Influence of pH on Protein Interactions and Microstructure of Process Cheese

S. MARCHESSEAU, E. GASTALDI, A. LAGAUDE, and J.-L. CUQ
Laboratoire de Génie Biologique et Sciences des Aliments, Université Montpellier II, Montpellier 34095 Cedex 5, France

ABSTRACT

During the manufacture of process cheese, biochemical characteristics (casein solubilization, peptization coefficient, and water-holding capacity) were investigated using a combination of microscopic and rheological techniques in order to understand the influence of pH. The contribution of ionic interactions to the stabilization of this structure was also studied.

Relationships were observed between pH variation and the characteristics of process cheese that demonstrated the importance of pH control during the manufacturing process. Optimal pH conditions during manufacture ranged from 5.7 to 6.0. Small changes in ionic composition and strength modified the protein interactions substantially and had important repercussions on the final structure and quality of the protein gel that was established during processing of cheese. In addition to ionic interactions, hydrogen and hydrophobic interactions appeared to be important in the structural stabilization of process cheese.
(**Key words**: process cheese, microstructure, scanning electron microscopy, ionic interaction)

Abbreviation key: **G′** = storage modulus, **G″** = loss modulus, **SEM** = scanning electron microscopy, **tan δ** = loss tangent.

INTRODUCTION

Process cheese represents an extremely delicate and complex system containing a wide variety of interacting components and a high water content. Its properties are affected by many variables, such as the composition and nature of the cheeses used as ingredients, the nature and the amount of emulsifying agents, the pH, and the manufacturing procedure (3, 23). Some researchers (13, 17) showed that the morphology and firmness of the process cheese network are greatly affected by pH. Marchesseau and Cuq (15) have previously shown a phenomenon of cooperativity in the various interactions created between protein polymers in the network of process cheese manufactured at a specific temperature (115°C). Thus, a basic understanding of the nature of the interactions among process cheese components, as affected by pH and ionic strength, is required to produce a product of good quality.

Microscopy has been employed extensively to study cheese structure (7, 8, 9, 10, 12, 25) to explain the physicochemical changes in dairy products that occur during manufacture and storage (11, 20, 22). Scanning electron microscopy (**SEM**) was applied to defatted process cheeses to permit us to observe the organization of the casein network and to compare the microstructure of process cheese that was manufactured at different pH values.

The objective of this study was to provide information about the effects of pH and ionic bonds on the microstructure of process cheese and on the nature of the protein interactions that stabilize the process cheese network. The correlations among structure as visualized by SEM, rheological behavior, and dissociation results obtained by the method previously presented by Marchesseau and Cuq (15) may contribute to the understanding of how various structural elements interact in the formation of the process cheese network at different pH values.

MATERIALS AND METHODS

Manufacture of Process Cheese

Process cheese products were manufactured in a cheese cooker (UMM-SK40; Stephan, Hameln, Germany); a mixture of cheeses, milk powder, butter, and water were melted in the presence of 30 g/L melting salts (polyphosphates; Joha-B.K. Ladenburg GmbH, Ladenburg, Germany). The cheese mixture was heated at 115°C for 1 min and then stirred at 95°C for 8 min. The hot melted cheese was conditioned in a hermetically sealed container to form a process cheese block (130 × 40 × 40 mm) and cooled immediately in a refrigerator at 4°C. After 15 d of

Received December 27, 1995.
Accepted January 23, 1997.

storage, samples were analyzed. A standard formula was used for the manufacture of process cheese. Solutions of HCl or NaOH (Sigma Chemical Co., St. Louis, MO) were added before cooking to obtain four process cheese samples at pH values of 5.2, 5.7, 6.1, and 6.7. Final pH values of process cheese were measured (PH N81 Tacussel pH meter; Tacussel, Lyon, France) with a glass electrode (Ingold; Ingold France SARL, Paris, France).

Analysis of Cheese and Cheese Fractions

The moisture contents of the cheese and pellet after ultracentrifugation (86,000 × *g*, for 25 min, at 20°C) were measured in duplicate; samples were dried to a constant weight (about 24 h at 100°C) in an oven at atmospheric pressure. Results were expressed as grams of H_2O per gram of sedimented protein. Fat content was determined in duplicate by the acidobutyric method of Van Gulik as described by Desfleurs and Baillaul (5). Total N, noncasein N, and NPN (the fraction of total N that was soluble in 120 g/L of trichloroacetic acid) were assayed by the Kjeldahl method (Büchi 322-342 apparatus; Büchi, Flawil, Switzerland). The peptization coefficient of process cheese, calculated by the technique of Lee et al. (14), represents the ratio between soluble casein N after centrifugation at 300,000 × *g* for 45 min at 20°C and total casein N. Protein contents in pellets were determined by the Lowry procedure as modified by Bensadoun and Weinstein (1).

Centrifugation Tests in Solutions of Different pH and Ionic Strength

Process cheese (9 g) was dispersed in 45-ml solutions of varying pH (pH 4.5 to 8.0) and varying ionic strength (μ = 0 to 2) and homogenized for 30 s (ultraturrax; T25-IKA-Labortechnik; Janke and Kundel, Staufen, Germany) at 9000 rpm. The pH value of the cheese dispersion was adjusted with 1 *M* HCl or 1 *M* NaOH and was measured using a pH meter (Corning Science Products, New York, NY). The NaCl and $CaCl_2$ were added at various concentrations to change the ionic strength of the aqueous solution and to increase the concentrations of Na^+ or Ca^{2+}. Dispersing agents and solutions were obtained from Sigma Chemical Co. The cheese dispersions (21 ml) were then ultracentrifuged (Beckman ultracentrifuge L7-65 with a Ti 70 rotor) at optimized centrifugation parameters (86,000 × *g* for 25 min at 20°C) (15). Pellet weight (or amount of sedimentation) represented cheese components that were still interacting in dispersion (different pH or presence of salts), and pellet weight was expressed as grams per kilogram of process cheese.

SEM

Process cheese samples were cut into small blocks (approximately 5 × 1 × 1 mm), fixed for 2 h at 20°C in 2.0% glutaraldehyde solutions, and buffered with 0.05 *M* phosphate buffer (6) at the pH value of the process cheese sample. Then, samples were dehydrated with a graded aqueous ethanol series (25, 50, 70, 80, 95, and 100%, in triplicate for 15 min each), defatted with chloroform (16), returned to ethanol, and dried to critical point with CO_2. After drying, samples were fractured under a dissecting microscope to show the inside surface and were cemented on aluminum stubs with silver conducting paint as described by Glaser et al. (6). Finally, samples were coated with gold (5 nm thick) and examined with a field emission SEM (JEOL JSM-6400F; JEOL Europe S.A., Croissy/Seine, France) operated at 15 kV.

Rheological Evaluation

All rheological properties were evaluated using a Carrimed (model CSL 100; Rheo, Champlan, France) and dynamic oscillating measurements as defined conditions (1-Hz oscillating frequency, amplitude of 10^{-3} rad, 2-mm measurement, and temperature of 20 ± 1°C). These conditions have been previously determined to be in a stable zone of amplitude and frequency. The loss tangent ($\tan \delta$) represents the ratio between the viscous or loss modulus (G'') and elastic or storage modulus (G'): $\tan \delta = G''/G'$; δ represents the phase difference between stress and strain.

Statistical Analysis

Experimental data were presented as the means of triplicate measurements from five experiments; data were subjected to ANOVA (Stat View, Abacus Concepts, Inc., Berkeley, CA). Fisher's protected least significant difference test was used to compare paired means, and differences between means were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effects of pH During Cheese Processing

Table 1 shows that peptization coefficients of process cheese and the water-holding capacity of the sedimented proteins were reduced as the pH was

lowered during manufacturing; the pellet weight increased. Under the conditions of the dispersion test, the value of the peptization coefficient gave an estimation of the quantity of proteins that were still interactive and indirectly permitted evaluation of the intensity of protein interactions in process cheese as a function of the manufacturing pH. Therefore, a process cheese that comprised a network stabilized by interactions that were easily disrupted during the peptization test yielded a high coefficient of peptization. A reduction in manufacturing pH could enhance the interactions of the proteins, which increased the amount of pellet and reduced the water-holding capacity of the sedimented proteins (Table 1).

The rheological analysis of products that were obtained at different pH is presented in Table 2. The G′ indicated a significant dependence of firmness on pH. Firmness of process cheese decreased as pH increased between 5.7 and 6.7 and decreased also between pH 5.7 and 5.2. Process cheese that was made at pH 5.7 had the firmest structure (G′ = 12,293 N/m^2); the process cheese that was made at pH 6.7 was softest (G′ = 187 N/m^2). Similar variations were observed for the changes in G″ versus pH. However, the relationship between G″ and G′ (tan δ) exhibited the lowest value at pH 5.7 and the highest at pH 6.7.

An understanding of the microstructure of process cheese, particularly the interaction among proteins in response to a change in manufacturing pH, would provide useful information that would assist in determining the constituents of a good quality product. The SEM photomicrographs in Figure 1 revealed significant changes in the organization of the protein matrix in process cheeses that were caused by small variations of pH. Kiely et al. (11) observed similar dramatic changes in Mozzarella curd structure when the pH of whey at drainage varied between 5.9 and 6.4. Subsequent discussion focuses on the relationships between the coefficient of peptization, the rheological properties, the water-holding capacity, the microstructure, and the pH of process cheese.

TABLE 1. Composition, peptization coefficient, and water-holding capacity[1] of process cheese that was manufactured at different pH values.

	pH			
Process cheese	5.2	5.7	6.1	6.7
DM, g/kg	426	425	427	421
Fat, g/kg DM	534	517	515	523
Total N, g/kg	97	100	103	101
Soluble N, g/kg	17	12	12	13
Peptization coefficient	2	22	59	83
Pellet weight, g/kg of cheese	820^d	530^c	300^b	180^a
Pellet solvation, g of H_2O/g of protein	4.3^d	4.5^c	5.9^b	6.6^a

^{a,b,c,d}Means (n = 5) within a row with no common superscript differ ($P < 0.05$).

[1]Obtained after ultracentrifugation of process cheese dispersion at 86,000 × g for 25 min at 20°C.

TABLE 2. Rheological characteristics[1] of process cheese manufactured at different pH values.

	pH			
	5.2	5.7	6.1	6.7
G′, N/m^2	913^c	12,293^a	1800^b	187^d
G″, N/m^2	515^c	3121^a	772^b	257^d
tan δ	0.56^b	0.25^d	0.40^c	1.39^a

^{a,b,c,d}Means (n = 5) in the same row with no common superscript differ ($P < 0.05$).

[1]G′ = Storage modulus, the measure of the energy stored and released per cycle of deformation, which represents the elastic part in the reaction of a material on a stress; G″ = loss modulus, the measure of the energy dissipated or lost as heat per cycle, which represents the viscous part in the reaction of a material on a stress; δ = loss angle is the phase difference between stress and strain (if effects of inertia are negligible); and tan δ = G″/G′.

Cheese preparation at pH 5.2. Figure 1a presents a highly distorted structure that includes the appearance of large spherical particles (3 to 10 μm) formed by a compact association of proteins. The peptization coefficient that was obtained for process cheese manufactured at pH 5.2 (Table 1) indicated a very high level of protein interactions, which could be correlated with the observed aggregation state of proteins near their isoelectric point. The formation of voluminous protein aggregates could be attributed to the facilitation of charge-charge attractions at pH values near the isoelectric point of casein. Visser et al. (24) also observed an aggregated corpuscular structure at about pH 5.2 in acidic skim milk gel. The rheological results that were obtained at pH 5.2 indicated a greater contribution of the elastic component. With the method of sample preparation that was used for SEM, the distribution of lipid globules was difficult to visualize at the lower pH; only some small globules (seen as empty cavities corresponding to their solubilization during sample preparation of SEM) could be visualized as included in the protein aggregates (Figure 1a). This observation confirmed the results of Lee et al. (13), who observed by transmission electron microscopy a greater dispersion of lipid globules when the pH decreased to less than 5.4. Organoleptic analyses of process cheese manufactured at pH 5.2 (data not shown) confirmed that the process cheese in this pH range was granular and not emulsified.

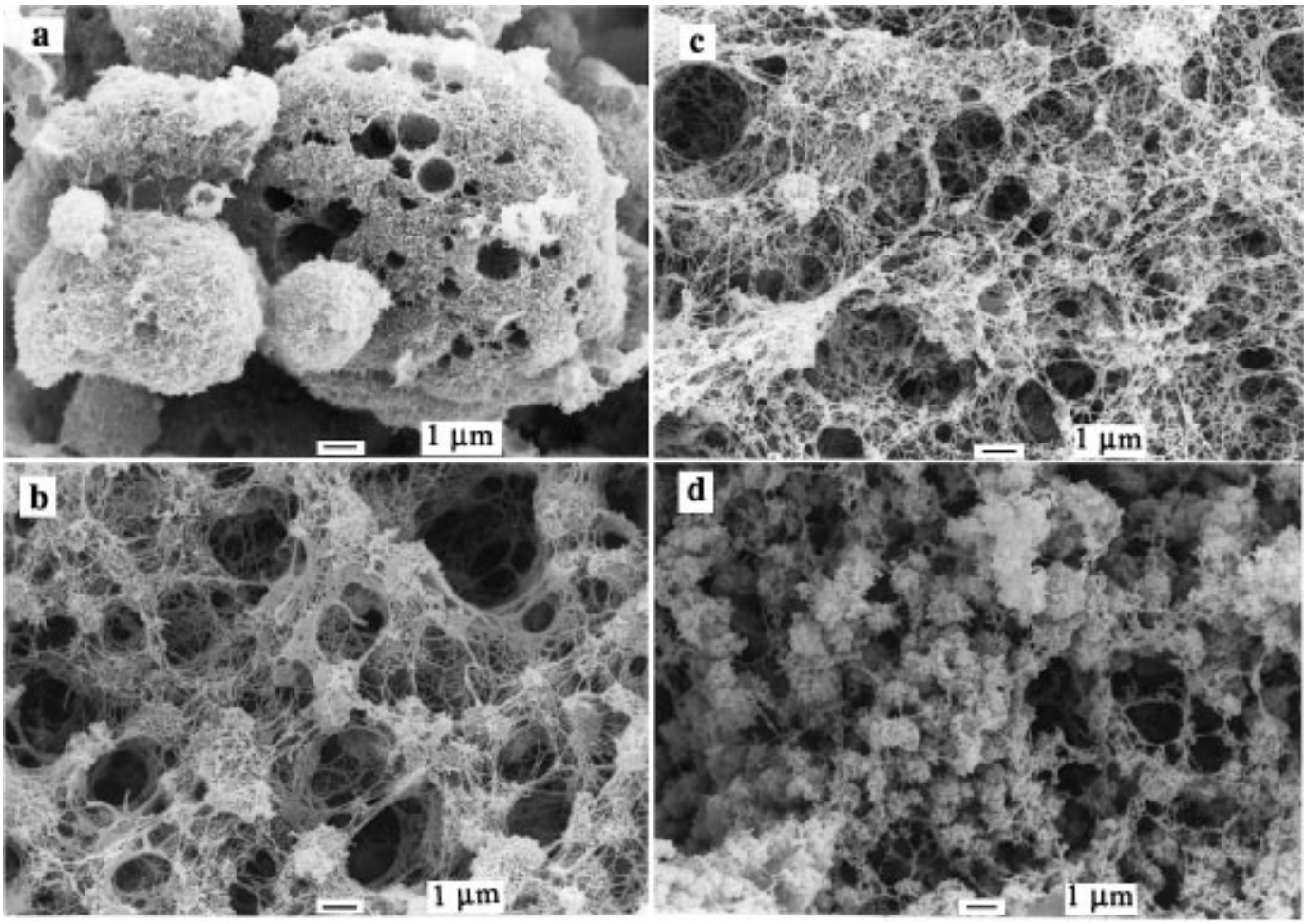

Figure 1. Scanning electron micrographs of process cheese cooked at 115°C for 1 min, stirred at 95°C for 8 min, and made at pH 5.2 (a), 5.7 (b), 6.1 (c), or 6.7 (d). Void spaces indicate presence of fat particles in original cheese. Bar = 1 μm.

Cheese preparation at pH 5.7. An increase in pH values to pH 5.7 resulted in the formation of a homogenous, dense network that had numerous protein interactions and clear separation between fat globules and the protein network (Figure 1b). Caseins were more negatively charged at pH 5.7 than at pH 5.2; the isoionic points of the major caseins are about 5.1 for α_{s1}-CN, 5.3 for β-CN, and 5.4 for κ-CN (21). These pH conditions permitted the formation of various types of interactions, including electrostatic bonds, calcium bonds, hydrophobic interactions, and hydrogen bonds. The diversity of the interactions created at this pH induced the formation of a homogeneous, three-dimensional protein network that had regular lipid distribution and substantial firmness. These results were in agreement with results of Rayan et al. (19), who reported that uniform protein network allowed a homogeneous distribution of stress and, thus, greater firmness.

Cheese preparation at pH 6.1. The distribution of protein particles appeared to be quite similar to the network distribution obtained at pH 5.7 (Figure 1c). Marchesseau and Cuq (15) described the formation of diversified and cooperative interactions between process cheese proteins around pH 5.9 to 6.1. Compared with attributes at pH 5.7, differences at pH 6.1 were slight; protein particles exhibited a more elongated shape, resulting in the formation of a string-like structure and a less compact microstructure. Although void spaces within the casein matrix remained spherical, they appeared to be less clearly defined than at pH 5.7. These results are in agreement with results of Kiely et al. (11) who observed that Mozzarella cheese curd, when drained between pH 5.9 and 6.15, resulted in an increase in the fusion of paracasein particles and produced a more continuous three-dimensional curd network.

TABLE 3. Effects of the ionic strength by addition of NaCl or $CaCl_2$ on the changes in pellet weight and pellet solvation.[1]

	NaCl				$CaCl_2$			
	0.1%	0.5%	1.0%	2.0%	0.1%	0.5%	1.0%	2.0%
	(μ)							
Salt concentration mol/L	0.1	0.5	1.0	2.0	0.03	0.17	0.33	1.50
pH of cheese dispersion	5.7	5.5	5.4	5.2	5.1	4.7	4.5	4.1
Pellet weight g/kg	520[a]	370[b]	320[c]	100[d]	580[x]	600[x]	540[x]	30[y]
Pellet solvation, g of H_2O/g of protein	5.6[a]	5.9[a]	5.2[a]	4.7[b]	3.9[x]	3.9[x]	3.8[x]	1.8[y]

[a,b,c,d;x,y]Means (n = 5) within the same row and within each type of salt with no common superscript differ ($P < 0.05$).

[1]After ultracentrifugation (86,000 × g for 25 min at 20°C) of process cheese dispersions (1:5, wt/vol) at different concentrations of NaCl or $CaCl_2$.

Between pH 5.7 and 6.7, the peptization coefficient increased, which suggested that protein interactions became less important in process cheese at higher pH values. The reduction in the compact microstructure as the pH increased (Figure 1, b and c) probably resulted from increased weakness in interactions among proteins (19). Changes in the microstructure over that pH range seemed to be related to the changes in the rheological properties of process cheese (decreases in elasticity and viscosity).

Cheese preparation at pH 6.7. At pH 6.7, small condensed aggregates of approximately 0.5 to 1 μm of diameter were observed; those aggregates appeared to disrupt the protein matrix. These results were in agreement with those of Buchheim and Jelen (2), who observed that the morphology of a three-dimensional network of milk protein varied greatly as pH varied; large aggregates (about 300 to 600 nm) appeared at pH 6.5. As shown in Table 1, the enhanced solubilization of casein led to a product that had low intensity of protein interactions, which yielded a unique network organization (Figure 1d). The increased solubilization of casein could be attributed principally to a reduction in electrostatic interactions because of the increase in the negative net charge of proteins. The high electrostatic repulsions could result in a rapprochement of uncharged polypeptidic chains that initially were distant from one another. The loss of electrostatic interactions could be compensated by the formation of new interactions, such as hydrogen bonds and hydrophobic interactions. The weakness of the resulting gel might be attributed to the creation of these interactions of lower energy and also to greater water sorption of the proteins (Table 2). These observations were in agreement with results given by Paquet (18), who obtained at pH 6.4 a very soft process cheese with an inconsistent structure. The soft nature of process cheese at higher pH values could be correlated to the high sorption of water by protein (Table 1), which acted as a plasticizer and reduced firmness of the process cheese (4).

A small variation in the manufacturing pH of process cheese had several repercussions on the nature of protein interactions and on the final microstructure and rheological properties of the process cheese network. These observations could be correlated to the model of the process cheese network formation that was proposed by Heertje et al. (8). In that model, the network was formed in two distinct stages: a dissociation stage and then a gelation stage. Dissociation might occur differently at different pH values because of the change in the net charge of proteins. Reassociation of these different fragments, as affected by pH, yielded very different structures at the two extreme pH values studied (5.2 and 6.7). The manufacture of process cheese in a pH range from 5.7 to 6.1 formed a structured and firm product with uniform distribution of the protein network.

Effects of pH and Ionic Strength on the Dissociation of Cheese Interactions

The firmest process cheese with the most homogeneous distribution of protein (the process cheese prepared at pH 5.7) was chosen to illustrate the importance of ionic interactions in the formation of the network. Figure 2 shows the changes in pellet weight and water binding to sedimented proteins as a function of the pH of the dispersion solution. When pH was near the isoelectric point of caseins, attractions between proteins increased, and interactions between protein and lipid seemed to be reinforced. The pellet weight was above 70 g/kg of cheese and

represented the proteins and the portion of the lipids that were still interacting under the conditions of dispersion. Pellet weight decreased markedly as the pH of the dispersion solution was raised above 5.3 (Figure 2). This effect of pH on protein solubilization corresponded essentially to a variation in the net charge of proteins. As pH increased above 5.3, carboxyl groups became negatively charged (pK_a of glutamic and aspartic acids are, respectively, 4.25 and 3.86) and induced the formation of sodium caseinates. Under these conditions of pH, the quantity of water that was bound to sedimented proteins increased (Figure 2). Importantly, interactions in process cheese were not eliminated as pH increased, and the enhancement of ionic repulsions in the cheese dispersion did not totally disperse proteins. Other interactions maintained the cheese structure, reinforcing the theory of the cooperativity between the different interactions existing in process cheese (15). Marchesseau and Cuq (15) have previously shown, using an ultracentrifugation test in the presence of various dissociating agents, that covalent bonds were not primarily responsible for process cheese gel cohesion.

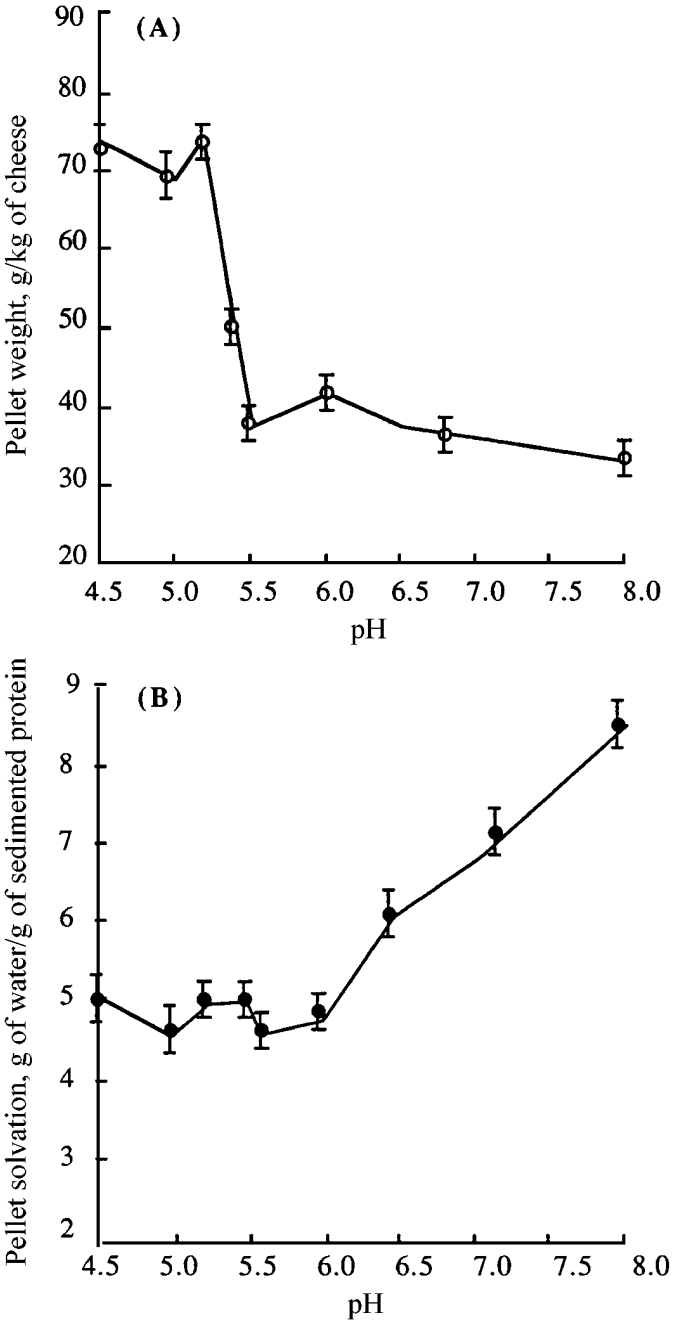

Figure 2. Changes in pellet weight (A) and pellet solvation (B) of process cheese cooked at 115°C for 1 min and stirred at 95°C for 8 min (pH 5.7) as a function of the pH of the dispersion aqueous solutions. The ionic strengths of the dispersion solutions were, respectively, 0.6, 0.1, 0.3, 0.7 at pH 4.5, 5.5, 6.5, 7.5. Ultracentrifugation conditions: dispersion (1:5, wt/vol) of cheese in aqueous solution at different pH values and ultracentrifugation at 86,000 × *g* for 25 min at 20°C.

The effects of the addition of different concentrations of NaCl and $CaCl_2$ on the rate of protein sedimentation were reported in Table 3. Although the addition of salts modified pH (particularly $CaCl_2$), results showed that both ionic strength and the nature of the added cations influenced the solubility of process cheese proteins. At conditions of similar ionic strength, the extent of protein sedimentation appeared to be affected more by the addition of monovalent cations (Na^+) than by addition of divalent cations (Ca^{2+}). After a similar increase in ionic strength (0.1 to 1), NaCl greatly increased protein solubility; solubility remained fairly constant when $CaCl_2$ was added. Such differences suggested that NaCl addition could lead to the formation of soluble sodium caseinates; $CaCl_2$ addition seemed to induce calcium bridging between proteins. However, at the higher ionic strength, when $CaCl_2$ was added, pellet weight decreased (Table 3). Under these conditions, the addition of $CaCl_2$ reduced the pH below the isoelectric point of casein. Greater protonation of the carboxylic groups imparted a net positive charge on the proteins and minimized the calcium bridging of divalent cations between the polypeptidic chains.

CONCLUSIONS

Minor variations in pH substantially affected the structure of process cheese. The emulsification, peptization, and ability of casein particles to interact in a heat-induced gel structure such as process cheese were largely dependent on the physicochemical state of the protein, which depended on pH and on ionic

composition and strength. Adjustment of pH values between 5.7 and 6.0 during the manufacture of process cheese produced a regular tridimensional network that entrapped spherical fat particles about 2 to 3 μm in diameter but avoided protein aggregation. In this pH range (5.7 to 6.0), the forces that were primarily responsible for gel cohesion appeared to be noncovalent bonds (hydrogen bonds, hydrophobic, and electrostatic interactions), which enhanced elasticity and springiness of the gel. Small changes in pH and ionic composition and strength induced substantial modifications during cheese processing and, consequently, had important repercussions on the final structure of the network that was formed with dissociated caseins.

ACKNOWLEDGMENTS

Financial support for this work was provided by the Direction Générale de l'Alimentation, Paris, France. The authors thank M. Martin for her contribution to a part of this work and M. Datas for the quality of the microphotographs.

REFERENCES

1 Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. Anal. Biochem. 70:241.
2 Buchheim, W., and P. Jelen. 1976. Microstructure of heat-coagulated whey protein curd. Milchwissenschaft 31:589.
3 Caric, M., M. Gantar, and M. Kalab. 1985. Effects of emulsifying agents on the microstructure and other characteristics of process cheese—review. Food Microstruct. 4:297.
4 Caric, M., and M. Kalab. 1993. Process cheese products. Page 339 *in* Cheese: Chemistry, Physics and Microbiology. P. F. Fox, ed. Elsevier Appl. Sci., London, England.
5 Desfleurs, M., and B. Bailleul. 1975. Quick determination of cheeses fat content. Lait 542:68.
6 Glaser, J., P. A. Carroad, and W. L. Dunkley. 1979. Surface structure of cottage cheese curd by electron microscopy. J. Dairy Sci. 62:1058.
7 Hall, D., and L. K. Creamer. 1972. A study of sub-microscopic structure of Cheddar, Cheshire and Gouda by electron microscopy. N.Z. J. Dairy Sci. Technol. 7:95.
8 Heertje, I., M. J. Boskamp, F. van Kleef, and F. H. Gortemaker. 1981. The microstructure of processed cheese. Neth. Milk Dairy J. 35:177.
9 Kalab, M., H. W. Modler, M. Caric, and S. Milanovic. 1991. Structure, meltability and firmness of process cheese containing white cheese. Food Microstruct. 10:193.
10 Kalab, M., J. Yun, and S. H. Yiu. 1987. Textural properties and microstructure of process cheese food rework. Food Microstruct. 6:181.
11 Kiely, L. J., P. S. Kindsted, G. M. Hendricks, J. E. Levis, J. J. Yun, and D. M. Barbano. 1993. Effect of draw pH on the development of curd structure during manufacture of Mozzarella cheese. Food Struct. 11:217.
12 Klostermeyer, H., and W. Buchheim. 1988. Microstructure of process cheese products. Kiel. Milchwirtsch. Forschungber. 40: 219.
13 Lee, B. O., G. Kilbertus, and C. Alais. 1981. Ultrastructural study on process cheese. Effect of different parameters. Milchwissenschaft 36:343.
14 Lee, B. O., D. Paquet, and C. Alais. 1979. Biochemical study of the cheese processing: peptization. Lait 589:589.
15 Marchesseau, S., and J. L. Cuq. 1995. Water-holding capacity and characterization of protein interactions in process cheese. J. Dairy Res. 62:479.
16 Mistry, V. V., and D. L. Anderson. 1993. Composition and microstructure of commercial full-fat and low-fat cheeses. Food Struct. 12:259.
17 Olson, N. F., D. G. Vakaleris, and W. V. Price. 1958. Acidity and age of natural cheese as factors affecting the body of pasteurized process cheese spread. J. Dairy Sci. 41:1005.
18 Paquet, D. 1988. Process cheeses: physico-chemical aspects. Page 227 *in* Functional Properties of Food Macromolecules. D. Lorient, B. Colas, and M. Le Mestre, ed. Les Cahiers de l'ENSBANA, Tec. Doc. Lavoisier, Paris, France.
19 Rayan, A. A., M. Kalab, and C. A. Ernstrom. 1980. Microstructure and rheology of process cheese. Scanning Electron Microsc. 3:635.
20 Rousseau, M., and C. Le Gallo. 1990. Scanning electron microscopic study of the structure of Emmental cheese during manufacture. Lait 70:55.
21 Swaisgood, H. E. 1992. Chemistry of the caseins. Page 63 *in* Advanced Dairy Chemistry. 1. Proteins. P. F. Fox, ed. Elsevier Appl. Sci., London, England.
22 Tamine, A. Y., M. Kalab, G. Davies, and M. F. Younis. 1990. Microstructure and firmness of process cheese manufactured from Cheddar cheese and skim milk powder cheese base. Food Struct. 9:23.
23 Tatsumi, K., S. Ohaba, I. Nakajima, K. Shinohara, and G. Kawanishi. 1975. The effect of emulsifying salts on the texture of process cheese. III. The effect of emulsying salts on the state of dispersion of casein. J. Agric. Chem. Soc. 49:481.
24 Visser, J., A. Minihan, P. Smits, S. B. Tjan, and I. Heertje. 1986. Effects of pH and temperature on the milk salt system. Neth. Milk Dairy J. 40:351.
25 Yiu, S. H. 1985. A fluorescence microscopic study of cheese. Food Microstruct. 4:99.