Changes in Flax (*Linum usitatissimum* L.) Seed Lipids During Germination

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ABSTRACT: Flax (*Linum usitatissimum* L.) seeds were germinated for 8 d under laboratory conditions, and changes in their lipid fraction were studied by various chemical and chromatographic methods. Total lipid content of the seeds was reduced fourfold at the end of the 8-d germination period as compared to ungerminated seeds on a fresh weight basis. The neutral lipids comprised the major fraction of seed lipids, and triacylglycerols predominated over all other lipid components even during the germination period. Both the spectrophotometric and thin-layer chromatography–flame-ionization detection methods of quantification showed a considerable increase in the content of free fatty acids. The glycolipid fraction of lipids increased, but the phospholipid fraction exhibited only minor changes. Lipase activity of flaxseed increased at the beginning of germination and then remained constant until the fifth day. Phosphatidylcholine was the major phospholipid of flaxseed lipids, and its content was reduced during the germination. The contents of lysophosphatidylcholine and phosphatidic acid increased from negligible amounts to 46% of the total phospholipids. Linolenic, linoleic, and oleic acids, respectively, were the predominant fatty acids of all the lipid fractions of flaxseed, and remained unchanged during the germination period. The glycolipid fraction had the lowest content of polyunsaturated fatty acids. Fatty acids $C_{14:0}$, $C_{20:0}$, $C_{24:0}$, $C_{20:1}$, $C_{22:1}$, and $C_{20:5}$ appeared after d 2 of germination in neutral, glyco- and phospholipid fractions.

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Germination of higher plant seeds involves a complex series of metabolic processes such as water imbibition, respiration, mobilization of food reserves, nucleic acid and protein synthesis, and cell differentiation and growth (1). Lipids of oleaginous seeds are either storage or structural components, and the stored lipids are mainly found in small discrete intercellular organelles called oil bodies (2,3). During germination of oilseeds, storage lipids are metabolized to supply the required energy for the high-energy demanding processes. Structural lipids also change quantitatively due to new membrane formation (4).

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Lipids comprise the largest fraction of flax (*Linum usitatissimum* L.) seed and range from 35.2 to 43.3%, on a dry weight basis (5), in seeds grown in North America, depending on the genetic and environmental factors. Among oilseeds, traditional flaxseed varieties contain a very high amount of polyunsaturated fatty acids, especially α-linolenic acid ($C_{18:3}$,ω-3; >50% of total fatty acids). The nutritional importance of polyunsaturated fatty acids (PUFA) of flaxseed oil was reviewed in humans (6–8), as well as farm animals (9–11) and cultured fish (12). Despite the long use of flaxseed as a food ingredient in different continents of the world, its nutritional properties were only recently studied and accepted. Studies from our laboratory reported several chemical and processing aspects of flaxseed in order to explore its novel applications (13–18). This paper discusses the behavior and fate of lipids of flaxseed during the first 8 d of germination under controlled conditions.

MATERIALS AND METHODS

Extraction and quantification of total lipids. Whole flaxseeds (variety, Somme) were germinated in the laboratory (temperature, 22 ± 1°C; relative humidity, 78 ± 2%; light supply, 375 lux), and samples of seedlings were withdrawn on d 0, 2, 4, 6, and 8. Details of the germination procedure were reported elsewhere (19).

Total lipids of germinated flaxseeds were extracted according to the Folch method (20). Each sample (1–2 g) was homogenized in 10 mL of methanol using a Polytron homogenizer (PT-3000; Brinkmann, Rexdale, ON, Canada) at 10,000 rpm for 1 min, followed by addition of 20 mL of chloroform and a further 2-min homogenization. The homogenate was then filtered under suction, and the residue was resuspended in 30 mL of chloroform/methanol (2:1, vol/vol) and homogenized for 3 min, followed by suction filtration. This procedure was repeated once more, and filtrates were combined and mixed well with a 0.88% (wt/vol) KCl solution (25% of the total volume of filtrate) in a separatory funnel. The lower chloroform layer was withdrawn and washed with a methanol/water (1:1, vol/vol, 25% of total volume of chloroform layer) mixture. The washed chloroform layer was passed through an anhydrous sodium sulfate bed (1 cm thick) into a preweighed 50-mL round-bottom flask and subsequent removal of the solvent took place using a rotary evaporator

(Rotavapor RE111; Buchi Laboratorium Technik, Schweiz, Switzerland). The weight of the extracted oil was determined by difference, and lipid content (%) of samples was then calculated.

Determination of total content of free fatty acids (FFA). Total extracted lipids [5–10 mg; (20)] were dissolved in 5 mL of chloroform in a 15-mL screw-capped precleaned glass vial. The FFA content of lipids was determined colorimetrically according to the method of Lowry and Tinsley (21). To the lipid solution in chloroform, 2.5 mL of copper reagent (1 M triethanolamine/1 M acetic acid/6.5%, wt/vol cupric nitrate trihydrate, 9:1:10, vol/vol/vol) was added and mixed for 2 min by vortexing. The mixture was then centrifuged for 5 min at $1500 \times g$. A clearly separated chloroform layer (3 mL) was carefully transferred to another tube in order to prevent contamination from the upper aqueous layer. The chloroform layer was mixed well with 0.5 mL of sodium diethyldithiocarbamate in 2-butanol (0.1%, wt/vol), and the absorbance of the chloroform layer was read at 440 nm using a reagent blank containing 3 mL of chloroform and 0.5 mL of sodium diethyldithiocarbamate in 2-butanol (0.1%, wt/vol). The total FFA content in the samples was determined as linoleic acid equivalents using the standard curve prepared with pure linoleic acid.

Separation of major lipid classes by column chromatography. Lipid classes were separated from total lipids extracted by the Folch method according to Christie (22). Samples (2.0 g) of total lipids were applied onto a silicic acid column (1.25 cm internal diameter and 20 cm height; 100 mesh silicic acid powder, Mallinckrodt Canada Inc., Point Claire, PQ). The neutral lipid (NL) fraction was first eluted with chloroform (48 times the column bed volume). Monogalactosyl diacylglycerols and digalctosyl diacylglycerols (GL) were then eluted with chloroform/acetone (50:50, vol/vol, 20 times the column bed volume) and acetone (48 times the column bed volume), respectively. Finally the phospholipid (PL) fraction was eluted with methanol (48 times the column bed volume). The solvents were removed from each fraction under vacuum using a rotary evaporator at 40°C. All fractions were weighed and their wt% calculated.

Analysis of fatty acid composition of lipids. Fatty acid methyl esters (FAME) of total lipids and lipid fractions, obtained from column chromatography, were prepared and quantified by gas chromatography as described elsewhere (23). Transmethylation of the fatty acids was performed by reacting the sample lipids with 6% (vol/vol) H_2SO_4 in 99.9 mol% methanol (high-performance liquid chromatography grade) at 65°C for 15 h. FAME were then extracted into hexane; removal of hexane and addition of carbon disulfide were followed by separation using a gas chromatograph (Hewlett-Packard 5890 Series II; Hewlett-Packard, Mississauga, ON, Canada) equipped with a fused-silica capillary column (Supelcowax 10, 0.25 mm × 60 m, 0.25 μm film thickness; Supelco, Oakville, ON, Canada), a flame-ionization detector (FID), and a split/splitless (split ratio 1:41) injector. The chromatographic parameters were: detector and injector temperatures, 250°C; oven temperature programming, held at 220°C for 10.25 min, then ramped to 240°C at 30°C/min followed by a hold period of 9 min. Total run time was 19.92 min, and helium was used as a carrier gas (15 mL/min). The injector and detector (FID) temperatures were set at 270°C. The FAME were identified by comparing their retention times with those of an authentic standard mixture (Supelco). The content of each identified fatty acid in the sample was calculated from the integration data of the chromatographed fatty acids and reported as wt% using methyl heptadecanoate ($C_{17:0}$) as an internal standard. HP3365 Series II Chemstation software (Hewlett-Packard) was used for data handling.

Thin-layer chromatography (TLC)–FID of lipids. The total lipids obtained from Folch extraction were chromatographed separately on Chromarod S-III and then analyzed on an Iatroscan® MK-5 (Iatron Laboratories Inc., Tokyo, Japan) analyzer equipped with an FID connected to a computer loaded with T-datascan software (Scientific Products and Equipment, Concord, ON, Canada) for data handling. The FID was operated using hydrogen and air flow rates of 160 and 2000 mL/min, respectively. The scanning speed of rods was 30 s/rod. The Iatroscan was fitted with a push-button switch to interrupt scanning when required, especially for partial scanning.

The chromarods were cleaned by soaking in concentrated nitric acid overnight, followed by thorough washing with distilled water and then acetone before use. The rods were scanned twice to burn off any impurities left on them. Chromarods were impregnated with boric acid by dipping in a 3% (wt/vol) boric acid solution for 5 min and then drying at 120°C for 5 min. Finally, rods were scanned twice to burn off any residual impurities.

A composite stock solution of neutral lipids (FFA, oleic acid), monoacylglycerol (MAG, monoolein), diacylglycerol (DAG, diolein), triacylglycerol (TAG, triolein), and phospholipids (LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; and PS, phosphatidylserine) were dissolved in chloroform/methanol (2:1, vol/vol) and stored under nitrogen at –20°C. Different dilutions of the stock solution, ranging from 0.1 to 10 μg/μL of lipid mixture, were used as working standards. Before making the composite standard mixture, each compound was developed individually and run on the Iatroscan-FID to determine its purity and R_f value. The samples dissolved in appropriate solvents were spotted on rods using Drummond microcap disposable pipettes (Drummond Scientific Co., Broomall, PA). As soon as the samples were spotted, solvents were dried off using a stream of cold air supplied by a blowdryer. The rods containing samples were placed in a humidity tank over saturated $CaCl_2$ for 10 min and then immediately transferred to the developing tank.

Aliquots of total extracted lipids from ungerminated and germinated flaxseeds were dissolved in chloroform/methanol

(2:1, vol/vol) in order to obtain a concentration of 1 μg lipid/μL. The sample (1 μL/rod) was applied on 9 out of the 10 rods, and a randomly selected rod was used for application of a standard mixture.

Three solvent systems were employed to obtain three separate chromatograms per rod. The first development of rods was carried out in benzene/chloroform/acetic acid [70:30:2, vol/vol/vol; (24)] for separation of neutral lipids. The chromarods were then dried at 110°C for 3 min and partially scanned to a point just after the MAG peak to reveal NL. The second development was carried out in acetone [100% (22)], chromarods were dried at 110°C for 3 min and then scanned to the lowest point after the acetone-mobile lipid peak. Final development of chromarods was carried out twice using chloroform/methanol/water [70:30:3, vol/vol/vol (25)] to separate polar lipids followed by drying at 110°C for 5 to 7 min. The chromarods were scanned completely to reveal PL and less mobile lipid components.

Peak areas of unknown compounds were calculated as wt% using conversion factors established with the calibration lines of the authentic standards for NL and PL. Quantification of glycolipids was not carried out. Each point on the calibration line was the mean value of 7 to 10 analyses.

Determination of lipase activity. Lipase or lipolytic activity of germinated flaxseeds was assessed according to the method described by Wanasundara (26) for microbial lipases.

Germinated flaxseeds were first washed with acetone (1:2, wt/vol) in a Büchner funnel. The washed seedlings were homogenized using a Polytron homogenizer with acetone (1:2, wt/vol) in a beaker. Residue was recovered by suction filtration and then washed three more times with acetone in order to remove any remaining lipids. Recovered residue was dried under vacuum (15 to 20 mbar) at 22°C for 24 to 36 h. Suspensions (50 mg/mL) of dried material so obtained were prepared in Tris-HCl buffer (pH 8.0, 50 mM) and mixed very well. These suspensions were centrifuged at $1000 \times g$ for 1 min, as particles interfere with pipetting, and supernatant was used for determining lipase activity.

Substrate-emulsion was freshly prepared by mixing 15 mL triolein (substrate) with 50 mL of the emulsifying mixture (17.9 g NaCl, 0.41 g KH_2PO_4, 400 mL demineralized water, 540 mL glycerol, and 6 g gum arabic mixed and diluted to 1 L with demineralized water) and 235 mL demineralized water in a 500-mL glass beaker using a Polytron homogenizer (1 min, 10,000 rpm).

A 20-mL aliquot of substrate-emulsion was transferred into the reaction vessel (4 cm diameter, 6.5 cm height) and preheated to 35°C for 3 to 4 min. The sample solution (1 mL of supernatant) was then added to the substrate-emulsion, and the reaction vessel was placed in the titration setup (Metrohm auto-titrator, Model SM, Titrino 702; Metrohm Ltd., Herisau, Switzerland). The pH of the mixture was then adjusted to 6.8–6.9 with 0.05 M NaOH in the autoburrette, and the pH-stat titration at 35°C was carried out while stirring at 200 rpm. The titration was stopped after 3 min of addition of alkali at a constant rate. The chart recorder speed used was 1 cm/min, and recorder amplitude was 1 cm/50 μL. Lipase activity was calculated as lipolytic activity units per gram dry matter (U/g).

Statistical analysis. All experiments carried out in this study were replicated three times. Mean values with standard deviations were reported when and where necessary. Analysis of variance (ANOVA) was performed and differences in mean values determined using Tukey's studentized test at $P < 0.05$ and employing ANOVA and TUKEY procedures of statistical analytical system, respectively (27).

RESULTS AND DISCUSSION

Under the conditions employed in this study, 95–98% germination of flaxseed was achieved. The content of dry matter decreased drastically as the seedlings grew to about 6.0 cm in length, and a 35% loss of dry matter was observed at the end of an 8-d germination period (Fig. 1). Most cotyledons were green at the termination stage of the germination experiment. During the initial stages of germination, seed reserves were utilized to support growth of new cells. The net loss of dry matter occurred as a result of oxidation and breakdown of the stored macromolecules (28), such as lipids and proteins of the seeds.

Lipids comprised 31.9 ± 1.0% of the soaked seeds (ungerminated). A quantitative decrease in the content of total lipids was observed as germination progressed (Fig. 1). At the end of the germination experiment, the lipid content of the seedlings was 8.6 ± 1.1% on a fresh weight basis. The content of FFA increased after d 2 of germination and on the d 8, FFA content was 2.2% of total lipids. However, this is a rather low value compared to the 10% increase reported by Zimmerman and Klostermann (29) for flaxseed.

Figure 2 shows quantitative changes of neutral NL, GL and PL of flaxseed during an 8-d germination period. At the beginning of germination (d 0), NL constituted 96.8% of the total lipids; their content was reduced significantly ($P < 0.05$) after d 4, and at the end of the experiment (d 8) reached 82.8%. In the fresh seedlings the content of NL changed from 30.7 ± 1.9 to 7.2 ± 3.0 g/100 g. The GL fraction showed a significant ($P < 0.05$) increase during germination and changed from 0.73 ± 0.13 to 1.17 ± 0.21 g/100 g seedlings (Fig. 2), equivalent to a change from 2.28 to 13.54% of total lipids. PL comprised the smallest fraction of flaxseed lipids prior to germination and throughout. At the termination of the experiment, PL content of fresh seedlings was 0.1 g/100 g. The ratio of simple (NL) to complex (GL + PL) lipids showed a considerable decrease, from 26.02 to 4.85 due to germination (Table 1). The decrease in simple lipids indicated their use as a respiratory substrate (30).

The lipase activity of germinated flaxseeds is given in Table 1. The water-imbibed seeds (d 0) had lipase activity of 160 U/g, which plateaued at d 2 of germination. Younis *et al.* (31) reported that lipolytic activity in flaxseeds increased con-

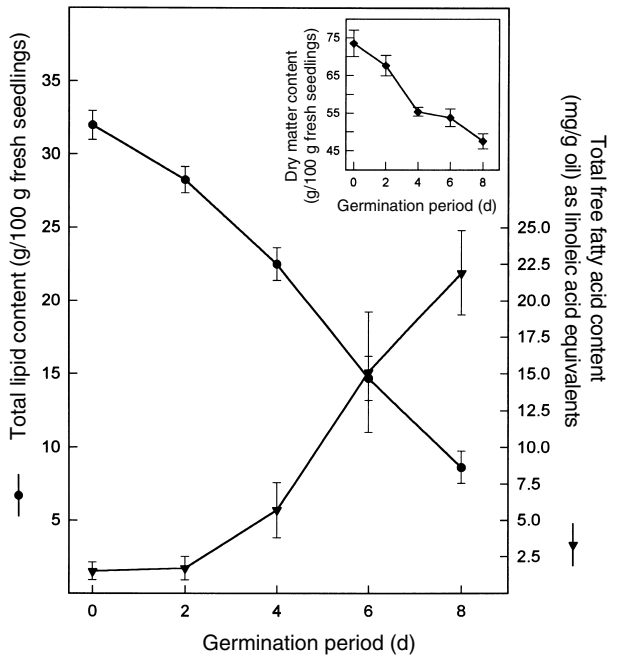

FIG. 1. Quantitative changes in total lipids and free fatty acids of flaxseeds during 8 d of germination. Inset: Changes of dry matter content during the same germination period.

siderably after d 2 and started to decline after d 6 of germination. The same study also indicated a very low lipase activity for flaxseeds when compared with other oleaginous seeds such as cottonseed (*Gossypium barbadense* L.) or castor (*Ricinus communis* L.). There are not many reports on lipolytic activity of germinating flaxseeds, however, Zimmerman and Vick (32) have reported the lipoxygenase activity of germinating flaxseeds.

The NL fraction of flaxseed lipids (Table 2) was composed of TAG, MAG, DAG, and FFA. Sterols (ST) were detected in minute quantities in some samples. TAG were the major component of flaxseed NL and their content decreased while that of other components showed minor fluctuation in the content during germination. The FFA content showed a similar trend of changing, as shown in Figure 1; however, lower numerical values were obtained using spectrophotometric quantitation when data were calculated on a fresh weight basis. It is also important to note that the reported changes in various lipid components of this study are for the whole seedling.

TABLE 1
Changes in the Lipase Activity and the Ratio of Simple to Complex Lipids of Flaxseed During Germination[a]

Germination period (d)	Lipase activity (U/g)	Simple/complex lipids[b]
0	160 ± 7	26.02
1	293 ± 5	n.d.
2	354 ± 6	19.00
3	343 ± 7	n.d.
4	340 ± 4	16.50
5	340 ± 3	n.d.
6	n.d.	9.20
7	n.d.	n.d.
8	n.d.	4.85

[a]Mean ± standard deviation (three replicates). Values are on dry weight basis. Abbreviations: U/g, units/gram dry matter; n.d., not determined.
[b]Simple lipids are neutral lipids and complex lipids are the sum of glyco- and phospholipids (values refers to Fig. 2).

PL constituted 1.4% of total lipids of flaxseed at the beginning of germination. TLC–FID separation of PL of flaxseed revealed that PC was the major PL present (Table 3). PS and PE eluted together, and their total content constituted the next largest fraction. The other components identified were PI and LPC, which were present in very small amounts (Table 3). PA and LPE were absent in the ungerminated (d 0) seeds but were detected in germinating seeds starting from d 2 and d 4, respectively. While the content of PC decreased during germination, the amount of LPC increased and that of PA increased drastically from a negligible level to 46% of the total PL fraction after 8 d of germination. The content of PI was also increased as germination proceeded.

Linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids were the predominant fatty acids of flaxseed lipids and constituted 80% of the total lipids (Table 4). Changes in fatty acid composition of total and NL (Table 5) followed a similar pattern. Approximately 60% of the fatty acids in the NL fraction was composed of linolenic acid, while the content of linoleic acid was approximately 13.5%. Oleic ($C_{18:0}$) acid was the predominant monounsaturated fatty acid (MUFA), and palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) were the main saturated fatty acids (SFA) present. The fatty acids $C_{14:0}$, $C_{20:0}$, $C_{24:0}$, $C_{16:1}$, $C_{20:1}$, $C_{22:1}$, and $C_{20:5}$, which were not detected in the NL fraction of ungerminated seeds, began to appear during the course of germination (Table 5).

PUFA constituted a major portion of fatty acids (67%) of flaxseed PL fraction and exhibited little change during germination (Fig. 3). Linolenic acid was the predominant fatty acid in the PL, and similar to other lipid fractions of flaxseed, its content was increased during germination. The only other PUFA found in PL fraction of flax was linoleic acid, the content of which was decreased during germination. Oleic acid, which was the predominant MUFA of PL, showed a decrease and then an increase during the germination period.

Linolenic, oleic, and palmitic acids were the dominant PUFA, MUFA, and SFA of the GL fraction of flax lipids, respectively. The contribution of linoleic acid to the total fatty acids of GL was comparatively less than that to NL or PL, and its content nearly doubled after 8 d of germination (Table 5). The content of SFA ($C_{14:0}$–$C_{24:0}$) of GL was higher (Table 5), and that of the total PUFA was lower, compared to NL or PL fractions. The MUFA present in the GL fraction on d 0 of germination were $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, $C_{20:1}$, and $C_{22:1}$, but their content decreased as a result of germination.

Figure 3 provides the total contents of PUFA, MUFA, and SFA of total lipid, NL, GL, and PL fractions of germinating

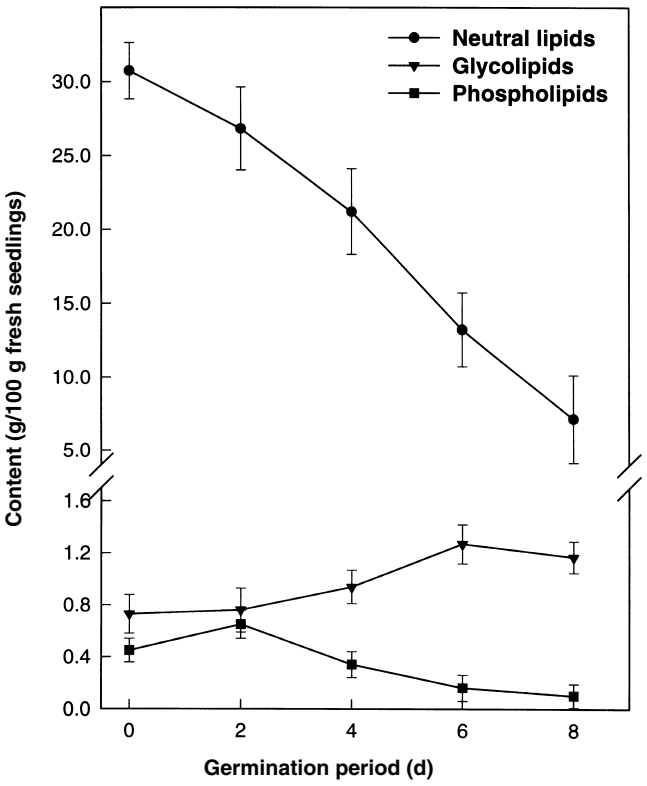

FIG. 2. Changes in the contents of neutral, glyco-, and phospholipids of the germinating flaxseeds.

flaxseeds. The GL fraction contained lower amounts of PUFA, but higher proportions of MUFA and SFA, than NL or PL. The total content of PUFA of NL did not decrease; however, there was a net decrease in total content of MUFA and an increase in the total content of SFA during the germination period. Meanwhile, the proportions of PUFA, MUFA, and SFA in the neutral lipids were similar to those of the total lipids. The total content of PUFA of the PL fraction first increased and then decreased, but the MUFA and SFA followed a decreasing and an increasing pattern, respectively, at the same time.

The initial step in lipid metabolism is the release of fatty acids from the reserve TAG, which is accomplished *via* hydrolysis due to increased lipolytic activity (30,33). The released fatty acids undergo β-oxidation (oxidation at the β position to the carboxyl group and sequential removal of carbon units) to produce the required energy (34) in the form of adenosine triphosphate (ATP). As the major energy reserve in flaxseed, lipids provide fatty acids that serve as an energy source to produce ATP and soluble carbohydrates for the growth of new cells during germination (35). Degradation of plant storage lipids is in the sequence of TAG → DAG → MAG → FFA (4). Decreased levels of TAG indicate that they are the major compounds involved in catabolism to provide substrate for oxidation during germination of flaxseed, as previously reported by Zimmerman (35). True plant lipases hydrolyze TAG as well as DAG and MAG; however, enzymes such as esterases and hydrolases degrade only DAG and MAG (33). The accumulation of DAG and/or MAG indicates the presence of various concentrations and/or types of hydrolytic enzymes in different tissues of seedlings (e.g., cotyledons, roots, etc.). Since in this study the whole seedlings were considered, a net increase in the content of DAG and MAG was observed (until d 6, Table 2). The increase in FFA content also suggests possible biosynthesis of fatty acids during the later stages of germination, similar to that observed for soybean and alfalfa (4). The content of glycolipids of flax increased significantly ($P < 0.05$) during seed germination (Fig. 2). Huang and Grunwald (4) suggested that glycolipids are important components of photosynthetic membranes and their increase during germination reflects chloroplast development and tissue greening. Occurrence of small amounts of minor fatty acids such as $C_{14:0}$, $C_{16:1}$, $C_{24:0}$, $C_{20:1}$, and $C_{22:1}$, especially in the NL fraction, was probably due to the fact

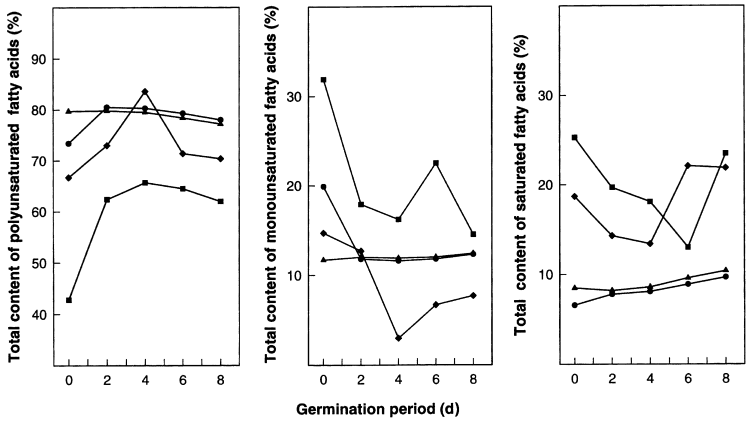

FIG. 3. Changing pattern of the total contents of polyunsaturated, monounsaturated and saturated fatty acids of total, neutral, glyco-, and phospholipid fractions of flaxseed during germination; ●, neutral lipids; ■, glycolipids; ◆, phospholipids; ▲, total lipids.

TABLE 2
Neutral Lipids of Flaxseed and Their Changes During Germination[a]

	Germination period (d)				
Neutral lipids	0	2	4	6	8
Monoacylglycerol	0.34 ± 0.02	0.64 ± 0.03	0.29 ± 0.03	0.83 ± 0.07	0.34 ± 0.04
Diacylglycerol	0.64 ± 0.03	1.03 ± 0.04	0.89 ± 0.02	0.69 ± 0.06	0.39 ± 0.04
Triacylglycerol	29.6 ± 0.63	23.7 ± 0.46	18.2 ± 0.42	10.4 ± 0.73	5.72 ± 0.40
Free fatty acids	0.42 ± 0.05	1.37 ± 0.04	1.33 ± 0.10	1.35 ± 0.04	0.71 ± 0.07
Sterols	Trace	n.d.	Trace	Trace	Trace

[a]As g/100 g fresh seedlings. Mean ± SD of three replicates. See Table 1 for abbreviation.

TABLE 3
Changes in the Contents of Phospholipids of Flaxseed During Germination[a]

	Germination period (d)				
Phospholipid	0	2	4	6	8
Phosphatidic acid	n.d.	132 ± 11	130 ± 31	65 ± 3	13 ± 2
Phosphatidylcholine	155 ± 20	348 ± 50	50 ± 16	27 ± 1	2 ± 1
Phosphatidylinositol	2 ± 1	5 ± 1	44 ± 2	9 ± 1	1 ± 1
Phosphatidylserine + phosphatidylethanolamine	220 ± 25	73 ± 10	59 ± 16	23 ± 1	2 ± 1
Lysophosphatidylcholine	67 ± 15	94 ± 2	52 ± 1	21 ± 9	7 ± 1
Lysophosphatidylethanolamine	n.d.	n.d.	2 ± 1	15 ± 8	1 ± 1

[a]As mg/100 g fresh seedlings. Mean ± SD of three replicates. See Table 1 for abbreviation.

TABLE 4
Fatty Acid Composition of Total Lipids of Flaxseed During Germination[a]

	Germination period (d)				
Fatty acid	0	2	4	6	8
$C_{14:0}$	0.6 ± 0.1	0.4 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
$C_{16:0}$	5.2 ± 0.5	5.1 ± 0.4	5.0 ± 0.3	5.5 ± 0.3	6.1 ± 0.3
$C_{18:0}$	2.7 ± 0.3	2.7 ± 0.4	2.8 ± 0.4	3.0 ± 0.3	3.1 ± 0.4
$C_{20:0}$	Trace	Trace	Trace	0.3 ± 0.1	0.3 ± 0.1
$C_{22:0}$	Trace	Trace	Trace	0.3 ± 0.1	0.2 ± 0.1
$C_{24:0}$	Trace	Trace	Trace	Trace	Trace
$C_{14:1}$	Trace	Trace	Trace	Trace	Trace
$C_{16:1}$	Trace	0.1 ± 0.1	0.2 ± 0.0	Trace	0.3 ± 0.1
$C_{18:1}$	11.7 ± 0.5	11.9 ± 0.3	11.7 ± 0.4	12.0 ± 0.3	12.1 ± 0.3
$C_{20:1}$	Trace	Trace	Trace	Trace	Trace
$C_{22:1}$	Trace	Trace	Trace	Trace	Trace
$C_{18:2}$	16.1 ± 0.9	16.1 ± 0.8	16.6 ± 0.8	18.0 ± 0.5	18.5 ± 0.3
$C_{18:3}$	63.6 ± 1.0	63.7 ± 0.9	62.8 ± 0.5	59.9 ± 0.7	58.1 ± 0.7
$C_{20:5}$	Trace	Trace	0.1 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
Total saturated fatty acids	8.5	8.2	8.6	9.6	10.3
Total monounsaturated fatty acids	11.7	12.0	11.9	12.0	12.4
Total polyunsaturated fatty acids	79.7	79.8	79.5	78.4	77.2

[a]As area percentage. Mean ± SD of three replicates.

TAG, which are the main component of NL, were in a dynamic state and were continuously hydrolyzed and resynthesized. Alternatively, catabolism and anabolism of TAG may occur simultaneously in two separate tissues within the seedling, e.g., oxidation in cotyledons and synthesis in the hypocotyl (root). In this study no odd carbon number fatty acids were observed, as reported by Zimmerman and Klostermann (29). SFA, MUFA, and PUFA with 20 or more carbon atoms appeared during the latter part of germination, indicating possible chain elongation and desaturation activities in the NL fraction. At the end of the germination period, flax seedlings contained 58% less lipid than the amounts present

TABLE 5
Fatty Acid Composition of Neutral, Glyco-, and Phospholipids of Flaxseed During Germination[a]

	Neutral lipids			Glycolipids			Phospholipids		
Fatty acid	0	4	8	0	4	8	0	4	8
$C_{14:0}$	0.0	0.06 (0.00)	0.09 (0.00)	7.48 (0.10)	1.71 (0.10)	3.21 (0.20)	1.82 (0.04)	0.47 (0.01)	0.55 (0.00)
$C_{16:0}$	4.35 (0.03)	4.90 (0.05)	5.52 (0.04)	12.27 (0.20)	10.71 (0.90)	13.36 (0.98)	11.78 (0.10)	10.13 (0.50)	15.21 (0.90)
$C_{18:0}$	2.29 (0.04)	2.71 (0.02)	3.02 (0.00)	4.32 (0.04)	3.55 (0.20)	3.97 (0.09)	3.71 (0.04)	2.83 (0.08)	4.89 (0.10)
$C_{20:0}$	0.0	0.14 (0.00)	0.28 (0.01)	0.73 (0.00)	0.0	0.36 (0.00)	0.69 (0.00)	0.0	0.0
$C_{22:0}$	0.0	0.0	0.0	0.0	0.0	1.74 (0.02)	0.68 (0.00)	0.0	1.23 (0.09)
$C_{24:0}$	0.0	0.33 (0.00)	0.78 (0.02)	0.0	2.09 (0.10)	0.94 (0.00)	0.0	0.0	0.0
$C_{14:1}$	9.96 (0.10)	0.0	0.0	4.97 (0.20)	0.0	2.50 (0.20)	0.0	0.0	0.23 (0.00)
$C_{16:1}$	0.0	0.06 (0.00)	0.09 (0.00)	8.81 (0.39)	0.57 (0.03)	1.25 (0.01)	0.20 (0.00)	0.0	1.42 (0.07)
$C_{18:1}$	9.96 (0.11)	11.40 (0.10)	12.00 (0.10)	13.82 (0.98)	12.54 (1.29)	10.76 (0.45)	13.76 (0.69)	2.99 (0.09)	5.48 (0.50)
$C_{20:1}$	0.0	0.15 (0.00)	0.16 (0.02)	0.77 (0.00)	0.0	0.0	0.0	0.0	0.0
$C_{22:1}$	0.0	0.0	0.04 (0.00)	3.52 (0.00)	2.92 (0.10)	0.0	0.69 (0.05)	0.0	0.59 (0.01)
$C_{18:2}$	13.34 (0.12)	16.07 (1.00)	17.83 (0.17)	13.34 (0.89)	17.55 (1.09)	13.62 (0.56)	29.86 (0.90)	10.37 (0.89)	15.62 (1.00)
$C_{18:3}$	59.59 (0.59)	64.07 (1.19)	59.85 (1.10)	29.18 (1.00)	47.08 (1.25)	48.39 (1.08)	36.80 (0.89)	73.21 (2.10)	54.82 (1.50)
$C_{20:5}$	0.0	0.12 (0.01)	0.39 (0.00)	0.28 (0.00)	1.08 (0.30)	0.0	0.0	0.0	0.0

[a]As area percentage. Mean (SD) of three replicates.

in the original seeds (approximately 18%), on a dry weight basis. Flax lipids after 8 d of germination were rich in PUFA (77%) and MUFA (12%), and contained only 10% of SFA.

REFERENCES

1. Harwood, J.L., and P.K. Stumpf, Fat Metabolism in Higher Plants, XL. Synthesis of Fatty Acids in the Initial Stage of Seed Germination, *Plant Physiol. 46*:500–508 (1970).
2. Applequist, L.Å., Biochemical and Structural Aspects of Storage and Membrane Lipids in Developing Oilseeds, in *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*, edited by T. Galliard and E.I. Mercer, Academic Press, London, 1975, pp. 247–286.
3. Huang, A.H.C., Oil Bodies and Oleosins in Seeds, *Annu. Rev. Plant Physiol. 43*:177–200 (1992).
4. Huang, L.-S., and C. Grunwald, Lipid and Fatty Acid Changes During Germination of Alfalfa Seeds, *Phytochem. 29*:1441–1445 (1990).
5. Bhatty, R.S., Nutrient Composition of Whole Flaxseed and Flaxseed Meal, in *Flaxseed in Human Nutrition*, edited by S.C. Cunnane and L.U. Thompson, AOCS Press, Champaign, 1995, pp. 22–42.
6. Cunnane, S.C., Metabolism and Function of α-Linolenic Acid in Humans, *Ibid.,* pp. 99–127.
7. Houlab, B.J., Platelet Composition and Function, *Ibid.*, pp. 128–137.
8. Kelly, D.S., Immunomodulatory Effects of Flaxseed and Other Oils Rich in α-Linolenic Acid, *Ibid.*, pp. 145–156.
9. Sim, J.S., and G.H. Qi, Designing Poultry Foods Using Flaxseed, *Ibid.*, pp. 315–333.
10. Kennely, J.J., Incorporation of Flaxseed Fatty Acids into Cow's Milk, *Ibid.*, pp. 334–347.
11. Romans, J.R., Flaxseed and the Composition and Quality of Pork, *Ibid.*, pp. 348–362.
12. Dick, T.A., and X. Yang, Flaxseed in Arctic Charr and Rainbow Trout Nutrition, *Ibid.*, pp. 295–314.
13. Wanasundara, P.K.J.P.D., R. Amarowicz, M.T. Kara, and F. Shahidi, Removal of Cyanogenic Glycosides of Flaxseed, *Food Chem. 48*:263–266 (1993).
14. Wanasundara, P.K.J.P.D., and F. Shahidi, Alkanol-Ammonia-Water/Hexane Extraction of Flaxseed, *Ibid. 49*:39–44 (1994).
15. Wanasundara, P.K.J.P.D., and F. Shahidi, Functional Properties and Amino Acid Composition of Solvent-Extracted Flaxseed Meal, *Ibid. 49*:45–51 (1994).
16. Wanasundara, P.K.J.P.D., and F. Shahidi, Optimization of Hexametaphosphate-Assisted Extraction of Flaxseed Proteins

Using Response Surface Methodology, *J. Food Sci. 61*:604–607 (1996).
17. Wanasundara, P.K.J.P.D., and F. Shahidi, Removal of Flaxseed Mucilage by Chemical and Enzymatic Treatments, *Food Chem. 59*:47–55 (1997).
18. Wanasundara, P.K.J.P.D. and F. Shahidi, Functional Properties of Acylated Flaxseed Protein Isolates, *J. Agric. Food Chem. 45*:2431–2441 (1997).
19. Wanasundara, P.K.J.P.D., F. Shahidi, and M.E. Brosnan, Changes in Nitrogenous Compounds of Flax (*Linum usitatissimum* L.) Seed During Germination, *Food Chem.* (1999), in press.
20. Folch, J., M. Lees, and G.H.S. Tanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1957).
21. Lowry, R.R., and I.J. Tinsley, Rapid Colorimetric Determination of Free Fatty Acids, *J. Am. Oil Chem. Soc. 53*:470–472 (1975).
22. Christie, W.W., Analysis of Complex Lipid, in *Lipid Analysis*, 2nd edn., Pergamon Press, New York, 1982, pp. 107–134.
23. Wanasundara, U.N., and F. Shahidi, Stabilization of Seal Blubber and Menhaden Oils with Green Tea Catechins, *J. Am. Oil Chem. Soc. 73*:1183–1190 (1996).
24. Fraser, A.J., D.R. Tocher, and J.R. Sargent, Thin-Layer Chromatography–Flame Ionization Detection and the Quantitation of Marine Neutral Lipids and Phospholipids, *J. Exp. Mar. Biol. Ecol. 88*:91–99 (1985).
25. Parrish, C.C., Separation of Aquatic Lipid Classes by Chromarod Thin-Layer Chromatography with Measurement by Iatroscan Flame-Ionization Detection, *Can. J. Fish Aquat. Sci. 44*:722–731 (1987).
26. Wanasundara, U.N., Marine Oils: Characterization, Stabilization and Omega-3 Fatty Acid Concentration, Ph.D. Thesis, Memorial University of Newfoundland, 1996, pp. 109–111.
27. *Statistical Analysis System User's Guide,* Version 6, SAS Institute Inc., Cary, North Carolina, 1990, Vols. 1,2.
28. Chavan, J.K., S.S. Kadam, and D.K. Salunkhe, Changes in Tannins, Free Amino Acids, Reducing Sugars, and Starch During Seed Germination of Low- and High-Tannin Cultivars of Sorghum, *J. Food Sci. 46*:638–641 (1981).
29. Zimmerman, D.C., and H.J. Klostermann, Lipid Metabolism in Germinated Flax Seeds, *J. Am. Oil Chem. Soc. 42*:58–62 (1965).
30. Huang, A.H.C., The Biochemistry of Plants, edited by P.K. Stumpf and E.E. Conn, Academic Press, New York, 1987, Vol. 9, pp. 97–116.
31. Younis, M.E., M.N.A. Hasaneen, and M.M. Nemet-Alla, Plant Growth, Metabolism and Adaptation in Relation to Stress Conditions IV. Effects of Salinity on Certain Factors Associated with the Germination of Three Different Seeds High in Fats, *Annals Botany 60*:337–344 (1987).
32. Zimmerman, D.C., and B.A. Vick, Hydroperoxide Isomerase: A New Enzyme of Lipid Metabolism, *Plant Physiol. 46*:445–453 (1970).
33. Gilliard, T., Degradation of Acyl Lipids: Hydrolytic and Oxidative Enzymes. Lipid Storage Functions, in *The Biochemistry of Plants*, edited by P.K. Stumpf, Academic Press, New York, 1985, Vol. 4, pp. 85–116.
34. Kindl, I.H., β-Oxidation of Fatty Acids by Specific Organelles, *Ibid.*, 1987, Vol. 9, pp. 31–53.
35. Zimmerman, D.C., Metabolism of Fatty Acids During Seed Germination, *Proceedings of the Flax Institute US. 41*:19–20 (1971).

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