Fluorescence Image Analysis of Process Cheese Manufactured with Trisodium Citrate and Sodium Chloride[1]

M. SUTHEERAWATTANANONDA,[2] R. G. FULCHER,[2]
F. B. MARTIN,[3] and E. D. BASTIAN[2]
University of Minnesota, St. Paul 55108

ABSTRACT

Fluorescence imaging and cryomicrotomy were used to study the size and shape distribution of fat particles in a model process cheese product. The technique generated data with reproducibility and consistency as confirmed by the statistical results. The process cheese product was manufactured with Cheddar cheese, NaCl, and trisodium citrate in a batch-type, twin-screw process cheese cooker with a 4.5-kg capacity. After reaching 65.5°C, samples were collected from each batch at cooking times of 0, 5, 10, and 15 min. The geometry of fat particles (circularity and diameter of the area equivalent circle) from both types of model process cheese was evaluated. Fat particles in the process cheese product that was manufactured with trisodium citrate were more circular than those in the process cheese manufactured with NaCl only. The diameter of the area equivalent circle of fat particles in process cheese containing trisodium citrate decreased and stabilized after 5 min of cooking time, but those in process cheese containing NaCl did not change.
(**Key words**: fluorescence microscopy, cheese microstructure, image analysis, fat emulsification)

Abbreviation key: **CLSM** = confocal laser scanning microscopy, $\mathbf{D_{ec}}$ = diameter of the area equivalent circle, **FITC** = fluorescein isothiocyanate, **SEM** = scanning electron microscopy, **TEM** = transmission electron microscopy.

INTRODUCTION

Fat globules in cheese have been studied extensively using various microscopic techniques: light microscopy, scanning electron microscopy (**SEM**), and transmission electron microscopy (**TEM**) (4, 5, 9, 10, 12, 13, 18, 23, 24). Image contrast is a common limiting factor for conventional light microscopy (31). For SEM, fat globules in cheese are subjected to organic solvents that are removed during the sample preparation, which may introduce artifacts (31). Although TEM offers high and low resolution to study cheese microstructure, samples are required to be dry, thin, and conductive (28). If present, fine air pockets in the structure may remain unidentified and may be interpreted as fat globules. In addition to the limited sample size and the lengthy sample preparation time, SEM and TEM are expensive and time consuming to use, making these techniques less appealing for cheese analysis (17, 31).

Confocal laser scanning microscopy (**CLSM**) also has been used to study fat structure in dairy products (3, 7). The CLSM produced better images with higher depth resolution than were produced by the conventional microscope. However, Vodovotz et al. (30) indicated that resolution is reduced when food emulsions are examined with CLSM, resulting in little advantage over conventional methods. When Nile Red and fluorescent isothiocyanate (**FITC**) were used to stain lipids and proteins in the same sample, the fluorescence of Nile Red and FITC could not be completely separated. Consequently, some protein fluorescence is captured in the lipid fluorescence image (3). Cheese that has been stained with Nile Red also undergoes severe photobleaching, resulting in rapid fading of the images within 1 to 2 s (7). The rhodamine B fluorescence dye could eliminate the photobleaching and distinguish fat particles from the protein matrices. However, any air pockets in cheese structure may not be distinguished from fat particles (7).

By comparison, fluorescence microscopy utilizes very sensitive and specific fluorescent probes to obtain chemical and structural data, provides relatively high resolution, and requires minimal sample preparation, making it flexible and efficient (31). However, fluorescence microscopy has one serious disadvantage: it is sensitive to environmental factors such as

Received March 18, 1996.
Accepted September 30, 1996.
[1]Published as Paper Number 22,283 of the contribution series of the Minnesota Agricultural Experiment Station and based on research conducted under Project 18-036. This research was sponsored, in part, by the Minnesota-South Dakota Dairy Foods Research Center and Dairy Management, Inc.
[2]Department of Food Science and Nutrition.
[3]Statistical Center, School of Statistics.

temperature, pH, and ionic strength (20). Meaningful data can be obtained only by carefully controlling these environmental factors. Several researchers (14, 15, 25) have used fluorescence microscopy to study fat and proteins in dairy products, but few have used this method for cheese analysis.

Nile Blue A has been reported as a possible fat stain for microscopy (2) and was commonly used as a fluorochrome for staining fat particles in cereals during the 1980s (8, 32). Yiu (31) showed the microstructure of fat and protein in both natural and process cheeses by using Nile Blue A as a fluorochrome and suggested that fluorescence microscopy with cryosectioning could be used as a procedure for monitoring cheese quality. The same technique was used to demonstrate structural differences between the protein matrices and fat globule characteristics of process cheese food (12).

Because cheese is a very heterogeneous material (13), a single electron micrograph showing a very small part (1 to 2 mm in diameters) may not represent the overall structure of the cheese. Large composite micrographs are needed to provide analytical information that is more reliable than can be obtained with small areas of a single micrograph (12). Microscopy coupled with image analysis could be used to evaluate several fields of a sample rather than a single image.

To study the size distribution of fat in process cheese, TEM and image analysis have been used (12). The diameter of fat particles decreased from >1 μm to about 0.35 μm after emulsification; fat particles showed irregular shapes at 30°C because milk fat is only 10% solid at that temperature. As the temperature is lowered from 30 to 10°C, the percentage of solidified fat increases from 10 to 60% (29). Everett et al. (7) used CLSM to study the structure of fat globules in cheese and found that the diameter and circularity of fat globules were not normally distributed. To date, no report has studied fat particle distributions in process cheese using fluorescence microscopy coupled with an image analysis system to monitor changes during processing. Our objective was to use fluorescence imaging to study the influence of cooking time on fat distribution and the extent of emulsification of process cheese manufactured with an emulsifying salt versus a nonemulsifying salt.

MATERIALS AND METHODS

Process Cheese Manufacture

Model process cheese was manufactured from 1-mo-old Cheddar cheese (Land O' Lakes, Arden Hills, MN), 5% (wt/wt) sweet whey powder (Davisco International, Inc., LeSueur, MN), 2% (wt/wt) NaCl or trisodium citrate, and water in a twin-screw cheese cooker (model CC-0010; Blentech Corp., Rohnert Park, CA). This system is only a model because whey protein is incorporated (which is not legally allowed in the standard of identity for process cheese) and because the cheese was formulated to contain the same moisture content as the original cheese to facilitate direct comparison of fat globules in natural and process cheeses. The 4.5-kg batch cheese cooker was equipped with direct and indirect culinary systems for steam injection. The cheese was manufactured with 103.42 kPa indirect steam heating and a screw speed of 110 rpm. The cheese formula was calculated to produce process cheese with moisture contents between 36 and 37%. After 65.5°C was reached and maintained, 450-g samples from each batch were collected after cooking times of 0, 5, 10, and 15 min. During this period, agitation was continued by maintaining a screw speed at 110 rpm. The experiment was repeated three times, providing 24 samples from six batches. Three batches contained trisodium citrate, and three were manufactured with NaCl. The samples with no cooking time were obtained when the cooking temperature reached 65.5°C. All samples were cooled immediately to 4°C.

Chemical Analysis

Moisture, protein, and fat contents were determined for each cheese sample using methods approved by AOAC (1). The total chloride content of process cheese was determined by using an automated chloride salt titrator (model 926 chloride analyzer; Ciba Corning Analytical, Halstead, Essex, England) following the procedure in the manual of the chloride titrator.

Sectioning and Staining

One cheese sample (0.5 cm^3) was cut from the center of each cheese block. The sample was placed on a cryostat stub that had been surfaced with Tissue-Tek O.C.T. (Miles Inc., Elkhart, IN) and frozen at –15°C. The sample was sectioned to approximately 8 μm in thickness. Six sections were collected from each sample, transferred onto microscopic slides at room temperature (25°C), air-dried, and stained with 0.3% (wt/vol) Nile blue A (Aldrich Chemical Co., Milwaukee, WI) for 2 min, rinsed briefly with distilled water, and mounted in water. The stained sections were held at 4°C and analyzed within 4 h.

Microscopic Analysis

A research microscope (Zeiss Universal; Carl Zeiss, Eching, Germany), fitted with fluorescence filters and a 200-W mercury arc lamp, was used to examine stained cheese sections. The filter set included an excitation barrier, and maximum excitation occurred at 450 to 490 nm. Fat particles appeared as bright yellow and were visualized through a 16× 0.40 Neofluar objective lens (Carl Zeiss). Images of fat particles were captured with a charged coupled device video camera module (model XC-77; Sony Co., Ltd., Tokyo, Japan) and analyzed by the Interaktives Bild-Analysen System Image Analysis System (Kontron Elektronik, Zurich, Switzerland). Localized heating of the samples was controlled by minimizing the exposure time to less than 5 s per field per measurement (7). The images were processed within 10 s in the following steps: contour smoothing, normalizing, emphasizing, multiplying, discriminating, identifying frame and objects, and measuring. All objects touching the measuring frame were automatically excluded from measurement. The field depth and other parameters were kept constant throughout the analysis. About 0.4 mm^2 were measured randomly from each sample. Size data were converted from individual area to the diameter of the area equivalent circle ($\mathbf{D_{ec}}$). Particle characteristics were evaluated as follows:

$$\text{Circularity (F circle)} = \frac{4\Pi \times A}{P^2}$$

where A = area, and P^2 = perimeter squared.

$$D_{ec} = 2\sqrt{\left(\frac{\text{Area}}{\Pi}\right)}.$$

Statistical Analysis

The split-plot ANOVA was used; 4.5-kg batches were used as the whole plot, and 450-g bricks of cheese were used as the split plots. The effects of NaCl, trisodium citrate, different cooking times (0, 5, 10, and 15 min), and differences among trials were tested. Significance was determined at $P = 0.05$. The preliminary results showed that the D_{ec} distributions of fat particles in process cheese that had been analyzed with an image analyzer were not normally distributed with nonconstant variances. We obtained 1500 data points from each sample. Because the data were not normally distributed, use of ANOVA directly on the data was not possible. When the data for each sample were sorted in ascending order, the D_{ec} values and circularity at specified percentiles for all 24 cheese samples could be used for ANOVA without violating the required assumptions. As confirmed by residual analysis, the percentile data were normally distributed with constant variances. The 25th, 50th, 75th, 90th, 95th, and 99th percentiles were selected for ANOVA (MACANOVA version 3.12) (21). Thus, for each variable (D_{ec} and circularity), six analyses were performed, one at each percentile. Each of the six analyses was done using 24 values (total of 23 df), and each value was taken from the cumulative distribution at the specified percentile. The model used for ANOVA was as follows: Y = replicate + salts + [replicate × salts(error 1)] + cooking times + [cooking times × salts] + error 2.

RESULTS AND DISCUSSION

Observations During Processing

During cooking, the model process cheese made with NaCl did not flow well, and the cheese formed a doughy mass in the cooker. Separation of oil from the cheese mass was not observed throughout cooking, and the texture of the cheese appeared to be dry, firm, and rubbery. Unlike process cheese made with NaCl, process cheese made with trisodium citrate melted as the temperature increased and oil was released and reincorporated back into the cheese mass. This release and emulsification of fat in the cheese also has been observed by other researchers (5, 34). Shimp (26) suggested that heating and agitation released fat and caused re-emulsification, but our results suggest that emulsifying salt is the major factor involved. Ellinger (6) suggested that emulsifying salts react with para-casein and its fragments to change the emulsifying property of the para-casein. Trisodium citrate is a strong chelator of micellar calcium resulting in the dispersion of para-casein (19, 24, 26). Because calcium ions are sequestered by emulsifying salts, protein solubility increases (34). When calcium is replaced by sodium ions, intermolecular bridges between casein molecules are broken, but, in our process cheese made with NaCl, this exchange was not sufficient to emulsify the fat.

Cheese Microstructure

Figure 1 shows fat particles in the young Cheddar cheese (a and b) that was used for manufacturing

process cheese, process cheese containing NaCl (c and d), and process cheese containing trisodium citrate (e and f). Fat particles in young Cheddar cheese varied in size and were clustered throughout the cheese matrix (Figure 1a). Natural cheese is composed of fused curds that consist of a protein matrix containing entrapped fat globules. Reduced sphericity of fat globules has been observed in natural cheese 1 d after

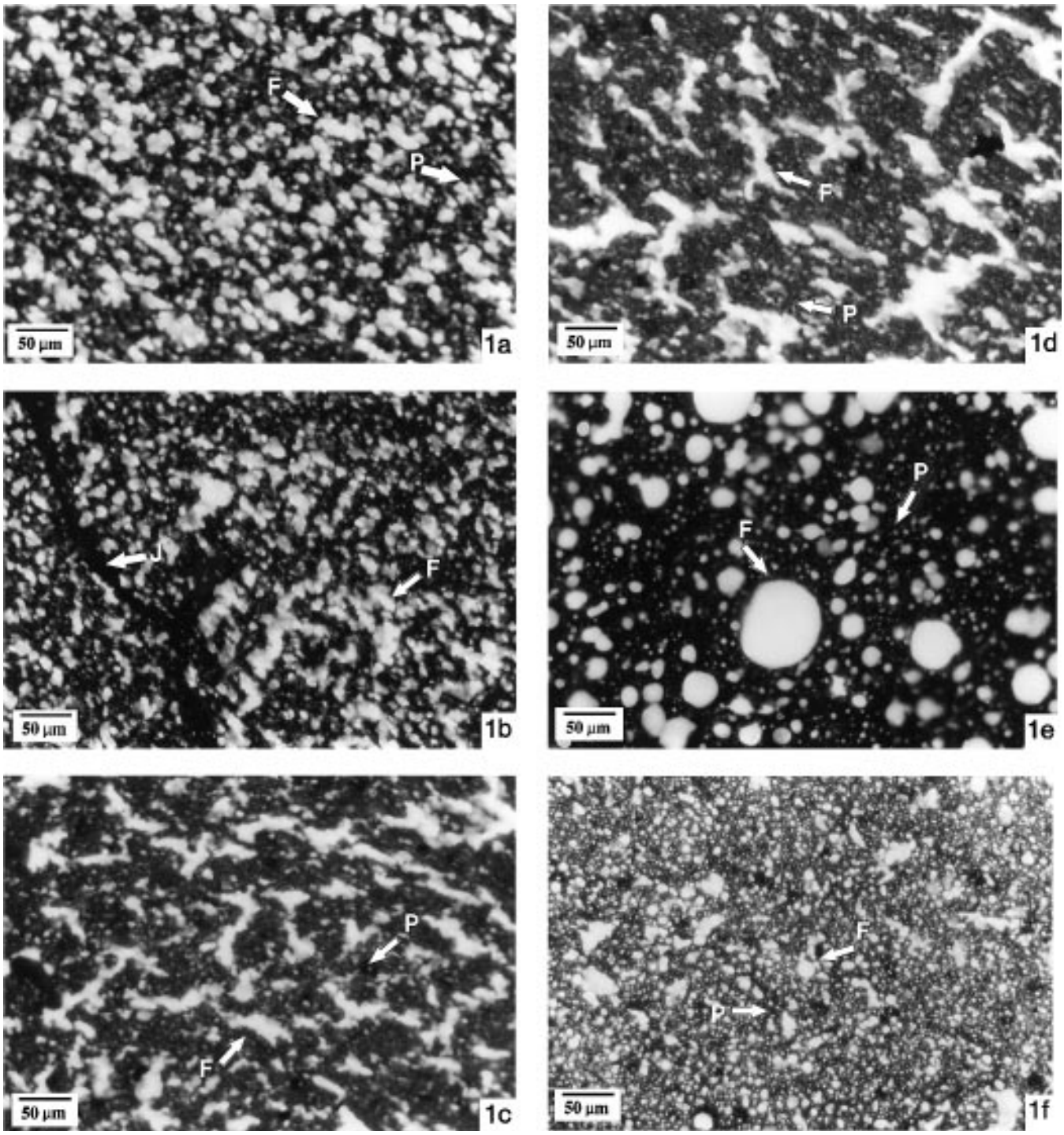

Figure 1. A section of young Cheddar cheese (a) showing cheese curd junctions (b), a section of process cheese manufactured with NaCl at 0 min (c) or 5 min (d) of cooking time, and a section of process cheese manufactured with trisodium citrate at 0 min (e) or 5 min (f) of cooking time. All samples were stained wth 0.3% (wt/vol) of Nile Blue A (Aldrich Chemical Co., Milwaukee, WI). Fat particles (F), protein (P), and curd junction (J) are indicated.

TABLE 1. Cheese composition.

Composition	Young Cheddar	Processed with NaCl	Processed with Trisodium citrate
		(%)	
Moisture after cooking			
15 min	36.00	35.99	36.00
0 min[1]		36.00	36.25
Fat	35.05	32.42	32.95
Protein	23.46	22.11	22.17
Chloride	0.93	1.66	0.97

[1]Prior to holding.

manufacture (7), and, although some fat globule membranes remain intact, the natural milk emulsion appears to be partially disrupted (5, 11). During ripening, the boundary between curd particles fuses together and becomes junctions that are mostly devoid of fat globules (Figure 1b) (5, 31).

Process cheese containing NaCl showed an intact protein structure that was similar to the structure of young Cheddar cheese; considerable numbers of small round fat particles were embedded in the matrix (Figure 1, c and d). Some large oblong fat particles were observed throughout the cheese structure. The protein matrices were partially disrupted and sheared by mechanical force during mixing, causing association of small fat particles. The distortion of the protein matrix and the coalescence of small fat particles resulted in a torn appearance. Prolonged cooking and mixing did not appear to change the overall cheese structure. Zehren (33) and Rao (22) hypothesized that lack of effect might be a result of the casein-phospholipid complex in natural state. This complex might be strong enough to resist physical shear in the process cheese cooker. Consequently, the mechanical force and the heat applied during processing did not allow fat separation and fat emulsification (Figure 1, c and d).

TABLE 2. The ANOVA of size and shape of fat particles in model process cheese.

Response	df	Circularity (F circle)	D_{ec}[1]
		P	
Replication	2	NS[2]	NS
Salts (S)	1	0.05	0.05
Cooking time (CT)	3	0.05	0.05
S × CT	3	0.05	0.05

[1]Diameter of the equivalent circle.

[2]$P > 0.05$.

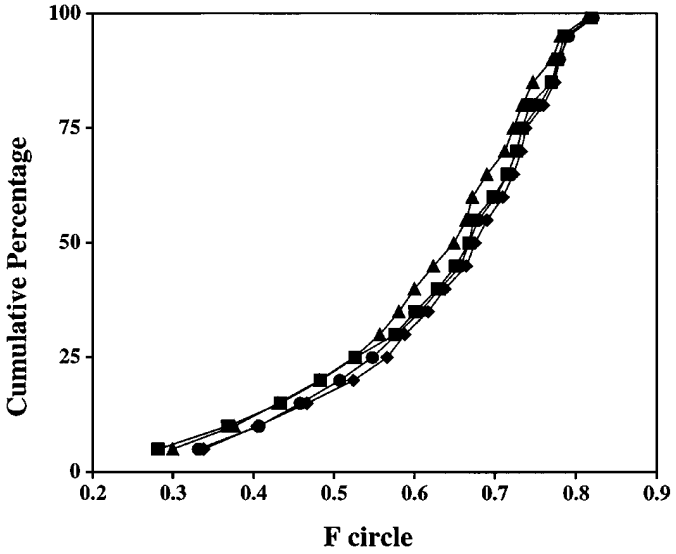

Figure 2. The cumulative distribution function of circularity (F circle) of fat particles in process cheese with NaCl at cooking times of 0 (■), 5 (♦), 10 (●), and 15 (▲) min.

In contrast, process cheese with trisodium citrate contained fat particles of various sizes (Figure 1, e and f) that became spherical and evenly distributed. The size of fat particles decreased as cooking time increased. Immediately after reaching the cooking temperature (Figure 1e), large, medium, and small spherical fat particles existed together. After 5 min of cooking (Figure 1f), the cheese structure was com-

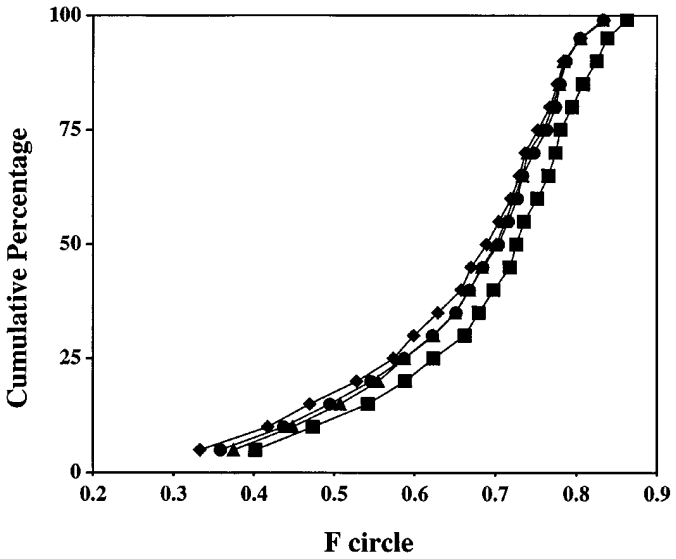

Figure 3. The cumulative distribution function of circularity (F circle) of fat particles in process cheese with trisodium citrate at cooking times of 0 (■), 5 (♦), 10 (●), and 15 (▲) min.

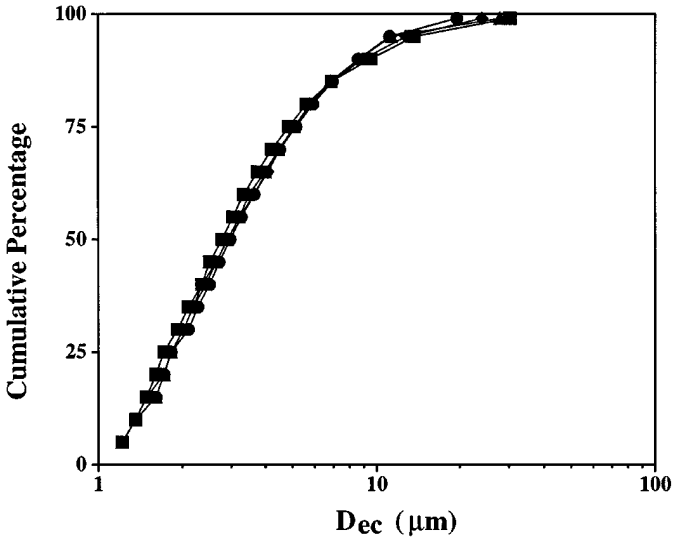

Figure 4. The cumulative distribution function of the diameter of the area equivalent circle (D_{ec}) of fat particles in process cheese with NaCl at cooking times of 0 (■), 5 (♦), 10 (●), and 15 (▲) min.

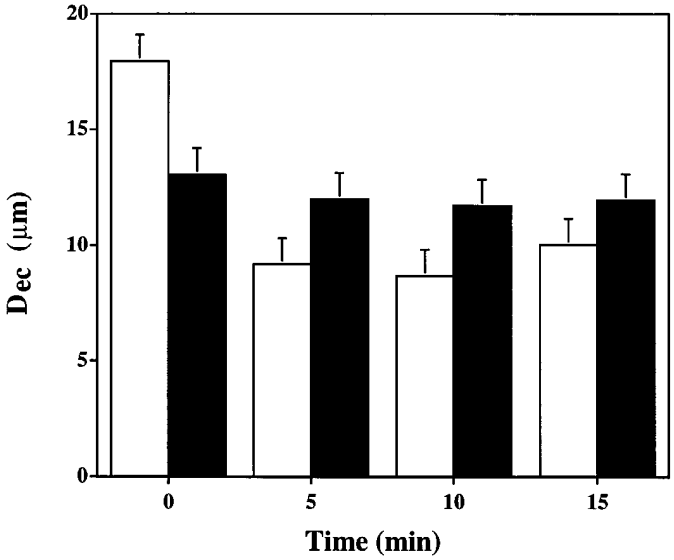

Figure 6. Least squares means (± SE) of the diameter of the area equivalent circle (D_{ec}) at the 95th percentile showing changes of fat particle size in process cheeses containing sodium citrate (open bars) and NaCl (closed bars) at the cooking times of 0, 5, 10, and 15 min.

posed of small and very small fat particles homogeneously mixed together. Cooking from 5 to 15 min (data not shown) did not appear to influence the size of fat particles. Similar observations on the emulsification of fat particles during the manufacture of process cheese with several emulsifying salts have been made by Rayan et al. (23) using SEM and TEM. Zehren and Nusbaum (34) suggested that stirring at elevated temperatures reduced the fat globule size.

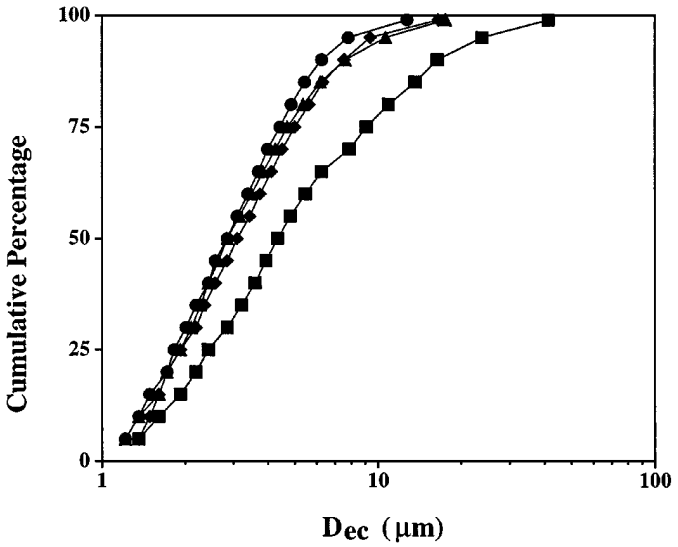

Figure 5. The cumulative distribution function of the diameter of the area equivalent circle (D_{ec}) of fat particles in process cheese with trisodium citrate at cooking times of 0 (■), 5 (♦), 10 (●), and 15 (▲) min.

Cheese Composition

Table 1 shows the chemical analysis of cheese and process cheese. All cheeses contained about 36% moisture. The difference in moisture contents between samples at 0 and 15 min of cooking time was within 0.5%. Fat and protein contents of process cheese were lower than those of young Cheddar cheese because of the addition of salts and sweet whey powder. The chloride contents of young Cheddar and process cheese made with trisodium citrate were equal to each other but were lower than that of process cheese containing NaCl.

Digital Image Analysis

The results from fluorescence image analysis were consistent and reproducible in this experiment; differences were not significant among trials. Table 2 shows the summary of ANOVA. Different salts and cooking times significantly influenced the circularity and D_{ec} of the fat particles. The interaction of salts and cooking times also influenced the fat size and shape. Figures 2 and 3 show the cumulative distribution for circularity of fat particles in process cheese made with NaCl and sodium citrate. The circularity of process cheese made with NaCl ranged from 0.28 to

0.81, indicating that none of the fat particles were truly circular. Most of the fat particles (from 25 to 100% of the data) were in the range of 0.5 to 0.8, an observation that correlates well with the circularity of fat particles in natural Cheddar cheese; Everett et al. (7) showed that, at 1 d after manufacture, fat particles in Cheddar cheese had sphericity (circularity) clustered around 0.66 to 0.70. Fat particles in process cheese made with trisodium citrate were more circular than those in process cheese made with NaCl (compare Figures 2 and 3). The circularity of fat particles was highest in process cheese containing trisodium citrate at the beginning of the cooking process (65.5°C). During mixing at the cooking temperature, the circularity of fat particles in process cheese made with trisodium citrate decreased, but circularity still remained higher than that in cheese containing NaCl.

Because Sinfort et al. (27) suggested that classic particle size analysis based on individual particle size probably would not be able to discriminate food products with clusters, D_{ec} was chosen as the variable for fat size because it minimized the effects of clustering (16, 28). The D_{ec} data exhibited the same skewness as observed by Everett et al. (7). The distribution of D_{ec} for process cheese containing NaCl did not change as the cooking time changed, but the fat particles for trisodium citrate cheese became smaller as the cooking time increased (Figures 4 and 5). After 5 min of cooking time, no further detectable changes occurred in the D_{ec} of fat particles in cheese made with trisodium citrate (Figure 6).

CONCLUSIONS

Characteristics of fat in process cheese can be quantitatively analyzed by a fluorescence imaging technique with minimum chemical manipulation and quick sample preparation. The technique takes advantages of fluorescence microscopy to distinguish fat particles from other cheese materials and offers good resolution. With the aid of a computerized image processing system, tedious manual counting and analysis can be done accurately within a short time and cover large areas of sample. Statistical comparison of different cheese samples is difficult because the data are not normally distributed and have nonconstant variances. Because cheese microstructure is heterogeneous, a large number of data points are required. The ANOVA of specific percentiles can be used to test for differences between population distributions. The results produced by the fluorescence imaging technique are consistent and reproducible, and there were no significance differences between replicates.

Using fluorescence imaging, we were able to show differences in fat globule size and shape between process cheese made with trisodium citrate and NaCl. The fat in cheese made with NaCl was not well emulsified, as indicated by lower circularity and unchanging size during cooking and mixing. In cheese made with trisodium citrate, fat globules were more circular and decreased in size during cooking and mixing.

ACKNOWLEDGMENTS

The authors thank Connie Chou, Research Assistant at the University of Minnesota Statistics Clinic, for assisting with experimental design and statistical analysis.

REFERENCES

1 Association of Official Analytical Chemists International. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.
2 Barnes, P. J., and R. G. Fulcher. 1989. Fluorometric measurement of fats. Page 1 *in* Fluorescence Analysis in Foods. L. Munck, ed. Longman Sci. Tech., New York, NY.
3 Blonk, J.C.G., and H. van Aalst. 1993. Confocal scanning light microscopy in food research. Food Res. Int. 26:297.
4 Brooker, B. E., D. G. Hobbs, and A. Turvey. 1975. Observations on the microscopic crystalline inclusions in Cheddar cheese. J. Dairy Sci. 42:341.
5 Càric, M., M. Gantar, and M. Kaláb. 1985. Effects of emulsifying agents on the microstructure and other characteristics of process cheese—a review. Food Microstruct. 4:297.
6 Ellinger, R. H. 1972. Phosphates as Food Ingredients. The Chemical Rubber Co. Press, Cleveland, OH.
7 Everett, D. W., K. Ding, N. F. Olson, and S. Gunasekaran. 1995. Applications of confocal microscopy to fat globule structure in cheese. Page 321 *in* Chemistry of Structure-Function Relationships in Cheese. E. L. Malin and M. H. Tunick, ed. Plenum Press, New York, NY.
8 Fulcher, R. G., and S. I. Wong. 1980. Inside cereals—a fluorescence microchemical view. Page 1 *in* Cereals for Food and Beverages. G. E. Inglett and L. Munck, ed. Academic Press, New York, NY.
9 Green, M. L., A. Turvey, and D. G. Hobbs. 1981. Development of structure and texture in Cheddar cheese. J. Dairy Res. 48:343.
10 Kaláb, M. 1977. Milk gel structure. VI. Cheese texture and microstructure. Milchwissenschaft 32:449.
11 Kaláb, M., and D. B. Emmons. 1978. Milk gel structure. 9. Microstructure of cheddared curd. Milchwissenschaft 33:670.
12 Kaláb, M., J. Yun, and S. H. Yiu. 1987. Textural properties and microstructure of process cheese food rework. Food Microstruct. 6:181.
13 Kimber, A., B. E. Brooker, D. G. Hobbs, and J. H. Prentice. 1974. Electron microscope studies of the development of structure in Cheddar cheese. J. Dairy Res. 41:389.
14 King, N. 1955. Fluorescence microscopy of fat in milk and milk powder. J. Dairy Res. 22:205.
15 King, N. 1958. Observations by fluorescence microscopy of casein in milk, curd and cheese. J. Dairy Res. 25:312.
16 Langton, M., and A. M. Hermansson. 1993. Image analysis determination of particles size distribution. Food Hydrocolloids 7(1):11.

17 Lee, S.-Y., and C. V. Morr. 1993. Fixation staining methods for examining microstructure in whipped cream by electron microscopy. J. Food Sci. 58:124.
18 Lee, Y. H., and R. T. Marshall. 1981. Microstructure and texture of process cheese, milk curds, and caseinate curds containing native or boiled soy proteins. J. Dairy Sci. 64:2311.
19 Morr, C. V. 1967. Some effects of pyrophosphate and citrate ions upon the colloidal caseinate-phosphate micelles and ultrafiltrate of raw and heated skimmilk. J. Dairy Sci. 50:1038.
20 Munck, L. 1989. Practical experiences in development of fluorescence analyses in an applied food research laboratory. Page 1 *in* Fluorescence Analysis in Foods. L. Munck, ed. Longman Sci. Tech., New York, NY.
21 Oehlert, G. W. 1993. MACANOVA Version 3.12: An Interactive Program for Statistical Analysis and Matrix Algebra. School Stat., Univ. Minnesota, St. Paul.
22 Rao, S. R. 1951. The study of the activity of lipase on milk fat. Ph.D. Diss., Univ. Wisconsin, Madison.
23 Rayan, A. A., M. Kaláb, and C. A. Ernstrom. 1980. Microstructure and rheology of process cheese. Scanning Electron Microsc. III:635.
24 Savello, P. A., C. A. Ernstrom, and M. Kaláb. 1989. Microstructure and meltability of model process cheese made with rennet and acid casein. J. Dairy Sci. 72:1.
25 Shimmin, P. D. 1982. Observations of distribution in cheese by incident light fluorescence microscopy. Aust. J. Dairy Technol. 37:33.
26 Shimp, L. A. 1985. Process cheese principles. Food Technol. 63(6):63.
27 Sinfort, N., V. Bellon, and F. Sevila. 1992. Image analysis for in-flow measurement of particle size. Food Control 3(2):84.
28 Tamime, A. Y., M. Kaláb, G. Davies, and M. F. Younis. 1990. Microstructure and firmness of processed cheese manufactured from Cheddar cheese and skim milk powder cheese base. Food Struct. 9:23.
29 Vasic, J., and J. M. deMan. 1965. Effect of temperature history on the solid fat content of milk fat. J. Dairy Sci. 48:1277.
30 Vodovotz, Y., E. Vittadini, J. Coupland, D. J. McClements, and P. Chinachoti. 1996. Bridging the gap: use of confocal microscopy in food research. Food Technol. 50(6):74.
31 Yiu, S. H. 1985. A fluorescence microscopic study of cheese. Food Microstruct. 4:99.
32 Yiu, S. H., R. G. Fulcher, and I. Altosaar. 1982. The microscopic structure and chemistry of rapeseed and its products. Food Microstruct. 1:135.
33 Zehren, V. L. 1954. The isolation and properties of casein. Ph.D. Diss., Univ. Wisconsin, Madison.
34 Zehren, V. L., and D. D. Nusbaum. 1992. Product testing and organoleptic evaluation. Page 38 *in* Process Cheese. D. Cooley & Co., Inc., New Holstein, WI.