for dimeric MHA free acid; $t_{1/2}$ = 1.6 days for trimeric MHA free acid. From eq 5 it can be calculated that it takes 6 days to have 90% of the dimeric MHA free acid split to the monomer (at 37 °C in 0.1 N HCl). These results show that dimeric and trimeric MHA free acids are rather stable toward hydrolysis under physiological conditions of pH and temperature.

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Electrophoretic Characterization of Adzuki Bean (*Vigna angularis*) Seed Proteins

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Seed proteins of Minnesota-grown adzuki beans (*Vigna angularis* L. cultivar Takara) were sequentially extracted with deionized distilled H_2O, 0.5 M NaCl, and 0.05 M Tris-HCl buffer (pH 8.6) containing 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol. The protein fractions were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), isoelectric focusing (IEF), and two-dimensional polyacrylamide gel electrophoresis using IEF in the first dimension and SDS–PAGE in the second dimension. The two-dimensional SDS–PAGE electrophoretogram of total seed proteins resolved about 60 polypeptides (spots). The polypeptides of different solubilities were well separated with little or no indication of cross contamination as determined from the electrophoretograms. Periodic acid–Schiff staining revealed two glycoproteins, corresponding to the α and β_1 subunits of the 7S globulin protein. The major seed protein of Takara adzuki bean is a glycoprotein that consists of two carbohydrate-containing subunits with a relative mass (M_r) of 55 000 and 35 000, respectively.

Adzuki beans have been used in desserts in Oriental countries, especially Japan and China, for over a thousand years. Adzuki beans are used primarily in paste "An" (Japanese for sweetened adzuki bean paste), which is used in desserts, confectionery items, and ice cream and as fillings in baked goods such as Manju and cakes (Hayakawa and Breene, 1982).

In 1977 adzuki beans were evaluated as a potential cash crop in Minnesota. They grow well in Minnesota with a satisfactory yield and excellent An quality. The potential of exporting adzuki beans or their products to Japan or of developing some adzuki bean products that might be acceptable to Western customers has been under serious consideration.

The molecular properties of proteins contribute to the functionality of food ingredients, i.e., specific proteins contribute to the select functional behavior of a food (Cherry et al., 1979). Protein functionality in foods is mainly determined by the molecular composition and structure of the individual proteins (Kinsella and Shetty, 1979). Thus, characterization of the individual seed proteins provides useful information for the isolation and utilization of legume proteins. Although adzuki beans are one of the most important edible legumes in the Oriental countries, little information is available on the properties of their seed proteins. Only the 7S protein of adzuki beans has been well characterized (Sakakibara et al., 1979).

In this study adzuki bean seed proteins were sequentially extracted in three aqueous systems and analyzed by isoelectric focusing and by one-dimensional and two-dimensional polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Dry seeds (11–12% moisture) of adzuki beans (*Vigna angularis* L. cv. Takara) grown in Minnesota were milled so as to pass through a 40-mesh screen.

Protein Extraction. Five grams of ground seed was stirred in 50 mL of doubly distilled water overnight at 4 °C. The slurry was centrifuged at 20000*g* for 30 min at 4 °C and the supernatant carefully removed. The residue was extracted 2 more times for 1 h each at 20 °C. The extracts were pooled and referred to as the water-soluble fraction. Following the third water extraction, the residue was extracted with 50 mL of 0.5 M NaCl (pH adjusted to 7.2 by 0.1 N NaOH) for 1 h with stirring at 4 °C and centrifuged at 20000*g* for 30 min. This extraction was also repeated 3 times. These pooled extracts were referred to as the salt-soluble fraction. The proteins remaining in the residue were extracted by stirring with 30 mL of 0.05 M Tris-HCl buffer (pH 8.6), containing 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol, for 1 h at room temperature (20 °C) and centrifuged at 20000*g* for 30 min to remove the residue. This extraction was repeated 3

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Table I. Percent of Total Protein Extracted in Three Aqueous Systems

fraction	protein content,[a] % of dry seed powder
H_2O soluble	2.3
NaCl soluble	15.0
SDS soluble	4.3
total	21.6

[a] Means of two separate extractions.

times; the pooled extracts were referred to as SDS-soluble fraction. Kjeldahl analysis indicated that more than 90% of the total seed nitrogen was removed after the sequential extraction. The protein extracts from each solvent system were dialyzed against distilled water at 4 °C for 24 h with six changes. The precipitates formed during dialysis were removed by centrifugation at 20000*g* for 30 min and used for subsequent electrophoretic analysis. The supernatant of the water-soluble fraction and the precipitates were freeze-dried and stored at −20 °C. Total seed proteins were extracted in 5 mL of 0.05 M Tris-HCl buffer (pH 8.6) containing 2% SDS and 5% 2-mercaptoethanol per g of ground seed for 1 h at room temperature with stirring. The extraction was repeated 3 times, and the 20000*g* supernatant was used directly for electrophoresis without dialysis.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). One-dimensional SDS–PAGE was performed following the procedure of Laemmli (1970) using 10% polyacrylamide gel slabs overlaid with a 4% stacking gel. Protein samples were solubilized in the sample buffer (0.1 M Tris-HCl, pH 6.8, containing 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol) by heating for 1 min in a boiling water bath. To each gel well 20 μg of protein was applied. Two-dimensional SDS–PAGE essentially followed the procedures of O'Farrell (1975). For each run, 100 μg of protein was applied at the anodic end of the IEF gel.

After electrophoresis, the gel slabs were stained with 0.03% Coomassie Brilliant Blue R-250 in 10% acetic acid and 25% 2-propanol and destained in 10% acetic acid until the background was colorless. The gels were stained with periodic acid–Schiff's reagent (Segrest and Jackson, 1972) in order to locate the glycoprotein bands. Phosphorylase *b* (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (20100), and lysozyme (14400) were used as molecular weight standards to estimate the relative mass (M_r) of the polypeptides.

Isoelectric Focusing (IEF). Water-soluble and salt-soluble peptides were separated by IEF in gels 115 mm long × 3 mm in diameter, containing 2% ampholine (pH 3.5–10). The gels contained 8 M urea when denatured proteins were isoelectric focused; however, nondenatured proteins were solubilized in 5% Nonidet P-40 and 2% ampholine (pH 3–10) and 100-μg samples were applied at the anodic end. The pH gradients established in the IEF gel were determined by slicing a gel and determining the pH of the water extracts (O'Farrell, 1975).

Protein Determination. Protein contents were determined by the improved Lowry method (Peterson, 1977), using bovine serum albumin (BSA) as the standard.

RESULTS

The percent of total protein extracted in the aqueous systems is shown in Table I. The water-soluble protein fraction, when separated by IEF without denaturation, revealed two major bands and several minor bands (Figure 1a). The isoelectric points (PI) of these two major bands

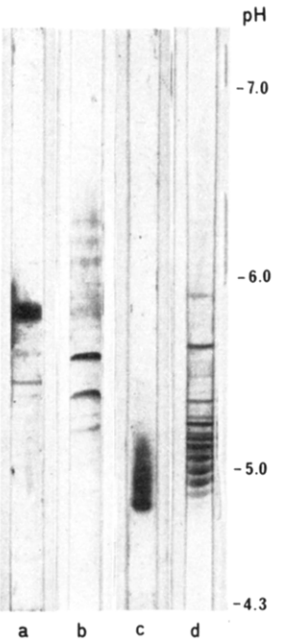

Figure 1. Isoelectric focusing profiles of H_2O-soluble (tracks a and b) and NaCl-soluble (tracks c and d) protein fractions from adzuki bean seed before (tracks a and c) and after (tracks b and d) denaturation with 8 M urea.

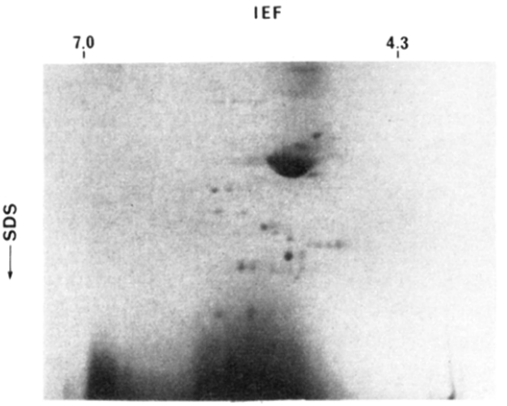

Figure 2. Two-dimensional polyacrylamide gel electrophoretogram of a total protein extract from the adzuki bean seed. The number on the top indicates the estimated isoelectric points.

were approximately 5.8 and 5.5. However, if the water-soluble fraction was focused in gels containing 8 M urea, two dominant bands (PI = 5.6 and 5.4) appeared plus several minor bands ranging from 5.0 to 5.3 and 5.8 to 6.3 (Figure 1b). The native proteins of the salt-soluble fraction could only be resolved as a diffuse broad band with a PI between 4.8 and 5.1 (Figure 1c). After denaturation in 8 M urea this diffuse band was separated into a series of well-defined bands with PI's ranging from 4.8 to 6.0 (Figure 1d).

Two-dimensional PAGE of the total protein extract resolved about 50 peptides (Figure 2). A large spot was found at PI = 4.9–5.2, with a M_r of about 55000 The majority of polypeptides fell in the PI range between 5 and 6; their M_r ranged from less than 20000 to over 90000. Two-dimensional PAGE of the water-soluble fraction revealed a major polypeptide with a M_r of approximately 30000 and PI = 5.5 (Figure 3A). Several other minor spots were also detected. The salt-soluble fraction was resolved by two-dimensional PAGE into one large spot and about 20 well-distinguished smaller spots (Figure 3B). The SDS-soluble peptides remaining in the residue after water and NaCl extraction are shown in two-dimensional PAGE

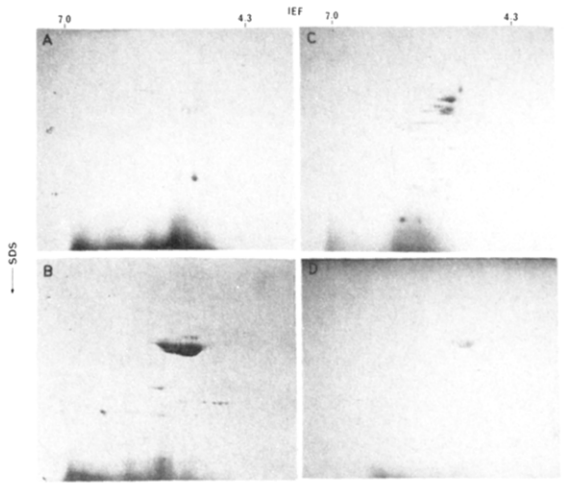

Figure 3. Two-dimensional polyacrylamide gel electrophoretograms of H_2O-soluble, (A), NaCl-soluble (B), and SDS-soluble (C) proteins stained with Coomassie brilliant blue R-250 and (D) NaCl-soluble proteins stained with periodic acid–Schiff's stain for glycoproteins.

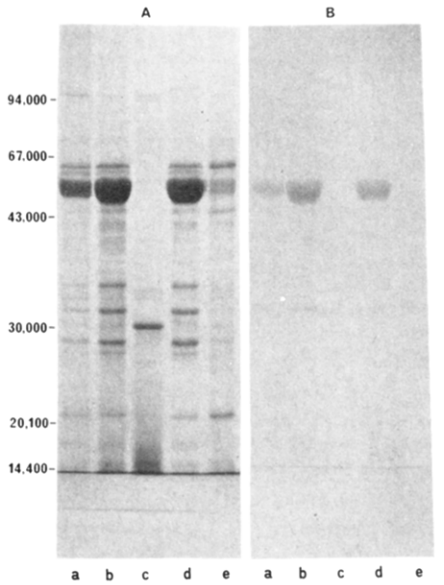

Figure 4. One-dimensional SDS–polyacrylamide gel electrophoretograms of adzuki proteins stained with Coomassie brilliant blue R-250 (A) and periodic acid–Schiff's reagent (B). (a) Total protein fraction. (b) Precipitate and (c) supernatant of H_2O-soluble fraction after dialysis against distilled H_2O. (d) NaCl-soluble fraction. (e) SDS-soluble fraction. The positions of M_r markers are indicated on the left.

gel (Figure 3C). The three solvents used in this study demonstrate the different solubilities of the peptides in adzuki bean seeds. Periodic acid–Schiff's staining of the salt-soluble fraction (Figure 3D) indicated that two of the polypeptides having a M_r of approximately 55 000 and 35 000, respectively, are glycoproteins.

The water-soluble, salt-soluble, and SDS-soluble fractions from adzuki bean proteins were also analyzed by one-dimensional SDS–PAGE (Figure 4). The total proteins were separated into about 30 bands by SDS–PAGE [Figure 4A (a)]. The water-soluble fraction after dialysis (precipitate removed by centrifugation) contained a major polypeptide, M_r approximately 30 000, plus many minor bands [Figure 4A (c)]. The precipitate of the water-soluble fraction showed a banding pattern [Figure 4A (b)] similar to that of the salt-soluble fraction [Figure 4A (d)]. The polypeptide pattern of the SDS-soluble fraction [Figure 4A (e)] is quite different from that of the water-soluble and salt-soluble fractions. The lanes in Figure 4A were stained with periodic acid–Schiff's stain for glycoprotein (Figure 4B), and two glycoprotein bands (M_r of approximately 55 000 and 35 000) were visualized.

DISCUSSION

Legume seeds are a rich source of proteins for humans and livestock. However, information about the total composition of legume seed proteins is rather scarce. The major seed protein fraction, the globulins, have been studied. However, only a few studies have been conducted on the other protein fractions (Bhatty, 1982). Most studies on legume seed proteins have stressed their nutritional aspects, such as amino acid composition (Bhatty, 1982; Hu and Esen, 1982; Johnson and Lay, 1974). With increasing consumption of formulated convenience foods, the need to develop ingredient proteins with reliable functional properties from plant sources has been emphasized (Kinsella and Shetty, 1979). Therefore, identification of legume protein components and characterization of their functional properties will provide fundamental information for developing new food products or improving the processing of existing products.

Two-dimensional PAGE analysis of total adzuki bean proteins and three solubility fractions in this study indicates that adzuki bean seed proteins are much less heterogeneous than other legume seed proteins. For example, Hu and Esen (1982) analyzed soybean protein by two-dimensional PAGE and found that several hundred spots could be detected in their electrophoretic map. In our study approximately 50 spots were detected. Hu and Esen (1982) used 400 μg of protein for each electrophoretic analysis whereas only 100 μg of protein was used in this study. Unfortunately, Hu and Esen (1982) did not show the results of two-dimensional PAGE analysis of total soybean protein prior to solubility fractionating. In our study, the spots on the electrophoretogram from the total protein extract and solubility fractions were compared to the spots on the electrophoretogram from proteins of each solubility fraction. Results indicate that sequential extraction separated the proteins quite well as indicated by the spots on the electrophoretogram. This relationship was confirmed by the distinct functional properties of each fraction (data not shown).

The data presented in this paper confirm that glycoproteins are the major storage protein in adzuki beans. As reported by Sakakibara et al. (1979), the 7S globulin protein of adzuki beans consists of three subunits, namely, α, β_1, and β_2. The estimated molecular weights were 55 000, 28 000, and 25 000, respectively. Our data show that the M_r of α and β_1, two of the glycoproteins among those three subunits, are 55 000 and 35 000, respectively. The difference in M_r of the β_1 subunit may be due to the different cultivars studied or to differences in the gel electrophoretic system used.

How the individual protein fractions contribute to the quality of An paste is not known. Studies to explore the functional properties of each solubility fraction are under way and will be reported in a separate paper.

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Quantitation of Individual and Total Aldehydes in Citrus Cold-Pressed Oils by Fused Silica Capillary Gas Chromatography

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Total aldehydes in cold-pressed oils from California and Florida citrus cultivars were quantified by fused silica capillary gas chromatography using a nonpolar bonded-phase fused silica column. Total aldehydes by GC were from 93 to 100% of the U.S.P. total aldehyde value and were within the limits of precision for the U.S.P. method. The major aldehydes quantified in oils were octanal and decanal, and these values were higher in oils with the higher total aldehydes with one exception. Most values for individual aldehydes were within or below aldehyde values reported earlier.

Volatile aldehydes are important to the flavor and aroma of citrus cold-pressed oils and total aldehydes are part of the standards of identity for determining oil quality (Kesterson et al., 1971). The current U.S.P. ("United States Pharmacopeia", 1965) method for total aldehydes is nonspecific, since the reagent used for color formation (hydroxylamine hydrochloride) reacts with any aldehyde or ketone carbonyl present in the oils. Thus, some of the compounds detected may not contribute in the same manner as the main aldehydes to the flavor or aroma of citrus oils.

A reliable gas chromatographic (GC) procedure would provide quantitative values for individual volatile aldehydes and ketones, total aldehydes, and quantitative values for many other desirable volatile flavor components in citrus oils. Early GC methods using packed analytical columns for quantifying volatile components of citrus oils met with limited success because of incomplete separation of individual components (Shaw, 1979). Development of glass capillary GC columns and improved instrumentation gave a method for analyzing complex mixtures where improved resolution and sensitivity were achieved (Jennings, 1979). Jennings (1980) discussed the advantages of high-resolution glass capillary chromatography for analyzing complex mixtures of volatiles in foods and essential oils. Wilson and Shaw (1980) reported the quantification of 32 constituents of Florida cold-pressed grapefruit oil using a Carbowax 20M glass capillary column. Vora et al. (1983) reported the concentration of 24 volatile flavor components from Florida Valencia and midseason cold-pressed oils using a nonpolar OV-101 fused silica column. They quantified most of the major aldehydes present but were unable to separate and quantify the major aldehyde, octanal. Staroscik and Wilson (1982a) quantified 37 components in a mixture of California and Arizona cold-pressed lemon oil with a nonpolar SE 54 glass capillary column and reported quantitative differences in lemon oil mixtures due to seasonal and regional variations (Staroscik and Wilson, 1982b). They found that SE 54 gave good separation of aldehydes and that it was superior to the widely used Carbowax 20M for analyzing citrus oils. Their value for citral determined by capillary GC was about 0.4% lower than the citral value determined by the hydroxylamine test. However, total aldehydes determined by capillary GC afforded up to 96% of the total aldehydes determined by the nonspecific U.S.P. method.

The current report describes the determination of total aldehydes in cold-pressed citrus oils from several California and Florida citrus cultivars using a bonded-phase fused silica capillary column and compares the results to those obtained by the U.S.P. method.

EXPERIMENTAL SECTION

Two commercial samples each of California navel orange, Florida Valencia orange, midseason orange, tangerine, and white graphefruit cold-pressed oils were obtained for this study. Each oil had a different U.S.P. total aldehyde value.

Oil samples were analyzed in triplicate on a Hewlett-Packard 5840A GC equipped with a fused silica capillary column with a bonded phase equivalent to SE 54 (DB-5, 30 m × 0.32 mm i.d., 1.0-μm film thickness, J & W Scientific, Inc., Rancho Cordova, CA). The oven temperature was held at 40 °C for 0.5 min, raised to 60 °C at 20 °/min, and then programmed to 250 °C at 4 °C/min. Injection port and detector temperatures were 250 and 350 °C, respectively. The carrier gas (H_2) flow (U) was 38 cm/s at 250 °C, and makeup gas (N_2) was 30 mL/min. the sample size for both oils and calibration mixtures was 0.1 μL with an injection port split ratio of 100:1. Compounds used as calibration standards were obtained from commercial sources (Aldrich Chemical Co., Milwaukee, WI; C. A. Aromatics, Floral Park, NY). Calibration mixtures for determining response factors were prepared as previously described (Wilson and Shaw, 1978). Ethyl cinnamate was

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