JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Formation and Stability of Foams Made with Sunflower (*Helianthus annuus*) Proteins

SERGIO GONZÁLEZ-PÉREZ,[†,§,#] JOHAN M. VEREIJKEN,[†] GERRIT A. VAN KONINGSVELD,[§] HARRY GRUPPEN,[§] AND ALPHONS G. J. VORAGEN*[,§]

Agrotechnology and Food Innovations B.V., P.O. Box 17, 6700 AA Wageningen, The Netherlands, and Laboratory of Food Chemistry, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Foam properties of a sunflower isolate (SI), as well as those of helianthinin and sunflower albumins (SFAs), were studied at various pH values and ionic strengths and after heat treatment. Less foam could be formed from helianthinin than from SFAs, but foam prepared with helianthinin was more stable against Ostwald ripening and drainage than foam prepared with SFAs. Foams made with SFAs suffered from extensive coalescence. The formation and stability of foams made from reconstituted mixtures of both proteins and from SI showed the deteriorating effect of SFAs on foam stability. Foam stability against Ostwald ripening increased after acid and heat treatment of helianthinin. Partial unfolding of sunflower proteins, resulting in increased structural flexibility, improved protein performance at the air/water interface. Furthermore, it was observed that the protein available is used inefficiently and that typically only ∼20% of the protein present is incorporated in the foam.

KEYWORDS: Sunflower; *Helianthus annuus*; helianthinin; albumin; protein; foam; denaturation

INTRODUCTION

Animal proteins are expensive in terms of both market price and environmental impact, for example, land use and pollution. In addition, consumer's confidence in animal proteins has decreased due to food safety problems related to diseases such as bovine spongiform encephalopathy and the use of animal hormones. Vegetable proteins may be economic and sustainable alternatives for animal proteins as functional ingredients in food formulations. Among these, sunflower proteins are particularly interesting. Sunflower seeds are familiar to both farmers and processors because of their functioning as a source for oil extraction. Compared to other sources of vegetable proteins, the seeds contain low amounts of antinutritional factors, such as protease inhibitors (*1*). Furthermore, sunflower proteins have been reported to have a high intrinsic solubility, which is a prerequisite for many functional properties (*2*, *3*). Therefore, a study was started to investigate the functional properties of sunflower proteins and establish relationships between their structure and functionality. Because sunflower seeds contain phenolic compounds, especially chlorogenic acid (CGA), that may interact with proteins (*1*, *4*), a procedure was developed to obtain native, CGA-free protein preparations (an isolate, termed SI, and the main protein fractions) from these seeds (*5*−*7*). The structure of these sunflower protein preparations in relation to pH, ionic strength, and heat has been studied as well as their solubility. The present investigation deals with the foam properties of these sunflower protein preparations.

The two main groups of sunflower proteins are 11S globulin, also known as helianthinin, and 2S albumins, or sunflower albumins (SFAs). It is generally accepted that helianthinin, at neutral pH, consists of six spherical subunits arranged into a trigonal antiprism (*8*). The individual subunits consist of an acidic (32−44 kDa) and a basic (21−27 kDa) polypeptide that are linked by a single disulfide bond. The arrangement of helianthinin subunits can be modulated by ionic strength and pH: helianthinin can occur as a monomer, trimer, or hexamer or in highly aggregated forms (*6*). SFAs are a diverse group of proteins, with a sedimentation coefficient of ∼2 S, of which some are rich in cysteine. They have been reported to be alkaline proteins and to have molecular masses ranging from about 10 to 18 kDa (*9*−*12*). In contrast to 2S albumins from other seed species (i.e., Brazil nut, rape seed, mustard seed, etc.), which consist of two chains linked by disulfide bonds, SFAs consist of a single polypeptide chain (*10*, *13*, *14*).

Foam formation and stability are considered to be important functional properties of food proteins and have a widespread applicability in many food products (*15*). During foaming, proteins adsorb at the air/water interface, thus lowering the interfacial tension (γ) and subsequently facilitating bubble breakup, which is opposed by the Laplace pressure ($P_{LP} = 4$

* Corresponding author (telephone +31317 485468; fax +31317 484893; e-mail fons.voragen@wur.nl; webpage www.foodchemistry.wur.nl).
[†] Agrotechnology and Food Innovations B.V.
[§] Wageningen University.
[#] Present address: Department of Bio-Organic Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

10.1021/jf0501793 CCC: $30.25
Published on Web 07/13/2005

γ/d, where d is the diameter). The most important role of the adsorbed proteins is, however, to prevent immediate recoalescence of the newly formed bubbles (*16*). Once at the interface, proteins may unfold to various extents, reorient, rearrange, and spread.

Several processes can destabilize foams and should, therefore, be monitored after foam formation. Because of the difference in density between air and water, gravitational (buoyancy) forces will tend to cause flow of the liquid out of the foam, which is called drainage. Coalescence is the merging of two bubbles into one bigger bubble due to the rupture of the liquid film (lamellae) between them. The presence of hydrophobic impurities, as fat or other insoluble material, large enough to touch both surfaces, is a common cause of coalescence (*17*). Ostwald ripening, the growing of large bubbles at the expense of smaller ones, is probably the most important type of instability in foams. The driving force is the Laplace pressure difference over a curved bubble surface, which results in a higher solubility of air in the liquid around a small bubble than around a larger one, as described by Henry's law. Proteins may stabilize foams against Ostwald ripening if they remain adsorbed on the shrinking bubble. Then, γ will decrease due to an increase in surface excess (Γ, mg/m^2). This decrease in γ will retard, or may theoretically even stop, Ostwald ripening (*18*).

The foam properties of sunflower proteins have been previously studied (*12*, *19*−*34*). However, most of the studies did not provide any information on the structure of the proteins under the conditions used, and the functionality tests were performed with protein products (meals, concentrates, isolates), of which the extent of denaturation and protein composition was marginally or not studied. In addition, some of the protein products investigated contained CGA, which is known to interact with proteins and hence likely affecting protein functionality. Therefore, despite the research performed, only limited information is available on the foam properties of native, CGA-free sunflower protein preparations and on the relationship between protein structure and functionality. The aim of this study is to provide this knowledge. Therefore, foam formation and stability were examined for individual sunflower protein fractions, that is, helianthinin and SFAs, combinations thereof, and isolate (SI). These properties were studied as a function of pH, ionic strength, and after heat treatment, thereby altering the structure and conformation of the proteins.

MATERIALS AND METHODS

Materials. Dehulled "Mycogen Brand" sunflower seeds were purchased from H. Ch. Schobbers B.V. (Echt, The Netherlands). All chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany).

Sunflower protein isolate (SI) was obtained as described by González-Pérez and co-workers (*5*). Helianthinin was obtained as previously described (*6*), but with omission of the last gel permeation chromatography (GPC) step. The resulting helianthinin preparation was mostly in the 11S and 7S forms (90%), in addition to ∼6% in its monomeric form and the presence of other protein impurities (<4%) as determined by GPC. Sunflower albumins were obtained as previously described (*7*). The resulting SFAs preparation contained other protein impurities (<4%), as determined by GPC.

Data were collected from a single preparation of the purified protein preparations in order to perform all of the experiments with the same batch of proteins. The degree of denaturation of these batches was monitored, and their yield allowed them to be regarded as a representative sample of the relevant sunflower proteins (*5*−*7*).

Preparations of the Protein Solutions. Protein dispersions (1.0−3.0 mg/mL) were prepared from bovine serum albumin (BSA), SI, SFAs, and helianthinin by dispersing these proteins in 22 mM Tris-HCl buffer (pH 7.1; I = 20 mM), 30 mM Tris-HCl buffer (pH 8; I = 20 mM) (for SI, SFAs, and helianthinin), 23 mM sodium phosphate buffer (pH 3; I = 20 mM) (for SI, SFAs, and helianthinin), and 30 mM sodium acetate buffer (pH 5; I = 20 mM) (for SFAs). When an ionic strength of 250 mM was used, 230 mM sodium chloride was added to the buffers. At pH 3 only ionic strengths of 20 and 100 mM were used for helianthinin and SI, because of the limited solubility of both protein preparations at higher ionic strength (*6*, *7*). Part of the helianthinin dispersion at pH 3 was adjusted (after it had been kept for 10−15 min at pH 3) to pH 7 by the addition of NaOH (0.1−1 M) and will be referred to as the pH 3→7 sample.

All protein dispersions prepared were stirred overnight at 16 °C. The pH was checked and, if necessary, adjusted with NaOH or HCl (0.1−1 M). Next, the protein dispersions were centrifuged (3000*g*, 30 min, 20 °C), and the supernatant was filtered over a filter with a pore size of 0.45 μm (Schleicher and Schuell, Dassel, Germany).

Helianthinin samples used for testing the effect of heat treatment were prepared by dispersing the protein in buffers of pH 3, 7, and 8, as described above. Samples were heated in a water bath for 30 min at 65 or 100 °C and subsequently cooled in ice water. Subsequently, the samples were centrifuged and the supernatant was filtered as described above.

The protein concentration of the final protein solutions was estimated using the method of Bradford (*35*). The final concentration was adjusted to 0.5 mg/mL using the corresponding buffer solution.

Protein mixtures of SFAs and helianthinin were prepared by mixing solutions of these proteins to obtain protein solutions with a final concentration of 0.5 mg/mL containing 10, 25, 50, 75, and 90% SFAs.

Foam Preparation and Characterization. Foam-forming and -stabilizing ability were tested using the whipping method described by Caessens and co-workers (*36*). A volume of 100 mL of a 0.5 mg/mL protein solution was placed in a graduated glass cylinder and whipped for 70 s at 2500 rpm using a small impeller. Foam volume was monitored for 1 h (at 2, 5, 10, 15, 30, 45, and 60 min after whipping had started) and calculated as the difference between the higher foam boundary and the lower foam boundary, as measured in the graduated glass cylinder. Foam quality (bubble size, coalescence, drainage, and Ostwald ripening) was evaluated visually. The extent to which Ostwald ripening occurred was arbitrarily ranked from 1 to 4. A higher number indicates faster Ostwald ripening. The effect of whipping speed on foam properties was tested using a whipping speed of 3500 rpm. All experiments were carried out at least in duplicate.

Composition of the Protein Solutions. GPC was carried out to assess the relative amount of helianthinin and SFAs in SI and the protein mixtures. The competitive adsorption of sunflower proteins to the air/water interface with the SFAs/helianthinin mixtures was investigated by comparing the protein composition of the original protein solution to that of the (drained) liquid after foam formation. GPC was performed using an Äkta Explorer System (Amersham, Pharmacia Biotech, Uppsala, Sweden). Protein samples (0.2 mL) were applied directly to a Superdex 200 HR 10/30 column and eluted with the same buffer used to form the foam at a flow rate of 0.5 mL/min at room temperature. The absorbance of the eluate was monitored at 214 and 280 nm.

RESULTS

The average standard deviation of the foam volume formed was calculated to be 3.5 mL. Therefore, differences in foam volume of <3.5 mL were considered not to be significant.

Foams Made at pH 7 (I = 20 mM). Table 1 displays the characteristics of sunflower protein foams (made at 2500 rpm, 70 s) at various conditions. BSA was used as a reference protein during the experiments. BSA formed foams that showed slow drainage, as ∼15% of the initial amount of liquid drained in 60 min. At pH 7 (I = 20 mM) foam volume was the highest for SFAs and SI and significantly less foam was formed with BSA and helianthinin. The volume decrease in time of foams made with helianthinin and BSA was, however, very low (∼10%), whereas a much faster decrease in volume was observed in

Table 1. Characteristics of Foams Made with Sunflower Protein Preparations at Various Conditions (2500 rpm, 70 s)

sample	pH	*I* (mM)	foam volume[a] (mL) V_{max} (2 min)	foam volume[a] (mL) V_{min} (60 min)	drainage[b] (%)	φ[c] (air)	coalescence[d]	Ostwald ripening[e]
BSA	7	20	35	31	15	0.67	–	****
helianthinin	8	20	44	31	25	0.68	–	****
	8	250	65	47	41	0.66	–	*
	7	20	32	28	21	0.70	–	****
	7	250	53	39	32	0.68	–	*
	3	20	55	43	39	0.63	–	*
	3	100	58	43	41	0.66	–	*
	3→7	20	60	49	41	0.63	–	*
	$8_{100°C}$[f]	20	74	61	40	0.68	–	*
	$8_{65°C}$[f]	20	53	43	32	0.64	–	**
	$3_{100°C}$[f]	20	59	48	39	0.68	–	*
	$3_{65°C}$[f]	20	55	41	40	0.62	–	*
SFAs	8	20	61	40	66	0.64	+	***
	8	250	66	40	66	0.66	+	***
	7	20	59	38	60	0.66	+	****
	7	250	66	42	68	0.66	+	****
	5	20	67	32	79	0.67	+	***
	5	250	64	0	100	0.67	+	****
	3	20	65	43	73	0.69	+	***
	3	250	68	38	71	0.67	+	***
SI	8	20	60	47	44	0.77	–	****
	8	250	64	47	50	0.64	–	***
	7	20	59	47	39	0.69	–	****
	7	250	56	38	50	0.68	–	***
	3	20	54	39	49	0.70	–	**
	3	100	56	44	41	0.66	–	**
SFAs/helianthinin mixtures[g] (% SFAs)								
10	7	20	47	40	18	0.70	–	****
25	7	20	50	42	28	0.67	–	****
50	7	20	62	46	48	0.65	–	****
75	7	20	60	41	61	0.64	–	****
90	7	20	69	44	61	0.67	+	****

[a] The average standard deviation of the foam volume is 3.5 mL. [b] Percent drained of liquid initially present in foam. [c] φ = volume fraction of air initially present in foam. [d] +, coalescence observed; –, coalescence not observed. [e] More asterisks indicate faster Ostwald ripening. [f] Subscripts indicate the temperature of the heat treatment. [g] Proportion of SFAs in the protein mixtures.

foams made with SFAs (36%) and SI (20%). Destabilization in foams made with SFAs at pH 7 was mainly due to coalescence. Coalescence was not observed in foams stabilized with helianthinin and SI.

Typical examples of foam volumes, and the upper and lower foam boundaries as a function of time, are displayed in **Figure 1**. The amount of liquid drained from the foam is related to the change in the lower foam boundary, whereas the upper foam boundary indicates the foam volume decrease caused by other instabilities. A pronounced foam volume decrease, mainly due to drainage, is observed in foams made from SFAs and SI at pH 7 (**Figure 1**). Drainage of foams made at pH 7 increased in the order BSA < helianthinin < SI < SFAs (**Table 1**).

For foams made with SFAs fast coalescence and continuous bursting of bubbles were observed. As a result, the final diameter of many bubbles was visibly >0.5 cm. Therefore, the volume decrease of foams made from SFAs should be interpreted carefully, as the bursting of a few bubbles after several minutes (5−10) later than the storage time shown in **Table 1** resulted in almost complete collapse of the foam.

Foams Made with Helianthinin at Various Conditions. The influence of pH on formation and stability of foams formed with helianthinin was studied at pH 3 and 8, in addition to pH 7. At $I = 20$ mM, foam formation for helianthinin was the highest at pH 3. Significantly less foam was formed at pH 8 and even less at pH 7 (**Table 1**). Foams made from helianthinin at pH 3, despite their higher stability against Oswald ripening, drained more quickly than those made at pH 7 and 8. When helianthinin was dispersed at pH 3 and subsequently adjusted to pH 7 (pH 3→7 sample), it formed twice as much foam as at pH 7. The pH 3→7 foam was clearly more stable against Ostwald ripening, but drained more quickly than the foam at pH 7.

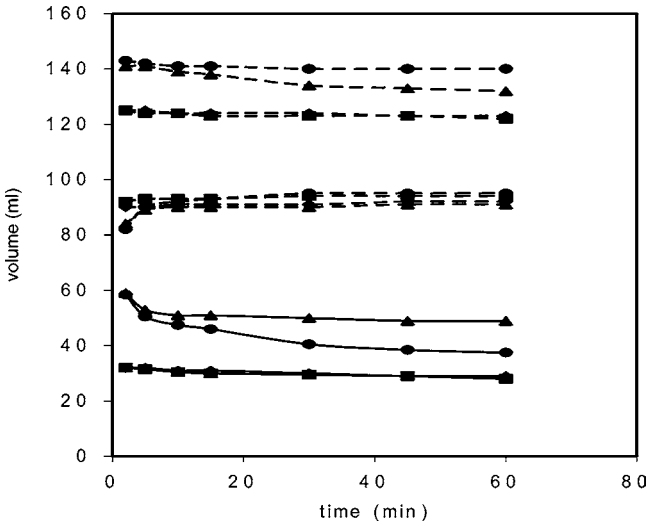

Figure 1. Foam volume (solid line) and upper and lower foam boundaries (dashed line) of foam formed at 2500 rpm (70 s) as a function of time, at pH 7 ($I = 20$ mM) with 0.5 mg/mL solutions of BSA (◆), helianthinin (■), SFAs (●), and SI (▲).

The effect of ionic strength (I) on the formation and stability of foams made from helianthinin is also displayed in **Table 1**. Increasing the ionic strength generally resulted in significantly higher foam volumes, independent of the pH. In addition, a higher I seems to be associated with faster drainage, slower Ostwald ripening, and a faster decrease in foam volume (**Table 1**). This faster decrease in foam volume for helianthinin foams is markedly higher at pH 8 and 7 (14%) than at pH 3 (3%).

Heat treatment improved foam formation and resulted in foams with a higher stability against Ostwald ripening. Foam volume for helianthinin (pH 8) increased by 20 and 70% when heated at 65 and 100 °C, respectively (**Table 1**). Foams from heated helianthinin contained smaller bubbles but drained more quickly than foams made with nonheated helianthinin. Similar improvements were obtained after heating at pH 7 (results not shown). Heating at pH 3 had little or no effect on both foam volume and foam stability (**Table 1**).

Foams Made with SFAs at Various Conditions. Foam formation and stability of foams made with SFAs were studied at pH 3, 5, 7, and 8. Changing the pH had only a minor effect on foams made from SFAs at $I = 20$ mM. All foams showed coalescence and Ostwald ripening, although the latter was almost obscured by the extremely fast coalescence observed at all tested pH values. Foam volume was somewhat smaller at neutral and alkaline pH values, but foams made at these conditions showed slower drainage than those made at acidic pH. Fast drainage was observed at all conditions and was the fastest at pH 5, with a loss of ~80% of the initial amount of liquid in 60 min (**Table 1**). SFAs solutions resulted, therefore, in coarse and dry foams upon whipping, which in most cases collapsed after 90 min of storage. Increasing the ionic strength from 20 to 250 mM slightly augmented foam volume. Foam volume, however, decreased more rapidly at high ionic strength at pH 5 and 7. At pH 5, salt addition even resulted in complete collapse of the foam after ~10 min.

Foams Made with SI at Various Conditions. Foam formation and stability of foams made with SI were studied at pH 3, 7, and 8. Changing the pH had much less effect on SI-stabilized foams than on foams made with helianthinin. At $I = 20$ mM, foam stability against drainage was better at pH 7 and 8 than at pH 3. The latter foam was, however, more stable against Ostwald ripening than foams made at pH 7 and 8. Increasing the ionic strength resulted in foams with a higher stability against Ostwald ripening but faster drainage, except at pH 3 (**Table 1**). No coalescence was observed in SI-stabilized foams.

Foams Made with Mixtures of Helianthinin and SFAs. Clear trends were found in foams made with protein mixtures of helianthinin and SFAs (10, 25, 50, 75, and 90% SFAs content) at pH 7 ($I = 20$ mM) (**Table 1**). Foam volume significantly increased with increasing SFAs content, but the foam volume reduction after 60 min and drainage were also more pronounced in foams with a higher SFAs content (**Table 1**; **Figure 2**). As illustrated in **Figure 2**, this reduction in foam volume was fastest in the first 15 min after whipping.

Effects of Whipping Speed on Foam Formation and Stability. The results presented above were obtained at a whipping speed of 2500 rpm. Foam formation and stability were also studied at a whipping speed of 3500 rpm (**Table 2**). **Figure 3** displays foam volume as a function of time for SFAs, helianthinin, and heat-treated helianthinin (100 °C) after whipping at 2500 and 3500 rpm. Increased whipping speed resulted in coagulation of BSA, as could be inferred from the turbidity of the solution upon whipping. At low ionic strength, the foam volume of foams made with helianthinin significantly decreased

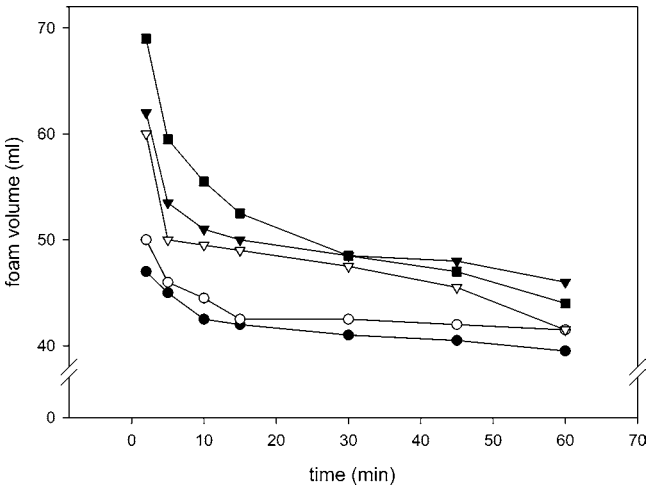

Figure 2. Foam volume as a function of time at pH 7 ($I = 20$ mM) using a whipping speed of 2500 rpm. Protein solutions were prepared with various helianthinin/SFAs mixtures with a final concentration of 0.5 mg/mL, containing 10 (●), 25 (○), 50 (▽), 75 (▼), and 90% (■) SAFs.

with increasing speed at pH 7 and 8 (**Tables 1** and **2**; **Figure 3**). These foams were visibly weaker and more unstable against Ostwald ripening than at lower speed. Also at high ionic strength, at pH 7 and 8, increasing whipping speed also resulted in a significant decrease in foam volume; however, these foams were rather stable against Ostwald ripening and drainage. The latter may be due to the high volume fraction of air (90%) contained in these foams at 3500 rpm (**Table 2**). In contrast, the foam volume of foams made with helianthinin at pH 3 increased, upon increasing the whipping speed, by 40 and 150% at ionic strengths of 20 and 100 mM, respectively (**Tables 1** and **2**). The effect of heating the helianthinin solutions prior to foam formation is also more evident at a higher whipping speed. The foam volume formed increased approximately by 135 and 225% for the helianthinin samples (pH 8) heated at 65 and 100 °C, respectively, as compared to foams formed at 2500 rpm (**Tables 1** and **2**; **Figure 3**). Helianthinin heated at pH 3 gave foam volume increases of 80% (65 °C) and 240% (100 °C) as compared to foams formed at 2500 rpm (**Tables 1** and **2**). Without heat treatments, the largest changes in foam volume with increasing whipping speed were observed with SFAs, with an average increase of ~230% in foam volume (**Tables 1** and **2**; **Figure 3**). This foam volume increase, however, resulted in even faster coalescence. Generally, the increase in foam volume involved the formation of much smaller bubbles for all protein solutions, but also resulted in faster drainage.

Foam volume decreased with increasing whipping speed for 10% SFAs mixtures, but increased by 25, 90, 160, and 190% for protein mixtures containing 25, 50, 75, and 90% SFAs, respectively (**Tables 1** and **2**). **Figure 4** displays the gel permeation chromatogram of a protein solution, containing approximately 25% SFAs and 75% helianthinin, at pH 7 before (original solution) and after (drained liquid) foam formation at 2500 and 3500 rpm. At higher whipping speeds, the volume of foam formed increased by ~25% for the latter protein mixture (**Tables 1** and **2**). This increase in foam volume resulted in a higher amount of protein incorporated in the foam (30%; **Figure 4**). In **Figure 4**, it can be also observed that all proteins were capable of adsorbing at the interface, as all peak areas are smaller after foam formation. The helianthinin monomer, however, seemed to be more readily adsorbed than the other proteins, as it appears to be absent from the drained liquid (**Figure 4**). At the lower whipping speed, the helianthinin

Table 2. Characteristics of Foams Made with Sunflower Protein Preparations at Various Conditions (3500 rpm, 70 s)

sample	pH	*I* (mM)	foam volume[a] (mL) 2 min	φ[b] (air)	coalescence[c]	Ostwald ripening[d]
helianthinin	8	20	30	0.77	–	*****
	8	250	50	0.86	–	low
	7	20	22	0.80	–	*****
	7	250	45	0.90	–	low
	3	20	77	0.70	–	**
	3	100	145	0.78	–	**
	$8_{100°C}$[e]	20	240	0.66	–	*
	$8_{65°C}$[e]	20	125	0.70	–	**
	$3_{100°C}$[e]	20	201	0.70	–	**
	$3_{65°C}$[e]	20	98	0.67	–	**
SFAs	8	20	220	0.67	+	****
	8	250	225	0.70	+	****
	7	20	213	0.69	+	****
	7	250	217	0.68	+	****
	5	20	210	0.67	+	****
	5	250	220	0.65	+	****
	3	20	215	0.68	+	****
	3	250	210	0.70	+	****
SFAs/helianthinin mixtures (% SFAs)[f]						
10	7	20	33	0.82	–	****
25	7	20	62	0.73	–	****
50	7	20	117	0.73	–	****
75	7	20	165	0.71	–	****
90	7	20	195	0.70	+	****

[a] The average standard deviation of the foam volume is 3.5 mL. [b] φ = volume fraction of air initially present in foam. [c] +, coalescence observed; –, coalescence not observed. [d] More asterisks indicate faster Ostwald ripening; "low" indicates that the destabilization is barely noticeable. [e] Subscripts indicate the temperature of the heat treatment. [f] Proportion of SFAs in the protein mixtures.

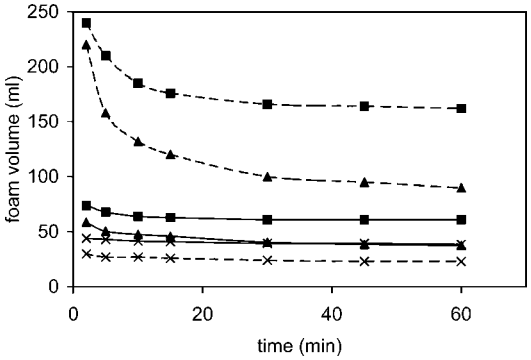

Figure 3. Foam volume as a function of time at pH 8 (I = 20 mM) using whipping speeds of 2500 rpm (solid line) and 3500 rpm (dashed line). Protein solutions were prepared with SFAs (▲), helianthinin (×), and helianthinin after heat treatment at 100 °C (■).

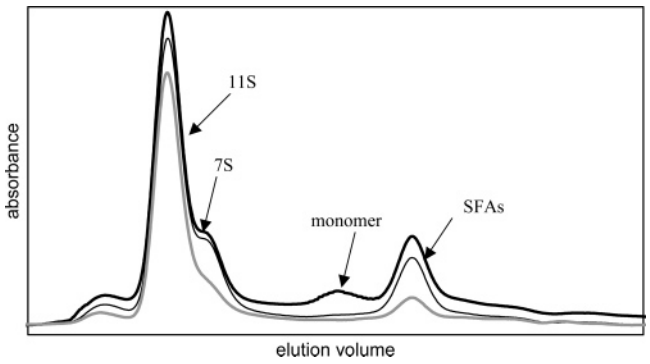

Figure 4. Gel permeation chromatography of a protein solution containing about 25% SFAs and 75% helianthinin at pH 7 (I = 20 mM), before foam formation (thick line), after foam formation at 2500 rpm (thin line), and after foam formation at 3500 rpm (gray line). The absorbance is monitored at 214 nm.

monomer adsorbed most readily at the interface (100%), followed by SFAs (30%) and finally the 7S and 11S forms of helianthinin (7%). The 7S form of helianthinin, however, seemed to adsorb in higher quantities (60%) than the 11S form (12%) at high whipping speed (**Figure 4**). It can also be observed that most of the protein remained in solution and only a minor part (~20% at 2500 rpm) is incorporated in the foam.

DISCUSSION

Foam Properties of SFAs. In SFAs-stabilized foams, destabilization is primarily the result of coalescence. Coalescence also brings about drainage of liquid from the foam (*3*). SFAs were, however, able to form high foam volumes. Foam formation requires from a protein the ability to quickly adsorb to the interface, thereby lowering the surface tension, to facilitate bubble breakup and formation of γ-gradients to stabilize newly formed bubbles against immediate coalescence. Hence, one of the most important factors for foam formation is the adsorption rate (*37*). However, the adsorption of proteins to the interface is not necessarily irreversible, and the loss of net energy upon adsorption may not be sufficient to maintain the protein adsorbed (*38*). SFAs seem to adsorb quickly, possibly due to their small size, but presumably unfold only slightly at the interface, as can be expected from their high conformational stability and compact structure (*7*). The fast adsorption to the interface seems to be confirmed by the increased foam volume at higher whipping speed, because at higher whipping speed the time available to adsorb is diminished. The coalescence observed in foams made with SFAs could have been induced by the presence of impurities. However, because SFAs were obtained by GPC and the protein solutions were filtered before use, this cause is highly improbable. So far, we do not have a plausible explana-

tion for the coalescence observed in SFAs-stabilized foams. These results are, however, in agreement with those reported by Guéguen and co-workers (*32*) and Popineau and co-workers (*12*), who also observed rapid degradation and little stability in foams made with SFAs.

Foam Properties of Helianthinin. Helianthinin produced low foam volumes at alkaline and neutral pH. This is probably due to its large size and closely packed globular conformation, which would cause it to adsorb slowly at the interface as compared to the time scales involved in foam formation. The decrease of foam volume at higher whipping speeds confirms this assumption. Once helianthinin is adsorbed, it will, due to its relatively large size, presumably not desorb easily. Protein-stabilized foams are often most stable against Ostwald ripening at their isoelectric pH (*3*, *38*, *39*). Because the isoelectric point of helianthinin is ~4−5.5 (*6*), it is observed that the further the pH from the isoelectric point of helianthinin, the lower is the stability of helianthinin foams against Ostwald ripening. However, possible structural changes due to exposure to low pH values must also be taken into account.

Effect of Heat and Acid Denaturation. Helianthinin dissociates at pH 3 into its monomeric form, which decreases its molecular size and results in a more flexible, unfolded protein (*6*). Proteins typically form and stabilize foams best under conditions at which the molecules are flexible and less compact (*38*−*40*). Dissociation probably also leads to increased surface hydrophobicity that favors protein adsorption (*41*). Hence, the higher foam volume at pH 3 may be explained by the fact the helianthinin subunits formed at this pH efficiently adsorb much more quickly than their multimeric counterparts. The higher stability against Ostwald ripening at pH 3 may be due to the unfolding of helianthinin: the unfolded helianthinin is likely to form strong intermolecular interactions at the interface, thus forming strong interfacial films and preventing desorption. These results are in line with the findings of Wagner and Guéguen (*41*, *42*) and Martin (*43*) for soy glycinin. The molecular structure of the acid-unfolded helianthinin at pH 7 resembles that at pH 3 (*6*). This explains the similarity in properties of helianthinin foams at pH 3 and foams at pH 7 of helianthinin that has been exposed to pH 3.

In addition to exposure to low pH, heat treatments also result in unfolding and dissociation of helianthinin (*6*). Both treatments resulted in foams with a high stability against Ostwald ripening. The relatively small increase in foam volume and stability against Ostwald ripening after the mild heat treatment (65 °C), as compared to treatment at higher temperature (100 °C), is probably due to the lower extent of unfolding and protein dissociation (*6*). That conformational changes and molecular size affect foam formation and stability has also been reported for soy glycinin and whey proteins (*42*−*44*).

Effect of Ionic Strength. Ionic strength significantly affected the foam properties of helianthinin (**Table 1**): both foam volume and stability against Ostwald ripening increased at pH 7 and 8 (**Table 1**). Helianthinin is negatively charged at the latter pH values. Addition of salt at these pH values will reduce charge repulsion, possibly allowing the protein to adsorb more easily, resulting in a faster lowering of the surface tension, that is, higher foam volume, and also a higher stability against Ostwald ripening. Similar results were found in foams made with BSA (*45*), potato (*46*), and soy protein (*47*). Increasing the ionic strength generally resulted in an increase in drainage rate (**Table 1**), a result also observed by other authors using other proteins (*45*, *46*). Higher drainage rates are generally correlated to a higher amount of liquid in the foam.

Mixtures of SFAs and Helianthinin. The experiments using mixtures of SFAs and helianthinin revealed the absence of synergetic or antagonistic effects on foam properties, contrasting previous studies on mixtures of proteins differing in their intrinsic properties (molecular size, isoelectric pH, conformational stability, etc.) (*38*, *48*−*50*). The reconstitution experiments rather showed an additive effect of helianthinin and SFAs, that is, higher volumes of foam with decreased stability when the proportion of SFAs in the protein mixtures was increased. The properties of SI-stabilized foams at pH 7 were quite in agreement with those of the reconstituted protein mixtures. The percentage of SFAs in the soluble fraction of SI at pH 7 ($I = 20$ mM) was estimated to be ~25−30%, which is consistent with the properties observed for foams made with mixtures having similar composition (**Table 1**). Coalescence, an important process occurring in SFAs foams, was observed only in mixtures containing as much as 90% SFAs. Coalescence, therefore, was effectively prevented by a small amount of helianthinin.

Sunflower proteins clearly differ in their ability to stabilize foams. The ability to stabilize foams that has been reported for sunflower products (*19*−*21*, *25*, *33*) must be mainly due to the presence of helianthinin and not SFAs, as is evident from our studies using protein mixtures. However, Booma and Prakash (*31*) reported that the foam properties of sunflower meal were better than those of helianthinin. Canella and co-workers (*34*) reported, in addition to a higher foam expansion (pH 2−10), as in our studies, also a higher stability (pH 2−6) for foams made with SFAs than for foams made with sunflower meal. These results may reflect the contribution of other constituents (fibers, carbohydrates, etc.), differences in the integrity and composition of the protein used, and the method utilized to make the foam. Furthermore, the latter authors tested foam properties with the total protein, that is, the soluble as well as the insoluble fractions. Although insoluble protein is accounted for in the total concentration, its contribution to protein functionality is usually very low. The solubility as a function of pH and ionic strength for helianthinin and SFAs is rather different (*7*). Therefore, conditions such as pH and ionic strength will determine the protein concentration of the soluble fraction, which could affect foam formation and stability to a higher extent than the physicochemical conditions themselves.

It can be concluded that the higher molecular flexibility and smaller molecular size of helianthinin, caused by heat treatment or low pH, resulted in improved foam properties. In addition, it was found that when sunflower proteins are used as foaming agent, the protein is not efficiently used and only a minor part of the available proteins is adsorbed to the interface.

ABBREVIATIONS USED

CGA, chlorogenic acid; SFAs, sunflower albumins; SI, sunflower isolate; GPC, gel permeation chromatography; BSA, bovine serum albumin; Γ, surface excess; γ, interfacial tension; P_{LP}, Laplace pressure; I, ionic strength; φ, volume fraction of air.

LITERATURE CITED

(1) Gassmann, B. Preparation and application of vegetable proteins, especially proteins from sunflower seed, for human consumption. An approach. *Nahrung* **1983**, *27*, 351−369.
(2) Kinsella, J. E. Functional properties of soy proteins. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242−258.
(3) Halling, P. J. Protein-stabilized foams and emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 155−203.

(4) Sastry, M. C. S.; Rao, M. S. N. Binding of chlorogenic acid by the isolated polyphenol-free 11S protein of sunflower (*Helianthus annuus*) seed. *J. Agric. Food Chem.* **1990**, *38*, 2103−2110.
(5) González-Pérez, S.; Merck, K. B.; Vereijken, J. M.; Van Koningsveld, G. A.; Gruppen, H.; Voragen, A. G. J. Isolation and characterization of undenatured chlorogenic acid-free sunflower (*Helianthus annuus*) proteins. *J. Agric. Food Chem.* **2002**, *50*, 1713−1719.
(6) González-Pérez, S.; Merck, K. B.; Vereijken, J. M.; Van Koningsveld, G. A.; Gruppen, H.; Voragen, A. G. J. Conformational states of sunflower (*Helianthus annuus*) helianthinin: effect of heat and pH. *J. Agric. Food Chem.* **2004**, *52*, 6770−6778.
(7) González-Pérez, S.; Vereijken, J. M.; Van Koningsveld, G. A.; Gruppen, H.; Voragen, A. G. J. Physico-chemical properties of 2S albumins and the corresponding protein isolate from sunflower (*Helianthus annuus*). *J. Food Sci.* **2005**, *70*, 98−103.
(8) Plietz, P.; Damaschun, G.; Muller, J. J.; Schwenke, K. D. The structure of 11-S globulins from sunflower and rape seed. A small-angle X-ray scattering study. *Eur. J. Biochem.* **1983**, *130*, 315−20.
(9) Kortt, A. A.; Caldwell, J. B. Low molecular weight albumins from sunflower seed: Identification of a methionine-rich albumin. *Phytochemistry* **1990**, *29*, 2805−2810.
(10) Anisimova, I. N.; Fido, R. J.; Tatham, A. S.; Shewry, P. R. Genotypic variation and polymorphism of 2S albumins of sunflower. *Euphytica* **1995**, *83*, 15−23.
(11) Raymond, J.; Robin Jean, M.; Azanza Jean, L. 11 S seed storage proteins from *Helianthus* species (*Compositae*): Biochemical, size and charge heterogeneity. *Plant Syst. Evol.* **1995**, *198*, 195−208.
(12) Popineau, Y.; Tatham, A. S.; Shewry, P. R.; Marion, D.; Guéguen, J. 2S sunflower albumins: functional properties of native and modified proteins. In *Plant Proteins from European Crops. Food and Non-food Applications*; Guéguen, J., Popineau, Y., Eds.; INRA Editions: Nantes, France, 1998; pp 131−135.
(13) Allen, R. D.; Cohen, E. A.; Vonder Haar, R. A.; Adams, C. A.; Ma, D. P.; Nessler, C. L.; Thomas, T. L. Sequence and expression of a gene encoding an albumin storage protein in sunflower. *Mol. Gen. Genet.* **1987**, *210*, 211−218.
(14) Shewry, P. R.; Pandya, M. J. The 2S albumins storage proteins. In *Seed Proteins*; Shewry, P. R., Casey, R., Eds.; Kluwer Academic Publishers: Amsterdam, The Netherlands, 1999; pp 619−664.
(15) Kinsella, J. E. Functional properties in foods: A survey. *CRC Crit. Rev. Food Sci. Nutr.* **1976**, *7*, 219−280.
(16) Walstra, P.; Smulders, P. A. E. Making emulsions and foams: An overview. In *Food Colloids: Proteins, Lipids and Polysaccharides*; Dickinson, E., Bergenståhl, B., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1997; pp 367−381.
(17) Dickinson, E. Foams. In *An Introduction to Food Colloids*; Dickinson, E., Ed.; Oxford Univerity Press: Oxford, U.K., 1992; pp 123−139.
(18) Lucassen, J. In *Anionic Surfactants*; Lucassen-Reijnders, E. H., Ed.; Dekker: New York, 1981; p 217.
(19) Huffman, V. L.; Lee, C. K.; Burns, E. E. Selected functional properties of sunflower meal (*Helianthus annuus*). *J. Food Sci.* **1975**, *40*, 70−74.
(20) Canella, M. Whipping properties of sunflower protein dispersions. *Lebensm.-Wiss. -Technol.* **1978**, *11*, 259−263.
(21) Rossi, M.; Germondari, I. Production of a food-grade protein meal from defatted sunflower. II. Functional properties evaluation. *Lebensm.-Wiss. -Technol.* **1982**, *15*, 313−316.
(22) Rossi, M.; Pagliarini, E.; Peri, C. Emulsifying and foaming properties of sunflower protein derivatives. *Lebensm.-Wiss. -Technol.* **1985**, *18*, 293−299.
(23) Lin, M. J. Y.; Humbert, E. S.; Sosulski, F. W. Certain functional properties of sunflower meal products. *J. Food Sci.* **1974**, *39*, 368−370.
(24) Kabirullah, M.; Wills, R. B. H. Foaming properties of sunflower seed protein. *J. Food Sci. Technol.* **1988**, *25*, 16−19.
(25) Raymond, J.; Rakariyatham, N.; Azanza, J. L. Functional properties of a new protein isolate from sunflower oil cake. *Lebensm.-Wiss. -Technol.* **1985**, *18*, 256−263.
(26) Khalil, M.; Ragab, M.; Abd El Aal, M. H. Foaming properties of oilseed proteins. *Nahrung* **1985**, *29*, 201−207.
(27) Khalil, M.; Ragab, M.; Hassanien, F. R. Some functional properties of oilseed proteins. *Nahrung* **1985**, *29*, 275−282.
(28) Claughton, S. M.; Pearce, R. J. Preparation and properties of acid-modified sunflower protein isolate. *J. Food Sci.* **1989**, *54*, 357−361.
(29) Rahma, E. H.; Rao, M. S. N. Removal of polyphenols from sunflower meal by various solvents: effects on functional properties. *J. Food Sci.* **1981**, *46*, 1521−1522.
(30) Venktesh, A.; Prakash, V. Functional properties of the total proteins of sunflower (*Helianthus annuus* L.) seed: effect of physical and chemical treatments. *J. Agric. Food Chem.* **1993**, *41*, 18−23.
(31) Booma, K.; Prakash, V. Functional properties of the flour and the major protein fraction from sesame seed, sunflower seed and safflower seed. *Acta Alimentaria* **1990**, *19* (*2*), 163−176.
(32) Guéguen, 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.
(33) Pawar, V. D.; Patil, J. N.; Sakhale, B. K.; Agarkar, B. S. Studies on selected functional properties of defatted sunflower meal and its high protein products. *J. Food Sci. Technol.* **2001**, *38*, 47−51.
(34) Canella, M.; Castriotta, G.; Bernardi, A.; Boni, R. Functional properties of individual sunflower albumin and globulin. *Lebensm.-Wiss. -Technol.* **1985**, *18*, 288−292.
(35) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(36) Caessens, P. W. J. R.; Gruppen, H.; Visser, S.; Van Aken, G. A.; Voragen, A. G. J. Plasmin hydrolysis of β-casein: foaming and emulsifying properties of the fractionated hydrolysate. *J. Agric. Food Chem.* **1997**, *45*, 2935−2941.
(37) Martin, A. H.; Grolle, K.; Bos, M. A.; Cohen Stuart, M. A.; van Vliet, T. Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability. *J. Colloid Interface Sci.* **2002**, *254*, 175−183.
(38) German, J. B.; Phillips, L. Protein interactions in foams. In *Protein Functionality in Food Systems*; Hettiarachy, N. S., Ziegler, G. R., Eds.; IFT Basic Symposium Series: Chicago, IL, 1991; pp 181−208.
(39) Kinsella, J. E. Functional properties of proteins: Possible relationships between structure and function in foams. *Food Chem.* **1981**, *7*, 273−288.
(40) Kinsella, J. E. *Protein and Fat Globule Modifications by Heat Treatment, Homogenization and Other Technological Means for High Quality Dairy Products*; IDF Special Issue 9303; International Dairy Federation: Brussels, Belgium, 1993; pp 67−72.
(41) Wagner, J. R.; Guéguen, J. Effects of dissociation, deamidation, and reducing treatment on structural and surface active properties of soy glycinin. *J. Agric. Food Chem.* **1995**, *43*, 3, 1993−2000.
(42) Wagner, J. R.; Guéguen, J. Surface functional properties of native, acid-treated and reduced soy glycinin. 1. Foaming properties. *J. Agric. Food Chem.* **1999**, *47*, 2173−2180.
(43) Martin, A. H. Mechanical and Conformational Aspects of Protein Layers on Water. Ph.D. Thesis, Wageningen University, 2003.
(44) Zhu, H.; Damodaran, S. Heat-induced conformational changes in whey protein isolate and its relation to foaming properties. *J. Agric. Food Chem.* **1994**, *42*, 846−855.
(45) Germick, R. J.; Rehill, A. S.; Narsimhan, G. Experimental investigation of static drainage of protein stabilized foams−comparison with model. *J. Food Eng.* **1994**, *23*, 555−578.
(46) van Koningsveld, G. A.; Walstra, P.; Gruppen, H.; Wijngaards, G.; van Boekel, M. A.; Voragen, A. G. Formation and stability

of foam made with various potato protein preparations. *J. Agric. Food Chem.* **2002**, *50*, 7651−7659.
(47) Yu, M.-A.; Damodaran, S. Kinetics of destabilization of soy protein foams. *J. Agric. Food Chem.* **1991**, *39*, 1563−1567.
(48) Matringe, E.; Phan Tan Luu, R.; Lorient, D. Functional properties of milk−egg mixtures. *J. Food Sci.* **1999**, *64*, 787−791.
(49) Sorgentini, D. A.; Wagner, J. R. Comparative study of foaming properties of whey and isolate soybean proteins. *Food Res. Int.* **2002**, *35*, 721−729.
(50) Aryana, K. J.; Haque, Z. Z.; Gerard, P. D. Influence of whey protein concentrate on the functionality of egg white and bovine serum albumin. *Int. J. Food Sci. Technol.* **2002**, *37*, 643−652.

Received for review January 25, 2005. Revised manuscript received May 27, 2005. Accepted June 7, 2005. This research was carried out with financial support from the EU. Marie Curie Fellowship: EU Contract FAIR-CT 98-5030.

JF0501793