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# Profiles of lipid components, fatty acid distributions of triacylglycerols and phospholipids in Jack beans (*Canavalia gladiata* DC.)

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ABSTRACT

Endogenous tocopherols in extracted lipids from Jack beans (*Canavalia gladiata* DC.) were determined by high-performance liquid chromatography (HPLC), and were investigated in relation to the fatty acids (FA) distribution of triacylgycerols (TAG) and phospholipids (PL). The dominant tocopherols were ⟨delta(δ)⟩-tocopherol (78.9–96.5 mg%) and ⟨gamma(γ)⟩-tocopherol (42.1–56.1 mg%) with much smaller amounts of ⟨alpha(α)⟩-tocopherol (1.1–1.3 mg%). The lipids of Jack beans comprised mainly TAG (34.6–38.6 wt.%) and PL (54.8–57.4 wt.%), and other components were also detected in minor proportions (0.3–3.8 wt.%). The PL components included phosphatidyl choline (46.2–48.7 wt.%), phosphatidyl inositol (23.4–29.6 wt.%) and phosphatidyl ethanolamine (18.5–21.2 wt.%). Comparison of these different beans showed, with a few exceptions, no significant differences ($P > 0.05$) in FA distribution. The FA distribution of TAG among the five beans was evident in the Jack beans: unsaturated FA (93.3–95.3 wt.%) were predominantly concentrated at the *sn*-2 position and saturated FA (33.6–34.4wt.%) primarily occupying the *sn*-1 position or *sn*-3 position. The results obtained from this work would provide useful information to both producers and consumers for manufacturing functional foods or beverages in Japan and elsewhere.



## 1. Introduction

Legumes are an inexpensive source of proteins with desirable characteristic such as abundance of carbohydrates, ability to lower the serum cholesterol, low fat (except oilseeds), high fibre, high concentration of polyunsaturated FA and a long shelf life. The legumes are also major sources of proteins and calories (Rockland & Nishi, 1979), and play a significant role in traditional diets in many regions of the world (Deshpande, 1992). They are known to contain certain bioactive compounds or phytochemicals whose beneficial effects need to be explored for nutritional and health benefits. The global production of food legumes in 1998 was 246 million tonnes (FAO, 1998). Research has to be geared to exploiting the unconventional legume resources to meet the protein requirements of developing countries. Under-explored legumes are important in terms of food security, nutrition, agricultural development, enhancement of economy and also as rotation crops (Ekanayake, Nair, Jansz, & Asp, 2003). Thus, little known legumes can play an important role in agriculture as they are protein plants, which contribute to the world food production due to their adaptation to adverse environmental conditions and high resistance to disease and pests.

Among legume seeds, some are used as vegetables and others as supplementary sources of protein in animal diets (Savage, 1988). The widespread use of legumes makes this food group an important source of lipids and FA in animal and human nutrition. Several publications dealing with the total lipids and FA compositions have been reviewed by several researchers (Grela & Gunter, 1995; Welch & Griffiths, 1984). Some beans are used as staple foods in many countries (Ciftci, Przybylski, Rudzinska, & Acharya, 2011) and are increasing in attention as protective products against coronary heart disease (Bazzano et al., 2001). The Jack bean (*Canavalia* (C.) *gladiata* DC., Japanese name "natamame") has been cultivated as a vegetable in Asian countries for feeding ruminants and widely

*Abbreivations:* DAG, diacylglycerols; DPG, diphosphatidyl glycerol; FA, fatty acids; FAME, fatty acid methyl esters; FFA, free fatty acids; GC, gas chromatography; HC, hydrocarbons; HPLC, high-performance liquid chromatography; MAG, monoacylglycerols; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PL, phospholipids; SE, steryl esters; TAG, triacylglycerols; TLC, thin-layer chromatography.

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studied for its nutritional value (Arun, Sridhar, Ravirajia, Schmidet, & Jung, 2003; Siddhuraju & Becker, 2001). The seeds of C. *gladiata* have been known to exhibit antiemetic and demulcent effects and to stop hiccups in Chinese traditional medicine (Sridhar & Seena, 2006). As chemical constituents of this plant, gibberellins (Tamura et al., 1967), an amino acid (Sridhar & Seena, 2006) and an enzyme (Akedo, Mori, Tanigaki, Shinka, & Morita, 1972) have been investigated. The composition of total FA is often the only information provided in studies on several bean lipids (Arun et al., 2003). The bean *C. gladiata* which is a leguminous plant is not eaten very frequently, but has the potential to become an important food source.

To the best of our knowledge, no literature search has been reported on the presence of endogenous tocopherols in relation to the distributions of FA of triacylglycerols (TAG) and phospholipids (PL). Therefore, the focus of the present study was to investigate the interrelationship between the content of these endogenous tocopherols and the distributions of FA in the triacylglycerols (TAG) and major phospholipids (PL) between the white and red Jack beans. The data obtained from the study would provide available information to both consumers and producers for manufacturing Jack bean foods.

## 2. Materials and methods

### 2.1. Jack beans

Samples of commercial Jack beans (*C. gladiata* DC.) were obtained from the following suppliers (the abbreviation in brackets following the name of the bean is used in this paper) Hachiouji (HJW), Gifu (GFW), Tanba (TBW) for white beans and Hachiouji (HJR), Gifu (GFR) for red beans which were grown in different districts of Japan during the summer of 2011. The beans were hand selected to eliminate cracked or otherwise damaged seeds. These seeds were sealed in polyethylene bags under nitrogen gas, respectively, and then stored in a stainless steel container at −35 °C until analysis.

### 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. The thin-layer chromatography (TLC) plates (silica gel 60 G, 20 × 20 cm, 0.25 mm thickness) were obtained from Merck (Darmstadt, Germany). Vitamin E homologues (α, β, γ and δ were acquired from Sigma–Aldrich Co. (St. Louis, MO, USA). All tocopherols were of the D-form (*RRR*-), and their purities were better than 98.5% as performed by high-performance chromatography (HPLC) using 2,2,5,7,8-pentamethyl-6-hydroxychroman as the internal standard and 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 purchased from Nacalai Tesque. Glyceryl-*sn*-1,3-dimyristate-*sn*-2- oleate (Sigma–Aldrich Chemical Co.; St. Louis, MO, USA) was used as the TAG standard for enzymatic hydrolysis. A PL kit from Serdary Research Laboratory (Missisauga, ON, Canada) was used as the PL standard for TLC. Lipase from porcine pancreas was obtained from Sigma–Aldrich Co., and used after purification with previously described methods (Yoshida & Alexander, 1983). The FA methyl esters (FAME) standards (F & OR mixture #3) were procured from Altech-Applied Science (State College, PA, USA). The internal standards, pentadecane and methyl pentadecanoate, were purchased from Merck, and then 100 mg of each was dissolved in *n*-hexane (20 ml). Boron trifluoride ($BF_3$) in methanol (14%; Wako Pure Chemical Inc., Osaka, Japan) was used to prepare the FAME.

### 2.3. Chemical analysis

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

### 2.4. Extraction of lipids

In order to obtain fine flour, beans (200 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 before extraction. Total lipids were extracted from 50 g of the flour in 300 ml chloroform/methanol (2:1, v/v) with vigorous shaking for 20 min at 0 °C three times, following the Folch procedure (Folch, Lee, & Sloane-Stanley, 1957). These solvents contained 0.01% butylated hydroxytoluene (Wako Pure Chemical Inc.) to inhibit oxidative degradation of lipids during analysis. The extraction was repeated three times, and the individual extracts were vacuum-filtered through defatted filter paper on a Buchner funnel. These filtrates were combined and dried in a rotary 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%) was added (Folch et al., 1957) and mixed vigorously. After phase separation, the chloroform layer was removed, dried over anhydrous $Na_2SO_4$, filtered, and the filtrate was concentrated under vacuum in a rotary evaporator at 35 °C. 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) and kept under nitrogen at −35 °C until further use.

### 2.5. Analysis of tocopherols

Analysis of tocopherols in the lipid extracts was performed by a HPLC (Shimadzu LC-10AD *vp*; Kyoto, Japan) according to the methods reported previously (Yoshida, Tomiyama, & Mizushina, 2010). Briefly, 250 mg lipid were weighed directly into a 2-ml volumetric flask and mixed with the mobile phase (*n*-hexane/1,4-dioxane/ethanol, 490:10:1, by volume) used for HPLC analysis. An aliquot (10 μl) from the sample solution was subjected to HPLC analysis (Yoshida et al., 2010), and the amount of each tocopherol was monitored with a fluorescence detector (Shimadzu RF-10 AXL) set at an excitation wavelength of 295 nm and an emission wavelength of 320-nm, and quantified as previously reported (Yoshida et al., 2010). The other HPLC conditions were as previously reported (Yoshida, Tomiyama, Yoshida, & Mizushina, 2009).

### 2.6. Lipid analysis

According to the previously outlined procedure (Yoshida et al., 2009), total lipids were separated by TLC into seven sufractions with a solvent system of *n*-hexane/diethyl ether/acetic acid (80:20:1, by volume). Bands corresponding to HC, SE, TAG, FFA, 1,3-DAG, 1,2-DAG, and PL were scraped into separate 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) except that pentadecane (10 μg) was used as the internal standard for HC analysis. The FAME were prepared from the isolated lipids by heating with