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Selective Thermal Denaturation as a Method of Preparative Isolation of 11S Globulins from Plant Seeds

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A method has been developed for isolation of 11S globulins from soybeans, broad beans, peas, sunflower seeds, and oats based on the principle of selective thermal denaturation. The preparation isolated from defatted soy meal contains 95–97% of 11S globulin according to sedimentation velocity measurements, while its yield amounts to 1–2 g/100 g of defatted meal. The preparation isolated from defatted meal of sunflower seeds contains 95–97% of 11S globulin, and its yield is 3–5 g/100 g of meal. The preparations of 11S globulin from broad beans, peas, and oats were isolated without preliminary defatting of the meal. The preparation of 11S globulin from oat seeds is of the same purity as those isolated from soybeans and sunflower seeds, while its yield accounts for 0.2–0.5 g/100 g of meal. The preparations of 11S globulins isolated from broad beans and peas contain no impurities, according to the sedimentation data. The yield of preparations is equal to 0.5 g/100 g of meal.

INTRODUCTION

The fractionation of proteins using the principle of selective thermal denaturation is well-known. Specifically, this principle is used for isolating enzymes (Kochetkov, 1980). It was also employed for isolating 11S globulin from soybeans (Osborne and Campbell, 1898), 13S globulin from sesame seeds (Hasegama et al., 1978), and 11S globulin from broad beans (Schlisier and Manteuffel, 1981). In the latter case it was shown that the isolated preparation is identical immunoelectrophoretically and serologically with the native 11S globulin.

EXPERIMENTAL SECTION

11S globulin from soybeans was isolated from defatted soy meal Soya Fluff 200 W (Central Soya International). 11S globulin from sunflower seeds was isolated from defatted meal (cultivar Yubileinyi-60). 11S globulins from broad beans (cultivar Orlovski-41), peas (cultivar Orlovski-3), and oats (unknown cultivar) were isolated from nondefatted meal.

Standard preparations of 2.8S, 7S, and 11S globulins were isolated from defatted soy meal according to the methods developed by Vaintraub and Shutov (1969) and Thanh and Shibasaki (1976). The preparation of 2.8S globulin was homogeneous according to velocity sedimentation, chromatography, and gel electrophoresis. The preparation of 7S globulin was sedimentationally homogeneous. The preparation of 11S globulin was purified by the chromatographic method on hydroxyl apatite (Wolf and Sly, 1965). According to sedimentation velocity measurements, it contained ~90% of 11S globulin, with an impurity having a sedimentation coefficient of 17S. The globulin fraction of soybeans was isolated by the isoelectric precipitation method (Wolf, 1972). According to sedimentation velocity measurements, this preparation contained 2S, 7S, 11S, and 15S components in a weight ratio 1:7.5:5.7:1.2. Chicken lysozyme (Type A, Biokhimreactiv; Olaine, USSR) with an activity of 26 000 units was used as calorimetric standard without additional purification.

Auxiliary materials (buffer salts, sodium chloride, guanidine hydrochloride (GuHCl), 2-mercaptoethanol, sodium azide) were of analytical grade. All the solutions were prepared with distilled water.

Preparation of Solutions. Stock aqueous solutions of 10% preparations of 11S globulins from soybeans and broad beans, containing sodium azide (0.02%), were stored in a dark place at room temperature in hermetically sealed vessels. Such storage provides invariability of thermodynamic and hydrodynamic characteristics of the preparations. Sedimentograms for the preparations of 11S globulins from sunflower seeds, peas, and oats were obtained immediately after their isolation. The concentration of globulins in the stock solutions was determined by drying to a constant weight at 105 ± 5 °C. The concentration of lysozyme in the solution was determined by spectrophotometry, assuming the extinction coefficient to be $E_{280}^{1\%}$ = 26.9 (Khechinashvili, 1977). Solutions for sedimentation, viscosimetric, and calorimetric investigations were pre-

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pared by diluting 10% stock solution with appropriate solvents.

Methods. *Preparative centrifugation* at all the stages of isolating of 11S globulins was carried out at 2500*g* on a K-70 centrifuge MLW. Clarification of the solutions at the final stage of isolation was performed at 200000*g* for 30 min on a UZP-60 centrifuge Biofispribor.

Sedimentation analysis was performed on a MOM 3170 B ultracentrifuge at 50 000 rpm and a temperature of 25 °C. The homogeneity of globulins was verified in phosphate buffer (2.6 mM KH_2PO_4, 32.5 mM K_2HPO_4, 0.4 M NaCl, pH 7.6, ionic strength 0.5) containing no mercaptoethanol. In order to determine small coefficients of sedimentation, a method of artificial boundary was employed. In each test six photographs were taken every 6–15 min. For 11S globulins from soybeans the obtained values for sedimentation coefficients were adjusted to the standard conditions (water, 25 °C) with consideration for the viscosity and density of the solvent. To approximate the concentration dependences of sedimentation coefficients, the known linear relationship was employed (Tanford, 1965a).

Viscosity measurements of diluted solutions of 11S globulin from soybeans were made on a Uberllode capillary viscosimeter. Prior to viscosity measurements the solutions were centrifuged (200000*g*, 30 min) and then filtered through a G-4 glass filter directly in the viscosimeter. The value for the relative viscosity (η_r) was determined as

$$\eta_r = \tau/\tau_0 \quad (1)$$

In the calculation of reduced viscosity the equation

$$\eta_{sp} = (\eta_r - 1)/C \quad (2)$$

was used where C is the protein concentration (g/dL); the value of correction for solvent density was ignored due to its insignificance (Ahmad and Salahuddin, 1974). The concentration dependence of the viscosity was approximated according to Huggins equation (Tanford, 1965)

$$\eta_{sp} = [\eta] + k_H[\eta]^2C \quad (3a)$$

in which $[\eta]$ is the intrinsic viscosity and k_H is the Huggins constant and to Mooney equation (Mooney, 1951)

$$\eta = \exp[SC/(1 - KS)] \quad (3b)$$

where the S parameter is equivalent to the intrinsic viscosity while the K parameter characterizes the effect excluded volume.

The molecular weights of the native forms of 11S globulin from soybeans (M) were estimated proceeding from the value for sedimentation constant ($S°_{20,w}$) according to the empirical equation

$$S°_{20,w} = (0.345 \times 10^{-2})M^{0.65} \quad (4)$$

obtained from the data of Tanford (1965b) and Serdyuk (1981) based on the values for $S°_{20,w}$ and molecular weights of 20 globular proteins. It should be noted that this equation is similar in form to the theoretical dependence ($S°_{20,w} \sim M^n$) for a model of hard spheres of equal density (Squire and Himmel, 1979) with the exponent n having almost a theoretical value ($^2/_3$).

The molecular weights of the denatured forms of the 11S globulin were estimated proceeding from the experimental values for the intrinsic viscosity according to a graph of empirical dependence $[\eta] = f(M)$, presented as the equation of Burchard (1961) and Stockmayer and Fixman (1963)

$$[\eta]M^{-0.5} = 0.095 + (4.286 \times 10^{-4})M^{0.5} \quad (5)$$

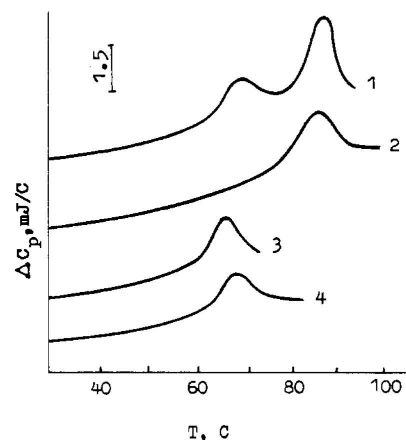

Figure 1. Thermograms of soybean globulin fraction (1) and 11S (2), 7S (3), and 2.8S (4) globulin solutions (protein concentrations 0.5%, 0.16%, 0.21%, and 0.15%, respectively; phosphate buffer (6.2 mM KH_2PO_4, 31.6 mM K_2HPO_4) with pH 7.6 and ionic strength 0.1, scanning rate 2 °C/min). Thermograms are arbitrarily displaced along the axis of ordinates.

where $[\eta]$ is expressed in cm/g^3.

This dependence was obtained in the work of Bikbov et al. (1981) based on Tanford's data (1968) on the values for and the molecular weights of 13 proteins in 4–7.5 M GuHCl at 25 °C.

Microcalorimetric studies have been carried out on a DASM-1M differential adiabatic scanning microcalorimeter of Privalov type (Special Design Office for Biological Instrument Making, USSR Academy of Sciences, Pushchino, USSR) in a temperature range from 20 to 110 °C and at a scanning rate of 2°/min and excess pressure of 1 atm. In each trial the heat capacity scale of the microcalorimeter was calibrated according to Joule–Lentz effect. The reliability of electric calibration was additionally checked with chicken lysozyme as the calorimetric standard. According to Khechinashvili's data (1977), the temperature and specific enthalpy of its denaturation equalled respectively 338.8 K and 34.8 J/g at pH 2.5 in 0.01 M glycine buffer. According to our data, these parameters are 338.8 K and 33.5 J/g. Such an agreement between our and Khechinashvili's (1977) data can be assumed to be quite satisfactory. The microcalorimetric investigations involved the use of 0.25% protein solutions in phosphate buffer (pH 7.6, ionic strength 0.1). The integration of thermograms in the denaturation range was carried out on a planimeter with consideration for a heat capacity jump at denaturation temperature. The heat capacity jump at denaturation temperature was estimated by extrapolating the linear branches of the thermograms before and after denaturation to denaturation temperature.

RESULTS AND DISCUSSION

Figure 1 illustrates thermograms of soybean globulin fraction (1), 11S globulin (2), 7S globulin (3), and 2.8S globulin (4). The thermogram of soybean globulin fraction has two denaturation maxima, while the thermograms of individual proteins have only one maximum. The position of the low-temperature maximum on the thermogram of globulin fraction approximately corresponds to that of maxima on the thermograms of 2.8S and 7S globulins. The position of the high-temperature maximum on the thermogram of globulin fraction practically coincided with that of the maximum on the thermogram of 11S globulin. These facts lead to the conclusion that the low-temperature maximum on the thermogram of globulin fraction corresponds to denaturation of 2.8S and 7S components, while the high-temperature one corresponds to denaturation of

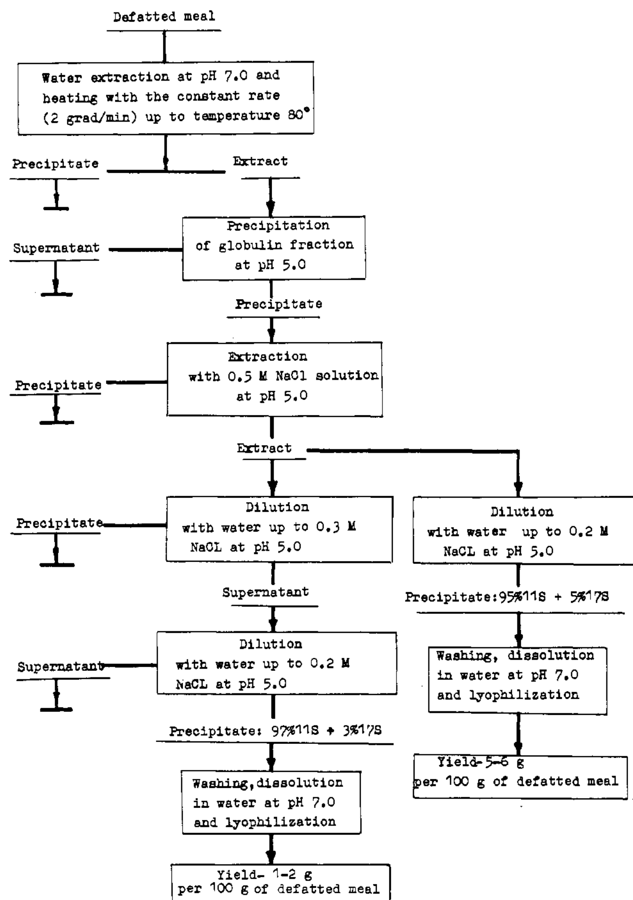

Figure 2. Scheme of isolating of 11S globulin from soybeans.

11S component. It is noteworthy that the denaturation temperature of 11S globulin is 16 °C higher than those of 2.8S and 7S components of globulin fraction. This circumstance makes it possible to use the principle of selective thermal denaturation for isolating of 11S globulin. The thermal denaturation of 2.8S and 7S globulins makes their separation from 11S globulin easier due to the fact that the denatured forms usually have a more hydrophobic accessible surface than the native forms (Kato et al., 1983; Townsend and Nakai, 1983). This difference promotes the separation of the indicated forms according to their solubility in media with low lipophilicity, for instance in salt solutions.

These data were used for development of the preparative method for isolating of 11S globulin from soybeans. Figure 2 presents a scheme of this method. The first stage involves the extraction of proteins with water (pH 7) from defatted meal at a meal/water weight ratio of 1 to 10 while the aqueous suspension of the meal is heated at a rate of 2 °C/min up to a temperature of 80 °C. Under these conditions the denaturation predominantly involves 2.8S and 7S globulins and partly 11S globulin, whereas the remaining amount of 11S globulin retains its nativity. The denaturation products obtained are not soluble in such lipophobic solvent as 0.5 M NaCl solution at pH 5, i.e. in the vicinity of the isoelectric point of globulin fraction. For this reason after precipitation of globulin fraction at pH 5, the use of 0.5 M NaCl as an extractant leads to extraction predominantly of 11S globulin from the residue. Apart from the 11S globulin, such an extract also contains a component with a sedimentation coefficient of about 15S.

Purification of 11S globulin from the secondary component is based on the difference of their molecular weights. It is known that a decrease in the solvent quality results at first in a precipitation of higher molecular

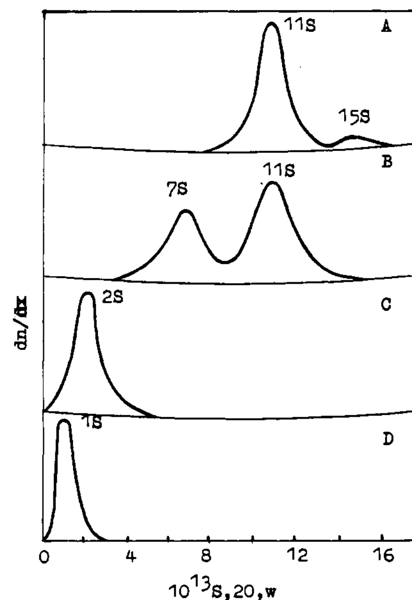

Figure 3. Schematic representation of composition of 11S globulin preparation isolated from soybeans by the method of selective thermal denaturation according to sedimentation velocity experiments in various solvents: A, phosphate buffer with pH 7.6 and ionic strength 0.5 (32.5 mM K_2HPO_4, 2.6 mM KH_2PO_4, 0.4 M NaCl); B, water with pH 7; C, 8 M GuHCl solution; D, 8 M GuHCl solution containing 0.01 M 2-mercaptoethanol. The scale is retained along the axis of ordinates.

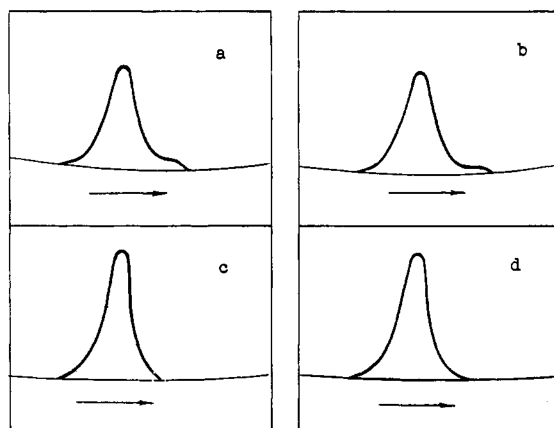

Figure 4. Sedimentograms of 11S globulins from sunflower seeds (a), oat seeds (b), broad beans (c), and peas (d) (protein concentration 0.5%, phosphate buffer with pH 7.6 and ionic strength 0.5 containing no 2-mercaptoethanol; photographs taken 22 min after the speed had reached 50 000 rpm).

fractions from polymer solutions (Tanford, 1965c). Therefore, a decrease of NaCl concentration to 0.3 M by dilution with water leads to precipitation of the greater part of 15S component. The last stage, i.e. the dilution of NaCl concentration up to 0.2 M with water, is intended for precipitation of 11S globulin. According to sedimentation velocity measurements, the isolated preparation contains 95–97% of 11S globulin (Figure 3A), and its yield accounts for 1–2 g/100 g of defatted meal. The yield of 11S globulin may be raised up to 5 g if the stage of isolating of 15S component is excluded. In this event the preparation contains ~95% of 11S globulin.

Table I presents comparative data on the thermodynamic parameters for denaturation of a "standard" preparation of 11S globulin, isolated by the method of Thanh–Shibasaki with subsequent chromatography on hydroxyl apatite, and the preparation isolated by the method of selective thermal denaturation. It can be seen

Table I. Comparison of Thermodynamic Parameters of Denaturation[a] of 11S Globulin Preparations Isolated from Soybeans by Different Methods

essence of method	denaturn temp, °C	sp enthalpy of denaturn, J/g
sel therm denaturn	85 ± 0.3	23 ± 1[b]
that of Thanh–Shibasaki (1976) and chromatogr on hydroxyl apatite	84 ± 0.3	21 ± 2[b]

[a] Data obtained in phosphate buffer, pH 7.6, ionic strength 0.1. [b] Confidence interval corresponds to significance level of 0.95.

Table II. Molecular Characteristics of Various Forms of 11S Globulin Preparation Isolated from Soybeans by the Method of Selective Thermal Denaturation

solvent[a]	globulin form	$10^{-13}S°_{20,w}$, s	$[\eta]$, cm^3/g	M, kDa
A	dodecamer	13.5	8.25[b]	340[c]
	cluster	17.5		960[d]
B	dodecamer	14.0	4.85[e]	356[c]
	hexamer	8.8	4.85[e]	174[c]
C	dimer	1.9	47[e]	58[f]
D	monomer	1.3	31[e]	32[f]

[a] The identification of solvents A–D is represented in the caption to Figure 3. [b] It is about 1.7 times higher than intrinsic viscosity of the dodecameric form of 11S globulin according to Koshiyama's data (1972). The difference may be caused by the cluster's admixture with the sedimentation constant 17S and intrinsic viscosity ~70 cm^3/g. [c] Calculated by eq 4 proceeding from the value of sedimentation constant. [d] Rough estimation, for a model of elongated ellipsoid, which does not contradict the values for intrinsic viscosity and sedimentation constant. [e] The result of averaged estimation of intrinsic viscosity according to equations of Huggins (eq 3a) and Mooney (3b). [f] Calculated by eq 5 proceeding from the value for intrinsic viscosity.

that both preparations are thermodynamically identical within the permissible experimental errors.

Figure 3 displays sedimentation velocity data on the preparation of 11S globulin isolated by the method of selective thermal denaturation in four solvents. In solvent A (phosphate buffer, pH 7.6, ionic strength 0.5) the preparation represents a component with a sedimentation constant of 13.5S and a secondary component with a sedimentation constant of about 17S. Solvent B (water, pH 7) shows two sedimentation components with sedimentation constants 8.8S and 14S. It is noteworthy that in this case no 17S component is found. It is apparently unstable in a media with a low ionic strength. The preparation is homogeneous in solvents C (8 M GuHCl) and D (8 M GuHCl and 0.01 M 2-mercaptoethanol). In this case it has sedimentation constants of about 2S and 1S, respectively.

Table II summarizes the values for sedimentation constants, intrinsic viscosities, and molecular weights of various preparation forms of 11S globulin isolated by the method of selective thermal denaturation. It is known (Wolf, 1972) that at neutral pH values with high ionic strength (solvent A) 11S globulin exists in the form of a dodecamer consisting of six acidic (A) and six basic (B) subunits. The dodecamer has a sedimentation constant of about 12–14S (Derbyshire et al., 1976) and an intrinsic viscosity of 4.85 cm^3/g (Koshiyama, 1972). The preparation of 11S globulin isolated by the method of selective thermal denaturation is represented under given conditions by two components. The sedimentation constant of the main component is 13.5S, and that of the secondary component is 17S. The value for sedimentation constant of the main component is located in the indicated range of sedimentation constants of 11S globulin. Therefore, the main component of the isolated preparation may be

Table III. Comparison between Experimental and Theoretical Values of Molecular Weights for Various Forms of 11S Globulin Preparation Isolated from Soybeans by the Method of Selective Thermal Denaturation

globulin form	M, kDa exptl	M, kDa theortl
dodecamer	348[a]	351[b]
hexamer	174[c]	175.5[d]
dimer	58	57.5[e]
monomer	32	31.8[b]

[a] Average estimation according to sedimentation velocity measurements in phosphate buffer, pH 7.6, ionic strength 0.5, and in water, pH 7. [b] Calculated by brutto formula of dodecamer A_6B_6 proceeding from molecular weights of acidic and basic subunits: M_A = 37.7 kDa and M_B = 20.8 kDa. [c] It should be noted that according to experimental data the ratio of molecular weights for dodecamer and hexamer is precisely equal to a theoretical value of 2. [d] Calculated by brutto formula of dimer AB proceeding from molecular weights of acidic and basic subunits indicated in the text. [e] Calculated by brutto formula of hexamer A_3B_3 proceeding from molecular weights of acidic and basic subunits indicated in the text. [f] Weight average molecular weight of equimolecular mixture of acidic and basic subunits.

identified with a dodecameric form of 11S globulin. The secondary component of the isolated preparation is apparently a cluster of denaturation products. Its presence considerably distorts the value for the intrinsic viscosity of the isolated preparation. It is about 1.7 times higher than the intrinsic viscosity of the dodecameric form of 11S globulin according to Koshiyama's data (1972). Assuming that the intrinsic viscosity of this form is 4.85 cm^3/g, then according to the known equation (Tanford, 1965d)

$$[\eta] = W_i[\eta]_i \quad (6)$$

linking the intrinsic viscosity of polymer mixture $[\eta]$ with a weight content (W_i) and intrinsic viscosities $[\eta]_i$ of its components, it is possible to evaluate the intrinsic viscosity of the cluster. It is equal to about 70 cm^3/g. Assuming that the cluster has the form of an elongated ellipsoid, it is possible to evaluate its axial relation ($a/b \sim 25$) and molecular weight (960 kDa) proceeding from the intrinsic viscosity and sedimentation constant (Tanford, 1965e).

This cluster is unstable in a medium with a low ionic strength since it is not found in a sedimentogram after replacing the buffer (solvent A) by water (solvent B). It may be assumed that at a low ionic strength the cluster is precipitated during acceleration of the ultracentrifuge rotor.

At neutral pH values and low ionic strength (solvent B) the obtained preparation of 11S globulin is represented by two components with sedimentation constants of 8.8S and 14S. These components may be identified with hexameric (A_3B_3) and dodecameric (A_6B_6) forms of 11S globulin (Wolf, 1972). It is noteworthy that when there is no cluster, the intrinsic viscosity of the preparation of exactly equal to that of 11S globulin, according to Koshiyama's data. This confirms our assumption that the unnaturally high intrinsic viscosity of the isolated preparation in solvent A stems from impurities of highly anisodiametric particles of the cluster. In solvents C (8 M GuHCl) and D (8 M GuHCl + 0.01 M 2-mercaptoethanol) the isolated preparation is homogenbous in terms of sedimentation. It is characteristic that the transition from solvent C to solvent D is accompanied by a decrease in the sedimentation constant and intrinsic viscosity. These results are consistent with the known data (Kitamura et al., 1976), in which 11S globulin is represented as dimers (AB) in denaturing media and as monomers, i.e. subunits (A + B), in denaturing media containing reducing agents. Table III compares experimental and theoretical values

of molecular weights for various forms of the isolated preparation. The molecular weights of dodecameric and hexameric forms of the isolated preparation hardly differ from those of these forms calculated by their brutto formulas (A_6B_6) and (A_3B_3) proceeding from the averaged molecular weights of acidic and basic subunits of 11S globulin (M_A = 37.7 kDa, M_B = 20.8 kDa) (Koshiyama, 1972; Catsimpoolas et al., 1971; Kitamura and Shibasaki, 1975; Draper and Catsimpoolas, 1977). The relationship of the molecular weights for dodecameric and hexameric forms of the isolated preparation is exactly equal to a theoretical value of 2. The molecular weight of the dimeric form practically coincides with that of this form calculated from its empirical formula (AB), proceeding from the molecular weights of acidic and basic subunits. And finally, the mean molecular weight of the monomeric forms also practically coincides with that of the equimolecular mixture of acidic and basic subunits (A + B), which are formed upon a complete dissociation of the tertiary structure of 11S globulin.

The obtained thermodynamic and hydrodynamic characteristics of the isolated preparation of 11S globulin lead to the conclusion that it corresponds to the native 11S globulin.

Considering that 11S globulins of legumes and oil seed cultures have a close amino acid composition and a similar subunit structure (Vaintraub, 1975; Derbyshire et al., 1976), hence apparently similar denaturation temperatures, it may be assumed that the principle of selective thermal denaturation could be used for isolation of 11S globulins from other cultures. Its application to globulins from sunflower seeds, broad beans, and peas as well as oats served as a typical example of this.

11S globulin from sunflower seeds was isolated from defatted meal. The first stage involved the extraction of proteins from the meal with 10% NaCl solution containing 0.1% of EDTA Schwenke and Raab, 1973; Vaintraub, 1984) in a meal to water ratio of 1 to 10 by weight while heating the aqueous suspension of the meal at a rate of 2 °C/min up to 80 °C. The suspension was cooled by twofold dilution with water, and the extract was clarified by centrifuging. Then, the extract was adjusted to pH 5.0 with 0.1 N HCl solution, and the ionic strength was reduced to 0.2 M by diluting with water to precipitate 11S globulin. After centrifuging, the precipitate was dissolved in water, adjusting to pH 7.0 with 0.1 N NaOH solution. According to sedimentation velocity measurements the isolated preparation contained 95–97% of 11S globulin and a cluster with a sedimentation coefficient of about 15S (Figure 4a). the yield of the preparation amounted to 3–5 g/100 g of defatted meal.

The isolation of 11S globulins from broad beans, peas, and oats was carried out without preliminary defatting of the meal. The extraction of proteins was performed for 30 min with 0.37 M NaCl solution at pH 5.0 and a meal to NaCl solution ratio of 1 to 10. Such extraction was shown to cause the transfer of only proteins into the solution, while lipids remain in the residue. The extract was clarified by centrifuging, and then the ionic strength of the solution was reduced by half by dilution with water to precipitate globulins. The residue obtained after centrifuging was dissolved in water, adjusting its to pH 7.0 with 0.1 N NaOH solution. The solution was heated to 80 °C at a rate of 2 °C/min and then cooled by a twofold dilution with water. The solution was adjusted to pH 5.0 with 0.1 N HCl solution to precipitate globulins. After centrifuging the residue was extracted by a 10-fold volume of 0.5 M NaCl solution. The extract was clarified by centrifuging, and then the ionic strength of the supernatant was reduced to 0.2 M by dilution with water. The precipitate obtained after centrifuging was dissolved in water, adjusting to pH 7.0 and 0.1 N NaOH solution.

The preparation of 11S globulin isolated from oat seeds, according to sedimentation velocity measurements, is 95–97% pure. The secondary component has a sedimentation coefficient of about 15S (Figure 4b). The yield of the preparation is 0.2–0.5 g/100 g of meal. The preparations of the 11S globulins isolated from broad beans and peas are homogeneous in terms of sedimentation (Figure 4c), and their yield is 0.2–0.5 g/100 g of meal.

Thus, using globulins from soybeans, broad beans, peas, sunflower seeds, and oats as an example, it has been shown that the principle of selective thermal denaturation is applicable for isolation of 11S globulins.

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Nutrient Composition of Retail Ground Beef

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In the U.S. 24% of all beef consumed is in the form of ground beef. Retail ground beef samples selected from 10 major cities representing five geographic regions were analyzed for protein, fat, and moisture by near-infrared reflectance (near-IR) spectroscopy and by traditional methods and for inorganic nutrients by atomic absorption spectroscopy (AAS). These data indicated large variability (coefficient of variation 14–27%) in the total fat content within various generic types. With regard to accuracy and precision, the near-IR technique compared favorably to traditional techniques in the analysis of fat and moisture. The accuracy of the near-IR for protein determination will require further study.

INTRODUCTION

In 1981, the per capita consumption of ground beef was 18.6 lbs, an increase of 1.9 lbs per capita since 1971. While total beef consumption has declined steadily since 1971, the consumption of ground beef has continue to increase. Ground beef accounts for 24% of all beef consumed, an increase of 4% since 1971 (American Meat Institute, 1982). Approximately 50% of all ground beef is purchased at the retail store and consumed in the home.

Ground beef is a formulated product prepared on a regional or local basis; the extent of variability in its nutrient composition is unknown. U.S. Department of Agriculture (USDA) regulations apply only to ground beef that is prepared in federally inspected establishments. USDA regulations state that "chopped beef" or "ground beef" shall consist of chopped fresh and/or frozen beef without the addition of beef fat as such, shall not contain more than 30% fat, and shall not contain added water, phosphates, binders, or extenders. Additional statements discuss the use of seasonings and limits on the use of beef cheek meat (9CFR319.115, 1983). Heart meat and tongue meat are not acceptable ingredients in chopped beef, ground beef, or hamburger (Hibbert, 1981). State or local government regulations or guidelines determine what the levels of fat should be in a ground beef product prepared at the retail site. In many cases the regulation or guideline is equivalent to the federal standard for ground beef. Labels such as "lean" and "extra lean" may be used on ground beef if the product has significantly less fat than expected in a similar product (Hibbert, 1984). State or local government regulations or guidelines determine what the levels of fat should be in a ground beef product prepared at the retail site. In many cases the regulation or guideline is equivalent to the federal standard for ground beef.

In general, ground beef that is labeled as prepared from a specific cut such as ground chuck is fabricated from that cut and adjusted to a fat level according to the policies of individual chains. In the retail trade, various cut designations seem to be synonymous with certain generic designations based on fat level. One chain may label according to the specific cut used while another may label according to relative fat content. These are ground chuck and lean ground beef; ground round and extra lean ground beef; and ground round and extra lean ground beef. Sometimes ground sirloin is considered to be similar to extra lean ground beef. Hamburger and regular ground beef are considered to be similar.

A telephone survey of the predominant grocery chains in 10 cities indicated that approximately half of those surveyed process ground beef at the warehouse level. Coarsely ground bulk products corresponding to the generic names are shipped to individual stores where they are reground with added meat trimmings free of visible fat and repackaged for retail sale. Ground beef prepared at the warehouse level is usually tested at intervals for fat content. Infrequent monitoring may or may not be done at the store level and is dependent upon the policies of specific chains. The other half of those chains surveyed prepare ground beef at the store level. Company guidelines are general and may be equivalent to the USDA standard or a slightly lower maximum total fat value. Some stores reported that their maximum value for total fat was 25 or 27% to ensure compliance with a state or local regulation or guideline for permissible maximum fat level. The formulation of the retail product was determined by a combination of the experience of the meat cutter within each store and product standards endorsed by chain managers. In some cases sporadic on-site checks of fat level were made by the district manager or his representative.

To obtain accurate and precise nutrient data that characterize a frequently consumed product, it is necessary to evaluate the inherent variability in that product. This is an important aspect of food composition studies that is often overlooked. This study was conducted to evaluate the sources and extent of variance in the nutrient composition of uncooked ground beef sold at the retail level in the U.S. The mean concentrations of protein, fat, ash,

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