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Profiles of Lipid Components, Fatty Acid Compositions and Triacylglycerol Molecular Species of Adzuki Beans (*Vigna angularis*)

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Abstract Fatty acid (FA) compositions and molecular species of triacylglycerols (TAG) isolated from total lipids extracted from adzuki beans (*Vigna angularis*) were determined with a combination of $AgNO_3$-TLC and GC, and were compared in relation to the content of endogenous antioxidants analyzed by HPLC. δ-Tocopherol was present in the highest concentration (53.7–89.3 mg/kg), and γ-tocopherol in small amounts (11.2–14.8 mg/kg). The main lipid components were phospholipids (72.2–73.4%) and TAG (20.6–21.9%), whilst other components were also present in minor proportions (0.1–3.4%). Eighteen different TAG molecular species were identified and quantified by successive applications of $AgNO_3$-TLC and GC. The main components were SMD (4.6–5.0%), S_2T (13.4–16.4%), SD_2 (11.8–14.3%), SMT (7.3–8.3%), SDT (9.9–10.6%), D_3 (6.9–7.9%), MT_2 (5.2–6.3%), D_2T (7.0–11.2%), DT_2 (7.4–7.6%) and T_3 (6.2–7.2%) (where S, M, D, and T denote a saturated FA, a monoene, a diene, and a triene, respectively). No marked difference ($P > 0.05$) in the molecular species composition could be observed among the five cultivars. The results could be useful to both consumers and producers for manufacturing traditional adzuki confectionaries in Japan and elsewhere.

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Introduction

Some beans are used as staple foods in many countries and are attracting increasing attention for protection against coronary heart disease [1]. Adzuki beans (*Vigna angularis*) are a very important food material in the Far East. They have been used in traditional Chinese medicine for various purposes, e.g., as a diuretic, an antidote, and a remedy for dropsy and beriberi, but mainly for the production of traditional confectionaries (*wagashi*), e.g., *youkan, manju*, and *amanatto* [2, 3], in Japan. Adzuki or small red beans serve as a rich source of carbohydrate, protein, fiber, vitamins and minerals [4, 5]; however, they also contain antinutritional factors. Therefore, when adzuki beans are used for confectionaries, they are boiled in a cooker and yield a hot water extract as a by-product. The extract is known to contain active ingredients [6], but is washed. It has been reported that the 40% ethanol fraction of the hot-water extract from adzuki beans suppresses not only the proliferation of human stomach cancer cells in culture but also benzo(α)pyrene-induced tumorigenesis in the mouse forestomach [7, 8]. Wu et al. [9] have shown recently that a water-soluble extract of the adzuki beans could inhibit acetaminophen-induced liver damage. Han et al. [10] have reported the protective action of an adzuki extract against acetaminophen-induced hepatotoxicity via a hepatic γ-glutamycysteinylglycine (GSH)-mediated antioxidation/detoxification system in rat liver after 4 weeks of feeding.

However, a literature search revealed that there is limited information on the lipid component and fatty acid (FA)

distribution of adzuki beans. The aim of the present study was to determine with respect to the tocopherol homologues, lipid class composition, the FA profile and the molecular species of triacylglycerols (TAG) from *V. angularis* beans, in an attempt to compare the composition and quality characteristics of the oils among the five cultivars.

Materials and Methods

Adzuki Beans

The mature adzuki beans (*V. angularis*) used in this study were from the five different Japanese cultivars—*Erimo, Otome, Roman, Akane* and *Toyomi*. These beans were harvested at Tokachi, Hokkaido in Japan during the summer of 2007. They were furnished by the Hokkaido Tokachi Area Regional Food Processing Technology Center. These beans were selected for uniformity based on a bean weight of 112–153 mg for *Eriomo* and *Otome,* 128–165 mg for *Roman,* 170–216 mg for *Akane* and 225–262 mg for *Toyomi,* respectively. The beans were hand-selected to eliminate those that were cracked or otherwise damaged. Beans of each cultivar were divided into groups and stored in separate stainless steel containers at 4 °C prior to the experiments.

Reagents and Standards

All solvents and chemicals used were of analytical grade (Nacalai Tesque, Kyoto, Japan), but diethyl ether was further purified to remove peroxides. TLC plates (silica-gel 60G F_{254}, 20 × 20 cm, 0.25 mm thickness) were obtained from Merck (Darmstadt, Germany). Vitamin E homologues (α, β, γ and δ) were obtained from the Eisai Co. (Tokyo, Japan). All tocopherols were of the D-form (*RRR*-), and their purities were better than 98.8% as determined by HPLC using 2,2,5,7,8-pentamethyl-6-hydroxychroman as the internal standard. The TLC standard mixture, containing monoacylglycerols (MAG), diacylglycerols (DAG), free fatty acids (FFA), triacylglycerols (TAG), steryl esters (SE) and hydrocarbons (HC), was obtained from Nacalai Tesque (Kyoto, Japan). Standard TAG (glycerol trimyristate, glycerol tripalmitate, glycerol tristearate, glycerol trioleate, glycerol trilinoleate and glycerol trilinolenate) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

FA methyl ester (FAME) standards (F & OR mixture #3) were obtained from Altech-Applied Science (State College, PA, USA). Methyl pentadecanoate (C15:0, 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 FAME.

Chemical Analysis

The AOAC [11] methods were used to determine the chemical composition of the beans. Samples were determined in triplicate for fat, protein and moisture content. Fat content was analyzed 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).

Extraction of Lipids

Adzuki beans (500 seeds) 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 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 KCl (0.75%) were added [12], and the phases were mixed vigorously. After phase separation, the chloroform layer was withdrawn, dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under vacuum. The extracted lipids were weighed to determine the lipid content of the beans and then transferred into a 25-mL brown glass volumetric flask with chloroform/methanol (2:1, v/v).

Analysis of Tocopherols

The tocopherols in the oils was analyzed by HPLC as described earlier [13]. The lipids (100 mg) were carefully transferred into a 2-mL volumetric flask, and the solvents were vaporized under a nitrogen stream at ambient temperature in a draft chamber. The residue was dissolved in mobile phase (*n*-hexane/1,4-dioxane/ethanol, 490:10:1, by volume) used for HPLC analysis. An aliquot (2 μL) from this sample solution was injected using the same method as described earlier [13], and the amount of each tocopherol was monitored with a fluorescence detector (Shimadzu RF-10 AXL, Kyoto, Japan) set at 295 nm excitation wavelength and 320 nm emission wavelength, and were quantified as previously described [13].

Lipid Analysis

The total lipids were fractionated by TLC into eight fractions [14]. Bands corresponding to HC, SE, TAG, FFA, 1,3-DAG, 1,2-DAG, MAG and PL were scraped off into test-tubes [105 × 16 mm; poly (tetrafluoroethylene)-coated screw caps]. Methyl pentadecanoate (10–100 μg) from a standard solution (5 mg/mL) was added to each tube as the internal standard with a 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 [15]. After cooling, 5 mL of *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 Na_2SO_4. The solvent was then vaporized under a gentle stream of nitrogen, and the residue (FAME) was determined by GC using a Shimadzu Model-14B GC (Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector (FID) and a capillary column (ULBO HE-SS-10 for FAME fused silica WCOT [serial no. PSC5481], cyanopropyl silicone, 30 m × 0.32 mm i.d.; Shinwa Chem. Ind., Ltd., Kyoto, Japan).

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. The oven temperature was programmed from an initial temperature of 180 °C (5 min hold), rising at 2 °C/min to 200 °C, and held isothermally (200 °C) for 15 min. The injection and detector temperatures were maintained at 230 and 250 °C, respectively. Identification was made by comparison of retention times to those of standard FAME. The other GC conditions were as described previously [14].

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 vol) as the mobile phase. PL classes were detected by iodine vapor and were consistent with the authentic standards. Bands corresponding to phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl inositol (PI) and others were carefully scraped off into test tubes. Then, FAME were prepared by the same method as described above and determined by gas chromatography (GC).

TAG Analysis

The TAG isolated by TLC was directly analyzed by GC according to the previously mentioned method [16], using a Shimadzu Model-14A GC equipped with a FID. A glass column (50 cm × 3.0 mm i.d.; Shimadzu, Kyoto, Japan) was packed with 2.0 wt.% OV-17 (phenyl methyl silicone; Nishio Co., Tokyo, Japan) supported on 80/100 mesh silanized Shimalite W. Helium was used as the carrier gas at a flow rate of 50 mL/min. The injection and detection port temperatures were set at 320 and 350 °C, respectively. The initial oven temperature was 285 °C. This temperature maintained for 5 min and then increased at a rate of 2 °C/min to 320 °C, which was held isothermally (320 °C) for 20 min. All samples were dissolved in *n*-hexane for injection. TAG peaks were identified and quantified by addition of a known weight (50 mg) of glyceryl trimyristate (trimyristin) as the internal standard, using an electronic integrator (Shimadzu C-R6A, Kyoto, Japan).

TAG Species Composition

Molecular species isolation from total TAG was carried out by silver nitrate/silica gel TLC according to the previously mentioned method [17]. TAG classes differing in unsaturation were isolated by $AgNO_3$-TLC using 1.5–4.0% (v/v) methanol in chloroform, depending on the differences in their degree of unsaturation [18]. In the case of adzuki TAG, three solvents of increasing polarity were required to isolate the lipid classes. The plates (20 × 20 cm) were streaked with 10–15 mg TAG using a microsyringe (Hamilton Co., Reno, NV, USA), developed by 1.5% (v/v) methanol in chloroform, and S_3, S_2M, S_2D, SM_2, M_3 and SMD (where S, M, and D denote a saturated FA, a monoene, and a diene, respectively) were easily isolated. The second TAG molecular species such as S_2T, SD_2, SMT, M_2D, MD_2 and SDT (where T denotes a triene) were isolated by developing the plate with 2.5% (v/v) methanol in chloroform. Finally, the most difficult to isolate were D_3, MDT, MT_2, D_2T, DT_2 and T_3, and these were separated by developing the plate using 4.0% (v/v) methanol in chloroform. To obtain a good separation, it was very important to use a small sample size (15 mg/TLC). This system was varied according to the temperature and humidity conditions.

Individual TAG bands were detected by spraying with 0.1% 2′,7′-dichlorofluorescein (Nacalai Tesque, Kyoto, Japan) in methanol and viewed under ultraviolet (254 or 365 mm) radiation. Each TAG subfraction was identified by comparison with the R_f values of a TAG standard. Bands were recovered from the plates by extraction with 3.5% aqueous HCl in the purified diethyl ether. The combined extracts with the diethyl ether were purified by alumina column chromatography (30 × 5.0 mm i.d., alumina column; Biomedicals, Eschwege, Germany) to remove the 2′,7′-dichlorofluorescein. In preliminary repeating experiments, it was confirmed that each TAG class was fully recovered (>98.0%) by passing TAG standards through the alumina column using this procedure.

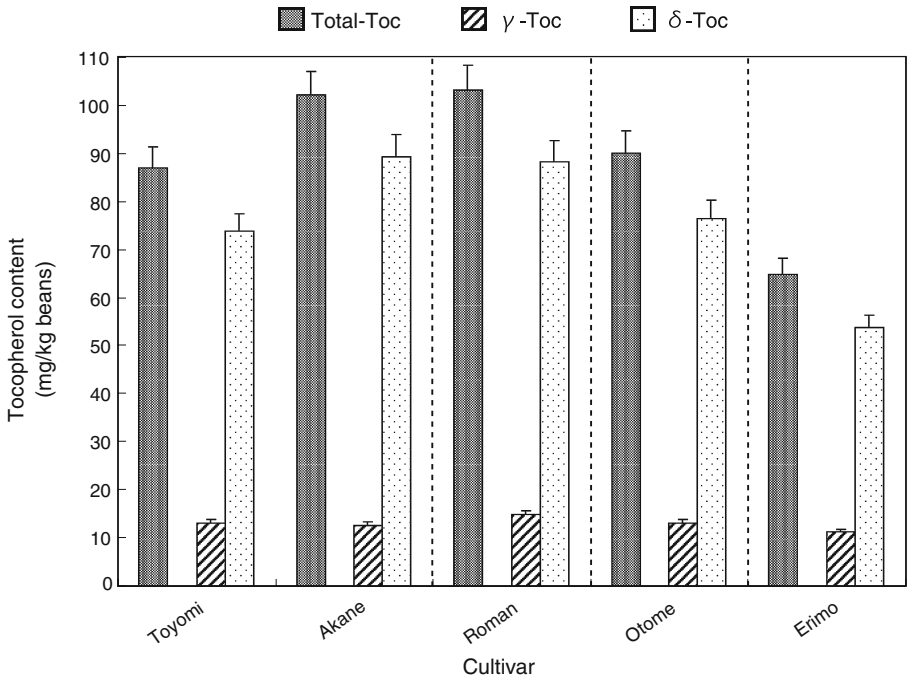

Fig. 1 Tocopherol contents in adzuki beans. Each value represents the average of three determinations. *Vertical bars* depict the standard error of the replicates

The solvent was then vaporized in small glass tubes under a gentle stream of nitrogen. Methyl pentadecanoate (10–50 μg) of the standard solution (2–10 μL) was added to each tube as the internal standard. Relative amounts of each TAG subfraction were quantified by GC as described in the preceding paragraphs and then by comparison of the FAME within each TAG fraction containing the internal standard.

Statistical Analyses

All preparations and measurements were performed in triplicate. Statistical analysis was performed using 2005 SAS (Version 9.1, SAS institute Inc. Cary, NC). Analysis of variance (ANOVA) was conducted, and Duncan's multiple range tests were used to determine significant differences at $P < 0.05$.

Results and Discussion

Tocopherol Composition

The main components were as follows: moisture 3.5–3.8%, fat 1.4–1.7% and protein 20.3–21.7% (dry basis), respectively. Adzuki beans are high in complex carbohydrates, protein, and fiber, yet are extremely low in fat [4, 5]. Previous studies [19] reported that vitamin E may reduce the risk of coronary heart disease by its strong biological antioxidant function. The tocopherol contents in the oils obtained from adzuki beans are illustrated in Fig. 1. The main tocopherol was δ-tocopherol (53.7–89.3 mg/kg), and γ-tocopherol (11.2–14.8 mg/kg) was a minor component. δ-Tocopherol contents varied significantly ($P < 0.05$) between the five cultivars as follows: *Akane* ≒ *Roman* > *Toyomi* ≒ *Otome* > *Erimo*. On the other hand, no significant difference ($P > 0.05$) was observed in γ-tocopherol content among the five cultivars. However, α- and β-tocopherols were not detected in adzuki beans. These distributions are very unique patterns and differ from other plant seeds such as peanuts [20] in which α- and β-tocopherols are usually detected. Contents of α- and γ-tocopherols decreased during the maturing period, whereas δ-tocopherol increased. δ-Tocopherol and γ-tocopherol are precursors to α- and β-tocopherols, respectively [21], but the factors that modulate transformation from one form to the other are not clear.

Lipid Class Composition

The compositional analyses carried out in this study included determination of the lipid classes and the FA compositions of the oils. Profiles of the lipid classes in the beans are shown in Table 1. Predominant components were PL (72.2–73.4%) and TAG (20.6–21.9%), followed by SE (2.4–3.3%), accompanied by very small amounts (0.2–1.8%) of others. No remarkable difference ($P > 0.05$) in the lipid components could be observed between the values estimated by a combination of TLC and GC using the internal standard (C15:0). The adzuki beans are not oil seeds but typical vegetable seeds [22]. Therefore, the PL content is quite significant, whilst glycolipids are present

Table 1 Lipid components in the oils obtained from adzuki beans[a]

Lipid class	Cultivar				
	Toyomi	*Akane*	*Roman*	*Otome*	*Erimo*
Hydrocarbons	23.7 (0.17)[a]	24.0 (0.17)[a]	56.8 (0.17)[c]	55.2 (0.34)[c]	47.1 (0.30)[b]
Steryl esters	373.5 (2.73)[a]	465.8 (3.33)[b]	433.0 (3.33)[b]	385.0 (2.37)[a]	392.5 (2.50)[a]
Triacylglycerols	2,999.8 (21.90)[a]	2,882.5 (20.60)[a]	3,608.1 (20.60)[c]	3,342.5 (20.58)[b]	3,343.7 (21.30)[b]
Free fatty acids	219.2 (1.60)[a]	237.9 (1.70)[b]	246.7 (1.70)[b]	277.2 (1.71)[c]	282.6 (1.80)[c]
1,3-Diacylglycerols	68.5 (0.50)[a]	84.0 (0.60)[b]	83.5 (0.60)[b]	81.5 (0.50)[b]	94.2 (0.60)[c]
1,2-Diacylglycerols	95.9 (0.70)[a]	111.9 (0.80)[b]	133.6 (0.80)[c]	146.7 (0.90)[d]	109.9 (0.70)[a,b]
Monoacylglycerols	27.4 (0.20)[a]	56.0 (0.40)[d]	50.0 (0.40)[c]	31.5 (0.19)[b]	47.0 (0.30)[c]
Phospholipids	9,889.2 (72.20)[a]	10,130.7 (72.40)[a,b]	12,027.0 (72.40)[c]	11,925.1 (73.41)[b,c]	11,381.1 (72.50)[b]

[a] Each value is the average of three determinations and expressed as mg lipid per kg beans. Values in parentheses are relative % content of individual lipids. Values in a low with different letters are significantly different between individual cultivars ($P < 0.05$)

Table 2 Major phospholipids in the oils obtained from adzuki beans[a]

Phospholipid	Cultivar				
	Toyomi	*Akane*	*Roman*	*Otome*	*Erimo*
Phosphatidyl ethanolamine	2,304.2 (23.3)[a]	2,380.7 (23.5)[a]	2,850.4 (23.7)[b]	2,945.5 (24.7)[c]	2,708.7 (23.8)[b]
Phosphatidyl choline	4,489.7 (45.4)[a]	4,680.4 (46.2)[a]	5,640.7 (46.9)[c]	5,461.7 (45.8)[b,c]	5,303.6 (46.6)[b]
Phosphatidyl inositol	2,551.4 (25.8)[a]	2,492.2 (24.6)[a]	2,946.6 (24.5)[b]	2,945.5 (24.7)[b]	2,811.3 (24.7)[b]
Others	543.9 (5.5)[a]	577.4 (5.7)[b,c]	589.3 (4.9)[c]	572.4 (4.8)[b,c]	557.7 (4.9)[a,b]

[a] Each value is the average of three determinations and expressed as mg lipid per kg beans. Values in parentheses are relative % content of individual lipids. "Others" include diphosphatidylglycerol, phosphatidic acid and phosphatidylglycerol. Values in a row with different letters are significantly different between individual cultivars ($P < 0.05$)

only trace amounts, indicating that PL forms the principal components of the cell membranes in the beans.

Table 2 shows the compositions of PL components among the five cultivars of adzuki beans. Similar distributions were shown in all PL, in which the original amounts of each PL were approximately 4,490–5,641 mg/kg (45.4–46.9%), 2,492–2,947 mg/kg (24.6–25.8%), 2,304–2,946 mg/kg (23.3–24.7%) and 544–589 mg/kg (4.8–5.7%) for PC, PI, PE and others, respectively. It is generally known that these PL are the essential components of the cell membranes in plants. Because 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 [23].

FA Composition of the Main Lipids

FA compositions of the main lipid components in the beans are illustrated in Fig. 2. The principal FA components of legumes are generally palmitic (16:0), stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, the composition of which varies according to these lipid classes. Moreover, long-chain saturated FA (20:0 and 22:0) were also detected at low percentages (0.8–3.2%) in these lipids. The samples had high amounts of total unsaturated FA (which consisted mainly of linoleic (18:2n-6) acid, followed by α-linolenic (18:3n-3) and oleic (18:1n-9) acids), representing 70.6–73.8%, 70.0–72.2% and 69.9–72.6% for total lipids, TAG and PL, respectively. In generally, the compositional distributions of FA in the individual lipid classes were very similar to each other among the five cultivars.

Some significant differences ($P < 0.05$) in the FA composition were found when comparing the three lipid classes. With a few exceptions, the percentage of palmitic acid (16:0) was higher ($P < 0.05$) in the PL, whilst α-linolenic acid (18:3n-3) was higher ($P < 0.05$) in the TAG as illustrated in Fig. 2. However, the percentage of linoleic acid (18:2n-6) was less ($P < 0.05$) in the TAG than that in the total lipids or PL. These characteristics in the FA distributions are not similar to the results reported in typical vegetable seeds such as kidney beans [22] or pea seeds [24]. The data for FA distributions of minor lipid components (<3.5%), such as SE, FFA, 1,3-DAG or 1,2-DAG and MAG as shown in Table 1, were omitted from Fig. 2 because the samples were too much small to obtain reliable results for these lipids.

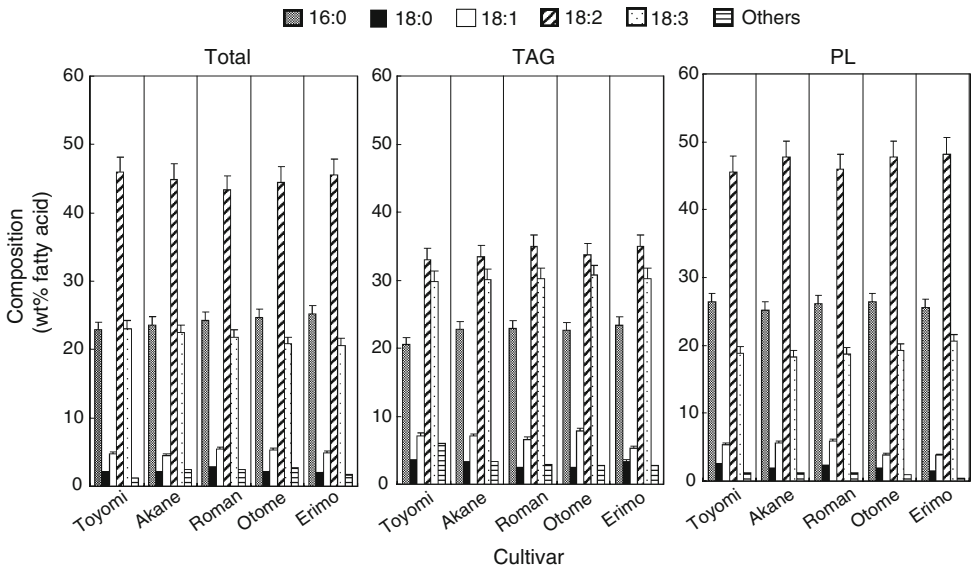

Fig. 2 Fatty acid distributions of major lipid components prepared from adzuki beans. Each value is the average of three replicates, and *vertical bars* depict the standard error of the replicates. Others minor fatty acids include 14:0, 16:1, 20:0 and 22:0

TAG Composition

The total carbon numbers (TCN) of FA in the TAG of the five adzuki cultivars ranged from 48 to 60 (Fig. 2). For example, in the case of tristearin, the TCN is 54. Predominant components consisted of 52 (11.8–12.6%), 54 (50.2–51.7%) and 56 (30.4–32.8%) TCN, followed by small amounts of 50 (1.2%), 58 (2.0–2.6%) and 60 (1.0–1.6%) TCN (data not shown). The 58 or 60 in the TAG suggested the presence of long-chain FA such as 20:0 and 22:0. These distribution patterns differ from the results reported previously for kidney beans [22]. These values may be due to the qualitative and quantitative differences of the TAG (Table 1) and molecular species of TAG (Fig. 3) among the five cultivars.

The FA compositions of the individual bands isolated by $AgNO_3$-TLC were determined by GC. According to these results, the distribution patterns of the individual TAG molecular species are illustrated in Fig. 3. Eighteen different molecular species were detected in the oil extracted from these adzuki beans. The three-letter designation does not suggest FA positional isomers in the TAG:P, palmitic (16:0); St, stearic (18:0); O, oleic (18:1n-9): L, linoleic (18:2n-6); and Ln, α-linolenic (18:3n-3) FA moieties. These molecular species were arranged according to the degree of unsaturation on the acyl chain-length of TAG (from left to right in Fig. 3). The main TAG species were SMD (POL or StOL), S_2T (PPLn or PStLn or StStLn), SD_2 (PLL or StLL), SMT (POLn or StOLn), SDT (PLLn or StLLn), D_3 (LLL), MT_2 (OLnLn), D_2T (LLLn), DT_2 (LLnLn) and T_3 (LnLnLn), followed by S_2M (PPO or PStO or StStO), S_2D (PPL or PStL or StStO), MD_2 (OLL) and MDT (OLLn). On the other hand (S_3: PPP or PPSt or PStSt or StStSt; SM_2: POO, StOO; M_3: OOO; and M_2D: OOLn) were minor components (less than ca. 56 mg: <2.0%). With a few exceptions, no remarkable difference ($P > 0.05$) in the molecular species composition could be observed among the five cultivars. Therefore, the data for *Akane* and *Erimo* were omitted from Fig. 3. However, these distribution patterns in the TAG molecular species differ from the results reported in typical vegetable seeds such as broad beans [14] or kidney beans [22].

Table 3 shows the FA contents (S, M, D and T) in the TAG isolated from the five cultivars of adzuki beans, expressed as milligram FA within the TAG in beans (1.0 kg) according to their degree of unsaturation on the acyl chain-length of the FA moieties. Briefly, the amounts of individual FA were summed up as S (14:0, 16:0, 18:0, 20:0 and 22:0), M (16:1n-9 and 18:1n-9), D (18:2n-6) and T (18:3n-3) from the results obtained by GC using methyl pentadecanoate as the internal standard. The theoretical contents of FA were calculated from the relative percentages of each TAG species based on the data in Fig. 3, and their distributions of each FA, which comprised the experimental value (Fig. 2: TAG). There were no qualitative or quantitative differences ($P > 0.05$) in the distribution patterns between the experimental and calculated (theoretical) values (data not shown).

The main components in adzuki beans were PL and TAG, whilst other lipid components were also present in minor proportions—less than 3.3%. δ-Tocopherol was present in the highest concentration and γ-tocopherol was detected in minor amounts. Tocopherol homologues may be directly associated with changes in the oil quality that are mediated by genetic and environmental influences on the concentration of α-linolenic (18:3n-3) acid [25]. The five cultivars contained linoleic (18:2n-6) and α-linolenic (18:3n-3) acids as their predominant FA. Thus, consumption

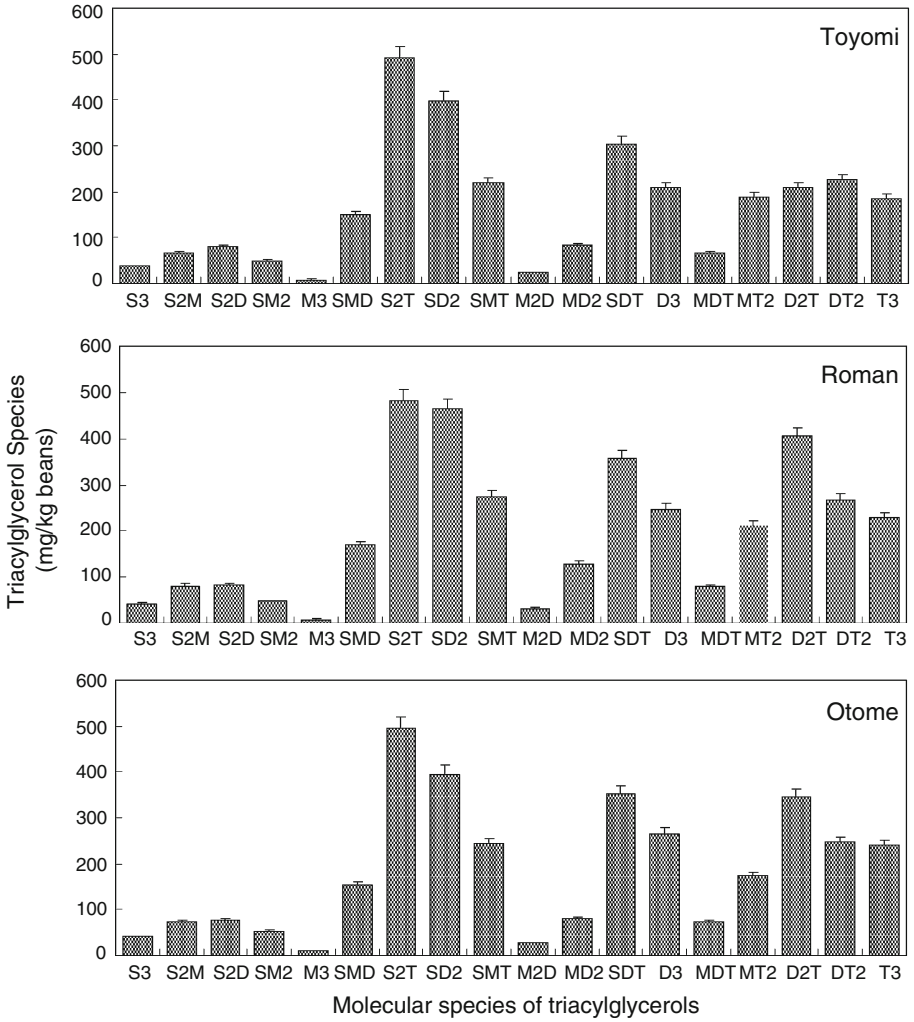

Fig. 3 Characteristics of the major molecular species of TAG prepared from adzuki beans. Saturated FA (S) consist of myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0) and behenic (22:0) acids. Unsaturated FA, palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3), are denoted as monoene (M), diene (D), and triene (T), respectively. Each value represents the average of three determinations. *Vertical bars* depict the standard error of the replicates

Table 3 Content of fatty acids in TAG isolated from adzuki beans[a]

Fatty acid	Cultivar				
	Toyomi	*Akane*	*Roman*	*Otome*	*Erimo*
Experimental					
S	845.9 (28.2)[a,b]	821.5 (28.5)[a]	970.6 (26.9)[c]	882.4 (26.4)[b]	946.3 (28.3)[c]
M	270.0 (9.0)[b]	230.6 (8.0)[a]	288.6 (8.0)[c]	307.5 (9.2)[d]	220.7 (6.6)[a]
D	989.9 (33.0)[a]	962.8 (33.4)[a]	1,259.2 (34.9)[c]	1,126.4 (33.7)[b]	1,167.0 (34.9)[b]
T	893.9 (29.8)[a]	867.6 (30.1)[a]	1,089.6 (30.2)[c]	1,026.1 (30.7)[b]	1,009.8 (30.2)[b]

[a] Experimental values were obtained by GC in comparison with a known amount of methyl pentadecanoate as the internal standard using from TAG isolated from adzuki beans. Each value is the average of three determinations and expressed mg FA per kg beans. Values in a row with different letters are significantly different between individual cultivars ($P < 0.05$). Values in parentheses are shown as total relative % of individual S, M, D and T, respectively. Abbreviations are shown in Fig. 3

of the beans of these cultivars, in addition to providing such nutrients as carbohydrates, protein, and minerals, much also impart some of the widely acclaimed health benefits of the n-3 and n-6 essential FA to the people of Japan. In particular, notwithstanding the low lipid content of the *V. angularis* cultivars, people in Japan obtain a good amount of α-linolenic (18:3n-3) acid from consuming these beans, which would help reduce the risk of coronary heat disease in this region [1, 26]. The data obtained from our work would be useful to both consumers and producers for manufacturing traditional adzuki confectionaries (*wagashi* or *an paste*) in Japan and elsewhere.

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