Flaxseed Hull: Chemical Composition and Antioxidant Activity during Development

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Abstract: Changes in the chemical composition and antioxidant activity of flaxseed hull during maturation were investigated. P129 hull variety was studied at four maturation stages (St₁, St₂, St₃, and St₄). Significant variation in proximate composition and flaxseed hull oil characteristics were observed. A significant increase in the carbohydrates content of the hull was observed during development. The main methyl esters were linolenic acid (48.95 - 51.52 %), oleic acid (20.27-23.41%) and linoleic acid (15.62-17.70%). The highest polyunsaturated fatty acids (PUFA) were found to be 67.14 % at the first stage of maturity (St₁). Flaxseed hull oil was of good quality, containing an abundance of omega-3 essential fatty acids. The iodine value increased, while the saponification value of oil decreased during seed development. The decrease in ascorbic acid content was steady. The maximum level of total phenolic acid content (128.3 mg/100 g oil) was reached at 7 DAF. The antioxidant activity of oilseed was assessed by means of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. Radical scavenging activity for green hull was 52.74% and mature hull was 69.32%.

Key words: hull oil, hull development, physicochemical properties, fatty acid, antioxidant activity

1 INTRODUCTION

Flaxseed are increasingly being used in cereal-based products, such as bread, muffins, bagels, cookies as well as other bakery products¹. Flaxseed's growing popularity is due to the rich content of lignan, especially Secoisolariciresinol diglucoside (SDG), and omega 3-fatty acids as well as dietary fiber². The separation of flaxseed into hull and cotyledon fractions has been the subject of several studies³⁻⁵. When the moisture content of the seed was adjusted to 20 g/kg prior to grinding, the yield of cotyledon was 718 g/kg with an increase of 20 and 28 g/kg for protein and oil, respectively³. The hull, on a full fat basis, comprised 41.4% of the seed, however, on an oil and moisture free basis; it constituted essentially 60% of the flaxseed meal. It had a nitrogen value approximately one-third that of the cotyledon fraction which contained 96.7% of the total oil³.

Flaxseed hulls represent 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 mechanically⁶. Flaxseed hull is difficult to digest and therefore hinders access to the lipids⁴. Oil content of flaxseed

hulls varies from 26% to 30% depending on processing conditions representing approximately 18% of the total seed oil⁷. 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 seed³. Flaxseed hull is enriched in SDG compared to the cotyledons². Commercial flaxseed hull oils were evaluated for physicochemical characteristics and antioxidant properties. Yield of oil ranged from 9 - 28% depending on extraction method. It was reported that the crude lipid contained 92.4% neutral lipids, 3.1% phospholipids, 2.4% acidic lipids and 2.1% free fatty acids. Oil extracted from flaxseed hull using supercritical CO₂ showed highest antioxidant capacity and the resultant defatted meal contained the highest (53 mg/g) secoisolariciresinol diglucoside which is an antioxidant phytochemical⁴.

There was no literature available on characteristics of flaxseed hull during development. Therefore, the objectives of the present study were to investigate the phytochemical profile and the antioxidant activity during hull development to increase its value and contribution to the develop-

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ment of new omega-3 products for the functional foods and nutraceutical applications.

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 (P129) was obtained from Institut National Recherche Agronomie Tunis (INRAT), Tunisia. The dehulled seed and hulls were separated by air aspiration. The color of hull was noted by visual inspection.

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 weight⁸⁾. The crude protein contents were calculated from nitrogen contents ($N \times 6.25$) obtained using the Kjeldahl method by⁸⁾. The crude fat contents were determined by continuous extraction in a Soxhlet apparatus for 5 h using petroleum ether as solvent⁸⁾. 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 desiccator⁸⁾. The carbohydrate contents were determined by difference that is by deducting the mean values of other parameters that were determined from 100. 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 analysis⁹⁾.

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.*¹⁰⁾: 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 procedure¹¹⁾.

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.*¹²⁾ modified by Lechvallier¹³⁾. 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 1 ml/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. The index of fatty acid unsaturation IU (Δ/mol) was calculated by the following formula¹⁴⁾:

$$IU = [1(\Sigma\% \text{ monoenes}) + 2(\Sigma\% \text{ dienes}) + 3(\Sigma\% \text{ trienes}) + 4(\Sigma\% \text{ tetraenes})]/100.$$

To evaluate the efficiency of the desaturation pathway during maturation process¹⁵⁾ 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:

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

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

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 *et al.*¹⁶⁾:

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

2.6 Physicochemical characteristics

Determination of 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 oils¹⁷⁾. The mean molecular mass (MMM) was estimated from the relationships $(560/\text{saponification value}) \times 100$ ¹⁸⁾. Oxidation value (OV) was calculated from Holm's equation, $\text{OV} = p\text{-AV} + 2(\text{PV})$, while theoretical flavor scores (F) were obtained from equation $F = 7.7 - 0.35(\text{OV})$ ¹⁹⁾. Oxidative stability was evaluated by the Rancimat method²⁰⁾. Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 743 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 1 g warmed to 100°C and air flow of 10 l/h. Higher Heating Value (HHV), known as the gross calorific value or gross energy, represents the heat released by the oxidation of a fuel in air. The HHV is the amount of heat produced by the complete combustion of a unit quantity of fuel. The HHV of flaxseed hull oil was calculated from the iodine value (IV) and saponification value (SV) derived using the following formula adopted by Demirbas²¹⁾:

$$\text{HHV} = 49.43 - (0.041 \times \text{SV}) - (0.015 \times \text{IV})$$

The cetane number of the oil was determined according to Bose²²⁾:

$$\text{CN} = 46.3 + 5458/\text{SV} - 0.225 \times \text{IV}$$

2.7 Thin-layer chromatography

Unsaponifiable lipids were determined by saponifying 5 g of lipid extracts with 50 mL ethanolic KOH 12% (w/v) heating at 60°C for 1.30 h. After cooling, 50 mL of H₂O was added and the unsaponifiable matter was extracted four times with 50 mL petroleum ether. The combined ether extract was washed with 50 mL of EtOH – H₂O (1:1). The ether extracted was dried over anhydrous Na₂SO₄ and evaporated. The dry residues were dissolved in chloroform for TLC analysis. The unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates (silica gel 60 G F254), using 1-dimensional TLC with hexane–Et₂O (65:35 by volume) as the developing solvent. The unsaponifiable (4 mg in 100 µl CHCl₃) was applied on the silica gel plates in 3 cm bands. After development the plate was sprayed with 20, 70-dichlorofluorescein and viewed under UV light. The identification of unsaponifiable classes was made by comparing their Retention Factor (RF) values with those of authentic standards chromatographed under the same conditions. The bands corresponding to unsaponifiable constituents were scraped off separately and each fraction was extracted three times with CHCl₃–Et₂O (1:1), filtered to remove the residual silica, dried in a rotary evaporator and the dry residue was weighted.

2.8 Vitamin C content

Vitamin C content was determined by application of the

2,6 dichloroindophenol volumetric method⁸⁾.

2.9 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.*²³⁾. 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:

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

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

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

2.10 Polyphenols contents

Extraction and determination of total phenolic acids and flavanoids contents were carried out according to the method of Gutfinger²⁴⁾.

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.*²⁵⁾. 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:

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

Where A₀ is the absorbance of the control, A₁ 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 DISCUSSION

3.1 Changes in Proximate composition during flaxseed hull development

The possible of explanation for the color change in flaxseed hull (Table 1) was associated with the enzymatic degradation of chlorophyll as hull ripeness increased²⁶⁾. The changes in proximate constituents (expressed as % of dry weight) during flaxseed development are presented in Table 2. The proximate composition during flaxseed hull development was significantly affected by maturity. Moisture content showed significant change during ripening. The oil content increased significantly. The most active period of oil accumulation occurred in the early development stage. In the last stages of maturity, oil biosynthesis was stopped which explains the regression of the total lipid at complete maturity of seed. Oomah *et al.* reported that flaxseed hull oil content was found in the range (9 - 28%) depending on extraction method⁴⁾. The study of the lipids accumulation during flaxseed hull development is important to decide the best moment for the harvest of flaxseed hull. The protein content increased significantly up to the third stage. The increase in protein content may be attributed to the synthesis of cell constituents and enzymes,

which lead to degradation of other constituents²⁷⁾. Therefore, the most important amounts of lipid and protein were observed at the third stage which corresponds to the best time of flaxseed hull harvest. Total carbohydrate significantly increased approximately 5 times greater than total carbohydrate in immature flaxseed hull. Osborne solubility-based protein fractionation (Table 2) data indicated that, at complete maturity, albumins (46% of the total soluble protein) were the most dominant, followed by globulins (41%) and glutelins (9%), and with a small quantity of prolamin also observed (4%). The dominance of albumins in the total hull protein is different to that observed for *Ailanthus excelsa* seeds (51.3%)²⁸⁾. Globulins fraction presents an accumulation profile similar to that of total storage proteins.

3.2 Evolution of the major fatty acid composition during flaxseed hull development

Table 3 shows that linolenic acid (C18:3) was the major fatty acid during flaxseed hull development. The high amount of C18:3 in flaxseed hull oil suggest an important activity of $\Delta 15$ desaturase. The fatty acids C18:1 and C18:2 were also well represented with mean values, respectively,

Table 1 Description of the selected maturity stages for sample collection of flaxseed hull (*Linum usitatissimum* L.).

Abbreviation	Maturity stages	Age days (DAF)	Color of hull	Dry weight (%)	Hull (%)
St ₁	immature	7	Green	21	47
St ₂	half-mature	21	Green	54	29
St ₃	pre-mature	42	Brown	83	22
St ₄	mature	56	Brown	93	27

Table 2 Proximate chemical composition of flaxseed hull at different maturities (expressed as % of dry weight).

DAF	St ₁	St ₂	St ₃	St ₄
Moisture (%)	78.22 ± 0.50	45.30 ± 0.44	16.78 ± 0.20	6.36 ± 0.12
Oils (%)	5.42 ± 0.32	12.43 ± 0.47	23.17 ± 0.19	20.32 ± 0.54
Protein (%)	4.34 ± 0.15	9.51 ± 0.33	21.64 ± 0.72	17.21 ± 0.64
Ash (%)	2.37 ± 0.07	2.80 ± 0.08	4.58 ± 0.10	5.40 ± 0.05
Total carbohydrate (%)	9.65 ± 0.36	29.96 ± 0.42	33.83 ± 0.29	50.71 ± 0.27
Energy (Kcal/100 g)	105.95 ± 0.59	273.50 ± 0.88	434.64 ± 0.61	460.90 ± 1.12
Energy (KJ/100 g)	443.60 ± 0.82	1145.07 ± 1.68	1819.75 ± 2.14	1929.70 ± 2.67
Osborn protein fraction				
Albumin (%)	48 ± 1.33	44 ± 1.18	41 ± 1.27	46 ± 1.40
Globulin (%)	14 ± 0.21	15 ± 0.17	38 ± 0.62	41 ± 0.54
Prolamin (%)	21 ± 0.28	19 ± 0.42	10 ± 0.14	4 ± 0.22
Glutelin (%)	17 ± 0.65	22 ± 0.36	11 ± 0.10	9 ± 0.35

Values given are the means of three replicates ± standard deviation.

Table 3 Major fatty acids (FAs) composition (% GC area) during flaxseed hull development.

FAs	St ₁	St ₂	St ₃	St ₄
C16:0	8.33 ± 0.14	7.84 ± 0.65	6.39 ± 0.32	7.76 ± 0.24
C18:0	3.12 ± 0.15	3.41 ± 0.21	3.81 ± 0.29	3.95 ± 0.16
C18:1	20.27 ± 0.88	21.74 ± 0.38	23.41 ± 1.06	20.54 ± 0.26
C18:2	15.62 ± 0.48	16.18 ± 0.64	16.07 ± 0.35	17.70 ± 0.40
C18:3	51.52 ± 0.23	49.35 ± 0.44	49.09 ± 0.46	48.95 ± 0.11
ΣSFA ^a	11.45 ± 0.29	11.25 ± 0.86	10.20 ± 0.61	11.71 ± 0.40
ΣUSFA ^b	87.41 ± 1.59	87.27 ± 1.46	88.57 ± 1.42	87.19 ± 0.77
ΣPUFA ^c	67.14 ± 0.71	65.53 ± 1.08	65.16 ± 0.81	66.65 ± 0.51
ΣPUFA/ΣSFA	5.86 ± 0.20	5.82 ± 0.36	6.38 ± 0.36	5.69 ± 0.44
omega 3/omega 6	3.30 ± 0.32	3.05 ± 0.27	3.05 ± 0.18	2.76 ± 0.30
Cox value	12.94 ± 0.87	12.54 ± 0.31	12.49 ± 0.62	12.60 ± 0.55
ODR	86.81 ± 0.37	75.08 ± 0.24	73.58 ± 0.21	76.44 ± 0.34
LDR	76.73 ± 0.62	75.30 ± 0.50	75.33 ± 0.42	73.44 ± 0.41
IU (Δ/ mol)	2.06 ± 0.14	2.02 ± 0.10	2.02 ± 0.10	2.03 ± 0.16

Fatty acids detected: C16:0 (Palmitic), C18:0 (Stearic), C18:1 (Oleic), C18:2 (Linoleic), C18:3 (Linolenic)

% GC area, mean of three measurements

^a Sum of major saturated fatty acids

^b Sum of major unsaturated fatty acids

^c Sum of major polyunsaturated fatty acids

^d Polyunsaturated fatty acids to saturated fatty acids ratio

Values given are the means of three replicates ± standard deviation.

of around 21 and 16% of total fatty acids. Hence, desaturase activity is important during maturity. A decrease was noticed for palmitic (C16:0) and stearic acid (C18:0) upon seed hull development. The differences in FA amount reflect the amounts and activities of enzymes catalyzing the various fatty acids synthetic processes. Higher amounts of unsaturated fatty acids (87–89%) were detected during flaxseed hull development. At the final stage of maturity (mature hull-St₄), flaxseed hull oil contained 67% polyunsaturated fatty acids (PUFA) and 12% saturated fatty acids (SFA). These results are similar to those of Oomah *et al.*³⁾. The highest PUFA/SFA ratio was reached at the third stage. PUFA are essential in the human diet and lower the risk of diseases related to cholesterol oxidation²⁹⁾. Calculated oxidizability (Cox) values were statistically the same during flaxseed hull development. Flaxseed hull oil was characterized by its higher polyunsaturated fatty acids (PUFA) which makes it particularly prone to oxidation. To evaluate the nutritional value of the present composition, the ratio n-3 to n-6 was used. As can be seen, this ratio varied from 2.76 to 3.30 during flaxseed hull development. These results suggest that flaxseed hull oils during maturation were of good nutritional quality according to the Japanese recommendations²⁹⁾. The index of fatty acid unsatura-

tion IU (Δ/mol) was found in the range of (2.02–2.06). Despite that there are no studies on the fatty acids biosynthesis during flaxseed hull development, it can be speculated that the increased amount of linolenic acid during maturation was presumably due to increased activity of linoleate desaturase. At this point, the values concerning the ODR and LDR which reflect the relative efficiency of the desaturation system from oleic to linoleic and from Linoleic to linolenic¹⁵⁾ showed higher value of ODR (73.58–86.81%) and that of LDR (76.73–82.9%). The higher value of LDR represented a considerably high amount of linolenic acid during maturation.

3.3 Changes in Physicochemical characteristics of flaxseed hull oils during development

The Changes in Physicochemical characteristics of flaxseed hull oils during development are presented in Table 4. A decrease was observed in saponification value as maturity progressed. The saponification values of flaxseed hull oil vary from 198 mg KOH/g oil to 178 mg KOH/g oil. As reported by Akbar *et al.*, high Saponification value indicates that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries³⁰⁾. Therefore, the value obtained for flaxseed hull oil in this study show

Table 4 Physicochemical and Fuel properties of flaxseed hull oils during development.

Parameters	St ₁	St ₂	St ₃	St ₄
Saponification Value (mg KOH g ⁻¹ oil)	198 ± 2.18	193 ± 1.30	184 ± 1.55	178 ± 1.08
Acid value (mg KOH g ⁻¹ oil)	3.2 ± 0.10	2.8 ± 0.15	1.9 ± 0.10	1.4 ± 0.08
FFA content (% as oleic acid)	2.40 ± 0.08	1.82 ± 0.07	1.66 ± 0.11	1.10 ± 0.05
Iodine value (g of I ₂ /100 g oil)	160 ± 0.93	164 ± 0.88	172 ± 1.16	170 ± 1.19
Peroxide value (mequiv O ₂ /kg oil)	1.28 ± 0.11	1.62 ± 0.08	4.24 ± 0.22	1.80 ± 0.14
<i>p</i> -Anisidine value	2.51 ± 0.19	2.18 ± 0.11	1.40 ± 0.10	1.23 ± 0.16
Oxidation value	5.07 ± 0.38	5.42 ± 0.51	9.88 ± 0.94	4.83 ± 0.47
Theoretical flavor scores (<i>F</i>)	5.92 ± 0.10	5.80 ± 0.08	5.24 ± 0.20	6.00 ± 0.14
K ₂₃₂	2.64 ± 0.16	2.50 ± 0.15	1.80 ± 0.10	1.45 ± 0.10
K ₂₇₀	0.55 ± 0.08	0.38 ± 0.04	0.30 ± 0.04	0.20 ± 0.02
Mean molecular mass (MMM)	282.8 ± 1.33	290.2 ± 1.48	304.3 ± 1.71	314.6 ± 1.18
Oil Stability (h)	1.8 ± 0.10	1.6 ± 0.04	1.2 ± 0.04	1.1 ± 0.06
Unsaponifiable matter (% w/w)	4.6 ± 0.36	3.2 ± 0.18	1.8 ± 0.20	1.2 ± 0.08
Sterols (mg/100 g oil)	1052 ± 5.23	688 ± 4.18	394 ± 3.71	260 ± 3.82
Aliphatic and triterpenic alcohols (mg/100 g oil)	621 ± 4.77	406 ± 3.42	310 ± 2.92	376 ± 2.27
Hydrocarbons (mg/100 g oil)	147 ± 2.51	130 ± 2.55	92 ± 2.78	88 ± 2.80
Ascorbic acid (mg/100 g)	3.20 ± 0.22	2.50 ± 0.14	1.70 ± 0.20	1.30 ± 0.10
Chlorophyll (mg/kg oil)	65.71 ± 1.48	32.40 ± 1.12	10.27 ± 0.55	2.34 ± 0.28
Carotenoids (mg/kg oil)	25.33 ± 0.73	47.15 ± 1.24	12.64 ± 0.58	7.52 ± 0.40
Total Phenolic acids, as gallic acid equivalents (mg/100 g oil)	128.3 ± 1.47	102.1 ± 1.16	96.0 ± 1.31	62.4 ± 0.89
Total Flavonoids, as luteolin equivalents (mg/100 g oil)	12.46 ± 0.83	17.85 ± 0.68	14.63 ± 0.37	12.27 ± 0.44
Fuel properties				
HHV (MJ/kg)	38.91 ± 0.96	39.06 ± 1.26	39.31 ± 0.72	39.58 ± 0.83
Cetane number	37.86 ± 1.14	37.68 ± 1.02	37.26 ± 1.10	38.71 ± 1.28

Values given are the means of three replicates ± standard deviation.

that it has high potential for use in the production of liquid soap and shampoos. Acid value and free fatty acid content are used as an indicator for oil edibility³¹⁾. During flaxseed hull development, acid value showed a dramatic decrease. It declined from 3.2 mg KOH/g oil on the first stage of maturity (St₁) to 1.4 mg KOH/g oil at maturity (St₄-56th DAF^o). The acid values were well within the limit of 4.0 mg KOH/g oil under Codex Alimentarius Commission (1999)³²⁾ for virgin oils and cold pressed fats and oils. In all samples studied, free fatty acid contents were less than 3 during all stages of flaxseed development. High FFA content causes problems in the transesterification process³³⁾. During flaxseed hull development, Iodine value was found in the range 160-170 (g of I₂/100 g oil). Flaxseed hull oil with high iodine value can be grouped as a drying oil. Higher unsaturated fatty acids tend to have better cold flow properties³³⁾. The Peroxide value (mequiv/kg of oil) and *p*-anisidine value (*p*-AV) measure hydroperoxides and secondary oxidation products, i.e. aldehydes, of the oils, respectively³⁴⁾. In this

study, flaxseed hull oil at early stages of maturity showed lower peroxide values (Table 4). Peroxide value was much lower than the upper limit of 10 meqO₂/kg oil. The *p*-AV values of 1.23 – 2.51 anisidine units suggest the presence of low amounts of secondary oxidation products in the test oil samples. The Oxidation value (OV) (4.83- 9.88) indicates that slight oxidative activity might be due to either lipoxygenase or autooxidation. The high content of PUFA would increase the susceptibility of the oils to oxidation and hence result in high OV. The Theoretical flavor scores (*F*) values (5.24- 6.00) are comparable to flavor score values reported for chickpea (*Cicer arietinum* L.)³⁵⁾. A gradual decrease of unsaponifiable content during flaxseed hull development. The changes in unsaponifiable constituents at different hull stages are reported. This result may be due to the decline of the amount of phytosterols, which are the most abundant compounds of the unsaponifiable fraction during flaxseed hull development. In fact, in immature seeds phytosterols are converted to brassinosteroids,

which are steroidal hormones that regulate the growth and development of immature tissues³⁶⁾. Ascorbic acid (mg/100 g) showed gradual decrease during flaxseed hull development. Most pronounced content begins 7 DAF, which is believed to be the active period of hull formation. The determination of UV-specific extinction permits an approximation of the oxidation process in unsaturated oils³⁷⁾. The values of K_{232} and K_{270} extinction coefficient showed a slight decrease during maturation, which confirm the good overall quality of this oil during development. Oil stability decreased during flaxseed hull development. This decrease (1.8 - 1.1) in stability is explained by the loss of natural antioxidants. Maximal chlorophyll content (89.72–130.5 mg/kg) was achieved at 7 DAF. The seeds in the first stages of development had the highest levels of chlorophyll. The highest level of carotenoids was detected at the second stage (47.15 mg/kg oil). At this stage, these lipid components could be used for economic and food industrial exploitation. Mean molecular mass (MMM) was found in the range of 282.8 - 314.6 during flaxseed hull development. During the early stages of flaxseed development, total phenolic acids content in flaxseed hull oil was higher (128.3 mg gallic acid/100 g oil) and then decreased during maturation to achieve the harvest values (62.40 mg gallic acid/100 g oil). Manach *et al.* reported that phenolic acids generally decrease during the course of maturation³⁸⁾. Indeed, phenolic acids are involved in the response of plants to different types of stress. Flavonoid amount began to increase from the second stage (17.85 mg/100g oil) with a slight decrease during hull maturation. Flavonoids content changes during flaxseed hull development according to Dihydroflavonol reductase (DFR) activity³⁹⁾. Flavonoids have been reported to have the potential to function as antioxidants in food lipid systems³⁹⁾. The fuel properties shown in Table 4 indicated that there was significant difference for HHV and the cetane number throughout maturation. The HHV, which is one of the most important properties of a fuel, is the amount of heat released during the combustion of 1 g of fuel to produce CO₂ and H₂O at its initial temperature and pressure. The HHV ranges from 38.91 to 39.58 MJ/kg during flaxseed hull development. Fuel properties including heat of combustion of 11 vegetable oils have been measured by Goering *et al.*⁴⁰⁾. The cetane number (CN) is one of the most commonly cited indicators of diesel fuel quality, especially the ignition quality⁴¹⁾. It measures the readiness of the fuel to auto-ignite when injected into the engine⁴²⁾. The CN of biodiesel fuels is considerably influenced by their fatty acid methyl ester composition⁴³⁾. The CN was quite high (37.26–38.71) in flaxseed hull oil during development. In the USA, No. 2 diesel fuel usually has a CN between 40 and 45 (World Wide Fuel Charter 2006). Higher CN are associated with greater combustibility, good ignition, and assist in easy engine starting, low temperature starting, low ignition pressures, and smooth operation with

lower knocking characteristics.

3.4 Changes in antioxidant activity of flaxseed hull oil during development

The DPPH test aims at measuring the capacity of the flaxseed hull oil to scavenge the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by donation of hydrogen atom or an electron. If the extracts have the capacity to scavenge the DPPH free radical, the initial blue/purple solution will change to a yellow colour due to the formation of diphenylpicrylhydrazine. The results of scavenging activity of this study were 52.74% to 78.55% during flaxseed hull development (Fig. 1). This result showed more ripened of hull had a higher of DPPH. All the samples showed significant difference. St₁ was lowest and St₃ was highest of DPPH value. Flaxseed hull oil exhibited higher antioxidant activity. This can be explained by the higher level of unsaturated fatty acids. The scavenging action of plant constituents has been found to relate to polyphenolic compounds⁴⁴⁾. 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 polyphenols⁵⁾ and other yet to be discovered antioxidant compounds. According to Wiesenborn *et al.* and Oomah *et al.*, lignan occurs mainly in the hull of flaxseed^{2, 4)}. The antioxidant activity of oils of soybean, sunflower, rapeseed, corn, grapeseed, hemp, rice bran and pumpkin oils has been studied with the DPPH radical-scavenging assay⁴⁴⁾. The results showed that the hemp oil exhibited the highest inhibition ratio (76.2 ± 4.5%), followed by the pumpkin oil (65.3 ± 3.1%) and then the rapeseed oil (51.2 ± 4.1%).

Fig. 1 Antioxidant activity of oil from flaxseed Hull on different stages of maturity assessed by DPPH radical scavenging assay.

Based on the obtained results, we can conclude that flaxseed hull may play potential roles as health-promoting agents with antioxidant activity in human diets, as well as providing valuable natural antioxidants for the pharmaceutical industry.

4 CONCLUSIONS

Proximate chemical composition and phenolic compounds changes during flaxseed hull development. This research describes also a wide range of physicochemical quality characteristics of flaxseed hull oil. Oil and protein contents were highly accumulated on the third stage. Whereas ascorbic acid, total phenolic acid and unsaponifiable contents reached their maximum on the first stage of flaxseed hull development. According to the results, more ripened stages of flaxseed hull oil resulted lower of total phenolic acid and flavanoids content but higher of DPPH value.

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