value (mg KOH/g)	180±0.62[a]	179±0.55[a]	176±0.50[b]	182±0.47[a]	174±0.53[b]
Acid value (mg KOH/g)	1.76±0.10[a]	1.68±0.08[a]	1.62±0.14[b]	1.42±0.12[b]	1.85±0.09[a]
Iodine value (g I2/100 g)					
Determined	175±0.78[a]	174±0.90[a]	170±0.88[b]	177±1.06[a]	163±0.65[b]
Calculated	174±0.57[a]	173±0.82[a]	171±0.59[b]	174±1.19[a]	161±0.84[b]
Unsaponifiable matter (% w/ w)	1.64±0.24[a]	1.47±0.18[a]	1.39±0.22[a]	1.78±0.26[a]	1.32±0.14[a]
[a] Triglyceride (%)	97.41±0.45[a]	97.63±0.82[a]	97.76±0.70[a]	97.40±0.60[a]	97.58±0.23[a]
Average molecular weight (g/mol)	935±3.62[a]	940±2.87[a]	956±3.14[a]	925±2.91[a]	967±2.76[a]

Average molecular weight = (3*56.1*1000)/saponification value. [a] Triglyceride (%) = 100 - {(free fatty acid, %) + (unsaponifiable matter, %)}. Values given are the means of three replicates ± standard deviation. Means with different letters (a–c) within a row are significantly different at ($p \leq 0.05$)

Table 4. Quality characteristics of flaxseed hull oil depending harvest year.

Years	2005	2006	2007	2008	2009
FFA (% of Oleic acid)	0.95±0.06[a]	0.90±0.10[a]	0.85±0.05[a]	0.82±0.08[a]	1.10±0.08[b]
Peroxide value (mequiv/kg)	2.10±0.22[a]	2.00±0.33[a]	1.90±0.16[b]	1.70±0.25[b]	2.20±0.18[a]
p-Anisidine value	1.70±0.12[a]	1.55±0.10[a]	1.35±0.14[b]	1.00±0.19[a]	1.20±0.15[b]
Oxidation value	5.90±0.54[a]	5.55±0.62[a]	5.15±0.33[b]	4.40±0.18[a]	5.60±0.44[b]
Flavor score	5.63±0.14[a]	5.75±0.15[a]	5.89±0.10[a]	6.16±0.13[a]	5.74±0.15[a]
Oil stability (h)	1.14± 0.05[a]	1.26±0.08[a]	1.35±0.10[b]	1.42±0.04[a]	1.10±0.04[a]
K_{232}	1.55±0.06[a]	1.50±0.08[a]	1.45±0.05[a]	1.60±0.06[a]	1.40±0.09[a]
K_{270}	0.25±0.02[a]	0.23±0.01[a]	0.22±0.01[a]	0.27±0.02[a]	0.20±0.03[a]
R-value (K_{232}/ K_{270})	6.2±0.08[a]	6.5±0.10[a]	6.6±0.06[a]	5.9±0.12[a]	7±0.08[a]

Values given are the means of three replicates ± standard deviation; Means with different letters (a–c) within a row are significantly different at ($p \leq 0.05$)

oil, the flavor scores (5.63 - 6.16) indicate that the oils from the test flaxseed hull samples would receive rather low acceptance as an edible oil without further refinement. The investigated cultivars not differed significantly with respect to theoretical flavor scores. The values are comparable to flavor score values reported for Phaseolus vulgaris seed oil (-3.2 to 3.9) (Mabaleha & Yeboah, 2004). The oil stability ranged from 1.10 to 1.42 h. The oxidative stability of the oils varied considerably and seemed to depend also on polyunsaturated fatty acid concentration. With respect to the absorbance at 232 and 272 nm, flaxseed hull oil seemed to show lower oxidation and were not affected by the harvesting year. There are few published data on the K_{232} and K_{270} for flaxseed hull.

Table 5. Bioactive and antioxidant compounds of flaxseed hull oil depending harvest year.

Years	2005	2006	2007	2008	2009
Phenolic acids					
ferulic acid equivalents (mg/ 100 g oil)	90±1.68a	91±1.05a	88±1.32b	94±1.46a	86±1.19b
Flavanoids					
luteolin equivalents (mg/100 g oil)	19±0.60a	15±0.87b	17±0.48a	14±0.74b	11±0.52b
Chlorophyll (mg /kg oil)	2.8±0.33a	2.4±0.20a	2.6±0.35a	2.2±0.27a	2.4±0.18a
Carotenoids (mg /kg oil)	8.2±0.37a	7.2±0.51a	7.6±0.35a	7.5±0.42a	7.8±0.24a
TS Content (% of oil)	1.15±0.12a	0.98±0.18b	0.92±0.10b	1.33±0.15a	0.80±0.10b
TT content					
(mg α-tocopherol/kg oil)	196±5.26a	210±6.10a	226±4.32b	239±6.08b	188±4.47a

Values given are the means of three replicates ± standard deviation; Means with different letters (a–c) within a row are significantly different at ($p \leq 0.05$).

3.3 Effect of harvest year on bioactive compounds and antioxidant activity

The bioactive compounds of flaxseed hull oil harvested from 2005 to 2009 are shown in Table 5. As can be seen in Table 5, harvesting year has a significant effect on the total phenolic acids, flavanoids, total sterols and total tocopherols contents. The Total phenolic acids content among flaxseed hull oils were significantly different with the greatest concentration for the crop cultivated in 2008 (94 mg/ 100 g oil), followed by the crop cultivated in 2006 (91 mg/ 100 g oil) and the crop cultivated in 2005 (90 mg/ 100 g oil). Although the interest in phenolics is related primarily to their antioxidant activity, they also show important biological activity in vivo and may be beneficial in combating diseases related to excessive oxygen radical formation exceeding the antioxidant defense capacity of the human body (Miguel et al., 2014). Flavanoids fall within a close range, from 11 to 19 mg/ 100 g oil. Flavanoids have been reported by Choo et al. (2007) to have the potential to function as antioxidants in food lipid systems. The results of chlorophyll and carotenoids content revealed that there were no significant differences with each other as far as pigments are concerned. As seen in Table 5, statistically significant differences were observed among the total sterol (TS) content of flaxseed hull oils studied (which was 1.26% on average). Phytosterols have attracted the interest of food oil chemists because of their perceived effects on cholesterol absorption. Their relative concentrations are also indicative of the genuineness of vegetable oils (Crane et al., 2005). While flaxseed hull sample cultivated in 2008 had the highest total tocopherol content (239 mg α-tocopherol/kg oil), the crop of the 2009 had the lowest total tocopherol content (188 mg α-tocopherol/kg oil). These values are lower than those of some apple seed oils with the TT content of about (1910 - 3790 mg kg^{-1} oil) (Górnaś, 2015). Tocopherols are particularly important functional constituents of the USM fraction of vegetable oils. The tocopherols have antioxidant properties and they are active as vitamin E, which makes them particularly important for human health. The antioxidant activity of flaxseed hull oils harvested from 2005 to 2009 is shown in Figure 1. The antioxidant activity varied between 54.57% (2009) to 61.38% (2008). All the samples shown significant difference. Flaxseed hull oil exhibited higher antioxidant activity. This can be explained by the higher level of unsaturated fatty acids. High tocopherol content was associated with a high resistance to oxidation of the oils. A linear relationship was found between tocopherol content and the antioxidant activity of flaxseed hull oils. Although the constituents of flaxseed hull oil, which show free radical scavenging action is still unclear, it is possible that the antioxidative activity of flaxseed hull oil are caused, at least in part, by the presence of polyphenols (Herchi et al., 2014a, 2014b).

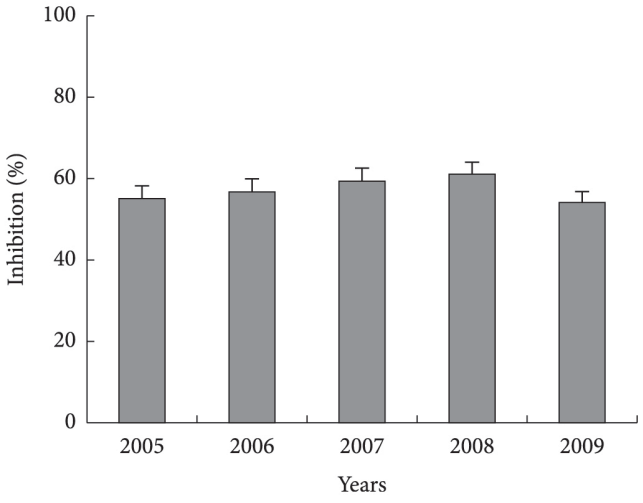

Figure 1. Influence of harvest year on antioxidant activity of flaxseed hull oils.

4 Conclusion

The total amount of proximate composition determined in flaxseed hull extracted from variety grown in Tunisia was harvest year depending. Regarding the fatty acid composition, Essential fatty acids varied between 57.84% and 64.62% according to year. On the basis of our physicochemical evaluation of flaxseed hull oils, we conclude that oil from each origin has its own special characteristics. The investigated parameters of flaxseed hull oils were significantly affected by the harvesting year. According to total phenolic acids, total tocopherol content and antioxidant activity, it is expected that flaxseed hull obtained in 2008 is the most resistant to lipid oxidation.

Acknowledgements

We thank the Ministry of Scientific Research, Technology and Competence Development of Tunisia for financially supporting this investigation.

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