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Characteristics of lipid components, fatty acid distributions and triacylglycerol molecular species of adzuki beans (*Vigna angularis*)

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ABSTRACT

Fatty acid (FA) distributions and molecular species of triacylglycerols (TAG) isolated from total lipids extracted from adzuki beans (*Vigna angularis*) were analysed by a combination of $AgNO_3$-TLC and GC, and were investigated in relation to the content of endogenous antioxidants determined by HPLC. δ-Tocopherol was present in the highest concentration (30.5 mg/kg beans), and γ-tocopherol in small amounts (12.8 mg/kg beans). The major lipid components were phospholipids (74.3%), TAG (13.5%), hydrocarbons (4.6%) and steryl esters (4.0%), whilst other components were also present in minor proportions (0.5–1.3%). Seventeen different molecular species were detected. The major TAG components were SMD (5.0%), S_2T (19.2%), SD_2 (13.7%), SMT (9.3%), MD_2 (4.5%), SDT (7.0%), D_3 (8.8%) and ST_2 (15.8%) (where S, M, D, and T denote a saturated FA, a monoene, a diene, and triene, respectively). These results would be useful to both consumers and producers for manufacture of traditional adzuki foods in Japan.

1. Introduction

Some beans are used as staple foods in many countries and are receiving increasing attention for protection against coronary heart disease (Bazzano, He, Ogden, Vupputuri, Myers, & Whelton, 2001). Adzuki or small red beans (*Vigna angularis*) are a popular ingredient in many confections in the orient. The consumption of adzuki beans is concentrated in Japan, where the bean has an important economic value. The predominant use of adzuki beans in traditional Japanese confections is in *wagashi* such as *youkan*, *manju* and *amanatoo* (Hoshikawa, 1985; Shi, 1988; Sperbeck, 1981). Adzuki beans are a rich source of carbohydrates, protein, minerals, vitamins and fibre (Tjahjadi, Lin, & Breene, 1988); however, they also contain antinutritional factors. α-Galactosides, phytates and trypsin inhibitors are among these factors, and their concentrations differ widely among different cultivars of adzuki beans. Therefore, when adzuki beans are used for confectionaries, they are boiled in a cooker and yield a hot water extract as a by-product, which is known to contain active ingredients, but is washed. It has been reported that the 40% ethanol adzuki fraction suppresses not only the proliferation of human stomach cancer cells in culture but also benzo-(α)-pyrene-induced tumorigenesis in the mouse forestomach (Itoh et al., 2002, 2004). Thus, the hot-water extract of adzuki beans has a number of functions. Wu, Wang, Lin, and Chang (2001) have shown recently that a water-soluble extract of the adzuki bean could inhibit acetaminophen-induced liver damage. Han et al. (2004) have reported the protective action of an adzuki extract against acetaminophen-induced hepatotoxicity *via* a hepatic γ-glutamylcysteinyl-glycine (GSH)-mediated antioxidation/detoxification system in rat liver after 4 weeks of feeding.

To the best of our knowledge, however, a literature search revealed that there is limited information on the lipid components and fatty acid (FA) distribution of adzuki beans. The objective of the present study was to determine with respect to the tocopherol homologues, lipid class composition, the FA profile and, most importantly, the molecular species of triacylglycerols (TAG), in an attempt to evaluate the composition and quality characteristics of the oils. These results would be useful to both consumers and producers for manufacturing traditional confectioneries in Japan.

2. Materials and methods

2.1. Adzuki beans

The commercially mature adzuki beans (*V. angularis* cv. Shionagon) used in this work were harvested at Tokachi, Hokkaido, Japan during the summer of 2007. The cultivar (Takii Seed Co., Kyoto,

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Japan) was selected for uniformity based on seed weight of 112–145 mg per bean. Beans were stored in separate stainless steel containers at 4 °C prior to experiments.

2.2. Reagents and standards

All chemicals and solvents used were of analytical grade (Nacalai Tesque, Kyoto, Japan), but diethyl ether was further purified to remove peroxides. TLC plates (silica-gel 60G F254, 20 × 20 cm, 0.25 mm thickness) were purchased from Merck (Darmstadt, Germany). Vitamin E homologues (α, β, γ and δ) were purchased from 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, as described in a later section. The TLC standard mixture containing monoacylglycerols (MAG), diacylglycerols (DAG), free fatty acids (FFA), triacylglycerols (TAG), steryl esters (SE) and hydrocarbons (HC) was from Nacalai Tesque (Kyoto, Japan). A phospholipids kit from Serdary Research Laboratory (Mississauga, Canada) was used as PL standard. Lipase was from a porcine pancreas and was used after purification with acetone and further with diethyl ether (Yoshida & Alexander, 1983).

FA methyl ester (FAME) standards (F & OR mixture #3) were procured 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 fatty acid methyl esters (FAME).

2.3. Chemical analysis

The AOAC (1997) methods were used to determine the chemical composition of the 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

The beans (1000 seeds) were extracted in a Maxim homogenizer (Nihonseiki Kaisha Ltd., Tokyo, Japan) at high speed for 10 min at 0 °C with 200 ml of chloroform/methanol (2:1, v/v) fortified with 0.01% BHT, which was added 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 rehomogenised 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%) was added (Folch, Lee, & Sloane-Stanley, 1957) and the phases were mixed vigorously. After phase separation, the chloroform layer was withdrawn, dried over anhydrous sodium sulphate and 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. Analysis of tocopherols

Analysis of tocopherols in oils was performed by HPLC as described earlier (Yoshida, Shougaki, Hirakawa, Tomiyama, & Mizushina, 2004). A mixture of *n*-hexane/1,4-dioxane/ethanol (490:10:1, v/v/v) was used as the mobile phase at a flow rate of 2.0 ml/min. A 0.2-ml portion of the sample, after removal of the extraction solvent under a stream of nitrogen, was placed in a 5-ml brown volumetric flask and diluted with the mobile phase. An aliquot (4 ml) from this sample solution was injected using the same method as described earlier, 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 (Yoshida, Kanrei, Tomiyama, & Mizushina, 2006).

2.6. Lipid analysis

Total lipids were fractionated by TLC into eight fractions (Yoshida et al., 2004). 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 (10–100 mg) 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 (AOCS, 1992). After cooling, 5 ml of *n*-hexane was added to each tube and washed several times with deionised water to remove BF_3 and silica-gel. The *n*-hexane layer containing the FAME was recovered and dried over anhydrous sodium sulphate. The solvent was then vaporised under a gentle stream of nitrogen, and the residue was quantified in a gas chromatograph (Shimadzu Model-14B, Kyoto, Japan) equipped with a hydrogen flame ionisation detector 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) at a column temperature of 180 °C.

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 injection and detector temperatures were held at 230 and 250 °C, respectively. The initial oven temperature was 180 °C and maintained for 5 min, and then increased at a rate of 2 °C/min to 200 °C, which was held for 15 min. All samples were dissolved in *n*-hexane for injection. The component peaks were identified and calibrated by comparison with those of standard FAME, 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 vol) as the mobile phase. PL classes were detected by iodine vapour and were consistent with the authentic standards. Bands corresponding to phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl inositol (PI) and others were carefully scraped into test-tubes. Then, FAME were prepared by the same method as described above and analysed by gas chromatography (GC).

2.7. Enzymatic hydrolysis of lipids

TAG hydrolysis *in vitro* was carried out according to the methods described previously (Yoshida & Alexander, 1983). The purified TAG (10 mg) were hydrolysed with 15 mg of pancreatic lipase at 37 °C in 5 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 (105 × 16 mm) as described above. A time period of 20 min was selected based on the results of preliminary experiments using the standard TAG (1,3-dilauroyl-2-myristoyl-*sn*-glycerol: Sigma Chemical Co.). After approximately 60% of the TAG was hydrolysed,

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 the *sn*-3 position within 60% hydrolysis (for 20 min). The reaction products were separated by TLC as already described (Yoshida & Alexander, 1983). The FFA and *sn*-2 MAG bands were carefully scraped off the plate and methylated (AOCS, 1992). The procedure was checked by comparing the FA compositions of the original TAG and the TAG remaining after partial hydrolysis.

2.8. TAG analysis

The TAG isolated by TLC was directly analysed by GC following the method (Matsui, Watanabe, & Ikekawa, 1973), using a Shimadzu Model-14A GC equipped with a hydrogen flame ionisation detector (FID). A glass column (500 × 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 silanised 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, 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 for 20 min. All samples were dissolved in *n*-hexane for injection. TAG peaks were identified by co-chromatography with known standards. Peak areas were calculated 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).

2.9. TAG species composition

Molecular species separation from total TAG was carried out by silver nitrate/silica-gel TLC according to the method (Bilky, Piazza, Bistline, & Haas, 1991). Briefly, plates were coated to 0.25 mm thickness with slurry of 45 g silica-gel 60 G (Merck) and 9.0 g $AgNO_3$ dissolved in 100 ml deionised water. Freshly prepared plates were activated at 120–125 °C for 12 h, then stored before use in a desiccator in the dark. TAG classes differing in unsaturation were separated by $AgNO_3$-TLC using 1.5–4.0% (v/v) methanol in chloroform, depending on differences in their degree of unsaturation (Jham et al., 2005). In the case of broad TAG, three solvents of increasing polarity were required to separate the lipid classes. The plates (20 × 20 cm) were streaked with 10–15 mg TAG using a microsyringe (Hamilton Co., Reno, NV, USA), developed with 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 separated. The second TAG molecular species such as S_2T, SD_2, SMT, MD_2 and SDT (where T denotes a triene) were separated by developing the plate with 2.5% (v/v) methanol in chloroform. Finally, the most difficult to separate were D_3, MDT, ST_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 plate). 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 (>98.5%) by passing TAG standards through the alumina column using this procedure.

The solvent was then vaporised in small glass tubes under a gentle stream of nitrogen. Methyl pentadecanoate of the standard solution (5–10 ml) 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.

2.10. Statistical analyses

Statistical analysis was performed by using the Proc ANOVA in SAS (Software version 8). Duncan's Multiple Range Test was used. For each sample three determinations have done.

3. Results and discussion

3.1. Tocopherol contents in the beans

The major chemical components were as follows: moisture 3.9%, fat 1.3% and protein 20.3%. Adzuki beans are high in complex carbohydrates, protein, and fibre, yet are extremely low in fat (Hsieh, Pomeranz, & Swanson, 1992). The major tocopherol was δ-tocopherol (30.5 mg/kg beans), and γ-tocopherol (12.8 mg/kg beans) was a minor component (data not shown). However, α- and β-tocopherols were not detected in adzuki beans. This distribution is a very unique pattern and differs from other plant seeds such as sunflower seeds (Yoshida, Hirakawa, Abe, & Mizushina, 2002) or peanuts (Yoshida, Hirakawa, Tomiyama, & Mizushina, 2003) in which α- and β-tocopherols are usually detected.

3.2. Lipid components in the beans

The compositional analyses carried out in this work included determination of the lipid classes and the FA compositions of the oils. Profiles of different lipid classes in the beans are shown in Table 1. Predominant components were PL (74.3%) and TAG (13.5%), followed by HC (4.6%) and SE (4.0%), accompanied by very small

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

Lipid class	mg/kg Beans	
Hydrocarbons	616 ± 23	(4.6)
Steryl esters	536 ± 21	(4.0)
Triacylglycerols	1809 ± 48	(13.5)
Free fatty acids	67.8 ± 2.1	(0.5)
1,3-Diacylglycerols	174 ± 3	(1.3)
1,2-Diacylglycerols	107 ± 3	(0.8)
Monoacylglycerols	134 ± 3	(1.0)
Phospholipids	9948 ± 238	(74.3)

[a] Mean values ± standard error. Each value represents the average of three determinations, and is expressed as mg/kg beans. Values in parentheses are relative wt% contents of the individual lipids in total lipids.

Table 2
PL contents in the oils prepared from adzuki beans.[a]

Lipid class	mg/kg Beans	
Phosphatidyl ethanolamine	2129 ± 96	(21.4)
Phosphatidyl choline	4735 ± 213	(47.6)
Phosphatidyl inositol	2656 ± 105	(26.7)
Others	428.0 ± 12.0	(4.3)

[a] Mean values ± standard error. Each value represents the average of three determinations, and is expressed as mg/kg beans. Values in parentheses are relative wt% contents of the individual lipids in PL. "Others" include diphosphatidylglycerol, phosphatic acid and phosphatidylglycerol.

Table 3
FA distributions of major lipid components prepared from adzuki beans.[a]

Lipid class	Fatty acid (wt%)					Others
	16:0	18:01	18:1	18:2	18:3	
Total Lipids	28.4 ± 1.2	2.5 ± 0.1	4.5 ± 0.2	44.4 ± 2.0	18.8 ± 0.6	1.4 ± 0.1
Triacylglycerols	28.0 ± 1.0	4.3 ± 0.2	6.9 ± 0.2	29.7 ± 1.1	25.3 ± 1.0	5.8 ± 0.2
2-Position	5.3 ± 0.2	0.8 ± 0.1	11.0 ± 0.6	54.4 ± 2.1	28.5 ± 1.1	0.1 ± 0.0
1,3-Position	39.4 ± 1.5	6.0 ± 0.3	4.9 ± 0.2	17.3 ± 0.7	23.7 ± 0.9	8.7 ± 0.3
Phospholipids	28.0 ± 1.0	1.7 ± 0.1	4.2 ± 0.2	46.8 ± 2.1	18.5 ± 0.7	0.8 ± 0.1
Phosphatidyl ethanolamine	32.5 ± 1.4	2.0 ± 0.1	4.5 ± 0.2	46.4 ± 2.0	13.5 ± 0.5	1.1 ± 0.1
Phosphatidyl choline	25.2±1.0	2.1 ± 0.1	5.8 ± 0.2	50.3 ± 1.9	15.3 ± 0.6	1.3 ± 0.1
Phosphatidyl inositol	48.2 ± 1.9	2.8 ± 0.1	4.7 ± 0.1	32.4 ± 1.3	10.5 ± 0.5	1.4 ± 0.1

[a] Mean values ± standard error. Each value represents the average of three determinations, and is expressed the relative wt% contents of individual FA. "Others" include minor FA such as C14:0, C16:1, C20:0 and C22:0.

amounts of 1,3-DAG (1.3%), MAG (1.0%), 1,2-DAG (0.8%) and FFA (0.5%). The adzuki beans are not oil seeds but typical vegetable seeds (Mabaleha & Yeboah, 2004). Therefore, the PL content is quite significant, whilst glycolipids are present only trace amounts, indicating that PL forms the principal components of the cell membranes in the beans.

Table 2 shows the profiles of PL components of the adzuki beans. The original amounts of each PL were approximately 4735 mg (47.6%), 2656 mg (26.7%), 2129 mg (21.4%) and 428 mg (4.3%) per kg beans for PC, PI, PE and others, respectively. Others include diphosphatidyl glycerol, phosphatidic acid and phosphatidyl glycerol. 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 (Alvarez-Ortega, Cantisan, Martinez-Force, & Garces, 1997).

3.3. Fatty acid composition of the major lipid components in the beans

FA compositions (expressed in terms of the esters by weight) of the major lipid components in the beans are shown in Table 3. The principal FA components of legumes are generally palmitic, stearic, oleic, linoleic and linolenic acids, the distribution 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 acid, followed by linolenic and oleic acids), representing 69.1% and 70.3% for total lipids and PL, respectively.

No significant differences ($p > 0.05$) in FA composition were found when comparing between total lipids and PL. The profiles of composition and positional distribution of FA in the TAG were compared. Unsaturated FA such as linoleic, linolenic and oleic were predominantly located in the 2-position of the TAG molecules, whilst saturated FA such as palmitic and stearic acids primarily occupied the 1-position or 3-position. The profiles of FA distribution in PE, PC and PI were compared among the three PL. The distribution patterns of FA were similar to each other between PE and PC in the beans. The percentage of linoleic and linolenic acids was higher in the PE and PC than that in the PI. Therefore, PI was unique in that it had the highest saturated FA content among the three PL. These FA profiles are not similar to the results observed in typical vegetable seeds such as peas (Miyazawa & Fujino, 1976) or kidney beans (Yoshida, Tomiyama, Kita, & Mizushina, 2005).

3.4. Distribution of triacylglycerol molecular species

The total carbon numbers (TCN) of FA in the TAG of adzuki beans ranged from 50 to 60 as illustrated in Fig. 1. For example,

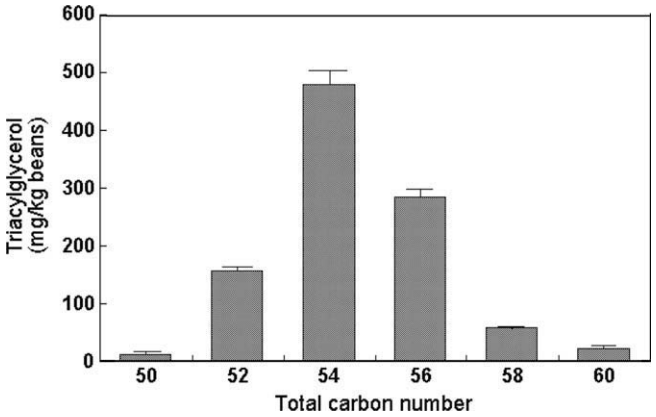

Fig. 1. TAG contents prepared from adzuki beans. The carbon number denotes the total length of the three acyl chain present in the TAG. For example, 54 are predominantly composed of 18:0, 18:1, 18:2 and 18:3. Each value represents the average of three determinations. Vertical bars depict the standard error of the replicates.

in the case of tristearin, the TCN is 54. Dominant components consisted of 52 (15.4%), 54 (47.8%) and 56 (28.2%) TCN, followed by small amounts of 50 (1.0%), 58 (5.6%) and 60 (2.0%) TCN. 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 (Yoshida et al., 2005). These values may be due to the qualitative and quantitative differences of the TAG (Table 1) and their FA (Table 2).

The FA composition 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 shown in Fig. 2. Seventeen different molecular species were detected in the oil extracted from adzuki beans. The three-letter designation does not suggest FA positional isomers in the TAG: P, palmitic; St, stearic; O, oleic: L, linoleic; and Ln, linolenic 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. 2). The major TAG species were SMD (POL or StOL), S_2T (PPLn or PStLn or StStLn), SD_2 (PLL or StLL), SMT (POLn or StOLn), MD_2 (OLL), SDT (PLLn or StLLn), D_3 (LLL) and ST_2 (PLnLn or StLnLn), followed by S_3 (PPP or PPSt or PStSt or StStSt), S_2M (PPO or PStO or StStO) and S_2D (PPL or PStL or StStO). On the other hand, the other species (SM_2: POO, StOO; M_3: OOO; MDT: OLLn; D_2T: LLLn; DT_2: LLnLn; and T_3: LnLnLn) were minor components (less than ca. 35 mg: <2.0%). These distribution patterns in the TAG molecular species are not similar to the results reported in typical vegetable seeds such as kidney beans (Yoshida et al., 2005) or broad beans (Yoshida, Tomiyama, Yoshida, Saiki, & Mizushina, 2008).

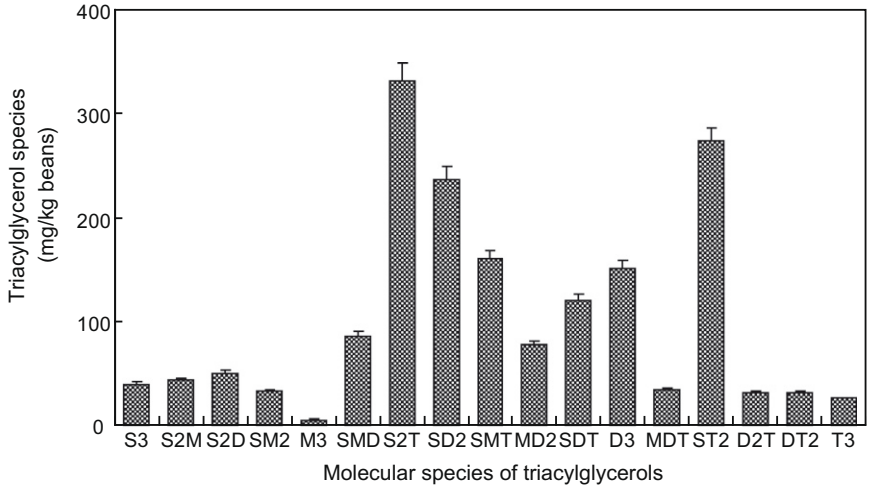

Fig. 2. Characteristics of the major molecular species of TAG prepared from adzuki beans. Saturated FA (S) consisted of myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0) and behenic (22:0) acids. Unsaturated FA, palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3) are 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 4
Contents of FA in TAG isolated from adzuki beans.[a]

	FA	mg/kg beans	
Experimental	S	638 ± 23	(35.8)
	M	161 ± 5.6	(8.9)
	D	537 ± 21	(29.7)
	T	463 ± 18	(25.6)

[a] Mean values ± standard error. Each value represents the average of three replicates, and is expressed as mg FA per kg beans. Experimental values are obtained by GC in comparison with a known amount of methyl pentadecanoate as the internal standard using TAG isolated from adzuki beans. Values in parentheses are shown as total relative wt% of the individual S, M, D and T, respectively. Abbreviations are shown in Fig. 2.

Table 4 presents the FA contents (S, M, D and T) in the TAG isolated from adzuki beans, expressed as milligram FA within the TAG per kg beans according to their degree of unsaturation on the acyl chain-length of the FA moieties. Briefly, the amounts of FA were summed as S (16:0, 18:0, 20:0 and 22:0), M (16:1 and 18:1), D (18:2) and T (18:3) from the results obtained by GC. The theoretical contents of FA were calculated from the relative percentages of each TAG species based on the data in Fig. 2, and the distributions of each FA were then composed with the experimental value (Table 3). There were no qualitative or quantitative differences ($p > 0.05$) in the distribution between the experimental and calculated (data not shown) values.

4. Conclusions

The dominant components in adzuki beans, were PL (74.3%) and TAG (13.5%), whilst other lipid components were also present in minor proportions less than 4.6%. δ-Tocopherol was present in the highest concentration (70.4%), and γ-tocopherol was detected in minor amounts (29.6%). PC was detected as the dominant component (47.6%), followed by PI (26.7%) and PE (21.4%). Some differences ($p < 0.05$) in the FA composition were observed when comparing the three lipid classes (total lipids, TAG, and PL). Linolenic acid has very high levels in the TAG (25.3%), total lipids (18.8%) and PL (18.5%).

Seventeen molecular species of TAG were detected in the beans (*V. angularis*). The main TAG species were S_2T (palmitostearolinolenin, dipalmitolinolenin, and distearolinolenin), ST_2 (palmitodilinolenin and stearodilinolenin), SD_2 (palmitodilinolein and stearodilinolein), SMT (palmitoleolinolenin and stearoleolinolenin), D_3 (trilenolein), SDT (palmitolinoleolinolenin and stearolinoleolinolein), SMD (palmitoleolinolein and stearoleolinolein) and MD_2 (oleodilinolein) in adzuki beans. There were no qualitative or quantitative differences ($p > 0.05$) in the distribution between the experimental and calculated values. The data obtained in this 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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