2015 norway
reviewed
11/2021

J. Dairy Sci. 98:5829–5840
http://dx.doi.org/10.3168/jds.2014-9039
© American Dairy Science Association®, 2015.

The composition and functional properties of whey protein concentrates produced from buttermilk are comparable with those of whey protein concentrates produced from skimmed milk

Sigrid Svanborg,*†[1] Anne-Grethe Johansen,*† Roger K. Abrahamsen,† and Siv B. Skeie†
*TINE SA R&D, Kalbakken, PO Box 7, Kalbakken, 0901 Oslo, Norway
†Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, PO Box 5003, 1432 Ås, Norway

ABSTRACT

The demand for whey protein is increasing in the food industry. Traditionally, whey protein concentrates (WPC) and isolates are produced from cheese whey. At present, microfiltration (MF) enables the utilization of whey from skim milk (SM) through milk protein fractionation. This study demonstrates that buttermilk (BM) can be a potential source for the production of a WPC with a comparable composition and functional properties to a WPC obtained by MF of SM. Through the production of WPC powder and a casein- and phospholipid (PL)-rich fraction by the MF of BM, sweet BM may be used in a more optimal and economical way. Sweet cream BM from industrial churning was skimmed before MF with 0.2-µm ceramic membranes at 55 to 58°C. The fractionations of BM and SM were performed under the same conditions using the same process, and the whey protein fractions from BM and SM were concentrated by ultrafiltration and diafiltration. The ultrafiltration and diafiltration was performed at 50°C using pasteurized tap water and a membrane with a 20-kDa cut-off to retain as little lactose as possible in the final WPC powders. The ultrafiltrates were subsequently spray dried, and their functional properties and chemical compositions were compared. The amounts of whey protein and PL in the WPC powder from BM (BMWPC) were comparable to the amounts found in the WPC from SM (SMWPC); however, the composition of the PL classes differed. The BMWPC contained less total protein, casein, and lactose compared with SMWPC, as well as higher contents of fat and citric acid. No difference in protein solubility was observed at pH values of 4.6 and 7.0, and the overrun was the same for BMWPC and SMWPC; however, the BMWPC made less stable foam than SMWPC.

Received October 29, 2014.
Accepted May 19, 2015.
[1]Corresponding author: sigrid.svanborg@tine.no

Key words: buttermilk, microfiltration, whey protein concentrate, phospholipid, milk fat globule membrane

INTRODUCTION

Over the past several years, the interest in high-protein ingredients such as whey protein concentrate (**WPC**) or isolate (**WPI**) has increased. Both WPC and WPI are usually produced from cheese whey. However, WPC can also be derived from the fractionation of skim milk (**SM**) by microfiltration. Skim milk WPC contains less minerals and has no caseinomacropeptides compared with traditional WPC from cheese whey. The functional properties of WPC from SM fractionation have recently been compared with those of cheese whey WPC by Coppola et al. (2014) and Evans et al. (2010). The WPC from SM fractionation have unique functionalities, such as excellent solubility, gelling after heat treatment, and foaming properties (Bacher and Køningsfeldt, 2000; Heino et al., 2007).

Buttermilk (**BM**), the by-product from the churning of butter, has a CN and whey protein ratio similar to that of SM (Corredig and Dalgleish, 1997), and spray-dried BM has a comparable nutritional value to that of SM powder (Morin et al., 2004). Govindasamy-Lucey et al. (2006) studied the use of BM in pizza cheese, and the solubility, foaming, and emulsifying properties of BM powders have been described in Sodini et al. (2006). However, a high fat content in BM may lead to sticky powder as well as increased risk of off-flavors from oxidation products. The fractionation of BM into a CN- and milk fat globule membrane (**MFGM**)-rich fraction and a whey protein fraction may increase the value of this by-product. Most of the BM produced today is used as animal feed (liquid) or for the production of BM powder, but it can also be exploited in a more optimal way to produce WPC, as the global demand for whey protein is increasing (Lafougère, 2014). Buttermilk is higher in MFGM protein and phospholipids (**PL**) than SM, which might alter the functional properties of its WPC.

Buttermilk is a rich source of MFGM components, such as proteins and PL (Rombaut et al., 2005), because parts of the MFGM are released from the fat globule into the serum phase during butter churning. Membrane technology is frequently used in the dairy industry to concentrate milk constituents, and by using microfiltration (**MF**), CN and MFGM can be separated from whey proteins. Several studies have focused on the separation and isolation of MFGM proteins or PL from BM by MF (Sachdeva and Buchheim, 1997; Corredig et al., 2003; Morin et al., 2006, 2007a,b) for the further purification of the PL (Astaire et al., 2003). Few studies, if any, have focused on the utilization of the whey protein fraction obtained from BM.

The high ratio of CN to total protein in BM (Corredig et al., 2003) gives a high CN recovery during MF. The retentate from the MF of sweet BM could be used for further extraction of the PL. Achieving high-purity PL from the MF of BM is difficult, as CN micelles and MFGM fragments have similar size distributions (Rombaut et al., 2006; Singh, 2006). However, a MF retentate from BM, which is rich in CN and MFGM components, can be used as a starting material for further separation and purification of PL or as a supplement in other dairy processes. However, several studies have reported the transmission of PL and MFGM proteins through the MF membranes (Astaire et al., 2003; Morin et al., 2007a), thus affecting the composition and functional properties of the products used from these permeates. Whey proteins and PL are applied in the food industry as ingredients for baking, as well as in pharmaceuticals and in cosmetics (Vanderghem et al., 2010) due to protein solubility, foaming, and emulsifying properties.

The major whey proteins, which are most important for the functional properties in most food applications, are β-LG and α-LA. Heat treatment does influence the functional properties of the whey proteins (Abd El-Salam et al., 2009) or the sizes of their aggregates (Schokker et al., 2000; De La Fuente et al., 2002). At approximately 67.5 to 78°C, the β-LG starts to unfold, and at higher temperatures of 78 to 82.5°C aggregation occurs (Sava et al., 2005). Denatured β-LG may aggregate with other β-LG molecules, CN (Donato and Guyomarc'h, 2009), α-LA (Dalgleish et al., 1997), or MFGM proteins (Ye et al., 2004). α-Lactalbumin has no free thiol group and contributes less to the aggregation caused by denaturation (Calvo et al., 1993).

The objective of the current study was to compare the final WPC powders obtained by MF fractionation and further UF and diafiltration (**DF**) concentration of BM and SM MF permeates. The BM used was of industrial origin with varying cream pasteurization histories, whereas the SM used was obtained from controlled pasteurization (73°C/15 s).

MATERIALS AND METHODS

Experimental Design

The study was carried out at a pilot-scale dairy processing plant at the Norwegian University of Life Sciences (**NMBU**), Department of Chemistry, Biotechnology and Food Science (Ås, Norway). The fractionation, concentration, and spray drying were carried out for each of 4 batches of BM and SM. To yield enough MF permeate to produce 1 batch of approximately 3 kg of WPC powder, the skimmed BM or SM of each powder production was MF on 2 consecutive days. The MF permeate from the first day was kept at 4°C and pooled with the MF permeate from the second day before further concentration by UF/DF and the final spray drying. The 2 pooled MF permeates originated from the same raw material batch (BM or SM) and were, therefore, treated as a single MF replicate block during further analysis. The UF and powder production were repeated 4 times (4 replicate blocks).

BM and Cream Treatment

Four batches of fresh sweet BM were collected from TINE Sandnessjøen (Sandnessjøen, Norway). The cream used for churning originated from different dairy plants in northern Norway. As the cream was obtained from different dairy plants, the cream had undergone different pasteurization routines prior transportation to TINE (Sandnessjøen, Norway). The cream was pasteurized upon arrival at TINE. Therefore, the heat treatment of the cream might have been repeated 2 to 3 times using the following conditions: 74.2°C/20 s (approximately 40% of the cream) or 86°C/5 s (approximately 60%) based on different routines at the supplying dairies. Each batch of BM was pasteurized once (76°C for 20 s) before transportation to the NMBU pilot plant. Including transportation, the BM was stored cold (4°C) for up to 5 d before MF. Upon arrival at the NMBU pilot-scale dairy processing plant, the BM was skimmed by a cream separator to 0.3% fat at 55°C (SA 1–01–175, Westfalia Separator AG, Oelde, Germany) to minimize the differences in the residual fat in the 4 batches investigated. The TS content of the BM after skimming was 7.50 ± 0.56 g/100 g, on average, and the pH was 6.84 ± 0.19. After skimming, the MF feed was kept at approximately 50°C for a maximum of 4 h before fractionation.

MF of BM and SM

The MF of skimmed BM was carried out using pilot plant equipment (APV UF/MF pilot MCC RV

00109921 RKA 01118340, APV, Silkeborg, Denmark) with a target volume concentration factor of 2.2 to 2.4 to achieve high yields of the permeate. A module containing 7 ceramic membranes with a pore size of 0.2 μm and total membrane surface area of 1.38 m^2 (OD25–19033–1016, Jiuwu, Nanjing, Jiangsu, China) was used. The processing conditions were the same as for the fractionation of pasteurized SM as described by Svanborg et al. (2014). The filtration temperature (55–58°C), permeate and retentate flow, and pressure differences on the permeate and retentate inlet and outlet were monitored to control fouling during fractionation. The target concentration factor was 2.2 to 2.5. The SM was pasteurized (73°C/15 s) before MF as described by Svanborg et al. (2014). The MF filtration of 650 L of SM was achieved in 3 h and 40 min, whereas the MF filtration of 800 L of BM took 4 h and 50 min (half the batch of the 1,600 L of skimmed BM).

UF and DF of BM Permeate and Pasteurized SM Permeate

The MF permeates from BM and SM were UF at 50°C using a pilot plant unit (UFS-4/6 Alfa Laval, Silkeborg, Denmark) with a single UF-membrane (cutoff value of 20 kDa, GR 62–6338/48P, Alfa Laval). Diafiltration was performed using pasteurized tap water until no remaining lactose was detected by a brix measurement (Atago N1 refractometer, Tokyo, Japan) of the UF concentrate. The amount of DF water used during UF of the different MF permeates was 350 to 500 and 650 to 1,075 L for SM and BM, respectively. The inlet and outlet pressures were held at 2.1 and 0.9 bar, respectively, for both types of permeates.

Production of WPC Powder from UF/DF Concentrates

The UF/DF concentrates of the MF permeates from of BM and SM were spray-dried (Niro Atomizer, Gea Niro, Søborg, Denmark) directly after concentration was finished. An inlet air temperature of 185 to 190°C and a constant outlet air temperature of 85°C were used. The spray drier rotary atomizer had a drying rate of 15 L of liquid/h.

Methods of Analysis

Total solids were analyzed according to IDF 21B:1987 (IDF, 1987b). Total nitrogen, casein nitrogen, noncasein nitrogen, and NPN were analyzed according to IDF 20–1:2001 (IDF, 2001b), IDF 20–4:2001 (IDF, 2001c), 20–5:2004 (IDF, 2001a), and 29–2:2004 (IDF, 2004), respectively. Organic acids and carbohydrates were analyzed using HPLC as described by Moe et al. (2013). The fat contents of skimmed BM, MF fractions, and UF/DF concentrates were analyzed according to the Gerber method (IDF, 2008) as an average of 2 measurements. The analysis of the fat contents in the powders was performed according to Röse-Gottlieb (IDF, 1987a). Sodium dodecyl sulfate-PAGE and sample preparation of 8% (wt/wt) protein WPC solutions followed the procedure described by Devle et al. (2014). Seven microliters of samples were applied to respective wells on 12% polyacrylamide separating gel (Mini-Protean TGX Precast Gel, Bio-Rad, Hercules, CA). In addition, 5 μL of a SDS-PAGE standard (Low range, Bio-Rad) was applied to one well. To identify some of the proteins visualized in the SDS-PAGE gels, the bands were cut and in-gel digested according to Devle et al. (2014), followed by identification by ultra-performance liquid chromatography and Q-extractive mass spectrometry as described by Islam (2014). The resulting peak list files were searched against the *Bos taurus* UniProt database by an in-house Mascot server (2014, version 2.4.1; Matrix Science, London, UK), containing a total of 24,205 protein sequences. Evaluation was carried out using the Exponentially Modified Protein Abundance Index (emPAI; Ishihama et al., 2005), with a protein threshold of 99.9% and a peptide threshold of 95% with a minimum of 5 peptides. Minerals in the milk fractions and powders were analyzed according to De La Fuente et al. (1997). The samples were digested by 65% concentrated HNO_3 at 250°C in a Milestone Ultra wave UltraClave III (Milestone, Sorisole, Italy) and were diluted to 10% HNO_3 before determination of the minerals by inductive coupled plasma optical emission spectrometry (Perkin Elmer Optima 5300 DV, Perkin Elmer, Shelton, CT). A commercial laboratory, Vitas AS (Oslo, Norway), analyzed phospholipids and α-tocopherol. The quantification of α-tocopherol was carried out using HPLC-fluorescence detection system as described by Berhe et al. (2007) with the following modifications: the separation was performed on a Zorbax SB-C18 50 × 4.6-particle size 1.8 μm column from Agilent (Santa Clara, CA) using methanol and water as the mobile phase. Identification of the unknowns was performed against standards from Alfa Aesar (Ward Hill, MA). For the PL analysis, the detection was carried out using a normal phase-HPLC with an evaporative light scattering detector. The samples were accurately weighed, dissolved in isopropanol, shaken, and centrifuged (4,000 × *g*, 10°C, 10 min). Then, the supernatants were transferred to new vials. The pellets were washed with isopropanol, centrifuged (4,000 × *g*, 10°C, 10 min), and the resulting supernatants were pooled with the first ones. The supernatants were evaporated to dryness and dissolved

in buffer A (hexane and isopropanol) before analysis. Separations were performed on a normal phase polyvinyl alcohol functionalized silica 250- × 4.6-mm HPLC column (YMC, Dinslaken, Germany) with buffers A and B (hexane, isopropanol, and water) as the mobile phase. The system used a linear gradient changing from 25 to 100% buffer B over 12 min, and then it was held for 15 min at 100% B before returning to the initial conditions. The total run time was 22 min. Analyses were calibrated against known standards from Lipoid GmbH (Lipoid GmbH, Köln, Germany) and Larodan (Larodan, Malmö, Sweden).

Functionality Tests of the Protein Solutions

The overrun was tested by preparing 200 mL of protein solutions (8% wt/vol protein) of WPC made from SMWPC or BMWPC and hydrated in deionized water by stirring for at least 30 min at ambient temperature. The solutions were kept overnight at 4°C for complete hydration and were adjusted to room temperature for 30 min before whipping with a Bosch MFQ35 hand mixer (Bosch, Gerlingen, Germany) at the maximum speed for 2 min the next day. The preparation of the solution and whipping was replicated at least 2 times for each batch of powder. Foam overrun was measured according to the methods of Phillips et al. (1987). Immediately after whipping, the foam was gently scooped into standard laboratory beakers and levelled off at 100 mL. The average of the foam weights of 10 measurements per whipping and the average initial weight of the protein solution, were used for the calculation of the overrun as follows:

$$\text{Overrun (\%)} = (\text{weight of 100 mL of solution} - \text{weight of 100 mL of foam/weight of 100 mL of foam}) \times 100\%.$$

The foam stability was measured as the time required to reduce the initial foam volume by 50% using a 250-mL graded cylinder, generating foam the same way as described above.

The suspension stability was measured in 1% (wt/wt) protein solutions of the powders as described by Sikand et al. (2011). Samples were adjusted to pH values of 7.0 and 4.6 using 5.0 or 1.0 *M* NaOH and 2.0 or 0.1 *M* HCl. The samples were centrifuged at 12,000 × *g* for 15 min at 4°C. The amount of soluble nitrogen in the samples was measured before centrifugation and in the corresponding supernatant using the Kjeldahl method (IDF, 2001b) taking the dilution effect due to pH adjustment into account in the calculation of the suspension stability.

Statistics

Due to differences in the TS in the UF concentrates, the results were balanced according to their TS contents before statistical analysis. One-way ANOVA was performed by using R statistical software (version 2.13.1 2011–07–08, R Development Core Team; http://www.r-project.org). The experimental factor for the different parts of the experiment (UF concentrates and powders) was the raw material (BM and SM) used for MF.

RESULTS

Fractionation of BM by MF

The microfiltration of SM has been described previously (Svanborg et al., 2014). During the microfiltration of BM, process observations were recorded 3 times per hour, and the average values from 8 filtration days are shown in Table 1. The initial batches of BM (1,600 L divided for use on 2 consecutive days) were larger than the batches of SM (650 L/MF); therefore, the flux was kept at a lower level to minimize time-dependent fouling when fractioning the BM. The fractionation of BM was comparable to the fractionation of pasteurized SM in terms of development of the transmembrane pressure (**TMP**). The TMP increased toward the end of filtration of both types of feed. Despite a higher concentration factor ($P < 0.05$) and permeate flux ($P < 0.001$), no significant difference in the TMP was observed between the 2 raw materials.

The mass balance of the 4 batches of BM (in total 8 filtrations) was calculated and is shown in Table 2. Approximately 62% of the TS were retained by the membrane, and around one-third of the whey proteins passed through the membrane. The majority of the minerals, fat, and almost all of the trace metals (Fe and Cu) were retained in the retentate. Most of the PL were retained by the membrane, except lysophosphatidylcholine (**LPC**), which was found in equal amounts in both fractions. Calcium and phosphorus followed the same

Table 1. Processing conditions during the microfiltration of skimmed buttermilk and skim milk given as means and their SD (n = 8)

	Skimmed buttermilk		Skim milk[1]	
Item	Mean	SD	Mean	SD
Volume concentration factor	2.43	0.06*	2.52	0.52
Flux (L × $h^{-1}min^{-2}$)	76.8	2.8***	82.9	4.6
Filtration temperature (°C)	56.2	0.6	56.2	1.2
Transmembrane pressure (kPa)	43	8	43	8

[1]Results from Svanborg et al. (2014).

*$P < 0.05$; ***$P < 0.001$.

Table 2. Mass balance after microfiltration (MF) of 1,000 kg of skimmed buttermilk (BM); values are expressed as means and their SD (n = 8)

Milk component[1]	BM (1,000 kg)		MF retentate from BM (411 kg)		MF permeate from BM (589 kg)	
	Mean	SD	Mean	SD	Mean	SD
TS (kg)	74.95	5.65	46.43	4.44	28.87	1.46
TN (kg)	3.51	0.40	3.22	0.32	0.12	0.03
NCN (kg)	0.76	0.07	0.46	0.05	0.40	0.09
NPN (kg)	0.29	0.05	0.11	0.01	0.16	0.01
TP (kg)	20.54	2.78	19.83	2.10	ND[2]	
Casein (kg)	15.67	3.10	16.57	2.21	ND	
Whey protein (kg)	3.05	0.46	2.37	0.19	1.55	0.57
Lactose (kg)	39.54	2.02	15.24	0.74	24.48	1.46
Fat (kg)	3.00	0.58	3.75	1.88	ND	
Ca (kg)	0.74	0.08	0.61	0.06	0.14	0.02
Cu (g)	0.02	0.04	0.60	0.10	0.00	0.00
Fe (g)	0.65	0.15	0.57	0.07	0.07	0.02
K (kg)	1.32	0.08	0.57	0.03	0.75	0.05
Mg (kg	0.09	0.01	0.05	0.03	0.04	0.00
Na (kg)	0.33	0.01	0.14	0.03	0.19	0.01
P (kg)	0.69	0.06	0.50	0.41	0.19	0.02
α-Tocopherol (mL)	40.7	7.4	58.5	31.0	ND	
PE (kg)	0.26	0.04	0.34	0.05	ND	
LPE (kg)	ND		ND		ND	
PC (kg)	0.47	0.05	0.44	0.05	0.03	0.00
PS + PI (kg)	0.36	0.07	0.56	0.08	ND	
SPM (kg)	0.05	0.00	0.07	0.02	ND	
LPC (kg)	0.30	0.03	0.17	0.04	0.17	0.02
Total PL (kg)	1.44	0.13	1.59	0.20	0.21	0.00

[1]NCN = noncasein nitrogen; LPC = lysophosphatidylcholine; LPE = lipophosphatidylethanolamine; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PI = phosphatidylinositol; PL = polar lipid (phospholipids); PS = phosphatidylserine; SPM = sphingomyelin; TN = total nitrogen; TP = total protein, (TN − NPN) × 6.38; whey protein = (NCN − NPN) × 6.38; casein = (TN − NCN − NPN) × 6.38.

[2]ND = not detected (below the threshold of the analysis).

trend as CN and fat during the fractionation of BM, whereas potassium and sodium were found in higher amounts in the permeate compared with the other minerals and trace metals. However, some discrepancy was noted between the somewhat low amounts of PL and α-tocopherol in the initial BM compared with the much higher summed amounts detected in the MF fractions of BM (Table 2).

UF Concentrate

The liquid WPC produced by UF/DF of MF permeates of BM and SM were compared on a TS basis to balance the data according to the initial difference in the TS in Table 3. In the liquid WPC from the BM, the fat content was rather high, whereas fat was not detected in the liquid WPC originating from SM. In addition, less total protein and lactose were observed in the BM liquid WPC compared with in the SM liquid WPC. The amounts of undenatured whey proteins were similar in the 2 concentrates. Meanwhile substantial amounts of CN were found in the liquid SM WPC, and no CN was detected in the liquid BM WPC. The concentrates also differed in their mineral compositions ($P < 0.01$), with less calcium and higher amounts of magnesium and iron in the liquid BM WPC than in the SM WPC. Only the BM WPC was analyzed for PL, and only phosphatidylcholine (0.18 ± 0.03 g/100 g) and PL (0.35 ± 0.08 g/100 g) were detected.

Composition and Functionality of WPC Powder

Results from the chemical analysis of BMWPC and SMWPC powders are shown in Table 4. As in the liquid WPC, the amounts of native whey proteins were similar in BMWPC and SMWPC. The BMWPC contained less total protein than SMWPC, although small amounts of caseins were also found in BMWPC. The samples of SMWPC powder showed higher CN contents compared with those of BMWPC, which was confirmed by SDS-PAGE as giving stronger bands in the area where CN is normally found (Figure 1). The BMWPC samples showed a strong band in the high molecular weight area of the SDS-PAGE gel (>97 kDa). This band was identified to be FA synthase by liquid chromatography-mass spectrometry, but it also contained an embryo-specific fibronecitin 1 transcript variant and complement C3. The same band was present in lanes loaded with

Table 3. Amounts of TS and components in the ultrafiltration/diafiltration (UF/DF) concentrates of the MF permeates from skimmed buttermilk (BM) and skim milk (SM) calculated on a DM basis[1]

	UF/DF concentrate of MF permeate from BM		UF/DF concentrate of MF permeate from SM	
Milk component[2]	Mean	SD	Mean	SD
TS (g)	5.51	0.49**	7.45	0.53
TN (g)	13.64	0.16**	14.43	0.30
NPN (g)	0.93	0.23	0.32	0.04
NCN (g)	12.88	0.19***	12.22	0.05
Total protein (g)	81.10	0.71***	90.00	1.74
CN (g)		0***	12.03	1.96
Whey protein (g)	76.23	1.44	75.89	0.40
Fat (g)	17.66	1.74*	ND[3]	
Ca (g)	0.591	0.045**	0.703	0.018
Cu (mg)	0.524	0.262	0.431	0.114
Fe (mg)	1.870	0.114**	1.071	0.287
K (g)	0.281	0.099	0.342	0.021
Mg (g)	0.078	0.005**	0.068	0.001
Na (g)	0.193	0.033	0.164	0.005
P (g)	0.303	0.029	0.315	0.007
Citric acid (g)	0.51	0.37	0.15	0.04
Lactose (g)	0.96	0.83*	2.48	0.56

[1]With the exception of TS given as per 100 g/sample, values are given as per 100 g/TS. Values are given as means and their SD (n = 4).

[2]NCN = noncasein nitrogen; TN = total nitrogen; TP = total protein (TN − NPN) × 6.38; whey protein = (NCN − NPN) × 6.38; CN = (TN − NCN − NPN) × 6.38.

[3]ND = not detected (below the threshold of the analysis).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

SMWPC, but it was weaker. Bands identified as xanthine dehydrogenase (**XDH**; ~97 kDa) were stronger in the BMWPC samples compared with in the SMWPC samples. The BMWPC bands between 14 and 22 kDa were not very well defined, but were identified to be calcineurin B homologous protein and glycosylation-dependent protein 1. Lactadherin (molecular weight 48 kDa) was identified from the BMWPC samples at approximately 45 kDa. Perilipin 2 and vitamin D-binding protein were identified in bands between 45 and 66 kDa, respectively, and were more pronounced in the SMWPC samples than in the BMWPC samples, whereas lactadherin, which appeared in the same area, was not apparent in the SMWPC samples. Bovine serum albumin was detected in all of the bands analyzed by liquid chromatography-mass spectrometry, and traces of CN, α-LA, and β-LG were detected in several of the other proteins.

Despite the higher fat content of BMWPC, the 2 powders had similar amounts of PL but differed in their PL composition (Table 4). Lysophosphatidylcholine was found in a smaller amount in BMWPC ($P < 0.01$) compared with in SMWPC and accounted for the majority of the PL in SMWPC. Sphingomyelin was only detected in BMWPC ($P = 0.0534$), whereas phosphatidylcholine was the dominating PL in BMWPC and was found in higher amounts compared with in SMWPC ($P < 0.05$). However, the somewhat higher amounts of phosphatidylethanolamine (**PE**), phosphatidylserine (**PS**), and phosphatidylinositol (**PI**) found in BMWPC were not significantly different from the levels found in SMWPC.

The functional properties of dispersions made from whey protein concentrates (BMWPC and SMWPC) are shown in Table 5. High overruns were observed for both powders tested: 835 ± 150 and 884 ± 230% for BMWPC and SMWPC, respectively. The SMWPC foam was, however, far more stable than the BMWPC foam; the foam of the BMWPC solution completely decomposed after only 5 min, whereas the SMWPC foam was stable for hours. The BMWPC foam had larger bubbles than the SMWPC foam (results not shown), whereas the SMWPC foam had a denser appearance than the BMWPC foam. Both powders showed high nitrogen solubility even at low pH values. At a pH of 7.0, the suspension stability was much higher than at a pH of 4.6, whereas some variation was observed in the suspension solubility between the powder batches at a pH of 4.6.

DISCUSSION

Filtration of BM can be challenging due to residual fat and MFGM fragments of various particle sizes. The largest particles (butter fragments and large fat globules) can be removed from BM using a cream separator

Table 4. Composition of powder made from whey protein concentrate from permeate after the microfiltration of buttermilk (BMWPC) and skim milk (SMWPC)[1]

	BMWPC		SMWPC	
Milk component[2]	Mean	SD	Mean	SD
TS (g)	94.50	1.29	94.50	0.58
Total protein (g)	82.12	0.98**	88.45	2.23
CN (g)	4.27	2.32**	10.90	2.03
Native whey protein (g)	72.73	0.70	75.15	3.80
Lactose (g)	0.22	0.21**	1.72	0.45
Citric acid (g)	0.62	0.30*	0.08	0.03
Fat (g)	1.48	0.22***	0.13	0.04
Ca (mg)	578.3	51.6*	662.5	13.0
K (mg)	272.8	95.6	318.8	16.1
Mg (mg)	188.8	35.2***	63.5	0.6
Na (mg)	76.0	5.8***	152.8	2.6
P (mg)	294.3	27.8	295.5	5.9
Cu (mg)	0.6	0.3	0.4	0.1
Fe (mg)	2.0	0.0***	1.1	0.2
α-Tocopherol (μg/mL)		ND	ND[3]	
PE (mg)	47.2	11.6	4.9	0.6
LPE (mg)			9.8	2.3
PC (mg)	86.5	44.3*	12.0	0.6
PS+PI (mg)	58.1	6.1	12.4	0.5
SPM (mg)	8.8	2.1	ND	
LPC (mg)	47.0	36.9**	227.9	58.7
Total PL (mg)	220.0	121.0	268.9	56.2

[1]All values are means given as per 100 g/sample. Values are given as means and their SD (n = 4).

[2]LPC = lysophosphatidylcholine; LPE = lipophosphatidylcholine; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PI = phosphatidylinositol; PL = polar lipid (phospholipids); PS = phosphatidylserine; SPM = sphingomyelin; TP = total protein (TN − NPN) × 6.38; whey protein = (NCN − NPN) × 6.38; CN = (TN − NCN − NPN) × 6.38.

[3]ND = not detected (below the threshold of the analysis).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(Fauquant et al., 2014); thereby, a decreased fouling during fractionation by MF may be obtained. Due to the larger batch volumes of BM, precaution during the MF of the skimmed BM was taken and the flux was lowered compared with SM fractionation. However, no difference in fouling in terms of increased TMP (above that expected from the time-dependent fouling) was observed between the MF of SM and BM. The CN-rich MF retentate contained the majority of the PL obtained, and the fractionation of BM could be carried out under similar conditions to those of the fractionation of SM. The BM was skimmed before fractionation to reduce some of the possible effects of fat content variations between the 4 batches of BM received. In spite of this, the MF permeate from BM was higher in fat than that from SM, and further concentration of the MF permeate resulted in a BMWPC that also contained higher amounts of fat than the SMWPC. A more severe skimming may have equalized the differences in the initial fat contents of BM and SM (0.3 and 0.04 g/100 g, respectively). Alternatively, a membrane with a smaller pore size would be able to retain more MFGM fragments and some of the fat globules. Although the MF membrane retained most of the fat during the MF of both the BM and SM, BMWPC contained higher amounts compared with SMWPC. One possible explanation is that the skimmed BM contained more fat initially compared with SM. Another possible explanation could be that the fat remaining in the skimmed BM had a higher content of free fat (Mulder and Walstra, 1974). Therefore, more fat was transferred through the membrane compared with in the fractionation of SM. Free fat cannot be separated from SM or BM by regular milk separators, and most of the triglyceride molecules are probably too small to be retained by the MF membranes. However, neither the fat globule size distribution nor the contents of the free fat were observed in the experiment reported here. The mass balance (Table 2) showed that the majority of the PL were retained in the retentate during MF. The origin of the PL obtained in the retentate would be the MFGM from intact fat globules, fragments of MFGM from disrupted fat globules, or complexes consisting of MFGM proteins and whey proteins or CN micelles, which were too large to pass through the membrane.

The temperatures during the filtrations in this study were all above 50°C. The filtration temperature is known to influence the transmission of PL over the

Table 5. Functional properties of dispersions made from the whey protein concentrates of microfiltered buttermilk (BMWPC) and microfiltered skim milk (SMWPC) given as means and their SD (n = 4)[1]

	BMWPC		SMWPC	
Functional property	Mean	SD	Mean	SD
SS (%) pH 7.0	98.00	2.39	98.09	2.22
SS (%) pH 4.6	79.11	9.01	79.44	4.86
TN (%) pH 7.0	99.50	2.18	98.26	2.14
TN (%) pH 4.6	85.73	2.93	84.59	5.28
Foam stability (min)	<2		>300	
Overrun (%)	884	230	835	150

[1]SS = suspension stability, TS in supernatant of 1% powder solution after centrifugation as a percentage of the TS in the sample; TN = soluble protein, total nitrogen in the supernatant of the sample solution as a percentage of the total nitrogen in sample; Overrun = [(wt protein solution − wt foam)/wt foam) × 100]. Foam stability is the time required to reduce the foam volume of an 8% protein solution by 50%.

MF membrane, and Morin et al. (2004) found that transmissions of the PL and total lipids were higher at high and intermediate temperatures of 50 and 25°C, respectively, than at 7°C. However, Morin et al. (2007b) found that the previous history of heat treatment of the BM and cream was the most important technological factor affecting the isolation of MFGM components by filtration technology. For this reason, the isolation of MFGM components from BM using membrane filtration could be improved if the heat denaturation of whey proteins, and possibly components in the MBGM membrane, was reduced by use of less harsh pasteurization conditions of the cream in the industry. Due to the transportation of cream between different dairy plants, the heating regimen of the butter cream in this experiment was representative for industrially obtained BM in Norway. From an industrial point of view, the diversity in cream treatment and BM handling would lead to a higher range in composition and functional properties of BMWPC compared with cheese WPC or SMWPC. To optimize the utilization of the whey protein fraction from BM, cream treatment and BM handling must be standardized between creameries, and the heat treatment controlled and documented. Severe heat treatment of BM could lead to formation of aggregates consisting of MFGM fragments and other milk

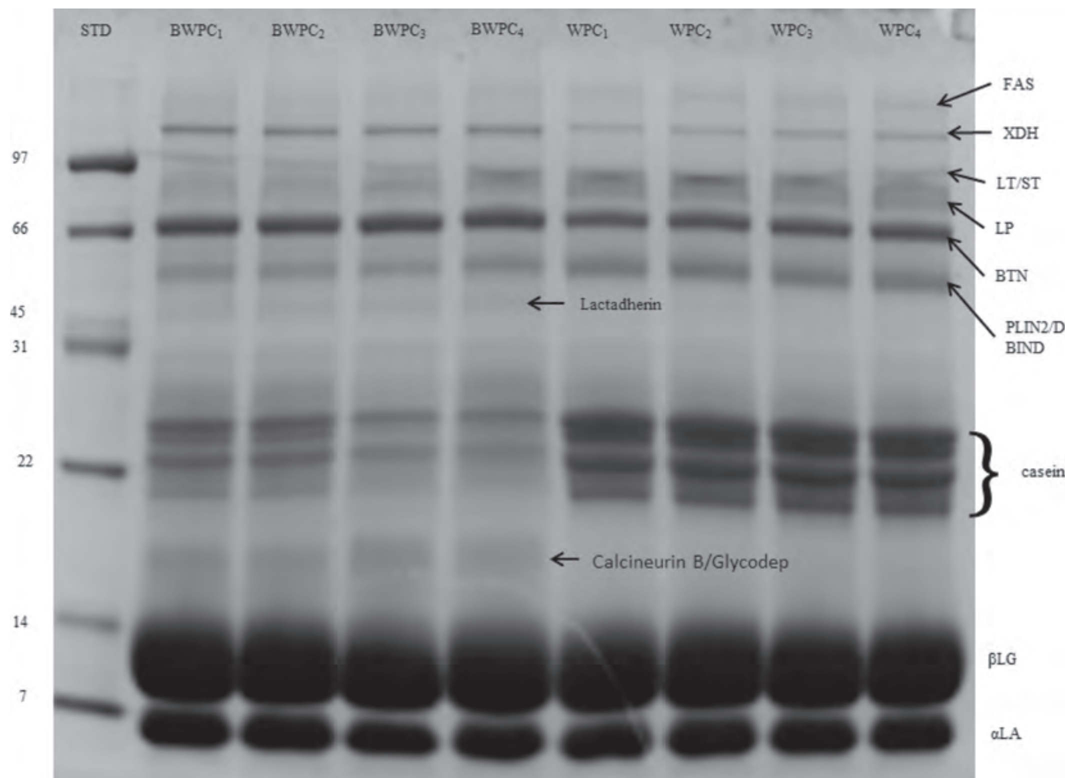

Figure 1. Sodium dodecyl sulfate-PAGE patterns of reconstituted whey protein concentrates (WPC) of buttermilk (BWPC) and skim milk. Subscripts indicate replicates. Lane STD was loaded with 5 μL of low-molecular-weight standard. Numbers to the left of lane STD indicate molecular weights (kDa). Lanes $BWPC_{1-4}$ and WPC_{1-4} were loaded with 7 μL of sample solution containing 8% protein (wt/wt). Identification of milk fat globule membrane and whey proteins by MS are indicated on the right side of the figure: BTN = butyrophilin; DBIND = vitamin D-binding protein; FAS = fatty acid synthase; Glycodep = glycosylation-dependent protein 1; LP = lactoperoxidase; LT = lactotransferrin; PLIN2 = perilipin 2; ST = serotransferrin; and XDH = xanthine dehydrogenase.

proteins (Ye et al., 2002), whey protein-CN interactions (Donato and Guyomarc'h, 2009), or aggregates of major whey proteins. The MF membrane retained the largest aggregates. However, some of the aggregates could be transmitted through the membrane, as the identified SDS-PAGE gel bands revealed aggregates containing casein, α-LA, and β-LG in both the BMWPC and the SMWPC samples.

Due to the analytical threshold, some PL classes could not be detected in the MF permeate. However, they may be identified in the more concentrated BMWPC powder, just as LPC was detected in the liquid WPC from BM. Variations in the sphingomyelin (**SPM**) content in milk have been reported, and the variation is mainly related to the size distribution of fat globules in the milk and thus to the amount of the MFGM available (Lopez et al., 2011). Sphingomyelin molecules, which are loosely bound to the outside of the MFGM membrane (Deeth, 1997), were not detected in the SMWPC. This is probably due to a lower initial fat content in the SM and to the fact that SPM tended to follow the fat fraction during separation (Spitsberg, 2005). The majority of SPM in the MF permeate from the fractionation of SM would be derived from intact fat globules that were small enough to be transported through the membrane. Phospholipids that originate from the smaller MFGM fragments and free fat in the BM are able to pass through the membrane (Astaire et al., 2003; Morin et al., 2004, 2006, 2007a). The PL distributions in BMWPC and SMWPC did not reflect the PL distribution usually found in the native MFGM present in milk, where phosphatidylcholine, phosphatidylethanolamine, and SPM are expected in higher quantities than the other varieties of PL (Vanderghem et al., 2010). Morin et al. (2007a) showed that the PL distribution was only slightly affected by MF and suggested that mainly whole MFGM fragments rather than dissociated PL were transmitted through the MF membrane during filtration. In milk, phosphatidylcholine, PI, and PS are buried in the inner layer of the MFGM (Danthine et al., 2000), preventing their interaction with other milk solids. When MFGM are no longer intact, both the inner and outer layers of the MFGM are exposed to the serum components of the BM. Morin et al. (2007b) showed that the intensity of heat treatment of the butter cream affected the MFGM protein aggregation and the solubility of BM solids more than the churning process itself. However, they found that spray drying of BM reduced the amount of phosphatidylethanolamine and to a much smaller extent the amounts of PI and PS. The functional properties of dairy products are partly affected by the state of the MFGM components after heat treatment (Keenan et al., 1983; Houlihan et al., 1992 a,b; Morin et al., 2008). In our study, protein denaturation caused by various and repeated cream pasteurization procedures did not significantly influence the soluble solids or the soluble total N in the final BMWPC compared with in the final SMWPC. However, a higher free fat content may reduce the solubility in the BMWPC (Schuck et al., 2012). Due to its high concentration of PL, BM may be easily oxidized (O'Connell and Fox, 2000). α-Tocopherol, a potential antioxidant to protect the polyunsaturated fat from oxidation (Nagachinta and Akoh, 2013), followed the CN, PL, and fat-rich fraction during MF and was therefore not detected in the MF permeates or in the final WPC powders. Therefore, the oxidative stability of BMWPC should be further investigated.

Whey proteins are known for their surface-active functionality. Undenatured β-LG and α-LA are recognized to have excellent foaming capacities and stabilities (Abd El-Salam et al., 2009). The large difference in foam stability observed between BMWPC and SMWPC was probably due to the difference in fat content, as the presence of lipids is known to depress the foaming properties of WPC (Morr, 1985). In an application where the WPC would have an emulsifying function, a higher lipid content would be favored; however, in most other applications, a fat reduction would be beneficial. A further improvement of the process to reduce the fat content prior membrane fractionation by a more intensive skimming or by using membranes with denser pore sizes (<0.2 μm) than used during MF in our study might enhance the foaming stability of BMWPC. No significant differences in the overrun between the WPC were observed despite the differences in the fat contents as well as in the PL and protein compositions. The total amounts of PL were similar in the 2 WPC, although higher levels of PL in the BMWPC was expected because of the PL following the MFGM fractions during churning.

The main difference in the nitrogen contents of BMWPC and SMWPC was the somewhat higher CN content in SMWPC and a corresponding higher content of whey proteins in the BMWPC samples. The reason for this is probably the low content of CN found in skimmed BM before MF. The BMWPC had a similar protein solubility to that of SMWPC at neutral and acidic pH values. The difference in the protein solubility in the WPC at a pH of 7.0 compared with at a pH of 4.6 may be explained by the acid precipitation of CN or aggregates of CN, denatured whey proteins, and MFGM proteins. The repeated cream pasteurization (74.2°C/20 s and 86°C/5 s) did not seem to influence the suspension stability or the protein solubility of the BMWPC, although we observed some variation in the suspension solubility under acidic conditions between the powder batches. This may possibly reflect the

variation in cream pasteurization procedures between the different BM batches.

The MFGM proteins, as well as the PL and sphingolipids in milk may have beneficial nutritional (Spitsberg, 2005; Britten et al., 2008; Dewettinck et al., 2008) and functional properties (Corredig and Dalgleish, 1997; Goudedranche et al., 2000). Traces of a wide spectrum of proteins and peptides were identified in the BMWPC and SMWPC, indicating the formation of aggregates during heat treatment of SM, BM, and cream before churning. Proteins account for 25 to 60% of the total mass of the MFGM, and membrane-specific proteins comprise approximately 1 to 2% of the total protein in milk (Mather, 2000). The MFGM proteins are known to react with β-LG during the heat treatment of milk (Kim and Jimenez-Flores, 1995). Ye et al. (2002) found that heating whole milk above 60°C resulted in larger protein complexes of XDH and butyrophilin (**BTN**), whereas lactadherin was more heat stable. In our study, the aggregates in the WPC consisted of MFGM-proteins (XDH, lactadherin, or BTN), CN, β-LG, and α-LA. The same compounds were detected in both BMWPC and SMWPC, with the exception of lactadherin, which was only identified in BMWPC. The XDH and BTN were, as expected, more pronounced in the BMWPC samples. Mucin 1, generally regarded as a major MFGM protein (Mather, 2000), was not detected in any of the samples. According to Le et al. (2012), a high resistance against trypsin and pepsin hydrolysis may influence the detection of mucin 1 during sample preparation due to its heavy glycosylation. Glycosylation-dependent cell adhesion molecule 1 was identified together with calcineurin B homologous protein, tetranectin, and Ras-related protein Rab-18. The latter protein was recently identified in BM by Le et al. (2013), who also found calcineurin B homologous protein associated with the MFGM fractions. However, a potential higher content of MFGM proteins in the BMWPC compared with in the SMWPC did not seem to influence the functional properties of the WPC, most likely due to the low levels of MFGM present in the final powders.

The level of lactose was lower in BMWPC compared with in the SMWPC. The reason for this is probably that during UF of the MF permeate from BM more water was used than during the UF/DF of the MF permeate from SM. The reason for the difference in volumes of DF water (350–500 and 650–1,075 L for the UF/DF of the SM and BM permeates, respectively) was the initial differences in the MF permeates produced. During the MF of SM, Ca and P tended to follow the CN fraction. The initial mineral composition of the feed influenced the mineral contents of the MF fractions. Buttermilk and its MF fractions contained less Ca compared with SM and SM fractions, most likely due to an initial lower CN content in the BM. Approximately 70% of the Ca in milk is associated with the CN micelle (Walstra et al., 2006). The higher amount of DF water used during UF/DF may have also influenced the mineral content of BMWPC. The transmission of serum-soluble minerals during the fractionation of BM resulted in a BMWPC richer in trace metals (Fe, Mg, and Cu) compared with the SMWPC. Evers (2004) reported a transfer of Cu from milk serum to MFGM due to heat treatment. Approximately 10% of Cu and nearly 50% of Fe is associated with the MFGM in milk (Walstra et al., 2006). The higher contents of Cu and Fe in BMWPC compared with in SMWPC are most likely a result of the higher initial fat content in BM and due to the storage of BM at 4°C for several days. Copper and Fe are present in approx. 0.3 and 0.01 mg/100 g of fat globules, respectively; but during cold storage, Cu may migrate irreversibly into the milk serum (Walstra et al., 2006). Conversely, the heating of cream above 60°C may cause considerable migration of Cu from the milk serum to the MFGM (Mulder and Walstra, 1974).

Iron is present in the MFGM of milk fat. Differences in the mineral composition can affect the functional properties of WPC and WPI (Lorenzen and Schrader, 2006). The BMWPC had a higher content of Mg but a lower content of Ca compared with SMWPC. Zhu and Damodaran (1994) showed that divalent cations (Ca^{2+} and Mg^{2+}) improved the foaming properties of WPI. The cations can link 2 negatively charged proteins and cause aggregation.

CONCLUSIONS

This study showed that a WPC powder, which has interesting composition (PL profile and protein composition) and solubility properties, could be produced from BM fractionation. The BMWPC was equivalent to SMWPC in its solubility, foaming properties, and chemical composition. The protein profiles of BMWPC and SMWPC were comparable, but SMWPC had a higher content of CN. Despite the comparable compositions of the WPC, WPC from BM showed a lower foam stability, probably due to its higher fat content. The SMWPC and BMWPC contained comparable amounts of total PL, but the compositions of the PL differed. All PL classes investigated, except for LPC, were found in significantly higher amounts in BMWPC than in SMWPC.

ACKNOWLEDGMENTS

This work was financially supported by TINE SA and The Research Council of Norway (Oslo) through

the Industrial PhD scheme. The authors thank the staff of the pilot plant and the laboratory at IKBM (Norwegian University of Life Sciences) as well as the staff of the laboratory of TINE R&D Måltidets Hus for their assistance and support during the experimental and analytical work. The authors also thank Morten Skaugen, Ellen Kathrine Ulleberg, and Irene Comi (Norwegian University of Life Sciences) for their kind assistance during protein analysis, Rahel Cristine Brändli (TINE R&D) for her support during the data analysis and Tom Hoffmann (TINE SA) for his assistance, inspiration, and support during the planning and testing period.

REFERENCES

Abd El-Salam, M. H., S. El-Shibiny, and A. Salem. 2009. Factors affecting the functional properties of whey protein products: A review. Food Rev. Int. 25:251–270. http://dx.doi.org/10.1080/87559120902956224.

Astaire, J. C., R. Ward, J. B. German, and R. Jimenez-Flores. 2003. Concentration of polar MFGM lipids from buttermilk by microfiltration and supercritical fluid extraction. J. Dairy Sci. 86:2297–2307.

Bacher, T., and P. Køningsfeldt. 2000. WPI by microfiltration of skim milk. Scand. Dairy Inf. 3:18–20.

Berhe, N., B. L. Halvorsen, T. E. Gundersen, B. Myrvang, S. G. Gundersen, and R. Blomhoff. 2007. Reduced serum concentrations of retinol and alpha-tocopherol and high concentrations of hydroperoxides are associated with community levels of S-mansoni infection and schistosomal periportal fibrosis in Ethiopian school children. Am. J. Trop. Med. Hyg. 76:943–949.

Britten, M., S. Lamothe, and G. Robitaille. 2008. Effect of cream treatment on phospholipids and protein recovery in butter-making process. Int. J. Food Sci. Technol. 43:651–657.

Calvo, M. M., J. Leaver, and J. M. Banks. 1993. Influence of other whey proteins on the heat-induced aggregation of α-lactalbumin. Int. Dairy J. 3:719–727.

Coppola, L. E., M. S. Molitor, S. A. Rankin, and J. A. Lucey. 2014. Comparison of milk-derived whey protein concentrates containing various levels of casein. Int. J. Dairy Technol. 67:467–473. http://dx.doi.org/10.1111/1471-0307.12157.

Corredig, M., and D. G. Dalgleish. 1997. Isolates from industrial buttermilk: Emulsifying properties of materials derived from the milk fat globule membrane. J. Agric. Food Chem. 45:4595–4600. http://dx.doi.org/10.1021/jf970531f.

Corredig, M., R. R. Roesch, and D. G. Dalgleish. 2003. Production of a novel ingredient from buttermilk. J. Dairy Sci. 86:2744–2750. http://dx.doi.org/10.3168/jds.S0022-0302(03)73870-3.

Dalgleish, D. G., V. Senaratne, and S. Francois. 1997. Interactions between α-lactalbumin and β-lactoglobulin in the early stages of heat denaturation. J. Agric. Food Chem. 45:3459–3464. http://dx.doi.org/10.1021/jf970113a.

Danthine, S., C. Blecker, M. Paquot, N. Innocente, and C. Deroanne. 2000. Evolution des connaissances sur la membrane du globule de gras du le lait: Synthese bibliographique. Lait 80:209–222. http://dx.doi.org/10.1051/lait:2000120.

De La Fuente, M. A., B. Carazo, and M. Juárez. 1997. Determination of major minerals in dairy products digested in closed vessels using microwave heating. J. Dairy Sci. 80:806–811. http://dx.doi.org/10.3168/jds.S0022-0302(97)76001-6.

De La Fuente, M. A., Y. Hemar, M. Tamehana, P. A. Munro, and H. Singh. 2002. Process-induced changes in whey proteins during the manufacture of whey protein concentrates. Int. Dairy J. 12:361–369. http://dx.doi.org/10.1016/s0958-6946(02)00031-6.

Deeth, H. C. 1997. The role of phospholipids in the stability of milk fat globules. Aust. J. Dairy Technol. 52:44–46.

Devle, H., E. K. Ulleberg, C. F. Naess-Andresen, E.-O. Rukke, G. Vegarud, and D. Ekeberg. 2014. Reciprocal interacting effects of proteins and lipids during ex vivo digestion of bovine milk. Int. Dairy J. 36:6–13. http://dx.doi.org/10.1016/j.idairyj.2013.11.008.

Dewettinck, K., R. Rombaut, N. Thienpont, T. T. Le, K. Messens, and J. Van Camp. 2008. Nutritional and technological aspects of milk fat globule membrane material. Int. Dairy J. 18:436–457. http://dx.doi.org/10.1016/j.idairyj.2007.10.014.

Donato, L., and F. Guyomarc'h. 2009. Formation and properties of the whey protein/kappa-casein complexes in heated skim milk—A review. Dairy Sci. Technol. 89:3–29.

Evans, J., J. Zulewska, M. Newbold, M. A. Drake, and D. M. Barbano. 2010. Comparison of composition and sensory properties of 80% whey protein and milk serum protein concentrates. J. Dairy Sci. 93:1824–1843. http://dx.doi.org/10.3168/jds.2009-2723.

Evers, J. M. 2004. The milkfat globule membrane—Methodologies for measuring milkfat globule (membrane) damage. Int. Dairy J. 14:747–760. http://dx.doi.org/10.1016/j.idairyj.2004.01.006.

Fauquant, J., E. Beaucher, C. Sinet, B. Robert, and C. Lopez. 2014. Combination of homogenization and cross-flow microfiltration to remove microorganisms from industrial buttermilks with an efficient permeation of proteins and lipids. Innov. Food Sci. Emerg. Technol. 21:131–141. http://dx.doi.org/10.1016/j.ifset.2013.10.004.

Goudedranche, H., J. Fauquant, and J. L. Maubois. 2000. Fractionation of globular milk fat by membrane microfiltration. Lait 80:93–98.

Govindasamy-Lucey, S., T. Lin, J. J. Jaeggi, M. E. Johnson, and J. A. Lucey. 2006. Influence of condensed sweet cream buttermilk on the manufacture, yield, and functionality of pizza cheese. J. Dairy Sci. 89:454–467. http://dx.doi.org/10.3168/jds.S0022-0302(06)72109-9.

Heino, A. T., J. Uusi-Rauva, P. R. Rantamäki, and O. Tossavainen. 2007. Functional properties of native and cheese whey protein concentrate powders. Int. Dairy J. 60:277–285. http://dx.doi.org/10.1111/j.1471-0307.2007.00350.x.

Houlihan, A. V., P. A. Goddard, B. J. Kitchen, and C. J. Masters. 1992a. Changes in structure of the bovine-milk fat globule-membrane on heating whole milk. J. Dairy Res. 59:321–329.

Houlihan, A. V., P. A. Goddard, S. M. Nottingham, B. J. Kitchen, and C. J. Masters. 1992b. Interactions between the bovine-milk fat globule-membrane and skim milk components on heating whole milk. J. Dairy Res. 59:187–195.

IDF. 1987a. Dried milk, dried whey, dried buttermilk and dried butter serum determination of fat content (Röse Gottlieb reference method). 9C:1987. International Dairy Federation, Brussels, Belgium.

IDF. 1987b. Milk cream and evaporated milk. Determination of total solid content. 21B:1987. International Dairy Federation, Brussels, Belgium.

IDF. 2001a. 20–5 Milk—Determination of nitrogen content. Part 5: Determination of protein-nitrogen content. 20–5:2005. International Dairy Federation, Brussels, Belgium.

IDF. 2001b. Determination of nitrogen content. Part 1: Kjeldahl method. 20–1 Milk:2001. International Dairy Federation, Brussels, Belgium.

IDF. 2001c. Determination of nitrogen content. Part 4: Determination of non-protein nitrogen. 20–4:Milk:2001. International Dairy Federation, Brussels, Belgium.

IDF. 2004. Milk—Determination of the casein-nitrogen content. Part 2: Direct method. 29–2:2004. International Dairy Federation, Brussels, Belgium.

IDF. 2008. Milk—Determination of fat content—Gerber butyrometers. 105:2008. International Dairy Federation, Brussels, Belgium.

Ishihama, Y., Y. Oda, T. Tabata, T. Sato, T. Nagasu, J. Rappsilber, and M. Mann. 2005. Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. Mol. Cell. Proteomics 4:1265–1272. http://dx.doi.org/10.1074/mcp.M500061-MCP200.

Islam, M. A. 2014. Principal components of buffalo and cow milk from Bangladesh: Proteolysis and lipolysis of buffalo, cow and omega-3

enriched milk by ex vivo digestion. PhD thesis. Norwegian University of Life Sciences, Ås, Norway.
Keenan, T. W., T.-W. Moon, and D. P. Dylewski. 1983. Lipid globules retain globule membrane material after homogenization. J. Dairy Sci. 66:196–203. http://dx.doi.org/10.3168/jds.S0022-0302(83)81777-9.
Kim, H. H., and R. Jimenez-Flores. 1995. Heat-induced interactions of skim milk proteins with milk fat globule membrane proteins. J. Dairy Sci. 78:24–35.
Lafougère, C. 2014. Supply and demand developments of whey in global perspective. Oral presentation. 7th International Whey Conference, Rotterdam, the Netherlands.
Le, T. T., G. Debyser, W. Gilbert, K. Struijs, J. Van Camp, T. Van de Wiele, B. Devreese, and K. Dewettinck. 2013. Distribution and isolation of milk fat globule membrane proteins during dairy processing as revealed by proteomic analysis. Int. Dairy J. 32:110–120. http://dx.doi.org/10.1016/j.idairyj.2013.05.002.
Le, T. T., T. Van de Wiele, T. N. H. Do, G. Debyser, K. Struijs, B. Devreese, K. Dewettinck, and J. Van Camp. 2012. Stability of milk fat globule membrane proteins toward human enzymatic gastrointestinal digestion. J. Dairy Sci. 95:2307–2318. http://dx.doi.org/10.3168/jds.2011-4947.
Lopez, C., V. Briard-Bion, O. Ménard, E. Beaucher, F. Rousseau, J. Fauquant, N. Leconte, and B. Robert. 2011. Fat globules selected from whole milk according to their size: Different compositions and structure of the biomembrane, revealing sphingomyelin-rich domains. Food Chem. 125:355–368.
Lorenzen, P. C., and K. Schrader. 2006. A comparative study of the gelation properties of whey protein concentrate and whey protein isolate. Lait 86:259–271. http://dx.doi.org/10.1051/lait:2006008.
Mather, I. H. 2000. A review and proposed nomenclature for major proteins of the milk-fat globule membrane. J. Dairy Sci. 83:203–247. http://dx.doi.org/10.3168/jds.S0022-0302(00)74870-3.
Moe, K. M., D. Porcellato, and S. Skeie. 2013. Metabolism of milk fat globule membrane components by nonstarter lactic acid bacteria isolated from cheese. J. Dairy Sci. 96:727–739. http://dx.doi.org/10.3168/jds.2012-5497.
Morin, P., M. Britten, R. Jimenez-Flores, and Y. Poulio. 2007a. Microfiltration of buttermilk and washed cream buttermilk for concentration of milk fat globule membrane components. J. Dairy Sci. 90:2132–2140. http://dx.doi.org/10.3168/jds.2006-832.
Morin, P., R. Jimenez-Flores, and Y. Pouliot. 2004. Effect of temperature and pore size on the fractionation of fresh and reconstituted buttermilk by microfiltration. J. Dairy Sci. 87:267–273.
Morin, P., R. Jimenez-Flores, and Y. Pouliot. 2007b. Effect of processing on the composition and microstructure of buttermilk and its milk fat globule membranes. Int. Dairy J. 17:1179–1187. http://dx.doi.org/10.1016/j.idairyj.2007.03.010.
Morin, P., Y. Pouliot, and M. Britten. 2008. Effect of buttermilk made from creams with different heat treatment histories on properties of rennet gels and model cheeses. J. Dairy Sci. 91:871–882. http://dx.doi.org/10.3168/jds.2007-0658.
Morin, P., Y. Pouliot, and R. Jimenez-Flores. 2006. A comparative study of the fractionation of regular buttermilk and whey buttermilk by microfiltration. J. Food Eng. 77:521–528. http://dx.doi.org/10.1016/j.jfoodeng.2005.06.065.
Morr, C. V. 1985. Composition, physicochemical and functional-properties of reference whey-protein concentrates. J. Food Sci. 50:1406–1411. http://dx.doi.org/10.1111/j.1365-2621.1985.tb10488.x.
Mulder, H., and P. Walstra. 1974. The Milk Fat Globule. Emulsion Science as Applied to Milk Products and Comparable Foods. Technical communication, Commonwealth Bureau of Dairy Science and Technology, UK.
Nagachinta, S., and C. C. Akoh. 2013. Spray-dried structured lipid containing long-chain polyunsaturated fatty acids for use in infant formulas. J. Food Sci. 78:C1523–C1528. http://dx.doi.org/10.1111/1750-3841.12243.
O'Connell, J. E., and P. F. Fox. 2000. Heat stability of buttermilk. J. Dairy Sci. 83:1728–1732.
Phillips, L. G., Z. Haque, and J. E. Kinsella. 1987. A method for the measurement of foam formation and stability. J. Food Sci. 52:1074–1077. http://dx.doi.org/10.1111/j.1365-2621.1987.tb14279.x.
Rombaut, R., J. V. Camp, and K. Dewettinck. 2005. Analysis of phospho- and sphingolipids in dairy products by a new HPLC method. J. Dairy Sci. 88:482–488. http://dx.doi.org/10.3168/jds.S0022-0302(05)72710-7.
Rombaut, R., V. Dejonckheere, and K. Dewettinck. 2006. Microfiltration of butter serum upon casein micelle destabilization. J. Dairy Sci. 89:1915–1925. http://dx.doi.org/10.3168/jds.S0022-0302(06)72259-7.
Sachdeva, S., and W. Buchheim. 1997. Recovery of phospholipids from buttermilk using membrane processing. Kiel. Milchwirtsch. Forsch. 49:47–68.
Sava, N., I. Van der Plancken, W. Claeys, and M. Hendrickx. 2005. The kinetics of heat-induced structural changes of β-lactoglobulin. J. Dairy Sci. 88:1646–1653. http://dx.doi.org/10.3168/jds.S0022-0302(05)72836-8.
Schokker, E. P., H. Singh, D. N. Pinder, and L. K. Creamer. 2000. Heat-induced aggregation of beta-lactoglobulin AB at pH 2.5 as influenced by ionic strength and protein concentration. Int. Dairy J. 10:233–240. http://dx.doi.org/10.1016/S0958-6946(00)00047-9.
Schuck, P., A. Dolivet, and R. Jeantet. 2012. Determination of total fat and free fat content. Pages 99–111 in Analytical Methods for Food and Dairy Powders. Wiley-Blackwell, Hoboken, NJ.
Sikand, V., P. S. Tong, S. Roy, L. E. Rodriguez-Saona, and B. A. Murray. 2011. Solubility of commercial milk protein concentrates and milk protein isolates. J. Dairy Sci. 94:6194–6202. http://dx.doi.org/10.3168/jds.2011-4477.
Singh, H. 2006. The milk fat globule membrane—A biophysical system for food applications. Curr. Opin. Colloid Interface Sci. 11:154–163. http://dx.doi.org/10.1016/j.cocis.2005.11.002.
Sodini, I., P. Morin, A. Olabi, and R. Jimenez-Flores. 2006. Compositional and functional properties of buttermilk: A comparison between sweet, sour, and whey buttermilk. J. Dairy Sci. 89:525–536. http://dx.doi.org/10.3168/jds.S0022-0302(06)72115-4.
Spitsberg, V. L. 2005. Bovine milk fat globule membrane as a potential nutraceutical. J. Dairy Sci. 88:2289–2294. http://dx.doi.org/10.3168/jds.S0022-0302(05)72906-4.
Svanborg, S., A.-G. Johansen, R. K. Abrahamsen, and S. B. Skeie. 2014. Initial pasteurisation effects on the protein fractionation of skimmed milk by microfiltration. Int. Dairy J. 37:26–30. http://dx.doi.org/10.1016/j.idairyj.2014.02.004.
Vanderghem, C., P. Bodson, S. Danthine, M. Paquot, C. Deroanne, and C. Blecker. 2010. Milk fat globule membrane and buttermilks: From composition to valorization. Biotechnol. Agron. Soc. Environ. 14:485–500.
Walstra, P., J. T. M. Wouters, and T. Geurts. 2006. Dairy Science and Technology. CRC/Taylor & Francis, Boca Raton FL.
Ye, A., H. Singh, M. W. Taylor, and S. Anema. 2002. Characterization of protein components of natural and heat-treated milk fat globule membranes. Int. Dairy J. 12:393–402. http://dx.doi.org/10.1016/S0958-6946(02)00034-1.
Ye, A. Q., H. Singh, M. W. Taylor, and S. Anema. 2004. Interactions of whey proteins with milk fat globule membrane proteins during heat treatment of whole milk. Lait 84:269–283. http://dx.doi.org/10.1051/lait:2004004.
Zhu, H. M., and S. Damodaran. 1994. Effects of calcium and magnesium-ions on aggregation of whey-protein isolate and its effect on foaming properties. J. Agric. Food Chem. 42:856–862. http://dx.doi.org/10.1021/jf00040a003.