Transmission Electron Microscopy of Milkfat Globules in the Low-Density Lipid Fraction of Swiss Cheese Whey

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ABSTRACT: A low-density lipid fraction (LDLF) was recovered from Swiss cheese whey (SCW) residual lipids by high-speed centrifugation. The present study was conducted to determine the microstructural properties of the extremely small-sized milkfat globules (MFG) in LDLF by: (i) freeze-fracture transmission electron microscopy (FF-TEM) and (ii) thin-section TEM (TS-TEM). FF-TEM results revealed that MFG in LDLF were ≤1 μm in size and were embedded in what appeared to be a smooth, protein-like matrix. The MFG in FF-TEM specimens exhibited either planarly cleaved fractions with smooth cores or peripherally cleaved fractions with surface laminations. TS-TEM results revealed that the MFG in LDLF were dispersed in an aggregated nonlipid matrix.

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KEY WORDS: Cheese whey, freeze-fracture transmission electron microscopy, residual whey lipids, thin-section transmission electron microscopy.

Cheese whey contains 0.1 to 0.2% residual lipids that are not removed by commercial clarification and separation processing treatments (1). Dried whey and whey protein concentrates contain in excess of 1% and 4 to 7% residual whey lipids (RWL), respectively, which impair their functional properties and flavor stability (2,3). Removal of RWL improves the flavor stability of cheese whey and whey protein concentrates, and also improves the functional properties of whey protein concentrates (4–6).

Three distinct fractions were produced (7) by high-speed centrifugation of Swiss cheese whey (SCW): (i) a low-density lipid fraction (LDLF), at the top turbid zone of the centrifuge tube, which contained the smallest-sized milkfat globules (MFG); (ii) a medium-density lipid fraction (MDLF), which was recovered in the middle clear zone; and (iii) a high-density lipid fraction (HDLF), which was recovered as a small gelatinous pellet at the bottom of the centrifuge tube. LDLF, MDLF, and HDLF contained about 95, 5, and 0.13% of the total SCW lipids, respectively.

LDLF constitutes only about 0.12% of SCW on a dry basis. Although LDLF is present in cheese whey in extremely small percentages, it contains the smallest-sized MFG and may have some potential as a new food ingredient. The correlation between microstructure and physical properties of dairy products and ingredients is well established (8). Information obtained in the present study may be useful for designing new and improved microfiltration membranes and centrifugal milk separation equipment for removing RWL from cheese whey (4,9). The present study was conducted to determine the microstructural properties of the smallest-sized MFG in the LDLF of SCW by freeze-fracture transmission electron microscopy (FF-TEM) and thin-section TEM (TS-TEM).

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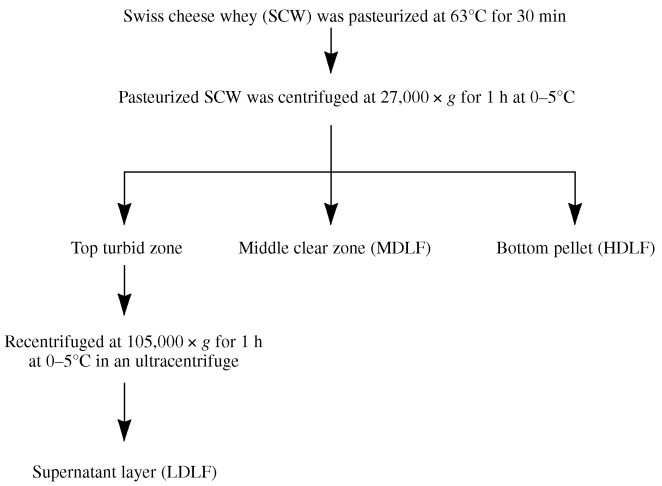
EXPERIMENTAL PROCEDURES

Recovery of LDLF of RWL from SCW. Pasteurized SCW was centrifuged for 1 h at 27,000 × *g* and 4°C in a Superspeed RC2-B centrifuge equipped with an SS-34 fixed-angle rotor (Sorvall Centrifuges, Newtown, CT) (Scheme 1). The top turbid zone was removed with a Pasteur pipet and recentrifuged for 1 h at 105,000 × *g* and 4°C in an L8-55 preparative ultracentrifuge, equipped with a Type 28 rotor (Beckman Instruments Inc., Brea, CA). The resulting top turbid zone of the centrifuge tubes, which was enriched with the smallest-sized low-density MFG, was recovered with a Pasteur pipet as LDLF (7).

TEM. The microstructural properties of the extremely small-sized MFG in LDLF were investigated by FF-TEM and TS-TEM.



SCHEME 1

FF-TEM. LDLF of approximately 0.5-mm cubed was loaded into a Balzer gold "hat," rapidly frozen in a nitrogen slush (–210°C), and stored in liquid nitrogen. The frozen sample was freeze-fractured with a Balzer 400T freeze-fracture machine (Balzers Aktiengesellshaft für Hochvakuumtechnik und Dunne Schichten, Principality of Liechtenstein). After loading the sample into the precooled machine, the sample table was warmed to –110°C. The frozen sample was microtomed, coated with platinum at a 45° angle, and rotary-coated with carbon. The sample was then removed from the machine, thawed, and digested to leave the replica, which was picked up on a 400-mesh grid and examined at 60 KV in a CM 12 electron microscope (Philips, Eindhoven, The Netherlands).

TS-TEM. One milliliter of LDLF was transferred to a 15,000-dalton MWCO dialysis bag (Spectrum Medical Industries, Inc., Houston, TX) and suspended in a 1% osmium tetroxide (OsO_4) solution for 12 h at 4°C. The conventional glutaraldehyde treatment was omitted because we were primarily concerned about the microstructural properties of MFG in LDLF. Preliminary experiments had demonstrated that this procedure provided satisfactory results. The dialysis bags were removed from the OsO_4 solution and washed by sequentially suspending them in fresh lots of distilled water for one 10-min and six successive 30-min treatments. The dialysis bags were then sequentially suspended in 30, 45, 60, and 70% vol/vol aqueous ethanol solutions for 30 min each. The gelled specimens were cut into small cubes and sequentially treated for 30 min each with 80, 95, 95, 100, and 100% vol/vol aqueous ethanol solutions. The dehydrated specimens were sequentially treated with two fresh lots of propylene oxide for 15 min each and sequentially embedded in 1:3, 1:1, and 3:1 vol/vol solutions of Spurr embedding media (10) in propylene oxide. The specimens were then treated with 100% Spurr for 3 h. Thereafter, the specimens were placed in Beem capsules (Polyscience Inc., Warrington, PA), covered with fresh Spurr, and held under vacuum for 4 h. The specimens were then held in a 60–70°C vacuum oven overnight to polymerize the Spurr. Specimens were thin-sectioned, placed on a grid, and viewed with the CM 12 electron microscope.

RESULTS AND DISCUSSION

FF-TEM. FF-TEM results (Figs. 1–3) revealed that the MFG in LDLF were predominantly ≤1 µm in diameter, falling in the lower range of the 0.1- to 10 µm diameter MFG present in milk (11). These MFG, which were either planarly fractured or peripherally fractured, were embedded in a smooth protein-like matrix. Figure 1 exhibits a planarly fractured MFG with a fine-textured core. Soderberg *et al.* (12) found that the lipid phase of MFG from milk and cream and the churned MFG from butter exhibited a fine structure when examined by FF-TEM. Figure 2 depicts a peripherally fractured MFG in LDLF, and Figure 3 exhibits a peripherally fractured MFG in LDLF with surface laminations. Buchheim and El-Nour (13) also observed planarly fractured MFG with an amorphous fine structure, and peripherally fractured MFG with crystalline layers in high-pressure-treated milk.

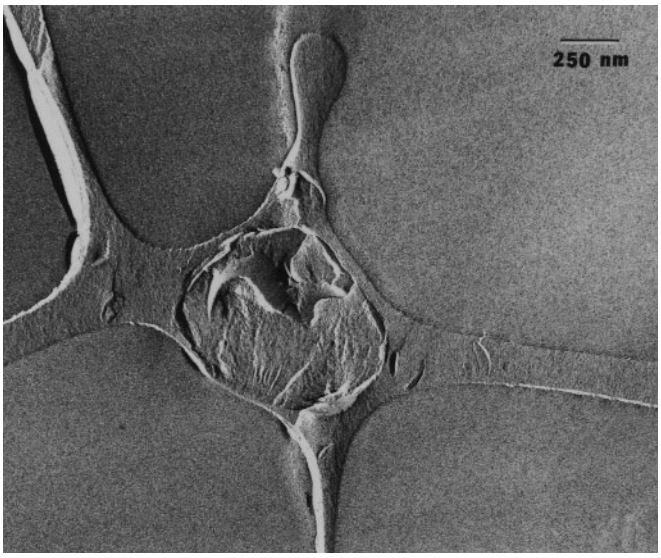


FIG. 1. A planarly fractured milkfat globule (MFG) in the low-density lipid fraction (LDLF) by the freeze-fracture transmission electron microscopy (FF-TEM) method.

TS-TEM. TS-TEM micrographs revealed that MFG in LDLF (Fig. 4) ranged in size from 250 nm to ≥1 µm and that they were embedded in what appeared to be an aggregated protein matrix. The aggregated particles in the background material are believed to be artifacts formed during specimen preparation. The dialysis treatments should have removed most of the nonprotein components, i.e., lactose and minerals, from the specimens. Some MFG were irregularly shaped, whereas others were spherically shaped. Some of the MFG were well-defined with whole, intact membranes, whereas others exhibited ruptured and incomplete membrane layers. Results from this

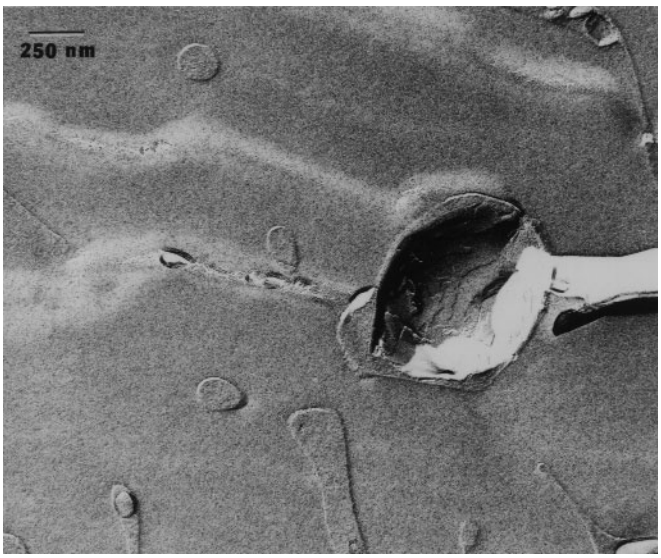


FIG. 2. A peripherally fractured MFG in LDLF by the FF-TEM method. For abbreviations see Figure 1.

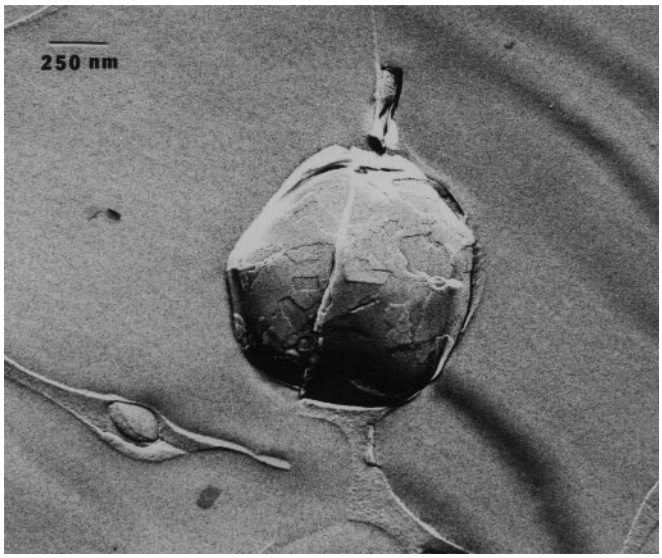


FIG. 3. Surface laminations on a peripherally fractured MFG in LDLF by the FF-TEM method. For abbreviations see Figure 1.

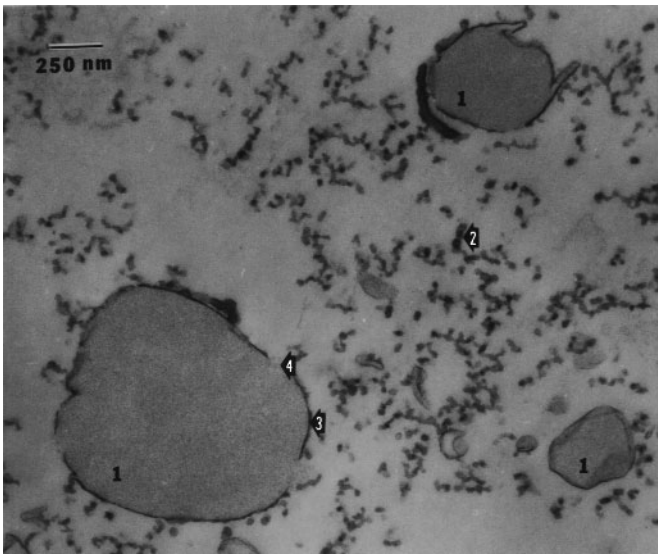


FIG. 4. MFG and matrix materials in LDLF by the thin-section TEM method. (1) MFG; (2) casein submicelles or aggregated matrix materials; (3) MFG membrane; and (4) ruptured MFG membrane. For abbreviations see Figure 1.

study confirm that the microstructure of the extremely small-sized MFG, recovered from the LDLF of RWL from SCW, is similar to that of MFG from milk and milk products.

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REFERENCES

1. Bassette, R., and J.S. Acosta, Composition of Milk Products, in *Fundamentals of Dairy Chemistry,* 3rd edn., edited by N.P. Wong, R. Jenness, M. Keeney, and E.H. Marth, Van Nostrand Reinhold, New York, 1988, pp. 39–79.
2. Morr, C.V., Whey Utilization, in *Whey and Lactose Processing,* edited by J.G. Zadow, Elsevier Applied Science, London, 1992, pp. 133–155.
3. Zadow, J.G., *Whey and Lactose Processing,* Elsevier Applied Science, London, 1992.
4. Karleskind, D., I. Laye, C.V. Morr, and T.W. Schenz, Emulsifying Properties of Lipid-Reduced and Calcium-Reduced Whey Protein Concentrates, *J. Food Sci. 61:*54–58 (1996).
5. Morr, C.V., and E.Y.W. Ha, Off-Flavors of Whey Protein Concentrates: A Literature Review, *Int. Dairy Journal 1:*1–11 (1991).
6. Laye, I., D. Karleskind, and C.V. Morr, Dynamic Headspace Analysis of Accelerated Storage Commercial Whey Protein Concentrate Using Four Different Adsorbent Traps, *Milchwissenschaft 50:*268–272 (1995).
7. Kamath, S.R., and C.V. Morr, Recovery and Composition of Swiss Cheese Whey Lipid-Containing Fractions, *J. Agric. Food Chem. 45:*4271–4274 (1997).
8. Buchheim, W., The Applicability of Electron Microscopy for Studying the Structure of Liquid and Solid Foods, *Procedures of the Fourth International Congress on Food Science and Technology,* 1974, Vol. 2, pp. 5–12.
9. Morr, C.V., Improving the Texture and Functionality of Whey Protein Concentrate, *Food Technol. 46:*110–113 (1992).
10. Spurr, A.R., A Low-Viscosity Epoxy Resin Embedding Medium for Electron Microscopy, *J. Ultrastruc. Res. 26:*31–43 (1969).
11. Walstra, P., and R. Jenness, *Dairy Chemistry and Physics,* John Wiley & Sons, New York, 1984.
12. Soderberg, I., L. Hernqvist, and W. Buchheim, Milk Fat Crystallization in Natural Milk Fat Globules, *Milchwissenschaft 44:*403–406 (1989).
13. Buchheim, W., and A.M. Abou El-Nour, Induction of Milk Fat Crystallization in the Emulsified State by High Hydrostatic Pressure, *Fat Sci. Technol. 10:*369–373 (1992).