Characterization of whey protein isolate obtained from milk microfiltration permeate

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Summary – Whey protein was concentrated from milk microfiltration permeate or Cheddar cheese whey using batch ultrafiltration and diafiltration. Permeate flux declined more rapidly for milk microfiltrate during concentration, but similar flux profiles were observed on both wheys during diafiltration. Protein contents in concentrates from milk microfiltrate and cheese wheys were respectively 95 and 74% on a dry matter basis, while their fat contents were respectively 0.2 and 15%. Protein solubility of the milk microfiltrate isolate was over 95% between pH 3 and 8, while protein solubility of cheese whey concentrate varied between 75 and 90% over the same pH range. β -Lactoglobulin in cheese whey concentrate showed extensive structural alteration as evidenced by the low proportion of SH groups in native position. β -Lactoglobulin structural alteration was less severe in protein isolated from milk microfiltrate, but still significant. Milk microfiltrate protein isolate showed better gelling properties than cheese whey protein concentrate. Cheese whey protein concentrate did not foam at all, while stiff and stable foams were produced from milk microfiltrate protein isolate. Gelation and foaming were very sensitive to the ionic environment and were controlled by a balance between the pH and the calcium concentration. Reduction of calcium content in milk microfiltrate protein isolate, using saline solution during diafiltration, had only slight effects of protein functional properties.

milk microfiltration / whey protein / ultrafiltration / heat set gelation / foaming property

Résumé – Caractérisation d'un isolat protéique de lactosérum obtenu à partir d'un perméat de microfiltration de lait. Les protéines d'un perméat de microfiltration de lait et d'un lactosérum de fromage Cheddar ont été concentrées par ultrafiltration en batch suivie d'une diafiltration. La comparaison des flux de microfiltration indique un colmatage plus rapide pendant la concentration du microfiltrat de lait. Les flux de perméation en fin de concentration et pendant la diafiltration étaient toutefois similaires. Les teneurs en protéines des concentrés protéiques de microfiltrat de lait et de lactosérum fromager étaient respectivement de 95 et 74%, alors que les concentrations lipidiques atteignaient respectivement 0,2 et 15,5 %. La solubilité des isolats de microfiltrat de lait était supérieure à 95 % entre pH 3 et 8, alors que la solubilité des concentrés de lactosérum fromager variait de 75 à 90 % sur la même fourchette de pH. La structure native de la β -lactoglobuline, évaluée par la position du groupement SH libre, était fortement altérée dans le concentré de lactosérum fromager. L'altération structurale de la β -lactoglobuline était moins importante dans l'isolat de microfiltrat de lait, mais toutefois significative. Les propriétés thermogélifiantes de l'isolat de microfiltrat de lait étaient supérieures à celles du concentré de lactosérum fromager. L'isolat de microfiltrat de lait formait des

mousses fermes et stables alors que le concentré de lactosérum fromager refusait de mousser. Les propriétés gélifiantes et moussantes étaient sensibles à l'environnement ionique et modulées par la combinaison du pH et de la disponibilité du calcium dans le milieu. La réduction de la teneur en calcium de l'isolat protéique, par l'utilisation d'une solution saline comme solvant de diafiltration, n'a cependant eu qu'une influence mineure sur ses propriétés fonctionnelles.

microfiltration / protéine du lactosérum / ultrafiltration / gélification thermique / propriété moussante

INTRODUCTION

Recent developments in membrane technology led to commercial uses of cross-flow microfiltration in dairy industry. Microfiltration is used to remove bacterial contaminants and suspended materials from milk or whey (Trouvé et al, 1991; Gésan et al, 1993; Mucchetti and Taglietti, 1993). Microfiltration makes possible also the selective concentration of native casein micelles (Fauquant et al, 1988). This concentrate is heat stable and well suited for cheese milk standardization and enrichment (Pierre et al, 1992). Furthermore, casein concentration through microfiltration produces a high value milk microfiltrate.

Milk microfiltration permeate is obtained from non-fermented milk, which brings important differences when compared to cheese whey. Milk microfiltrate is almost free from bacteria and bacteriophages (Gautier et al, 1994). It requires then very mild heat treatment to ensure salubrity and to be used as in the formulation of cultured products. Milk microfiltrate is also free from degradation products related to starter culture and milk coagulation enzyme activities. Since milk is not acidified before whey separation, the mineral equilibrium is not altered and remains similar to that of milk. Compared to cheese whey, lower amounts of calcium and phosphorus and almost no organic acids are expected. Finally, milk microfiltrate is expected to contain only traces of fat. Clarification treatments to remove lipids are not required to produce ingredients with good functional properties.

The purpose of this study was to concentrate protein from milk microfiltration permeate and cheese whey and to compare their functional properties.

MATERIALS AND METHODS

Preparation of protein concentrates from milk microfiltrate and cheese whey

Fresh raw milk was skimmed and microfiltered, with the MFS-7 Alfa-Laval equipment (Alfa-Laval, Lund, Sweden) operated at 50 °C. An initial filtration using a 1 P 19 Membralox (SCT, Tarbes, France) 1.4 µm pore size cartridge (0.85 m length; internal diameter of channels: 4 mm; area 0.2 m²) was performed according to Trouvé et al (1991), to reduce bacterial contamination. A second filtration using a 0.1 µm pore size cartridge was performed according to Pierre et al (1992), to concentrate native phosphocaseinate. The permeate of this second microfiltration, containing whey proteins, lactose and minerals, was used as starting material. A 120 kg mass of milk microfiltrate was batch-concentrated up to weight concentration factor 16 with the UFS-1 Alfa-Laval equipment fitted with a PM 10 polysulfone hollow fibre (internal diameter of fibres: 1.5 mm; area 1.3 m²) ultrafiltration cartridge (Romicon Corp, Woburn, MA, USA). Temperature during concentration was maintained at 50°C. Inlet and outlet pressure were maintained at 1.8 and 0.6 bar, respectively. Permeate flux was measured and permeate samples were withdrawn at weight concentration factors (CF) 1, 1.5, 2, 3, 5, 8, 12, and 16. Following concentration, the retentate (7.5 kg) was diafiltered by the addition of 37.5 kg of deionized water in continuous mode, which corresponded to a final mass dilution rate (α = kg added water/kg retentate) of 5. Permeate flux was measured and permeate samples were with-

drawn at dilution rates (α) 0, 1, 2, 3, 4 and 5. The retentate was then freeze-dried and stored at -20°C until further analysis. A second concentrate was produced by the same process but during diafiltration, sodium chloride solution (0.3% w/w) was used instead of water up to $\alpha = 3$, in order to further reduce the calcium content of the retentate. Excess sodium was removed using deionized water from $\alpha = 3$ to $\alpha = 5$. A third concentrate was obtained from pasteurized Cheddar cheese whey (from a local cheese factory). The same process was applied to cheese whey, with the use of sodium chloride solution in the early stages of diafiltration. Each protein concentrate was produced three times with starting material from independent batches (raw milks or cheese wheys).

Composition analysis

Ultrafiltration permeate samples were analyzed for total nitrogen (Kjeldahl), ashes (5 h, 550°C) and calcium (adapted from Pearce, 1977). In addition, freeze-dried protein concentrates were analyzed for non-casein nitrogen (NCN) and non-protein nitrogen (NPN) (Rowland, 1938). The main protein fractions were analyzed by reverse phase HPLC using C4 bonded silica column (Vydac 214TP, The Separations Group, Hesperia, CA, USA) according to Kim et al (1987). Total lipids were determined from methanol/chloroform (2:1 v/v) extraction according to Pierre et al (1994). Lactose was spectrophotometrically measured (Dubois et al, 1956) and the minerals (Ca, Mg, Na, K) were detected using inductively coupled plasma spectrometry (ICP spectrometer model 3510, Applied Research Laboratories, Sunland, CA, USA).

Retention coefficients

The averaged retention coefficient of solutes during concentration was calculated from changes in retentate composition, according to Glover (1985):

$$C = C_0 \cdot C F^R \quad (1)$$

where C_0 is the initial concentration of solute, C , the solute concentration at concentration factor CF and R , the averaged retention coefficient. The R value was the slope of $\log C/C_0$ vs $\log CF$ and was obtained through linear regression analysis.

Average retention coefficient during diafiltration was also determined from changes in retentate composition (Glover, 1985).

$$C_0 = C \cdot e^{(1-R)\alpha} \quad (2)$$

where α is the dilution rate. The R value was obtained from the slope $(1-R)$ of $\ln C_0/C$ vs α and was obtained through linear regression analysis.

Free SH groups determination

Total free SH groups were determined spectrophotometrically from reaction with DTNB. According to Shimada and Cheffell (1989), the SH group in the native state of β -lactoglobulin (position 119/121) reacts more slowly with DTNB in presence of SDS than other SH groups resulting from SH/S-S interchange reactions. The kinetic data of the reaction (reacting SH groups vs time) were then fitted to a double exponential equation. The first exponential represented the contribution from the slow reacting SH groups and the second, the contribution from the fast reacting SH groups:

$$SH_t = SH_s \cdot (1 - e^{-0.0034t}) + SH_f \cdot (1 - e^{-0.0461t}) \quad (3)$$

where SH_t is the concentration of SH group reacting with DTNB at time t , SH_s , the concentration of slow reacting SH groups in the protein sample and, SH_f , the concentration of fast reacting SH groups in the protein sample. Rate constant of the first exponential (-0.0034) was obtained from kinetic data of whey protein isolated from acidified fresh raw milk and fitted to a single exponential model. β -Lactoglobulin in fresh raw milk was assumed to contain exclusively slow reacting SH groups. The rate constant for the second exponential (-0.0461) was obtained from kinetic data of denatured whey protein isolate (1% solution heated at 90°C for 20 min), fitted to a double exponential with -0.0034 used as the rate constant of the first exponential. SH group concentrations are reported as μmol per g of β -lactoglobulin (according to the protein composition (table I), the theoretical contribution of BSA to the free SH groups content of the isolates was less than 1% and was neglected in calculations).

Solubility of protein concentrates

Protein solubility was determined from whey protein concentrates suspended in deionized water.

Table I. Composition of whey protein concentrates on dry matter basis (g kg^{-1}).*Composition des concentrés protéiques sur base sèche (g kg^{-1}).*

Concentrate from	MM	MM-LC*	CW
Protein	958 ^{a**}	962 ^a	779
NCN ($\times 6.38$)	895 ^a	890 ^a	660
β -lactoglobulin	794 ^a	788 ^a	591
α -lactalbumin	71 ^a	78 ^a	54 ^a
BSA	30 ^a	24 ^a	14 ^a
NPN ($\times 6.38$)	9 ^a	8 ^a	38
Lipids	2 ^a	2 ^a	155
Lactose	13.4 ^a	14.2 ^a	22.2
Ash	18 ^a	20 ^a	23 ^a
Ca	3.3	1.0 ^a	1.1 ^a
Mg	0.36	0.09 ^a	0.10 ^a
Na	0.7 ^a	4.4 ^a	3.0 ^a
K	1.5	0.4 ^a	0.2 ^a
P	0.5 ^a	0.3 ^a	0.5 ^a

*Low calcium (diafiltered with NaCl solution).
Faible teneur en calcium (diafiltré avec une solution de NaCl).

**Means in a same row, followed by the same letter are not significantly different ($\alpha = 0.05$).

Les moyennes d'une même ligne suivies de la même lettre ne sont pas significativement différentes ($\alpha = 0,05$).

The effect of pH and added calcium on the solubility were tested through a 11×4 completely randomized design. The pH of the solution was adjusted (between 3 and 8 with 1 mol L⁻¹ HCl or 1 mol L⁻¹ NaOH) and CaCl₂ was added at four different levels (0, 5, 10 and 15 mmol L⁻¹). Final protein concentration was adjusted to 10 g L⁻¹. Protein dispersions were centrifuged at 20 000 g for 15 min. Solubility was obtained from the absorbance ratio between the supernatant and the dispersion before centrifugation. Absorbance was measured at 280 nm on a sample aliquot diluted 1:10 (v/v) in dissociating buffer (50 mmol L⁻¹ EDTA; 8 mol L⁻¹ urea; pH 10).

Gelling properties of protein concentrates

Whey protein concentrates were dispersed in deionized water. The effect of pH (4.0, 6.0 and 8.0)

and added calcium (0, 5, 10 and 15 mmol L⁻¹) on gelling properties were tested through a split plot factorial design, with the whey concentrates and the pH in the main plot and the level of added calcium in the subplot. Final protein concentration was adjusted to 75 g L⁻¹. A 2-ml sample of protein solution was placed in a 11-mm internal diameter test tube and heated at 90 °C for 30 min. Gels were cooled in an ice bath for 5 min and allowed to stand 2 h at 22 ± 1 °C before analysis. Strain-deformation curves were monitored at the same temperature using a texture analyzer (model TA-XT2, Texture Technologies Corp, Scarsdale, NY, USA). The probe was a flat cylinder (6-mm diameter) which penetrated the gel at a speed of 0.8 mm s⁻¹. Maximal deformation was set to 15 mm. Gel strength was associated with the force required to fracture the gel (maximum on strain-deformation curve). Broken gels were then centrifuged (2500 rpm, 15 min), and syneresis index was calculated from the volume proportion of free serum. All gels were prepared and analyzed in triplicates.

Foaming properties of protein concentrates

Whey protein concentrates were dispersed in deionized water. The effect of pH (4.0, 6.0 and 8.0) and added calcium (0, 5, 10 and 15 mmol L⁻¹) on foaming properties were tested through a split plot factorial design, with the whey concentrates and the pH in the main plot and the level of added calcium in the subplot. Final protein concentration was adjusted to 50 g L⁻¹. A 100-mL sample was whipped at 22 ± 1 °C with an electrical mixer (model Mixmaster, Sunbeam Corporation, Toronto, Canada) operating at maximum speed for 5 min. Foam expansion factor was calculated from the density ratio between the solution and the foam. Foam firmness was measured with a Brookfield viscometer (Model DVII) mounted on a Helipath support and fitted with a 'T' type spindle. Spindle rotational speed was set to 12 rpm, while vertical displacement was fixed at 2.5 cm min⁻¹. Apparent viscosity was measured after 1-cm penetration. Foam drainage stability was defined as the time required to drain half the foam weight. For that purpose, a 50-mL funnel ('combitip' from Brinkmann, Rexdale, Canada) was filled with freshly prepared foam and placed over a balance. The balance was connected to a computer which collected weight data of drained liquid as a function of time.

Statistical analyses

Analysis of variance was used to determine if the factors and their interactions had a significant effect on the measured properties (SAS Institute Inc, Cary, NC, USA). Statistical analyses were performed at an $\alpha = 0.05$. Composition and physicochemical properties of whey protein concentrates were compared through multiple comparisons of least square means at controlled α level of 0.05. Contrast analyses were used to compare the gelling and foaming behaviours of whey protein concentrates. Error bars on graphs represent the standard error obtained from the statistical models.

RESULTS AND DISCUSSION

Concentration of whey protein

Cheese whey and milk microfiltrate were batch-concentrated up to CF 16. As a consequence of membrane fouling, the permeate flux rapidly decreased with increasing CF (fig 1a). In the early stages of concentration, flux declined faster with milk microfiltrate than with cheese whey ($P = 0.0001$). At CF greater than 10, permeate fluxes values were similar and averaged $18 \text{ L h}^{-1} \text{ m}^{-2}$. Considering the generally accepted relationship between ultrafiltration flux decline and residual lipids in whey (de Wit and de Boer, 1975; Merin and Gordin, 1983; Piot et al, 1984; Daufin et al, 1992), lower flux observed during concentration of milk microfiltrate was not expected. However, lipid content is not the only composition factor controlling permeate flux. Calcium phosphate precipitation during concentration of dairy fluids is responsible for severe flux decline (Merin and Cheryan, 1980; Glover, 1985). The pH of milk microfiltrate was slightly higher than that of cheese whey (6.6 vs 6.2), which could have promoted mineral precipitation. In order to reduce mineral fouling, it would be appropriate to slightly reduce the pH of milk microfiltrate or to operate the ultrafiltration unit at a temperature lower than the temperature used during milk microfiltration.

Fig 1. Permeate flux during (a), batch-concentration (●, milk microfiltrate; ■, cheese whey) and (b), diafiltration of whey.

Flux de perméation pendant (a), la concentration en batch (●, microfiltrat de lait; ■, lactosérum de fromage) et (b), la diafiltration du lactosérum.

The average retention coefficients (R) for nitrogen compounds during concentration of milk microfiltrate and cheese whey were not significantly different ($P = 0.4221$) and averaged 0.83 (data not shown). Calcium retention during concentration was slightly higher for cheese whey ($R = 0.18$) than for milk microfiltrate ($R = 0.16$) ($P = 0.0401$). This difference was attributed to the portion of non-permeable calcium which increased with thermal treatments (pasteurization) of cheese milk and whey (Brulé and Fauquant, 1981).

Diafiltration of whey protein

After concentration (CF = 16), cheese whey (CW) and milk microfiltrate (MM) were diafiltered in continuous mode up to $\alpha = 5$. The permeate flux was not affected either by the type of whey or the diafiltration solvent (water vs NaCl solution) ($P = 0.3658$). However, the permeate flux showed variations associated with dilution rate (α) ($P = 0.0213$) (fig 1b). From $\alpha = 0$ to $\alpha = 3$, the flux gradually increased up to 120% of its initial value. Flux increase was attributed to the viscosity decrease of UF-permeate resulting from the elimination of lactose (Peri et al, 1973). Further diafiltra-

Fig 2. Change in calcium concentration during diafiltration of whey concentrate. ■, MM-concentrate diafiltered with deionized water; ○, MM-concentrate diafiltered with NaCl solution up to $\alpha = 3$, followed with deionized water; ●, CW-concentrate diafiltered with NaCl solution up to $\alpha = 3$, followed with deionized water.

Évolution de la concentration en calcium pendant la diafiltration du concentré de lactosérum.

■, concentré de microfiltrat de lait diafiltré avec l'eau déionisée; ○, concentré de microfiltrat de lait diafiltré avec une solution de NaCl jusqu'à $\alpha = 3$, puis avec l'eau déionisée; ●, concentré de lactosérum de fromage diafiltré avec une solution de NaCl jusqu'à $\alpha = 3$, puis avec l'eau déionisée.

tion reversed the trend, with permeate flux almost back to its initial value at $\alpha = 5$, suggesting further fouling of the UF membrane.

Average retention for nitrogen compounds was not affected either by the type of whey or the diafiltration solvent ($P = 0.6241$) with a coefficient of 0.95. Calcium retention coefficient increased during diafiltration as evidenced by the non-linear relationship between $\ln(Co/C)$ vs α curves (fig 2). This deviation from linearity was attributed to the portion of non-permeable calcium. Non-permeable calcium, expressed on a protein basis averaged 3.4 mg g^{-1} in milk microfiltrate protein isolate. It was reduced by 70% when sodium chloride was used in the early stages of diafiltration. Use of sodium chloride during diafiltration of cheese whey led to a similar result.

Composition of whey protein isolates

Compositions of MM-isolates and CW-concentrate are presented in table I. Protein concentration of MM-isolates averaged 96% on a dry matter basis. The CW-concentrate showed lower protein content (77.9%) due to the significant amount of lipids concentrated with proteins during ultrafiltration. In MM-isolates, the pH 4.6-insoluble fraction averaged 7.0% of total protein. Pierre et al (1992) found a similar proportion in milk microfiltration permeate (6.4%) and associated it to the presence of caseins. The pH 4.6-insoluble protein fraction in CW-concentrate was higher (15.3%). Denaturation of whey proteins, induced by pasteurization steps during cheese making would explain the difference. The proportion of the various whey proteins was altered by the concentration process. The weight ratio between β -lactoglobulin and α -lactalbumin, which is slightly higher than 4:1 in milk serum (Marshall, 1982), increased to at least 10:1 in ultrafiltration concentrate. Polymeric membranes, such as those used in the present study are generally characterized by a diffuse cut-off and allow slight protein permeation according to molecular size (Cheryan, 1986). A lower extent of proteolysis in milk microfiltrate compared to cheese whey would explain the lower NPN content in MM-isolates. Lipid content of MM-isolates was much lower than in CW-concentrate. The mineral composition of MM-isolate was modified by the use of sodium chloride instead of water in the early stages of diafiltration: Ca, Mg and K concentrations were reduced by about 70%, while sodium increased from 0.7 g kg^{-1} to 4.4 g kg^{-1} .

Protein denaturation

The kinetics of the reaction between free SH groups and DTNB reagent was used to determine the extent of SH/S-S interchange of β -lactoglobulin (table II). The

Table II. SH groups characteristics in whey protein concentrates ($\mu\text{mol g}^{-1}$ β -lactoglobulin). *Caractéristiques des groupements SH dans les concentrés protéiques ($\mu\text{mol g}^{-1}$ β -lactoglobuline).*

	SHinterchanged	SH _{native}	SH _{tot}
MM-isolate	9.6 ^{a*}	28.6 ^{a,b}	38.2 ^a
MM-isolate LC**	10.1 ^a	28.7 ^a	38.8 ^a
CW-concentrate	23.3 ^a	6.6	29.9

*Means in a same row, followed by the same letter are not significantly different ($\alpha = 0.05$). **Low calcium (diafiltered with NaCl solution).

*Les moyennes d'une même ligne suivies de la même lettre ne sont pas significativement différentes ($\alpha = 0,05$). **Faible teneur en calcium (diafiltré avec une solution de NaCl).

slow-reacting SH group content of β -lactoglobulin in MM-isolates was close to $29 \mu\text{mol g}^{-1}$ and represented 75% of total SH groups. The concentration process induced significant structure alteration through SH/S-S interchange reactions resulting in 25% decrease of native β -lactoglobulin content. In the present study, whey was batch-concentrated. It was then submitted to pumping and was maintained at 50°C for a long period of time. Such conditions have been shown to induce partial protein unfolding (Harris et al, 1989). In order to preserve the native structure of β -lactoglobulin, it would be appropriate to use a system configuration with shorter residence time and reduce filtration temperature. β -Lactoglobulin from CW-concentrate showed a lower level of slow reacting SH groups which represented 22% of total SH groups. This proportion of SH group in native position is close to the expected equilibrium value (20%) assuming an extensive and random interchange reaction.

According to its molecular mass (18 362 Da; Swaisgood, 1982) the total free SH group content of native β -lactoglobulin is

$54.5 \mu\text{mol g}^{-1}$. A lower free SH group content reflects the formation of intermolecular disulphide bonds or other oxidation products (Donovan and Mulvihill, 1987). In table II, it can be seen that the total SH group content of β -lactoglobulin in MM-isolates averaged $38.5 \mu\text{mol g}^{-1}$, corresponding to 29.4% of the original SH being oxidized. Batch ultrafiltration, performed at 50°C , was likely to induce significant alteration of whey protein structure. A lower SH content of β -lactoglobulin was found in CW-concentrate, where 45.1% of the original content of SH groups had been oxidized.

Solubility of whey protein isolates

Solubility of protein isolates was measured at various pHs. Solubility was not influenced by the addition of calcium ($P = 0.2601$) and results presented in figure 3 are the averages of solubility measured at the four calcium levels. The solubility of MM-isolates averaged 97% at pH values below 4.5 and higher than 5.5. A slight decrease was observed at the isoelectric point of β -lactoglobulin (IP = 5.3). The CW-concentrate showed lower solubility than MM-iso-

Fig 3. Solubility of whey protein concentrates. ■, MM-Isolate; ○, low calcium MM-isolate; ●, CW-concentrate.

Solubilité des concentrés protéiques de lactosérum. ■, isolat de microfiltrat de lait; ○, isolat de microfiltrat de lait faible en calcium; ●, concentré protéique de lactosérum de fromage.

lates at any pH tested. Furthermore, solubility in the low pH portion of the profile did not improve compared to the solubility at the isoelectric point of β -lactoglobulin. The solubility profile suggests that proteins in cheese whey suffered from severe treatment probably associated with the cheese making practices or whey pasteurization conditions.

Gelling properties

Thermal gels were produced from whey protein solutions. The effects of pH adjustment and calcium addition on gel properties were tested. The gel strength was significantly affected by both variables and the type of whey protein concentrate ($P = 0.0001$) (fig 4). Cheese whey concentrate produced very soft gels with maximum gel strength values around 50 g under optimum conditions (pH 4.0). Presence of fat in the concentrate (Morr et al, 1993) and evidence of severe protein denaturation (Mangino et al, 1987) could explain the poor gelling performance.

Fig 4. Effect of pH and added calcium on whey protein gel strength. a, pH 4; b, pH 6; c, pH 8; ■, MM-isolate; ○, low calcium MM-isolate; ●, CW-concentrate.

Effet du pH et de l'ajout de calcium sur la fermeté des gels protéiques. a, pH 4; b, pH 6; c, pH 8; ■, isolat de microfiltrat de lait; ○, isolat de microfiltrat de lait faible en calcium; ●, concentré protéique de lactosérum de fromage.

Protein isolate from milk microfiltrate gelled at the three pHs tested. From contrast analysis, it was shown that the use of sodium chloride during the early stages of diafiltration did not influence the gel strength ($P = 0.8524$). It seems that calcium complexes did not affect gelation. However, the addition of ionic calcium increased the gel strength and this effect was more important as the pH increased. Calcium ions promoted protein-protein interactions through cross-linking negatively charged residues. Increasing pH increased the proportion of residues involved in calcium bridges. It has been shown that the strength of thermal gels from clarified whey protein concentrates increased with pH increasing from 5 to 9 (Gault et al, 1990). At low pH, the gel strength was essentially explained by non-specific interactions between protein molecules, while calcium bridges and disulphide bond formation explained gel strength at higher pH (Schmidt et al, 1979). A combination of high pH and high ionic calcium content produced the strongest gels.

Whey protein gels were centrifuged and syneresis was monitored (fig 5). All the variables studied (pH, calcium, protein source) and their statistical interactions had significant effects on gel syneresis ($P = 0.0001$). As a general trend, gels from MM-isolates showed lower levels of syneresis than gels from CW-concentrate. Again, the use of sodium chloride during the early steps of diafiltration did not affect syneresis ($P = 0.1500$). Minimum syneresis was observed on gels obtained at pH 4.0. The aggregated protein matrix formed at this pH was soft (fig 4) and highly hydrated. Addition of calcium for gels produced at pH 8.0 decreased syneresis. Under alkaline conditions, calcium ions contributed to the formation of strong gel matrix (fig 4) which resisted deformation upon centrifugation. Under these conditions, low syneresis was related to the gel matrix mechanical properties.

Fig 5. Effect of pH and added calcium on whey protein gel syneresis. a, pH 4; b, pH 6; c, pH 8; ■, MM-isolate; ○, low calcium MM-isolate; ●, CW-concentrate.

Effet du pH et de l'ajout de calcium sur la synerèse des gels protéiques. a, pH 4; b, pH 6; c, pH 8; ■, isolat de microfiltrat de lait; ○, isolat de microfiltrat de lait faible en calcium; ●, concentré protéique de lactosérum de fromage.

Foaming properties

Foaming properties of whey protein solutions were determined at various pHs and different levels of added calcium. Cheese whey protein concentrate did not foam at all over the entire range of conditions tested. The lipid content of CW-concentrate (table I) was responsible for deficient foaming behaviour (Maubois, 1988; Joseph and Mangino, 1988). Protein isolates from milk microfiltrate showed much better foaming properties (fig 6). Foam expansion was slightly lower for low calcium isolate (use of sodium chloride in the early steps of diafiltration) ($P = 0.0069$), and the difference remained constant over the range of conditions tested. The addition of calcium to the protein solution had no significant effect on foam expansion ($P = 0.2354$). It suggests that transport and spreading of protein molecules at the air-water interface during the foaming process are not influenced by calcium concentration. However, foam expansion of MM-isolates

Fig 6. Effect of pH and added calcium on whey protein foam expansion factor. a, pH 4; b, pH 6; c, pH 8; ■, MM-isolate; ○, low calcium MM-isolate; ●, CW-concentrate.

Effet du pH et de l'ajout de calcium sur le facteur d'expansion des mousses protéiques. a, pH 4; b, pH 6; c, pH 8; ■, isolat de microfiltrat de lait; ○, isolat de microfiltrat de lait faible en calcium; ●, concentré protéique de lactosérum de fromage.

was influenced by the pH of the solution. As pH increased, the foam expansion factor decreased. Alkaline conditions increased the net negative charge of protein molecules. Transport to the air-water interface was then slowed down by increased electrostatic repulsion between proteins in solution and proteins already adsorbed at the interface (Kinsella and Whitehead, 1989). Increased charge of protein molecules also promoted intra-molecular repulsions and spreading at the interface. These two factors were associated with the formation of thin and unstable protein films, susceptible to collapse during whipping.

Addition of calcium, and pH adjustment had various effects on foam firmness (fig 7). Increasing both calcium concentration and pH increased the firmness of foams from MM-isolates. These conditions promoted the formation of calcium bridges leading to a more rigid membrane at the air-water interface. It was noticed that the use of sodium chloride during diafiltration (low calcium isolate) reduced

Fig 7. Effect of pH and added calcium on whey protein foam firmness. a, pH 4; b, pH 6; c, pH 8; ■, MM-isolate; ○, low calcium MM-isolate; ●, CW-concentrate.

Effet du pH et de l'ajout de calcium sur la fermeté des mousses protéiques. a, pH 4; b, pH 6; c, pH 8; ■, isolat de microfiltrat de lait; ○, isolat de microfiltrat de lait faible en calcium; ●, concentré protéique de lactosérum de fromage.

Fig 8. Effect of pH and added calcium on whey protein foam stability. a, pH 4; b, pH 6; c, pH 8; ■, MM-isolate; ○, low calcium MM-isolate; ●, CW-concentrate.

Effet du pH et de l'ajout de calcium sur la stabilité des mousses protéiques. a, pH 4; b, pH 6; c, pH 8; ■, isolat de microfiltrat de lait; ○, isolat de microfiltrat de lait faible en calcium; ●, concentré protéique de lactosérum de fromage.

the firmness of foams produced at pH 8.0. Higher level of monovalent cations in the MM-isolate diafiltered with saline solution (table I) could have masked some residues which otherwise would have participated in calcium bridges. From these results, it seems that foam firmness is controlled by the combined effect of pH, monovalent cations and available ionic calcium on the electrostatic repulsions and calcium bridge formation between protein molecules at the air-water interface.

Foam stability results reflected also the combined effect of the variables under study on foaming properties (fig 8). At low pH, foam was stable and not affected by the addition of calcium. As pH increased, foam stability tended to decrease, but was improved by the addition of calcium. There was no significant effect associated with the use of sodium chloride during the early stage of diafiltration ($P = 0.4804$).

CONCLUSION

Concentration of proteins from milk microfiltrate led to a high value protein isolate with improved solubility, gelling and foaming properties (compared to cheese whey). However, it has been shown that the structure of β -lactoglobulin had been altered by the concentration conditions used in the present study. To further improve the quality of the isolate, the filtration conditions should be revised in order to reduce protein structure alteration.

The functional properties of the isolate were strongly dependent on the ionic environment (pH, calcium content). In order to develop the desired properties, the quality of the ionic environment is as important as the quality of the protein ingredient. The balance between pH and calcium content should be controlled to ensure optimal behaviour of whey protein isolates in complex food systems.

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