Interfacial and Emulsifying Properties of Whey Peptide Fractions Obtained with a Two-Step Ultrafiltration Process

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Interfacial and emulsifying properties of whey protein concentrate (WPC), heat-treated WPC (90 °C, pH 2.5, 10 min), and their tryptic or chymotryptic peptide fractions obtained by ultrafiltration were measured in conditions similar to a salad dressing emulsion (pH 4.0; $\mu = 0.6$). The rate of adsorption of protein components at the air/water interface as well as emulsifying capacity (EC) was determined. The type of enzyme and UF separation of small components from the hydrolysates were significant treatments for both parameters studied. A correlation was found between the square root of the rate of adsorption and EC ($r = 0.98$, $P < 0.0001$). A combination of heat treatment, tryptic hydrolysis, and UF separation of small components from the hydrolysate gave a fraction having better interfacial properties than WPC, which may lead to improved emulsifying properties for food applications.

INTRODUCTION

Whey proteins are utilized extensively in specialty products because of their outstanding nutritional quality (Maubois and Brulé, 1982; McDermott, 1987). Although whey protein concentrates have good solubility characteristics, their ability to stabilize emulsions and foams is poor (Morr, 1979) and may be a limiting factor for their use as food ingredients.

To modify functional properties of whey protein concentrates, heat treatments and enzymatic hydrolysis have been used (Vuillemard et al., 1989). Heat treatment under acidic conditions (Modler and Harwalkar, 1981; Paquet et al., 1978) caused the partial denaturation of whey proteins with little aggregation and resulted in improvement of the foaming properties (Paquet et al., 1978, 1979).

Enzymatic hydrolysis of proteins produced peptides with smaller molecular sizes and less secondary structure than proteins. Thus, functional properties of hydrolysates are different: increased solubility over pH range, decreased viscosity, and significant changes in the foaming, gelling, and emulsifying properties (Fox et al., 1982; Adler-Nissen, 1986). For the improvement of emulsifying properties by enzymatic hydrolysis, hydrophobicity (Chaplin and Andrews, 1989) and large molecular weight (Jost and Monti, 1982; Lee et al., 1987; Adler-Nissen and Olsen, 1979) of the resulting peptides are reported to be important factors. These factors may be controlled by different ways. The use of highly specific proteases may have an impact on the hydrophobicity and on the size of peptides (Whitaker and Puigserver, 1982; Chobert et al., 1988). Also, larger peptides can be produced by limiting the degree of protein hydrolysis (Adler-Nissen and Olsen, 1979) or by separation of hydrolysates using an ultrafiltration (UF) reactor system (Deeslie and Cheryan, 1988).

Whey peptide fractions with different ranges of molecular weight were previously produced (Turgeon and Gauthier, 1990) by combined effects of (1) heat treatment under acidic conditions, (2) enzymatic hydrolysis, and (3) UF separation of hydrolysates. The objective of the present investigation was the evaluation of the interfacial and emulsifying properties of those whey peptide fractions in conditions (pH 4.0, $\mu = 0.6$) similar to a salad dressing emulsion (Inglett and Inglett, 1982).

MATERIALS AND METHODS

Materials. Commercial whey protein concentrate (35% w/w protein) was obtained from a cheese plant (Saputo, St-Hyacinthe, Québec). Trypsin (bovine pancreas, type III-S) and α-chymotrypsin (bovine pancreas, type II) were purchased from Sigma Chemical Co. (St. Louis, MO). All other products were of reagent grade.

Preparation of Whey Peptide Fractions. Peptide fractions were obtained by ultrafiltration of tryptic or chymotryptic hydrolysates of whey protein concentrate (WPC) or heat-treated WPC (H-WPC; 90 °C, pH 2.5, 10 min) as described by Turgeon and Gauthier (1990). The substrate was suspended in distilled water (10% w/w, pH 8.0, 40 °C), and the enzyme solution (trypsin or chymotrypsin, 5% w/v in 0.001 N HCl) was added (enzyme/substrate ratio 1:200). After 45 min of hydrolysis, the mixture was pumped to the hollow fiber membrane module and the proteolytic products were removed continuously by ultrafiltration using a membrane with a molecular weight cutoff of 30 000 (HF1-43-PM30, Romicon Inc., Woburn, MA). The permeate and retentate of the first ultrafiltration were named total hydrolysate (TH) and reaction mixture (RM), respectively. After completion of the first ultrafiltration, the TH was further partitioned by a second ultrafiltration with a smaller cutoff membrane of 1000 (HF1-43-PM1, Romicon) to remove amino acids and small peptides. The retentate of the second ultrafiltration was named mixture of polypeptides (MP). The permeate fraction was composed of amino acids and small peptides (AA). Samples were taken from each fraction and freeze-dried.

Kinetics of Adsorption. The surface tension measurements were made by using the Wilhelmy plate method (Adamson, 1982; Laliberté et al., 1988). The surface tension was determined with an Autotensiomat surface tension analyzer (Model 215, Fisher Scientific, Ste-Foy, Québec) and a glass lamella. The glass lamella (22 × 22 × 0.5 mm) and all the glassware were carefully cleaned with a commercial cleaner solution (No-Chromix, Godax Inc., New York) and rinsed with double-deionized water. Protein solutions (0.005% w/v) were prepared in McIlvaine's (1921) phosphate–citrate buffer (pH 4.00, $\mu = 0.6$) containing 0.01% sodium azide. An aliquot (25 mL) of the protein solution was poured into a dish, and the initially formed film was removed by aspiration of the surface. The surface tension of the protein solution was monitored continuously with a Fisher 5000 recorder (Fisher Scientific) measuring the apparent mass of the lamella, which is proportional to the surface tension. The initial interfacial tension of the buffer solution was 71 mN m^{-1}. The interfacial tension of WPC, H-WPC, and their peptide fractions obtained by tryptic or chymotryptic hydrolysis was plotted as a function of time up to 60 min. The values of rate of surface tension decrease were calculated as illustrated in Figure 1. From the surface

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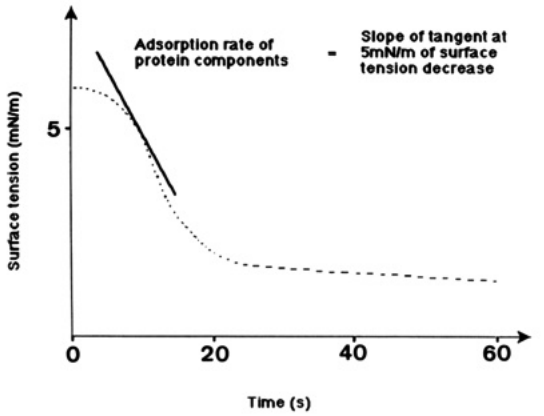

Figure 1. Surface tension depression curve and calculation of rate of adsorption of protein components.

tension curve, the slope of the tangent is calculated at a surface pressure drop of 5 mN m^{-1} and corresponds to the rate of adsorption of protein components (mN m^{-1} s^{-1}). The data represent a mean value of at least triplicate analyses.

Emulsifying Capacity. Emulsifying capacities (EC) of WPC, H-WPC, and their peptide fractions were determined according to the method of Vuillemard et al. (1990). Preliminary studies on the effect of protein concentration on emulsifying capacity (EC) of native WPC were done to determine the concentration giving the maximum EC as suggested by Vuillemard et al. (1990). A protein concentration of 0.02% gave EC_{max} (4.6 g of oil/mg of protein) under our experimental conditions (pH 4.0; $\mu = 0.6$), and this concentration was used to evaluate the peptide fractions.

Protein dispersions (0.02%) were prepared in McIlvaine's buffer (pH 4.00, $\mu = 0.6$) and homogenized by using an Ultra-Turrax PT18/10 homogenizer with a 525N-25F generator (Janke-Kunkel, Staufen, Germany). Mixing speed was determined by a tachometer (Ika-Tron DZM 5) and maintained at 15000 rpm by raising manually the power input as the viscosity of the emulsion increased. Pure corn oil (Best Foods, Etobicoke, Ontario) was added continuously (about 25 mL min^{-1}) into the stirred mixture. EC was determined by the increase of the electrical resistance at the inversion point detected by two electrodes connected to a multimeter (Simpson Electric Company, Elgin, IL). The amount of oil added for emulsification was determined by the difference in weight. The EC is defined as $(O_s - O_b)/P$, where O_s is the amount (grams) of oil added to reach the inversion point of the sample, O_b is the amount (grams) of oil added to reach the inversion point of the blank (solution without proteins), and P is the amount (milligrams) of protein in the sample.

Statistical Analysis. Effects of heat treatment, source of enzyme, and separation on the kinetics of adsorption and emulsifying capacity at pH 4.0 were analyzed as a 2 (heat treatment) × 2 (source of enzyme) × 3 (fractions obtained by UF) factorial experiment by analysis of variance. The same data were also treated as single-factor products and analyzed by one-way analysis of variance and Duncan's multiple comparisons among the means. The model system data were also subjected to regression analysis.

RESULTS

Kinetics of Adsorption. The rates of adsorption of WPC, H-WPC, and their peptide fractions on the basis of surface tension changes are reported in Figure 2. As shown by analysis of variance, the rates of adsorption were significantly affected by acidic heat treatment ($P < 0.0001$), type of enzyme ($P < 0.0001$), and UF separation ($P < 0.0001$). Heat treatment (0.646 mN m^{-1} s^{-1}) slightly decreased the rate of adsorption of WPC (0.759 mN m^{-1} s^{-1}), while it increased the adsorption rates of peptide fractions, particularly those obtained by tryptic hydrolysis. Trypsin hydrolysis provided peptide fractions (TH, MP, AA) with higher rates of adsorption than similar fractions obtained by chymotrypsin hydrolysis. For both

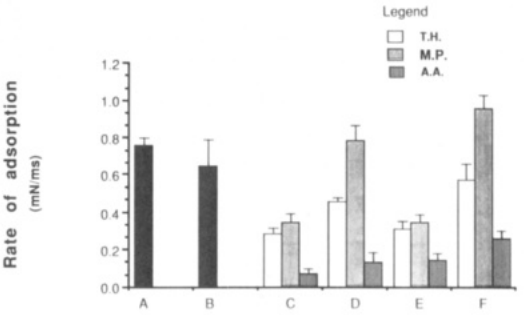

Figure 2. Rates of adsorption of WPC, heated WPC (90 °C, pH 2.5, 10 min), and their peptide fractions obtained by proteolysis with chymotrypsin or trypsin. (A) WPC; (B) H-WPC; (C) chymotrypsin hydrolysis of WPC; (D) trypsin hydrolysis of WPC; (E) chymotrypsin hydrolysis of H-WPC; (F) trypsin hydrolysis of H-WPC.

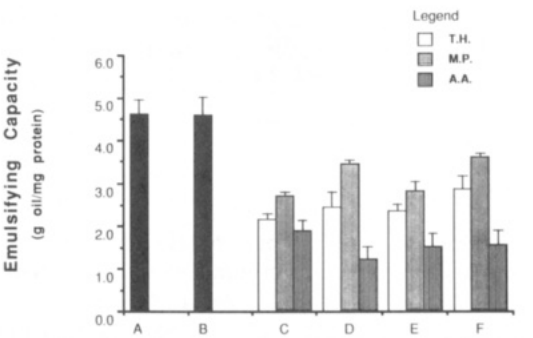

Figure 3. Emulsifying capacities of WPC, heated WPC (90 °C, pH 2.5, 10 min), and their peptide fractions obtained by proteolysis with chymotrypsin or trypsin. (A) WPC; (B) H-WPC; (C) chymotrypsin hydrolysis of WPC; (D) trypsin hydrolysis of WPC; (E) chymotrypsin hydrolysis of H-WPC; (F) trypsin hydrolysis of H-WPC.

enzymes, TH fractions have lower adsorption rates than substrates (WPC or H-WPC). UF separation of amino acids and small peptides from hydrolysates provided MP fractions with improved adsorption rates in comparison with their TH ($P < 0.0001$), and this effect is more pronounced on tryptic fractions. Multiple-comparison analysis showed that the tryptic MP from heated WPC had the highest rate of adsorption ($P < 0.05$), being an even better surface tension depressor (0.967 mN m^{-1} s^{-1}) than WPC (0.759 mN m^{-1} s^{-1}). All AA fractions had low rates of adsorption.

Emulsifying Capacity. Heat treatment did not modify EC of WPC (Figure 3), but it increased slightly EC of peptide fractions from H-WPC ($P < 0.0638$). Proteolysis (TH) decreased the EC of WPC, tryptic fractions having better EC than chymotryptic fractions ($P < 0.0004$). UF separation of amino acids and small peptides from hydrolysates improved EC for all the MP fractions ($P < 0.0001$), but contrary to adsorption rates (Figure 2), EC values remained lower (20%) than those of substrates (WPC or H-WPC). As for rates of adsorption, the lowest EC values were obtained for AA fractions.

Relationship between EC and Adsorption Rates. Since similar trends were observed by analysis of adsorption rates and EC results, the relationships between those methods have been studied. The relationship between EC and adsorption rates is shown in Figure 4. A linear correlation between EC and the square root of adsorption rates is obtained for WPC, H-WPC, and their peptide fractions. The equation for the line is $Y = 3.942X^{1/2}$. The correlation coefficient is 0.98 and is highly significant ($P < 0.0001$).

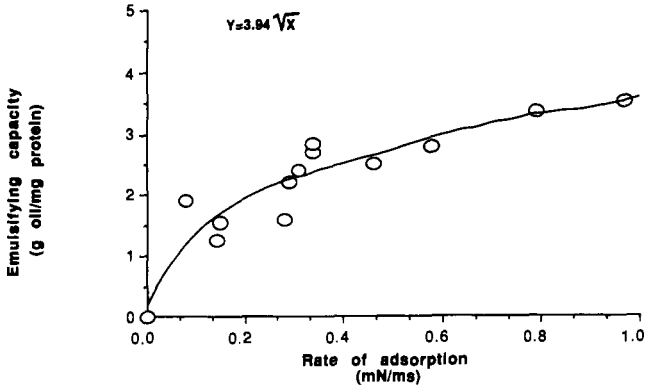

Figure 4. Correlation between rate of adsorption of whey peptide fractions (TH, MP, AA) and emulsifying capacity.

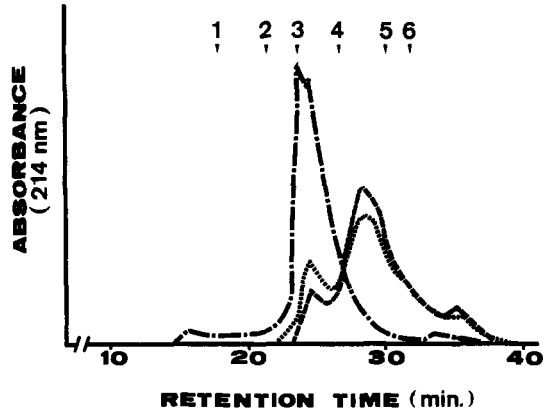

Figure 5. Molecular weight distribution profiles of (- - -) whey protein concentrate, (– –) tryptic mixture of polypeptides (MP) obtained from WPC, and (- - -) H-WPC by high-performance size exclusion chromatography (TSK 2000 SW). Markers: (1) BSA (MW 66 200); (2) ovalbumin (MW 43 000); (3) β-lactoglobulin (MW 18 400); (4) ACTH (MW 4390); (5) bacitracin (MW 1411); (6) L-leucyl-L-leucyl-L-leucine (MW 358).

DISCUSSION

Kinetics of Adsorption. Heat treatment under acidic conditions was chosen for its specific effects on whey proteins (Harwalkar and Modler, 1981; Harwalkar and Kalab, 1985). Under acidic conditions, heating of whey proteins causes denaturation of a part of the proteins into aggregates of varying sizes and single molecules of various degrees of unfolding with intermolecular interaction of hydrophobic nature. Thus, the decrease of adsorption rate of H-WPC (Figure 2B) is explained by a slower diffusion rate to the interface of the unfolded heated proteins in comparison to the native proteins (WPC).

Proteolysis (TH fractions) decreased considerably the adsorption rate of WPC (Figure 2). TH fractions are composed of protein components of different molecular weights, and although small peptides diffuse rapidly and adsorb at the interface, these small molecules are less efficient in decreasing surface tension because they cannot unfold and spread like proteins upon adsorption at the interface. Furthermore, smaller peptides can be desorbed from the interface by larger peptides having more affinity with the interface. Depending on the specificity of the enzyme, the nature of the peptides (hydrophobicity, charge density) will be different. Better surface depression properties were observed for tryptic hydrolysates (Figure 2), and the same result was obtained by Jost and Monti (1982). Previous characterization (Turgeon and Gauthier, 1990) of the hydrolysates indicated that chymotrypsin produced peptides with a wider range of molecular weight having a slightly higher proportion of smaller digestion products, and these products have the lowest adsorption rates (AA fractions). Furthermore, chymotrypsin hydrolyzes at the carboxyl end of aromatic amino acids (Trp, Tyr, Phe), resulting probably in peptides having less hydrophobic amino acids within their structure than tryptic peptides.

Separation of amino acids and small peptides from the TH improved rates of adsorption of MP fractions. This is related to the enrichment of MP in higher molecular weight peptides (Turgeon and Gauthier, 1990) resulting in less competition for the interface. For tryptic MP from heated WPC, the elimination of short peptides is a part of the explanation of the performance of this fraction (better adsorption rate than WPC). By size exclusion chromatography (HPSEC), we found a larger proportion of higher molecular weight components when compared to MP obtained from WPC (Figure 5). Those components are intact protein and/or peptide aggregates probably provided by different cleaving patterns of H-WPC and resulting in components with more amphipathic structure and higher surface activity. Shimizu et al. (1986) have found improved surface activity by synergistic effect of coexistent casein peptides. Our results suggest that the molecular weight of peptides, their hydrophobicity, and the presence of intact proteins and/or peptide aggregates play an important role in the surface active properties of MP fractions. The combination of heat treatment, tryptic hydrolysis, and UF separation of the hydrolysate gave a fraction with better interfacial properties than WPC.

Emulsifying Capacity. Emulsifying capacity (EC) is evaluated in a dynamic system in which diffusion rate and shape of molecules are less effective parameters. This probably explains that protein denaturation resulting from acidic heat treatment of WPC did not affect the EC. As for the rate of adsorption, proteolysis (TH fractions) decreased EC, and UF separation of small peptides and amino acids from hydrolysates improved EC. These results are in agreement with the literature and the fact that there is an optimum mean molecular size of peptide to provide good emulsifying properties. This was demonstrated by Adler-Nissen and Olsen (1979) by controlling the degree of protein hydrolysis and by Ochiai et al. (1982) by fractionation of hydrolysates.

The importance of enzyme specificity was also emphasized by EC results. Trypsin hydrolysis resulted in higher molecular weight peptides, probably having a more amphipathic nature, and gave higher EC values than chymotrypsin fractions. Jost and Monti (1982) also obtained higher emulsifying properties with tryptic hydrolysate of whey proteins.

While some peptide fractions gave equal or higher values of adsorption rate than WPC, the EC values remain lower than those of substrates. This result indicates that peptide solutions covered lower oil surface than proteins, probably by the formation of a more condensed film at the interface resulting from better molecular flexibility, compared to the globular proteins (WPC), which gave steric hindrance. These results showed that even with a lower EC, the more flexible MP fractions possess better surface activity, which should lead to an enhancement of the emulsion stability.

Relationship between EC and Adsorption Rates. Heat treatment and proteolytic degradation result in changes in conformation of proteins, molecular flexibility, molecular weight distribution, and hydrophobicity. As seen from the highly significant correlation, those factors affect adsorption rates and emulsifying capacity in the same way. Adsorption rate measurement is a more

powerful method to evaluate the structural changes in protein and seems a rapid and simple method to compare emulsifying properties of proteins and peptide fractions. An interesting fact is the small quantities needed to perform a test (0.005% protein content). However, some research is still needed to verify the correlation with EC under other conditions (pH, μ) with other proteins and peptide fractions. Furthermore, as emulsifying stability is an important measure for utilization of ingredients in various products, it will be interesting to verify the relationship between adsorption rates and emulsifying stability.

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