

# Lipid Components of Borage (*Borago officinalis* L.) Seeds and Their Changes During Germination

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**ABSTRACT:** The changes in composition of total and neutral lipids (NL) as well as glycolipids (GL) and phospholipids (PL) of borage (*Borago officinalis* L.) seeds, germinated in the dark at 25°C for 10 d, were studied. Total lipids constituted 34.0% of the dry matter of borage seeds. During germination, the content of total lipids was decreased by 95%. NL accounted for 95.7% of total lipids prior to germination and were composed of triacylglycerols (TAG; 99.1%), diacylglycerols (DAG; 0.06%), monoacylglycerols (MAG; 0.02%), free fatty acids (FFA; 0.91%), and sterols (0.02%). The content of TAG was significantly ( $P \leq 0.05$ ) decreased, while that of other components, such as MAG and FFA, significantly ( $P \leq 0.05$ ) increased during germination. However, the content of DAG did not change. GL and PL accounted for 2.0 and 2.3% of total lipids, respectively, and their contents significantly ( $P \leq 0.05$ ) increased as germination proceeded. The thin layer chromatography–flame-ionization detection studies showed that phosphatidylcholine (PC; 69.7%) was the major PL present. The total content of phosphatidylserine (PS) and phosphatidylethanolamine (PE), which were coeluted, was 18.2%; phosphatidic acid (PA) was present at 11.2% of the total PL fraction. Lysophosphatidylcholine was detected at 0.9%. The proportion of PC, PS, and PE significantly ( $P \leq 0.05$ ) decreased during germination, but that of PA increased ( $P \leq 0.05$ ) markedly. The fatty acid composition of lipid fractions changed as germination proceeded. The predominant fatty acids of total lipids, NL, and GL were linoleic and linolenic acids, while those of PL were linoleic and palmitic acids. The present study demonstrated that the overall changes of lipids seen in borage seeds during germination agree well with results for other oilseeds. Changes in lipid compositions during germination result from the formation of tissues and metabolic interconversion of lipid classes. Rapid changes in lipid composition during seed germination may enhance the nutritional value of the sprouts.

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**KEY WORDS:** Borage oil, glycolipids,  $\gamma$ -linolenic acid, neutral lipids, phospholipids.

Borage (*Borago officinalis* L.) is commonly found in southern Europe, in North Africa, and in the wastelands of many temperate areas. It is mainly cultivated for its seeds, which contain 30 to 33% of an oil that is the most concentrated natural source of  $\gamma$ -linolenic acid (GLA; 18:3n-6) (1,2).

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Borage oil is frequently used in nutritional and clinical supplements where impaired or inadequate  $\Delta$ -6 desaturase activity may be involved in the initiation and progression of several diseases. This impairment may be alleviated by dietary supplementation with GLA (3). GLA is the  $\Delta$ 6-desaturation product of linoleic acid (18:2n-6) in the metabolic pathway of essential n-6 fatty acids (4). The physical and chemical properties, as well as the nutritional and medicinal importance of GLA, have recently been reviewed (4,5).

Lipids are important components of seeds and can be classified as storage and structural constituents. During germination, storage lipids are metabolized to supply the required energy for this process, and thus their content decreases with a concurrent increase in the content of structural lipids due to membrane formation (6–8). Thus, in studies involving the analysis of plant tissues, any change in lipid content and composition may reflect the occurrence of one or both of these processes. In oil-rich seeds, where lipids provide an important source of energy, a decrease in triacylglycerols (TAG) and an increase in the total amount of free fatty acids (FFA) is noticed (9–12). This indicates that lipase action is not usually limiting in the degradation process. Lipase activity increases sharply during the early stages of germination and results in stepwise hydrolysis of TAG to diacylglycerols (DAG), monoacylglycerols (MAG), and finally free glycerol and FFA (13). The net formation of membranes paralleled an increase in the relative contents of glycolipids (GL) and phospholipids (PL) of borage seeds during germination. Such changes are easily seen in lipid-poor seeds, such as peas (14), but are also visible in lipid-rich seeds, such as soybeans (15,16). In particular, large changes in the PL composition of seeds are often seen, presumably due to membrane formation. The major GL are the monogalactosyl DAG and digalactosyl DAG, and these are found universally in higher plant tissues (17). Linolenic acid is the chief fatty acid associated with the DAG moiety (8). GL exist in large amounts in the chloroplast; they are, however, not exclusive to chloroplasts (17).

Changes in storage and structural lipids, as well as changes in their fatty acid composition, that accompany seed germination have been studied in a number of plant species (8,18,19). In this paper, lipid composition of germinating borage seeds and changes in their MAG, DAG, and TAG, FFA, GL, PL, and fatty acid compositions are reported.

## MATERIALS AND METHODS

**Reagents.** Standards of neutral lipids (NL) (triolein, diolein, monoolein, and oleic acid) and PL [phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), lysophosphatidylcholine (LPC), and lysophosphatidylethanolamine (LPE)] were purchased from Sigma (St. Louis, MO). In each instance, purity checks revealed a single peak on the thin-layer chromatography–flame-ionization detection (TLC–FID) chromatogram. Silicic acid (100 mesh) for column chromatography was also obtained from Sigma. All other chemicals and organic solvents used were of analytical grade.

**Germination of borage seeds.** Seeds of borage were obtained from Bioriginal Food & Science Corp. (Saskatoon, Canada). The seeds were surface-sterilized with 8-hydroxyquinoline sulfate (0.3%) for 10 min and rinsed with deionized water. The seeds were then soaked in water overnight at room temperature and allowed to germinate on top of moist paper towels at room temperature in the dark. In germination studies, seeds were selected for uniformity before use and for length of hypocotyl after germination. Samples of seedlings were withdrawn on days 0, 2, 4, 6, 8, and 10 for further analyses; day 0 was designated for seeds after pretreatment.

Moisture content of the seedlings was determined by drying in a forced-air oven at  $104 \pm 1^\circ\text{C}$  for 18 h until a constant weight was reached. Moisture content was calculated as percentage weight loss (moisture) of the samples after drying (20).

**Extraction and quantification of total lipids.** Total lipids of borage seeds were extracted according to the method of Folch *et al.* (21). The weight of the extracted oil was determined by difference, and lipid content (% dry weight basis) of samples was then calculated.

**Separation of major lipid classes by column chromatography.** The total lipid fraction was further fractionated as described by 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). The NL fraction was first eluted with chloroform (10 times column volume). Monogalactosyl DAG and digalactosyl DAG were then eluted with chloroform/acetone (50:50, vol/vol, 8 times column bed volume) and acetone (10 times column bed volume), respectively. Finally, the PL fraction was eluted with methanol (10 times column bed volume). The solvents were removed from each fraction under vacuum using a rotary evaporator at  $40^\circ\text{C}$ . All fractions were weighed and their weight percentages calculated.

**FFA content.** The FFA content of the lipid fractions was determined colorimetrically according to Kwon and Rhee (23). The total content of FFA in the samples was determined as oleic acid equivalents using a standard curve.

**TLC–FID.** The lipid fractions obtained from column chromatography were chromatographed separately on Chromarod S-III and then analyzed on an Iatroscan MK-5 (Iatron Laboratories Inc., Tokyo, Japan) analyzer equipped with a FID connected

to a computer loaded with T-dascan software (Scientific Products and Equipment, Concord, Ontario, Canada) for data handling. The FID was operated using a hydrogen flow rate of 160 mL/min and an air flow rate of 2,000 mL/min. The scanning speed of rods was 30 s/rod.

**Calibration of chromarods.** A composite stock solution of NL [FFA (oleic acid), MAG (monoolein), DAG (diolein), TAG (triolein), and PL (PC, PE, LPC, LPE, PA, PI, and PS)] were dissolved in chloroform/methanol (2:1, vol/vol) and stored under nitrogen at  $-20^\circ\text{C}$ . Different dilutions of the stock solution, ranging from 0.1 to 10  $\mu\text{g}/\mu\text{L}$  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 blow dryer. The rods containing samples were placed in a humidity tank over saturated calcium chloride for 10 min and then immediately transferred to the developing tank.

**Chromarod development.** Total lipids and lipid fractions obtained from borage were dissolved in chloroform/methanol (2:1, vol/vol) in order to obtain a concentration of 1  $\mu\text{g}$  lipid/ $\mu\text{L}$ . The sample (1  $\mu\text{L}/\text{rod}$ ) was applied on 9 out of 10 rods and a randomly selected rod was used for the standard mixture.

Three different solvent systems were used to obtain three chromatograms per rod. The first development of rods was carried out for 45 min in benzene/chloroform/acetic acid (70:30:4, vol/vol/vol) (24). The chromarods were then dried at  $110^\circ\text{C}$  for 3 min and partially scanned to a point just beyond the MAG peak to reveal NL. The chromarods were then developed for 30 min in 100% acetone (25), dried at  $110^\circ\text{C}$  for 3 min, and then scanned partially to the lowest point beyond the acetone–mobile lipid peak. Finally, chromarods were developed twice for 40 min in chloroform/methanol/water (70:30:3, vol/vol/vol) (26), dried at  $110^\circ\text{C}$  for 5–7 min, and scanned completely to reveal PL and less mobile lipid components. Upon completion of the three-stage development process and the drying of the rods, the rods were scanned in the Iatroscan TLC–FID analyzer. This procedure was repeated three times for each sample.

Peak areas of unknown compounds were calculated as weight percentages using conversion factors established with the calibration lines of the authentic standards for NL and PL. Each point on the calibration line was the mean value of six analyses.

**Fatty acid composition of lipids.** The fatty acid composition of total lipids and lipid fractions was analyzed by gas chromatography as described elsewhere by Wanasundara and Shahidi (27).

**Statistical analysis.** One-way analysis of variance (ANOVA) and Tukey's studentized range test (28) were used to determine differences in mean values based on data collected from three replications of each measurement. ANOVA was performed using the Statistical Analysis System (29). Significance was established at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

Lipids constituted 34.0% of the dry matter of borage seeds and provided a major portion of the energy required for the growth of seedlings. Quantitative changes in the content of total lipids, NL, GL, and PL, as well as FFA of borage occurred during a 10-d germination period (Table 1). A highly significant ( $P \leq 0.05$ ) decrease was observed in the total content of lipids after 6 d of germination. Lipid contents were lowered by 14 and 87% on days 2 and 8 of germination, respectively. A significant ( $P \leq 0.05$ ) decrease was observed in the content of the NL fraction (Table 1). At the beginning of germination (day 0), GL constituted 2.0% of the total lipids; their content increased up to 7.3% at the termination of the experiment (day 10). The PL fraction also showed a significant ( $P \leq 0.05$ ) increase during germination and changed from 2.3 to 8.6% of total lipids. The content of FFA increased by sixfold until day 8 of germination (Table 1) and may reflect fatty acid biosynthesis during the latter stages of germination.

The primary stage in lipid metabolism is the release of fatty acids from reserve TAG accomplished *via* hydrolysis under the action of lipase (10,30–35). The released fatty acids undergo  $\beta$ -oxidation to produce the required energy (36,37) in the form of ATP. As the major energy reserve in the seed, lipids provide fatty acids that serve as a source of energy to produce ATP and soluble carbohydrates for the growth of new cells during germination (38).

NL account for nearly 96% of total lipids of borage seeds

prior to germination (Table 1). The NL fraction isolated from borage seeds was composed of TAG (99.1%), DAG (0.06%), MAG (0.02%), FFA (0.9%), and sterols (0.02%) (Table 2). The TAG that provide reserve fuel for the process of germination may be stored in cotyledons, endosperm, or both, depending on the species (39). The content of TAG decreased while MAG and FFA increased during germination (Table 2). However, the DAG content did not increase significantly ( $P < 0.05$ ). During 10 d of germination, the content of TAG decreased by approximately 13%. A similar decrease in TAG was observed for other plant species (6–8,10,14,20), and reflects the increase in lipase activity during seed germination (30,40). The decreased level of TAG indicates that they are the major seed components involved in catabolism which provide the substrate for oxidation during germination (38). Degradation of plant storage lipids follows the sequence: TAG  $\rightarrow$  DAG  $\rightarrow$  MAG  $\rightarrow$  FFA (20,41). Plant lipases not only hydrolyze TAG, but also DAG and MAG; however, enzymes such as esterases and hydrolases degrade DAG and MAG, but not TAG (31).

Linoleic acid (18:2n-6) was the major fatty acid of total lipids of borage seeds. Changes in the fatty acid composition of total and NL (Tables 3 and 4, respectively) followed a similar pattern. Linoleic acid constituted approximately 37% of the fatty acids in the total lipid and NL fractions, whereas GLA was approximately 21%. The percentage of linoleic acid decreased, while that of  $\alpha$ -linolenic acid (18:3n-3) increased slightly during germination.

**TABLE 1**  
Changes in the Total Lipid Content, Free Fatty Acids, and Major Lipid Classes of Borage Seeds During Germination<sup>a</sup>

Germination period (d)	Total lipid content (%)	FFA content (%) <sup>b</sup>	Major lipid classes (% of total)		
			NL	GL	PL
0	34.0 $\pm$ 1.5 <sup>d</sup>	2.3 $\pm$ 1.4 <sup>a</sup>	95.7 $\pm$ 1.2 <sup>c</sup>	2.0 $\pm$ 1.5 <sup>a</sup>	2.3 $\pm$ 1.7 <sup>a</sup>
2	29.2 $\pm$ 2.1 <sup>c</sup>	4.9 $\pm$ 2.1 <sup>b</sup>	92.0 $\pm$ 2.8 <sup>b,c</sup>	4.1 $\pm$ 1.2 <sup>a,b</sup>	3.9 $\pm$ 1.0 <sup>a,b</sup>
4	26.6 $\pm$ 2.2 <sup>b,c</sup>	5.0 $\pm$ 1.0 <sup>b</sup>	91.2 $\pm$ 1.5 <sup>b,c</sup>	3.5 $\pm$ 1.5 <sup>a,b</sup>	5.3 $\pm$ 1.2 <sup>a,b,c</sup>
6	23.8 $\pm$ 1.4 <sup>b</sup>	7.5 $\pm$ 0.9 <sup>c</sup>	88.1 $\pm$ 1.2 <sup>a,b</sup>	4.6 $\pm$ 1.7 <sup>a,b</sup>	7.3 $\pm$ 1.7 <sup>b,c</sup>
8	3.2 $\pm$ 1.0 <sup>a</sup>	12.9 $\pm$ 2.2 <sup>e</sup>	89.2 $\pm$ 2.0 <sup>a,b</sup>	5.7 $\pm$ 1.6 <sup>a,b</sup>	5.1 $\pm$ 1.8 <sup>a,b,c</sup>
10	1.7 $\pm$ 0.9 <sup>a</sup>	8.9 $\pm$ 2.0 <sup>d</sup>	84.1 $\pm$ 2.5 <sup>a</sup>	7.3 $\pm$ 1.0 <sup>b</sup>	8.6 $\pm$ 2.3 <sup>c</sup>

<sup>a</sup>Mean  $\pm$  SD ( $n = 3$  germination experiments). Means within a column with different roman superscript letters are significantly different  $P \leq 0.05$ . Values are on dry weight basis. FFA, free fatty acid; GL, glycolipids; NL, neutral lipids; PL, phospholipids.

<sup>b</sup>As oleic acid equivalents.

**TABLE 2**  
Neutral Lipids of Borage Seeds and Their Changes During Germination<sup>a</sup>

NL	Germination period (d)					
	0	2	4	6	8	10
Triacylglycerols (TAG)	99.1 $\pm$ 0.5 <sup>b</sup>	97.1 $\pm$ 2.6 <sup>b</sup>	95.0 $\pm$ 1.5 <sup>b</sup>	87.3 $\pm$ 1.2 <sup>a</sup>	86.2 $\pm$ 2.6 <sup>a</sup>	86.0 $\pm$ 1.5 <sup>a</sup>
Diacylglycerols (DAG)	0.06 $\pm$ 0.1 <sup>a</sup>	1.6 $\pm$ 2.2 <sup>a</sup>	1.2 $\pm$ 1.0 <sup>a</sup>	1.0 $\pm$ 1.0 <sup>a</sup>	0.5 $\pm$ 0.4 <sup>a</sup>	0.24 $\pm$ 1.0 <sup>a</sup>
Monoacylglycerols (MAG)	0.02 $\pm$ 0.1 <sup>a</sup>	0.4 $\pm$ 0.5 <sup>a</sup>	0.3 $\pm$ 0.6 <sup>a</sup>	2.6 $\pm$ 0.7 <sup>b</sup>	3.53 $\pm$ 0.7 <sup>b</sup>	8.9 $\pm$ 0.5 <sup>c</sup>
Free fatty acids (FFA)	0.91 $\pm$ 0.8 <sup>a</sup>	0.84 $\pm$ 0.9 <sup>a</sup>	3.4 $\pm$ 1.0 <sup>a,b</sup>	9.1 $\pm$ 0.8 <sup>c</sup>	9.47 $\pm$ 0.4 <sup>c</sup>	4.78 $\pm$ 1.5 <sup>b</sup>
Sterols (ST)	0.02 $\pm$ 0.1 <sup>a</sup>	0.06 $\pm$ 0.7 <sup>a</sup>	0.1 $\pm$ 0.3 <sup>a</sup>	ND	0.3 $\pm$ 0.2 <sup>a</sup>	0.08 $\pm$ 0.6 <sup>a</sup>

<sup>a</sup>As a weight percentage of total NL. Mean  $\pm$  SD of triplicate determinations. Means within a row with different roman superscript letters are significantly different ( $P \leq 0.05$ ). ND, not detected; for other abbreviation see Table 1.

**TABLE 3**  
Fatty Acid Composition of Total Lipids of Borage Seeds During Germination<sup>a</sup>

Fatty acid	Germination period (d)					
	0	2	4	6	8	10
16:0	10.8 ± 0.1	10.5 ± 0.1	10.5 ± 0.1	10.5 ± 0.1	10.4 ± 0.1	10.9 ± 0.5
18:0	4.7 ± 0.1	4.7 ± 0.2	4.6 ± 0.3	4.6 ± 0.1	4.8 ± 0.2	4.9 ± 0.2
16:1	ND	0.2 ± 0.1	0.2 ± 1.3	0.2 ± 0.1	0.2 ± 0.5	ND
17:1	0.3 ± 0.3	0.2 ± 0.2	0.4 ± 0.7	ND	ND	0.8 ± 0.7
18:1	17.8 ± 0.4	17.8 ± 0.4	16.8 ± 0.7	16.6 ± 0.1	15.2 ± 0.4	15.1 ± 0.4
20:1	4.1 ± 0.4	4.1 ± 0.2	4.2 ± 0.4	4.3 ± 0.3	4.4 ± 0.1	4.3 ± 0.3
22:1	2.4 ± 0.2	2.5 ± 0.2	2.6 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
24:1	1.6 ± 0.2	1.6 ± 1.6	1.6 ± 0.1	1.6 ± 0.2	1.8 ± 0.3	1.8 ± 0.7
18:2n-6	37.2 ± 0.7	37.2 ± 0.5	36.8 ± 1.6	36.8 ± 0.3	34.0 ± 0.2	35.3 ± 0.2
18:3n-6	20.5 ± 0.4	20.5 ± 0.2	20.4 ± 1.7	21.4 ± 0.1	21.8 ± 0.3	21.9 ± 0.3
18:3n-3	ND	ND	0.7 ± 0.2	0.9 ± 0.4	2.6 ± 0.1	2.3 ± 0.2
Total saturated fatty acids	15.6	15.2	15.2	15.1	15.2	15.8
Total monounsaturated fatty acids	26.1	26.3	25.8	25.1	24.3	23.8
Total polyunsaturated fatty acids	57.7	57.7	57.9	59.2	58.4	59.6

<sup>a</sup>As area percentage. Mean ± SD of triplicate determinations. For abbreviation see Table 2.

The contents of saturated and some monounsaturated fatty acids in the total and NL fractions remained nearly constant during germination.

GL constituted 2% of the total lipids of borage seeds (Table 1). During germination, the content of glycolipids was significantly ( $P \leq 0.05$ ) increased, in agreement with previous reports for other germinating seeds (6–8,20). The increase in GL is probably due to the formation and transformation of membranes during germination (11,42). GL are important components of photosynthetic membranes, and their increase during germination reflects chloroplast development and tissue greening.

The changes in fatty acid composition of GL during germination are shown in Table 5. Linoleic acid and GLA made up 50% of the GL of borage lipids. The polyunsaturated fatty acid (PUFA) content of glycolipids increased, but that of monounsaturated and saturated fatty acids decreased as germination proceeded (Table 5). Of particular interest is the rapid increase of

$\alpha$ -linolenic acid, an increase that probably reflects fatty acid biosynthesis during the development and greening of foliar tissues (43).

PL constituted 2.3% of the total lipids of borage seeds (Table 1). The total PL content did not change significantly ( $P > 0.05$ ) over the first 4 d, but then reached a maximum on day 10 after germination. A similar increase in PL was observed by Ichihara and Noda (8) and Huang and Grunwald (19) in safflower and alfalfa seeds, respectively. This indicates that the increase in PL during germination plays a biologically important role and as essential components in the formation of membrane systems.

The individual PL were characterized and their levels estimated in borage seeds during germination (Table 6). TLC–FID separation of PL revealed that PC was the major PL (70% of total) of borage seeds prior to germination (Table 6). PC is also the major PL in seeds of alfalfa (*Medicago sativa*) (20), pea (*Pisum sativum*) (14,44), barley (*Hordeum vulgare*) (19), saf-

**TABLE 4**  
Fatty Acid Composition of the Neutral Lipids of Borage Seeds During Germination<sup>a</sup>

Fatty acid	Germination period (d)					
	0	2	4	6	8	10
16:0	10.7 ± 0.1	10.4 ± 0.1	10.8 ± 0.4	10.9 ± 0.2	10.5 ± 0.2	10.9 ± 0.2
18:0	4.7 ± 0.2	4.6 ± 0.1	4.8 ± 0.1	5.2 ± 0.3	4.9 ± 0.1	5.4 ± 0.2
18:1	17.9 ± 0.1	17.9 ± 0.2	17.6 ± 0.2	17.4 ± 0.1	16.5 ± 0.2	17.4 ± 0.1
20:1	4.1 ± 0.2	4.1 ± 0.6	4.4 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	5.1 ± 0.2
22:1	2.5 ± 0.2	2.5 ± 0.8	2.6 ± 0.1	2.9 ± 0.1	2.8 ± 0.2	3.2 ± 0.1
24:1	1.6 ± 0.1	1.5 ± 0.5	1.7 ± 0.2	1.9 ± 0.1	1.9 ± 0.2	2.1 ± 0.4
18:2n-6	36.8 ± 0.1	37.1 ± 0.3	38.1 ± 1.2	36.2 ± 0.6	35.0 ± 0.2	33.3 ± 0.3
18:3n-6	20.2 ± 0.2	20.2 ± 0.3	20.4 ± 0.7	20.6 ± 0.2	22.2 ± 0.2	19.2 ± 0.1
18:3n-3	ND	ND	1.4 ± 0.2	ND	1.2 ± 0.4	1.3 ± 0.2
Total saturated fatty acids	15.5	15.0	15.6	16.1	15.4	16.3
Total monounsaturated fatty acids	26.0	26.0	26.1	26.9	25.9	27.7
Total polyunsaturated fatty acids	57.0	57.2	59.9	56.8	58.4	53.8

<sup>a</sup>As area percentage. Mean ± SD of triplicate determinations. See Tables 1 and 2 for abbreviations.

**TABLE 5**  
**Fatty Acid Composition of Glycolipids of Borage Seeds During Germination<sup>a</sup>**

Fatty acid	Germination period (d)					
	0	2	4	6	8	10
14:0	1.8 ± 0.5	ND	ND	ND	ND	ND
16:0	13.3 ± 0.2	10.7 ± 0.1	10.7 ± 1.0	10.1 ± 0.2	4.2 ± 1.0	10.3 ± 0.2
18:0	4.9 ± 0.7	4.9 ± 0.1	4.8 ± 0.3	4.1 ± 0.1	ND	4.8 ± 0.1
18:1	16.2 ± 0.1	27.6 ± 0.7	9.3 ± 0.4	7.3 ± 0.1	10.0 ± 0.3	8.5 ± 1.0
18:2n-6	31.9 ± 0.1	25.3 ± 0.5	26.6 ± 0.1	30.1 ± 0.2	25.7 ± 1.1	26.8 ± 0.2
18:3n-6	17.7 ± 0.4	7.1 ± 0.7	23.9 ± 0.1	20.8 ± 0.2	15.3 ± 1.0	16.8 ± 0.1
18:3n-3	ND	ND	17.0 ± 0.5	23.3 ± 0.1	22.9 ± 2.6	26.9 ± 0.1
Total saturated fatty acids	20.0	15.6	15.4	14.1	4.2	15.0
Total monounsaturated fatty acids	16.2	27.6	9.3	7.3	10.0	8.5
Total polyunsaturated fatty acids	49.6	32.5	50.5	74.2	63.9	70.6

<sup>a</sup>As area percentage. Mean ± SD of triplicate determinations. For abbreviation see Table 2.

**TABLE 6**  
**Changes in the Composition of Phospholipids of Borage Seeds During Germination<sup>a</sup>**

PL	Germination period (d)					
	0	2	4	6	8	10
Phosphatidic acid	11.2 ± 0.1 <sup>a</sup>	20.2 ± 5.8 <sup>a</sup>	59.7 ± 3.1 <sup>b</sup>	77.2 ± 1.0 <sup>c</sup>	88.3 ± 4.0 <sup>d</sup>	88.7 ± 5.9 <sup>d</sup>
Phosphatidylcholine	69.7 ± 0.4 <sup>e</sup>	59.2 ± 5.7 <sup>d</sup>	28.3 ± 1.5 <sup>c</sup>	10.2 ± 2.5 <sup>b</sup>	1.3 ± 1.1 <sup>a</sup>	9.9 ± 0.6 <sup>b</sup>
Phosphatidylserine + phosphatidylethanolamine	18.2 ± 0.4 <sup>b</sup>	15.5 ± 8.4 <sup>b</sup>	3.9 ± 1.0 <sup>a</sup>	ND <sup>a</sup>	0.9 ± 0.4 <sup>a</sup>	1.1 ± 0.5 <sup>a</sup>
Phosphatidylinositol	ND	ND	ND	ND	1.6 ± 0.6	ND
Lysophosphatidylcholine	0.9 ± 0.4 <sup>a</sup>	4.8 ± 3.9 <sup>b,c</sup>	7.6 ± 0.5 <sup>c</sup>	13.2 ± 0.4 <sup>d</sup>	7.6 ± 0.3 <sup>c</sup>	0.1 ± 0.5 <sup>a</sup>
Lysophosphatidylethanolamine	ND	0.6 ± 0.1 <sup>a</sup>	0.3 ± 0.5 <sup>a</sup>	ND	0.4 ± 0.5 <sup>a</sup>	ND

<sup>a</sup>As area percentage. Mean ± SD of triplicate determinations. For abbreviations see Tables 1 and 2.

**TABLE 7**  
**Fatty Acid Composition of Phospholipids of Borage Seeds During Germination<sup>a</sup>**

Fatty acid	Germination period (d)					
	0	2	4	6	8	10
16:0	17.8 ± 0.2	18.2 ± 0.2	20.8 ± 0.1	22.4 ± 0.1	23.9 ± 0.1	22.0 ± 0.2
18:0	4.8 ± 0.4	4.5 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.9 ± 0.4	4.0 ± 0.4
18:1	10.9 ± 0.1	6.2 ± 0.4	6.5 ± 0.4	6.9 ± 0.6	8.8 ± 0.2	8.1 ± 0.1
18:2n-6	52.3 ± 0.1	44.5 ± 0.1	42.0 ± 0.4	41.9 ± 0.3	38.3 ± 0.7	33.3 ± 0.4
18:3n-6	14.1 ± 0.6	17.6 ± 0.1	19.4 ± 0.1	17.5 ± 0.1	19.3 ± 0.1	19.9 ± 0.4
18:3n-3	ND	ND	6.8 ± 0.7	3.8 ± 0.5	4.6 ± 0.1	4.7 ± 0.1
Total saturated fatty acids	22.6	22.7	25.2	27.0	28.8	26.0
Total monounsaturated fatty acids	10.9	6.2	6.5	6.9	8.8	8.1
Total polyunsaturated fatty acids	66.4	62.1	68.2	63.2	62.3	57.9

<sup>a</sup>As area percentage. Mean ± SD of triplicate determinations. For abbreviations see Tables 1 and 2.

flower (*Carthamus tinctorius*) (8), and soybean (*Glycine max*) (42). PS and PE were coeluted and their total made up the next-largest fraction. Although the content of PC decreased during germination, the content of LPC increased moderately and that of PA increased drastically from 11 to 89% of the total content of after 10 d of germination. An increase in PA might be expected since it constitutes a key intermediate in PL synthesis. Increase in the percentage of PA was also observed with germinating soybean (42). LPE was not detected in ungerminated bor-

age. However, its content began to increase 2 d after germination. The content of LPE fluctuated during germination, but this change was not significant ( $P \leq 0.05$ ). In germinating soybean (7) and pea (14), the total PL content increased, but composition of individual PL remained unchanged. Based on these data, no consistent pattern was observed for changes in individual PL during germination.

The changes in fatty acid composition of PL during germination are shown in Table 7. The percentage of palmitic acid

(16:0) increased, whereas that of oleic acid (18:1) decreased as germination proceeded. PUFA constituted a major fraction of the fatty acids (66%) of PL borage, which exhibited little change during germination (Table 7). Linoleic acid was the predominant fatty acid in the PL fraction of borage, and its content was decreased during germination. However, the contents of  $\alpha$ - and  $\gamma$ -linolenic acids were increased. These observations were in agreement with the results obtained from analyses of germinating soybean (42). Palmitic, oleic and linoleic acids were found predominantly among the saturated fatty acids, monounsaturated fatty acids, and PUFA of all the lipid fractions (total, neutral, GL, and PL) of borage seeds, similar to that in a number of other germinating seeds (7,15,20).

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