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Effects of germination on chemical composition and antioxidant activity of flaxseed (*Linum usitatissimum* L) oil

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SUMMARY: The present study was carried out to determine the changes in proximate composition and physicochemical characteristics of flaxseed during germination. Flaxseed was germinated for 4 days and observations were taken every day throughout the study. Changes in the seed reserve and antioxidant activity were determined during germination. The oil content of the cultivar decreased from 35.10 to 27.22%. During the germination period, the total protein content increased to 23.84%. Germinated flaxseed showed significantly higher unsaturated as compared to saturated fatty acid ratios and higher calculated oxidizability (Cox) values. The Saponification value ranged from 182 to 192 mg KOH·g^{-1} oil during germination. The highest peroxide value (2.4 mequiv O_2·kg^{-1} oil) was observed at the end of germination. The unsaponifiable contents ranged from 1.62 to 1.18%. The oxidation value of the oil samples were statistically in the same range (4.1–6.4%). After 4 days of germination, oil stability was reduced to 1.0 h. The increase in ascorbic acid content was steady. Total phenolic acid contents differed significantly. The greatest concentration was detected in non germinated flaxseed oil. Germinated Flaxssed oil showed an important free radical scavenging activity towards 1-1-diphenyl-2-picrylhydrazyl (DPPH) free radicals.

KEYWORDS: *Antioxidant activity; Germination; Oil; Oil stability; Physicochemical characteristics*

RESUMEN: ***Efecto de la germinación sobre la composición química y la actividad antioxidante de aceites de linaza*** **(Linum usitatissimum *L*).** El presente estudio se llevó a cabo para determinar los cambios en la composición y características físico-químicas de aceites de linaza durante la germinación. La linaza se germinó durante 4 días y el estudio se realizó todos los días durante este proceso. Se determinaron los cambios en la reserva de las semilla y la actividad antioxidante. El contenido de aceite de los cultivos disminuyó de 35,10 a 27,22%. Durante este periodo, el contenido de proteína total aumentó a 23,84%. La linaza germinada mostró valores significativamente más altos de la relación de ácidos grasos insaturados frente a saturados y mayor facilidad de oxidación (Cox). El índice de saponificación varió desde 182 hasta 192 mg KOH·g^{-1} de aceite durante la germinación. El índice de peróxido más alto (2,4 mequiv O_2·kg^{-1} de aceite) se observó al final de la germinación. El contenido de materia insaponificable varió desde 1,62 hasta 1,18%. La oxidación de las muestras de aceite fueron estadísticamente del mismo rango (4.1 a 6.4%). Después de 4 días de germinación, la estabilidad del aceite se redujo a 1,0 h. El aumento en contenido de ácido ascórbico fue estable. Los contenidos totales de ácidos fenólicos diferían significativamente. La mayor concentración se detectó en el aceite de linaza no germinado. El aceite de linaza germinado mostró una importante actividad de eliminación de radicales libres hacia 1-1-difenil-2-picrilhidrazil (DPPH).

PALABRAS CLAVE: *Aceite; Actividad antioxidante; Características físico-químicas; Estabilidad de aceite; Germinación*

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1. INTRODUCTION

Flaxseed (*Linum usitatissimum* L.) is a globally important agricultural crop grown both for its seed oil as well as its stem fiber. Flaxseed is used as a food source and has many valuable nutritional qualities. The seed oil also has multiple industrial applications such as in the manufacture of linoleum and paints and in preserving wood and concrete (Vaisey-Genser and Morris, 2001). Nutritionally, flaxseed has multiple desirable attributes. It is rich in dietary fiber and has a high content of essential fatty acids, vitamins and minerals. The seeds are composed of ~45% oil, 30% dietary fiber and 25% protein. Around 73% of the fatty acids in flaxseed are polyunsaturated. Approximately 50% of the total fatty acids consist of α-linolenic acid (ALA), a precursor for many essential fatty acids in the human diet (Sebei *et al.*, 2007). Flaxseed is also a rich source of the lignan component secoisolariciresinol diglucoside (SDG). In addition to having anti-cancer properties, SDG also has antioxidant and phytoestrogen properties (Touré and Xueming, 2010). Flaxseed contains about 400 $g \cdot kg^{-1}$ total dietary fiber. This seed fiber is rich in pentosans and the hull fraction contains 2–7% mucilage (Vaisey-Genser and Morris, 1997). The other major constituents of flaxseeds are storage proteins that can range from 10–30% (Sebei *et al.*, 2007).

Seeds generally consist of the embryo, endosperm tissue, and seed coat. One of the main purposes of the endosperm is to serve as a nutrient source for the germinating embryo (Linkies *et al.*, 2010). Germination is the result of different physiological processes and the germination process is initiated by water uptake. The imbibition of seeds induces other physiological processes, resulting in the breakdown of reserves, the mobilization and utilization of the broken-down products, and the growth and expansion of the embryo. Germination is assumed to be completed when the radicle emerges from the endosperm and seed coat (Bewley and Black, 1994). The germination of seeds mobilizes reserves from the seed to the growing seedling; increased metabolic activities in turn result in chemical changes in the macromolecules (Wanasundara *et al.*, 1999a). Although the germination effect on flaxseed composition has been studied (Wanasundara *et al.*, 1999a; Wanasundara *et al.*, 1999b; Sebei *et al.*, 2007), no information is available, in the literature, on the effect of germination on the physicochemical properties and antioxidant activity of flaxseed oil.

The purpose of the present investigation is to study the changes in proximate constituents during germination. Changes in oil characteristics including physicochemical properties, Fuel properties, and antioxidant activity are also reported.

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. Germination of flaxseed

The seeds of flaxseed (*L. usitatissimum* L.) variety, "I61" were supplied by the "Institut National de la Recherche Agronomique de Tunis" (INRAT, Tunisia). Whole flaxseeds (400 g) were germinated on sterile filter paper in Petri dishes. The dishes were incubated for 4 days in the dark at room temperature (20 ± 2°C). The germinated flaxseeds were dried in a dryer at 40 °C and were ground and packed in air tight bags for further analysis.

2.3. Proximate composition

The dry matter contents of flaxseed were determined by drying in an oven at 105 °C for 24 h to constant weight (AOAC, 1990). The crude protein contents were calculated from the nitrogen contents (N × 6.25) obtained using the Kjeldahl method by AOAC (1990). The crude fat contents were determined by continuous extraction in a Soxhlet apparatus for 5 h using petroleum ether as solvent (AOAC, 1990). The total ash contents were determined by incinerating flaxseed (2 g) in a furnace at 550 °C for 6 h, then weighing the residue after cooling to room temperature in a desiccator (AOAC, 1990). The carbohydrate contents were determined by difference which is by deducing the mean values of other parameters that were determined from 100.

2.4. Gas chromatography–flame ionization detection

The quantification of fatty acid 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 an FID detector on a capillary column coated with Supelcowax™ 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 $1 mL \cdot min^{-1}$. The 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 regards to the total area. To evaluate

the efficiency of the desaturation pathway during the 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:

ODR = [% C18: 2 + % C18: 3 / % C18: 1 + % C18: 2 + % C18: 3] × 100

LDR = [% C18: 3 / % C18: 2 + % C18: 3] × 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 enzyme activities during seed germination. The Cox value of the oils was calculated based on the percentage of unsaturated C18 fatty acids, applying the formula proposed by Fatemi and Hammond (1980):

Cox value = [1 (18: 1%) + 10.3 (18: 2%) + 21.6 (18: 3%)] / 100

2.5. Physicochemical characteristics

Determinations 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 were carried out by standard IUPAC methods for the analysis of fats and oils (Dieffenbacher and Pocklington 1987). Oxidation value (OV) was calculated from Holm's equation, OV = *p*-AV + 2 (PV), while theoretical flavor scores (F) were obtained from the equation F = 7.7–0.35 (OV) (List *et al*., 1974). Oxidative stability was evaluated by the Rancimat method (Gutiérrez, 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 with an air flow of 10 $L \cdot h^{-1}$.

2.6. Total chlorophyll and carotenoids

A 1.5 g sample of germinated flaxseed 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 the carotenoid fraction. The values of the coefficients of the 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:

Chlorophyll $(mg \cdot kg^{-1}) = (A_{670} \times 10^6) / (613 \times 100 \times d)$

Carotenoid $(mg \cdot kg^{-1}) = (A_{470} \times 10^6) / (2{,}000 \times 100 \times d)$

Where A is the absorbance and d is the spectrophotometer cell thickness (1 cm). The data reported is based on oil weight ($mg \cdot kg^{-1}$ flaxseed oil).

2.7. Polyphenols contents

The extraction and determination of total phenolic acid and flavonoid contents were carried out according to the method of Gutfinger (1981).

2.8. Fuel properties

The 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 germinated flaxseed oil was calculated from the iodine value (IV) and saponification value (SV) derived using the following formula adopted by Demirbas (1998):

HHV = 49.43 − (0.041 × SV) − (0.015 × IV)

The cetane number of the oil was determined according to Bose (2009):

CN = 46.3 + 5458/SV – 0.225 × IV

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 the 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:

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

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

2.10. Statistical analysis

The analyses were performed with three replicates. All data are reported as means ± standard deviation of three samples. Differences were tested for significance using the ANOVA procedure, using a significance level of $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Changes in proximate composition and fatty acid contents of flaxseed during germination

The moisture content of flaxseed was significantly ($p<0.05$) affected by germination (Table 1). It ranged from 5.22% to 11.27% during the germination period. During the study period, the oil content

Table 1. Proximate composition and fatty acids contents of flaxseed during germination

	Duration of germination (days)				
Component	**0**	**1**	**2**	**3**	**4**
Proximate composition (%)					
Moisture (%)	5.22±0.36a	10.50±0.42b	11.27±0.40b	10.29±0.24b	9.42±0.28b
Oil (%)	35.10±1.18a	33.61±2.04a	30.14±1.83a	28.74±1.33b	27.22±1.15b
Protein (%)	22.65±1.24a	23.12±1.39a	23.44±1.00a	23.60±1.12a	23.84±0.90a
Ash (%)	2.90±0.10a	3.17±0.22a	3.10±0.16a	2.86±0.20a	2.91±0.18a
Total carbohydrate (%)	34.12±0.78a	29.60±1.08b	32.05±0.62a	34.51±0.86a	36.61±1.14a
Fatty acid contents (%)					
C16:0	6.62±0.05a	6.71±0.06a	6.27±0.04a	6.40±0.08a	6.69±0.07a
C18:0	5.81±0.08a	5.72±0.04a	5.40±0.06a	5.67±0.05a	5.66±0.05a
C18:1	28.36±0.20a	30.29±0.27b	30.74±0.15b	28.74±0.11a	28.91±0.19a
C18:2	15.77±0.12a	14.93±0.10a	15.59±0.14a	15.06±0.12a	15.30±0.20a
C18:3	42.53±0.25a	41.70±0.18a	41.07±0.33a	43.24±0.30b	41.46±0.21a
ΣSFA[a]	12.43±0.13a	12.43±0.10a	11.67±0.10b	12.07±0.13a	12.35±0.12a
ΣMUFA[b]	28.36±0.20a	30.29±0.27a	30.74±0.15a	28.74±0.11a	28.91±0.19a
ΣPUFA[c]	58.30±0.27a	56.63±0.28a	56.66±0.47a	58.30±0.42a	56.76±0.41a
TU	86.66±0.47a	86.92±0.56a	87.40±0.62a	87.04±0.53a	85.67±0.60a
TU/TS[d]	6.97±0.04a	7.00±0.08a	7.48±0.06b	7.21±0.06b	6.93±0.06a
n-3/n- 6	2.69±0.02a	2.79±0.06a	2.63±0.08a	2.87±0.04b	2.71±0.02b
Cox value	11.09±0.24a	10.84±0.14a	10.78±0.18a	11.17±0.20a	10.82±0.32a
ODR	67.27±0.27a	65.15±0.19b	64.82±0.33b	66.98±0.35a	66.25±0.17a
LDR	72.95±0.30a	73.63±0.21a	72.48±0.18a	74.16±0.24b	73.04±0.33a

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 monounsaturated fatty acids; [c]Sum of major polyunsaturated fatty acids; [d]total unsaturated fatty acids to total saturated fatty acids ratio. Values given are the means of three replicates ± standard deviation. Means with different letters (a–c) within a row are significantly different at $p \leq 0.05$.

of flaxseed progressively decreased, suggesting that oils are the major source of energy during germination and the early periods of seedling growth (Graham, 2008). The protein content increased during germination and showed no significant difference ($p<0.05$). The increase in protein content may be attributed to the synthesis of cell constituents and enzymes, which lead to the degradation of other constituents (Lee and Karunanithy, 1990). The ash content remained at the same level before and after germination. During germination, the ash content showed no significant difference ($p<0.05$). The carbohydrate content increased after 4 days of germination, reaching 37 mg per 100 g, which is significantly ($p<0.05$) higher than that of sesame (30 mg·100 g^{-1}) (Hahm *et al*., 2009). Hahm *et al*., (2009) reported that the degradation of reserve nutrients (lipids and carbohydrates) during germination is a process whose essential purpose is to provide the energy required for protein synthesis in plant growth. As shown in Table 1, the ratio between unsaturated and saturated fatty acids (USFA/SFA ratio) and the Cox value of flaxseed were statistically ($p<0.05$) the same during the germination period. Germinated Flaxseed oil was characterized by its higher polyunsaturated fatty acids (PUFA) (linoleic acid, C18:2) and lower SFA percentages (Palmitic acid, C16:0, and stearic acid, C18:0), which make it particularly prone to oxidation. Indeed, the higher value of LDR indicates higher linolenic acid production during the germination period. This suggests that the biosynthetic pathway of fatty acids is efficient in the formation of linolenic acid, justifying therefore the higher amount of the latter fatty acid. The higher ratio of n-3/n-6 (2.63 vs. 2.87) indicated that flaxseed oil had greater nutritional value.

3.2. Changes in physicochemical characteristics of Flaxseed during germination

The physicochemical characteristics of flaxseed oils and their values in the literature are shown in Tables 2A and 2B, respectively. The saponification value is an index of average molecular weight (or chain length) of all the fatty acids present. The observed saponification value increased with

TABLE 2A. Physicochemical Characteristics of Flaxseed oil during germination

	Duration of germination (days)				
Properties	**0**	**1**	**2**	**3**	**4**
Saponification Value (mg KOH·g^{-1} oil)	182±1.42a	183±1.17a	188±0.96b	190±0.82b	192±0.47b
Acid value (mg KOH·g^{-1} oil)	0.94±0.05a	1.36±0.08ab	1.72±0.06b	2.10±0.04c	2.48±0.08c
FFA content (% as oleic acid)	0.50±0.02a	0.74±0.02a	0.94±0.03b	1.17±0.04b	1.33±0.03b
Iodine value (g of I_2·100 g^{-1} oil)	172±1.10a	170±0.90a	167±1.15b	163±1.10b	160±1.38c
Peroxide value (mequiv O_2·kg^{-1} oil)	1.6±0.18a	1.8±0.22a	2.0±0.16b	2.3±0.24b	2.4±0.16b
p-Anisidine value	0.9±0.16a	1.1±0.20a	1.2±0.14a	1.5±0.31b	1.6±0.25b
Oxidation value	4.1±0.16a	4.7±0.18a	5.2±0.20b	6.1±0.16c	6.4±0.18c
Theoretical flavor scores (F)	6.3±0.10a	6.0±0.09a	5.9±0.12a	5.6±0.10b	5.5±0.12b
K_{232}	1.46±0.18a	1.55±0.12a	1.63±0.14a	1.79±0.10b	2.12±0.10b
K_{270}	0.24±0.10a	0.28±0.14a	0.32±0.11b	0.36±0.10b	0.44±0.12c
Oil Stability (h)	1.4±0.24a	1.4±0.20a	1.3±0.14a	1.2±0.18a	1.0±0.10b
Unsaponifiable matter (% w/w)	1.62±0.15a	1.44±0.17a	1.35±0.10b	1.30±0.22b	1.18±0.28b
Triglyceride (%)[a]	97.88±1.14a	97.82±0.83a	97.71±0.90a	97.53±1.15a	97.49±1.22a
Ascorbic acid (mg·100 g^{-1})	1.35±0.12a	2.18±0.32b	2.38±0.19b	3.45±0.35b	2.74±0.26a
Total phenolic acids, as ferulic acid equivalents (mg·100 g^{-1} oil)	108.58±7.45a	97.35±6.28a	88.14±5.93b	82.69±6.85b	73.11±4.29b
Total Flavanoids, as luteolin equivalents (mg·100 g^{-1} oil)	10.64±1.84a	12.36±2.27a	14.10±2.68b	11.61±2.52a	11. 82±1.20a
Carotenoids (mg·kg^{-1} oil)	2.23±0.05a	4.11±0.04b	5.30±0.06b	6.19±0.08c	6.27±0.07c
Chlorophyll (mg·kg^{-1} oil)	4.45±0.12a	5.13±0.17a	5.72±0.24a	6.20±0.32b	7.37±0.28b

[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≤0.05.

germination (182–192 mg KOH·g^{-1} oil) and there were statistically significant differences among them (p<0.05). These values indicate the absence of lauric acid in the investigated flaxseed oils, and this range is indicative of oils characterized by medium chain-length FAs. Acid value varied significantly (p<0.05) during the germination period. The lower acidity value of germinated flaxseed oil indicates that the oil has a better quality and longer shelf life. The acid values of all the extracted samples in this study were not different from those reported by Teh and Birch (2013) and Choo *et al*. (2007), and higher than those determined for hemp oil and canola oil. Peroxide value was found between 1.4 and 2.6 (mequiv O_2·kg^{-1} oil) and showed significant differences (p<0.05). These results are in accordance with those reported by Teh and Birch (2013) and Choo *et al*. (2007) and were not different from the results reported for hemp oil and canola oil. According to the Codex Alimentarius Commission (2006) standard for virgin oils and cold pressed fats and oils, a good quality oil should have a peroxide value of less than 10 mequiv O_2·kg^{-1} oil. Lower acid and peroxide values have indicated that germinated flaxseed oil was more suitable as an edible oil. The iodine value, which indicates the degree of unsaturation of an oil, decreased during the germination period. The IV (172–160) obtained in this study indicates that the oils contain appreciable level of unsaturated FAs, which is confirmed by the FA profile of the germinated flaxseed investigated. The *p*-anisidine values of germinated flaxseed oils showed slight increases. These may be attributed to the light increase in carbonyl compounds. A good quality oil should have a *p*-anisidine value of less than two (Subramanian *et al*., 2000). All the oil samples from the literature had low *p*-anisidine values (Table 2B), reflecting the *p*-anisidine value of a good quality oil. The oxidation value of flaxseed oils ranged from 4.1 to 6.4% and showed no significant difference among all the samples (p>0.05). These relatively higher oxidation values prompted a separate study for the lipoxygenase activity of chickpea oil. These values were similar to those reported in the literature (Table 2B). The values of K_{232} and K_{270} extinction coefficient showed a slight increase during germination. Oil stability decreased during flaxseed germination. This decrease (1.4 h−1.0 h) in stability is explained by the loss of natural antioxidants. The relative decrease in the unsaponifiable content observed during the germination period may possibly be due to the initial loss lipids and other major reserves of the seed. A parallel decrease in the content of triglycerides was also observed. The ascorbic

TABLE 2B. Physicochemical characteristics of flaxseed oil, hemp and canola seed oils from the literature (Teh and Birch (2013), (Choo *et al*., 2007)

	Valued in the literature***			
Properties	**Flaxseed oil**	**Flaxseed oil**	**Hemp oil**	**Canola oil**
Saponification Value (mg·KOH g^{-1} oil)	–	–	–	–
Acid value (mg KOH·g^{-1} oil)	0.50–2.50	1.49	1.76	1.43
FFA content (% as oleic acid)	0.25–0.98	0.75	0.89	0.72
Iodine value (g of I_2·100 g^{-1} oil)	–	–	–	–
Peroxide value (mequiv O_2·kg^{-1} oil)	0.70–3.00	2.04	1.94	2.39
p-Anisidine value	0.36–0.74	0.52	0.62	0.27
Oxidation value	1.30–6.00	4.6	4.5	5.05
Theoretical flavor scores (F)	7.24–5.60	6.09	6.12	5.93
K_{232}	1.8–2.8	2.08	1.53	2.21
K_{270}	0.20–0.40	0.02	0.02	0.02
Oil Stability (h)	–	–	–	–
Unsaponifiable matter (% w/w)	0.39–0.71	0.40	0.26	0.54
Triglyceride (%) [a]	99.36–98.31	98.85	98.85	98.74
Ascorbic acid (mg·100 g^{-1})	–	–	–	–
Total phenolic acids, as ferulic acid equivalents (mg·100 g^{-1} oil)	76.8–307.3	136.93	188.23	57.17
Total Flavanoids, as luteolin equivalents (mg·100 g^{-1} oil)	12.7–25.6	18.75	19.50	16.41
Carotenoids (mg·kg^{-1} oil)	–	0.6	–	2.2
Chlorophyll (mg·kg^{-1} oil)	0.80–5.76	6.78	75.21	0.86

[a]Triglyceride (%) = 100 - {(free fatty acid, %) + (unsaponifiable matter, %)}.
***Teh and Birch (2013), (Choo *et al*., 2007).

acid of flaxseed increased in the early stages of germination and then remained constant until the fourth day of germination. A significant decrease in total phenolic acid content was observed during germination (108.58−73.11 mg·g^{-1}). The total phenolic acid content showed no significant difference (p<0.05). The decrease in TPC has also been attributed to enzymatic activity during germination (Gujral *et al*., 2011). Randhir *et al*. reported that germination causes a decrease in the total phenolic content in Green mung. All the respective values were similar to those reported from the literature (Table 2B). In addition, these results are lower than those reported for hemp oil and higher than those reported for canola oil. The Total flavanoid content increased to 14.10 mg·g^{-1} on the second day then dropped down at the end of day 3. The highest level of chlorophyll and carotenoids was detected by the end of the germination period (6.27−7.37 mg·kg^{-1} oil, respectively). The fuel properties shown in Fig. 1 indicate that there was no significant difference for HHV and the cetane number during germination. 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 CO_2 and H_2O at its initial temperature and pressure. The highest heating value (39.39 MJ·kg^{-1}) was obtained from ungerminated flaxseed oil. The heating values are the same for most of the oils (39–40 MJ·kg^{-1}) except for castor oil (37.3 MJ·kg^{-1}). The cetane number (CN) is one of the most commonly cited indicators of diesel fuel quality, especially the ignition quality (Bamgboye and Hansen, 2008). The highest CN (37.87) was obtained for

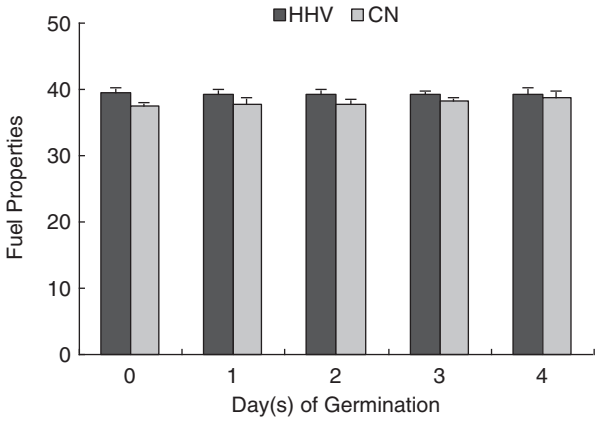

FIGURE 1. Effect of germination on Fuel properties.

germinated flaxseed oil (1 day of germination). The values of CN of soybean oil-derived biodiesel ranged from 45 to 60, whereas those of rapeseed oil-derived biofuel ranged from 48 to 61.2 (Bamgboye and Hansen, 2008). Higher CNs 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 (Aminul Islam *et al*., 2012). Germinated Flaxseed oils with good physicochemical properties will have potential to be biodiesel feedstocks.

3.3. Changes in the antioxidant activity of Flaxseed oil during germination

Antioxidant activity is expressed as percent DPPH radical scavenging activity with higher values indicating greater antioxidant activity. The antioxidant activity ranged from 40.14 to 52.48% (Fig. 2) with the highest activity exhibited by ungerminated flaxseed and the lowest exhibited by germinated flaxseed oil at the end of day 3. During germination, the antioxidant activity significantly decreased ($p<0.05$) up to 3 days of germination and then further increased upon 4 days of germination. The decreasing trend of antioxidant activity during germination is similar to the trend observed in lentils (*Lens culinaris*) while the increasing trend of antioxidant activity is similar to beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*) (López-Amorós *et al*., 2006). Wong *et al*. (2006) stated that one of the possible reasons for the decreased value obtained from the DPPH assay for plant samples could be due to the presence of compounds which are not reactive towards DPPH free radicals. Polyphenols may be more efficient as reducing agents in reducing ferric iron but some may not scavenge DPPH free radicals as efficiently due to stearic hindrance. All the samples showed significant difference ($p<0.05$).

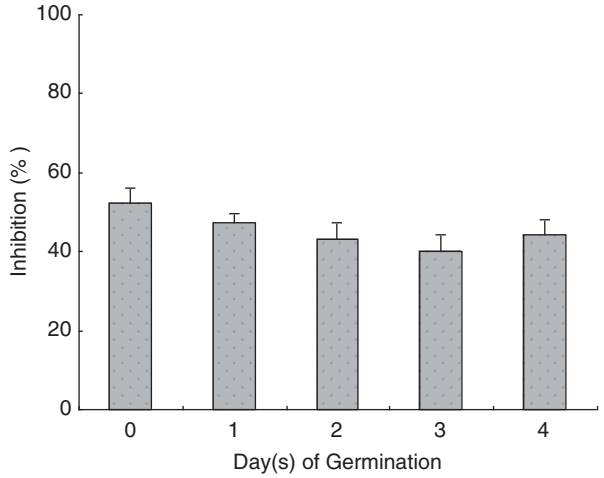

FIGURE 2. Effect of germination on antioxidant activity.

The scavenging action of plant constituents has been found to relate to polyphenolic compounds (Siger *et al*., 2008). Although the constituents of germinated flaxseed oil, which show free radical scavenging action are still unclear, it is possible that the antioxidative activity of germinated flaxseed oil is caused, at least in part, by the presence of polyphenols (Azhari *et al*., 2014; Anwar *et al*., 2013; Gujral *et al*., 2011) and other yet to be discovered antioxidant compounds.

4. CONCLUSIONS

Phytochemical contents changed during flaxseed germination. The crude fat content was reduced significantly from 35% in ungerminated seeds to less than 28% after germination. The ash and crude protein content remained at the same level before and after germination. Cox value and LDR showed no significant difference ($p>0.05$). The total phenolic acids content was highly accumulated on non-germinated flaxseed. On the basis of our physicochemical evaluation of flaxseed oil we conclude that oil originating from each day of germination has its own special characteristics. According to the test carried out on the crude oil in order to assess the efficiency of the DPPH method of antioxidant evaluation, this oil can be a source for use in the food, cosmetics and pharmaceutical industries.

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