6

Guar Foaming Albumin – A Foam Stabilizer

Ami Shimoyama and Yukio Doi
Department of Food and Nutrition, Kyoto Women's University, Higashiyama-ku, Kyoto, Japan

1. Introduction

Various surface active agents are used as food additives in food processing when a decrease in surface tension is required e.g., in production and stabilization of all kinds of dispersions, which include emulsion, foams, aerosols and suspensions. Emulsions and foams are of particular interest in food processing and the basic principles involved in their formation and maintenance are very similar: foaming agents and emulsifiers, due to their amphipathic nature, form interface films and thus prevent the disperse phases from flowing together (1).

Protein stabilized foam is important to the structure and texture of many food products, including various cakes, confections, meringues, etc. (2). To produce stable foams the following abilities of the protein responsible for foam formation become important: 1) the ability to adsorb rapidly at the air-water interface, 2) the ability to denature promptly at the interface for maintaining the appropriate balance between hydrophobicity and hydrophilicity, and 3) the ability to interact mutually among the proteins that unfold at the interface and form a strong cohesive, viscoelastic film that can withstand thermal and mechanical agitation (1).

The molecular characteristics of a protein surface and the conformational flexibility determine the mode of adsorption at the interface (3). Flexible disordered proteins like beta-casein can undergo rapid conformational changes at the interface, being excellent foaming proteins. On the other hand, rigid structured globular proteins such as lysozyme and soy protein cannot undergo extensive conformational changes at the interface, being less foaming proteins. The mechanical strength of a protein film at the interface depends on cohesive intermolecular interactions and the stiffness is due to small bubble size and high viscosity. In particular, the formation of sufficiently strong protein film is needed for baked foam products to endure rupture and collapse due to heat expansion of air. Thus the gelling properties in addition to foaming properties are also required for this purpose (1).

Proteins from egg white and milk are widely used for many processed foods and foam-type products. Egg white contains various globular proteins with enough flexibility to make strong cohesive interactions, being a prime foam stabilizer for a variety of baked foam products. In fact, egg white appears to be only protein suited to producing baked foam products; other protein-stabilized foams such as gelatin (used for marshmallow) and whipped whey isolate cannot endure the thermal agitation upon heating and are melt away when baked. In spite of its excellent stabilizing ability to form heat resistant bubbles, egg

white has a serious drawback, strong allergenicity. Egg white contains ovomucoid and ovalbumin, which make it the major food allergen (4).

Recently we isolated an albumin fraction with high foaming ability and foam stabilizing ability from guar meal, and designated guar foaming albumin (GFA) (5). The foaming activity of GFA was 10 times higher than that of egg white at low protein concentrations. GFA mainly composed of a simple protein with the molecular mass of 13 kDa. As a plant protein, GFA has a rather high nutritional value, and would be best suited to allergic patients against animal proteins. These features of GFA make it a promising candidate as a foaming agent in lieu of egg white. Guar meal is a byproduct during extraction of a guar gum, a galactomannnan gum, widely used as stabilizer in various processed food (6). Although proteins isolated from guar meal are characterized in some extent (7-10), GFA was first identified as a protein responsible for their foaming property.

With the particular attention to the high foaming ability and foam stabilizing ability of GFA, in this chapter we would like to investigate its foaming functionality, especially from the perspective of its application for baked food products. We focused on possible application of GFA to substitute egg white, a major food allergen.

2. Materials and methods

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

Materials. GFA was prepared according the method we reported elsewhere (5). The GFA solution was dialyzed against 0.1 M phosphate buffer (pH 6.8) for assessing foaming properties. To examine the effect of added sucrose and NaCl, sample is dialyzed against 5 mM phosphate buffer (pH 6.8), and diluted with using the same buffer if necessary. For a control experiment, thin albumen (EW), obtained from fresh egg white strained through fine gauze, was diluted with an appropriate buffer.

Foaming Studies. The sample (8 g each) diluted to 5 ~ 70 mg/ml of protein concentration was placed in a bowl (14.5 cm in diameter and 9.5 cm in depth), and whipped with a two-blade hand mixer (model HF-230, Hitachi Appliance, Inc.) for 2 min at the lowest speed setting of 1, followed for 2.5 min at speed 5 and finally for 0.5 min at speed 1. To determine the effect of sucrose and NaCl, samples were whipped for 2 min at speed 1 followed for 7.5 min at speed 5 and for 0.5 min at a speed setting of 1. The concentration of sucrose was adjusted by adding an appropriate amount of saturated sucrose solution to sample solutions in order to achieve a mild and complete mixing. The addition of sucrose and NaCl was carried out before whipping. To measure the specific volume, the foam prepared was transferred to a glass container (2.7 cm in diameter and 2.0 cm in depth) by using a plastic spatula and the overflowed foam was removed by sliding the spatula along the edge of the container without pressing foam. The precise volume of the container was previously obtained by measuring the weight of the container filled with water. The specific volume of foam was obtained by dividing the foam volume by the foam weight.

For baking, the foam in a bowl were transferred to a plastic tube (2.5 cm in diameter and 2.0 cm in height) which was placed on cooking paper by using a spatula without squeezing foam. After removing the tube off by carefully lifting it up (no foam left attached to the

tube), the shaped foam samples were baked in an electric oven (model EMO-VA4, Sanyo Appliance, Inc.) for 20 min at 100°C. To measure the volume of a baked foam sample, a plastic tube big enough to cover the whole sample without touching it was placed surrounding the sample. Then a clump of rapeseed was carefully introduced into the tube without damaging the sample just to fulfill the tube, and the weight of the rapeseed was measured. The volume of the baked foam was calculated by subtracting the displaced rapeseed volume from the total tube volume. Control experiments were carried out in the same manner using EW as samples. All determinations in the foam volumes were performed in triplicate samples of at least two independent experiments.

Bubble size measurement. A portion of the foam sample, usually around 0.5 cm^3, was carefully placed on a microscope slide and observed through an inverted microscope equipped with a digital camera (Olympus, C5060). The protein concentrations of samples were 5 and 40 mg/ml in 0.1 M phosphate buffer (pH 6.8). All pictures were taken within 3 min after the formation of foam. The image analysis to measure the size distribution of foam was carried out by using ImageJ (version 1.43b, NIH) program and statistical analysis by Excel program (Microsoft).

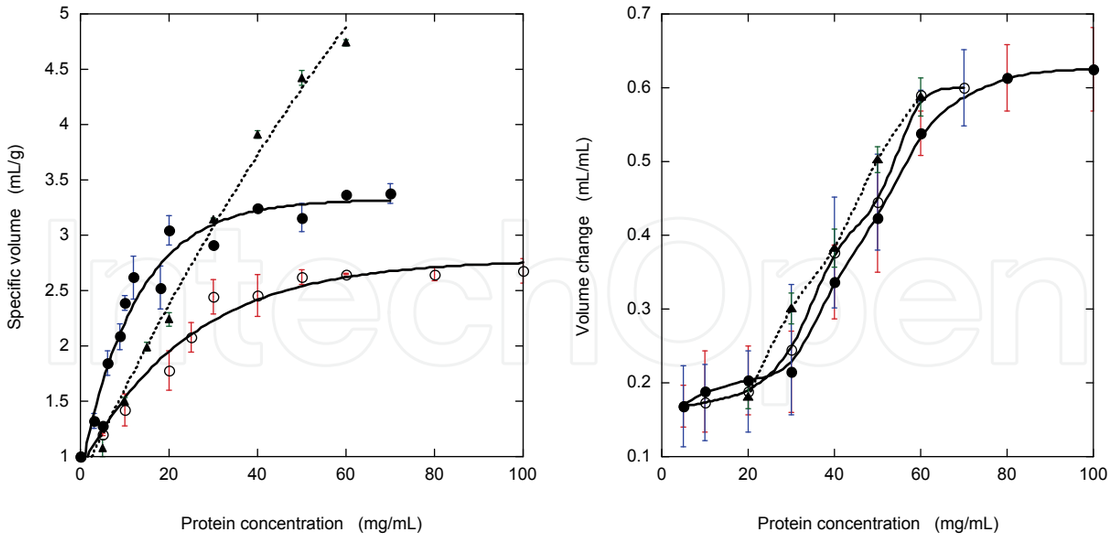
Enzyme-linked immunosorbent assay. An enzyme-linked immunosorbent sassy kit to determine the peanut protein content was purchased from Morinaga (Tokyo, Japan). The kit is manufactured according to the guideline for detecting food allergens, approved by Ministry of Health, Labour and Welfare in Japan. The antibody used in the kit was elicited by using a mixture of peanut proteins containing Ara h2, which is one of major peanut allergenic proteins.

Other Analytical Procedures. Protein concentrations were determined according to the Bradford method (11) with bovine serum albumin as a standard.

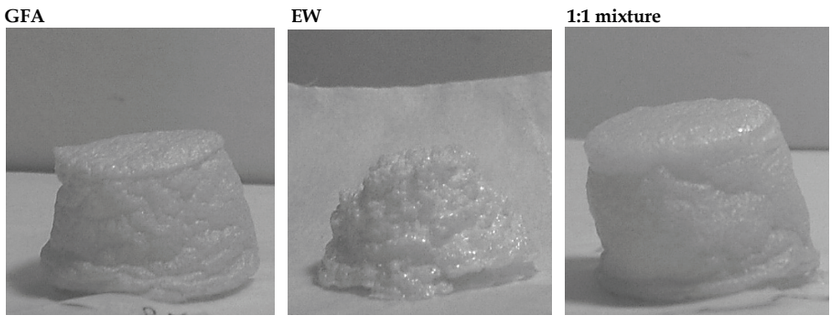
3. Results and discussion

Protein concentration dependence. When the concentration dependence of the foam volumes were examined, the specific foam volumes of GFA increased as the protein concentration increased to 20 mg/ml and plateaued thereafter (Figure 1 A). The specific volumes of GFA were 1.2-1.9 times higher than those of egg white when compared at the same protein concentrations. It should be mentioned that in the previous study (5) we showed 10 times higher foaming ability of GFA than that of EW where the foamability was assessed at much lower protein concentrations, less than 0.1 mg/ml. In the present study the foaming ability as well as stability were assessed at much higher protein concentrations. Interestingly, the foam volumes of the 1:1 mixture of GFA:EW were close to those of EW at lower than 20 mg/ml, but they became close to those of GFA as the protein concentration increased. At high protein concentrations (> 40 mg/ml), the foam volumes of the 1:1 mixture much exceeded those of GFA, indicating a synergistic effect of GFA on the foaming activity at high protein concentrations. It should be noted that the protein concentration here represents that of the total protein, i.e., the protein concentration of GFA and EW is 30 mg/ml each for the 1:1 mixture sample at 60 mg/ml.

It is known that basic proteins such as lysozyme and clupeine improve the foaming properties of acidic proteins such as bovine serum albumin and β-lactoglobulin due to enhanced electrostatic interactions at the bubble surface (12). In these cases, the difference in



(A) (B)

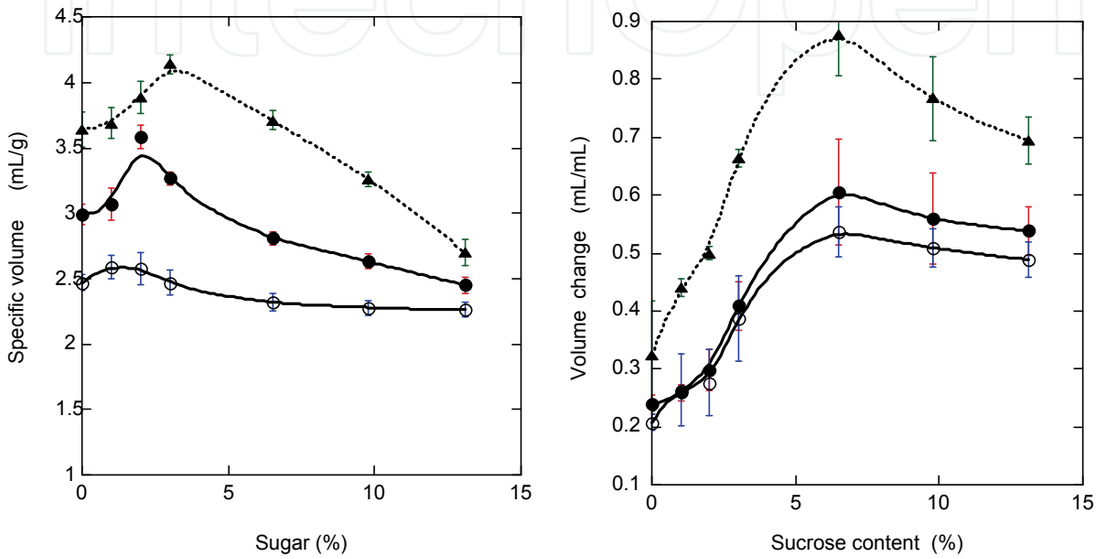


(C)

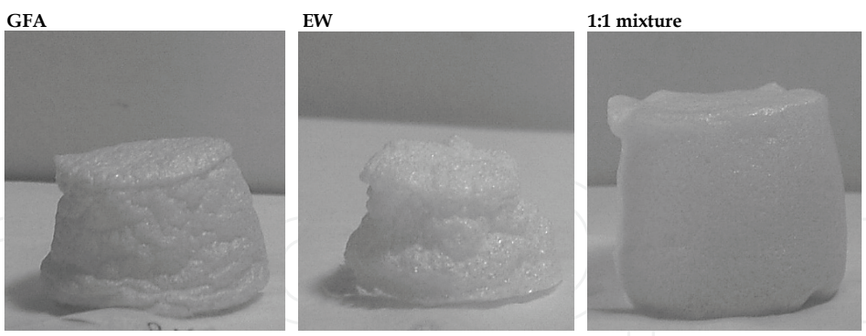
Fig. 1. (A) Changes in the specific volume at various protein concentrations of GFA, EW and the 1:1 mixed sample. Samples were dialyzed against 0.1 M phosphate buffer (pH 6.8), and GFA, EW and the 1:1 mixed sample were whipped by a hand mixer for 5 min. The specific foam volumes calculated were plotted against protein concentrations. GFA (●), EW (○) and the 1:1 mixed sample (▲). Bars indicate the standard deviation. (B) Change in the foam volumes before and after baking of GFA, EW and the 1:1 mixed sample. The samples prepared in Figure 1A were baked for 20 min at 100 °C using an electric oven. The volumes of baked foams were always smaller than the foams before baking. GFA (●), EW (○) and the 1:1 mixed sample (▲). Bars indicate the standard deviation. (C) Appearance of the baked foam produced by GFA, EW and the 1:1 mixture. The protein concentration used was 50 mg/ml each

isoelectric points (pI) between the acidic and basic proteins must be sufficiently large so that at intermediate pHs, interactions are strong enough to yield good foaming properties. GFA is prepared by the acid precipitation at its pI of 4 and most proteins of egg white have their pIs at the acidic region. Therefore, the synergistic effect observed here may not be explained merely by a simple electrostatic interaction between oppositely charged proteins. It is also interesting to mention that mixtures of two dissimilar proteins exhibit thermodynamic incompatibility upon mixing resulting in phase separation at the air-water interface (13).

The phase separation occurred at high protein concentrations (10-20% w/v) then brings about the instability of the film since the high interfacial energy between the phase-separated regions may act as zones of instability. Considering the increased foaming ability observed in the 1:1 mixtures at high protein concentrations, GFA must be a protein that is compatible to most of proteins present in EW. Any intermolecular disulfide interactions between proteins, if any, existed in the 1:1 mixture appears not to be involved in the synergistic effect observed here since a similar augmented foam formation was also observed in the presence of beta-mercaptoethanol (data not shown). Although exact nature



(A) (B)



(C)

Fig. 2. (A) The effect of sucrose on the specific volumes of GFA, EW and the 1:1 mixed sample. The protein concentration used was 32 mg/ml each. Samples were prepared as described in Figure 1 A, except sucrose was added to each solution before foaming by a hand mixer for 10 min. GFA (●), EW (○) and the 1:1 mixed sample (▲). Bars indicate the standard deviation. (B) The effect of sucrose on the volume changes before and after baking. The protein concentration used was 32 mg/ml each. Samples were baked as described in Figure 1 B. GFA (●), EW (○) and the 1:1 mixed sample (▲). Bars indicate the standard deviation. (C) Appearance of the baked foam produced in the presence of 5% sugar by GFA, EW and the 1:1 mixture. The protein concentration used was 50 mg/ml each

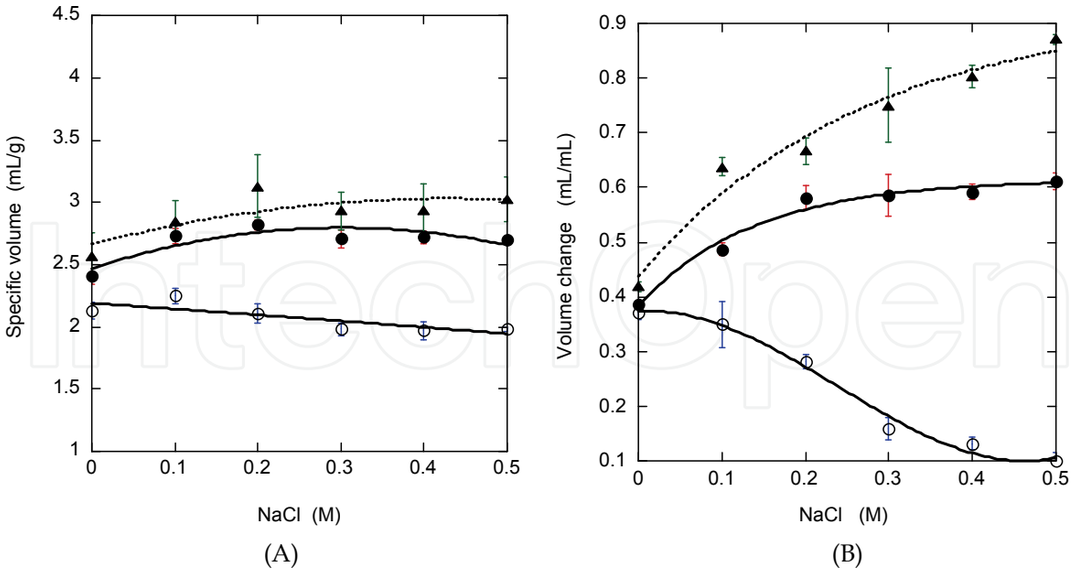
of the synergistic effect remains to be investigated, some specific, weak interaction operative only at high protein concentrations might be involved.

The ability of proteins to stabilize foams is mainly determined by the rheological properties of protein adsorption layers: viscoelastic behaviour, irreversible structures and rheological changes under compression and expansion (14). An extreme expansion of foams may occur upon heating, for example, during baking meringue. Since GFA was shown to possess better foam stability than EW (5), changes in foam volume upon baking were examined. In all samples of GFA, EW and the 1:1 mixture, the foam volumes after baking reduced to 15% to 65% of the original volumes depending on the protein concentrations and the protein concentration dependence of the volume reduction were similar among the samples (Figure 1 B). Therefore, the stability of the GFA adsorbed film upon baking is comparable to that of the EW film. It should be noted, however, that the actual foam volume after baking in the present experiment was maximum in the 1:1 mixture at 60 mg/ml (Figure 1 C). The fact that the strength of the GFA adsorbed film remained even after baking is in contrast to that of β-casein, for example, whose foams are easily ruptured upon baking.

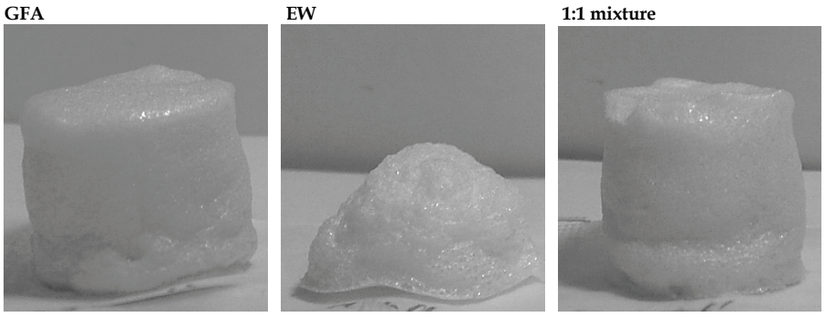
Effect of sucrose addition. The addition of sucrose up to 13% to EW did not significantly affect its foaming ability (Figure 2 A). The addition of 2-3% sucrose to GFA slightly enhanced the foam specific volume but decreased at higher sucrose concentrations. In the 1:1 mixture, the positive effect of sucrose was observed between 2-6%, but at higher concentrations sucrose affected adversely. In fact sucrose appears to damage the foaming ability of GFA more severely than that of EW as the foam volume of GFA reduced considerably in the presence of 45% sucrose in contrast to EW, which did not show an appreciable reduction (data not shown).

In general, addition of sugars to protein solutions often impairs foamability due to enhanced stability of protein structure, but improves foam stability due to increased viscosity (2). When the foams prepared with various sucrose concentrations were baked, stabilizing effect of sucrose is pronounced (Figure 2 B, C). Both GFA and EW foams were similarly stabilized and the maximum stability was observed at around 5% sucrose. The stabilizing effect of sucrose in the 1:1 mixture was similar but more conspicuous, suggesting that the synergistic interaction between GFA and EW was effectively augmented by the presence of small amount of sucrose. The increase in viscosity by the addition of sucrose may not contribute significantly to the observed stability.

Effect of NaCl addition. The addition of NaCl to EW decreased the foam specific volume only slightly (Figure 3 A). In contrast, the foaming ability of GFA as well as the 1:1 mixture were slightly enhanced by the NaCl addition. The synergistic interaction between GFA and EW appears not to be influenced by charge neutralization upon addition of salt ions. Upon baking, however, the addition of NaCl notably influenced the foam stability (Figure 3 B, C). EW reduced its foam volume upon heating as increased the NaCl concentration. On the contrary, in both GFA and the 1:1 mixture, the foam volume reduction upon heating was restrained by the addition of NaCl. Especially, the foams produced by the 1:1 mixture were greatly stabilized by NaCl, as was the case by sucrose, implying that both electrostatic interactions and hydrogen bondings between proteins in the film may contribute to exhibiting the synergistic effect.



(A) (B)



(C)

Fig. 3. (A) The effect of NaCl on the specific volumes of GFA, EW and the 1:1 mixed sample. The protein concentration used was 25 mg/ml each. Samples were prepared as described in Figure 1 A, except NaCl was added to each solution before foaming and samples were dialyzed against 5 mM phosphate buffer (pH 6.8). GFA (●), EW (○) and the 1:1 mixed sample (▲). Bars indicate the standard deviation. (B) The effect of NaCl on volume changes before and after baking. The protein concentration used was 25 mg/ml each. Samples were baked as described in Figure 1 B. GFA (●), EW (○) and the 1:1 mixed sample (▲). Bars indicate the standard deviation. (C) Appearance of the baked foam produced in the presence of 0.3 M NaCl by GFA, EW and the 1:1 mixture. The protein concentration used was 50 mg/ml each

Bubble size distribution. Since the foam produced by GFA seemed much smoother in appearance than that by EW (5), the size distribution of the GFA bubbles was examined through microscopic observation (Figure 4). GFA can produce smaller and more uniform bubbles than EW; the average bubble size of GFA is half of that of EW with a much narrower size distribution (Table 1). The average bubble size obtained with the 1:1 mixture was between those with GFA and EW. A similar difference in size distribution was also observed at lower protein concentration (5 mg/ml); the average bubble areas with GFA and EW were 0.025 and 0.052 mm², respectively.

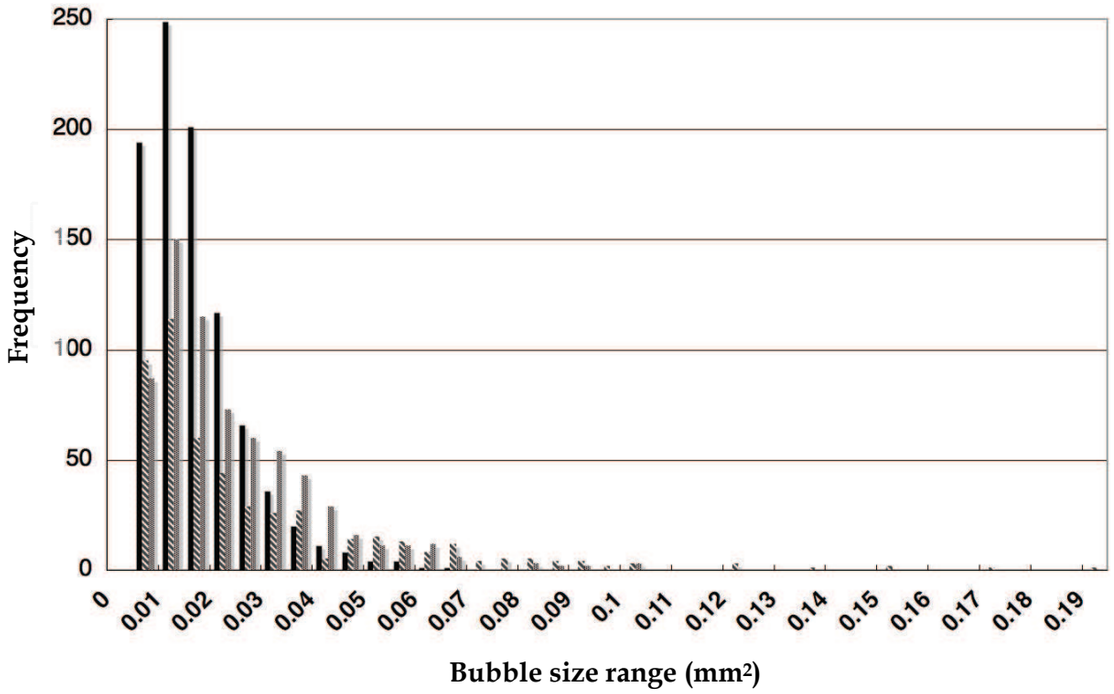


Fig. 4. Bubble size distribution of foams made by GFA (solid), EW (oblique) and the 1:1 mixture (shaded). The protein concentration in all samples was 40 mg/ml

	GFA	EW	1:1 mixture
Bubble area, Mean x 10^{-3} (mm^2) ± SD	12.5 ± 9.31	23.3 ± 26.4	19.4 ± 16.2
Bubble area, Median x 10^{-3} (mm^2)	10.3	13.1	14.2
Number of Bubbles (mm^{-2})	43.4	23.7	32.3

Table 1. Summary of the bubble size distribution in foams obtained with GFA, EW and the 1:1 mixture

According to the Laplace principle, the internal pressure of a bubble is greater than the external pressure, and the pressure difference is inversely proportional to radius of the foam bubble (1). Therefore, smaller foam bubbles can withstand heavier load than larger bubbles; the smaller the bubble sizes, the stiffer and stronger the foams become. The observed small bubble sizes of GFA substantiate the stability of GFA foams. However, the fact that the average foam size of the 1:1 mixture is larger than that of GFA did not appear to be in line with the synergistic effect observed in the mixture (Figure 1 A). The mechanism for the synergistic effect should be further investigated in many respects.

Immunological analysis. Previously no apparent immunoreactivity of GFA was observed against the antisera obtained from the allergic patients to egg, wheat and soybean (5). Since GFA was obtained from guar (*Cyamopsis tetragonolobus*) which belongs to the pea family (*Fabaceae*), the possible immunological relation to peanut proteins (*Arachis*

hypogaea) was investigated by using an enzyme-linked immunosorbent assay kit, which is manufactured according to the guideline for detecting food allergens approved by Ministry of Health, Labour and Welfare in Japan. In Table 2 were listed the amount of protein in various nuts detected by the antibody against peanut proteins. The content of the protein in guar beans reactive to the anti-peanut antibody were less than 10^{-5} fold compared with that found in peanut. In the protein isolate of GFA, the amount of protein detected by the present method was 10^{-5} μg/g, which could be negligible when GFA was used in processed food as foam enhancer. It should be pointed out that an allergic warning against peanuts such as "contains peanuts" should be labelled properly on the surface of processed food products according to Japanese Food Sanitation Act if food contains the peanut protein more than 10 ppm.

Sources	Reactive Protein (μg/g)*
Peanut (*Arachis hypogaea*)	62880
Guar (*Cyamopsis tetragonolobus*)	5.9
Makadamia nut (*Macadamia integrifolia*)	0.9
Soybean (*Glycine max*)	0.5
Common bean (*Phaseolus vulgaris*)	0.5
Azuki bean (*Vigna angularis*)	0.3
Pistachio (*Pistacia vera*)	0.2
GFA	10.0

*The amount of the protein reactive to the anti-peanut antibody is calculated on the basis of mass of nut (bean), except for GFA which is based on the total protein content measured by the Bradford method (11).

Table 2. The amount of protein detected by ELISA using the antibody against peanut proteins

Abbreviations Used: EW, egg white (thin albumen); GFA, guar forming albumin; pI, isoelectric point

4. References

[1] 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; p.277-281.

[2] Campbell, G.M.; Mougeot, E. Creation and characterisation of aerated food products. *Trends Food Sci. Technol*. 1999, 10, 283-296.

[3] Dickinson, E. Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloid Surface B: Biointerfaces*. 1999, 15, 161-176.

[4] Davis, P.J. Historical and cultural background to plant food allergies. *In Plant Food Allergens*; Mills, E.N.C.; Shewry, P.R., Eds; Blackwell: Oxford, 2004; p1-15.

[5] Shimoyama, A.; Kido, S.; Kinekawa, Y.; Doi, Y. Guar foaming albumin: a low molecular mass protein with high foaming activity and foam stability isolated from guar meal. *J. Agric. Food Chem.* 2008, 56, 9200–9205.

[6] 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.

[7] 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.

[8] 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.

[9] 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.

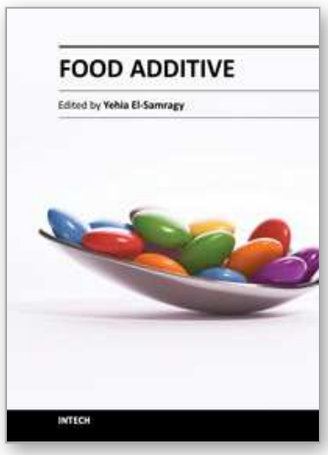
[10] M. Biochemical and technological studies on the production of isolated guar protein. *Nahrung/Food* 2001, 45, 21-24.

[11] 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.

[12] Poole, S.; West S.I.; Walters C.L. Protein-protein Interactions: Their importance in the foaming heterogeneous protein systems. *J. Sci. Food Agric.* 1984, 35, 701-711.

[13] Razumovsky, L.; Damodaran, S. Incompatibility of mixing of proteins in adsorbed binary protein films at the air-water interface. *J. Agric. Food Chem.* 2001, 49, 3080-3086.

[14] Yampolskaya, G.; Platikanov, D. Proteins at fluid interfaces: Adsorption layers and thin liquid films. *Adv. Colloid Interface Sci*. 2006, 128-130, 159-183.



Food Additive

Edited by Prof. Yehia El-Samragy

ISBN 978-953-51-0067-6
Hard cover, 256 pages
Publisher InTech
Published online 22, February, 2012
Published in print edition February, 2012

A food additive is defined as a substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value. Food additives are natural or manufactured substances, which are added to food to restore colors lost during processing. They provide sweetness, prevent deterioration during storage and guard against food poisoning (preservatives). This book provides a review of traditional and non-traditional food preservation approaches and ingredients used as food additives. It also provides detailed knowledge for the evaluation of the agro-industrial wastes based on their great potential for the production of industrially relevant food additives. Furthermore the assessment of potential reproductive and developmental toxicity perspectives of some newly synthesized food additives on market has been covered. Finally, the identification of the areas relevant for future research has been pointed out indicating that there is more and more information needed to explore the possibility of the implementation of some other materials to be used as food additives.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen