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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,