Turbidimetry for Crystalline Fractionation of Lard

Feng-Sheng Wang and Chin-Wen Lin*

Laboratory of Chemistry and Technology of Animal Products, Department of Animal Science, National Taiwan University, Taipei, Taiwan, Republic of China

ABSTRACT: Development of specific properties of lard, a well-known edible animal by-product and one which plays an important role in Chinese-style foods, was studied by means of multiple-step crystalline fractionation. A method for monitoring the crystallization temperature and crystallization time of lard by spectral turbidity was also studied. The capabilities of the proposed method were experimentally demonstrated through recovery of the fat crystal mass. Six significant changes in turbidity spectra, which correspond to the formation of fat crystals, were observed at various temperatures while cooling the melted lard from 50°C to the final vessel temperature at a constant cooling (0.5°C/min) and agitator rate (50 rpm). Crystallization time for each lard fraction was determined while the peak in turbidity was observed in the process of cooling at a specific temperature. Determination of crystallization time by means of turbidimetry correlated with the increase in deposition of fat crystals. No significant increase in fat crystal mass was observed when cooling prolongation after the turbidity peak for the sample was measured. Attributes of lard fractions were characterized by iodine value, saponification value, fatty acid composition, and melting profile of crystallization temperature. Based on the results, turbidimetry might be suggested as a fast and inexpensive method for monitoring the crystallization temperature and crystallization time for the routine crystalline fractionation process.

JAOCS 72, 585–589 (1995).

KEY WORDS: Crystallization, fractionation, lard, turbidimetry.

Lard contributes a unique flavor and function to some Chinese-style bakery foods and plays a major role in Chinese cooking culture. The annual production of edible lard is 120 million kg in Taiwan. However, health concerns about saturated fat and cholesterol have limited consumption of this product. Recently, utilization of edible animal by-products and the development of lard to meet the requirements of consumers and specific food processing uses has been strongly encouraged.

Fractionation that separates fats into fractions with various physical and chemical properties has been accomplished by several methods, such as short-path distillation, supercritical carbon dioxide extraction, and crystallization (1–5). Crystalline fractionation is based on crystallization of part of the fat, followed by separation of the crystals from the mother liquor. The specific nutritional and physicochemical properties of fat fractions are then determined (6). Theoretically, crystallization involves various stages of complicated solid–liquid equilibrium, phase transformation, and heat/mass transfer (7). As far as the control of fat crystalline fractionation is concerned, many conditions may affect the physicochemical characteristics of fat fractions in practice, e.g., composition of fat, agitation, and temperature programming (8–11). Although many studies have focused on studying fat polymorphism by X-ray diffraction spectroscopy (12) and the kinetics of fat crystallization by means of polarized-light microscopy, laser light diffraction spectrophotometry, or Fourier transform infrared spectroscopy (13,14), the enormous expenditure for equipment and the time-consuming nature of the determination might not suit the on-line process. There is, in contrast, little information about a handy analytical method and low-cost apparatus available for monitoring the bulk system during fractionation or routine on-line practice. Utilization of an easy measurement to observe the change in the overall appearance of samples to predict the process conditions is useful for practical purpose. Turbidimetry is experimentally simple, can be used for a wide range of particle sizes, and does not disturb the system under investigation. Furthermore, this method is fast, reproducible, and inexpensive (15). These advantages make it an attractive method for the determination of particle size, distribution or volume in latex polymerization processing (16,17), oil-in-water emulsification (18), and heat-induced aggregation of food protein dispersions (19).

The objective of this study was to monitor the crystallization temperature, crystallization time, and fractions of lard during the crystalline fractionation process by means of turbidimetry and to determine its feasibility. The attributes of lard fractions were also evaluated.

MATERIALS AND METHODS

Preparation of lard. Porcine backfat was excised from fresh, pre-chilled, slaughtered porcine carcasses and then finely ground with a meat grinder (Maschinenfabrik Seydelmann

* To whom correspondence should be addressed at 50, Lane 155, Keelung Rd. Sec. 3, Taipei, Taiwan, Republic of China.

KG, Stuttgart, Germany) fitted with a 5-mm stainless-steel plate. To provide consistency in fat composition for all experiments, a batch of 1000 kg of raw material was rendered in a steam-jacketed rotary dry-rendering cooker (Harrslev Maskinfabrik A/S, Bogense, Denmark) at 95°C for 2.5 h. After rendering, the lard oil was collected sequentially by means of filtering the dregs and horizontal centrifugation. The refining of the lard was accomplished at a local fat rendering plant. Crude lard oil was mixed with 0.1% (vol/vol) concentrated phosphoric acid and neutralized by stirring with 3 M sodium hydroxide at 80°C for 30 min. The lard oil was centrifugated after washing with 60°C water. The neutralized lard oil was decolored in a vacuum pan that contained 0.5% (wt/vol) active white clay, stirred, and heated at 380 mmHg, 80°C for 1 h, and filtered. The refined lard was stored at 4°C for further studies.

Crystalline fractionation of lard. Multiple-step crystalline fractionation (5) of the lard was prepared by pouring 1.9 L of molten lard (60°C) into a 2-L stainless-steel jacketed vessel (250 mm height × 100 mm inner diameter), and water was circulated in a refrigerated water bath (LAUDA K-20; Lauda Dr. R. Wobser GmbH, Lauda-Konigshofen, Germany). A marine-type impeller, consisting of 45° pitched propeller blades (Bel-Art Products Co., VWR Scientific Co., South Plainfield, NJ), was attached to a drive unit (EYELA DC-RT; Tokyo Rikaikikai Co. Ltd., Tokyo, Japan) and maintained at the same motor speed during the process. The lard was equilibrated to 50°C and cooled at continuous, 50-rpm agitation. The change in temperature of the water and the molten lard in the vessel was monitored with thermocouples positioned 15 mm from the center, and 15 and 120 mm from the bottom of the vessel. The thermocouples were connected to a digital recorder (Yokogawa Electronic Co., Osaka, Japan). After crystallization, the solid fat crystals of each fraction were separated from the liquid fraction by vacuum filtration through Whatman No. 1 filter paper in a chamber that was maintained at the crystallization temperature. This process was repeated at consecutively lower temperatures.

Turbidimetry for crystalline fractionation. To determine the temperature of fat crystal formation in the bulk system and the time that should be taken to deposit sufficient fat crystals at a specific temperature before the separation of the fat crystals, changes in the turbidity of the system and the fat crystal mass were measured in the process of cooling. The ultraviolet/visible turbidity spectra of bulk systems were recorded with a Beckman (Palo Alto, CA) DU-65 spectrophotometer equipped with a thermoelectric cell holder and a temperature controller with temperature programming capabilities. The wavelength was chosen by determining the transmittance peak scanned at 200–900 nm for the molten lard in a quartz cuvette with a light path of 1 cm. The crystallization temperature was determined by recording the temperature for the sample while progressively increasing turbidity in the sample during cooling. Crystallization time was measured by determining the changes in turbidity and in fat crystal mass of the sample by collecting 5-mL samples at 30-min intervals during crystallization. The correlation of turbidity and fat crystals in the sample is based on a ray of monochromatic light that passes through a solution in a transparent vessel, where the major proportion of light is absorbed and some is scattered by particles in the solution. The Beer–Lambert law states that when a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the length and concentration of the absorbing medium increases:

$$I = I_0\, e^{-k\,c\,l} \qquad [1]$$

where I_0 and I represent intensities of the incident and transmitted beam, respectively, and c and l represent the concentration of the absorbing solution and the pathlength of the absorbing medium, respectively. When logarithms are taken of Equation 1, the overall absorbance, A, of the system can be expressed as:

$$A = \log_e I_0 / I = k\,c\,l \qquad [2]$$

$$A = \log_{10} I_0/I = k'c\,l/\,2.303 \qquad [3]$$

For a suspension of spherical, isotropic particles in the absence of multiple scattering, k and k' represent the scattering coefficient, which is related to the relative size of the particle to the wavelength of the light in the medium. We hypothesize that lard crystals are isotropic particles of similar size. If Equation 3 is obeyed and l is kept constant (1cm), then the absorbance is proportional to the concentration of particles and the particle size distribution in the solution. Therefore, Equation 3 can be modified as:

$$A = C/K \qquad [4]$$

where C and K represent the concentration of the fat crystal mass and the particle size distribution in the suspension. In the process of molten lard crystallization, the deposition rate of fat crystals for time, t, at a specific temperature can be derived from Equation 4:

$$A/t = C/Kt \qquad [5]$$

$$dA/dt = K_c\,(dC/dt) \qquad [6]$$

where K_c is related to the extinction of light. Equation 6 hypothesizes that the change in turbidity of the solution is well matched to the deposition of the fat crystal mass during crystallization if the fat particle size, and particle size distribution are constant.

Deposition of fat crystals was measured by centrifugation of a 5-mL sample at 3000 × g for 5 min at the crystallization temperature. After centrifugation, liquid oil was absorbed as completely as possible with a strip of Whatman No. 1 filter paper, and then the filter paper and fat crystal cake in the centrifuge tube were weighed. To avoid crystallization or melting, the centrifuge tube was kept in a water bath set at the

crystallization temperature during absorption of the liquid oil. The deposition of fat crystals was expressed as a percentage of the sample.

Analysis of lard fraction attributes. Iodine values, saponification values, peroxide values (POV), and melting points were determined by methods Cd 1-25, Cd 3-25, Cd 8-53 and Cc 1-25, respectively, of the Americn Oil Chemists' Society (20). The fatty acid composition of each fraction was determined after conversion of the fatty acids into corresponding methyl esters (21). Gas–liquid chromatographic (GLC) analysis was accomplished with a Hitachi (Tokyo, Japan) 263-50 gas chromatograph equipped with a flame-ionization detector. GLC conditions were: a fused-silica capillary column DB-WAX (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Inc., Rancho Cordova, CA); temperature programming from 120°C for 4 min to 250°C for 6 min at 2°C/min; injector 260°C; detector 280°C; and carrier gas, nitrogen at 25 mL/min. The chromatograms were recorded on a Hewlett-Packard (Palo Alto, CA) 3396 II integrator. Correction factors were determined by analysis of an oil standard reference mixture of fatty acid methyl esters (AOCS No. 6; Sigma Chemical Co., St. Louis, MO) with a composition that resembles an average lard sample.

Statistical analysis. Analyses of variance were performed on the mean values of three replications. Probability values less than 0.05 ($P < 0.05$) were significant. Comparison of the means was based on Duncan's multiple range test. Correlation between turbidity and the fat crystal mass of molten lard was obtained by a linear regression analysis from the Statistical Analyses System.

RESULTS AND DISCUSSION

Spectra of melted lard. The spectrum of molten lard (50°C) is shown in Figure 1. The peak transmittance of the sample was observed at 530–550 nm. Therefore, the wavelength (540 nm) was chosen to determine the turbidity of the samples in the cooling process. The changes in turbidity during cooling are shown in Figure 2. The turbidity of each lard fraction markedly increased at 35, 31, 27, 24, 21, and 15°C in the process of multiple-step fractionation. The turbidity of the samples increased rapidly if the cooling system was not kept at a constant temperature. As small particles formed and joined together to form aggregates in the solution (molten lard), the coagulated or flocculated particles scattered more light than isolated particles, and the turbidity of the solution increased. Initial significant changes in turbidity at a given temperature, as shown in Figure 2, might be defined as "the temperature for the formation of lard crystals" or "the apparent crystallization temperature". Crystallization can be broken down into nucleation, the formation of crystal lattices, and aggregation with other crystal nuclei (7). The observations reported for nucleation and crystallization temperatures for fat crystallization were mainly accomplished by using polarized-light microscopy and differential scanning calorimetry, (DSC), respectively; however, these methods are time-consuming (22). The crystallization temperature of edible fat crystalline fractionation was related to the physicochemical properties of the products. However, the conditions reported for measurement may not be consistent with general practice (2,23). Although results did not show any evidence of nucleation in the sample, the fat crystals did not grow in the absence of a nucleating surface (13). Turbidimetry has the potential to measure the particle size in the submicrometer region and is easily implemented in a plant environment (24). Furthermore, this method can be operated rapidly in the process of fractionation; hence, turbidimetry in addition to DSC is suggested to determine the temperature for formation of lard crystals during cooling.

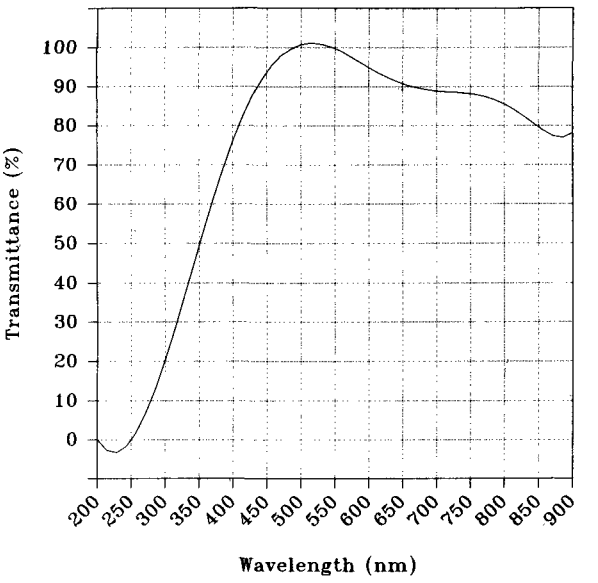

FIG. 1. Spectrum of molten lard.

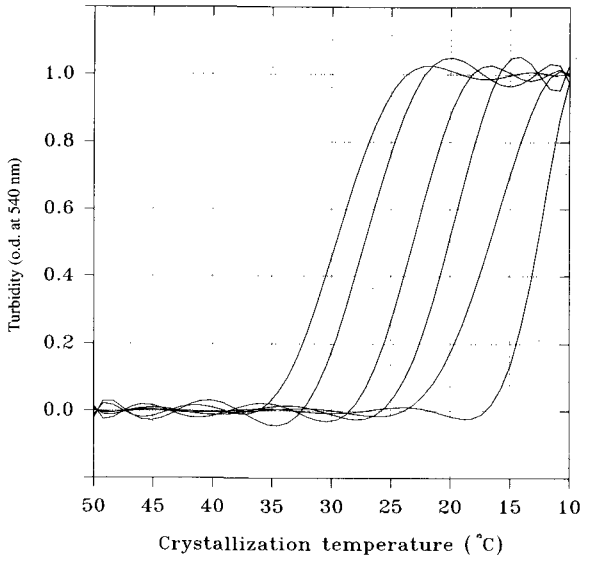

FIG. 2. Changes in turbidity of lard during multiple-step crystalline fractionation.

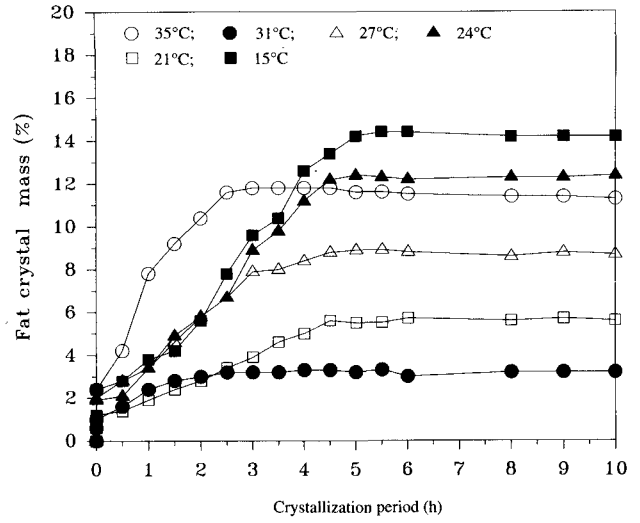

FIG. 3. Changes in fat crystal mass of lard during crystalline fractionation.

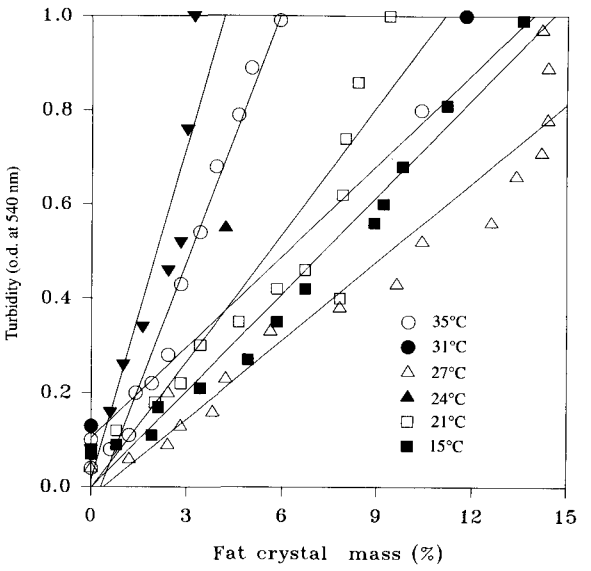

FIG. 5. Correlation between turbidity and fat crystals for molten lard crystallization at various temperatures.

Crystallization time for lard fractionation. In the process of crystalline fractionation, a conventional method to determine the proper moment for separating the fat crystal mass, i.e., crystallization time determined by turbidimetry, is necessary for on-line use. The changes in the fat crystal mass fractionated at specific temperatures are shown in Figure 3. More than four hours should be taken to form a sufficient fat crystal mass at each crystallization temperature. No further increase in the percentage of fat crystals was observed in the samples by prolonging the time. The crystallization time for each fraction was 4, 4, 6, 6, 6, and 8 h. Increases in the percentages of fat crystals in all samples parallel the change in turbidity during crystallization. In the 35°C fraction, for instance (Fig. 4), the percentage of fat crystals and the turbidity of the sample increased with crystallization time, whereas no significant increase in the percentage was observed after turbidity reached its peak. There is excellent correlation (R^2 = 0.96–0.92) between the increase in percentage of fat crystals and change in turbidity for each fraction during the estimated crystallization time (Fig. 5). Similar results were observed in kinetics of butterfat crystallization (11). Therefore, measurement of turbidity might be suggested to determine the crystallization time for fractionation of lard.

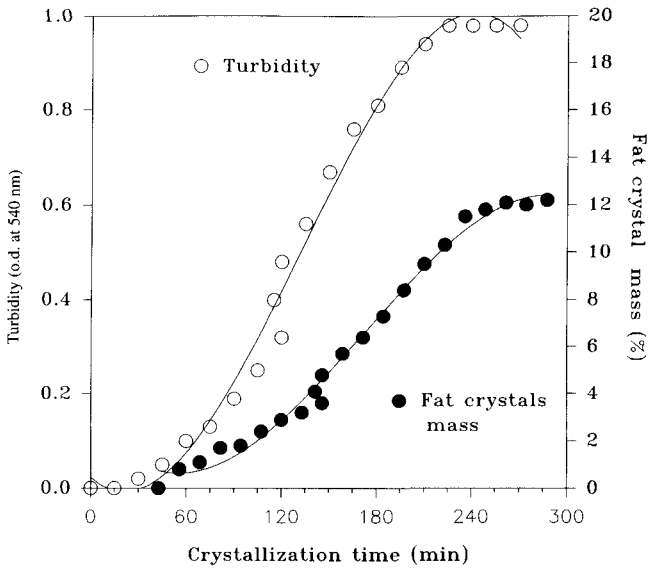

FIG. 4. Changes in turbidity and fat crystal mass of lard fraction during crystallization at 35°C.

Attributes of lard fractions. The POV values of the original lard (unfractionated) and the solid fraction obtained were measured to evaluate the effect of the various processes on oxidative stability. No significant difference of POV was observed among samples, and the values for all samples were lower than 0.1 meq O_2/ kg fat (data is not shown). Iodine values of the samples varied with crystallization temperature and increased with lower temperatures, except at 35°C (Table 1). Saponification values for the fractions also increased with the lowering of crystallization temperature, which may result from the lower molecular weights of these fractions than of the original lard. The iodine values for lard fractions correlated well with the fatty acid composition. The major fatty

TABLE 1
Attributes of Lard and Its Crystalline Fractions

Fractionation temperature (°C)	Iodine number	Saponification number	Melting point (°C)
Original lard	65.8	196.9	38.6
35	44.6	192.4	46.3
31	49.5	204.8	36.4
27	52.5	209.3	30.3
24	56.6	210.8	28.9
21	65.4	214.5	24.2
15	73.7	217.3	12.6

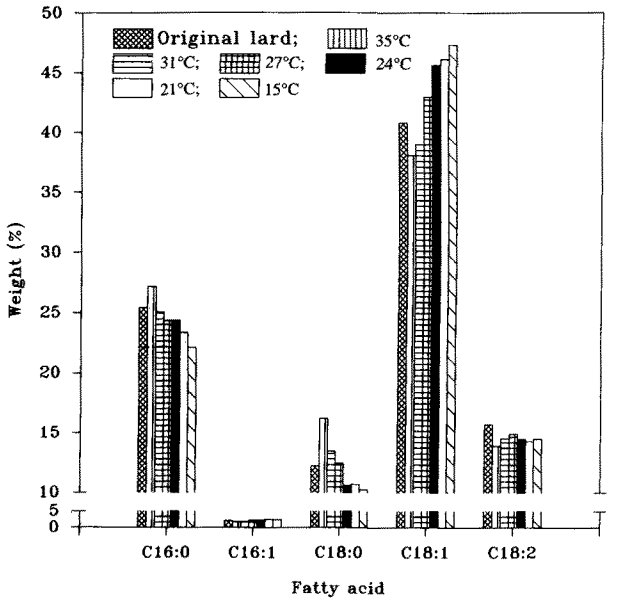

FIG. 6. Fatty acid composition in lard fractions after fractionation at various temperatures.

acids in the fractions, observed by GLC analysis, were palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). In the 35°C fraction, the major saturated fatty acids, C16:0 and C18:0, were significantly ($P < 0.05$) greater than in the original lard and in fractions obtained at lower temperatures. On the other hand, the content of unsaturated fatty acids, C16:1, C18:1, and C18:2, decreased (Fig. 6). The content of C16:0 and C18:0 decreased with crystallization temperature. The extent of decrease of C16:0, compared with the original lard, was 8.2% for the 24°C fraction and 13.2% for the 15°C fraction. In addition, the content of C18:0 decreased 12.6% for the 24°C fraction and 16.4% for the 15°C fraction. However, the content of C16:1, C18:1, and C18:2 increased with the crystallization temperature. The content of oleic acid, the major unsaturated fatty acid in lard, for the 31 and 15°C fractions increased 5.37 and 15.2% compared with the original lard. The unsaturated fat of the lard fractions significantly ($P < 0.05$) increased with crystallization temperature except for the 35°C fraction. As fractionation progressed to lower temperatures, the increasing iodine values parallel to the increasing content of unsaturated fatty acids. However, the melting point for the fractions decreased with crystallization temperature, except at 35°C (Table 1). The lard fractionated at 35°C can serve as a shortening and/or hardening agent for margarine, while the softer solid lard fractionated at 31–24°C might serve as a substitute for the spreadable glazing agent in Chinese-style baking. The clear appearance of the liquid lard fractionated at 24–15°C could be used as a cooking oil. Design of an automatic measurement system to determine the fat crystal mass in on-line routine operations, experimentation toward utilization of the lard fractions and evaluation of the oxidative stability of these products for various food processes should proceed.

ACKNOWLEDGMENTS

This work was supported by grants from the Council of Agriculture, Taiwan, Republic of China. We gratefully acknowledge the equipment assistance of the Meat Processing & Fat Rendering Plant, Frozen Foods Section, Lee-Tah Farming Industry Ltd. Co., Kaoshiung, Republic of China on Taiwan.

REFERENCES

1. Arul, J., A. Boudreau, J. Makhlouf, R. Tardif and T. Bellavia, *J. Am. Oil Chem. Soc. 65*:1642 (1988).
2. Hierro, M.T.G., and G. Santa-Maria, *Food Chem. 45*:189 (1992).
3. Versteeg, C., *Food Res. Quat. 51*:32 (1991).
4. Grompone, M.A., *J. Am. Oil Chem. Soc. 66*:253 (1989).
5. Kaylegian, K.E., and R.C. Lindsay, *J. Dairy Sci. 75*:3307 (1992).
6. D'Souza, V., L. deMan and J.M. deMan, *Ibid. 68*:153 (1991).
7. Hartel, R.W., in *Physical Chemistry of Foods*, edited by H.G. Schwartzberg, and R.W. Hartel, Marcel Dekker, Inc. New York, 1992, p. 42.
8. Herrera, M.L., J.A. Segura, G.J. Rivarola and M.C. Anon, *J. Am. Oil Chem. Soc. 69*:898 (1992).
9. van Putte, K.P.A.M., and B.H. Bakker, *Ibid. 64*:1138 (1987).
10. D'Souza, V., L. deMan and J.M. deMan, *Ibid. 69*:153 (1992).
11. Grall, D.S., and R.W. Hartel, *Ibid. 69*:741 (1992).
12. deMan, J.M., *Food Res. Int. 25*:471 (1992).
13. Ozilgen, S., C. Simoneau, J.B. German, M.J. McCarthy and D. S. Reid, *J. Sci. Food Agric. 61*:101 (1993).
14. Kellens, M., W. Meeussen and H. Reynaers, *J. Am. Oil Chem. Soc. 69*:906 (1992).
15. Kourti, T., J.F. MacGregor and A.E. Hamielec, in *Particle Size Distribution II*, edited by T. Provder, Americn Chemical Society, Washington, D.C., 1991, p. 1.
16. Chen, S-A., and S-T. Lee, *Macromolecules. 24*:3340 (1991).
17. Kourti, T., and J.F. MacGregor, in *Particle Size Distribution II*, edited by T. Provder, American Chemical Society, Washington, D.C., 1991, p. 64.
18. Walstra, P., *J. Colloid Interface Sci. 74*:163 (1975).
19. Dickinson, E., and G. Stainsby, in *Colloids in Food*, Applied Science Publishers Ltd. London, 1990, p. 196.
20. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by R.C. Walker, American Oil Chemists' Society, Champaign, Methods Cd 1-25, Cd3-25, Cd8-53, Co 1-25.
21. Christie, W.W., in *Lipid Analysis*, edited by W.W. Christie, Robert Maxwell, New York, 1982, p. 23.
22. deMan, J.M., and A.M. Beers, *J. Texture Studies 18*:303 (1987).
23. Yap, P.H., J.M. deMan and L. deMan, *J. Am. Oil Chem. Soc. 66*:1792 (1989).
24. Elicabe, G., and L.H. Garcia-Rubin, *J. Call. Interphase Sci. 129*:1 (1989).

[Received May 27, 1994; accepted February 17, 1995]