Emulsifying and Foaming Properties of Native and Chemically Modified Peptides from the 2S and 12S Proteins of Rapeseed (*Brassica napus* L.)

Christophe Malabat[a], Ra l S nchez-Vioque[a], Claude Rabiller[b], and Jacques Gu guen[a,*]

[a]Laboratoire de Biochimie et Technologie des Protéines, Institut National de la Recherche Agronomique (I.N.R.A.), 44316 Nantes Cedex 03, France, and [b]Faculté des Sciences et des Techniques, Unité de Recherche en Biocatalyse (UPRES 2161), 44322 Nantes Cedex 03, France

ABSTRACT: The 2S and 12S proteins of rapeseed were isolated and subsequently hydrolyzed by pepsin or a combination of pepsin plus trypsin. The resulting hydrolysates had a 15% degree of hydrolysis and were purified by gel filtration chromatography in order to obtain homogeneous peptide fractions. Three major fractions, having an average peptide chain length of 7.5–11 amino acids, were recovered. Purified peptide fractions were acylated with butyric anhydride and sulfamidated with *p*-toluenesulfonyl chloride. The degree of modification was always higher than 90%. Emulsifying and foaming properties of native and chemically modified peptides were studied and compared to those of sodium dodecyl sulfate (SDS) as standard. A peptide fraction from the 15% hydrolysis of the 12S protein exhibited the best foaming properties. After sulfamidation, this peptide fraction showed a foam formation similar to that of SDS. Whereas the attachment of toluene groups generally improved the surface properties, the incorporation of an aliphatic chain of four atoms of carbon was detrimental in most of the cases. On the other hand, none of the native or hydrophobized peptide fractions was able to form a stable emulsion.

Paper no. J9679 in *JAOCS 78,* 235–241 (March 2001).

KEY WORDS: Chemical modification of peptides, cruciferin, enzymatic hydrolysis of proteins, foaming and emulsifying properties, napin.

Proteins are recognized to be much more than a simple source of nutrients. Because of their amphiphilic nature, proteins are also involved in functional aspects of foods, such as the formation of emulsions and foams. Proteins can be adsorbed at oil–water and air–water interfaces, decreasing surface tension values, and, hence facilitating the formation of emulsions and foams. This adsorption is believed to occur in three distinct steps. First, protein molecules diffuse to the subsurface just below the interface; second, they are adsorbed; and finally, they unfold at the interface to adopt a thermodynamically optimized conformation. In addition, proteins form a continuous viscoelastic film around the oil droplets or air bubbles that stabilize emulsions and foams (1). Therefore, the formation of a stable foam or emulsion is a complex phenomenon that depends on the physicochemical characteristics of the protein, such as net charge, solubility, hydrophobicity, flexibility, etc. (2). Protein structure can be intentionally modified in order to improve these surface properties. Such protein modifications can be easily performed by enzymatic or chemical treatments.

Enzymatic hydrolysis improves the solubility of proteins, even if the degree of hydrolysis is low. The increase of ionic groups after hydrolysis makes the peptides more soluble with respect to the original protein throughout the pH range (3,4), and consequently enhances the diffusion of the protein and facilitates the formation of emulsions (5). In addition, enzymatic hydrolysis can increase the surface hydrophobicity of peptides by exposing hydrophobic groups that are generally buried in the core of native proteins (5,6). The positive effect of hydrophobicity on the surface properties of proteins and peptides has been demonstrated in several works (7–9). Nevertheless, emulsifying and foaming properties are only improved if the enzymatic hydrolysis is limited and the resulting peptides maintain a size large enough to form a stable film around the dispersed phase (10).

Chemical modification also constitutes a useful and well-known procedure to modify the functional properties of proteins. Acylation, succinylation, esterification, oxidation, reduction, glycosylation, phosphorylation, and alkylation are the most common chemical reactions used for this purpose (11). Emulsifying and foaming properties of different chemically modified proteins are widely described in the literature. Chemical treatments were used in the following ways: to improve the flexibility of proteins either by reduction of disulfide bonds (12) or glycosylation (13); to increase their solubility by either deamidation (14) or grafting polar molecules (15); and to enhance the hydrophobicity of proteins by either dissociation and reduction (16) or grafting hydrophobic groups by esterification (17), mainly by acylation and succinylation of amino groups (18–20).

Nevertheless, the combination of enzymatic hydrolysis plus chemical modification is little used, and only few works deal with the preparation of chemically modified peptides. A procedure for the succinylation of hydrolysates from soy, peanut, cottonseed, corn, rapeseed, and sesame is patented (21). Acylated dipeptides were prepared from the synthetic reaction between *N*-lauroyl-arginine and free amino acids, ob-

*To whom correspondence should be addressed at Laboratoire de Biochimie et Technologie des Protéines, Institut National de la Recherche Agronomique (I.N.R.A.), rue de la Géraudière B.P. 71627, 44316 Nantes Cedex 03, France. E-mail: gueguen@nantes.inra.fr

tained commercially or from the total hydrolysis of collagen (22,23). These dipeptides showed good surfactant and antimicrobial properties. The proteolysis of esterified β-lactoglobulin was performed by pepsin and the resulting peptides identified, but their surface properties were not evaluated (24).

Rapeseed (*Brassica napus* L.) is the most important oilseed crop in Europe, and there is excess oil production in some countries, such as France. Rapeseed is also an excellent source of proteins (20–25%), although its high content of some antinutritional constituents such as phytic acid, glucosinolates, and phenols limits the extraction and utilization of rapeseed proteins in food products (25). For these reasons, rapeseed proteins are used mainly for animal feeding. Nevertheless, the production of biodegradable and so-called green peptide surfactants from these proteins might constitute an alternative for increasing the value of rapeseed. Such surface-active peptides might be considered as potential substitutes for current chemical surfactants in some nonfood applications.

The objective of this work was to investigate the emulsion and foam properties of native and chemically treated peptides obtained from rapeseed. With this aim, the major proteins of rapeseed, 2S (napin) and 12S (cruciferin) were purified, hydrolyzed, and subsequently modified by acylation and sulfamidation. Both reactions were recently optimized in our laboratory and applied to bovine serum albumin (26) and the 12S and 2S proteins from rapeseed (27). These reactions are based on the nucleophile reaction between the free amino groups of peptides and butyric anhydride or *p*-toluenesulfonyl chloride to form an amide or a sulfonamide bond, respectively. As a result of these reactions, amino groups of peptides are hydrophobized with an aliphatic chain (butyric anhydride) or with an aromatic ring (*p*-toluensulfonyl chloride).

EXPERIMENTAL PROCEDURES

Materials. Rapeseed (var. Lirajet) was dehulled, ground, and extracted with hexane under mild conditions to remove most of the fat. Pepsin (EC 3.4.23.1; 4,500 U/mg; from porcine stomach mucosa), trypsin [EC 3.4.21.4; 11,000 U Nα-benzoyl-L-arginine ethyl ester (BAEE)/mg; type XIII from bovine pancreas], *o*-phthaldialdehyde (OPA), *p*-toluenesulfonyl chloride, L-leucine, *n*-hexadecane, and butyric anhydride were purchased from Sigma Chemical Co. (St. Louis, MO). Phenylisothiocyanate (PITC) and dimethyl-2-mercaptoethylammonium chloride (DMMAC) were obtained from Pierce (Rockford, IL) and Merck (Darmstadt, Germany), respectively. All other chemicals were of analytical grade.

Amino acid analysis. Quantification of amino acids was performed according to Bidlingmeyer *et al*. (28). Proteins and peptides were hydrolyzed with 6 N hydrogen chloride at 110°C for 24 h. Resulting free amino acids were then derivatized with PITC and separated by high-performance liquid chromatography (HPLC) using a PICO TAG C18 (150 × 3.9 mm) reversed-phase column (Waters, Milford, MA). Separations were by a binary gradient system using 140 mM sodium acetate, 0.1% (vol/vol) triethylamine buffer, pH 6.4 (solvent A), and a 60% aqueous solution of acetonitrile (solvent B). Solvents were delivered to the column as follows: time 0–2 min, linear gradient from 10 to 13% solvent B; time 2–6 min, linear gradient from 13 to 18% solvent B; time 6–14 min, linear gradient from 18 to 38% solvent B; time 14–20 min, linear gradient from 38 to 62% solvent B; time 20–21 min, linear gradient from 62 to 100% solvent B; time 21–29 min, elution with 100% solvent B. Temperature of the column was maintained at 50°C. Flow rate was 1 mL/min and elution was monitored at 254 nm. Percentage of each amino acid was calculated from calibration curves.

Determination of amino groups. Free amino groups were determined according to the modified OPA method described by Frister *et al*. (29). Proteins or peptides were dissolved in 12.5 mM sodium tetraborate, 2% sodium dodecyl sulfate (SDS) buffer, pH 8.5. Fifty microliters of this solution was added to 1 mL of a reagent made up of the following: 50 mL 0.1 M sodium tetraborate buffer, pH 9.3, plus 1.25 mL of 20% SDS, plus 100 mg of DMMAC, plus 40 mg of OPA dissolved in 1 mL of methanol. After 2 min, the absorbance of the mixture was measured at 340 nm. The number of amino groups was calculated from a standard curve of L-leucine.

Extraction of rapeseed proteins. Defatted rapeseed flour (250 g) was suspended in 2,500 mL of 22 mM sodium phosphate, 0.3% sodium bisulfite buffer, pH 7.0, and extracted by stirring for 1 h at room temperature. Sodium bisulfite was used to avoid the darkening of the proteins. The suspension was centrifuged at 8,000 × *g* for 15 min. The supernatant was filtered and eluted with the extraction buffer in a Sephadex GH-25 (Pharmacia, Uppsala, Sweden) column to remove polyphenols and other nonprotein compounds.

Purification of 2S and 12S proteins from rapeseed. Protein rapeseed extract was loaded on a SP Sepharose Big Bead (Pharmacia) ion exchange column. Protein purification was carried out using a binary gradient system. Solvents were 22 mM sodium phosphate, 0.3% sodium bisulfite, pH 7.0 (buffer A), and buffer A plus 1 M sodium chloride (buffer B). Solvents were delivered to the column as follows: time 0–130 min, elution with 100% buffer A; time 130–230 min, linear gradient from 0 to 25% buffer B; time 230–250 min, linear gradient from 25 to 100% buffer B; time 250–265 min, elution with 100% buffer B. Flow rate was 70 mL/min. Protein fractions were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). The 12S- and 2S-enriched fractions were eluted, respectively, in the range of 0–25% and 100% buffer B. These fractions were recovered, concentrated, and further purified using a Sephadex C-25 gel filtration column equilibrated in buffer A. Napin and cruciferin purified fractions were recovered and desalted on a Sephadex GH-25 column equilibrated with 0.1% ammonium carbonate solution. Finally, protein samples were freeze-dried.

Enzymatic hydrolysis of 2S and 12S proteins. Cruciferin (250 mg) was dissolved in distilled water (125 mL) at 37°C and pH 2.5 adjusted by 6 N HCl. Two milliliters of pepsin (2.5 mg/mL) was added, and the mixture was stirred for 1 h. Napin was successively hydrolyzed with pepsin and trypsin.

Pepsin hydrolysis was carried out for 24 h as described above and then stopped by increasing the pH to 7.8 with 6 N NaOH. Subsequently, trypsin (2 mL at 2.5 mg/mL) was added and the hydrolysis continued up to 120 h. Hydrolysis was stopped by adjusting the pH to 5 and freeze-drying. Cruciferin was hydrolyzed under nondenaturing conditions, whereas napin was reduced with 2% (vol/vol) 2-mercaptoethanol at 80°C for 10 min prior to the enzymatic hydrolysis.

Determination of the degree of hydrolysis (DH) and average peptide chain length (PCL). DH, defined as the percentage of peptide bonds cleaved, was calculated using the following formula:

$$\mathrm{DH} = \left(N_h - N_O / N_T\right) \times 100 \qquad [1]$$

where N_O and N_h are the number of free amino groups in the native and hydrolyzed protein, respectively, determined by OPA reaction (29); N_T is the total number of peptide bonds: 2637 for the 12S globulin (30) and 125 for the 2S albumin (31).

Average PCL was calculated according to Adler-Nissen (3):

$$\mathrm{PCL} = 100/\mathrm{DH} \qquad [2]$$

Separation of rapeseed peptides. Rapeseed protein hydrolysates were fractionated on a Superdex 30 TM prep grade 600 × 160 mm gel filtration column (Pharmacia) (Fig. 1). Peptides were eluted with 1.5% ammonium carbonate at a flow rate of 0.8 mL/min. Elution was monitored at 280 nm.

Chemical modification of rapeseed peptides. Peptide fractions (30 mg) were dissolved in distilled water (10 mL), and the pH was adjusted to 10.5. Butyric anhydride (3 equivalents per amino group) or *p*-toluensulfonyl chloride (2 equivalents per amino group) was added, and the pH was maintained at 10.5 by 1 N sodium hydroxide using a pH-stat. The reaction medium was stirred at room temperature until a constant pH was reached. After the reaction, side-reaction products were removed by washing the peptide solution three times with hexane. Modified peptides were recovered in the aqueous phase and then freeze-dried (26). Peptide fractions (X) are referenced as X-B and X-S for butylated or sulfamidated, respectively.

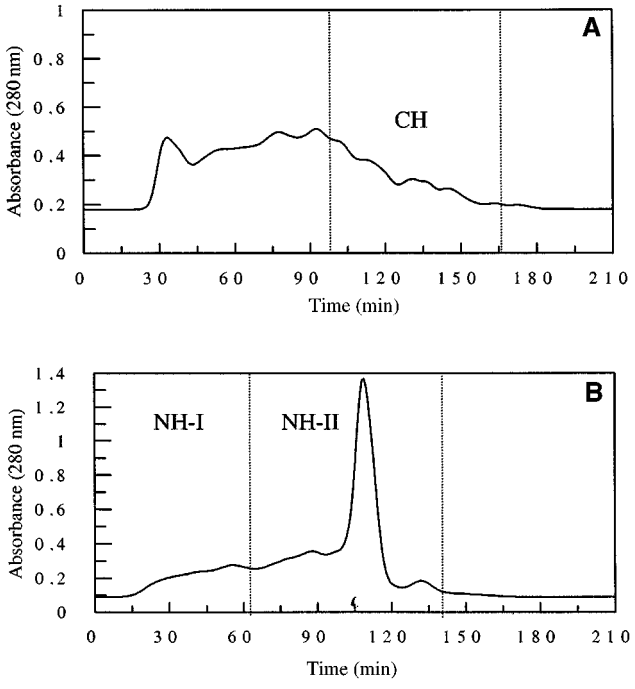

FIG. 1. Fractionation by gel filtration chromatography of 15% degree of hydrolysis hydrolysates prepared from (A) 12S protein (cruciferin) and (B) 2S protein (napin). Recovered fractions are between dotted lines.

Determination of the degree of modification. The percentage of modified peptides was calculated using the following formula:

$$\text{modified peptides (\%)} = \left(N_O - N_m / N_O\right) \times 100 \qquad [3]$$

where N_O and N_m are the number of free amino groups, determined by OPA reaction (29), in the nonmodified and modified samples, respectively.

Foaming properties. Foam formation was characterized using the foam analyzer apparatus described by Fains *et al.* (32). Samples (8 mL at 1 mg/mL) dissolved in 1% ammonium carbonate buffer, pH 9.0, were placed in a glass column (200 × 30 mm). Foam was generated by sparging air into the peptide solution through a porous metal disk (2 µm pore diameter) at a rate of 15 mL/min. At the base of the column, a pair of electrodes measured by conductimetry the quantity of liquid under the foam. The volume of liquid incorporated into the foam was determined by comparison with the initial volume of peptide solution. The foam volume in the glass column was detected by a uniline CCD camera (model OM-1024; Schmersal, Wuppertal, Germany) linked to a microcomputer. Bubbling stopped automatically when the volume of foam reached 35 mL. The formation and drainage of the foam was recorded for 20 min. Foams were compared on the basis of the maximal liquid incorporated into the foam and half drainage times ($t_{1/2}$), which is defined as the time required for draining half of the volume of liquid released at the end of the analysis.

Emulsifying properties. Emulsions were evaluated by the study of the creaming–flocculation kinetics (33). Samples (0.8 mg/mL) were dissolved in 1% ammonium carbonate buffer, pH 9.0. Then 25 mL of this solution was placed into a glass cylinder containing two electrodes at the base, and 15 mL of *n*-hexadecane was gently added over the aqueous solution. The system was stirred at 20,000 rpm for 30 s by a Polytron PT-3000 (Littau, Switzerland). The volume of the aqueous phase was continuously monitored by conductivity measurements. Results were expressed as the volume fraction of dispersed oil in the emulsion and were calculated from

$$\phi = 1 - \left[25/15 \times \left(1 - C_t / C_O\right)\right] \qquad [4]$$

where C_O is the conductivity of the peptide solution before emulsification and C_t is the conductivity of the emulsion at time t; 25 and 15 are the volumes of the aqueous and oil

phases in milliliters, respectively.

RESULTS AND DISCUSSION

Enzymatic hydrolysis of the 2S and 12S proteins from rapeseed. The 12S protein was preferably hydrolyzed by pepsin as a sole enzyme, because at the optimal pH for pepsin activity (pH 2.5) the 12S globulin is dissociated into its αβ subunits favoring the accessibility to the cleavage sites. Conversely, the proteolysis of 12S globulin at a neutral pH is generally limited because the protein is organized as close-packed hexamers (34). For this reason, the hydrolysis by another enzyme with an optimal pH around neutrality was not assayed. The DH of 12S globulin after 1 h of hydrolysis with pepsin was 15%.

The 2S albumin showed a high resistance to enzymatic hydrolysis by pepsin. This is probably due to the compact globular conformation of this protein characterized by a rigid tridimensional structure linked by four disulfide bonds (35). Therefore, the hydrolysis of 2S albumin was performed under denaturing conditions in order to cleave these disulfide bonds and facilitate the action of the proteases. In addition, we used two enzymes, pepsin and trypsin, with different specificities. The former is a protease that hydrolyzes peptide bonds of aromatic amino acids, whereas trypsin has a specificity for basic amino acids. With this combination of enzymes it is possible to increase the DH of hydrolysates by lowering the effect of the specificity of individual enzymes (36). By using pepsin plus trypsin on 2S albumin, we obtained a DH of 15% after 120 h of hydrolysis.

Separation, characterization, and lipophilization of rapeseed peptides. Rapeseed hydrolysates were fractionated by gel chromatography in order to obtain peptides with a homogeneous size (Fig. 1). Peaks were not detected in the chromatograms corresponding to the nonhydrolyzed cruciferin or napin, which indicates the efficiency of the enzymatic hydrolysis. Cruciferin hydrolysate was characterized by a profile without separated peaks that suggests the presence of peptides quite heterogeneous in size. On the contrary, napin hydrolysate showed a well-defined peak eluted at around 110 min. Five fractions were separated but only three major fractions were recovered and studied, one from the cruciferin hydrolysate (CH) and two from napin hydrolysate (NH-I and NH-II). Other fractions were not kept because they either represented a minor percentage or lacked homogeneity. The PCL of CH, NH-I, and NH-II peptide fractions were 7.5, 11.0, and 8.0 amino acids, respectively. The hydrophobic/hydrophilic balance of the peptide fractions was also evaluated because of the importance of this parameter on the surface properties of peptides (Fig. 2). The hydrophobic amino acid content of CH and NH-I were 57 and 54%, respectively, which were very close to those of 2S and 12S proteins (around 58%). On the other hand, this percentage was significantly lower in the NH-II fraction (36%). In order to increase their initial hydrophobicity and to obtain more surface active compounds, rapeseed peptides were modified using either butyric anhydride or *p*-toluenesulfonyl chloride. Because of the small size of peptides, amino groups were more accessible to reagents than those of proteins, and the chemical modification was almost complete. When butyric anhydride was used, the percentages of modified amino groups of CH, NH-I, and NH-II were 92, 91, and 93%, respectively, whereas in the case of *p*-toluenesulfonyl chloride, they reached 94, 93, and 98%, respectively.

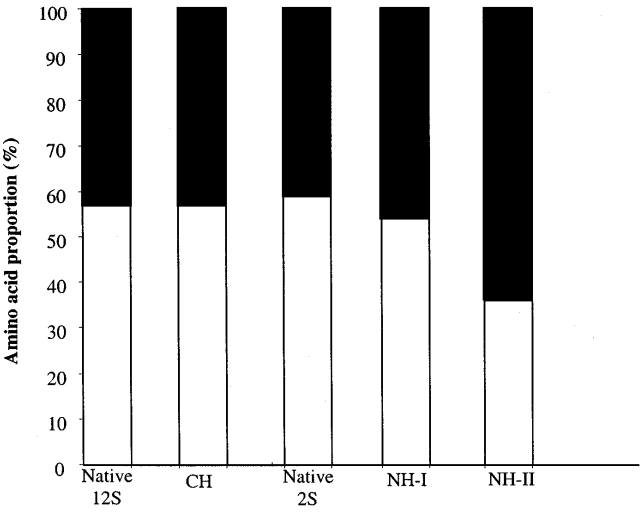

FIG. 2. Percentages of hydrophobic (□) and hydrophilic amino acids (■) of the purified peptide fractions. CH = cruciferin hydrolysate; NA = napin hydrolysate (fractions I and II).

Foaming properties of native and chemically modified rapeseed peptides. The foaming and emulsifying properties of rapeseed peptides, before and after chemical modification, were compared to those of SDS as the standard surfactant. Foam formation profiles of native peptide fractions are shown in Figure 3, and the main parameters characterizing the foams are in Table 1. The best results were obtained with SDS,

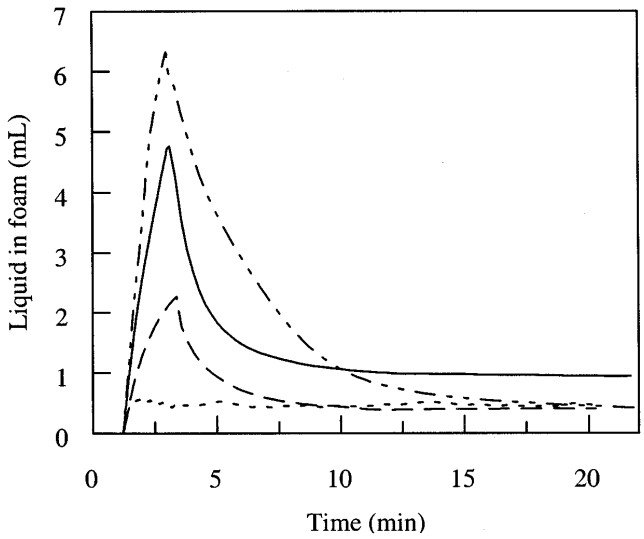

FIG. 3. Foam formation curves of native peptide fractions CH (—), NH-I (— —), and NH-II (----), SDS (— -- —) at 1.0 mg/mL and pH 9.0. See Figure 2 for abbreviations.

TABLE 1
Maximum Liquid Volume and Half Drainage Times ($t_{1/2}$) of Foams Formed from Native (CH and NH), Butylated (B), and Sulfamidated (S) Rapeseed Peptide Fractions

	Maximum liquid (mL)	$t_{1/2}$ (s)
SDS[a]	6.3	176
CH	4.8	60
CH-B	3.5	50
CH-S	4.9	201
NH-I	2.3	91
NH-I-B	1.8	—
NH-I-S	2.0	—
NH-II	—	—
NH-II-B	—	—
NH-II-S	2.1	—

[a]SDS, sodium dodecyl sulfate. —, no foam formation or extremely unstable foam formation.

which formed a foam with a maximal liquid incorporation of 6.3 mL and a $t_{1/2}$ of 176 s. Fraction CH also formed a rather stable foam with a maximal liquid incorporation of 4.8 mL and a $t_{1/2}$ of 60 s. Peptide fractions obtained from napin, NH-I and NH-II, showed poor foaming properties. The amount of liquid that fraction NH-I incorporated into foam was only 2.3 mL, whereas NH-II did not show foam formation. The different foam formation characteristics observed in CH and NH-II fractions could be due to their amino acid compositions. Thus, although both fractions had the same PCL, fraction NH-II was more hydrophilic (64% of polar amino acids) than CH (43%) and NH-I (46%). This higher hydrophilicity of fraction NH-II could hinder its adsorption at the air–water interface, because it seems that only peptides having a high hydrophobicity are able to be adsorbed into the interfacial layers, whereas hydrophilic peptides generally stay in the aqueous phase (4). Nevertheless, the hydrophobicity of peptides cannot explain the differences found between the foaming properties of CH and NH-I. NH-I had a higher PCL than CH and a very similar percentage of hydrophobic amino acids, but its foaming properties were significantly lower. A possible hypothesis is that CH has a more favorable amino acid sequence, with polar and apolar amino acids grouped in separate regions in order to form an amphiphilic molecule (37).

In spite of the fact that grafting of aliphatic chains of four atoms of carbon was reported as a valuable means to prepare proteins with improved foaming properties (38), acylation of rapeseed peptides using butyric anhydride was detrimental for foaming formation. Thus, fractions CH-B, NH-I-B, and NH-II-B exhibited poorer foaming properties than native peptides (Fig. 4 and Table 1). Maximal liquid incorporated in the foam decreased from 4.8 mL of CH fraction to 3.5 mL after butylation (CH-B fraction). NH-I-B also showed a slight decrease in its foaming properties with respect to unmodified NH-I, and NH-II-B did not show foam formation.

Unlike butylation, sulfamidation, in general, improved the foaming properties of peptide fractions (Fig. 5 and Table 1). Sulfamidation of fraction CH did not improve the amount of liquid incorporated into the foam (4.9 mL) with respect to the

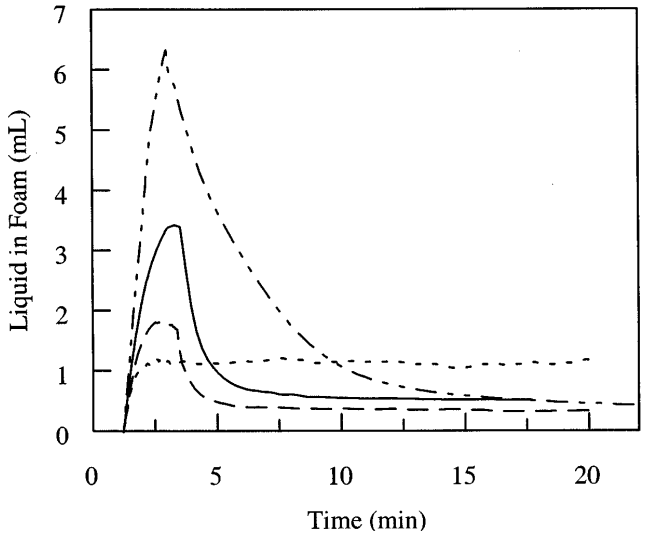

FIG. 4. Foam formation curves of butylated peptide fractions CH-B (—), NH-I-B (— —), and NH-II-B (----), SDS (— -- —) at 1.0 mg/mL and pH 9.0. See Figure 2 for abbreviations.

original fraction, but the destabilizing rate of CH-S foam was much slower than for CH, as indicated by a $t_{1/2}$ of 201 s. This value is even superior to that of SDS, although the amount of liquid into foam was higher in the standard surfactant. Sulfamidation also improved the foaming properties of NH-II, although the maximal liquid incorporated in the foam remained low (2.1 mL). As in the case of butylation, sulfamidation did not improve the foaming properties of NH-I fraction.

Emulsifying properties of native and chemically modified rapeseed peptides. Native rapeseed peptides exhibited very poor emulsifying properties (Fig. 6). The creaming flocculation kinetics showed that emulsions formed were not stable and creamed rapidly after their formation. Only CH fraction showed a more gradual creaming, although after 50 min the emulsion

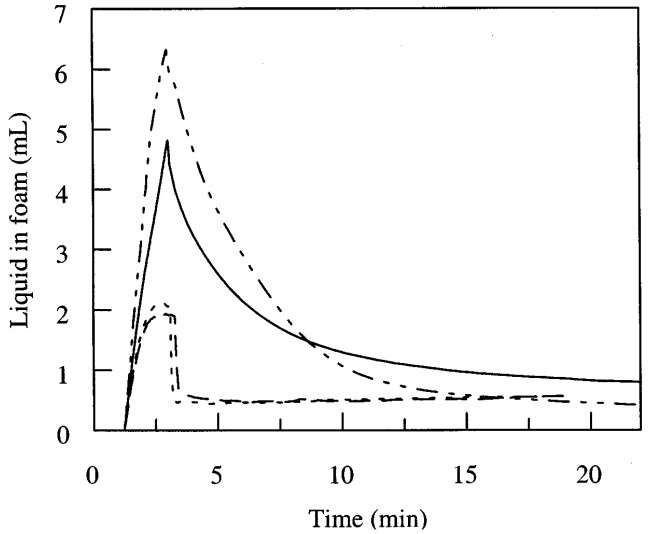

FIG. 5. Foam formation curves of sulfamidated peptide fractions CH-S (—), NH-I-S (— —), and NH-II-S (----), SDS (— -- —) at 1.0 mg/mL and pH 9.0. See Figure 2 for abbreviations.

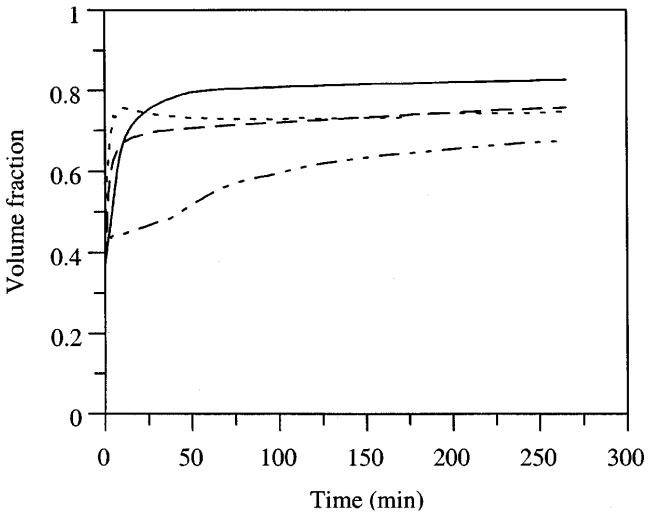

FIG. 6. Creaming flocculation kinetics of native peptide fractions CH (—), NH-I (— —), and NH-II (----), SDS (— -- —) at 0.8 mg/mL and pH 9.0. See Figure 2 for abbreviations.

had been creamed from the aqueous phase. Unlike the case of foaming properties, no significant improvement of emulsifying properties was obtained by acylation or sulfamidation.

ACKNOWLEDGMENTS

This study is a part of the EU-Fair CT-95-0260 project, which is funded by the EU Directorate General XII, Division Agro-Industrial Research, DG XII-E-2, Brussels, Belgium.

REFERENCES

1. Magdassi, S., and A. Kamyshny, Introduction: Surface Activity and Functional Properties of Proteins, in *Surface Activity of Proteins. Chemical and Physicochemical Modifications*, edited by S. Magdassi, Marcel Dekker Inc., New York, 1996, pp. 1–38.
2. Phillips, L.G., D.M. Whitehead, and J. Kinsella, Protein Films, in *Structure-Function Properties of Food Proteins*, edited by S.L. Taylor, Academic Press Inc., San Diego, 1994.
3. Adler-Nissen, J., Some Fundamental Aspects of Food Protein Hydrolysis, in *Enzymic Hydrolysis of Food Proteins*, edited by J. Adler-Nissen, Elsevier Applied Science Publishers, Essex, 1986, pp. 9–24.
4. Chobert, J.M., L. Briand, J. Guéguen, Y. Popineau, C. Larré, and T. Haertlé, Recent Advances in Enzymatic Modifications of Food Proteins for Improving Their Functional Properties, *Nahrung 40*:177–182 (1996).
5. Qi, M., N.S. Hettiarachchy, and U. Kalapathy, Solubility and Emulsifying Properties of Soy Protein Isolates Modified by Pancreatin, *J. Food Sci. 62*:1110–1115 (1997).
6. Kato, A., K. Komatsu, K. Fujimoto, and K. Kobayashi, Relationship Between Surface Functional Properties and Flexibility of Proteins Detected by the Protease Susceptibility, *J. Agric. Food Chem. 33*:931–934 (1985).
7. Keshavarz, E., and S. Nakai, The Relationship Between Hydrophobicity and Interfacial Tension of Proteins, *Biochim. Biophys. Acta 576*:269–279 (1979).
8. Kato, A., and S. Nakai, Hydrophobicity Determined by a Fluorescence Probe Method and Its Correlation with Surface Properties of Proteins, *Ibid. 624*:13–20 (1980).
9. Nakai, S., Structure-Function Relationship of Food Proteins with an Emphasis on the Importance of Protein Hydrophobicity, *J. Agric. Food Chem. 31*:676 (1983).
10. Adler-Nissen, J., and U.S. Olsen, The Influence of Peptide Chain Length on Taste and Functional Properties of Enzymatically Modified Protein, in *Functionality and Protein Structure*, edited by A. Pour-El, ACS Symposium Series 92, American Chemical Society, Washington, DC, 1979.
11. Kinsella, J.E., and D.M. Whitehead, Emulsifying and Foaming Properties of Chemically Modified Proteins, in *Advances in Food Emulsions and Foams*, edited by E. Dickinson and G. Stainsby, Elsevier Applied Science, London, 1988, pp. 163–188.
12. Kim, S.H., and J.E. Kinsella, Surface Active Properties of Food Proteins: Effects of Reduction of Disulphide Bonds on Film Properties and Foam Stability of Glycinin, *J. Food Sci. 52*: 128–131 (1987).
13. Le Meste, M., B. Colas, D. Simatos, B. Closs, J.-L. Courthaudon, and D. Lorient, Contribution of Protein Flexibility to the Foaming Properties of Casein, *Ibid. 55*:1445–1447 (1990).
14. Matsudomi, N., T. Sasaki, A. Kato, and K. Kobayashi, Conformational Changes and Functional Properties of Acid-Modified Soy Protein, *J. Agric. Food Sci. 49*:1251–1256 (1985).
15. Baniel, A., D. Caer, B. Colas, and J. Guéguen, Functional Properties of Glycosylated Derivatives of the 12S Storage Protein from Pea (*Pisum sativum* L.), *J. Agric. Food Chem. 40*:200–205 (1992).
16. Wagner, J., and J. Guéguen, Effects of Dissociation, Deamidation, and Reducing Treatment on Structural and Surface Active Properties of Soy Glycinin, *Ibid. 43*:1993–2000 (1995).
17. Halpin, M.I., and T. Richardson, Selected Functionality Changes of β-Lactoglobulin upon Esterification of Side-Chain Carboxyl Groups, *J. Dairy Sci. 68*:3189–3198 (1985).
18. Franzen, K.L., and J.E. Kinsella, Functional Properties of Succinylated and Acetylated Soy Proteins, *J. Agric. Food Chem. 24*: 788–795 (1976).
19. Haque, Z., and M. Kito, Lipophilization of αs1-Casein. 1. Covalent Attachment of Palmitoyl Residue, *Ibid. 31*:1225–1230 (1983).
20. Guéguen, J., S. Bollecker, K.D. Schwenke, and B. Raab, Effect of Succinylation on Some Physicochemical and Functional Properties of the 12S Storage Protein from Rapeseed (*Brassica napus* L.), *Ibid. 38*:61–69 (1990).
21. Fulger, C., and J. Dewey, Preparation of Acid Soluble Acylated Protein from Oleaginous Proteins, U.S. Patent 4138500 (1979).
22. Molinero, J., M.R. Juliá, P. Erra, M. Robert, and M.R. Infante, Synthesis and Properties of *N*-Lauroyl-L-arginine Dipeptides from Collagen, *J. Am. Oil Chem. Soc. 65*:975–978 (1988).
23. Infante, M.R., J. Molinero, P. Bosch, M.R. Juliá, and P. Erra, Lipopeptidic Surfactants: I. Neutral *N*-Lauroyl-1-arginine Dipeptides from Pure Amino Acids, *Ibid. 66*:1835–1839 (1989).
24. Chobert, J.M., L. Briand, V. Grinberg, and T. Haertlé, Impact of Esterification on the Folding and Susceptibility to Peptic Proteolysis of β-Lactoglobulin, *Biochim. Biophys. Acta 1248*: 170–176 (1995).
25. Jones, J.D., Rapeseed Protein Concentrate Preparation and Evaluation, *J. Am. Oil Chem. Soc. 56*:716–719 (1979).
26. Gerbanowski, A., C. Rabiller, C. Larré, and J. Guéguen, Grafting of Aliphatic and Aromatic Probes on Bovine Serum Albumin: Influence on Its Structural and Physicochemical Characteristics, *J. Protein Chem. 18*:325–336 (1999).
27. Gerbanowski, A., C. Malabat, C. Rabiller, and J. Guéguen, Grafting of Aliphatic and Aromatic Probes on Rapeseed 2S and 12S Proteins: Influence on Their Structural and Physicochemical Properties, *J. Agric. Food Chem. 47*:5218–5226 (1999).
28. Bidlingmeyer, B.A., S.A. Cohen, and T.L. Tarvin, Rapid Analysis of Amino Acids Using Precolumn Derivatization, *J. Biochem. 336*:93–104 (1984).

29. Frister, H., H. Meisel, and E. Schlimme, OPA Method Modified by Use of *N,N*-Dimethyl-2-mercaptoethylammonium Chloride as Thiol Component, *Fresinius Z. Anal. Chem. 330*:631–633 (1988).
30. Schwenke, K.D., B. Raab, K.J. Linow, W. Pähtz, and J. Uhlig, Isolation of the 12S Globulin from Rapeseed (*Brassica napus* L.) and Characterization as a "Neutral" Protein. On Seed Proteins. Part 13, *Nahrung 25*:271–280 (1981).
31. Monsalve, R.I., and R. Rodriguez, Purification and Characterization of Proteins from the 2S Fraction from Seeds of the Brassiceae Family, *J. Exp. Bot. 41*:89–94 (1990).
32. Fains, A., D. Bertrand, A. Baniel, and Y. Popineau, Stability and Texture of Protein Foams: A Study by Video Image Analysis, *Food Hydrocolloids 11*:63–69 (1997).
33. Guéguen, J., Y. Popineau, I.N. Anisimova, R.J. Fido, P.R. Shewry, and A.S. Tatham, Functionality of the 2S Albumin Seed Storage Proteins from Sunflower (*Helianthus annuus* L.), *J. Agric. Food Chem. 44*:1184–1189 (1996).
34. Schwenke, K.D., B. Raab, P. Plietz, and G. Damaschun, The Structure of the 12S Globulin from Rapeseed (*Brassica napus* L.), *Nahrung 27*:165–175 (1983).
35. Schwenke, K.D., B. Drescher, D. Zirwer, and B. Raab, Structural Studies on the Native and Chemically Modified Low Molecular Mass Storage Proteins (napin) from Rapeseed (*Brassica napus* L.), *Biochem. Physiol. Pflanzen 183*:219–224 (1988).
36. Hsu, W.D., D.L. Vavak, L.S. Satterlee, and G.A. Miller, A Multienzyme Technique for Estimating Protein Digestibility, *J. Food Sci. 42*:1269–1273 (1977).
37. Caessens, P.W.J.R., H. Gruppen, C.J. Slangen, S. Visser, and A.G.J. Voragen, Functionality of β-Casein Peptides: Importance of Amphipathicity for Emulsion-Estabilizing Properties, *J. Agric. Food Chem. 47*:1856–1862 (1999).

38. Toledano, O., and S. Magdassi, Formation of Surface Active Gelatin by Covalent Attachment of Hydrophobic Chains, *J. Colloid Interface Sci. 193*:172–177 (1997).

[Received June 26, 2000; accepted November 8, 2000]