

Emulsifying and Foaming Properties of Gluten Hydrolysates with an Increasing Degree of Hydrolysis: Role of Soluble and Insoluble Fractions

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ABSTRACT

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Gluten solubility was improved by enzymatic proteolysis at moderate acidic pH level. Reversed-phase HPLC analysis of gluten hydrolysates with a degree of hydrolysis (DH) in the range of 0–5% showed that both hydrophilic and hydrophobic soluble peptides were released. Emulsifying and foaming properties of hydrolysate dispersions at 3.75 mg/mL decreased with the increasing DH at all pH levels and salt conditions investigated.

On the other hand, the soluble fractions separated from those hydrolysate dispersions exhibited good functional properties, independently of the initial DH. The proportion of hydrophilic and hydrophobic peptides in the soluble fractions depended on DH, pH level, and salt concentration. Nevertheless, these soluble fractions were characterized by an excellent capacity to stabilize both oil-water and air-water interfaces.

The solubility properties of proteins, which depend on intrinsic physicochemical characteristics and on the pH level and salt conditions of the medium, sometimes limit their use in formulated food systems (Panyam and Kilara 1996). The improvement of the functional properties of proteins by enzymatic or chemical modifications has been extensively studied (Hardwick and Glatz 1989, Hamada 1992, Guillerme et al 1993, Guéguen et al 1995, Popineau et al 1995, Chobert et al 1996).

Wheat gluten, a by-product of the wheat starch industry, is a typical water-insoluble protein. Much research focused on chemical or enzymatic modifications has resulted in the enhancement of its solubility, foaming, and emulsifying properties (Yang and McCalla 1968, Batey 1985, Bollecker et al 1990, Thébaudin 1990, Kato et al 1991, Mannheim and Cheryan 1992, Mimouni et al 1994, Babiker et al 1996). Adler-Nissen (1986) reported that extensive protein hydrolysis produced short peptides unable to form stable films at the oil-water or air-water interfaces. Thébaudin (1990) showed that limited hydrolysis of wheat proteins by various proteases improved solubility and that gluten hydrolysates with a degree of hydrolysis (DH) in the range of 1–2% exhibited good emulsifying and foam-forming properties. However, the foam-stabilizing properties of gluten hydrolysates were very limited (Thébaudin 1990, Manneheim and Cheryan 1992). Proteolysis of gluten by several enzymes (pepsin, neutrase, alcalase) confirmed these results (Mimouni et al 1994). According to this author, a pepsin hydrolysate with 1.25% DH exhibited a solubility of 80% but poor foam-stabilizing properties at pH 7. This showed that high solubility is not the only requirement to improve other functional properties. It is important to note that many studies were performed with total hydrolysates characterized by high solubility but only a few reported the effect of the insoluble fraction. Only Velev et al (1993) compared the emulsifying properties of tomato seed protein isolate with its water-soluble fraction and noted a positive effect of the insoluble fraction.

The objectives of this research were 1) to study the hydrolysis of gluten proteins with a commercial enzymatic preparation at moderate acidic pH level; 2) to determine the effect of the insoluble fraction on the emulsifying and foaming properties of the hydrolysates in relation to degree of hydrolysis; and 3) to identify the peptides adsorbed at the oil-water interface.

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MATERIALS AND METHODS

Materials

Gluten (75% [w/w] protein, dry basis) was provided by Amylum Belgium N.V. (Aalst, Belgium). The protease (Corolase PN.L, 220 UHb/g at pH 6.8) was supplied by Röhm Enzyme (Darmstadt, Germany).

Production of Gluten Hydrolysates

Four gluten hydrolysates with increasing degrees of hydrolysis (LS1, LS2, LS3, and LS4) were produced. The protease-to-substrate ratios were 1/500 for LS1 (0.44 UHb/g of gluten), 1/100 for LS2 and LS3 (2.2 UHb/g of gluten), and 1/50 for LS4 (4.4 UHb/g of gluten). Reaction time was 2 hr for LS1 and LS2, 6 hr for LS3 and LS4.

Dried gluten (40 g) was dispersed in 400 mL of 50 mM acetic acid and incubated in a water bath at 50°C for 10 min. When the gluten dispersion reached 50°C, the protease was added. The pH of the initial solution was 4.3 but it was not controlled during the enzymatic reaction. After digestion, the hydrolysate solution was introduced into Teflon acid digestion bombs, designed to retain working pressures up to 1,200 psi. The enzymatic reaction was stopped by microwave heating to reach and stay at 90–100°C by applying the power sequence: 1 min at 600W, 1 min at 400W, 2 min at 100W. The four hydrolysates were freeze-dried. Protein contents of gluten hydrolysates were determined by Kjeldahl procedures and were 75% ± 2.

Determination of Degree of Hydrolysis

The degree of hydrolysis of gluten hydrolysates was measured by the *o*-phthalaldehyde (OPA) method (Frister et al 1988). The gluten hydrolysate powder was solubilized at 1.25 mg/mL in 12.5 mM Na borate buffer, pH 8.5, 2% (w/v) SDS. A 50-μL portion of this solution was mixed with 1 mL of reagent (50 mL of 0.1M Na borate buffer, pH 9.3; 1.25 mL of 20% (w/v) SDS solution; 100 mg of N,N-dimethyl-2-mercaptoethylammonium chloride [DMMAC]; and 40 mg of OPA dissolved in 1 mL of methanol). The mixture was allowed to stand for 2 min before measuring the absorbance at 340 nm. The number of amino groups was determined with reference to the L-leucine standard curve (0.5–5 mM). The increase in amino groups between native gluten and hydrolysates was attributed to proteolysis, and the degree of hydrolysis was calculated by the equation:

$$\text{DH (\%)} = [(\alpha - n_i)/n_T] \times 100 \quad (1)$$

where n_T is the total number of amino groups in native gluten after total hydrolysis, n_i is the number of amino groups in native gluten, and α is the number of free amino groups measured in the gluten hydrolysate. The degree of hydrolysis expressed for each sample was the mean of six determinations.

Size-Exclusion HPLC

The SE-HPLC was performed on a Kontron system and a Pharmacia HR (10/30) column packed with Superose 6 Prep Grad gel was used. The eluant was 12.5 mM Na borate buffer, pH 8.5, 0.1% (w/v) SDS, and the flow rate was 0.3 mL/min. The eluant buffer was filtered through a 0.2- μ m Millipore membrane and degassed under vacuum before use. Gluten and gluten hydrolysates were dispersed (1 mg/mL) in the same buffer containing 2% SDS and were sonicated at 6W for 30 sec (Microson ultrasonic cell disruptor) to ensure complete solubilization. Samples were then centrifuged at $8,800 \times g$ for 15 min and an aliquot (100 μ L) was loaded onto the column. The protein and polypeptides in the column effluent were monitored by absorbance at 220 nm. Chromatograms were divided into five zones: P1 > 500,000; excluded peak; 500,000 > P2 > 75,000; 75,000 > P3 > 25,000; 25,000 > P4 > 15,000; and P5 < 15,000. The area of each zone was calculated. All SE-HPLC elution profiles were duplicated.

Reversed-Phase HPLC

The RP-HPLC was performed on a Kontron system and a C₁₈ column (Nucleosil 300 – 5, pore diameter 300Å, particle size 5 μ m, 250 \times 4 mm) from Macherey-Nagel (Hoerd, France). Elution was achieved at 1 mL/min by a gradient formed from solvent A (0.1% [v/v] TFA in water) and B (0.08% [v/v] TFA, 80% [v/v] acetonitrile in water): 5% B = 4.5 min, 5–75% B > 58 mins, 75–100% B > 1 min. Gluten hydrolysates were solubilized at 2.5 mg/mL in solvent A and were centrifuged at $8,800 \times g$ for 15 min before injecting an aliquot (80 μ L) onto the column. The column effluents were monitored at 220 nm. Chromatograms were divided into four zones: 20 min < M1 < 35 min ; 35 min < M2 < 40 min; 40 min < M3 < 45 min ; 45 min < M4 < 55 min and integrated. All RP-HPLC elution profiles were duplicated.

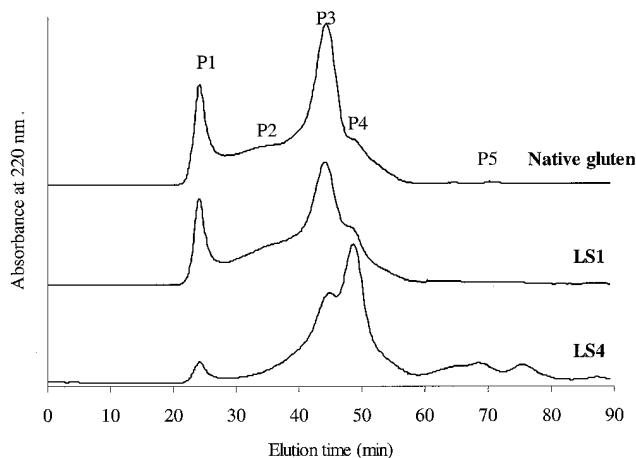


Fig. 1. Size-exclusion HPLC elution profiles of native gluten and gluten hydrolysates LS1 and LS4. Samples solubilized at 1 mg/mL in 12.5 mM Na borate buffer, pH 8.5, 2% SDS. P1–5 = molecular size distribution zones.

Solubility of Gluten Hydrolysates

Functionality was studied at pH 4 and 6.5 with 0.2 or 2% of added salt (NaCl) to cover a wide range of food system conditions. Solubility of gluten hydrolysates was determined by analyzing the soluble fraction by SE-HPLC. Gluten hydrolysates were suspended in 50 mM acetic acid (pH 4) or 0.1 mM acetic acid (pH 6.5) overnight. Before adjusting to the desired pH level with NaOH, NaCl was added to obtain 0.2 or 2% (w/v). The final sample concentration was 2 mg/mL (w/v). After pH adjustment, samples were centrifuged at $12,500 \times g$ for 15 min to separate the insoluble fraction. The supernatant was diluted (v/v) in 25 mM Na borate buffer, pH 8.5, 4% (w/v) SDS, and was centrifuged at $8,800 \times g$ for 10 min before injecting an aliquot (100 μ L) onto the SE-HPLC column (Superose 6). The total area of the chromatogram was calculated, and the hydrolysate solubility was expressed as the ratio of the area obtained for the supernatant to that obtained for the reference sample (solubilized in 12.5 mM Na borate buffer, pH 8.5, 2% [w/v] SDS). The solubility expressed for each sample was the mean of two measurements.

The hydrophobicity profile of the soluble fractions at all pH levels and salt conditions was obtained using aliquots of the supernatant described previously but without dilution on SDS buffer. Chromatograms obtained on the C₁₈ column were analyzed as described previously.

Emulsifying Properties

Droplet-size distribution. Emulsions were formed by mixing together 15 mL of aqueous solution and 9 mL of purified *n*-hexadecane (volume fraction $\phi = 0.375$) using a Polytron PT3000 homogenizer (30 sec at 20,000 rpm). The aqueous solution was

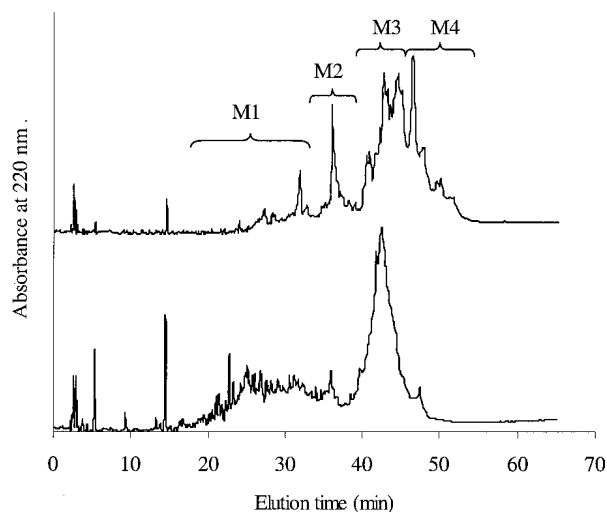


Fig. 2. Reversed-phase HPLC elution profiles of gluten hydrolysates LS1 and LS4. Samples solubilized at 2.5 mg/mL in solvent A (0.1% [v/v] trifluoroacetic acid in water). M1–4 = hydrophobicity profiles of eluted peptides.

TABLE I
Biochemical Characteristics of Total Gluten Hydrolysates Integrating Size-Exclusion HPLC and Reverse-Phase HPLC Elution Profiles^a

	SE-HPLC Elution Profiles					RP-HPLC Elution Profiles			
	P1 (%)	P2 (%)	P3 (%)	P4 (%)	P5 (%)	M1 (%)	M2 (%)	M3 (%)	M4 (%)
Native gluten	16.4	23.7	46.8	13.1	0	nd ^b	nd	nd	nd
Hydrolysates									
LS1 0.5%	15.1	21.0	45.2	16.4	2.3	12.6	14.0	44.1	29.4
LS2 1.4%	8.7	18.3	37.8	30.3	5.0	22.9	12.2	47.1	17.9
LS3 2.8%	5.9	16.2	31.2	37.7	9.2	29.5	10.7	48.4	11.5
LS4 4.7%	4.1	8.7	27.1	44.1	16.2	36.8	9.5	45.9	8.0

^a P1–5 = molecular size distribution zones; M1–4 = hydrophobicity profiles of eluted peptides. Each population (%) determined by the ratio of the area of each peak to the total area. Measurements were performed in duplicate.

^b Impossible to determine in the same conditions as for gluten hydrolysates.

composed of LS1 or LS4 dispersions at 3.75 mg/mL or of the soluble fractions. Just after emulsification, 2 mL of creamed phase was dispersed in 25 mL of 50 mM Tris-HCl, pH 8, 1% SDS buffer, to stop the emulsion destabilization (Agboola et al 1998, Caessens et al 1999). Distilled water was used to disperse the diluted creamed phase. The droplet-size distribution was measured using the Mastersizer IP laser particle sizer (Malvern Instruments Ltd.), with optical parameters defined by the manufacturer's presentation code 0403, and a 100-mm lens (reverse Fourier optics, 0.5–180 μm diameter range). Measurements were made on four replicates and results were expressed as average size of emulsion droplets [d(4.3)].

Flocculation-creaming kinetics. The creaming of the emulsion was followed by a conductivity method (Sarker et al 1999). For each measurement, 25 mL of hydrolysate solution and 15 mL of purified *n*-hexadecane ($\phi = 0.375$) were mixed in a glass tube in which rod-like electrodes were located at the bottom, using the same Polytron apparatus as for droplet size measurement (30 sec at 20,000 rpm). The average phase volume (ϕ_{av}) of the emulsion in the top 15 mL was calculated as :

$$\phi = 1 - \{(A/B) \times [1 - (C_t/C_{sol})]\} \quad (2)$$

where *A* is the volume of the aqueous phase (25 mL of hydrolysate solution), *B* is the volume of the apolar phase (15 mL of purified hexadecane), C_{sol} is the conductivity of the buffered test solution before emulsification, and C_t is the emulsion conductivity at time *t*. The emulsification decreased the conductivity to a minimum corresponding to an oil volume fraction of 0.375 in emulsion, and the creaming of droplets was related to the conductivity increase as a function of time (during 5 hr). The emulsions were compared on the basis of 1) the time to increase the oil volume fraction in the creamed phase to 0.1 ($\Delta t_{0.1}$ in min) which is related to the initial speed of creaming; 2) the oil volume fraction in the creamed phase 5 hr after emulsification (ϕ_5); and 3) the oil volume fraction in the creamed phase 20 hr after emulsification (ϕ_{eq}).

Resistance to coalescence. The quantity of released oil after several centrifugation cycles applied to the emulsions was determined. Emulsions were formed as for droplet-size measurements. Emulsions were then centrifuged at $500 \times g$ for 10 min (three cycles), then at $2,000 \times g$ for 10 min (three cycles), and at $8,000 \times g$ for 10 min (three cycles). After each centrifugation cycle, the *n*-hexadecane layer that appeared on the top of the samples was weighed. The percentage of released oil (expressed as: [released oil weight/weight of 9 mL of oil] $\times 100$) was followed as a function of the cumulative acceleration ($G = g \times \text{min}$).

Three types of emulsion were prepared from each gluten hydrolysate: 1) emulsions stabilized with hydrolysate dispersions at 3.75

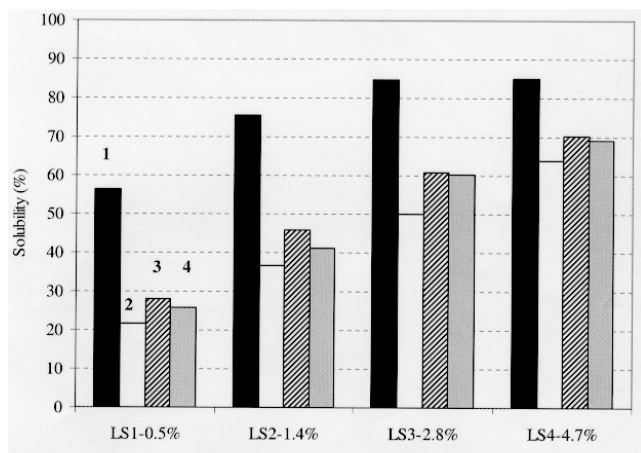


Fig. 3. Influence of degree of hydrolysis on solubility of gluten hydrolysates LS1–4 at different pH levels and salt concentrations: 1) pH 4 and 0.2% NaCl; 2) pH 4 and 2% NaCl; 3) pH 6.5 and 0.2% NaCl; 4) pH 6.5 and 2% NaCl.

mg/mL; 2) emulsions containing only the soluble peptides: the hydrolysate dispersion was centrifuged at $12,500 \times g$ for 15 min to eliminate the insoluble fraction before forming the emulsion (the protein concentration depended on the solubility of the gluten hydrolysates); 3) emulsions where the concentration of the soluble peptides was adjusted to 1 mg/mL. Emulsifying properties were measured in 50 mM acetic acid adjusted to pH 4 with 0.2 or 2% NaCl, or 0.1 mM acetic acid adjusted to pH 6.5 with 0.2 or 2% NaCl. All reported values are the mean of two duplicates which were very close to each other.

Foaming Properties

An original apparatus for foam analysis, based on conductimetric measurement of the foaming solution, coupled with an optical detection of foam volume was used (Loisel 1992, Baniel et al 1997, Fains et al 1997). The foam was generated in a transparent plastic column (20 cm height, 3 cm diam) by sparging air into 8 mL of sample solution through a porous metal disk (2 μm diam) at a rate of 15 mL/min. When the level of foam reached 35 mL, the bubbling was automatically stopped. A constant pressure was applied under the porous disk to avoid liquid flow back through the disk. The formation and drainage were analyzed for 20 min by measuring the conductivity as a function of time (C_t). The volume of liquid entrapped in the foam (V_L) was calculated (Sarker et al 1998):

$$V_L = V_{init} \times [1 - (C_t/C_{init})] \quad (3)$$

where V_{init} is the volume of sample solution (8 mL) introduced into the apparatus and C_{init} the conductivity of the initial protein solution. Foams were compared on the basis of 1) maximal liquid incorporated into the foam at the end of the sparging period (L_{max}); 2) half drainage times ($t_{1/2}$, in minutes) which is the time taken to drain half of the maximal liquid incorporated; and 3) drainage rate ($vd_{1/2}$, in mL/min) which is related to $t_{1/2}$. The maximal liquid

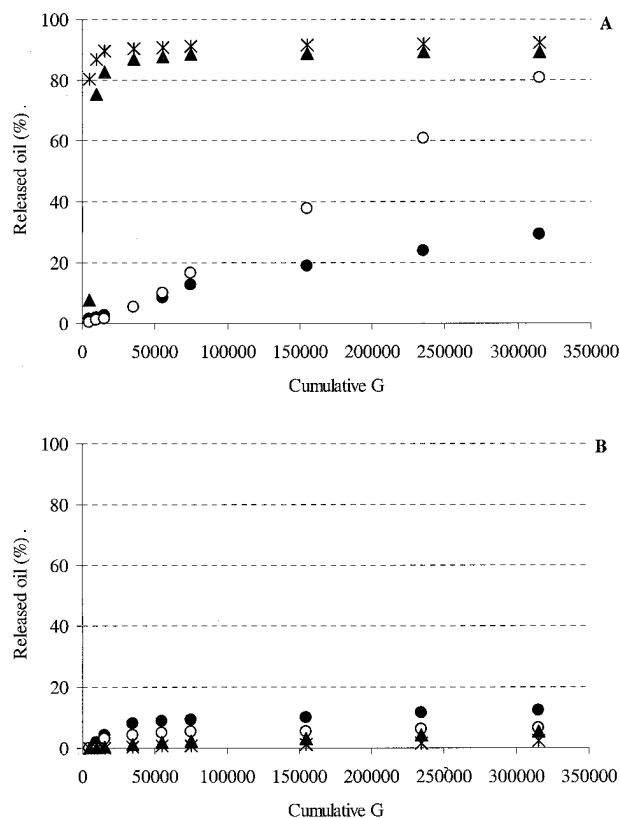


Fig. 4. Resistance to coalescence of emulsions stabilized with hydrolysate dispersions LS1 (●), LS2 (○), LS3 (▲), and LS4 (*) at pH 4 and 2% NaCl. A, 3.75 mg/mL; B, soluble fractions.

incorporated into the foam is related to the foaming capacity of the sample. The half drainage time and the drainage rate give information about the stability of the foam.

As for emulsifying properties, the same three foam types were studied for each gluten hydrolysate. All foam analyses were conducted in 0.1 mM acetic acid with 0.2 or 2% NaCl adjusted to pH 6.5. All reported values are the mean of two duplicates which were very close to each other.

Statistical Analysis

Determination of solubility, flocculation-creaming kinetics, resistance to coalescence, and foaming properties were made in duplicate. Consequently, a variance analysis was performed on each experiment to determine the effect of DH, pH level, salt concentration, and insoluble fraction at the 99% confidence level.

RESULTS AND DISCUSSION

Changes in Biochemical Properties by Enzymatic Modification of Gluten

Degree of hydrolysis. Determination of amino groups and molecular size by SE-HPLC (results not shown) enabled conditions to be selected for the production of four gluten hydrolysates with increasing degrees of hydrolysis in the range of 0–5%. The degree of hydrolysis (DH) of LS1, LS2, LS3, and LS4 was 0.5% ± 0.1, 1.4% ± 0.2, 2.8% ± 0.2, and 4.7% ± 0.1, respectively.

Molecular size distribution. The molecular size distribution of native gluten, LS1 and LS4, is reported in Fig. 1 and was divided into five zones: P1, high M_r glutenin polymers; P2, low M_r glutenin polymers; P3, gliadins; P4, peptides with M_r 25,000 to 15,000; and P5, peptides with M_r <15,000. The increase in DH induced a shift of the major population toward lower molecular size and peptides with a M_r <15,000 appeared (Table I). The

decrease in molecular size as a function of DH showed that glutenin polymers, although highly aggregated under the reaction conditions, were sensitive to proteolysis. These results are in agreement with those of Mimouni et al (1994).

Hydrophobicity profile. Elution profiles of the four gluten hydrolysates can be grouped into four categories according to the increasing hydrophobicity of the eluted peptides (M1–M4). (Fig. 2, Table I). The hydrophobic-to-hydrophilic peptide ratio, expressed as (M3+M4)/(M1+M2), decreased when the degree of hydrolysis increased and was 2.8, 1.9, 1.5, and 1.2 for LS1, LS2, LS3, and LS4, respectively. Amounts of the most hydrophobic peptides (M4) were reduced by the prolongation of proteolysis, inducing a decrease in the apparent peptide hydrophobicity. Nevertheless, the percentage of M3 peptides remained constant during proteolysis.

Solubility of Gluten Hydrolysates as a Function of pH Level and Salt Concentration

Solubility level. Solubility of gluten hydrolysates as a function of pH level and salt concentration is presented in Fig. 3. The cleavage of only a few covalent bonds greatly enhanced the solubility at all pH levels and salt concentrations, and the increase in DH also improved the solubility of hydrolysates ($P < 0.01$). At pH 4, the increase in salt concentration had a noticeable effect on the solubility of the least hydrolyzed sample, whereas at pH 6.5, the solubility of each sample was not influenced by salt concentration at all ($P < 0.01$).

RP-HPLC analysis of soluble fractions from hydrolysate dispersions at 3.75 mg/mL. The comparison of the hydrolysates and their soluble fractions showed that, independent of the hydrolysis level, all peptides were very soluble at pH 4 with 0.2% NaCl. The increase in salt concentration greatly decreased the solubility of the hydrophobic peptides (M3 and M4), but the increase in hydrolysis improved their solubility. At pH 4 with 2% NaCl, only

TABLE II
Emulsifying Properties of Hydrolysate Dispersions at 3.75 mg/mL and of Soluble Fractions Separated from Hydrolysate Dispersions^a

	pH 4, 0.2% NaCl			pH 4, 2% NaCl			pH 6.5, 0.2% NaCl			pH 6.5, 2% NaCl		
	$\Delta t_{0.1}$ (min)	ϕ_5	ϕ_{eq}	$\Delta t_{0.1}$ (min)	ϕ_5	ϕ_{eq}	$\Delta t_{0.1}$ (min)	ϕ_5	ϕ_{eq}	$\Delta t_{0.1}$ (min)	ϕ_5	ϕ_{eq}
Hydrolysate dispersions												
LS1	20.4	0.70	0.70	11.2	0.67	0.67	13.8	0.68	0.68	12.1	0.72	0.75
LS2	11.7	0.71	0.72	15.7	0.68	0.68	16.7	0.72	0.72	14.6	0.75	0.88
LS3	9.8	0.78	0.84	18.1	0.70	0.80	19.6	0.72	0.74	16.1	0.82	0.95
LS4	8.5	0.83	0.91	16.3	0.81	0.95	19.1	0.85	0.94	10.2	0.99	1
Soluble fractions												
LS1	5.4	0.71	0.71	3.8	0.74	0.75	5.5	0.77	0.77	6.0	0.76	0.76
LS2	12.5	0.70	0.71	6.0	0.74	0.75	5.8	0.75	0.74	12.8	0.75	0.75
LS3	12.9	0.70	0.71	12.3	0.69	0.69	14.7	0.74	0.74	13.1	0.76	0.76
LS4	13.9	0.72	0.71	10.8	0.75	0.75	18.5	0.75	0.75	13.5	0.78	0.78

^a Flocculation-creaming kinetics at various pH levels and salt concentrations. Measurements performed in duplicate. $\Delta t_{0.1}$ = initial speed of creaming; ϕ_5 = oil volume fraction 5 hr after emulsification; ϕ_{eq} = oil volume fraction 20 hr after emulsification.

TABLE III
Foaming Properties of Hydrolysate Dispersions at 3.75 mg/mL and of Soluble Fractions Separated from Hydrolysate Dispersions^a

	pH 6.5, 0.2% NaCl			pH 6.5, 2% NaCl		
	L_{max} (mL)	$t_{1/2}$ (min)	$V_{d1/2}$ (mL/min)	L_{max} (mL)	$t_{1/2}$ (min)	$V_{d1/2}$ (mL/min)
Casein ^b	5.8	3.4	0.86	6.9	5.2	0.67
Hydrolysate dispersions						
LS1	4.4	2.4	0.96	5.7	3.6	0.80
LS2	5.2	2.8	0.95	5.8	4.0	0.73
LS3	5.6	2.7	1.07	6.0	3.0	0.99
LS4	5.9	1.9	1.61	6.0	2.0	1.47
Soluble fractions						
LS1	3.7	2.1	0.89	6.2	5.2	0.60
LS2	5.4	4.1	0.65	6.6	4.8	0.69
LS3	5.7	4.6	0.62	6.7	5.4	0.63
LS4	6.3	5.0	0.62	6.8	5.4	0.62

^a Measurements performed in duplicate. L_{max} = maximal liquid incorporated into foam; $t_{1/2}$ = half drainage time; $V_{d1/2}$ = drainage rate.

^b Casein concentration 5 mg/mL (w/w) in dry matter, corresponding to 4.5 mg/mL of protein concentration (protein content 90%).

8.2% of those hydrophobic peptides present in LS1 were solubilized, whereas 50.4% of those peptides were solubilized for LS4. The same observation was made at pH 6.5 with 0.2 or 2% NaCl.

The increase in gluten solubility by Corolase hydrolysis is in agreement with results obtained with different proteases (Yang and McCalla 1968, Batey 1985, Thébaudin 1990, Mimouni et al 1994, Chobert et al 1996). Moreover, the selected protease released soluble hydrophobic peptides that tend to exhibit surface active properties (Nakai 1983, Panyam and Kilara 1996). According to SE-HPLC profiles, most of the peptides had M_r 15,000–25,000, even for the gluten hydrolysate with 4.7% DH.

Emulsifying Properties of Hydrolysate Dispersions and Effect of Insoluble Fractions

Droplet-size distribution. The droplet size of emulsions formed with LS1 and LS4 were measured at pH 6.5 with 2% NaCl. The droplet-size distributions of emulsions stabilized with hydrolysate dispersions at 3.75 mg/mL or with their soluble fractions were monomodal with an average droplet size of $20 \mu\text{m} \pm 3$ showing that the insoluble fraction had no influence on the emulsion-forming ability of gluten hydrolysates.

Flocculation-creaming properties. The results of flocculation-creaming kinetics are reported in Table II. They give information about the evolution of the resting emulsion. Statistical analysis of the values of $\Delta t_{0.1}$ showed no effect of the DH on the initial rate of creaming ($P > 0.25$) although some large variations were observed at pH 4 with 0.2% NaCl. Under all conditions, the increase of DH had a negative effect on the creaming level at equilibrium (ϕ_{eq}) of emulsions prepared with hydrolysate dispersions at 3.75 mg/mL ($P < 0.01$). For the least hydrolyzed sample, creaming reached a maximum 5 hr after emulsification, whereas the process continued beyond this time for the most hydrolyzed sample (LS4).

The increase in pH level or salt concentration increased the creaming level at equilibrium ($P < 0.01$). As observed at pH 6.5, the creaming level increased when salt was added, even though the solubility did not change ($P < 0.01$). This showed that solubility was a necessary but not a sufficient condition for peptides to stabilize emulsions. The net charge of the protein and consequently of the adsorbed protein layer is dependent on pH level. At pH 6.5, which is close to the isoelectric point of gluten, the ionic

strength had a greater influence on their emulsifying properties.

The study of the flocculation-creaming properties of the soluble fractions separated from hydrolysate dispersions at 3.75 mg/mL gave information on the contribution of the insoluble fraction to the emulsion stability (Table II). For the least hydrolyzed sample (LS1), the removal of insoluble fractions induced an increase in the initial rate of creaming ($P < 0.01$) but had no significant effect on volume fraction at equilibrium ($P > 0.01$). For the most hydrolyzed sample, the insoluble fraction had almost no influence on the initial rate of creaming ($P > 0.01$) but its removal had a significant effect on the long-term stability ($P < 0.01$); the values of ϕ_{eq} were lowered when emulsions were prepared with the soluble fractions of LS4.

Resistance to coalescence. Resistance to coalescence of emulsions is indicative of the mechanical strength of the interfacial film of emulsions. Statistical analysis was done for two values of cumulative G : after the third cycle of centrifugation which corresponds to $15,000 \times g \times \text{min}$ and at the end of the experiment which corresponds to $315,000 \times g \times \text{min}$. As pH level and salt conditions had no significant effect on the percentage of released oil, only one combination of pH level and salt condition is reported here (Fig. 4). Among the four hydrolysates tested, LS1 had some weak stabilizing properties but the increase in DH induced a noticeable decrease in the resistance to coalescence of emulsions ($P < 0.01$). The removal of insoluble fractions greatly improved the resistance of all the emulsions ($P < 0.01$). In all conditions, the soluble peptides were tightly adsorbed at the oil-water interface and were in sufficient quantity to stabilize the emulsion. The aggregated fraction in hydrolysate dispersions could interact with the soluble peptides and weaken the film resistance by modifying the interfacial organization.

Foaming Properties of Hydrolysate Dispersions and Effect of Insoluble Fractions

The foam-forming and foam-stabilizing properties of the gluten hydrolysates were studied at pH 6.5 with 0.2 or 2% NaCl (Table III) and a commercial casein was taken as reference because it produced stable foams (Guillerme et al 1993, Rodríguez Patino et al 1995, Fains et al 1997). These properties were not investigated at pH 4 because acidic food foams are not common.

TABLE IV
Composition of Soluble Fractions (1 mg/mL) and Adsorbed Peptides at the Oil-Water Interface^a

	Soluble Fractions (%) ^b				Adsorbed Peptides (%) ^c			
	1	2	3	4	1	2	3	4
LS1								
M1	15.6	39.5	25.7	31.4	0	0	0	0
M2	14.7	28.4	23.9	26.5	3.6	38.7	23.1	33.2
M3	47.2	28.2	38.7	35.5	60.3	54.7	57.5	57.4
M4	24.7	3.8	11.7	6.6	36.1	6.6	19.4	9.4
LS2								
M1	23.6	47.2	42.2	39.7	0	0	0	0
M2	13.7	19.3	13.9	18.0	4.6	28.4	20.4	19.6
M3	46.0	29.1	37.1	36.9	68.5	62.3	69.1	71.7
M4	16.7	4.4	6.7	5.4	26.9	9.3	10.5	8.7
LS3								
M1	29.0	45.8	41.4	37.9	0	0	0	0
M2	12.1	15.9	12.2	15.1	4.4	20.4	15.9	16.7
M3	46.2	34.2	39.8	41.8	74.7	71.4	74.2	75.4
M4	12.7	4.1	6.6	5.2	20.9	8.2	10.0	7.9
LS4								
M1	34.7	44.7	44.5	39.7	0	0	0	0
M2	11.5	13.3	10.5	12.0	4.0	12.5	9.7	10.3
M3	45.3	38.1	39.0	42.5	81.0	79.6	80.0	80.3
M4	8.5	4.0	5.9	5.7	15.0	7.9	10.3	9.4

^a Determined by RP-HPLC analysis. 1) pH 4, 0.2% NaCl; 2) pH 4, 2% NaCl; 3) pH 6.5, 0.2% NaCl; 4) pH 6.5, 2% NaCl. Each group of peptides (%) was determined by the ratio of the area of each peak to the total area. LS1–4 = gluten hydrolysates; M1–4 = hydrophobicity profiles of eluted peptides.

^b Mean area of elution profiles of soluble fractions adjusted to the same concentration $322 \text{ mV} \times \text{min} \pm 33$.

^c Calculated as difference between the elution profiles of soluble fractions and the aqueous phase of emulsions 20 hr after emulsification.

With 0.2% NaCl, the liquid incorporated into the foam (L_{\max}), which gives information about the foam-forming properties of samples, increased with DH ($P < 0.01$). An increase in the salt concentration induced a larger increase in L_{\max} for the least hydrolyzed sample (LS1) than for the others ($P < 0.01$). In all cases, the values obtained for L_{\max} reflected good foam-forming properties. The foam ageing was observed through liquid drainage. The increase in DH induced a significant decrease in the half drainage time and consequently an increase in the drainage rate ($P < 0.01$). The foam-stabilizing properties of gluten hydrolysate dispersions were very limited. Our results obtained on the foaming properties of hydrolysate dispersions are in agreement with other studies (Mannehein and Cheryan 1992, Mimouni et al 1994, Chobert et al 1996).

Statistical analysis performed on the characteristics of foams formed with LS1 showed that the insoluble fraction had no significant effect on foaming ability and foam-stabilizing properties. On the other hand, with the most hydrolyzed sample (LS4), the removal of the insoluble fraction induced a noticeable improvement in foaming ability, especially with 2% NaCl, and in the foam stability ($P < 0.01$). The characteristics of foams formed with soluble fractions as a whole were close to those of foams made with caseins (Table III).

Functional Properties of Soluble Fractions

To avoid any difference due to the quantity of soluble peptides as a function of DH, pH level, and salt concentration, emulsifying and foaming properties of soluble fractions were compared at the same concentration (1 mg/mL).

Emulsifying properties. The initial rate of creaming ($\Delta t_{0,1}$) obtained for soluble fractions was not influenced by DH ($P > 0.50$) but was dependent on pH level and salt concentration ($P < 0.01$). The values of $\Delta t_{0,1}$ were longer at pH 4 with 0.2% NaCl (12.5–13.9 min) than for other conditions (3.2–7.3 min). For all emulsions, creaming reached a plateau 5 hr after emulsification but the creaming level at equilibrium was slightly influenced by pH level and salt concentration as a function of the soluble fraction used. At pH 4 with 0.2% NaCl, the values of ϕ_{eq} were 0.68 with soluble fractions of LS1 and reached 0.75 with soluble fractions of LS4. For other conditions, differences were very small and not related to DH (the values of ϕ_{eq} were 0.71–0.75). These observations could be related to the proportion of hydrophobic peptides in soluble hydrolysates as a function of pH level and salt concentration. The higher proportion of hydrophobic peptides at pH 4 with 0.2% NaCl than for other conditions seemed to limit the creaming process.

When coalescence experiments were performed on those soluble fractions at 1 mg/mL, the final quantity of released oil was <12.9%. All emulsions were strongly stabilized without any effect of pH level, salt concentration, or the soluble fraction used ($P > 0.01$).

Analysis of soluble fractions and nonadsorbed peptides at the oil-water interface by RP-HPLC. To determine the adsorbed peptides at the oil-water interface, the composition of the initial soluble fractions at 1 mg/mL and the aqueous phase of emulsions 20 hr after emulsification were compared by RP-HPLC as for the total hydrolysates (Table IV). The elution profiles were integrated in four populations as for the elution profiles of total gluten hydrolysates. The higher the DH, the less the composition of soluble fraction was influenced by pH level and salt concentration. This can be related to the solubility of the hydrophobic peptides as a function of DH.

Independent of the pH level and salt conditions, $51\% \pm 6$ of material was adsorbed in the creamed phase. Of this material, $90\% \pm 7$ of the more hydrophobic peptides (M3 and M4) were adsorbed, and the more hydrophilic peptides (M1) remained in the aqueous phase in all studied conditions. Moreover, a negative correlation was found between the amount of M3 and M4 peptides in the soluble fraction and the percentage of M2 peptides present

in the creamed phase (Fig. 5). When the more hydrophobic peptides (M3 and M4) were present in sufficient quantity to stabilize the oil-water interface, the M2 peptides were not adsorbed. The decrease in the quantity of hydrophobic peptides in soluble fractions induced an increase in the adsorption of peptides defined by M2 to cover the oil-water interface.

Foaming properties. The analysis of the foaming properties of soluble fractions adjusted to 1 mg/mL showed that at one salt concentration the foam-forming and foam-stabilizing properties of the different soluble fractions were equivalent ($P > 0.01$), even though the biochemical characteristics varied with the DH. On the other hand, the salt concentration influenced the quantity of incorporated liquid and the rate of drainage ($P < 0.01$). With 0.2% NaCl, L_{\max} values were 4.1–4.6 mL; the increase in salt concentration improved L_{\max} up to 6.0 mL. On the other hand, the rate of drainage was slightly slower with 0.2% NaCl (0.50–0.59 mL/min) than with 2% NaCl (0.61–0.67 mL/min). However, all foams were stable, and these results show the great ability of the soluble fractions to stabilize the air-water interface.

CONCLUSIONS

Functional hydrolysates were produced from wheat gluten by limited enzymatic hydrolysis at acid pH levels. The selected protease released soluble hydrophobic peptides. Moreover, a majority of peptides had M_r 15,000–25,000 when DH was <5%.

Emulsifying and foaming properties of hydrolysate dispersions were in agreement with all published results. Increased DH induced a decrease in the emulsion- and foam-stabilizing properties. However, this study shows the important negative effect of the insoluble fraction even if this accounts for <20% of total hydrolysate. Whatever the degree of hydrolysis, the soluble fractions of hydrolysates exhibited a strong capacity to stabilize oil-water and air-water interfaces at all pH levels and salt conditions. Moreover, the analysis of adsorbed peptides at the oil-water interface showed the capacity of the less hydrophobic soluble peptides to stabilize emulsions when the more hydrophobic peptides were not present in sufficient quantity to cover the interface.

Interest was focused on LS4 which was characterized by a solubility >64% for all pH levels and salt conditions. This sample exhibited the highest proportion of soluble peptides with surface active properties. This point should be exploited to produce soluble peptides with good emulsifying and foaming properties.

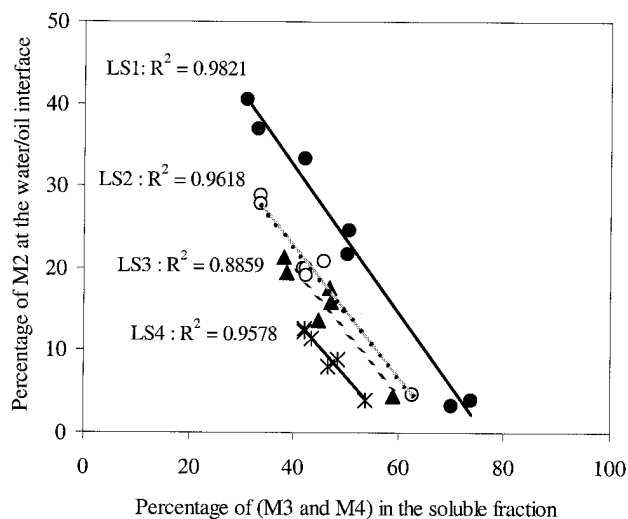


Fig. 5. Correlation between % of (M3 + M4) peptides of soluble fractions and % of M2 peptides recovered in creamed phase. Hydrolysate dispersions: LS1 (●), LS2 (○), LS3 (▲), LS4 (*). Concentration of peptide solution 1 mg/mL.

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