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Guar Foaming Albumin: A Low Molecular Mass Protein with High Foaming Activity and Foam Stability Isolated from Guar Meal

AMI SHIMOYAMA,[†] SHOKO KIDO, YOH-ICHI KINEKAWA,[‡] AND YUKIO DOI*

Department of Food and Nutrition, Kyoto Women's University, Higashiyama-ku, Kyoto 605-8501, Japan

The water extract of guar meal (*Cyamopsis tetragonolobus*) was examined for its foamability. Compared with egg white, the extract showed an extraordinary foam stability: no drainage after 3 h of standing in contrast to 65% drainage for egg white at the same protein concentration. The acid-precipitated protein from the extract was responsible for the high foamability and designated guar foaming albumin (GFA). The foaming activity of GFA was 20 times higher than that of egg white. GFA consisted of two subunits with molecular masses of 6 and 11 kDa linked to each other through disulfide bonds. The cleavage of disulfide bonds in GFA affected the foamability only slightly. GFA remarkably decreased the surface tension of water at low protein concentrations. Immunoblotting analysis demonstrated that GFA did not react to the antisera from allergic patients against plant food. These results suggest that GFA serves as an effective food additive in developing protein-stabilized foam.

KEYWORDS: Guar meal; protein-stabilized foam; foaming protein; albumin; *Cyamopsis tetragonolobus*

INTRODUCTION

Guar bean (*Cyamopsis tetragonolobus*), an annual legume with a large endosperm, contains a galactomannan gum, which is extracted, refined, and used as a stiffener in soft ice cream, as a stabilizer for cheeses, as a whipped cream substitute, and as a meat binder. Guar beans are used as a vegetable for human consumption, and the crop is also grown for cattle feed in Asia (*1*). Guar meal, composed of germ and hull residues, is a byproduct produced during extraction of the gum and contains approximately 45% protein. Although early research with guar meal suggested the presence of toxic factors such as trypsin inhibitor, saponins, and residual gum, which may cause deleterious effects on chicken growth, a recent study showed that the trypsin inhibitor activity in guar meal was negligible and not considered to be a significant factor limiting its use in poultry feeds (*2*).

With attention to the high protein content of guar meal, several studies have been conducted to characterize its protein (*3*−*7*). Nath and Narasinga-Rao (*6*) reported that the fraction isolated with 1 M NaCl revealed proteins of molecular masses ranging from 1 to 250 kDa (*4*) and noted that it possessed considerable foaming ability. The pH dependence of foaming ability showed that the guar protein has the highest foaming activity at pH 2.0, with 1.3 times higher activity than soy protein. Khalil et al. (*7*) showed that the best protein yield was obtained when guar meal was extracted with 0.1 M NaOH/1 M NaCl, and the recovered protein fraction with a high content of lysyl residue had high foamability. We have previously examined the guar proteins isolated according to the method of Osborne (*8*) and showed that the water soluble proteins exhibited much higher foamability than the NaCl extract (*9*).

In this study the water extract containing low molecular mass proteins was further examined to clarify their foaming properties with their possible application as food additives in mind.

MATERIALS AND METHODS

All chemicals were of analytical grade and were used as supplied. Commercial guar meals imported from Pakistan were provided by Daiichikasei Co. Ltd. (Kyoto, Japan).

Delipidation of Guar Meal. Guar meal was ground in an electric blender, and the powder (200 g) was refluxed with *n*-hexane (500 mL) for 12 h, dried completely, and stored in a freezer. The delipidation step is useful for removing a characteristic odor.

Preparation of the Water Extract. To the delipidated meal (10 g) was added 500 mL of water, and the suspension was mixed for 3 min at the speed setting of 3 over 7 in a blender (model 7012S, Waring Products, New Haven, CT). Then the suspension was transferred to a beaker and stirred overnight at 4 °C after the addition of 1000 mL of water. The suspension was centrifuged at 8000*g* for 25 min, and the supernatant was obtained as a water extract. Most of the residual guar gum left in guar meal formed a viscous gel by the addition of water and was removed in the sediment by the centrifugation. A portion of the sediment was then extracted with 1 M NaCl for obtaining the globulin fraction of guar meal, which was used for a sample in SDS-PAGE.

* Author to whom correspondence should be addressed (telephone +81 75-531-7131; fax +81 75-531-7170; e-mail doi@kyoto-wu.ac.jp).
[†] Permanent address: Osaka Kun-ei Women's College.
[‡] Permanent address: Daiichi-Kasei Co. Ltd.

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Preparation of the Acid-Precipitated Fraction. Because the preliminary experiment indicated that the maximal amount of protein isolate was recovered at pH 4.0, the water extract was adjusted to pH 4.0 with 4 N HCl. After 1 h of standing, an acid-precipitated fraction was recovered by centrifugation (15000*g*, 4 °C), leaving the acid soluble fraction in the supernatant. The precipitate was suspended to 10 mM Tris-HCl (pH 7.0) and the pH adjusted to 7.0 with 1 N NaOH. The clear, slightly brownish solution was dialyzed against 10 mM Tris-HCl (pH 7.0) overnight for the measurement of foam stability and foamability.

Foaming Studies. To examine the foam stability of the water extract, it was first concentrated in a dialysis bag with a molecular cutoff of 8000 (BioDesign) that was covered with Sephadex G-200 powder until the protein concentration reached over 20 mg/mL. Ten milliliters each of the water extract at various protein concentrations in plastic containers was vigorously mixed with a two-blade hand mixer (model HF-230, Hitachi Appliances, Inc.) at the speed setting of 1 over 3 for 1, 6, and 12 min, and the resultant foam was transferred completely to the funnel, which was placed on a graduated cylinder (10 mL) to measure the volume of drainage. In a control experiment, fresh egg white strained through fine gauze was diluted to 20 mg of protein/mL with saline (0.15 M NaCl, pH 7.2) and used to examine foam stability. To observe the appearance of foam formed, 2 mL instead of 10 mL of samples was used.

To determine the foaming activity of guar proteins at various protein concentrations, 1 mL each of sample with protein concentrations ranging from 0 to 0.3 mg/mL was vortexed vigorously in a graduated test tube for 90 s, and immediately after, the volume of foam formed was recorded. As controls, fresh egg white diluted with water was used at the same protein concentrations, and their pH values ranged from 7.2 to 7.8. Foaming activity (FA) is defined as the slope of the initial concentration dependence of foam volume:

$$\mathrm{FA\ (mL^2/mg)} = \frac{\text{foam vol (mL)}}{\text{protein concn (mg/mL)}} \quad (1)$$

Surface Tension. Surface tension (γ) was measured according to the drop weight method (*10*) at 23 °C using a stalagmometer with the outer diameter of 0.33 cm. Two milliliters of the acid precipitate fraction or ovalbumin at various concentrations was introduced to the apparatus, and the weight (m) of a falling drop was calculated from the weight of 10 droplets. Surface tension was calculated by using the equation

$$\gamma = \frac{mg}{2\pi r} \times \phi \quad (2)$$

where g, ϕ, and r represent gravitational force, a correction factor for estimating the ideal drop at the instance of release and the capillary radius of the stalagmometer, respectively. Φ is a function of $r/V^{1/3}$, where V represents the volume of the drop, and its numerical values were given by Harkins and Brown (*10*). In this experiment $\phi = 1.624$ was employed.

SDS-PAGE and Immunoblotting. Proteins were separated by SDS-PAGE on 10% gel following standard protocols (*11*) and visualized with Coomassie brilliant blue. To examine the total protein profile contained in guar meal, the meal was extracted with 2% SDS overnight at room temperature, and the supernatant obtained after centrifugation was subjected to SDS-PAGE. To determine the relative amount of protein in each stained band, a digitalized photograph of the elution pattern was analyzed by NIH Image (*12*).

For Western blotting, proteins were transferred onto polyvinylidine difluoride membranes in transfer buffer containing 0.1% SDS. After being washed with phosphate buffer saline three times, the membranes were blocked with 5% nonfat dry milk in 0.05% Tween 20 in Tris-buffered saline (TTBS) for 30−60 min and incubated with the primary antiserum at a 1:10 dilution in the blocking buffer containing 0.1% bovine serum albumin for 1 h. After being washed with TTBS five times followed with Tris-buffered saline (TBS) once, the membranes were incubated with a 1:5000 dilution of secondary antibody in TTBS for 1 h. The membrane was washed with TBS once, with TTBS five times, and with TBS once and stained with BCIP-NBT (5-bromo-4-chloro-3-indolyl phosphate, nitro blue tetrazolium) solution. The primary antibodies used were sera from allergic patients against wheat and soybean (purchased from Morinaga & Co., Ltd.), and the secondary antibodies were alkaline phosphatase-conjugated goat anti-human IgE (American Qoalex A116AN).

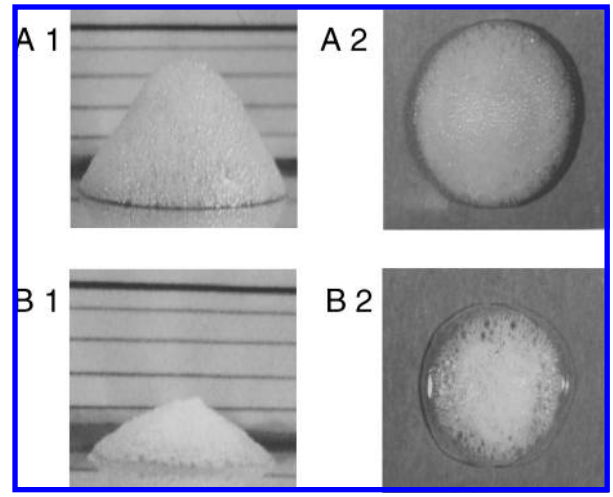

Figure 1. Appearance of foam formed by the water extract and egg white. Two milliliters of the water extract (**A**) or diluted egg white (**B**) was mixed vigorously with a hand mixer for 6.5 min, and the resultant foam was transferred onto a glass plated to be observed from the side (**A1** and **B1**) and from the top (**A2** and **B2**). The protein concentration used was 20 mg/mL in each case.

Amino Acid Analysis. The water extract, the acid precipitate after solubilization at pH 7.0, and the acid soluble fraction were thoroughly dialyzed against water and lyophilized. A portion of each sample (about 10 μg) was taken in a sample tube, and vapor phase hydrolysis was carried out on a Pico-Tag workstation (Waters) by using 6 N HCl for 24 h at 106 °C. After removal of HCl by adding triethylamine, sample was dried under vacuum and dissolved in 200 μL of 0.02 N HCl. A portion of the sample solution after filtration through membrane filter was applied to the cation exchange column in a Hitachi amino acid analyzer (model L-8500) equipped with the ninhydrin detector system.

Other Analytical Procedures. Protein concentrations were determined according to the Bradford method (*13*), with bovine serum albumin as the standard. Carbohydrate content was determined according to a phenol−sulfonic acid method (*14*).

RESULTS

Foam Stability of the Water Extract. When the water extract from guar meal containing 2% protein was mixed vigorously with a mixer, smooth, bulky foam was obtained without drainage (**Figure 1A1**, **A2**). Egg white diluted to the same protein concentration, in contrast, produced less bulky foam with coarse texture, and some drained water was observed (**Figure 1B1**, **B2**). The foam volume of the extract, roughly estimated from the size of circular cone, was 9 times larger than that of egg white.

To analyze the foam stability, the water extract was mixed for various periods of time, and the volume of drainage was measured (**Figure 2A**). Although the foam obtained after 1 and 6 min of mixing drained 60 and 50% of water after standing for 30 min, respectively, the foam obtained after 12 min of mixing did not drain at all, even after 3 h of standing. In contrast, the egg white foam obtained in the same manner drained 60% of water after 30 min of standing regardless of the mixing period of time (**Figure 2B**).

Isolation of the Acid-Precipitated Fraction. To the water extract was added HCl to pH 4.0, and the precipitate was recovered by centrifugation (**Table 1**). The acid-precipitated fraction in which 9% of total protein was recovered exhibits

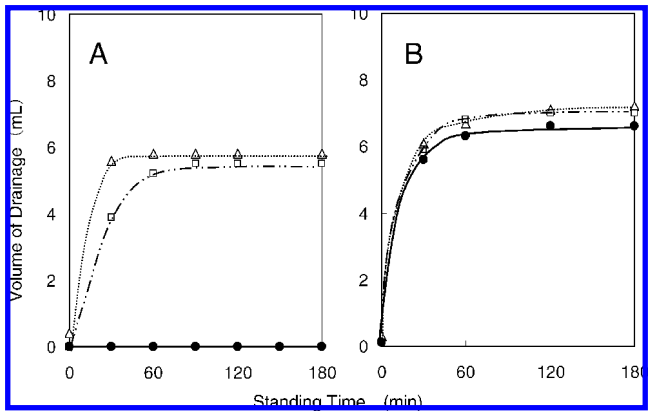

Figure 2. Stability of the foam produced with various times of mixing durations. The water extract (**A**) or diluted egg white (**B**) was vortexed vigorously for 1 min (△), 6 min (□), or 12 min (●). Immediately after, 10 mL of each foam was transferred into a measuring cylinder, and the subsequent change in the drain volume was measured for 180 min. The protein concentration used was 20 mg/mL in each case.

Table 1. Fractionation of the Foaming Activity from the Water Extract

fraction	vol (mL)	carbohydrate concn (mg/mL)	protein concn[a] (mg/mL)	total protein (mg)	protein yield (%)	foaming activity[b] (mL^2/mg)	total foaming activity[c] (mL^2)
water extract	1460	0.26	1.4	2044	100	7	14300
acid precipitate	55	0.04	3.3	182	9	18	3276
acid soluble	1430	0.18	0.4	572	28	30	17160

[a] Protein concentration was determined according to the Bradford method (*13*). [b] Foaming activity was obtained from the protein concentration dependence of foamability as depicted in **Figure 4** and defined as follows: FA (mL^2/mg) = (foam volume, mL)/(protein concentration, mg/mL). [c] Total foaming activity (mL^2) = (foaming activity, mL^2/mg) × (protein, mg).

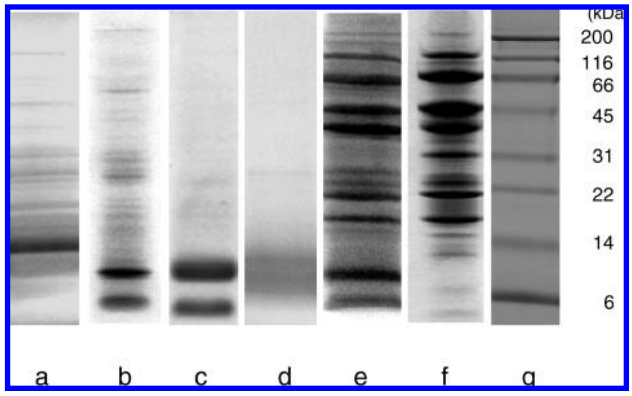

Figure 3. SDS-PAGE. Samples applied to 14% polyacrylamide gel are the water extract fraction (**a**, **b**), the acid precipitate fraction (**c**), the acid soluble fraction (**d**), the total guar protein extracted with 1% SDS (**e**), the globulin fraction extracted with 1 M NaCl after the water extraction (**f**), and molecular mass standard (**g**). The sample preparation buffer contains 100 mM mercaptoethanol in all cases except the one in lane **a**. The amounts of protein applied were 20 μg (**a**−**d**, **f**, **g**) and 50 μg (**e**). Molecular masses indicated in kilodaltons at the right were obtained by using prestained standard (Bio-Rad).

2.6 times higher foaming activity than the water extract, having 23% of the total foaming activity in the water extract. The acid precipitate contains a negligible amount of carbohydrate. On the other hand, the acid soluble fraction also demonstrates 4.3 times higher foaming activity and total activity exceeding the original activity.

Table 2. Amino Acid Compositions of the Foam Active Fractions

amino acid	fractions[a] water extract	acid precipitate	acid soluble
Asp	12.2	12.0	12.4
Thr	4.7	3.8	3.0
Ser	6.7	6.4	4.9
Glu	20.3	20.5	16.1
Gly	9.7	8.2	12.6
Ala	9.2	7.0	22.1
Val	3.5	3.4	4.0
Met	0.6	1.8	0.8
Ile	2.9	3.7	1.9
Leu	5.9	7.5	3.4
Tyr	4.2	2.7	2.8
Phe	3.5	2.7	2.6
Lys	4.6	6.7	5.7
His	2.0	1.7	0.7
Arg	6.1	8.6	2.8
Pro	3.9	3.3	4.2

[a] Data are the average values (mole percent) obtained in triplicate samples.

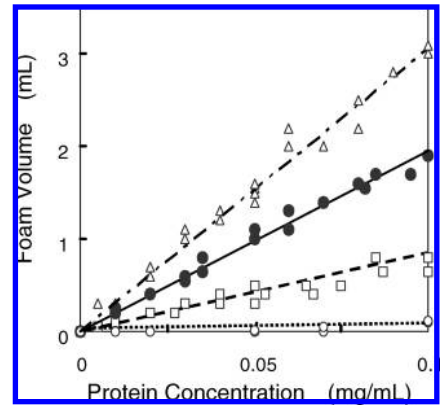

Figure 4. Foaming activity of guar proteins and egg white. One milliliter each of the water extract (□), the acid precipitate fraction (●), the acid soluble fraction (△), and egg white (○) in a graduated test tube was vortexed vigorously for 90 s, and immediately after, the volume of foam was recorded. The foaming activity is defined by the slope.

Amino Acid Composition. The amino acid compositions of the three fractions with foaming activity were determined after hydrolysis (**Table 2**). The compositions of the acid precipitate as well as the water extract are characterized by high amounts of acidic amino acids. The amino acid pattern of the acid soluble fraction is considerably different from those of the acid precipitate and the extract.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE of the water extract reveals two main protein bands with molecular masses of 6 and 11 kDa, as well as some minor protein bands (**Figure 3b**). The acid precipitate mainly consists of these two polypeptides (**Figure 3c**), which appear to link together through disulfide bonds because the SDS-PAGE pattern without a reducing agent shows a single protein band with an apparent molecular mass of 13 kDa (**Figure 3a**). Hereafter, this protein is designated guar foaming albumin (GFA). The total protein profile of guar meal, which was obtained by extracting guar meal with 2% SDS, demonstrates the presence of proteins with molecular masses ranging from 6 to 74 kDa (**Figure 3e**). The acid soluble fraction consists of several proteins around 20 kDa (**Figure 3d**), implying that the acid treatment of the water extract precipitates most of the GFA. Indeed, the globulin fraction, which was a 1 M NaCl extract of the residues obtained after the water extraction, hardly contains GFA (**Figure 3f**).

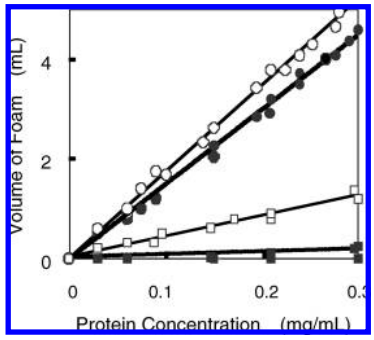

Figure 5. Effect of reduction on the foam activity. One milliliter each of acid precipitate fraction (○, ●) and egg white (□, ■) was vortexed vigorously for 90 s, and immediately after, the volume of foam was recorded. Open symbols represent the samples with the addition of 10 mM mercaptoethanol, and solid symbols represent samples without the addition. The slope defines the foaming activity.

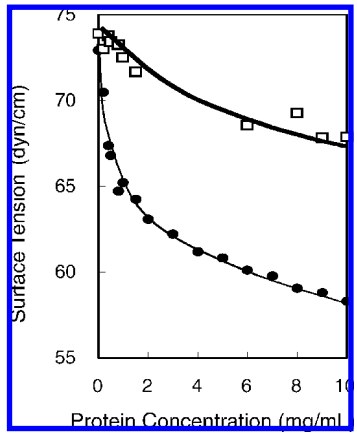

Figure 6. Surface tension at various protein concentrations. Surface tensions of GFA (●) or ovalbumin (□) at various concentrations were determined by the drop weight method at 23 °C using a stalagmometer.

Foaming Activity. Figure 4 shows changes in foam volumes of GFA, the acid soluble fraction, the water extract, and egg white at various protein concentrations. When the foaming activity is defined by the slope in the initial concentration dependence of foam volume, the foaming activity of GFA is 20 times higher than that of egg white. In contrast, the foaming activity of the water extract is only 8 times higher than that of egg white. On the other hand, the acid soluble fraction demonstrates 30 times higher foaming activity than egg white. This apparent high activity may be due to the overestimation of the activity, which is defined on the basis of protein content.

Because the two subunits of GFA appear to bind together through disulfide bonds, the effect of mercaptoethanol on the foaming activity was examined (**Figure 5**). The increase in foaming activity of GFA was only 1.1-fold upon reduction, whereas that of egg white was 5-fold after the addition of mercaptoethanol.

Surface Tension. Figure 6 represents the reduction in the surface tension of water at various concentrations of GFA and ovalbumin. GFA remarkably decreases the surface tension at low concentrations (<1 mg/mL), whereas ovalbumin reduced it gradually. At the highest concentration examined, the surface tension of water (73 dyn/cm) decreases to 58 and 68 dyn/cm in GFA and ovalbumin solutions, respectively.

Immunoblotting. Immunoreactivity of GFA was investigated because some plant proteins are known to be allergens. **Figure 7** shows typical data obtained by using the antisera reactive to

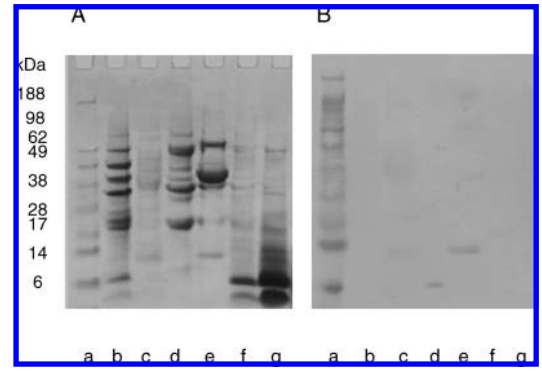

Figure 7. Immunoblotting analysis. Protein staining pattern of SDS-PAGE (**A**) and the corresponding immunoblotting pattern stained with allergic patient's serum and antihuman IgE (**B**). Samples applied were a molecular mass marker (**a**), total guar protein (**b**), total wheat protein (**c**), total soybean protein (**d**), total egg white protein (**e**), the water extract of guar meal (**f**), and the acid precipitate fraction of guar meal (**g**). Total proteins were obtained by incubation with 1% SDS solution overnight. Molecular masses indicated in kilodaltons at the left were obtained by using SeeBlue prestained standard (Invitrogen). The amounts of protein applied were 20 μg (**a**, **b**, **d**−**g**) and 5 μg (**c**).

both wheat and soybean proteins. GFA does not react at all against the antiserum (**Figure 7B**, lane g), although the water extract shows a faint reactive band with a molecular mass around 17 kDa (**Figure 7B**, lane f). The total guar protein faction shows almost no reactive bands (**Figure 7** B, lane b). As expected, wheat and soybean have some reactive proteins with 40 and 13 kDa and 14 kDa, respectively (**Figure 7B**, lanes c and d), although the amount of wheat protein applied on the gel was not adequate (**Figure 7A**, lane c). Unexpectedly, the antiserum gives a strongly stained band at 14 kDa (**Figure 7B**, lane e) against egg white, which appears to correspond to lysozyme. Five other sera obtained from the patients allergic to wheat and soybean proteins gave a similar reactive pattern.

DISCUSSION

We found that most of the foaming activity in guar protein is attributed to the albumin fraction. In previous papers the foaming activity of guar meal was found in globulin and glutelin fractions, although proteins responsible for high foamability were not identified (*6, 7*). We have examined foaming activity in the globulin fraction, which contains several different proteins with molecular masses ranging from 6 to 50 kDa (**Figure 3f**) and found that the globulin fraction possesses only 20% of the foaming activity found in GFA; the total foaming activity in the water extract was 14300 mL^2 (**Table 1**), whereas that in the globulin was 260 mL^2, which was obtained in a separate experiment. Therefore, GFA is responsible for the high foaming activity and foam stability shown in guar protein. Nath and Narasinga-Rao (*6*) examined the functional properties of guar protein isolate obtained after the alkaline treatment at various pH values and found the foaming activity was almost diminished around pH 4, indicating that proteins responsible for the foam formation are precipitated at pH 4. The result is in consistent with our finding.

A significant amount of the foaming activity in the water extract was recovered in GFA by the acid treatment. With a view to industrial applications, it is a great advantage to be able to isolate and concentrate the foaming proteins by a single step of acidification. In the present experiment we employed a rather large volume of water (1.5 L/10 g pf guar meal) to extract

albumin extensively as indicated by a negligible amount of GFA left in the globulin fraction (**Figure 3f**). For maximal recovery of GFA, optimal extraction conditions such as pH, temperature, and time duration should be investigated further. After the acid treatment, a considerable amount of foaming activity remained in the acid soluble fraction as the activity was assessed on the basis of protein concentration (**Table 1**). In fact, the total activity of the acid soluble fraction exceeded that of the water extract. The overestimation in the foaming activity may be due to saponin, which exists in abundance in guar meal, 100 g/kg (*15*). A rather high content of carbohydrate in the acid soluble fraction appears to reflect the presence of legume saponin, which comprises an aglycone of pentacyclic triterpene and a carbohydrate chain (*16*). The low protein content in the acid soluble fraction and its amino acid composition being different from that of GFA substantiate this notion. In addition, the foam formed by the acid soluble fraction with a coarse texture differed from the smooth foam produced by GFA. Furthermore, when the foaming activity of quillaja saponin was measured, it coincided with that of the acid soluble fraction (data not shown).

In the development of the protein-stabilized foams, proteins function as surfactants by being adsorbed at the freshly created air/water interface during bubble formation. This adsorption lowers the interfacial tension, which promotes bubble formation. Immediately after and during the initial adsorption, protein−protein attractions at the interface can result in network formation, which promotes bubble stability (*17*). Although the functional properties of proteins at the interface have not been explained as satisfactorily as those of detergents due to their large molecular size and complex structure, the first critical event that has to occur is the reduction of surface tension by the adsorption. For rapid concentration and subsequent denaturation at the surface, the protein should be soluble, flexible, and, above all, bear an appropriate hydrophobic−hydrophilic balance upon denaturation. The proteins undergo conformational changes at the surface by unfolding to expose more hydrophobic regions of the molecules that facilitate the association of the polypeptides (*18, 19*). In this paper the foaming activity was determined at low protein concentrations so that the critical event of the protein adsorption and the immediate lowering of surface tension can be directly assessed without being interfered with by subsequent secondary stabilizing effects such as a viscosity increase of lamellae due to self-association of denatured proteins. Therefore, the high foaming activity exhibited by GFA may reflect their intrinsic hydrophobic−hydrophilic balance suitable for lowering the surface tension as well as their high diffusion rate to the air/water interface due to their relatively small molecular size. Their extraordinary foamability is also reflected in their high reducing power of the surface tension (**Figure 6**).

Generally, proteins displaying good foamability do not have the ability to stabilize foam (*17*). In the case of GFA, it possesses both a remarkable foamability and foam stability (**Figure 1**). In fact, when meringue was prepared from GFA at the protein concentration of 40 mg/mL, the foam volume was 1.2 times larger than that of egg white, and a foam with a finer texture than that of egg white was obtained upon baking. Moreover, the addition of GFA to egg white at various protein ratios increased the volume of meringues 1.3−1.5 times, regardless of their lower protein contents compared with egg white alone (data not shown).

It is well-known that the foamability of proteins increases considerably upon the reduction due to increased flexibility and hydrophobicity (*20*) as was the case in egg white (**Figure 5**). However, GFA exhibited only a slight increase in the foaming activity by rupture of disulfide bonds. It is possible that only one of the two polypeptide chains consisting of GFA has a foaming ability and that the release of the other peptide does not affect the foamability. It is also conceivable that a stable hydrophobic interface furnished by disruption of the intermolecular linkage cancels out an additional ability lowering the surface energy, which is provided by the increased molecular flexibility due to breakage of intramolecular disulfide bonds. Although the increased flexibility of a protein sometimes has an adverse effect on the foam stability, the foam produced by GFA in the presence of mercaptoethanol was stable as well.

Guar meal is composed of germ (25%) and hull (75%) fractions. During guar meal manufacture from pulverized seed, the hull fraction, but not the germ fraction, is processed at 100 °C and then separated from the endosperm fraction containing guar gum (*2*). Therefore, it is possible that GFA is denatured or cleaved products of the comparable native protein contained in guar beans. When unprocessed guar beans, instead of guar meal, were used to extract total guar protein and analyzed by SDS-PAGE, a protein pattern identical to that observed with guar meal (**Figure 3e**) was obtained, indicating that GFA is present in raw beans and not artificial end products produced during guar meal manufacture. Denaturation of protein is known to increase its foaming capacity to a great extent (*21*).

The content of GFA in guar meal was estimated from the intensity of stained bands of the total guar protein (**Figure 3e**) and found to be 20%. This means that 1 kg of guar meal contains 90 g of GFA provided that the protein content of guar meal is 45% (*2*). With regard to the protein recovery in the acid precipitate (182 mg of protein from 10 g of guar meal; **Table 1**), 18 g of GFA can be isolated from 1 kg of guar meal. From a nutritional point of view, plant proteins in general are mediocre in quality because of low contents in lysine and sulfur-containing amino acids. The amino acid composition of GFA, however, demonstrates a rather high chemical score of 82, with valine as the most limiting amino acid (*22*), compared with usual plant proteins such as gluten, which has a chemical score of 47. GFA showed no immunoreactivity to sera from allergic patients with radio-allergosorbent test scores over 4 against wheat and soybean (**Figure 7**). These properties together with its high foamability make GFA appropriate substitutes for egg white in food manufacture requiring protein-stabilized foam. In this context, it is interesting to examine the emulsifying properties of GFA.

Several foam-stabilizing proteins of low molecular mass have been found in plant seeds. Puroindolines in wheat endosperm are cationic, cystine-rich, lipid-binding proteins of 13 kDa (*23*). They exhibit unique foaming properties and appear to be responsible for the stabilized foam structure of dough (*24*). Barley lipid transfer protein becomes responsible for beer foaming through its glycation by Maillard reaction and after unfolding by reduction (*25*). Some structural similarities are indicated to exist between plant nonspecific lipid transfer protein and puroindoline (*26*). The 2S albumins from sunflower show good emulsifying characteristics, although they have poor foamability (*27*). Although the amino acid composition of GFA did not indicate a strong correlation with them, further investigations are necessary for identifying the molecular structure of GFA. Purification and sequencing analysis of GFA are currently under way in our laboratory.

In conclusion, GFA consists of two subunits of 6 and 11 kDa, both having high foaming activity and good foam stability. A considerable amount of GFA is easily prepared from guar meal, which is a byproduct obtained after guar gum manufacture.

Because GFA has also a good nutritional value without apparent allergenicity, it is a promising candidate as a foam agent in food processing.

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LITERATURE CITED

(1) Undersander, D. J.; Putnam, D. H.; Kaminski, A. R.; Kelling, K. A.; Doll, J. D.; Oplinger, E. S.; Gunsolus, J. L. Guar In Alternative Field Crops Manual; University of Wisconsin—Extension; http://www.hort.purdue.edu/newcrop/afcm/guar.html, accessed July 2008.

(2) Lee, J. T.; Connor-Appleton, S.; Haq, A. U.; Bailey, C. A.; Cartwright, A. L. Quantitative measurement of negligible trypsin inhibitor activity and nutrient analysis of guar meal fractions. *J. Agric. Food Chem.* **2004**, *52*, 6492–6495.

(3) Nath, J. P.; Subramanian, N.; Narasinga-Rao, M. S. Extraction and separation of guar seed proteins. *J. Agric. Food Chem.* **1978**, *26*, 1243–1246.

(4) Nath, J. P.; Subramanian, N.; Narasinga-Rao, M. S. Isolation and characterization of the major fraction of guar proteins. *J. Agric. Food Chem.* **1980**, *28*, 844–847.

(5) Nath, J. P.; Subramanian, N.; Narasinga-Rao, M. S. Effect of detoxification treatments on the proteins of guar meal. *J. Agric. Food Chem.* **1981**, *29*, 529–532.

(6) Nath, J. P.; Narasinga-Rao, M. S. Functional properties of guar proteins. *J. Food Sci.* **1981**, *46*, 1255–1259.

(7) Khalil, M. M. Biochemical and technological studies on the production of isolated guar protein. *Nahrung/Food* **2001**, *45*, 21–24.

(8) Osborne, T. B. *The Proteins of the Wheat Kernel*; The Carnegie Institution of Washington: Washington, DC, 1907; pp 1−119.

(9) Shimoyama, A.; Takeshige, T.; Tanaka, Y.; Yoshioka, R.; Kido, S. Properties of guar meal proteins with reference to soybean proteins. *Kyoto Joshi Daigaku Shokumotsu Gakkaishi* **2003**, *58*, 1–8.

(10) Harkins, W. D.; Brown, F. E. The determination of surface tension (free surface energy), and the weight of falling drops: the surface tension of water and benzene by the capillary height method. *J. Am. Chem. Soc.* **1919**, *41*, 499–524.

(11) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

(12) NIH image. http://rsb.info.nih.gov/nih-image/download.html, accessed July 2008.

(13) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248–245.

(14) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for the determination of sugars. *Nature* **1951**, *168*, 167.

(15) Fenwick, D. E.; Oakenfull, D. Saponin content of food plants and some prepared foods. *J. Sci. Food Agric.* **1983**, *34*, 186–191.

(16) Belits, H. D.; Grosch, W.; Schieberle, P. Legumes *Food Chemistry*, 3rd rev ed.; translated by Burghagen; Springer: Tokyo, Japan, 2004; p 765.

(17) Damodaran, S. Amino acids, peptides, and protein In *Fennema's Food Chemistry*, 4th ed.; Damodaran, S., Parkin, K. L., Fennema, O. R., Eds.; CRC Press: New York, 2007; pp 277−281.

(18) Dickinson, E. Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids Surf. B. Biointerfaces* **1999**, *15*, 161–176.

(19) Murray, B. S. Interfacial rheology of food emulsifiers and proteins. *Curr. Opin. Colloid Interface Sci.* **2002**, *7*, 426–431.

(20) Townsend, A. A.; Nakai, S. Relationships between hydrophobicity and foaming characteristics of food proteins. *J. Food Sci.* **1983**, *48*, 588–594.

(21) Hagolle, N.; Relikin, P.; Popineau, Y.; Dominique, B. Study of the stability of egg white protein-based foams: effect of heating protein solution. *J. Sci Food Agric.* **2000**, *80*, 1245–1252.

(22) Boutrif, E. Recent developments in protein quality evaluation. FAO corporate document repository; http://www.fao.org/DOCREP/U5900T/U5900T07.HTM, accessed July 2008.

(23) Blochet, J. E.; Chevalier, C.; Forest, E.; Pebay-Peyroula, E.; Gautier, M. F.; Joudrier, P.; Pezolet, M.; Marion, D. Complete amino acid sequence of puroindoline, a new basic and cystine-rich protein with a unique tryptophan-rich domain, isolated from wheat endosperm by Triton X-114 phase partitioning. *FEBS Lett.* **1993**, *329*, 336–340.

(24) Gan, Z.; Graaf, J.; Leonard, S. A.; Brooker, B. E.; Parker, M. L.; Schofield, J. D. The role of wheat proteins and polar lipids in the stabilisation of the foam structure of dough. In *Bubbles in Food*; Campbell, G. M., Webb, C., Pandiella, S. S., Niranjan, K., Eds.; Eagan Press: St. Paul, MN, 1999; pp 89−93.

(25) Perrocheau, L.; Bakan, B.; Boivin, P.; Marion, D. J. Stability of barley and malt lipid transfer protein 1 (LTP1) toward heating and reducing agents: relationships with the brewing process. *J. Agric. Food Chem.* **2006**, *54*, 3108–3113.

(26) Dubriel, L.; Vie, V.; Beaufils, S.; Marion, D.; Renault, A. Aggregation of puroindoline in phospholipid monolayers spread at the air−liquid interface. *Biophys. J.* **2003**, *85*, 2650–2660.

(27) Gueguen, J.; Popineau, Y.; Anisimova, I. N.; Fido, R. J.; Shewry, P. R.; Tatham, A. S. Functionality of the 2S albumin seed storage proteins from sunflower (*Helianthus annuus* L.). *J. Agric. Food Chem.* **1996**, *44*, 1184–1189.

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