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Tocopherol Content and Fatty Acid Distribution of Peas (*Pisum sativum* L.)

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Abstract The positional distribution of fatty acids (FA) of triacylglycerols (TAG) and major phospholipids (PL) prepared from four cultivars of peas (*Pisum sativum* L.) were investigated as well as their tocopherol contents. The lipids extracted from these peas were separated by thin-layer chromatography (TLC) into seven fractions. The major lipid components were PL (52.2–61.3%) and TAG (31.2–40.3%), while the other components were also present in minor proportions (5.6–9.2%). γ-Tocopherol was present in the highest concentration, and α- and δ-tocopherols were very small amounts. The main PL components isolated from the four cultivars were phosphatidylcholine (42.3–49.2%), followed by phosphatidylinositol (23.3–25.2%) and then phosphatidylethanolamine (17.7–20.5%). Small but significant differences ($P < 0.05$) in FA distribution existed when different pea cultivars were determined. However, the principal characteristics of the FA distribution in the TAG and the three PL were evident among the four cultivars; unsaturated FA were predominantly located in the *sn*-2 position, and saturated FA primary occupied the *sn*-1 or *sn*-3 position in the oils of the peas. These results suggest that the regional distribution of tocopherols and fatty acids in peas is not dependent on the climatic conditions and the soil characteristics of the cultivation areas during the growing season.

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Keywords Cultivar · Fatty acid · Phosphatidylcholine · Phosphatidylethanolamine · Phosphatidylinositol · Positional distribution · Peas (*Pisum sativum* L.) · Tocopherol homologs · Triacylglycerols

Introduction

Legumes are an important food source and play a significant role in traditional diets in many regions of the world. Peas are one of the most important legumes because they also contain 20–30% protein as a storage form. Peas rank fourth in the world production of food legumes next to soybeans, peanuts and dry beans [1]. The world production has increased in the last few decades, especially in Europe [2]. Peas are consumed by humans principally as green immature seeds. However, in recent years, dry peas have been thoroughly investigated because of their importance in animal nutrition as an alternative to soybeans [2]. As a result, there are many available cultivars, and their cultivation has also experienced considerable improvement. All these factors have contributed to placing the cultivation of legume beans such as peas at the same economic level as that of cereals, with the added value that legume cultivation is more environmentally friendly.

Peas are a rich source of protein, complex carbohydrates, dietary fibre, vitamins and minerals in the diet [3, 4]. However, they also include certain antinutritional factors, such as protease inhibitors, lectins, raffinose-series oligosaccharides, tannins and phytates [5]. One proteinaceous component of peas that has received considerable attention as a quality determinant is that which complexes with the intestinal protease trypsin; the component is thus referred to as a trypsin inhibitor [6]. A number of protease inhibitors have been isolated from peas. To our knowledge, no data

have been reported on the presence of tocopherols as well as the positional distribution of FA in the TAG and PL isolated from *Pisum sativum*. The composition of total FA is often the only information provided in studies of seed lipids. Therefore, the aim of the present study was to develop knowledge of the tocopherol content and the positional distribution of FA in the TAG and major PL, and, further, to compare the results among the four cultivars. These data will be valuable for producers and industry in their effort to enhance the nutritional quality of peas, both for human consumption and animal feed, and to identify needs for future work.

Materials and Methods

Samples

The commercially available mature peas (*Pisum sativum* L.) seeds used in this work were from four different cultivars-Gurume, Nankaimidori, Kurumeyutaka and Usui—grown in Thailand, Japan, France and New Zealand, respectively, during the summer of 2006. These cultivars (Takii Seed Co, Kyoto, Japan) were selected for uniformity based on a seed weight of 200–230 mg for Gurume, 290–330 mg for Nankaimidori, 300–340 mg for Kurumeyutaka, and 370–410 mg for Usui. All the peas were divided into groups for storage in stainless-steel containers at 4 °C prior to the experiments.

Reagents and Standards

All chemicals and solvents used were of analytical grade (Nacalai Tesque, Kyoto, Japan). 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). The TLC standard mixture, containing hydrocarbons (HC), steryl esters (SE), free fatty acids (FFA), diacylglycerols (DAG) and TAG, was from Nacalai Tesque (Kyoto, Japan). For phospholipid standards, a phospholipid kit (Serdary Research Laboratory, Ontario, Canada) was used.

The lipase was from porcine pancreas, and was used after purification with acetone and then diethyl ether as previously reported by Yoshida and Alexander [7]. Phospholipase A_2 was from bee (*Apis mellifera*) venom. Both enzymes (lipase and phospholipase A_2) were procured from Sigma Chemical Co. (St Louis, MO). Pentadecanoic acid methyl ester (C15:0, 100 mg; Merck) was dissolved in *n*-hexane (20 mL) and used as the internal standard for quantitative analyses. Boron trifluoride (BF_3) in methanol (14%; Wako Pure Chemical Inc., Osaka, Japan) was used to prepare fatty acid methyl esters (FAME).

Chemical Analysis

The AOAC methods [8] were used to determine the chemical composition of the peas. The peas were analyzed in triplicate for fat, protein and moisture content according to the standard methods. Fat was determined by solvent extraction (Method 991.36), protein by a Kjeldahl method (Method 981.10) and moisture by oven-drying to constant weight at 105 °C (Method 925.40).

Extraction of Lipids

In order to obtain fine flour, peas (100 g) were ground to pass through a 0.5 mm sieve, using a Maxim homogenizer (Nihonseiki Kaisha Ltd Tokyo, Japan) at high speed for 10 min at 0°C and extracted with 200 mL chloroform/methanol (2:1, vol/vol). 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 chloroform/methanol (2:1, vol/vol); then, 20 mL aqueous potassium chloride (0.75%) was added [9], 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 peas and then transferred to a 25-mL brown glass volumetric flask with chloroform/methanol (2:1, vol/vol).

Analysis of Tocopherols

The lipids (400 mg) were carefully transferred to a 5-mL brown volumetric flask, and the solvents were vaporized under a nitrogen stream at ambient temperature in a draft chamber. The residue was dissolved in the mobile phase (*n*-hexane/1,4-dioxane/ethanol, 490:10:1, by volume) used for high-performance liquid chromatography (HPLC) analysis. The chromatographic system consisted of a normal-bonded-phase Shim-pack CLC-SIL (M) column (5 μm, 250×4.5 mm i.d.; Shimadzu) protected by a 1-cm guard column (Shim-pack G-SIL) and was operated at a flow rate of 2.0 mL/min. An aliquot (5–10 μL) of these solutions was injected into the HPLC system with a microsyringe (Hamilton Co., Reno, NY, USA). Each tocopherol was monitored with a fluorescence spectrometer (Shimadzu

RF-10 AxL; Shimadzu Instruments Inc., Kyoto, Japan) set at 295 nm excitation wavelength and 320 nm emission wavelength, and were quantified as previously reported [10].

Lipid Analysis

Using previously reported methods [11], the total lipids were fractionated by TLC into seven fractions. Bands corresponding to HC, SE, TAG, FFA, 1,3-DAG, 1,2-DAG and PL were scraped into test-tubes [105 × 16 mm, poly (tetrafluoroethylene)-coated screw caps]. Methyl pentadecanoate (5 or 20 μL) of a standard solution (5 mg/mL) was added to each tube as the internal standard with the microsyringe. 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 [12]. After cooling, 5 mL *n*-hexane was 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 vaporized 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 ionization detector and a capillary column (ULBO HE-SS-10 for Fames fused-silica WCOT, cyanopropyl silicon, 30 m × 0.32 mm i.d.; Shinwa Chem. Ind, Ltd., Kyoto, Japan) at a column temperature of 180 °C. The injection and detection temperatures were held at 220 and 250 °C, respectively. 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 & 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 FA for each FAME in a 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, by volume) as the mobile phase. PL classes were detected by iodine vapor and were consistent with the authentic standards. Bands corresponding to phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) and others were carefully scraped into test tubes. Then, methyl pentadecanoate (5 μL) of a standard solution was added to each tube as internal standard. FAME were prepared by the same method as described above and analyzed by GC.

Enzymatic Hydrolysis of Lipids

TAG hydrolysis in vitro was carried out according to the methods previously reported [7]. The purified TAG (20 mg) were hydrolyzed 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 (0.1%) in a 10 mL test tube. A time period of 20 min was selected based on the results of preliminary experiments using a standard TAG (2-myristoyl-1,3-dilaurin; Merck, Darmstadt, Germany). After approximately 60% of the TAG was hydrolyzed, adding 0.5 mL of 6 M HCl and 1 mL of ethanol stopped the reaction. No FA (myristic acid) in the *sn*-2-position of TAG is transferred to the *sn*-1- or *sn*-3-position within 60% hydrolysis (for 20 min). The reaction products were separated by TLC as already reported [7]. The FFA and *sn*-2 monoacylglycerol bands were scraped off the plate and transmethylated. The procedure was checked by comparing the FA composition of the original TAG and those remaining after partial hydrolysis.

The positional distribution of FA in each of the PE, PC and PI samples isolated by preparative TLC was determined by phospholipase A_2 hydrolysis [13]. Briefly, 3–7 mg of each PL was suspended in 0.5 mL of 0.25 M Tris buffer (pH 7.5) containing 0.01 M $CaCl_2$ in a 10 mL Erlenmeyer flask. To this suspension, 5 mg of phospholipase A_2 and 10 mL of diethyl ether were added. The reaction mixture was incubated at 28 °C for up to 10 h with continuous shaking under a nitrogen atmosphere. The hydrolysis was almost complete (>98%) in this period, as judged from a preliminary experiment using a standard phospholipid (L-3-phosphatidylcholine 1-palmitoyl-2-oleoyl; Sigma, Chemical Co. St. Louis, MO, USA). Diethyl ether was evaporated under nitrogen, and samples were extracted with chloroform/methanol (2:1, vol/vol). To obtain the different lipid fractions, the lipid extracts were subjected to one-dimensional TLC with chloroform/methanol/deionized water (65:25:4, by volume). The spots were visualized with iodine vapor, and the bands corresponding to the free FA and lysophospholipids were separately scraped into text tubes. The constituent FA were analyzed with GC after methylation as described above.

Statistical Analysis

All preparations and determinations were carried out in triplicate, and the data were subjected to analysis of variance. Analysis variance was performed using the ANOVA procedure [14]. Multiple comparison tests were performed to determine any significant differences ($P < 0.05$) among treatments [15].

Results and Discussion

The major chemical components were as follows: moisture 4.2–4.8%, fat 2.1–3.7%, and protein 21.4–23.1%. There were small significant differences ($P < 0.05$) in these contents among the four cultivars.

Tocopherol Distribution

Most publications deal with tocopherol concentrations in soybean oil. There is little information in the literature about tocopherol concentrations in legume seeds [16]. There is no α-tocopherol noted in common bean (*Phaseolus vulgaris*) and kidney bean (*Phaseolus coccineus*). The amount of total tocopherols present in Usui and Gurume was 94.3–97.3 mg/kg seeds, followed by Kurumeyutaka and Nankaimidori (90.4–92.3 mg/kg seeds). As shown in Fig. 1, no significant differences ($P > 0.05$) in the individual tocopherol contents were observed among the four cultivars. The major tocopherol homologs in all cultivars was γ-tocopherol (>85.4%), and α- and δ-tocopherols were very small components (<6.7% for α and <8.0% for δ, respectively). Low levels of α- and δ-tocopherols have been reported in other seeds [17]. However, β-tocopherol was not detected by HPLC in all cultivars. γ-Tocopherol is a more potent antioxidant in oils than other tocopherols, but it has a lower vitamin E value in biological systems than α-tocopherol [17]. Therefore, γ-tocopherol in legume seeds would not be a function for the biological activity of vitamin E but that for the biological antioxidants [18].

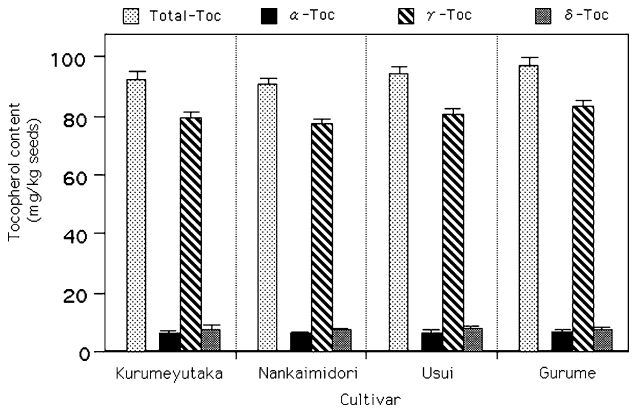

Fig. 1 Tocopherol (*Toc*) distributions in the oils obtained from peas. Each value shows the average of three replicates, and *vertical bars* depict the mean SD

Lipid Components

The lipid yields for the four cultivars ranged from 2.1 to 3.7%, and these values are in close agreement with the results reported by other researchers [19]. Peas are high in complex carbohydrates, protein, and fiber, yet are extremely low in fat. 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 acyl lipid classes in the peas are shown in Table 1. In all four cultivars, predominant components were PL (52.2–61.3%) and TAG (31.2–40.3%), with very little amounts of HC (0.5–0.9%), SE (0.8–2.4%), FFA (1.3–2.7%), 1,3-DAG (1.0–1.8%) and 1,2-DAG (1.0–2.2%). Therefore, these minor components were designated as "others" in Table 1. The distribution patterns for Kurumeyutaka and Nankaimidori were very similar and different to the other two cultivars (Usui and Gurume). As shown in Table 1, however, the amount of PL was always higher than those of TAG in all cultivars. These results agree with the fact that the peas are not oilseeds such as soybean, peanut or sesame seeds but typical vegetable seeds such as kidney beans [20].

FA Composition of Total Lipids and PL

FA compositions (expressed in terms of the esters by weight) of total lipids and PL in the peas were compared among the four cultivars (Fig. 2). The principal FA components of legumes are generally palmitic (15.4–18.2%), stearic (3.2–4.6%), oleic (22.5–28.0%), linoleic (48.7–50.9%) and linolenic (3.8–7.1%) acids [16, 21]. These FA distribution patterns were very similar to each other in both

Table 1 Lipid components in the oils prepared from pea seeds

Cultivar	Total lipids (mg)	Triacylglycerols (mg)	Phospholipids (mg)	Others (mg)
Kurumeyutaka	3457.0^{b}	1393.2^{c} (40.3)	1842.6^{b} (53.3)	221.2^{b} (6.4)
Nankaimidori	3419.8^{b}	1320.0^{b} (38.6)	1785.1^{b} (52.2)	314.6^{c} (9.2)
Usui	2086.6^{a}	651.0^{a} (31.2)	1279.1^{a} (61.3)	156.5^{a} (7.5)
Gurume	3702.4^{c}	1358.8^{b} (36.7)	2136.2^{c} (57.7)	207.3^{b} (5.6)

Each value is the average of three determinations and expressed as mg lipid per 100 g seeds. Values in parentheses are relative percentage content of the individual lipids in total lipids. Others include minor lipid components such as steryl esters, free fatty acids and diacylglycerols. Values in the same column with different letters are significantly different from those for individual varieties ($P < 0.05$)

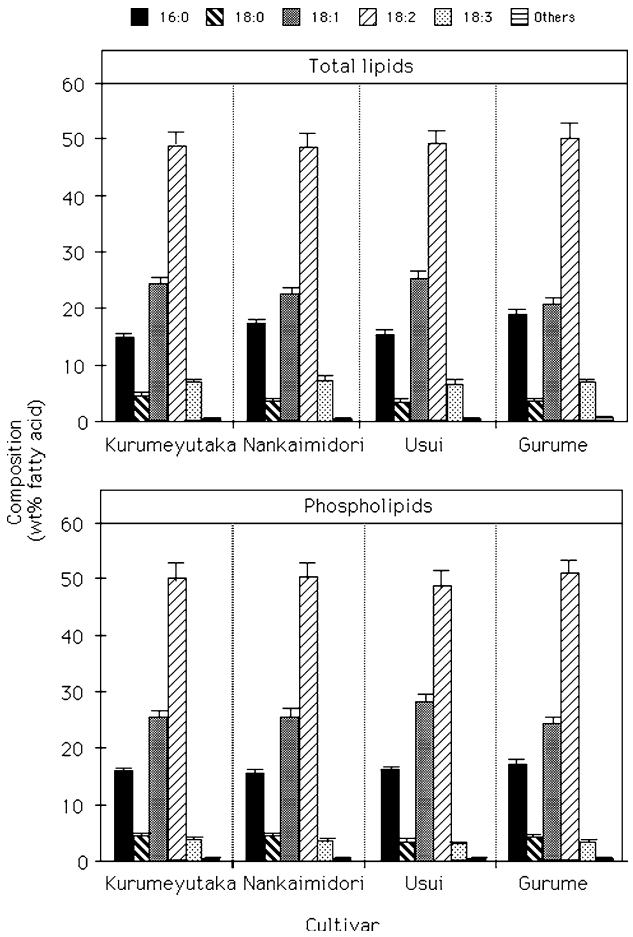

Fig. 2 Fatty acid distribution of total lipids and phospholipids obtained from peas. Each value shows the average of three replicates, and *vertical bars* depict the mean SD. Other minor FA include C14:0, C16:1, C17:0, C20 and C22:0

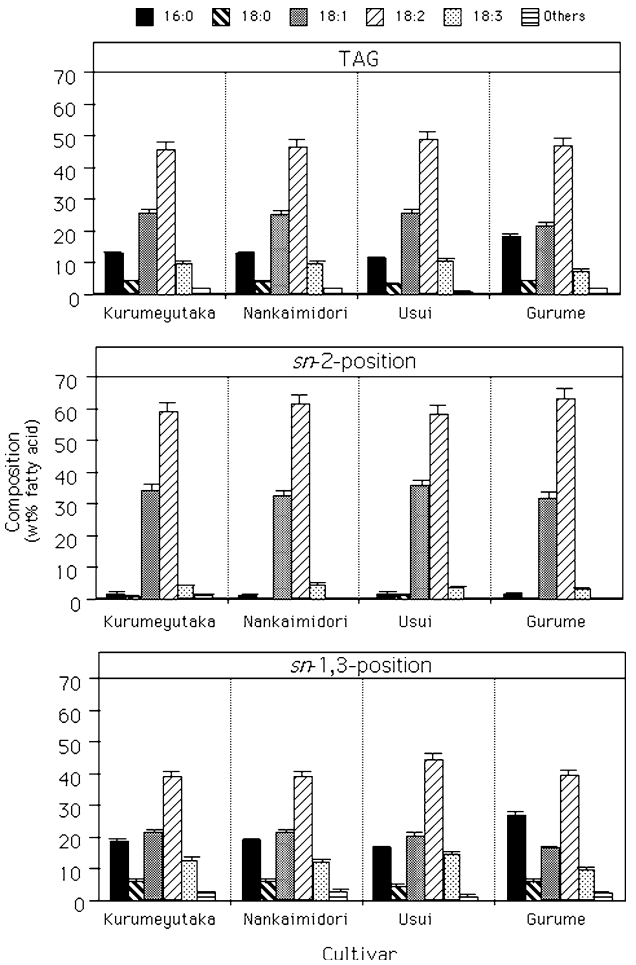

Fig. 3 Composition and positional distribution of FA of TAG obtained from peas. Each value shows the average of three replicates, and *vertical bars* depict the mean SD. Other minor FA include C14:0, C16:1, C17:0, C20:0 and C22:0

lipid classes among the four cultivars. All lipid samples had large amounts of total unsaturated FA (which consisted mainly of linoleic acid, followed by oleic acid), representing 77.8–81.0% of the total lipids and PL. With a few exceptions, no significant differences ($P > 0.05$) were observed in the FA distributions for both lipids among the four cultivars (Fig. 2).

Positional Distribution of FA in the TAG

The profiles of composition and positional distribution of FA in the TAG were compared among the four cultivars (Fig. 3). In general, no significant differences ($P > 0.05$) existed in the FA distributions for TAG among the four cultivars; the dominant component was linoleic, followed by oleic, palmitic, linolenic and stearic acids. Unsaturated FA such as linoleic and oleic were predominantly located in the *sn*-2 position of the TAG molecules, whereas saturated FA such as palmitic and stearic acids primary occupied the *sn*-1 or *sn*-3 position. No significant differences ($P > 0.05$) occurred in the FA distributions at the *sn*-2 position of TAG among the four cultivars. The positional distribution patterns in the FA of TAG were very similar to the results obtained from other seed oils such as corn and soybean [22, 23].

Positional Distribution of FA in the Major PL

To clarify the distribution of PL in peas, 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. Table 2 shows the profiles in the PL fraction of the peas. The original amounts of the each PL were approximately 226.4–437.9, 553.9–906.6 and 322.5–497.7 mg per 100 g seeds for PE, PC and PI, respectively. PC was detected as the dominant component (42.3–49.2%), followed by PI (23.3–25.2%) and then PE (17.7–20.5%).

Table 2 The content of major phospholipids in the oils obtained from pea seeds

Cultivar	Phosphatidylethanolamine (mg)	Phosphatidylcholine (mg)	Phosphatidylinositol (mg)	Others (mg)
Kurumeyutaka	333.5[b] (18.1)	906.6[c] (49.2)	429.3[b] (23.3)	173.2[b] (9.4)
Nankaimidori	332.0[b] (18.6)	864.0[b] (48.4)	448.1[b] (25.1)	141.0[a] (7.9)
Usui	226.4[a] (17.7)	553.9[a] (43.3)	322.3[a] (25.2)	176.5[b] (13.8)
Gurume	437.9[c] (20.5)	903.6[c] (42.3)	497.7[c] (23.3)	296.9[c] (13.9)

Each value is the average of three determinations and expressed as mg lipid per 100 g seeds. Values in parentheses are relative percentage content of the individual lipids in total lipids. Others include diphosphatidylglycerol, phosphatidic acid, and phosphatidylglycerol. Values in the same column with different letters are significantly different from those for the individual varieties ($P < 0.05$)

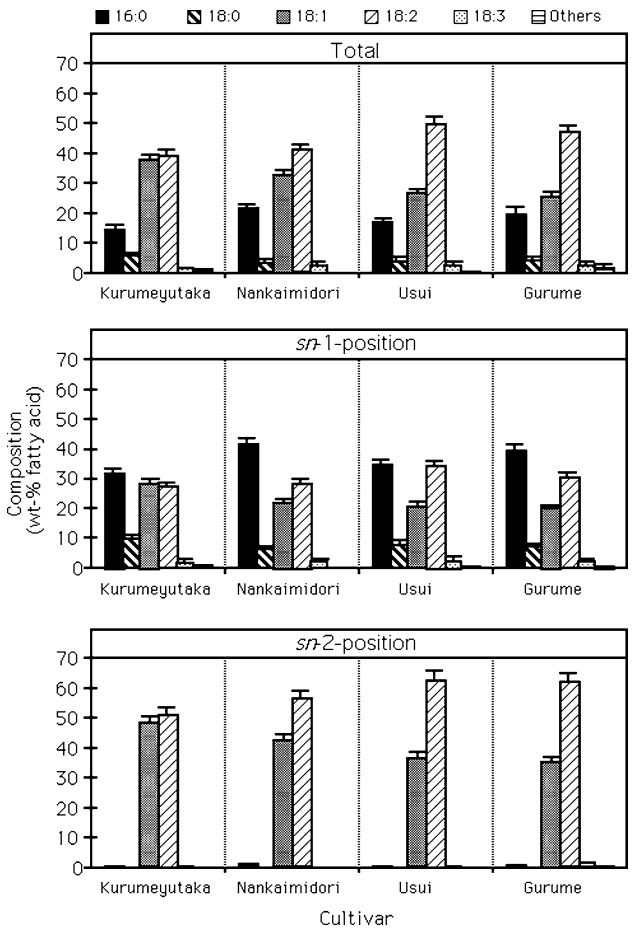

Fig. 4 Positional distribution of FA of PE obtained from peas. Each value shows the average of three replicates, and *vertical bars* depict the mean SD. Other minor FA include C14:0, C16:1, C17:0, C20:0 and C22:0

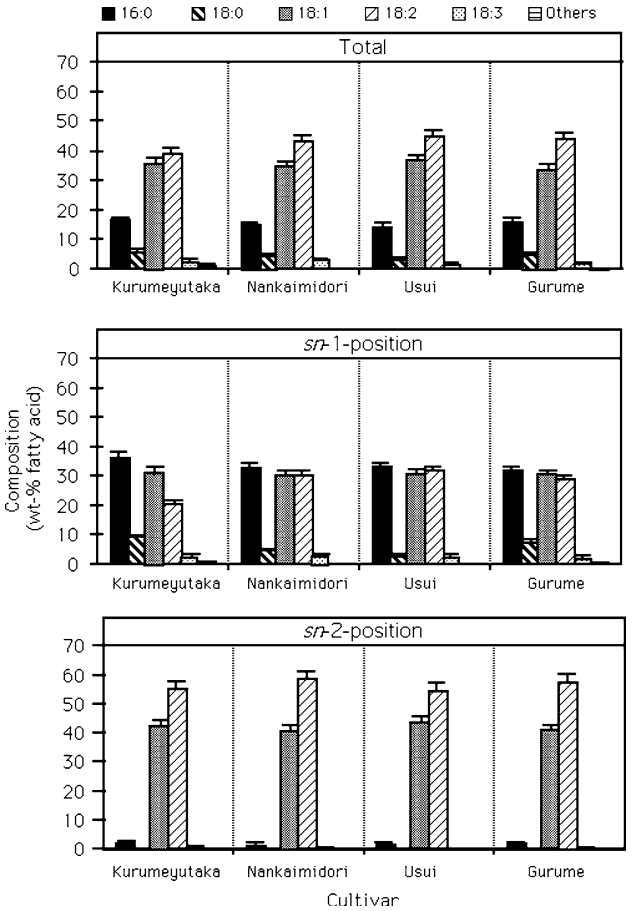

Fig. 5 Positional distribution of FA of PC obtained from peas. Each value shows the average of three replicates, and *vertical bars* depict the mean SD. Other minor FA include C14:0, C16:1, C17:0, C20:0 and C22;0

Peas are typical lower oil-bearing legumes and PL form the principal components of the cell membranes in the peas. 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 [24]. This may explain why the FA composition in newly differentiated tissue was conserved regardless of genotype.

The profiles of composition and positional distribution of FA in the PE, PC and PI were compared among the four cultivars (Figs. 4, 5, 6). The major FA in the three PL were commonly palmitic, stearic, oleic, linoleic and linolenic acids. There were significant differences ($P < 0.05$) in the positional distribution of FA in PE among the four cultivars. Moreover, PI was unique in that it had the highest saturated fatty acid content among the three PL. The concentration of saturated acids in the *sn*-1 position of PI was

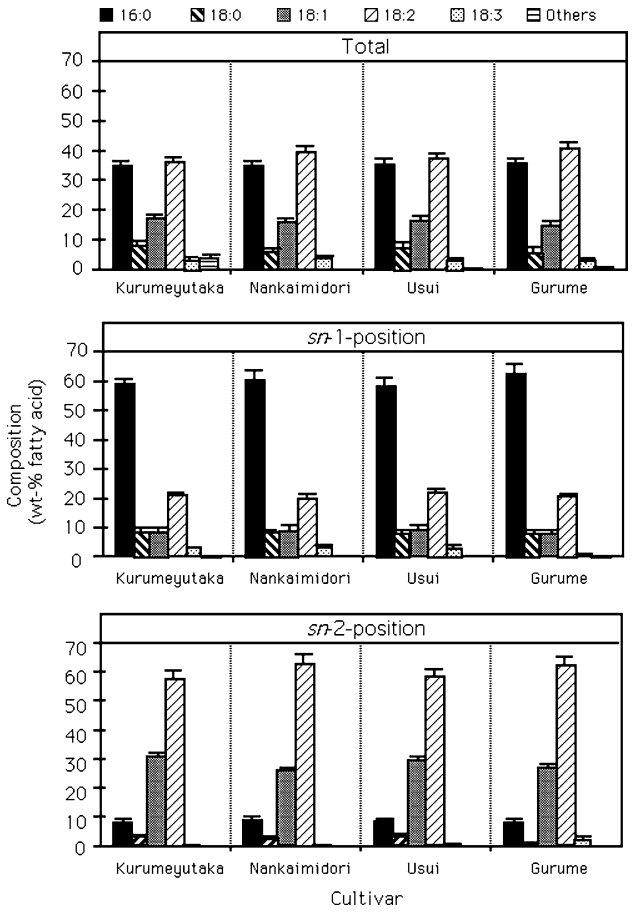

Fig. 6 Positional distribution of FA of PI obtained from peas. Each value shows the average of three replicates, and *vertical bars* depict the mean SD. Other minor FA include C14:0, C16:1, C17:0, C20:0 and C22:0

markedly higher than that of PE or PC. However, the positional distribution of FA in PE, PC and PI was observed in all PL:saturated FA were mostly located in the *sn*-1 position whereas unsaturated FA predominantly occupied the *sn*-2 position of these molecules. These results were similar to the positional FA distributions in TAG or PL among the plant seeds reported in the literature [25, 26].

Consequently, the major lipid components in the peas were PL and TAG, whereas the other were also present in minor proportions. γ-Tocopherol was present in the highest concentration, and α- and δ-tocopherols were very small amounts. The principal characteristics of FA distributions in the TAG were evident among the four cultivars; unsaturated FA, especially linoleic and oleic acids, were predominantly concentrated in the *sn*-2-position, and saturated FA, especially palmitic and stearic acids, primarily occupied the *sn*-1 or *sn*-3 position in the oils. The positional distribution of FA in PE, PC and PI was also observed in all PL:unsaturated FA predominantly occupied the *sn*-2 position. It seems that the four cultivars were highly related to each other with regard to the FA distribution pattern of the TAG and PL as well as the tocopherol contents. These results should be useful to both producers and consumers for manufacturing peas foods.

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References

1. Adsule RN, Kadam SS (1989) Proteins. In: Salunkhe DK, Kadam SS (eds) Handbook of world food legumes: nutritional chemistry, processing technology and utilization, vol II, edn. CRC, Boca Raton, pp 75–97
2. Hedley CL (2001) Carbohydrates in grain legume seeds. Improving nutritional quality and agronomic characteristics. CABI Publishing, Wallingford
3. Paul AA, Southgate DAT (1988) The composition of foods, 4th edn. In: McCanc RA, Widdwson EM (eds) Elsevier Applied Science, Amsterdam, pp 175–177
4. Wang N, Daun JK (2004) Effect of variety and crude protein content on nutrients and certain antinutrients in field peas (*Pisum sativum*). J Sci Food Agric 84:1021–1029
5. Vidal-Valverde C, Frias J, Hernandez A, Martin-Alvarez PJ. Sierra I, Rodriguez C, Blazquez I, Vicente G (2003) Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*) seeds. Ibid 83:298–306
6. Wang XF, Warkentin TD, Briggs CJ, Oomah BD, Campbell CG, Woods S (1998) Trypsin inhibitor activity in field pea (*Pisum sativum* L.) and grass pea (*Lathhyrus sativus* L.). J Agric Food Chem 46:2620–2623
7. Yoshida H, Alexander J (1983) Enzymatic hydrolysis in vitro of thermally oxidized sunflower oil. Lipids 18:611–616
8. AOAC (1984) Official methods of analysis. Association of Official Analytical Chemists, Washington
9. Folch J, Lee M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
10. Yoshida H, Takagi S (1997) Effect of seed roasting temperature and time on the quality characteristics of sesame (*Sesamum indicum*) oil. J Sci Food Agric 75:19–26
11. Yoshida H, Tomiyama Y, Mizushina Y (2005) Lipid classes, fatty acid composition and triacylglycerol molecular species of kidney beans (*Phaseolus vulgaris* L.). Eur J Lipid Sci Technol 107:307–315
12. AOCS (1992) Official methods Ce 1b-89. Fatty acid composition of GLC. Marine oils. AOCS, Champaign
13. Yoshida H, Abe S, Hirakawa Y, Takagi S (2001) Roasting effects on fatty acid distributions of triacylglycerols and phospholipids in sesame (*Sesamum indicum*) seeds. J Sci Food Agric 81:620–626
14. Steel RCD, Torrie JH, Dickey DA (1997) Principles and procedures of statistics. 3rd edn. McGraw-Hill, New York
15. Baker BJ (1980) Multiple comparison tests. Can J Plant Sci 60:325–327
16. Savage GP (1988) The composition and nutritive value of lentils (*Lens culinaris*). Nutr Abst Rev A 59:66–88

17. Grela ER, Gunter KF (1995) Fatty acid composition and tocopherol content of some legume seeds. Anim Feed Sci Technol 52:325–331
18. Tsuda T, Osawa T, Nakayama T, Kawakishi S, Ohshima K (1993) Antioxidant activity of pea bean (*Phaseolus vulgaris* L.) extract. J Am Oil Chem Soc 70:909–913
19. Daveby YD, Abrahamsson M, Aman P (1993) Change in chemical composition during development of three different types of peas. J Sci Food Agric 63:21–28
20. Lee KG, Mitchell AE, Shibamoto T (2000) Determination of antioxidant properties of aroma extracts from various beans. J Agric Food Chem 46:4817–4820
21. Miyazawa T, Ito S, Fujino Y (1975) Fatty acid composition of glycerides and stereospecific analysis of triglyceride in pea seeds. J Nutr Sci Vitaminol 21:137–142
22. Arcos JA, Garcia HS, Hill CG Jr (2000) Regioselective analysis of the fatty acid composition of triacylglycerols with conventional high-performance liquid chromatography. J Am Oil Chem Soc 77:507–512
23. Slabas AR, Simon JW, Elborough KM (1995) Information needed to create new oil crops. Int News Fats Relat Mater 6:159–166
24. Alvarez-Ortega R, Cantisan S, Martinez-Force E, Garces R (1997) Characterization of polar and nonpolar seed lipid classes from highly saturated fatty acid sunflower mutants. Lipids 32:833–837
25. Miyazawa T, Fujino Y (1976) Phospholipids in pea seed. Nippon Nogeikagaku Kaishi 50:169–174
26. Vichi S, Pizzale L, Conte LS (2007) Stereospecific distribution of fatty acids in triacylglycerols of olive oils. Eur J Lipid Sci Technol 109:72–78