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Fatty acid distribution in triacylglycerols and phospholipids of broad beans (*Vicia faba*)

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ABSTRACT

Fatty acid distribution of triacylglycerols (TAG) and phospholipids (PL) obtained from the four cultivars of broad beans (*Vicia faba*) was investigated. Total lipids extracted from the beans were separated by thin-layer chromatography into eight fractions. The major lipid components were TAG (47.7–50.1%) and PL (47.5–50.5%), while hydrocarbons, steryl esters, free fatty acids, diacylglycerols (1,3-DAG and 1,2-DAG) and monoacylglycerols were present in minor proportions (1.8–2.4%). The major PL components were phosphatidylcholine (56.4–58.4%), phosphatidylethanolamine (20.3–21.7%) and phosphatidylinositol (16.6–18.6%). Phosphatidylinositol was unique in that it had the highest saturated fatty acid content among the three PL. No significant differences ($P > 0.05$) in fatty acid distribution existed when the different cultivars were compared. The fatty acid distributions in the TAG were evident among the four cultivars: unsaturated fatty acids were predominantly concentrated in the *sn*−2 position, and saturated fatty acids primarily occupied the *sn*−1 or *sn*−3 position in the oils. These results could be useful to both consumers and producers for manufacture of traditional foods in Japan and elsewhere.

1. Introduction

Legumes are an important food source and play a significant role in traditional diets in many regions of the world. Among the legume seeds, some are used as vegetables and others as supplementary sources of protein in animal diets (Savage, 1988). The widespread use of legumes makes this food group an important source of lipid and fatty acids in animal and human nutrition. Some publications dealing with the total lipid and fatty acid composition are reviewed by a few researchers (Grela & Gunter, 1995; Welch & Griffiths, 1984). *Vicia faba* beans (VFB) are consumed by man and domestic animals as an important source of protein, especially in countries with a shortage of high-quality protein sources. Several reports (Ford & Hewitt, 1979; Nitsan, 1971) suggest that VFB have a deleterious effect on rats, whereas others report good results, with no apparent ill effects. With chicks receiving a high proportion of VFB in the diets, Bletner, Chalhoub, and Goff (1963) obtained poor growth, feathering and feather pigmentation, but this was improved considerably by addition of a source of good quality protein. Some beans are used as staple foods in many countries and are receiving increasing attention as preventive products against coronary heart disease (Anderson, Story, Sieling, Chen, Petro & Story, 1984; Bazzano et al., 2001). *V. faba* beans are a rich source of carbohydrate, protein, fibre, vitamins and minerals (Grela & Gunter, 1995); however, their contents include certain antinutritional factors, such as protease inhibitors, lectins, raffinose-series oligosaccharides, tannins and phytic acid (Hickling, 2003; Wang & Daun, 2004).

Poor growth after receiving diets containing *V. faba* beans is attributed, by some investigators, to a deficiency in, or an imbalance of, some of the essential amino acids, and to the presence of growth inhibitors associated with the protein (Ford & Hewitt, 1979; Nitsan, 1971). The negative effect of VFB has also been attributed to contamination of the beans with poisonous substances, such as castor seeds or croton seed residues. The contradictory results described in the literature may also be due to differences in the nutritive value as a result of variability in the composition of different cultivars of the bean. However, a literature search revealed that there is limited information on the lipid components and fatty acid distribution of broad beans (Grela & Gunter, 1995).

The purpose of the present study was to determine the composition and fatty acid distribution of the triacylglycerols (TAG) and phospholipids (PL) obtained from *V. faba* beans, in an attempt to compare the composition and quality characteristics of the oils among the four cultivars. The data obtained would be useful

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information to both producers and consumers for manufacturing traditional confectioneries in Japan and elsewhere.

2. Materials and methods

2.1. Broad beans

The commercially available mature broad beans (*V. faba*) used in this study were from Minpo, Sanuki, Nintoku and Sanren cultivars (Takii Seed Co. Kyoto, Japan) grown in different districts of Japan during the summer of 2006. These seeds were selected for uniformity based on seed weights of 1.06–1.10 g for Minpo, 1.31–1.35 g for Sanuki, 2.21–2.30 g for Nintoku, and 2.31–2.35 g for Sanren. The beans were hand-selected to eliminate those that were cracked or otherwise damaged. Broad beans of each cultivar were divided into groups and stored in separate stainless steel containers at 4 °C prior to the repeated experiments.

2.2. Reagents and standards

All solvents and chemicals used were of analytical grade (Nacalai Tesque, Kyoto, Japan). However, diethyl ether was further purified to remove peroxides. Thin-layer chromatography (TLC) precoated silica gel 60 plates (10 × 20 or 20 × 20 cm, 0.25 mm layer thickness) were purchased from Merck (Darmstadt, Germany). A TLC standard mixture containing monoacylglycerols (MAG), diacylglycerols (DAG), free fatty acids (FFA), triacylglycerols (TAG), steryl esters (SE) and hydrocarbons (HC), was purchased from Nacalai Tesque. A phospholipid kit from Serdary Research Laboratory (Mississauga, ONT, Canada) was used as phospholipid standard. Lipase from porcine pancreas was obtained from Sigma Chemical Co (St. Louis, MO, USA), and used after purification with acetone and then diethyl ether as described previously (Yoshida & Alexander, 1983). 1-Myristoyl-1,3-dilaurin (Sigma Chemical Co) was used as TAG standard for enzymatic hydrolysis. Methyl pentadecanoate (100 mg; Merck, Darmstadt, Germany) was dissolved in *n*-hexane (20 ml) and used as the internal standard. Boron trifluoride (BF_3) in methanol (14%; Wako Pure Chemical Inc., Osaka, Japan) was used to prepare fatty acid methyl esters (FAME).

2.3. Chemical analysis

The AOAC (1997) methods were used to determine the chemical composition of the broad beans. Samples were analysed in triplicate for fat, protein and moisture contents according to the standard methods. Fat content was determined by solvent extraction (Method 991.36), protein content by a Kjeldahl method (Method 981.10) and moisture content by oven-drying to constant weight at 105 °C (Method 925.40).

2.4. Extraction of lipids

In order to obtain fine flour, beans (50 seeds) were ground to pass a 0.5 mm sieve, using a Maxm homogenizer (Nihonseiki Kaisha Ltd. Tokyo, Japan) at high speed for 10 min at 0 °C and extracted with 300 ml of chloroform/methanol (2:1, v/v). The lipids were further extracted by vigorous shaking of triplicate samples. These solvents contained 0.01% butylated hydroxytoluene (BHT) to inhibit the oxidative degradation of lipids during analysis. The homogenate was vacuum-filtered through defatted filter paper on a Buchner funnel, and the filter residue was rehomogenized with a second volume of chloroform/methanol. The filtrates were combined and dried in a rotary vacuum evaporator at 35 °C. The residue was dissolved in 100 ml of chloroform/methanol (2:1, v/v); then, 20 ml aqueous potassium chloride (0.75%) were added (Folch, Lee, & Sloane-Stanley, 1957), and the phases were vigorously mixed. After phase separation, the chloroform layer was withdrawn, dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated under vacuum. The extracted lipids were weighed to determine the lipid content of the beans and then transferred to a 25 ml brown glass volumetric flask with chloroform/methanol (2:1, v/v).

2.5. Lipid analysis

Using previously described methods (Yoshida, Shougaki, Hirakawa, Tomiyama, & Mizushina, 2004), the total lipids were fractionated by TLC into eight fractions with *n*-hexane/diethyl ether/acetic acid (80:20:1, v/v/v). Bands corresponding to HC, SE, TAG, FFA, 1,3-DAG, 1,2-DAG, MAG and PL were scraped into test-tubes [105 × 16 mm; poly (tetrafluoroethylene)-coated screw caps]. Methyl pentadecanoate (2 or 20 μl) from a standard solution (5 mg/ml) was added to each tube as internal standard with the microsyringe (Hamilton Co., Reno, NV, USA). With the exception of HC, FAME were prepared from the isolated lipids by heating with silica-gel for 30 min at 80 °C in BF_3/methanol on an aluminium block bath (AOCS, 1992). After cooling, 5 ml of *n*-hexane were added to each tube and washed several times with deionized water to remove BF_3 and silica-gel.

The *n*-hexane layer containing the FAME was recovered and dried over anhydrous sodium sulfate. The solvent was then vaporised under a gentle stream of nitrogen, and the residue was quantified on a Shimadzu Model-14B GC (Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionisation detector and a capillary column (ULBO HE-SS-10 for FAME fused-silica WCOT, cyanopropyl silicone, 30 m × 0.32 mm i.d.; Shinwa Chem. Ind., Ltd., Kyoto, Japan) at a column temperature of 180 °C. The injection and detector temperatures were held at 220 and 250 °C, respectively. The initial oven temperature was 180 °C. This temperature was maintained for 5 min and then increased at a rate of 2 °C/min to 200 °C, which was held for 15 min. Helium was used as the carrier gas, at a flow rate of 1.5 ml/min, and the GC was operated under a constant pressure of 180 kPa. All samples were dissolved in *n*-hexane for injection. The component peaks were identified and calibrated by comparison with standard FAME (F and OR mixtures No. 3; Altech-Applied Science, State College, PA, USA), using an electronic integrator (Shimadzu C-R4A). The detection limit was 0.05 wt% of total fatty acids for each FAME in the FAME mixture, and the results are expressed as wt% of total FAME.

Samples of the extracted polar lipids, obtained as described above, were further separated by TLC into several fractions with chloroform/methanol/acetic acid/deionized water (170:30:20:7, v/v/v/v) as the mobile phase. PL classes were detected by iodine vapour and were consistent with authentic standards. Bands corresponding to phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) and others were carefully scraped into test tubes. Then, methyl pentadecanoate of a standard solution (5 μl) was added to each tube as the internal standard. FAME were prepared by the same method as described above and analysed by GC.

2.6. Enzymatic hydrolysis of lipids

TAG hydrolysis in vitro was carried out according to the methods described previously by Yoshida and Alexander (1983). The purified TAG (20 mg) was hydrolysed with 20 mg of pancreatic lipase at 37 °C in 6 ml of 0.25 M Tris buffer (pH 7.5) containing 0.1 ml of 0.01 M $CaCl_2$ and 0.25 ml of deoxylcholate (0.l%) in a 10 ml test tube. A time period of 20 min was selected, based on the results of preliminary experiments using standard TAG

(2-myristoyl-1,3-dilaurin). After approximately 60% of the TG was hydrolysed, adding of 0.5 ml of 6 M HCl and l ml of ethanol stopped the reaction. In that study, it was confirmed by TLC and GC that no fatty acid (myristic acid) in the *sn*−2 position of standard TAG is transferred to the *sn*−1 or *sn*−3 position within 60% hydrolysis (for 20 min). The reaction products were separated by TLC with *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v) as already described (Yoshida & Alexander, 1983). The free fatty acid and *sn*−2 MAG bands were separately scraped into test tubes. The constituent fatty acids were analysed by GC after methylation, as described above.

2.7. Statistical treatment

All preparation and measurements were carried out in replicates, and the data were subjected to analysis of variance. Analysis variance was performed using the ANOVA procedure (Steel, Torrie, & Dickey, 1995). Multiple comparison tests were performed to determine any significant differences ($P < 0.05$) among treatments (Baker, 1980).

3. Results and discussion

3.1. Lipid components in the beans

The major chemical components were as follows: moisture 3.5–3.7%, fat 2.3–2.9% and protein 24.8–25.6%. There were no significant differences ($P > 0.05$) in these contents among the four cultivars. Broad beans are rich in complex carbohydrates, protein, and fibre, yet are extremely sparse in fat (Nitsan, 1971). The compositional analyses carried out in this study included determination of the lipid classes and the fatty acid compositions of the oils. Profiles of the different lipid classes in the beans were compared among the four cultivars (Table 1). Predominant components were TAG (47.7–50.1%) and PL (47.5–50.5%), accompanied by very small amounts (less than 2.4%) of other lipid components. These minor components are designated as 'others' in Table 1. The broad beans are not oilseeds but typical vegetable seeds (Lee, Mitchell, & Shibamoto, 2000). Therefore, the PL content is quite significant, while glycolipids are present in only trace amounts, indicating that PL form the essential components of the cell membranes in the beans.

3.2. Fatty acid composition of total lipids and phospholipids

Fatty acid compositions (expressed in terms of the esters by weight) of total lipids and PL in the beans were compared among the four cultivars (Fig. 1). The principal fatty acid components of legumes are generally palmitic, oleic and linoleic acids, the distribution of which varies according to these lipid classes. However, these fatty acid distribution patterns were very similar to each other among the four cultivars. The samples had high amounts of total unsaturated fatty acids (which consisted mainly of linoleic acid, followed by oleic acids), representing 81.0–81.7% and 77.6–79.7% for total lipids and PL, respectively.

Some differences ($P < 0.05$) in fatty acid composition of cultivars existed when comparing them by the two lipid classes. The percentage of palmitic and oleic acids was higher ($P < 0.05$) in the PL (Fig. 1, right) whereas linoleic acid was higher ($P < 0.05$) in the total lipids (Fig. 1, left). However, the percentages of stearic and linolenic acids were almost the same. These fatty acid profiles are not similar to the results observed in typical vegetable seeds, such as kidney beans (Mabaleha & Yeboa, 2004) or peas (Yoshida, Tomiyama, Tanaka, & Mizushina 2007).

Table 1
Lipid components in the oils obtained from broad beans[A]

Cultivar	Total lipids	Triacylglycerols	Phospholipids	Others
	(mg/100 g beans)			
Minpo	2367 ± 111[a]	1181 ± 57[a] (49.9)	1141 ± 53[a] (48.2)	45.0 ± 2.0[b] (1.9)
Sanuki	2313 ± 106[a]	1129 ± 53[a] (48.8)	1147 ± 55[a] (49.6)	37.0 ± 1.6[a] (1.6)
Nintoku	2925 ± 126[c]	1466 ± 70[c] (50.1)	1390 ± 67[c] (47.5)	70.2 ± 3.2[c] (2.4)
Sanren	2595 ± 119[b]	1238 ± 59[b] (47.7)	1311 ± 61[b] (50.5)	46.7 ± 2.2[b] (1.8)

[A] Mean values ± standard error. Each value represents the average of three replicates, and is expressed as mg lipid per 100 g of beans. Values in parentheses are relative wt% contents of individual lipids in total lipids. "Others" include minor components such as hydrocarbons, steryl esters, free fatty acids, diacylglycerols and monoacylglycerols. Values in the same column with different superscripts are significantly different from those of individual cultivars ($P < 0.05$).

3.3. Positional distribution of fatty acids of triacylglycerols

The profiles of composition and positional distribution of fatty acids in the TAG were compared among the four cultivars (Table 2). No significant differences ($P > 0.05$) existed in the fatty acid distributions for TAG among the four cultivars; the major components were palmitic, oleic and linoleic acids, followed by stearic and linolenic acids. Unsaturated fatty acids, such as oleic and linoleic (>95.0%), were predominantly concentrated in the *sn*−2 position of the TAG molecules, while saturated fatty acids, such as palmitic and stearic acids, were primarily located in the *sn*−1 or *sn*−3 positions. However, oleic acid was almost evenly distributed in the *sn*−1, 2, or 3 positions, as other researchers have also reported (Reske, Siebrech, & Hazebroek, 1997). Therefore, the positional distribution patterns in the fatty acids of TAG were very similar to the results obtained for other seed oils, such as corn and soybeans (Arcos, Garcia, & Hill, 2000).

3.4. Fatty acid distribution of major phospholipids

To clarify the distribution of individual PL in the broad beans, further separation of the PL fraction into several fractions (PE, PC, PI and others) was carried out on TLC in the presence of authentic standards. Comparisons were made of the profiles of PE, PC, PI and others among the four cultivars (Table 3). Regardless of these cultivars, PC, PE and PI were the principal PL in the broad beans, and the highest content was observed for PC (56.4–58.4%), followed by PE (20.3–21.7%) and PI (16.6–18.6%). The original amounts of individual PL were approximately 233–283 mg of PE, 626–795 mg of PC and 190–257 mg of PI per 100 g of beans. Among the four cultivars, PC was detected as the dominant component, followed by PE and PI. Broad beans are typical low-oil-bearing legumes, and PL form the principal components of the cell membranes in the beans. Since membrane lipids are involved in such fundamental cell processes as ion transport, energy generation and biological reactions, they are highly conserved in terms of both quantity and quality (Arcos et al., 2000). This may explain why the fatty acid composition in newly differentiated tissue was conserved, regardless of genotype.

Table 4 shows typical fatty acid distributions of PE, PC and PI among the four cultivars. The major fatty acids in the three PL were commonly palmitic, stearic, oleic and linoleic acids. These fatty acid distributions were very similar to each other in the individual PL among the four cultivars. However, significant differences ($P < 0.05$) in these fatty acid distributions for PC were observed among the four cultivars. When comparing the three PL among the four cultivars, the percentage of linoleic acid was significantly ($P < 0.05$) higher in PE than that in PC, while the percentage of oleic

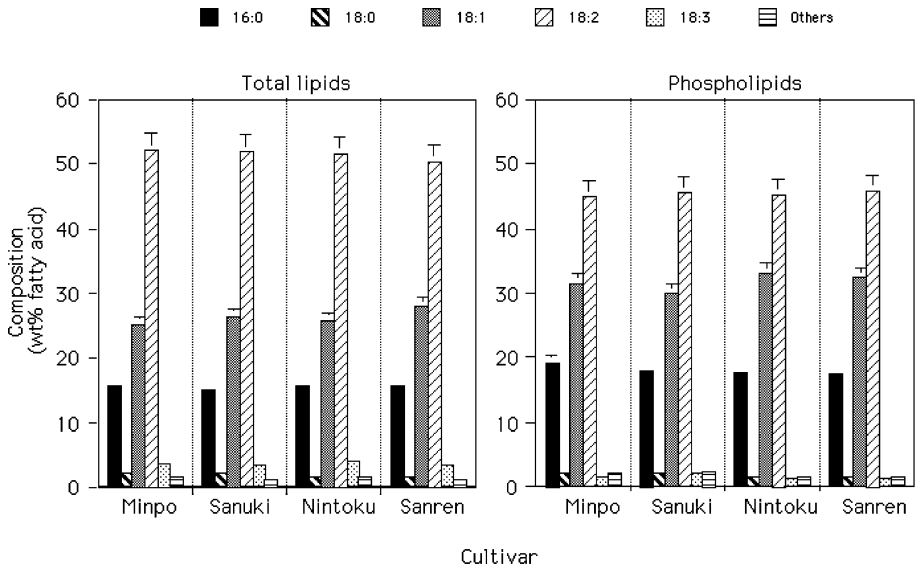

Fig. 1. Fatty acid distributions of total lipids and phospholipids prepared from broad beans. Each value represents the average of three replicates, and vertical bars depict the mean standard deviation. Other minor fatty acids include 14:0, 16:1, 20:0 and 22:0.

Table 2
Composition and positional distribution of fatty acids in the triacylglycerols obtained from broad beans[A]

Cultivar	Position	Fatty acid (wt%)					
		16:0	18:0	18:1	18:2	18:3	Others
Minpo	Total	14.0 ± 0.5^{a}	2.0 ± 0.1^{a}	22.6 ± 1.0^{a}	54.3 ± 2.3^{b}	5.1 ± 0.1^{c}	2.3 ± 0.1^{a}
	2	1.5 ± 0.1^{a}	0.1 ± 0.0^{a}	26.0 ± 1.1^{a}	69.8 ± 3.1^{a}	2.3 ± 0.1^{a}	0.4 ± 0.0^{a}
	1,3	20.3 ± 0.8^{a}	4.0 ± 0.1^{a}	20.9 ± 0.8^{a}	46.3 ± 2.0^{b}	6.3 ± 0.2^{c}	2.2 ± 0.1^{a}
Sanuki	Total	15.2 ± 0.6^{b}	2.0 ± 0.1^{a}	23.8 ± 1.0^{a}	52.5 ± 2.2^{a}	4.0 ± 0.1^{a}	2.5 ± 0.1^{a}
	2	1.2 ± 0.1^{a}	0.1 ± 0.0^{a}	27.6 ± 1.2^{b}	67.8 ± 3.0^{a}	2.5 ± 0.1^{a}	0.8 ± 0.0^{b}
	1,3	22.8 ± 1.0^{b}	4.2 ± 0.1^{b}	21.8 ± 1.0^{a}	43.6 ± 1.8^{a}	5.4 ± 0.2^{b}	2.4 ± 0.1^{b}
Nintoku	Total	16.5 ± 0.6^{c}	2.3 ± 0.1^{b}	23.0 ± 1.0^{a}	50.8 ± 2.1^{a}	4.7 ± 0.1^{b}	2.7 ± 0.1^{b}
	2	2.0 ± 0.1^{b}	0.1 ± 0.0^{a}	26.8 ± 1.2^{a}	68.3 ± 3.0^{a}	2.5 ± 0.1^{a}	0.3 ± 0.0^{a}
	1,3	23.5 ± 1.0^{b}	3.8 ± 0.1^{a}	20.3 ± 0.8^{a}	44.1 ± 1.8^{a}	6.2 ± 0.2^{c}	2.1 ± 0.1^{a}
Sanren	Total	14.1 ± 0.5^{a}	2.4 ± 0.1^{b}	23.5 ± 1.0^{a}	53.2 ± 2.2^{a}	3.8 ± 0.1^{a}	3.0 ± 0.1^{c}
	2	2.1 ± 0.1^{b}	0.1 ± 0.0^{a}	28.1 ± 1.2^{b}	67.1 ± 3.0^{a}	2.3 ±0.1^{a}	0.3 ± 0.0^{a}
	1,3	20.8 ± 0.8^{a}	5.5 ± 0.2^{c}	20.8 ± 0.8^{a}	46.1 ± 2.1^{b}	4.2 ± 0.1^{a}	2.5 ± 0.1^{b}

[A] Mean values ± standard error. Each value represents the average of three replicates, and is expressed relative wt% contents of individual fatty acids. "Others" include minor fatty acids such as 14:0, 16:1, 20:0 and 22:0. Values in the same column with different superscripts are significantly different from those of individual cultivars ($P < 0.05$).

Table 3
The content of major phospholipids in the oils obtained from broad beans[A]

Cultivar	Phosphatidylethanolamine	Phosphatidylcholine	Phosphatidylinositol	Others
	(mg/100 g beans)			
Minpo	241 ± 11^{a} (21.7)	626 ± 30^{a} (56.4)	190 ± 9^{a} (17.1)	53.3 ± 2.1^{a} (4.8)
Sanuki	233 ± 11^{a} (20.3)	649 ± 31^{a} (56.6)	213 ± 10^{b} (18.6)	51.6 ± 2.4^{a} (4.5)
Nintoku	283 ± 12^{c} (20.4)	795 ± 36^{b} (57.2)	257 ± 11^{c} (18.5)	54.2 ± 2.3^{a} (3.9)
Sanren	266 ± 12^{b} (20.3)	766 ± 35^{b} (58.4)	218 ± 10^{b} (16.6)	61.6 ± 2.8^{b} (4.7)

[A] Mean values ± standard error. Each value is the average of three replicates, and is expressed as mg lipid per 100 g of beans. Values in parentheses are relative wt% contents of individual lipids in phospholipids. "Others" include minor phospholipid components such as diphosphatidylglycerol, phosphatidic acid and phosphatidylglycerol. Values in the same column with different superscripts are significantly different from those of individual cultivars ($P < 0.05$).

acid was significantly ($P < 0.05$) higher in PC than that in PE, respectively. Furthermore, PI was unique in that it had the highest saturated fatty acid content among the three PL, although their distribution patterns were very similar among the four cultivars. Particularly, the percentage of palmitic acid was significantly ($P < 0.05$) higher in PI than in PC or PE among the four cultivars.

Table 4
Fatty acid distribution of major phospholipids obtained from broad beans[A]

Phospholipid	Cultivar	Fatty acid (wt%)				
		16:0	18:0	18:1	18:2	Others
Phosphatidylethanolamine	Minpo	18.4 ± 0.8[b]	2.8 ± 0.1[b]	29.6 ± 1.2[a]	47.7 ± 2.1[a]	1.7 ± 0.1[a]
	Sanuki	17.4 ± 0.8[a]	2.9 ± 0.1[b]	31.8 ± 1.2[a]	46.0 ± 2.1[a]	1.9 ± 0.1[a]
	Nintoku	17.0 ± 0.8[a]	2.3 ± 0.1[a]	33.0 ± 1.3[b]	46.0 ± 2.1[a]	1.7 ± 0.1[a]
	Sanren	17.8 ± 0.8[a]	2.4 ± 0.1[a]	32.5 ± 1.3[b]	45.7 ± 2.1[a]	1.6 ± 0.1[a]
Phosphatidylcholine	Minpo	12.1 ± 0.4[a]	1.5 ± 0.1[a]	45.3 ± 2.0[a]	39.5 ± 1.6[b]	1.6 ± 0.1[b]
	Sanuki	12.6 ± 0.4[a]	1.6 ± 0.1[b]	47.2 ± 2.1[a]	37.2 ± 1.6[b]	1.4 ± 0.1[a]
	Nintoku	12.5 ± 0.4[a]	1.8 ± 0.1[c]	48.6 ± 2.1[b]	35.6 ± 1.5[a]	1.5 ± 0.1[a]
	Sanren	12.2 ± 0.4[a]	1.9 ± 0.1[c]	49.6 ± 2.1[b]	34.8 ± 1.4[a]	1.5 ± 0.1[a]
Phosphatidylinositol	Minpo	41.1 ± 1.8[a]	3.0 ± 0.1[c]	12.0 ± 0.4[a]	42.1 ± 2.0[a]	1.8 ± 0.1[c]
	Sanuki	41.7 ± 1.8[a]	2.0 ± 0.1[a]	13.3 ± 0.4[b]	41.1 ± 2.0[a]	1.9 ± 0.1[c]
	Nintoku	40.5 ± 1.8[a]	2.5 ± 0.1[b]	14.2 ± 0.4[b]	41.4 ± 2.0[a]	1.4 ± 0.1[a]
	Sanren	40.0 ± 1.8[a]	2.5 ± 0.1[b]	15.4 ± 0.5[c]	40.5 ± 2.0[a]	1.6 ± 0.1[b]

[A] Mean values ± standard error. Each value represents the average of three replicates, and is expressed as wt% content of individual fatty acids. "Others" include minor fatty acids such as 14:0, 16:1, 20:0 and 22:0. Values in the same column with different superscripts are significantly different from those of individual cultivars ($P < 0.05$).

4. Conclusions

Major lipid components in broad beans were PL and TAG, while HC, SE, FFA, DAG and MAG were also present in minor proportions. The principal profiles of fatty acid distributions in the TAG were evident in the beans: unsaturated fatty acids (>95.0%), especially linoleic and oleic acids, were predominantly concentrated in the *sn*−2 position, while saturated fatty acids, especially palmitic and setaric acids, primarily occupied the *sn*−1 position or *sn*−3 position in the TAG. The fatty acid distribution in PI differed significantly ($P < 0.05$) from that of PE or PC among the four cultivars, presumably owing to differences in their biosynthetic pathways (Vogel & Browse, 1996). The lipid components and fatty acid distributions were almost the same in the four cultivars and not influenced by genetic variability and planting location. The data obtained may be valuable for producers and industry in their effort to enhance the nutritional quality of broad beans, both for human consumption and animal feed, and to identify needs for future work.

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