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## Effect of seed maturation stages on physical properties and antioxidant activity in flaxseed (*Linum usitatissimum* L.)

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### Abstract

The changes in flaxseed constituents at different stages of maturity are reported. The physical properties and antioxidant activity of flaxseed oil during flaxseed development have been evaluated. Continuous decrease in total polyphenol content during flaxseed development. All the results showed no significant differences between HPLC-MS and TLC for quantitative determination of phospholipids classes. The fatty acid compositions of individual phospholipids were also reported. The antioxidant activity of oilseed was assessed by means of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay. The test demonstrated that the antioxidant activity of the flaxseed oil was found to be harvesting time-dependant.

**Keywords:** physical properties; antioxidant activity; total polyphenol content; flaxseed oil; HPLC-MS; maturation.

**Practical Application:** Practical application for the study entitled “Effect of seed maturation stages on physical properties and antioxidant activity in flaxseed (*Linum usitatissimum* L.)” regard the possibility of analyzing phospholipids contents and antioxidant activity during flaxseed development. The changes in the constituents of flaxseed during development and maturity have been a subject of continuing interest for many investigators. From this study, valuable information will be provided for flaxseed cultivations and help in developing functional food resources.

## 1 Introduction

Flaxseed is the seed from the flax plant (*Linum usitatissimum* L.), which is a member of the Linaceae family. As the source of linen fibre, flaxseed has been cultivated since at least 5000 BC; today it is mainly grown for its oil (Liu et al., 2016). The spherical fruit capsules contain two seeds in each of five compartments. The seed itself is flat and oval with a pointed tip. Seed colour is determined by the amount of pigment in the outer seed coat the more pigment, the darker the seed (Coskuner & Karababa, 2007). Its' seeds containing about 36 to 40% of oil, have long been used in human and animal diets and in industry as a source of oil and as the basic component or additive of various paints or polymers (El-Beltagi et al., 2007). Flaxseed oil is the richest plant source of linoleic (omega-6) and linolenic (omega-3) polyunsaturated fatty acids (PUFA), which are essential for humans since they cannot be synthesized in the organism and must be ingested in food (El Beltagi et al., 2007). Flaxseed oil is qualitatively different from the more common vegetable oils with high PUFA proportions, such as soya oil, sunflower oil, rape oil, olive oil, etc and it has a relatively low glucosinolate content (El-Beltagi et al., 2007). The protein and fibre content in the seed are also important nutritional parameters: the crude protein content in the seed ranges from 25% to 35%, while the crude fibre content is about 28% (Bilek & Turhan 2009).

The effectiveness of lipid unsaponifiable matters in retarding oil deterioration has been demonstrated by many investigators (Herchi et al., 2014a). In flaxseed, lipids are protected against

oxidation by various mechanisms, for example, the presence of antioxidants such as lignans, phenols, tocopherols and flavanoids (Herchi et al., 2015). In addition to preventing fat rancidity, these antioxidants could increase commercial value of food products and have beneficial effects on human health. The antioxidant ability of lignans, tocopherols and flavanoids is related to the presence of OH groups which may directly bind to free radicals and chelate metals (Pengkumsri et al., 2015). Flaxseed oil is cited as potentially useful by the American Heart Association in the prevention of cardiovascular diseases, including reduction of serum cholesterol, platelet aggregation, and inflammatory markers, improving glucose tolerance and acting as an antioxidant (Santos et al., 2014).

To the best of our knowledge, the chemical composition and oxidative stability during flaxseed hull development have been investigated (Herchi et al., 2014b) but no work have been reported on the physicochemical properties and antioxidant activity of whole flaxseed from different stages of maturity. The objective of this research was to analyze physical properties, phospholipids contents and antioxidant activity during flaxseed development.

## 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).

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## 2.2 Plant materials

The variety of flaxseed “P129” was obtained from Institut National Recherche Agronomie Tunis (INRAT), Tunisia. This variety of flaxseed (*L. usitatissimum* L.) was grown in restricted zones (15 m × 3 m) on the Agronomy farm of the INRAT from the middle of November 2006 until the end of June 2007. Each sample was collected at intervals after a number of days of flowering (DAF) ranging from 7<sup>th</sup> DAF to 56<sup>th</sup> DAF. Moisture and seeds weights were determined by weighing 100 seeds before and after drying to constant weight in a vacuum oven at 80 °C for 72 h. Capsule diameter was measured, using a digital caliper, at approximately weekly intervals for all 10 capsules between 7 and 56 DAF.

## 2.3 Physical properties

Seed dimensions (length (L), width (W) and Thickness (T)) were carried out with a digital caliper to an accuracy of 0.01 mm. Seed weights in milligrams were determined using an electrical balance. Geometric mean diameter ( $D_g$ ), Arithmetic Mean diameter ( $D_a$ ), Aspect ratio ( $R_a$ ), Surface area (S), Sphericity ( $\phi$ ), Seed volume (V), Oblate spheroid ( $V_{osp}$ ) and Ellipsoid shapes ( $V_{ellip}$ ) values were found using the following formula (Calisir et al., 2005):

$$D_g = \text{Geometric Mean diameter} = (L.W.T)^{1/3}$$

$$D_a = \text{Arithmetic Mean diameter} = (L + W + T) / 3$$

$$R_a = (W / L) \times 100$$

$$\pi = 3.14 \text{ mathematical constant (Pi).}$$

$$S = \pi \cdot D_g^2$$

$$\phi = (L.W.T)^{0.333} / L$$

$$V = \pi B^2 L^2 / 6 (2L - B) \text{ Where } B = (WT)^{0.5}.$$

$$V_{osp} = 4\pi/3 (L/2) (W/2)^2 \text{ Oblate spheroid}$$

$$V_{ellip} = 4\pi/3 (L/2) (W/2) (T/2) \text{ Ellipsoid shapes}$$

## 2.4 Lipid extraction

The total lipids were extracted by the method of Folch et al. (1957) modified by Bligh & Dyer (1959). Flaxseeds (40 g) were washed with boiling water for 5 min to denature the phospholipases (Douce, 1964) and then crushed in a mortar with a mixture of  $\text{CHCl}_3$  - MeOH (2:1, v/v). Fixing water was added and the homogenate was centrifuged at 3000 rpm for 15 min. The lower chloroformic phase containing the total lipids was dried in a rotary evaporator at 40 °C.

## 2.5 Lipid class separation by Thin-Layer Chromatography

Lipid classes were separated by TLC using glass plates (20 × 20 cm) covered with silica gel (G60, Merck) at a thickness of 0.25 mm. For this, the plates were activated at 120 °C for 2 h immediately before use, and approximately 30 mg of total lipids per gram of adsorbent was fractionated. Phospholipids (PLs) were separated using a mixture of chloroform– acetone – methanol–acetic acid

– water (50:20:10:10:5, v/v/v/v/v) as described by (Lepage, 1967). Lipid spots were detected after a brief exposure of the plates to iodine vapors saturating a tightly closed vat. The identification of lipid classes was made by comparing their Retention Factor (RF) values with those of authentic standards chromatographed under the same conditions. After the detection of the lipid classes, the plates were submitted to a nitrogen stream in order to eliminate iodine, and individual bands were scraped from the plates and corresponding phospholipids were recovered from the silica gel by elution with 5 mL of hexane.

## 2.6 Phospholipids (PL) extraction (Diol SPE)

The SPE cartridge was preconditioned with 20 mL chloroform, 20 mL chloroform/methanol (9:1, v/v), and 20 mL chloroform. Lipid extracts dissolved in 2 mL chloroform were loaded onto the cartridge and eluted with the following solvents: 30 mL chloroform to remove neutral lipids, 25 mL acetone to remove glycolipids, and 30 mL methanol plus 10 mL of chloroform/methanol (1:1 v/v) to recover phospholipids. Lipid fractionation using this solvent system has been previously described (Herchi et al., 2011b). The recovered fraction was dried in a rotary evaporator and weighted in order to determine the PL amount in the oil. The PL fraction was re-dissolved in 1 mL of methanol for LC-MS analysis.

## 2.7 Determination of PL molecular species by HPLC-MS

LC-MS experiments were performed using a method described in detail by Herchi et al. (2011b). The method employed an Agilent1200 LC system (Agilent Technologies, Palo Alto, CA) coupled to an Applied Biosystem /MDS Sciex 3200 QTRAP LC/MS/MS with a Turbo Ion Spray source. The phospholipid fractions from flaxseeds were separated using an Ascentis Express HILIC column (15 cm × 2.1 mm, 2.7 mm). The mobile phase consisted of 92/8 acetonitrile/125 mM ammonium formate pH3.0 (A) and 10 mM ammonium formate in water with 0.2% formic acid pH3.0 (B). The solvent gradient initiated at 0% B, increased to 16% B in 12 min, to 70% B in 0.1 min and maintained at 70% B for 5 min, and returned to initial solvent composition in 0.1 min and reequilibrated for 20 min prior to next injection. The injection volume was 2  $\mu\text{L}$  and flow rate was 200  $\mu\text{L}/\text{min}$ . MS analysis of phospholipids was performed using ESI in the negative ion mode. Nitrogen was used as curtain gas, nebulising gas, and turbo gas. The instrumental settings were as follows: spray voltage 4000 V; curtain gas (CUR) 25; nebulizer gas (GS1) 40; turbo gas (GS2) 30; and ion source temperature 400 °C. Analyst 1.4.2 software was used for data acquisition and analysis. Relative concentrations of each phospholipid class and species were estimated using uncorrected peak areas of extracted ion chromatograms (EIC). The MS/MS data was obtained during HILIC-LC separations using information dependent acquisition (IDA) enhanced product ion (EPI) scan modes which automatically trigger MS/MS acquisition as molecular species are detected. The total ion chromatogram (TIC) together with the extracted ion chromatograms (EICs) of PL classes from flaxseed oil is presented by Herchi et al. (2011b).

## 2.8 Total polyphenol content

The content of total polyphenol was determined by using the Folin-Ciocalteu colorimetric method, based on the reaction of the reagent with the functional hydroxyl groups of phenols.

A one-gram oil sample was weighed, dissolved in 10 mL hexane and transferred to a separatory funnel. Then, 20 mL of a methanol–water mixture (80:10 v/v) was added. After 3 min of shaking the lower methanol–water layer was removed. The extraction was repeated twice and the methanol–water phases were combined. The methanol–water extract was driven to dryness in a rotary evaporator under a vacuum at 40 °C. The dry residue was then dissolved in 1 mL of methanol. The extraction procedure described above was performed three times (Parry et al., 2005). An aliquot (0.2 mL) of the methanolic extract was placed in a volumetric flask (10 mL). Diluted Folin–Ciocalteu reagent (0.5 mL) was added. After 3 min, saturated sodium carbonate (1 mL) was added. The flask was filled with water up to 10 mL. After 1 h, the absorbance at 765 nm was measured using a UV–vis spectrophotometer (LKB–pectronic 20 D+) with a 1-cm cell. Total phenolic compounds were determined after preparation of a standard curve. Gallic acid was used as a standard. Results are expressed as mg of gallic acid equivalent per kg of oil.

## 2.9 Determination of antioxidant activity

The 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 1:

$$\text{Inhibition percentage} = (I \%) = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

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

## 2.10 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 Morphology and physical properties during flaxseed development

A summary of the physical properties of flaxseed during development is shown in Table 1. We did not find any study in the literature on the effect of maturation on the physical properties of flaxseed. All the properties except from sphericity increased slightly with maturity. Capsule diameter increased rapidly from 7.2 mm to 12.8 mm at 28 DAF, and then increased more slowly with maturation to around 13.4 mm at approximately 42 DAF (Table 1). Thereafter, capsule diameter decreased slightly to 12.4 mm at maturity. Table 1 indicates that the seeds expand in length, width, thickness, arithmetic and geometric mean diameter within

**Table 1.** Physical properties during flaxseed development.

Properties/ DAF	17	14	21	28	35	42	49	56
Length (mm)	1.94±0.02 <sup>a</sup>	2.30±0.02 <sup>a</sup>	3.26±0.02 <sup>a</sup>	5.00±0.03 <sup>a</sup>	5.24±0.03 <sup>a</sup>	6.00±0.04 <sup>a</sup>	6.10±0.04 <sup>a</sup>	6.10±0.05 <sup>a</sup>
Width (mm)	1.10±0.01 <sup>a</sup>	1.13±0.01 <sup>a</sup>	1.66±0.01 <sup>a</sup>	2.60±0.02 <sup>a</sup>	2.70±0.01 <sup>a</sup>	3.00±0.02 <sup>a</sup>	3.00±0.02 <sup>a</sup>	3.00±0.02 <sup>a</sup>
Thickness (mm)	0.42±0.01 <sup>a</sup>	0.76±0.01 <sup>a</sup>	0.80±0.01 <sup>a</sup>	0.84±0.01 <sup>a</sup>	0.86±0.01 <sup>a</sup>	0.92±0.01 <sup>a</sup>	0.94±0.01 <sup>a</sup>	0.96±0.02 <sup>a</sup>
Dg	0.96±0.01 <sup>a</sup>	1.25±0.01 <sup>a</sup>	1.63±0.01 <sup>a</sup>	2.22±0.02 <sup>b</sup>	2.30±0.02 <sup>b</sup>	2.55±0.02 <sup>b</sup>	2.58±0.02 <sup>b</sup>	2.60±0.02 <sup>b</sup>
Da	1.15±0.01 <sup>a</sup>	1.40±0.01 <sup>a</sup>	1.91±0.02 <sup>a</sup>	2.81±0.02 <sup>b</sup>	2.93±0.02 <sup>b</sup>	3.31±0.02 <sup>b</sup>	3.35±0.03 <sup>b</sup>	3.35±0.03 <sup>b</sup>
Aspect ratio ( $R_a$ )	56.70±2.18 <sup>a</sup>	41.85±1.43 <sup>b</sup>	50.92±1.62 <sup>a</sup>	52.00±2.48 <sup>a</sup>	51.53±1.54 <sup>a</sup>	50.00±1.78 <sup>b</sup>	49.18±1.49 <sup>b</sup>	49.18±1.25 <sup>b</sup>
Seed Weight (mg)	1.60±0.02 <sup>a</sup>	2.80±0.04 <sup>a</sup>	5.20±0.04 <sup>a</sup>	6.40±0.02 <sup>a</sup>	9.40±0.02 <sup>b</sup>	10.70±0.06 <sup>b</sup>	10.60±0.05 <sup>b</sup>	10.50±0.02 <sup>b</sup>
Seed count (seeds/kg)	589000 <sup>a</sup>	358000 <sup>a</sup>	193000 <sup>b</sup>	157000 <sup>b</sup>	107000 <sup>b</sup>	94000 <sup>b</sup>	96000 <sup>b</sup>	97000 <sup>b</sup>
Surface Area (S) (mm <sup>2</sup> )	2.89±0.03 <sup>a</sup>	4.91±0.03 <sup>a</sup>	8.34±0.04 <sup>a</sup>	15.48±0.05 <sup>b</sup>	16.61±0.04 <sup>b</sup>	20.42±0.06 <sup>b</sup>	20.90±0.05 <sup>b</sup>	21.23±0.05 <sup>b</sup>
Sphericity (Ø)	0.50±0.01 <sup>a</sup>	0.54±0.01 <sup>a</sup>	0.50±0.02 <sup>a</sup>	0.44±0.01 <sup>a</sup>	0.44±0.01 <sup>a</sup>	0.43±0.01 <sup>a</sup>	0.42±0.02 <sup>a</sup>	0.42±0.01 <sup>a</sup>
Seed volume (V) (mm <sup>3</sup> )	0.29±0.04 <sup>a</sup>	0.65±0.03 <sup>a</sup>	1.37±0.03 <sup>a</sup>	3.36±0.07 <sup>b</sup>	3.71±0.08 <sup>b</sup>	5.02±0.06 <sup>b</sup>	5.22±0.08 <sup>b</sup>	5.36±0.10 <sup>b</sup>
Oblate spheroid ( $V_{osp}$ ) (mm <sup>3</sup> )	1.23±0.03 <sup>a</sup>	1.54±0.02 <sup>a</sup>	4.70±0.03 <sup>a</sup>	17.69±0.08 <sup>b</sup>	20.00±0.10 <sup>b</sup>	28.26±0.14 <sup>b</sup>	28.26±0.14 <sup>b</sup>	28.26±0.14 <sup>b</sup>
Ellipsoid shapes ( $V_{ellip}$ ) (mm <sup>3</sup> )	0.47±0.01 <sup>a</sup>	1.02±0.02 <sup>a</sup>	2.27±0.02 <sup>a</sup>	5.72±0.04 <sup>b</sup>	6.37±0.04 <sup>b</sup>	8.67±0.08 <sup>b</sup>	9.00±0.12 <sup>b</sup>	9.20±0.10 <sup>b</sup>
Capsule diameter (mm)	7.2±0.2 <sup>a</sup>	8.4±0.2 <sup>a</sup>	10.2±0.3 <sup>b</sup>	12.8±0.4 <sup>b</sup>	13.2±0.4 <sup>b</sup>	13.4±0.4 <sup>b</sup>	12.2±0.3 <sup>b</sup>	12.4±0.4 <sup>b</sup>

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

maturity. Similar results have been reported by some researchers (Coskuner & Karababa, 2007). The geometric mean diameter of flaxseed is lower than for okra seeds (Calisir et al., 2005) and higher than that of sesame seeds (Tunde-Akintunde & Akintunde 2004). Oblate spheroid and Ellipsoid shapes showed an increase during flaxseed development. The data obtained from the work may be of interest to people seeking information on physical properties of flaxseed to aid in design and fabrication of processing equipment for the product.

### 3.2 Comparative analysis of phospholipids (PLs) using HPLC-MS and TLC

Table 2 shows comparative analysis of the individual phospholipids contents in total phospholipids using TLC (Thin layer chromatography) and HPLC-MS. TLC revealed the presence of six phospholipids at different stages of maturity, phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC) and lysophosphatidylcholine (LPC). Similar phospholipids classes were detected by HPLC-MS (Herchi et al., 2011b). Using TLC, The total phospholipid content was high (46%). Both methods shows continuous decrease in the total phospholipid content from about (46%-35%) at 7 DAF to (4%-2%) at 56 DAF. All the results showed significant differences between HPLC-MS and TLC for quantitative determination of phospholipids classes. Herchi et al. (2012) have studied the change of fatty acids (FAs) composition of the total phospholipid mixture, but information on the changes of fatty acids composition in each class of phospholipids in flaxseed at different stages of seed development, using HPLC-MS, is still insufficient or limited. Practically no literature data are available on the modifications of each molecular species of each PL during development, which play a pivotal role for the study of the change of the nutritional quality of flaxseed oil. Table 3 gives the evolution of fatty acids composition in each individual phospholipid. The major fatty acids in all the six phospholipids were found to be linolenic, linoleic, oleic and palmitic acids. The highest amount of linolenic acid (31%) was observed in PG. Linoleic acid content

in PE was found to be the maximum (51%) compared to other phospholipids. It was found that PG contained a higher amount of palmitic acid and a lower amount of oleic acid, whereas PC contains a lower amount of palmitic acid and a higher amount of oleic acid. It was observed that the unsaturated fatty acids were mostly located in PC, whereas the saturated fatty acids predominantly occupied PG compared to other phospholipids. Rao et al. (2009) reported that the phospholipids fraction of jatropha seeds oil was further characterized and quantified and found to contain phosphatidyl choline (PC) 60.5%, phosphatidyl inositol (PI) 24% and phosphatidyl ethanolamine (PE) 15.5%. They mentioned that linoleic acid content in PE was found to be the maximum (41%) compared to other phospholipids. The highest amount of phosphatidylcholine was observed at 49 DAF. Phosphatidylcholine as well as its essential fatty acid (omega-6 fatty acid) (12%) and choline components is required for many vital functions in the cardiovascular, reproductive, immune, and nervous systems. This characterization can be proposed as an application for the study of specific technology markers in the flaxseed processing industry.

### 3.3 Changes in total polyphenol content and antioxidant activity during flaxseed development

The change in total polyphenol content during flaxseed development is presented in Figure 1. Total polyphenol content decreased steadily from early to late development stage. A similar kind of decrease in Total phenolic compounds was reported by Herchi et al. (2011a). The decrease in Total polyphenol content has been attributed to the oxidation of polyphenols by polyphenoloxidase during fruit maturity (Eiberger & Matthes, 2011). The total polyphenol content of flaxseed oil, as reported by Zhang et al. (2007) ranges from about 77 to 115 mg / kg oil, as gallic acid equivalents. The antioxidant capacity of flaxseed oil at different stages of maturity was assessed with the DPPH radical-scavenging assay. The change in antioxidant activity during flaxseed development is presented in Figure 2. To our knowledge, no data are available on the changes in antioxidant

**Table 2.** Changes in phospholipids composition (%) during flaxseed development.

DAF	7	14	21	28	35	42	49	56
<b>SPE-HPLC-MS</b>								
PLs (g/100g oil)	34.50±2.28 <sup>a</sup>	28.63±1.75 <sup>a</sup>	20.28±1.42 <sup>a</sup>	12.54±1.33 <sup>b</sup>	7.33±0.44 <sup>b</sup>	4.12±0.71 <sup>b</sup>	3.74±0.50 <sup>b</sup>	1.85±0.26 <sup>b</sup>
PG (%)	45	12	22	2	3	1	2	4
PA (%)	19	63	60	68	13	70	50	6
PE (%)	2	1	1	8	47	14	19	27
PI (%)	17	6	3	12	29	8	16	32
PC (%)	7	4	3	5	5	6	11	9
LPC (%)	10	14	11	5	3	1	2	22
<b>TLC</b>								
PLs (g/100g oil)	46.28±2.80 <sup>a</sup>	32.14±2.52 <sup>a</sup>	24.60±2.11 <sup>a</sup>	16.47±1.86 <sup>a</sup>	8.62±1.47 <sup>b</sup>	6.83±0.91 <sup>b</sup>	5.10±0.24 <sup>b</sup>	3.38±0.17 <sup>b</sup>
PG (%)	50	10	24	6	8	3	5	6
PA (%)	21	60	56	72	18	64	44	5
PE (%)	2	2	2	4	50	16	23	23
PI (%)	14	8	10	14	22	5	18	34
PC (%)	5	2	5	3	8	10	8	12
LPC (%)	8	18	3	1	2	2	2	20

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



**Table 3.** Fatty acids composition in phospholipids classes during flaxseed development.

Phospholipids class/DAF	7	14	21	28	35	42	49	56
<b>PG</b>								
16:0	41	45	45	41	44	39	40	35.5
18:0	-	-	-	-	-	-	-	-
18:1	2	20	23	12	23.5	10	16.5	11
18:2	26	15	17	20	14.5	19	23	25.5
18:3	31	20	15	27	18	32	25.5	28
SFA	41	45	45	41	44	39	40	35.5
USFA	59	55	55	59	56	61	60	64.5
n-3/n-6	1.19	1.33	0.88	1.35	1.24	1.68	1.08	1.09
<b>PA</b>								
16:0	30	36.5	36	16	26.5	14	13.5	14.5
18:0	-	-	-	-	-	-	-	-
18:1	26	26.5	26	22	47.5	22.5	22	18.5
18:2	22	20	22.5	40	20.5	40.5	42	42
18:3	22	17	15.5	22	5.5	23	22.5	25
SFA	30	36.5	36	16	26.5	14	13.5	14.5
USFA	70	63.5	64	84	73.5	86	86.5	85.5
n-3/n-6	1	0.85	0.69	0.55	0.26	0.56	0.53	0.59
<b>PE</b>								
16:0	39	24.5	20.5	15.5	18	15	15.5	15.5
18:0	-	-	-	-	-	-	-	-
18:1	21.5	22	25.5	26.5	37	25.5	27.5	26.5
18:2	38	46	45.5	50	40.5	50.5	49.5	54
18:3	1.5	7.5	8.5	8	4.5	9	7.5	4
SFA	39	24.5	20.5	15.5	18	15	15.5	15.5
USFA	61	75.5	79.5	84.5	82	85	84.5	84.5
n-3/n-6	0.1	0.16	0.18	0.16	0.11	0.18	0.15	0.1
<b>PI</b>								
16:0	36	43	32.5	33	36	31	32	28
18:0	-	-	-	-	-	-	-	-
18:1	17	10.5	22	19	31.5	19	22	28.5
18:2	25	21.5	23	27	23	29.5	27.5	32.5
18:3	22	25	22.5	21	9.5	20.5	18.5	11
SFA	36	43	32.5	33	36	31	32	28
USFA	64	57	67.5	67	64	69	68	72
n-3/n-6	0.88	1.16	1	0.78	0.41	0.69	0.67	0.34
<b>PC</b>								
16:0	-	-	-	-	-	-	-	-
18:0	13.5	20	20.5	6	15.5	3.5	6	7
18:1	51	58.5	57.5	38	59	32	41.5	53.5
18:2	19.5	17	13	41	14	45	40.5	32.5
18:3	16	17.5	9	15	11.5	19.5	12	7
SFA	13.5	20	20.5	6	15.5	3.5	6	7
USFA	86.5	80	79.5	94	84.5	96.5	94	93
n-3/n-6	0.82	1	0.69	0.36	0.82	0.43	0.30	0.21
<b>LPC</b>								
16:0	18	16	11	25	13	28	20	7
18:0	20	14	17	22	30	15	13	11
18:1	6	3	4	24	37	22	33	35
18:2	36	37	46	19	12	13	23	34
18:3	20	30	22	10	8	22	11	13
SFA	38	30	28	47	43	43	33	18
USFA	62	70	72	53	57	57	67	82
n-3/n-6	0.55	0.81	0.48	0.52	0.66	1.69	0.48	0.38

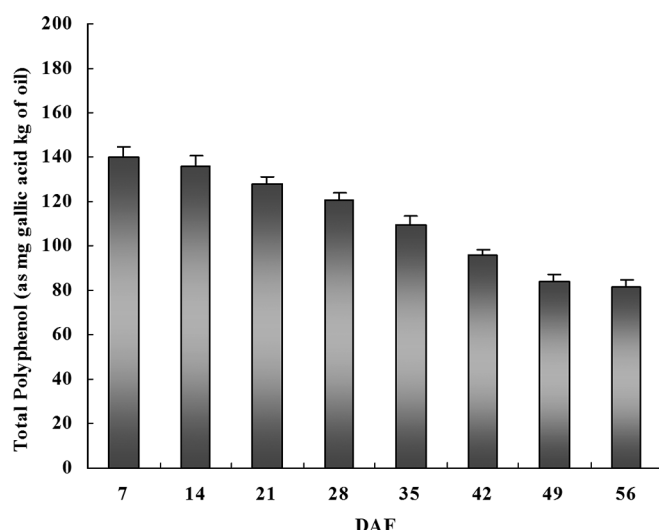


Figure 1. Changes in total polyphenol content during flaxseed development.

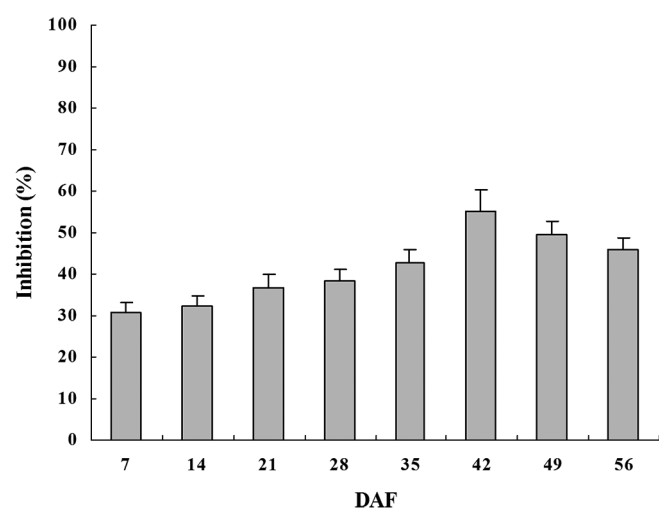


Figure 2. Changes in antioxidant activity during flaxseed development.

activity of flaxseed oil during development. The results of scavenging activity of this study were 30.82% to 55.37% during flaxseed development. This result showed more ripened of flaxseed had a higher of DPPH. Flaxseed oil exhibited higher antioxidant activity. The highest value of antioxidant activity was found in ripe flaxseed (42 DAF). In fact, it appears that there is no important correlation between antioxidant activity and total polyphenol amounts. At 7 DAF where a high content of total polyphenol was detected (140 mg / kg oil), the antioxidant activity was weak (30.82%) in comparison to other stages such as at the 42 DAF (96 mg / kg oil) and the antioxidant activity was 55.37%. Although the constituents of flaxseed oil, which show free radical scavenging action is still unclear, it is possible that the antioxidative activity of flaxseed oil are caused, at least in part, by the presence of polyphenols (Herchi et al., 2014a, 2015) and other yet to be discovered antioxidant compounds. The trend of a decreasing antioxidant capacity during ripening was also observed in durian fruits by (Haruenkit et al., 2010).

## 4 Conclusion

Physical characteristics changes during flaxseed development. The results of this study have shown that each phospholipids class has a specific fatty acids composition at different maturity stages. Linoleic acid content in PE was found to be the maximum (51%) compared to other phospholipids. It was observed that the unsaturated fatty acids were mostly located in PC, whereas the saturated fatty acids predominantly occupied PG compared to other phospholipids. Total polyphenol content were highly accumulated on the 7<sup>th</sup> DAF and mature stages of flaxseed resulted high antioxidant activity. The result of this study will provide valuable information for flaxseed breeders and growers in developing and producing functional food resources and products.

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