Temperature Effect on Structure-Opacity Relationships of Nonfat Mozzarella Cheese¹

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ABSTRACT

Our objective was to determine the effect of heating on the structure of nonfat Mozzarella cheese and then to relate changes in structure to changes in cheese opacity. Cheese was made according to a direct-acid, stirred-curd procedure. Cheese samples, at 4°C, were taken on d 1 and placed into glass bottles, which were sealed and heated. Once the cheese reached 10°C or 50°C, the bottles were placed on a scanner and color values measured. Samples were also taken on d 1 for chemical, micro, and ultrastructural analyses. Applying heat increased cheese opacity. At 50°C the cheese was more opaque than at 10°C. The increase in temperature induced changes in cheese structure. Larger high-density protein aggregates and increased protein concentration in the protein matrix were observed in cheese at 50°C. Applied heat would favor hydrophobic interactions, and possibly, re-association of β -casein and calcium with the protein matrix, promoting protein-to-protein interactions. Thus, the protein matrix contracts, occupying less cheese matrix area, and microphase separation occurs, causing serum pockets to grow in size, and microstructural heterogeneity to increase. It is proposed that the increased size of aggregates and heterogeneity of the cheese at 50°C promote light reflection, thus increasing cheese opacity. We concluded that applying heat alters protein interactions in the cheese matrix, manifested as changes in cheese structure. Such changes in structure help provide an understanding of changes in cheese opacity.

(Key words: hydrophobic interactions, protein matrix, color, whiteness)

Abbreviation key: SEM = scanning electron microscopy, TEM = transmission electron microscopy.

INTRODUCTION

The opacity or whiteness of cheese is the manifestation of light scattered by aggregates, molecules, and atoms that compose the cheese. The scattering of light by the cheese, or any other system, is related to its heterogeneity at the molecular or even atomic level (Bohren and Huffman, 1983; Zemb, 1990). When a particle, molecule or atom is stroked by an electromagnetic wave, electric charges are set into oscillatory motion and the particle radiates electromagnetic energy in all directions (Bohren and Huffman, 1983). Therefore, the study of cheese opacity deals with the study of cheese structure and heterogeneity. The ionic environment of the cheese, influenced by variables such as calcium, moisture, and salt (sodium chloride) content, affects the status of the protein matrix by altering protein interactions, which may alter the structure of the cheese (Paulson et al., 1998). However, physical factors may also affect protein interactions. Interactions under entropic control, such as hydrophobic interactions, are promoted by an increase in temperature (Payens, 1979) and are considered the major source of favorable association free energy in the formation of protein aggregates (Bloomfield, 1979). Thus, heat constitutes a driving force promoting protein-to-protein interactions, which could in turn affect cheese structure. Previous studies (Metzger et al., 2000; Dave et al., 2001), on the effect of temperature on the opacity of reduced and nonfat cheeses suggest that changes in the serum phase of the cheese could account for the differences in cheese opacity. However, the protein matrix of the cheese, comprising most of the cheese matrix, was not analyzed as a possible contributor to cheese opacity. Therefore, our objective was to define how the temperature of the cheese affects cheese ultra- and microstructure, and then to determine whether changes in cheese structure could explain changes in cheese opacity.

MATERIALS AND METHODS

Cheese

Nonfat Mozzarella cheese made using a direct-acid, stirred-curd procedure, as previously described (Dave et

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al., 2001) was used for this study. The composition of the cheese was 0.2% fat, 37.0% protein, 60.0% moisture, 1.5% salt, and 0.6% calcium. The pH of the cheese was 5.3. Cheese was obtained 1 d after manufacture and its opacity determined by measuring L^* values using a color scanner with LAB Smart software (Westcor, Logan, UT). Cylindrical cheese samples (2.5 cm diameter and 4 cm height), at 4°C, were placed into flat-bottom glass bottles, which were then sealed with rubber stoppers and heated. Once the cheese reached either 10°C or 50°C, the samples were placed in the scanner and color values determined. Cheese samples at 10°C and 50°C were also prepared for structural analysis.

Electron Microscopy

Scanning electron microscopy (SEM) was used to prepare low magnification (3000×) micrographs for image analysis of bulk arrangements of the cheese matrix, whereas transmission electron microscopy (TEM) was used to prepare both low (3000×) and high (85,000×) magnification micrographs. The high magnification images were then used to characterize protein-to-protein arrangements within the protein matrix.

SEM. Cheese samples, (approximately 1 mm × 1 mm × 10 mm), were taken at 10°C and 50°C on d 1, and fixed in fresh 3% glutaraldehyde for 3 h (also at 10°C and 50°C, respectively). The glutaraldehyde solution was changed every 30 min. Once fixed, the samples were stored in new glutaraldehyde solution at 4°C until further processed according to McManus et al. (1993). Samples were frozen in liquefied Freon 22 (−159°C; Mallinckrodt Inc., Paris, KY), transferred to liquid nitrogen, cryofractured perpendicular to their long axis, and thawed in 2% glutaraldehyde. They were then dehydrated in a graded ethanol series followed by fat extraction with Freon 113 (Mallinckrodt Inc., Paris, KY). After overnight storage in Freon 113 at 4°C, the samples were rehydrated by reversing the graded ethanol series, and washed with a 0.1 M sodium cacodylate buffer (Electron Microscopy Sciences, Fort Washington, PA), pH 7.2. The samples were then postfixed for 2 h with a solution containing 1% OsO₄ (Electron Microscopy Sciences, Fort Washington, PA) and 1.5% K₄Fe(CN)₆·3H₂O (Fisher Scientific Co., Fair Lawn, NJ). This solution was replaced by a 2% tannic acid (Mallinckrodt Inc., Paris, KY) solution in cacodylate buffer, and the samples were left for 3 h at 20°C. The tannic solution was then replaced with osmium tetroxide-potassium ferrocyanate solution, and samples left for 4 h. This solution was later replaced with an aqueous solution of 1% hydroquinone (Mallinckrodt Inc., Paris, KY), and samples left overnight. After postfixing, the samples were washed with distilled water, dehydrated in a graded ethanol series, and critical-point dried in

a critical-point drier (Model 1200; Polaron, Waterford, England) with CO₂. Samples were viewed in a field emission scanning electron microscope (Model S-4000T FESEM; Hitachi Scientific Instruments, Mountain View, CA) operated at 3 kV. Images from each sample, at 3000× magnification, from two randomly selected fields were recorded on Kodak TMX 120 film.

TEM. Cheese samples, approximately 1 mm³, were obtained from previously taken and fixed samples for SEM. The samples were stored in new glutaraldehyde solution at 4°C until further processed according to Paulson et al. (1998). Samples were then placed into 1% OsO₄ (Electron Microscopy Sciences, Fort Washington, PA) in 0.2 M cacodylate buffer for 1 h (Electron Microscopy Sciences, Fort Washington, PA). After dehydration in a graded ethanol series, the samples were infiltrated with Spurr's epoxy overnight, transferred to Beem capsules filled with Spurr's epoxy, and heated to 70°C for 24 h. Sections, 70 to 80 nm thick, were cut on an Ultracut ultramicrotome (Leica Inc., Deerfield, IL), transferred to 300-hex mesh grids, and counterstained with uranyl acetate and lead citrate. They were then viewed on an electron microscope (Model 902; Carl Zeiss, Inc. Thornwood, NY) at an accelerating voltage of 80 kV. Transmission electron micrographs, at 3000× and 85,000× magnifications, from two randomly selected fields were then recorded on Kodak SO 163 film.

Image Analysis

SEM micrographs. Negatives of scanning electron micrographs were scanned and their digital images recorded. The images were then uploaded into Adobe Photoshop 4.0 (Adobe Systems Inc., San Jose, CA), and their grayscale values analyzed. Dark areas (corresponding to areas previously occupied by serum) were differentiated from light areas (corresponding to protein matrix) by applying the threshold function. A threshold level of either 60 or 75 was selected for each micrograph, depending on the individual image characteristics, so that a precise differentiation between dark and light areas was obtained. The proportions of dark and light pixels were then determined by applying the histogram function.

TEM micrographs. Negatives of transmission electron micrographs taken at low and high magnification were scanned and their digital images recorded. High-magnification images were then uploaded into Adobe Photoshop 4.0, and a representative 512 × 512-pixel area was selected by applying the crop function. The cropped images were then uploaded into NIH Image 1.60 (National Institutes of Health, Washington, DC), and the threshold function, at a level equal to the mean density value of each image, was applied to allow for differentia-

tion between high- (dark) and low- (light) density regions. Regions of the micrograph with a high-density of electrons were considered to represent protein aggregates within the protein matrix. The percentage of area of the micrographs representing protein aggregates and their mean density value were determined by applying the measure function of the software, which computes the area and mean gray value of the current selection. Density was measured in the grayscale from 0 to 255, with 0 corresponding to white and 255 to black. Aggregate size (area) was determined in a 250×250 -pixel image area, cropped from the 512×512 -pixel image, by applying the measure function. Then, by assuming the aggregates to be spheres, their mean volume was determined. The number of aggregates was determined in a 170×170 -pixel image by applying the measure function. Considering the width of the cheese section to be 75 nm in average, and assuming no superposition of aggregates within the three-dimensional section, the concentration, or number of aggregates per unit volume of protein matrix could be calculated. Then, by considering the aggregates to be evenly distributed within the section, the distance between adjacent aggregates was determined. Also, the volume of the section occupied by them was determined.

Experimental Design and Statistical Analysis

The experiment was conducted in quadruplet with treatments, cheese temperatures of 10°C and 50°C, randomly assigned. Two replications were considered for SEM and TEM image analysis. For TEM, two samples from each replicate were analyzed to determine the number, size, and area occupied by high-density protein aggregates. When determining size, up to 200 aggregates per sample were measured, and values greater than four standard deviations were disregarded. The mean aggregate size from each sample was then considered for analysis. Statistical analysis, ANOVA, was performed using SAS (1991).

RESULTS

Cheese Opacity

At 10°C the cheese was translucent, and it had a color L^* value of 78.0. In contrast, at 50°C, the cheese was opaque and had a color L^* value of 91.5 ($P < 0.001$).

Cheese Microstructure

Differences between cheeses at 10°C and 50°C could be visually observed in images obtained by SEM (Figure

1) and low-magnification TEM (Figure 2). In the TEM images, cheese at 10°C (Figure 2a) had a less dense protein matrix with fewer and smaller serum pockets present in the cheese matrix, as compared with cheese at 50°C (Figure 2b). This was also observed in scanning electron micrographs. In cheese at 10°C (Figure 1a), spherical serum pockets, ranging in size between 0.6 and 1.0 μm in diameter, were observed in the cheese matrix occupying 1% of the matrix area (Figure 1c). In contrast, at 50°C (Figure 1b), the cheese matrix had bigger pockets, ranging in size between 0.6 and 7.0 μm in diameter and occupying 31% of the cheese matrix area (Figure 1d). There was, however, no difference in number of pockets observed. In terms of protein matrix, there was a corresponding reduction in area, with cheese at 50°C having a reduced area occupied by protein matrix, as compared with cheese at 10°C, 69% versus 99% ($P = 0.022$).

Cheese Ultrastructure

Differences between high-magnification TEM images of cheese at 10°C and 50°C could be observed visually (Figure 3). At 10°C (Figure 3a), the protein matrix appeared less dense and was more homogenous than at 50°C (Figure 3b). The lower temperature was also associated with the presence of a higher number of aggregates and smaller mean aggregate size (Table 1). Even though the largest aggregates were observed in cheese at 10°C, a higher frequency of small aggregates led to their lower mean size. These aggregates were located 15 nm apart and occupied 48% of the area of the protein matrix (Table 1). In contrast, at 50°C, a bigger size but lower number of aggregates in the protein matrix was observed (Table 1). This resulted in an increase of area (+15%) and volume (+40%) of protein matrix occupied by aggregates in cheese at 50°C. On average, the aggregates were located 24 nm apart. Also, the mean density value was higher for cheese at 50°C than at 10°C (Table 1). Assuming the aggregates to be spheres and uniformly distributed within the protein matrix, a graphical two-dimensional representation of their arrangement in a $100 \times 100 \times 75$ -nm section of matrix was developed (Figure 4). At 10°C the aggregates were smaller and located closer together (Figure 4a) as compared with aggregates at 50°C (Figure 4b). Figure 4c shows that if the aggregates at 10°C were drawn to the same size as observed at 50°C there would be insufficient protein material to account for the increased size of protein aggregates observed at 50°C. Thus, at 50°C there was more protein material per unit volume of protein matrix than at 10°C.

Figure 1. Scanning electron micrographs of nonfat cheese at 10°C (A) and at 50°C (B) and their corresponding binary images after thresholding (C) and (D).

Figure 2. Transmission electron micrographs of nonfat cheese at 10°C (A) and 50°C (B).

Figure 3. Transmission electron micrographs of nonfat cheese at 10°C (A) and 50°C (B) showing selected fields for image analysis. Aggregate size was determined using a 250 × 250-pixel field, and aggregate number was determined by using a 170 × 170-pixel field, as outlined in A and B. The corresponding binary images of selected fields after thresholding, (C) and (D), are also shown.

DISCUSSION

Cheese Structure

When the temperature of the cheese increased from 10°C to 50°C, larger high-density protein aggregates

Table 1. Comparison of the characteristics of protein aggregates that comprise the protein matrix of nonfat cheese at 10°C and 50°C.

Parameter measured	Cheese temperature		<i>P</i> ^d
	10°C	50°C	
Aggregate area ^a (%)	48	55	0.034
Aggregate density ^b	91	157	0.008
Aggregate size (nm ²)	1.9	7.0	0.074
Aggregate number ^c	181	37	0.045

^aArea of protein matrix occupied by high-density aggregates.

^bGray value in the 0 to 255 grayscale.

^cDetermined in a 170 × 170-pixel area, as shown in Figures 3c and 3d.

^dProbability that mean values for cheese at 10°C and 50°C were not different.

were present in the protein matrix. This increase in aggregate size suggests that protein-to-protein interactions were promoted as the temperature was raised. At the same time as bigger aggregates were formed, the number of aggregates in the protein matrix decreased (Table 1). Although we did not determine aggregate composition, it can be assumed that the composition of the aggregates was probably different, with aggregates at 50°C containing more β -casein. Among the caseins, β -casein has the greatest average hydrophobicity and its solubility is greatly affected by temperature (Bingham, 1971). The decreased solubility of β -casein as it is heated is thought to be the major parameter affecting heat-induced opacity of cheese (Metzger et al., 2000; Dave et al., 2001). Dissociation of β -casein from the micelle occurs at low temperature as a consequence of weakening of hydrophobic interactions and removal of colloidal calcium phosphate (Pierre and Brule, 1981). This dissociation is reversible and dependent on temperature. Disso-

Figure 4. Schematic diagram of relative size and number of electron-dense aggregates per unit volume of protein matrix, based upon a $100 \times 100 \times 75$ -nm section of cheese matrix, showing the increase in aggregate size and spacing between aggregates that occurs when cheese is heated from 10°C (A) to 50°C (B). The resultant contraction of the protein matrix is shown in C.

ciation of β -casein from the protein matrix at 10°C possibly contributed to the occurrence of a higher number of smaller aggregates observed at that temperature.

In analyzing electron micrographs, dark areas have high electron density and light areas have low electron density. This contrast is based on binding of heavy metal atoms (Os, Ur, and Pb) by proteins. Thus, heavy metals are used for “staining” the protein fraction of the cheese matrix. The higher the retention of heavy metals, the more electrons that are scattered, and consequently, the darker the micrograph image. Therefore, the higher electron density of cheese at 50°C (Table 1) was indicative of more protein present per unit volume of protein matrix. To obtain this increased concentration of protein requires a contraction of the protein matrix.

As hydrophobic interactions become more important with temperature increase, there would be a reassociation of β -casein (and possibly calcium) with the other proteins. Calcium promotes protein-to-protein interactions (Bloomfield, 1979), and its reassociation at the higher temperature may have also contributed to the occurrence of bigger aggregates by affecting interactions involving α_{s1} - and α_{s2} -casein as well as β -casein. This promotion of aggregation at the molecular level is then manifested as changes in cheese structure. Contraction of the protein matrix increases the overall density of the matrix, and serum pockets throughout the cheese matrix become larger (Figure 1b compared to 1a). Thus, changes in cheese structure, as the temperature of the cheese increases, are ultimately brought about by changes in the extent and nature of protein interactions.

Cheese Opacity

A nonfat cheese can be considered to be composed of two interacting phases, the serum and the protein matrix. The effect of changes in the serum phase of cheese during heating and cooling on cheese whiteness was suggested by Metzger et al. (2000). Based on in vitro formation of a white β -casein gel from cheese serum, they suggested that formation of such a gel during heating of cheese would increase the whiteness of the cheese. However, it has not been demonstrated that such a gel would be formed in vivo within the cheese matrix. When cheese is heated, β -casein would be surrounded by other proteins, and formation of a β -casein homopolymeric gel would have to compete with reassociation of β -casein with other proteins in the cheese matrix. Such association of β -casein with α_{s1} -, α_{s2} -, and para- κ -casein would deplete the serum of protein, and a serum gel would not form.

In the present study, no evidence of such a separate serum gel was found either in scanning or transmission electron micrographs of the cheese. If a protein gel were formed, it should have been at least partially retained by means of fixation as a consequence of sample preparation for electron microscopy. Also, when the ultrastruc-

ture of the cheese was examined, no apparent discontinuity in the protein matrix was observed, as would be the case when two different gel matrices were present. Therefore, the contribution of such a heat-induced serum gel to the observed increased opacity of cheese does not appear feasible.

The structure of the protein matrix at 50°C can be considered similar to the particulate type of gel that can be formed by aggregation of whey proteins. In Langton and Hermansson's study (1992) of the effect of pH on the formation of β -lactoglobulin and whey protein gels, the occurrence of such a gel network was associated with the observation of a white rather than a translucent gel. A translucent gel was observed when a fine-stranded network was formed. At pH 4.0, a white β -lactoglobulin gel was formed that had an open and irregular network, with increased density fluctuations as compared with translucent gel, which was formed at pH 3.5. Accordingly, the protein matrix of cheese at 50°C appeared less uniform, with more density fluctuations and bigger openings, as was observed by Langton and Hermansson (1992), when a white β -lactoglobulin gel was formed.

In their study, bigger aggregates were present in the white gel than in the more translucent gel. Similarly, larger aggregates were observed in the protein matrix of cheese at 50°C. Therefore, in agreement with previous observations, the occurrence of larger aggregates and more density fluctuations in the protein matrix was associated with increased light reflection and opacity of the cheese. Whether such changes in protein aggregation contribute to differences in reflection of light should be considered according to light scattering principles.

The scattering of light by a small particle (longest dimension being less than $\lambda/20$; i.e., spherical particles with diameter less than 25 nm, for an average wavelength of light of 500 nm) is determined by Rayleigh's equation:

$$\frac{I_{\theta}}{I_{o,u}} = \frac{9\pi^2(n_1^2 - n_0^2)^2}{2\lambda^4 r^2(n_1^2 + 2n_0^2)^2} \nu^2 N_p (1 + \cos^2\theta) \quad (\text{Eq. 1})$$

where $I_{o,u}$ is the initial intensity of unpolarized light of wavelength λ in the surrounding medium; I_{θ} is the intensity of scattered light; θ is the angle measured in the plane of the initial and scattered beam; r is the distance from the particle at which the scattered light is measured; n_0 is the refractive index of the surrounding medium; n_1 is the refractive index of the particle; ν is the particle volume; and N_p is the number of particles per unit volume (Hunter, 1989).

Protein matrix. In the case of a solid, a large number of small scattering centers will scatter light, and the waves will interfere either constructively or destructively, depending on the phase difference (Dickinson and

Stainsby, 1982). However, in practice, an interparticle distance about three times the radius of the particle may be considered as a sufficient condition for independence (Zemb, 1990). This means that if the particles are sufficiently apart from one another their scattering properties can be considered without reference to the surrounding scatterers. Therefore, based upon the small size of the aggregates ($< \lambda/20$), and the distance between them (> 3 radii), they can be considered to behave independently as Rayleigh scattering elements. According to Eq. 1, and considering that λ , θ , and r would remain constant when cheese is heated, the intensity of scattered light becomes a function of the difference in refractive index of the particles and surrounding medium (n_1 and n_0), the volume of the particles (ν), and the number of particles per unit volume (N_p). Consequently, the bigger the size of the particle and the higher its concentration, the more light will be scattered.

When cheese temperature increased from 10°C to 50°C, the size of the aggregates increased from 1.6 nm to 3.0 nm in diameter. This was accompanied by a decrease in aggregate concentration from 181 to 37 aggregates per $7.5 \times 10^5 \text{ nm}^3$ -protein matrix section. However, when the change in aggregate volume and concentration were considered in Eq. 1, the product $\nu^2 N_p$ was bigger for cheese at 50°C than at 10°C (9.1×10^{-3} versus 9.5×10^{-4} , respectively). Thus, the increase in average size of the aggregates more than compensates for the decrease in particle concentration and would contribute to increased scattering of light. And since the particles are far enough apart to be considered independent light scatterers, they increase the opacity of the cheese when heated to 50°C.

In addition to the changes in aggregate size that promote light scattering of cheese at 50°C, changes in refractive index should also be considered. The refractive index of the continuous and dispersed phases of the protein matrix (serum and protein aggregates, respectively) was not determined, but any changes would have also contributed to increased light scattering. If β -casein moves from very soluble to insoluble as cheese is heated, its concentration in the serum would decrease, thus decreasing the refractive index of the serum. At 50°C, the protein aggregates were more compact (i.e., had higher electron density), so if anything, their refractive index would increase. Therefore, as aggregation was promoted, differences in chemical composition and physical properties between the serum and protein aggregates probably increased. This would have resulted in an increased difference between the refractive index of the continuous and dispersed phases, thus further promoting light scattering in accordance with Eq. 1.

Serum pockets. Another consequence of increasing protein-to-protein interactions as cheese was heated was

the increase in size of serum pockets in the cheese matrix (Figures 1 and 2). This can be considered a manifestation of microphase separation. Such phase separation has been recognized to occur in the formation of turbid or opaque gels from globular proteins (Clark, 1998). As the proteins approach their isoelectric point, or are subject to high ionic strength, a gel is formed which normally releases water and is characterized by presenting a heterogeneous microstructure. Also, when heating cycles are applied to globular protein gels, the system may become progressively demixed and phases may separate.

In the present experiment, as the continuous phase (serum) became increasingly different in composition and physical properties with respect to the dispersed phase (protein aggregates), phase separation occurred. Thus, serum pockets grew in size from a maximum size of 1.0 μm in diameter at 10°C to 7.0 μm at 50°C (Figure 1). This indicates that increased protein-to-protein interactions, promoted by an increase in cheese temperature, results in a decreased capacity of proteins to interact with water. As a consequence, protein hydration decreases and serum separates from the protein matrix, increasing the size of the serum pockets. Such an increase in size of serum pockets increases the heterogeneity of the system at the microstructural level. This increases the difference between the refractive index of the continuous (protein matrix) and dispersed (serum pockets) phases as they separate from one another. Consequently, and according to Eq. 1, this would also promote light scattering.

Serum pockets themselves may also be considered scattering elements. According to the Mie scattering theory, as particles grow to larger than the wavelength of light, all wavelengths of light tend to be equally scattered, which usually results in a white sample appearance (Waldman, 1983). Therefore, as serum pockets grow in size they could promote scattering of light, scattering all wavelengths equally. As we did not collect data regarding either the composition or refractive index of the serum, the actual independent contribution of the serum pockets to light scattering remains uncertain. Still, in general, the increase in heterogeneity of the system brought about by the increase in size of the serum pockets would have contributed to promoting light scattering and opacity of the cheese.

CONCLUSIONS

Increasing cheese temperature from 10 to 50°C induced changes in both the ultra- and microstructure of the cheese. Heating cheese promotes hydrophobic interactions and reassociation of β -casein and calcium with the other caseins in the protein matrix. These increased interactions between proteins result in larger and denser

protein aggregates, as well as an increase in protein concentration in the protein matrix. Also, as the ability of the proteins to interact with water decreases, serum is released from within the protein matrix, the matrix contracts and microphase separation would occur. As the phases, serum and protein matrix, separate from one another, the serum pockets in the cheese matrix grow in size. These changes in cheese structure were associated with changes in cheese opacity. Both an increase in size of protein aggregates and heterogeneity of the system help promote light reflection that results in increased opacity of the cheese.

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REFERENCES

- Bingham, E. W. 1971. Influence of temperature and pH on the solubility of α_{s1} , β , and κ -casein. *J. Dairy Sci.* 54:1077–1080.
- Bloomfield, V. A. 1979. Association of proteins. *J. Dairy Res.* 46:241–252.
- Bohren, C. F., and D. R. Huffman. 1983. Pages 3–4 in *Absorption and Scattering of Light by Small Particles*. John Wiley & Sons, New York, NY.
- Clark, A. H. 1998. Gelation of globular proteins. Pages 117–118 in *Functional Properties of Food Macromolecules*. S. E. Hill, D. A. Ledward, and J. R. Mitchel, ed. Aspen Publishers Inc., Gaithersburg, MD.
- Dave, R. I., D. J. McMahon, J. R. Broadbent, and C. J. Oberg. 2001. Reversibility of the temperature-dependent opacity of nonfat Mozzarella cheese. *J. Dairy Sci.* 84:2364–2371.
- Dickinson, E., and G. Stainsby. 1982. *Experimental methods*. Pages 201–202 in *Colloids in Foods*. Applied Science Publishers, New York, NY.
- Hunter, R. J. 1989. Behaviour of colloidal dispersions. Pages 72–73 in *Foundations of Colloid Science*. Vol. 1. Oxford University Press, New York, NY.
- Langton, M., and A. M. Hermansson. 1992. Fine-stranded and particulate gels of β -lactoglobulin and whey protein at varying pH. *Food Hydrocolloids* 5:523–539.
- McManus, W. R., D. J. McMahon, and C. J. Oberg. 1993. High resolution scanning electron microscopy of milk products: A new sample preparation procedure. *Food Struct.* 12:475–482.
- Metzger, L. E., D. M. Barbano, M. A. Rudan, P. S. Kindstedt, and M. R. Guo. 2000. Whiteness change during heating and cooling of Mozzarella cheese. *J. Dairy Sci.* 83:1–10.
- Paulson, B. M., D. J. McMahon, and C. J. Oberg. 1998. Influence of sodium chloride on appearance, functionality, and protein arrangements in nonfat Mozzarella cheese. *J. Dairy Sci.* 81:2053–2064.
- Payens, T. A. J. 1979. Association of caseins and their possible relation to structure of the casein micelle. *J. Dairy Sci.* 49:1317–1324.
- Pierre, A., and G. Brule. 1981. Mineral and protein equilibria between the colloidal and soluble phases of milk at low temperature. *J. Dairy Res.* 48:417–428.
- SAS User's Guide: Statistics, Version 6.1 Edition. 1991. SAS Inst., Inc., Cary, NC.
- Waldman, G. 1983. Introduction to light. Page 174 in *The Physics of Light, Vision, and Color*. Prentice Hall Inc., New York, NY.
- Zemb, T. 1990. Scattering of connected networks. Pages 3–4, 303 in *Neutron, X-Ray and Light Scattering: Introduction to an Investigative Tool for Colloidal and Polymeric Systems*. P. Lindner and T. Zemb, ed. North-Holland, The Netherlands.