Recovery and Composition of Swiss Cheese Whey Lipid-Containing Fractions

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Cheese whey contains 0.1−0.2% residual lipids. The present study was conducted to develop a high-speed centrifugation procedure for fractionating and recovering residual whey lipids (RWL) from commercial Swiss cheese whey (SCW) and to determine the yields and compositions of the resulting fractions. Three RWL fractions were obtained: (1) a low-density lipid-containing fraction (LDLF) at the top of the centrifuge tubes; (2) a medium-density lipid-containing fraction (MDLF) in the clear, middle zone; and (3) a high-density lipid-containing fraction (HDLF) in the small, compact, and gelatinous pellet at the bottom of the centrifuge tubes. LDLF, MDLF, and HDLF contained about 95, 5, and 0.13% of the total SCW lipids, respectively. Palmitic acid was the major fatty acid, and oleic, stearic and myristic acids were present in lower concentrations. The major phospholipid components of all three SCW lipid fractions were phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin.

Keywords: *Swiss cheese whey; residual whey lipids; centrifugation; fractionation*

INTRODUCTION

Cheese whey, which contains about 50% of the nutrients originally present in milk (Bassette and Acosta, 1988), is processed and used widely as a nutritional ingredient in animal feeds and food products (Zall, 1992; Morr, 1992a; Zadow, 1992). It is estimated that in excess of 524 million pounds of dried whey and some 48 million pounds of whey protein concentrates (WPC) containing ≥35% protein are manufactured in the United States annually (Morr, 1984, 1992a).

Conventional processing schemes for manufacturing dried whey and WPC include a centrifugal clarification and separation step for removing "whey cream" or residual whey lipids (RWL) and fine curd particles from the whey. However, commercial dried whey typically contains ≥1.1% RWL (Bassette and Acosta, 1988) and WPC contains 4−7% RWL (Morr and Foegeding, 1990). RWL inhibits some of the important functional properties as well as the flavor stability of these products (Williams et al., 1991). WPC produced from microfiltered cheese whey to contain ≤1% RWL exhibits substantially improved functional properties and flavor stability (Morr, 1992b; Morr and Ha, 1993; Karleskind et al., 1995a−c, 1996; Laye et al., 1995).

Since commercial milk clarifiers and separators are not capable of removing the small-sized RWL particles from cheese whey, the industry is currently considering microfiltration membrane processing technologies for this purpose (Rinn et al., 1990; Morr, 1992b; Karleskind et al., 1995a). Commercial application of these more efficient whey delipidization processes should result in the production of significant amounts of the RWL-enriched fraction for use by the food industry. The present study was conducted to develop a laboratory-scale centrifugation method for fractionating and recovering sufficient quantities of RWL from commercial Swiss cheese whey (SCW) and to determine their yields and composition. This information should be useful for developing alternative schemes for recovering and utilizing RWL on a commercial scale.

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MATERIALS AND METHODS

Four lots of clarified and separated SCW were obtained from Holmes Cheese Co. (Millersburg, OH) on different days extending over a period of 15 months.

Recovery and Fractionation of Residual Whey Lipids. The SCW was pasteurized by heating at 63 °C for 30 min, cooled to 0−5 °C, and stored in a laboratory freezer at −10 to −20 °C (Figure 1). Frozen SCW was thawed overnight at 4 °C and centrifuged for 1 h at 27000*g* and 4 °C in a Superspeed RC2-B centrifuge equipped with a SS-34 fixed-angle rotor (Sorvall Centrifuges, Newtown, CT). Three distinct zones were formed in the centrifuged SCW: (1) a top, turbid zone that contained the smallest sized, residual milkfat globules (MFG) with densities of 0.92−0.93 g mL^{-1} [p 273 of Walstra and Jenness (1984)]; (2) a clear middle zone that contained the medium-density lipid fraction (MDLF); and (3) a small, gelatinous pellet that formed on the bottom of the tube that contained the high-density lipid fraction (HDLF). The top, turbid zone was withdrawn from the centrifuged SCW with a Pasteur pipet and recentrifuged for 1 h at 105000*g* and 4 °C in an L8-55 preparative ultracentrifuge equipped with a Type 28 fixed-angle rotor (Beckman Instruments Inc., Brea, CA). The supernatant turbid zone, which contained the smallest sized, low-density MFG, was identified as the low-density lipid fraction (LDLF). The infranatant layer contained the MDLF and was combined with the clear middle zone from the 27000*g* centrifugation step. The fractionation procedure was repeated several times for each lot of SCW to obtain sufficient amounts of each lipid fraction for analysis. SCW and the three lipid fractions were shell-frozen by suspending them in a dry ice−ethanol bath, freeze-dried in a Labconco Freeze-Dry System/Lyph Lock 4.5 freeze-drier (Labconco Corp., Kansas City, MO), and stored in the freezer until they were analyzed.

Compositional Analyses. *Total Solids, Ash, Protein, and Lactose.* Total solids and ash contents of freeze-dried SCW

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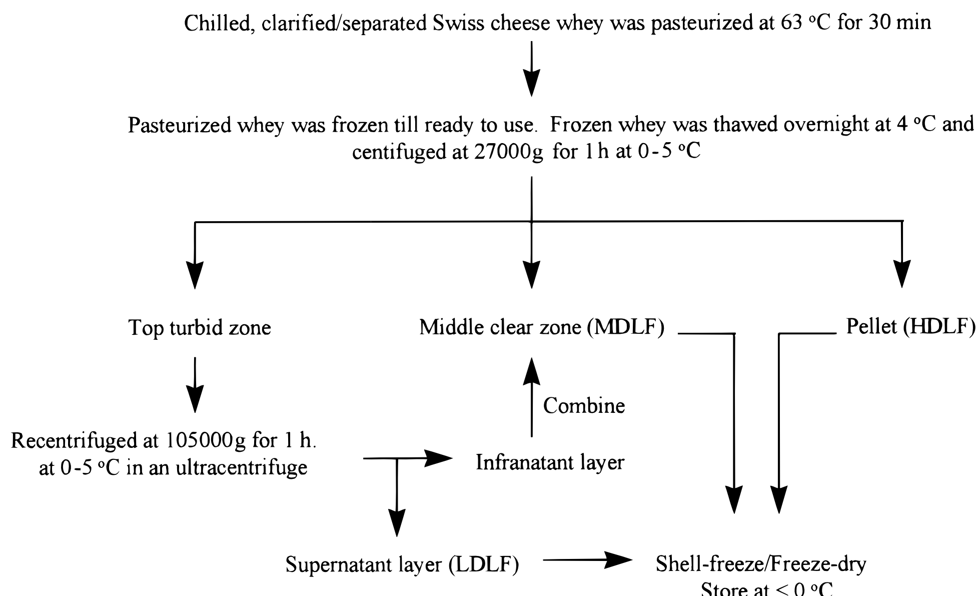

Figure 1. Flow diagram of the procedure to fractionate and recover RWL.

and its centrifugal fractions were determined according to AOAC methods (AOAC, 1984). Protein was determined according to the Kjeldahl method of Prasad (1995) using an Auto Sampler System 1035 analyzer (Hoganas, Sweden) and a nitrogen conversion factor of 6.38. Lactose composition was determined by difference using the equation

$$\% \text{ lactose} = \% \text{ total solids} - (\% \text{ ash} + \% \text{ protein} + \% \text{ total lipids})$$

Total Lipids. Two gram samples of freeze-dried SCW and each of the lipid fractions were weighed into separate 250 mL Erlenmeyer flasks following the procedure of Karleskind et al. (1996). Thirty-two milliliters of 0.05 M NaCl solution, 80 mL of methanol (Fisher Scientific, Fair Lawn, NJ), and 40 mL of chloroform (Fisher Scientific) were added sequentially, and the resulting suspension was stirred for 15 min on a magnetic stirrer. Forty milliliters each of distilled water and chloroform were added to the suspension, and stirring was continued for an additional 15 min. The suspension was centrifuged for 20 min at 1500*g* and 4 °C, and after the top aqueous layer was removed with a Pasteur pipet, the insoluble materials were removed from the chloroform layer by filtering with Whatman No. 1 filter paper. Two hundred thirty-two milliliters of a 2:2:1.8 (v/v/v) mixture of chloroform, methanol, and distilled water (80 mL:80 mL:72 mL) was added to the resulting filtrate, and the suspension was thoroughly mixed by stirring on a magnetic stirrer. The suspension was again centrifuged for 20 min at 1500*g* and 4 °C, and the two layers were separated as before. The clear chloroform layer was transferred into a clean, dry, preweighed 125 mL flat-bottom, round vacuum flask and evaporated to dryness with a Brinkmann Rotavapor R110 rotary vacuum evaporator (Brinkmann Instruments, Westbury, NY). The outside surface of the flask was wiped dry, and it and its contents were dried overnight in a vacuum desiccator and reweighed. The total lipids content of the sample was determined as the difference in weight of the vacuum flask before and after use.

Fatty Acid Analyses. The dried total lipids extracts recovered in the 125 mL flat-bottom, round vacuum flasks as previously described were dissolved separately in 6 mL of benzene (Fisher Scientific). One hundred microliters of each solution and 50 μL of a 15 mg mL^{-1} C_{17} fatty acid (Sigma Chemical Co., St. Louis, MO) solution as internal standard were pipetted into separate 4 mL amber glass vials. One hundred microliters of Meth Prep II (Alltech Associates Inc., Deerfield, IL) was added as methylating agent. One microliter of the fatty acid methyl ester was injected into a 5890 Series II gas chromatograph (GC) (Hewlett-Packard, Wilmington, DE) equipped with a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-Wax capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (Hewlett-Packard). Ultrapure helium (99.995%) was supplied at a flow rate of 40 cm s^{-1} as the carrier gas. Injector and detector temperatures were 250 and 300 °C, respectively. The oven temperature was programmed at 50 °C for 2 min, increased to 250 °C at a rate of 10 °C min^{-1}, and held at 250 °C for 8 min as recommended by the column manufacturer. Fatty acids as methyl esters were tentatively identified by comparing their retention times with those of fatty acid ester standards (Sigma Chemical Co.). Fatty acid profiles were computed as the percent of the total peak areas of the major fatty acids.

Total Phospholipids. The method of Laye (1993) was used to fractionate and quantitate the phospholipids contained in the total lipids fraction. The dried total lipids extracts recovered in 125 mL vacuum flasks as previously described were dissolved separately in 2−3 mL of chloroform and fractionated on a PrepSep-Si column (Fisher Scientific) that had been preconditioned by passing 20 mL of chloroform through it. Neutral lipids were eluted with 20 mL of chloroform, and the total phospholipids were subsequently eluted with 20 mL of methanol. The phospholipid fraction was transferred quantitatively to a clean, dry, preweighed 125 mL flat-bottom, round vacuum flask and evaporated to dryness with the rotary evaporator. The outside surface of the flask was wiped dry, and it and its contents were dried in a vacuum desiccator overnight and reweighed. Total phospholipids were determined as the difference in weight of the flask before and after use.

Phospholipid Analyses. The dried total phospholipids fractions obtained by the previously described procedure were dissolved in chloroform to provide a concentration of 1.5 mg mL^{-1} and fractionated according to the method of Stahl (1965). Phospholipid standards, i.e., phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SPH), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), and phosphatidylserine (PS) from Sigma Chemical Co., were prepared in chloroform at the same concentration. The chloroform solutions of the total phospholipids fractions and phospholipid standards were fractionated on Silica G precoated thin layer chromatographic (TLC) plates (Universal Scientific Inc., Atlanta, GA) using a 71:26:3 (v/v/v) mixture of chloroform, methanol, and water as mobile phase. Fractionated phospholipid zones were visualized by treating the TLC plates with iodine vapors and tentatively identified by comparing their migration distances with those of the standard phospholipids.

Statistical Treatment of Data. The compositional data were subjected to analysis of variance (ANOVA) by the program of the SAS Institute, Inc. (1988). Mean values were compared using the least significant difference test.

Table 1. Composition (Percent) of Freeze-Dried SCW and Its Lipid Fractions[a]

fraction	total solids[b]	ash[b]	protein[b]	total lipids[b]	total phospholipids[c]	lactose[d]
SCW	96.44[c]	7.84[a]	12.75[b]	2.16[c]	0.41[b]	74.59
LDLF	98.07[b]	6.39[b]	12.10[c]	11.71[a]	0.38[b]	68.09
MDLF	96.30[c]	7.00[ab]	12.44[bc]	1.50[c]	0.36[b]	75.64
HDLF	100[a]	4.64[c]	51.08[a]	5.63[b]	2.03[a]	37.82

[a] Means within the same column with different superscripts differ ($P < 0.05$). [b] Means of triplicate determinations. [c] Means of duplicate determinations. [d] Determined by difference.

RESULTS AND DISCUSSION

Yield of RWL Fractions. About 6−7, 90−91, and 0.19−0.22% of the total weight of SCW was recovered in the top, middle, and bottom fractions by centrifugation of SCW for 1 h at 27000*g* and 4 °C. About 14−17% of the total weight of SCW was recovered in the LDLF by centrifuging the top, turbid layer from the 27000*g* centrifugation treatment for 1 h at 105000*g* and 4 °C. The percent yield on a dry basis (total solids content) of LDLF, MDLF, and HDLF accounted for 0.12, 0.06, and 0.02% of SCW, respectively.

Composition of RWL Fractions. The total solids, ash, protein, total lipids, total phospholipids, and lactose compositional data for freeze-dried, pooled fractions prepared from the four lots of SCW are given in Table 1. The total solids, ash, and protein contents of SCW, LDLF, and MDLF were generally similar but differed from those of HDLF. The total solids content of freeze-dried LDLF was ∼2% higher than for SCW and MDLF, whereas the total solids content was 100% for HDLF, which was significantly higher than for the other lipid fractions. The ash contents of SCW and MDLF were higher than for LDLF, which was also much higher than for HDLF, which contained ∼4.6% ash. The protein content of SCW and MDLF were higher than for LDLF but considerably lower than for HDLF, which contained ∼51% protein. The high protein content of HDLF was probably due to sedimentation of residual casein-containing cheese curd particles that were too small to be removed by centrifugal clarification and separation processing treatment of the SCW (Morr, 1973). The total lipids content of LDLF of 11.71% was significantly higher than for the HDLF fraction of 5.6% and the other fractions that contained ∼1.5−2.2% total lipids. Although these findings confirmed that, as expected, LDLF contained the highest concentration of lipids, the lipid contents of MDLF and HDLF were higher than expected. The lipids that were recovered in the lower centrifugal fractions are likely lipoprotein in nature with apparent density values >1.035 g mL^{-1}, the density of skim milk (Sherbon, 1988). The total phospholipids content of HDLF was much higher than for SCW and the other fractions, accounting for roughly 36% of its total lipids content. The lactose content of these fractions, which was determined indirectly, reveals that, as expected, HDLF contained the lowest value of ∼38% compared to values ranging from ∼68 to 75.6% for SCW and the other fractions.

These results demonstrate clearly that most of the residual whey lipids were recovered in LDLF and HDLF and that most of the phospholipids and proteins were recovered in HDLF. The relatively high protein and phospholipid content of HDLF suggests that the lipids were in the form of phospholipoprotein complexes (Houlihan and Goddard, 1991) rather than small cheese curd particles that contained physically entrapped MFG. The latter would not be expected to result in the high phospholipid contents that were displayed in this study for HDLF.

Table 2. Fatty Acid Composition (Percent of Total Chromatographic Peak Area) of Freeze-Dried SCW and Its Lipid Fractions

fatty acid	SCW	LDLF	MDLF	HDLF
4:0	3.91	2.31	17.37	1.75
6:0	1.16	1.62	0	1.31
8:0	1.07	1.12	0	1.00
10:0	2.58	2.53	tr[a]	2.5
12:0	3.29	3.02	0	3.24
14:0	11.11	11.16	11.05	10.61
14:1	tr	0.91	0	0.62
16:0	32.62	31.59	31.05	31.21
16:1	1.87	1.73	0	1.56
18:0	16.18	14.79	18.42	15.11
18:1	21.24	26.26	22.11	25.09
18:2	3.73	2.76	tr	5.31
18:3	1.24	0	0	0.69

[a] tr, trace.

Fatty Acid Profiles. Table 2 provides information on the major fatty acids recovered from SCW and its centrifugal fractions on the basis of percentage of total gas chromatographic peak area. SCW, LDLF, and HDLF contained generally similar fatty acid profiles. MDLF contained fewer minor fatty acids present at ≤10% of the total fatty acids than SCW and its other lipid fractions. The four major fatty acids that were present at concentrations of ≥10% of the total fatty acids of SCW, LDLF, and HDLF included 14:0, 16:0, 18:0, and 18:1, whereas MDLF contained five major fatty acids including 4:0, 14:0, 16:0, 18:0, and 18:1. The 18:1 fatty acid was the only major unsaturated fatty acid recovered from SCW and its lipid fractions. Palmitic acid (16:0) was present in the highest concentrations of ≥31% of the total fatty acids in SCW and its lipid fractions, which agrees with results for milkfat from cow's milk (Kurtz, 1978). Other fatty acids recovered from SCW residual lipids that are also important in cow's milkfat include myristic (14:0) and oleic (18:1) acids. Our results are at variance with those of Bangela et al. (1987), who reported that oleic (18:1) acid was the major fatty acid in Gouda cheese whey. The relatively low concentrations of unsaturated fatty acids in SCW residual lipids suggest that it should not be highly susceptible to off-flavor development by lipid oxidation mechanisms.

Phospholipid Composition. Typical TLC results for the separation of phospholipid standards and phospholipids recovered from SCW and its lipid fractions are given in Figure 2. The direction of migration of the phospholipids during separation is from the bottom of the figure to the top. The most polar phospholipids migrate to near the top of the TLC plate, and the less polar lipids migrate shorter distances during separation. SCW and its lipid fractions exhibited similar phospholipid profiles, with PC, PE, and SPH being the principal phospholipids. All fractions exhibited trace amounts of LPC. These results agree well with those of Mangold (1965), who investigated the composition of animal phospholipids using mobile and stationary phases similar to those in the present study. Brunner (1978) also reported that PE, PC, and SPH were the major phospholipids recovered from milkfat globule membranes. The phospholipids recovered from SCW and its lipid fractions may be contributed by MFG that were so small that they were not removed during centrifugal clarification and separation of the whey. Alternatively, the phospholipids may be membrane fragments of larger MFG formed during curd formation, cooking, or draining steps of the cheese making process.

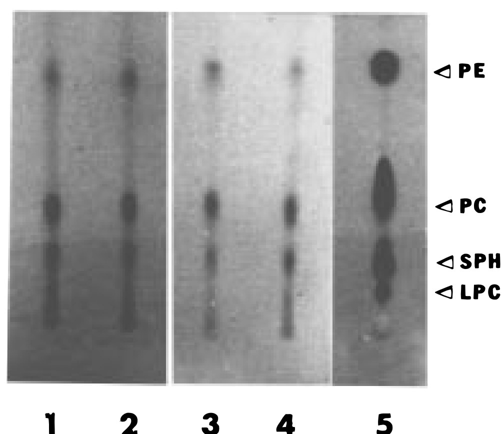

Figure 2. Separation of major phospholipids by TLC: (1) LDLF; (2) SCW; (3) HDLF; (4) MDLF; and (5) standard containing PE, PC, SPH, and LPC.

Conclusions. Results from this study should provide a basis for developing and exploiting more effective centrifugal fractionation processing technologies capable of recovering the extremely small sized MFG and phospholipoprotein complexes from cheese whey on a commercial scale. High capital and operating costs for such equipment and processing schemes may make this approach relatively unappealing to the whey processing industry. However, the potential for manufacturing dried whey and WPC with vastly improved functional properties and flavor stability should make this approach worthwhile. Information presented here on the composition of the RWL fractions should allow food processors to decide on their potential value as ingredients in various specialized food product applications.

ABBREVIATIONS USED

RWL, residual whey lipids; SCW, Swiss cheese whey; WPC, whey protein concentrates; LDLF, low-density lipid fraction; MDLF, medium-density lipid fraction; HDLF, high-density lipid fraction; PC, phosphotidylcholine; PE, phosphatidylethanolamine; SPH, sphingomyelin; LPC, lysophosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine.

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