Microfiltration of Raw Whole Milk to Select Fractions with Different Fat Globule Size Distributions: Process Optimization and Analysis

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ABSTRACT

We present an extensive description and analysis of a microfiltration process patented in our laboratory to separate different fractions of the initial milk fat globule population according to the size of the native milk fat globules (MFG). We used nominal membrane pore sizes of 2 to 12 μm and a specially designed pilot rig. Using this process with whole milk [whose MFG have a volume mean diameter (d_{43}) = $4.2 \pm 0.2 \mu\text{m}$] and appropriate membrane pore size and hydrodynamic conditions, we collected 2 extremes of the initial milk fat globule distribution consisting of 1) a retentate containing large MFG of d_{43} = 5 to 7.5 μm (with up to 250 g/kg of fat, up to 35% of initial milk fat, and up to 10% of initial milk volume), and 2) a permeate containing small MFG of d_{43} = 0.9 to 3.3 μm (with up to 16 g/kg of fat, up to 30% of initial milk fat, and up to 83% of initial milk volume and devoid of somatic cells). We checked that the process did not mechanically damage the MFG by measuring their ζ -potential. This new microfiltration process, avoiding milk aging, appears to be more efficient than gravity separation in selecting native MFG of different sizes. As we summarize from previous and new results showing that the physicochemical and technological properties of native milk fat globules vary according to their size, the use of different fat globule fractions appears to be advantageous regarding the quality of cheeses and can lead to new dairy products with adapted properties (sensory, functional, and perhaps nutritional).

Key words: microfiltration, milk fat globule, particle size, cheese

INTRODUCTION

Fat is known to contribute greatly to the sensory and functional characteristics of many dairy products such as cheese, yogurt, or whipped cream. Fat is present in

milk in the form of small (from 0.15 to 15 μm) droplets: the milk fat globules (MFG; Mulder and Walstra, 1974). There are numerous small fat globules (SFG) representing a small fraction of the fat but a tremendous surface area, and very few large ones (LFG) comprising a larger fat percentage (Mulder and Walstra, 1974). These globules are naturally surrounded by their native milk fat globule membrane (MFGM), which is composed mainly of phospholipids, cholesterol, proteins, and enzymes (Mather, 2000). In many dairy products, fat composition and structure cannot be adjusted easily because these native MFG from raw milk must be used. Indeed, reducing the fat globule size by homogenization results in small fat droplets that are disrupted and covered by caseins and whey proteins, thereby promoting their interactions with the cheese casein matrix (Michalski et al., 2002). Consequently, in some cheeses, milk homogenization adversely affects the structure of the rennet gel, resulting in poor functional properties (Gilles and Lawrence, 1981; Green et al., 1983; Jana and Upadhyay, 1992).

Due to the use of native MFG being of tremendous importance in some cheese making processes, technologies have been developed to separate native MFG of different sizes. Indeed, the small native fat globules are expected to alter the functionality because they contain more MFGM and would differ slightly in composition (Timmen and Patton, 1988). However, the technologies proposed to date are gravity separation (Ma and Barbano, 2000; O'Mahony et al., 2005) and centrifugation (Timmen and Patton, 1988), which cannot accurately separate MFG of different sizes. Indeed, the "cream" fraction enriched in larger fat globules cannot be devoid of the numerous smallest ones, and the semiskimmed fraction containing small globules can hardly contain the smallest ones and they cannot be selectively separated.

The aim of the present article is to present the thorough description and analysis of a microfiltration (MiFi) process patented in our laboratory (Goudédranche et al., 1998). This process, formerly of a proprietary nature, was briefly described by Goudédranche et al. (2000) and enables one to obtain native milk fat globule fractions sterically selected from whole milk, with di-

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ameters significantly smaller or larger than the original unseparated MFG population (so-called regular MFG). The optimization and operating hydrodynamic conditions of the process will be presented as well as its enhanced performance compared with gravity separation. A review of the physicochemical and technological properties of the SFG and LFG fractions will also be presented.

MATERIALS AND METHODS

Crossflow Microfiltration System

Membrane Properties and Cleaning. We used 0.24 m² or 0.72 m² of tubular mineral multichannel membrane made of alumina, with 19 channels of average diameter = $4 \cdot 10^{-3}$ m and membrane length = 1.02 m (Membralox P1940, Pall Exekia, Tarbes, France). Different mean nominal pore diameters were studied as suggested in the patent, from 2 to 12 μ m, and the process was optimized for industrial use with a pore size of 5 μ m. Before each run, the membrane was chemically cleaned using bleach [48° chloride (Pintaud, Mansle, France), 0.12% vol/vol, 20°C, 20 min, retentate extraction and permeation flux (J_P) = 416 L/h per m², batch circulation, flow rate of the retentate in the rig (F_R) = 6 m³/h], alkaline solution [Ultrasil 25F, Henkel-Ecolab, SNC, Issy-les-Moulineaux, France, 1% vol/vol, 65°C, 20 min, retentate extraction and J_P = 416 L/h per m², batch circulation, F_R = 6 m³/h], and HNO₃ [purity 58% (Carlo Erba Reagenti, Val de Rueil, France), 1% vol/vol, 50°C, 20 min, retentate extraction and J_P = 416 L/h per m², batch circulation, F_R = 6 m³/h] with water rinsing (filtered at 5 μ m) between each step until neutral pH was reached. For each pore size, all experiments were performed with the same cleaned membranes. The hydraulic resistance of the 5- μ m membrane was 2×10^{-10} m⁻¹. After each run, the membrane was rinsed with water at 50°C, then cleaned with Ultrasil 25 F (1% vol/vol, 65°C, 10 min without permeation and with retentate extraction at 416 L/h per m², then 10 min with retentate extraction and J_P = 416 L/h per m², F_R = 6 m³/h), HNO₃ (1% vol/vol, 50°C, 10 min without permeation and with retentate extraction at 416 L/h per m², then 10 min with retentate extraction and J_P = 416 L/h per m², F_R = 6 m³/h) and bleach (0.12% vol/vol, 20°C, 20 min, retentate extraction and J_P = 416 L/h per m², batch circulation, F_R = 6 m³/h).

Pilot Rig. The MiFi system (TIA, Bollène, France) used in this study is described in Figure 1 and was set up to ensure minimal physical damage to the MFG during the process. It was set up after the experiments described previously that were performed with a less suitable rig (Goudedranche et al., 2000). In this new system, the small dead volume of 4 L allows the resi-

dence time of the retentate in the system to be minimized [e.g., maximum 14 min at a volume reduction factor (**VRF**) = 10] and there were only 3 elbows at 90°. The filtration pilot rig operated at uniform transmembrane pressure. The raw whole milk (Compagnie Laitière Européenne, Montauban-de-Bretagne, France) was collected at the outlet of the tanker to prevent fat globule damage in the factory pipes and was stored at 4°C. To be microfiltered, milk was heated continuously at 50°C with a plate exchanger and driven by a volumetric pump (PCM Moineau, Vanves, France) maintaining the entrance pressure in the retentate compartment at 0.1 MPa. A centrifuge pump (Fristam S.A., Noisy-le-Sec, France) for retentate recirculation ensured the tangential shear stress at the wall (τ_w). A centrifuge pump (Fristam) allowed a cocurrent permeate recirculation to ensure uniform transmembrane pressure. The concentric region between the filter and the steel casing contained polypropylene beads to provide the necessary pressure drop. Retentate and permeate extractions were performed through membrane valves to limit shear stress on the MFG: the valve for retentate extraction was from Burkert Contromatic (Villé, France), and the valve for the regulation of permeate extraction was from Samson Régulation (Vaulx-en-Velin, France). Pressure gauges (Endress and Hauser, Humingue, France) were fitted at the inlet and outlet ports of both permeate and retentate. A temperature gauge (PT100, Endress and Hauser) controlled the temperature in the retentate compartment. Electromagnetic flow meters (Endress and Hauser) were located at the circulation and extraction steps of the retentate and permeate.

Principles of Microfiltration Optimization. To develop and optimize the process described in the patent (Goudedranche et al., 1998), the new pilot rig was set up as described above. Process optimization aimed to: 1) confirm process feasibility, 2) determine optimal operating conditions of the process, and 3) estimate process productivity (J_P) and efficiency (fat transmission, fat globule size distribution, avoidance of MFG damage) for later economical evaluation and scaling up of the process. The main drawback of membrane microfiltration is fouling, which is impossible to predict quantitatively. This is why pilot-scale assays have to be performed and are linked to the knowledge of membrane performance depending on operating parameters (hydrodynamic conditions, forces linked to pressure and flux, and volume reduction factor). The optimization of the MiFi process applied to the fractionation of milk fat globules by size consisted of characterizing the hydrodynamic parameters to obtain fractions of native MFG of different sizes with sufficient fat content, while avoiding damaging the MFG and minimizing fouling. To achieve these goals, 3 hydrodynamic parameters

Figure 1. Set-up of the microfiltration (MiFi) system. The electromagnetic flow meters, pumps, and temperature and pressure gauges are connected to the digital control platform where process run data is stored and exported.

were studied: τ_w , J_p , and VRF. Particularly, we used the ratio $J_p \cdot \text{VRF} / \tau_w$ as an index of the competition between convection of particles and erosion at the interface between milk and the membrane (Le Berre and Daufin, 1996). Indeed, J_p and VRF would tend to promote possible cake build-up by MFG, whereas above a threshold value, τ_w discourages fouling (Le Berre and Daufin, 1996).

Process Operation. For each run of the MiFi process, 1) operating parameters were set up with water, followed by a continuous transition with milk wherein operating parameters were adjusted due to increased viscosity (this procedure is similar to industrial practice); 2) volumes of permeate and retentate corresponding to 3 dead volumes of each compartment were eliminated before collecting significant fractions for the given hydrodynamic conditions; and 3) permeate and retentate fractions were finally collected continuously.

Fractions Collected. During process optimization and characterization with each membrane pore size,

all permeates and the corresponding retentates were collected for fat globule size distribution and composition analyses. Then, to characterize further the MFG with different sizes (fatty acid composition, thermal behavior, rheological and technological functionalities), either the retentate (LFG) or the microfiltrate (SFG) was collected. When the SFG fraction was collected, the retentate containing the remaining fat globules was discarded and vice versa for the LFG fraction. This way, only 2 extremes of the initial MFG size distribution were collected. In the following, small and large MFG referred to with the same subscript (e.g., $\text{SFG}_{\text{April}}$, $\text{LFG}_{\text{April}}$) originated from the same milk but were not collected from the same batch, to collect only the extreme fractions.

Calculations. The wall shear stress was determined experimentally according to: $\tau_w = D \cdot \Delta P / 4L$, where ΔP is the longitudinal pressure drop in Pa (Haritonidis, 1989). The shear rate at the wall can be calculated as $\dot{\gamma}_w = 8v/D$ (Kromkamp et al., 2006), where v is the

crossflow velocity (m/s). The volume reduction factor was calculated as $VRF = 1 + Q_P/Q_R$, where Q_P and Q_R are the extraction flows (m³/h) of the permeate and of the retentate, respectively. Transmission (**Tr**) of fat = $(C_P/C_R) \times 100$, where C_P and C_R are the fat concentrations (g/kg) in the permeate and in the retentate, respectively. The fat yield (recovery of fat in a given milk fraction compared with original unseparated milk) was calculated as **Yield** = $[(VRF - 1) \times C_P \times 100]/(VRF \times C_{milk})$ for the permeate; and **Yield** = $(C_R \times 100)/(VRF \times C_{milk})$ for the retentate, in which C_{milk} is the fat concentration in the initial milk. The volume percentage of each fraction compared with the original unseparated milk volume was calculated as **Volume** = $[(VRF - 1)/VRF] \times 100$ for the permeate and **volume** = $100/VRF$ for the retentate.

Physicochemical Analyses

Composition. The fat content of milk fractions was determined using the acid butyrometric method of van Gulick (FIL-IDF, 1997). Total nitrogen (**TN**) and TS of some of the fractions were measured with an infrared analyzer (DairyLab, Foss, Nanterre, France). The total mesophilic flora and the coliform flora were counted in unseparated milk and in permeate and retentate during a 6-h assay at 50°C with a 5- μ m membrane (FIL-IDF, 1991, 1998, 2001). Somatic cells were also counted by fluorescent microscopy in unseparated milk, permeate, and retentate using a Fossomatic 360 apparatus (Foss Electric; standard method performed in Liliad, formerly Cinterliv, Châteaugiron, France).

Fat Globule Size. The MFG size distribution was measured by laser light scattering (MasterSizer2000, Malvern Co., Malvern, UK) after dissociation of the casein micelles as described in detail previously (Michalski et al., 2001a, 2004a). The following fat globule size distribution parameters were calculated by the MasterSizer software: modal diameter (d_{mod} ; diameter at the peak maximum of the main population), volume mean diameter (d_{43}) = $\Sigma(v_i \times d_i)/\Sigma v_i$ (where v_i is the volume of globules in a size class i of average diameter d_i), specific surface area (**S**) = $6 \cdot \rho^{-1} \cdot d_{32}^{-1}$, where ρ is the milk fat density (0.92 at 20°C) and d_{32} is the volume-surface average diameter $[\Sigma v_i/\Sigma(v_i/d_i)]$.

ζ -Potential. To check that no deep physical damage such as homogenization occurred to the MFG in the pilot rig, the apparent ζ -potential of MFG was measured by laser doppler electrophoresis (ZetaSizer 3000HS, Malvern) using the method set up by our team (Michalski et al., 2001b). Indeed, a significant increase of ζ -potential indicates that caseins are incorporated into the MFGM. This must be avoided in the MiFi process in which native small MFG covered with their MFGM

are expected. According to the present method, the damage occurring to the MFGM can be calculated as its surface coverage with caseins (Φ); the latter is estimated by $\Phi = [1 - \exp(-1.082 \times 10^{-3} \times RI_c^2)] \times 100$, where RI_c is the relative increase (%) of the ζ -potential of MFG compared with original raw unseparated milk (Michalski et al., 2001b). Using this technique, MFG can be considered as having been damaged for values of calculated $\Phi > 1.5\%$. Such a value never occurred using our new MiFi process rig.

Biochemical and Functional Properties. Previous articles indicate the methods used for: 1) fat extraction, fatty acid, and conjugated linoleic acid analysis by gas chromatography (Briard et al., 2003; Fauquant et al., 2005; Michalski et al., 2005); 2) analysis of thermal behavior and crystallization temperature measurement by differential scanning calorimetry (Michalski et al., 2004a); 3) rheological measurement of the storage modulus of rennet and acid milk gels containing 150 g/kg of SFG or LFG (Michalski et al., 2002); and 4) manufacture and characterization of Camembert and Emmental cheeses containing SFG and LFG (Briard and Michalski, 2004; Michalski et al., 2003, 2004b, 2006). The firmness of the rennet gels was further characterized by calculating the penetration shear stress, from the force measured at 13 mm depth using a universal testing machine (Instron, model 4501 with IX series software, Guyancourt, France) equipped with a 10-N load cell and a 90° cone moving down at 60 mm/min (Korolczuk and Mahaut, 1988). After creaming of SFG fractions (which did not damage MFG) and adjustment of fat content of regular, SFG, and LFG creams, whipped creams were produced with a laboratory-scale foaming device (Sanomat, Vaihinger GmbH, Bad Camberg, Germany); their storage stability at 4°C in a graduated cylinder was calculated by measuring the ratio of liquid cream that had melted to the initial whipped cream volume.

Statistical Analysis

Results are expressed as mean $\pm$ standard error of the mean (SEM). Results with homogeneous variance were compared using a paired Student's t -test and differences between means were considered significant at $P < 0.05$. Very highly significant differences such as $P < 10^{-6}$ were often obtained regarding the size difference of fat globules in the permeate (SFG fraction) vs. the retentate (LFG fraction).

RESULTS AND DISCUSSION

Optimization of the Microfiltration Process with Nominal Membrane Pore Size of 5 μ m

Effect of τ_w . Hydrodynamic parameters to be used with the 5- μ m membrane were optimized: first, we per-

Figure 2. Fat transmission (Tr, $\blacklozenge$), fat content in the permeate (C_p , $\square$) and modal diameter (d_{mod}) of the milk fat globules in the permeate ($\circ$) and in the retentate ($\bullet$), as a function of the shear stress at the wall τ_w , at volume reduction factor = 4, permeation flux = 1,041 L/h per m^2 , and 50°C. Error bars represent standard deviations.

formed a stepwise increase of τ_w from 50 to 200 Pa (upper operating limit of the pump) with 7 stages of 10 min, at VRF = 4 and J_p = 1,042 L/h per m^2 to characterize the best shear stress regarding fat transmission and fouling prevention (Figure 2). Transmembrane pressure (TMP) increased regularly from 15 to 80 kPa without any fouling at low stress, and this low increase indicates that the possible fouling layer forms slowly. Fat transmission decreased sharply for τ_w in the range 70 to 90 Pa and remained stable at 10.7% from 110 Pa. The fat content that could be reached in the permeate below the critical τ_w for transmission drop was C_p = 15.1 ± 0.9 g/kg for an average d_{mod} = 2.9 ± 0.1 μm . The ζ -potential was not significantly different between unseparated raw milk (-11.5 ± 0.2 mV), the permeate at τ_w = 90 Pa (-11.7 ± 0.2 mV) or 200 Pa (-11.0 ± 0.3 mV), and the retentate at τ_w = 200 Pa (-11.3 ± 0.2), indicating that the MFGM was not damaged during the process (no reduction of fat globule size due to shear or cavitation; $\phi < 0.3\%$). Therefore, the tangential shear stress at the wall did not damage MFG. High τ_w inhibits the onset of a possible fouling layer; however, it also reduces fat globule transmission through the membrane. Therefore, τ_w = 80 Pa was chosen as usual operating shear stress; not only shear-induced diffusion but also inertial lift may play a role as back-transport mechanism, especially for the largest MFG (Kromkamp et al., 2006). This τ_w corresponded to v = 4.6 m/s and $\dot{\gamma}_w$ = 9,200 s^{-1} for the production of SFG with the 5- μm membrane.

Effect of J_p and VRF. Different filtrations were performed at τ_w = 80 Pa with stepwise increase of VRF (from 4 to 20 with 5 stages of 10 min) for each value of J_p at 1,041, 1,350, and 1,875 L/h per m^2 . The ζ -potential

Figure 3. Influence of permeation flux (J_p) and volume reduction factor (VRF) on fat transmission (Tr), permeate fat concentration (C_p) and retentate fat concentration (C_R) at shear stress at the wall = 80 Pa, 50°C: (--- $\diamond$ ---) Tr at J_p = 1,041 L/h per m^2 ; (— $\blacklozenge$ —) Tr at J_p = 1,350 L/h per m^2 ; (--- $\diamond$ ---) Tr at J_p = 1,875 L/h per m^2 ; (--- $\square$ ---) C_p at J_p = 1,041 L/h per m^2 ; (--- $\square$ ---) C_p at J_p = 1,350 L/h per m^2 ; (--- $\square$ ---) C_p at J_p = 1,875 L/h per m^2 ; (--- $\blacksquare$ ---) C_R at J_p = 1,041 L/h per m^2 ; (--- $\blacksquare$ ---) C_R at J_p = 1,350 L/h per m^2 ; and (--- $\blacksquare$ ---) C_R at J_p = 1,875 L/h per m^2 .

of fat globules in initial milk was -12.0 ± 0.3 mV vs. -12.0 ± 0.2 mV in the permeates and -12.2 ± 0.2 mV in the retentates, which indicates that MFG were not mechanically damaged ($\phi < 0.3\%$). Figure 3 shows the influence of J_p and VRF on Tr, C_p , and C_R . For a given J_p , C_R and C_p increased with VRF, although a plateau value seemed to be reached. Fat transmission increased when increasing J_p , and for a given J_p , Tr decreased with increasing VRF. To choose hydrodynamic parameters for the process, a compromise must be found between fat transmission and fat globule size. Figure 4A shows examples of fat globule size distributions at VRF = 4 and 16, and Table 1 presents the modal diameters obtained at the various J_p and VRF. In both permeate and retentate, d_{mod} increased significantly with VRF, whereas J_p presented no significant influence on d_{mod} (0.1- μm increase between each plateau of J_p at 1,041, 1,350, and 1,875 L/h per m^2). For a given membrane, various fat contents and MFG sizes can thus be reached depending on the parameters of the process. This result cannot be explained by the fractionation action of the microfiltration membrane, nor to deposition of fat globules onto the membrane. Rather, it highlights the importance of process hydrodynamics in governing the separation of polydisperse colloidal particles such as MFG, particularly via inertial lift. Indeed, in a process engineering study about the effects of particle size segregation on crossflow microfiltration performance, Kromkamp et al. (2006) explicitly used our fat globule MiFi process with a 5- μm tubular membrane at VRF = 2.5 to demonstrate the practical relevance of their find-

Figure 4. A) Fat globule size distribution in raw whole milk (—), and in the following fractions obtained at shear stress at the wall (τ_w) = 80 Pa, 50°C, permeation flux (J_p) = 1,041 L/h per m^2 : (---○---) permeate at volume reduction factor (VRF) = 4; (---○---) permeate at VRF = 16; (---●---) retentate at VRF = 4; (---●---) retentate at VRF = 16. B) Influence of the ratio $J_p \cdot VRF / \tau_w$ (expressed in m/h per Pa) on the modal diameter (d_{mod}) of milk fat globules in the permeate (○) and in the retentate (●) obtained from raw whole milk with $d_{mod} = 3.7 \pm 0.2 \mu m$. Trend line for fat globule diameter in the permeate: $d_{mod} = 2.10 \times (J_p \cdot VRF / \tau_w) + 2.83$; $R^2 = 0.82$. Trend line for fat globule diameter in the retentate: $d_{mod} = 5.46 \times (J_p \cdot VRF / \tau_w) + 4.16$; $R^2 = 0.84$.

ings with polystyrene particles. They observed, for $J_p = 250$ to $2,000$ L/h per m^2 and $\dot{\gamma}_w = 5,100$ to $15,400$ s^{-1} , that no deposition of fat globules had taken place. This is consistent with the absence of fouling that we observed at $\dot{\gamma}_w = 9,200$ s^{-1} . Moreover, their Tr decreased with increasing v , which is consistent with Figure 2. Finally, they observed that C_p increased with J_p at $v = 2.6$ m/s, which is consistent with Figure 3 at VRF = 4 and $v = 4.6$ m/s. Their d_{mod} hardly increased with J_p for $v = 7.7$ m/s, as we also observed at $v = 4.6$ m/s. Kromkamp et al. (2006) highlight that MFG are clearly separated on size using our process.

Table 1. Modal diameter in permeate and in retentate obtained by microfiltration of milk at 50°C, with tangential shear stress of 80 Pa, according to the volume reduction factor (VRF)¹

VRF	Modal diameter (μm)	
	Permeate	Retentate
4	$2.9^{Aa} \pm 0.1$	$4.4^{B,a} \pm 0.0$
8	$3.2^{A,b} \pm 0.1$	$5.0^{B,b} \pm 0.1$
12	$3.3^{A,bc} \pm 0.1$	$5.4^{B,c} \pm 0.1$
16	$3.4^{A,cd} \pm 0.1$	$5.8^{B,d} \pm 0.1$
20	$3.5^{A,d} \pm 0.1$	$6.0^{B,e} \pm 0.0$

^{A,B}Means in a row with different superscript symbols are significantly different ($P < 0.001$).

^{a-e}Means in a column that do not share a common superscript letter are significantly different ($P < 0.05$).

¹Permeate flux (J_p) had no significant effect on modal diameter in the range $J_p = 1,042$ to $1,875$ L/h per m^2 . Results are mean $\pm$ SEM ($n = 3$).

Figure 4B summarizes the effect of the ratio $J_p \cdot VRF / \tau_w$ on d_{mod} . Depending on the MFG fraction desired (small or large globules), it is possible to choose an optimum ratio $J_p \cdot VRF / \tau_w$: operating at $J_p \cdot VRF / \tau_w < 0.05$ m/h per Pa allows to obtain fat globules of $d_{mod} = 2.8 \pm 0.1 \mu m$ in the permeate at a fat content $C_p = 14.2 \pm 1.0$ g/kg; whereas operating at $J_p \cdot VRF / \tau_w > 0.3$ allows to obtain fat globules of $d_{mod} = 5.8 \pm 0.1 \mu m$ in the retentate at a fat content $C_R = 173.0 \pm 10.9$ g/kg. Values of d_{mod} of small and large MFG obtained by this process are significantly different from the initial MFG size ($P < 0.001$) and both fractions contain sufficient fat to be valid for use in dairy industrial applications.

Long-Term Operation of the Process. Figure 5 presents the evolution of TMP during runs of 9 h for the production of SFG and 8 h for LFG, with the corresponding evolution of d_{mod} , which had not been performed previously. Assays were performed using raw whole milk continuously heated at 50°C, with VRF = 4 and $J_p = 1,042$ L/h per m^2 for SFG and VRF = 15 and $J_p = 1,475$ L/h per m^2 for LFG. During the production of SFG fractions, TMP increased very slowly (19-kPa increase after 9 h). This indicates that no fouling occurred, which is also confirmed in the study of Kromkamp et al. (2006) showing that no deposition of fat globules had taken place onto the membrane; in these hydrodynamic conditions, permeation flux appears to be controlled by the membrane resistance solely. Fat concentration in the permeate decreased from 18.5 g/kg after 1 h of microfiltration to 15.2 g/kg at the end of the run (9 h), corresponding with slightly smaller globules (Figure 5). The ζ -potential of SFG was -12.8 ± 0.1 mV throughout the run vs. -12.5 mV in unseparated milk, indicating the absence of MFG damage. The TMP increase was higher during the production of LFG (Figure 5; 24-kPa increase after 8 h), due to the higher fat concentration in the rig, but remained within accept-

Figure 5. Evolution of the transmembrane pressure (TMP) and modal diameter of the native milk fat globules (d_{mod}) during long runs of the crossflow microfiltration process with nominal membrane pore size of 5 μm : TMP (□) and d_{mod} (○) during the collection of small milk fat globules in the permeate at permeation flux (J_p) = 1,041 L/h per m^2 , volume reduction factor (VRF) = 4, and shear stress at the wall (τ_w) = 80 Pa; (■) TMP and d_{mod} (●) during the collection of large milk fat globules in the retentate at J_p = 1,475 L/h per m^2 , VRF = 15, and τ_w = 80 Pa.

able limits because the fat transmission remained stable during the entire microfiltration assay. Fat concentration in the retentate increased from 203.6 g/kg after 1 h of microfiltration to 226.4 g/kg at the end of the run (8 h). The ζ -potential of LFG was -11.7 ± 0.1 mV throughout the run vs. -11.9 mV in unseparated milk: MFG were not damaged. Because particle size segregation occurs during this MiFi process due to inertial lift (Kromkamp et al., 2006), smaller fat globule sizes appear in the region near the wall. This can contribute to the absence of irreversible fouling, making the process suitable for long runs in industrial applications.

During a 6-h MiFi run at VRF = 15 of raw whole milk with a coliform flora of 91 cfu/mL at 50°C, this population was 37 ± 18 cfu/mL in the permeate and 28 ± 11 cfu/mL in the retentate. Total mesophilic flora was $7,250 \pm 724$ cfu/mL in the permeate and $8,075 \pm 645$ cfu/mL in the retentate vs. 4,800 cfu/mL in unseparated milk at 50°C. Therefore, no deleterious bacterial development appeared during the process. Regarding SCC, during a 2-h MiFi run at VRF = 4 of raw whole milk with $\text{SCC} = 193 \times 10^3$ cell/mL at 50°C for the production of SFG, we found $\text{SCC} = 0$ in the permeate. Therefore, one advantage of native SFG obtained by microfiltration is that this fraction is devoid of somatic cells. During a 2-h MiFi at VRF = 15 of raw whole milk with $\text{SCC} = 208 \times 10^3$ cell/mL at 50°C for the production of LFG, we found $\text{SCC} = 2,761 \times 10^3 \pm 62 \times 10^3$ cell/mL in the retentate with $C_R = 204 \pm 3$ g/kg. Then, we measured $390 \times 10^3 \pm 2 \times 10^3$ cell/mL in the LFG fraction

when standardized at 29 g/kg of fat for cheese-making processes.

To test the technological aptitudes of the small and large MFG, 24 different MiFi runs with a 5- μm membrane were performed in a 2-yr period. The MFG populations were more deeply characterized in terms of volume mean diameter (d_{43}), specific surface area (S), TN, and fat content, as detailed in Table 2. Fat globule size distribution parameters were highly significantly different among milk fractions. The smaller diameter of the fat globules in the permeate increases the specific surface area by 28%. The diameter and fat content of SFG fractions were similar among assays, whereas fat content depends more on the duration of the run for the production of LFG. Even during 10-h microfiltration, no mechanical damage of the MFG was observed because the ζ -potential remained constant (Table 2; $\Phi < 1\%$).

Different Fractions of Milk Fat Globules Obtained with 2-, 3-, and 12- μm Pore Sizes

To obtain MFG populations of extreme sizes for the purpose of scientific studies, the MiFi process was operated with different membrane pore sizes from 2 to 12 μm and various hydrodynamic conditions. Particularly, VRF down to 2 was used to obtain the smallest fat globules with the 2- and 3- μm membranes, whereas VRF up to 25 was used to obtain the largest globules with the 12- μm membrane.

Table 3 shows the physicochemical characteristics of extreme milk fractions that were obtained with this process. Some of the corresponding fat globule size distributions are shown Figure 6. Except for the smallest MFG obtained with a 2- μm membrane, for which the milk fraction obtained is almost skimmed, the small and large fat globule fractions usually represent ~15 to 35% of the initial milk fat content. Unlike the suggestion of Kromkamp et al. (2006), our results with different MiFi membranes show that the pore size of the membrane does not have to be larger than the largest MFG for the fractionation to be efficient; although smaller pores result in lower fat yield in the permeate and smaller SFG. No retention of proteins (mainly casein micelles) was observed in the retentate, even with the 2- μm membrane (Table 3). The use of membrane pore sizes of 2 μm (permeate) and 12 μm (retentate) that can further be diafiltered with skimmed milk to increase d_{43} further while keeping composition constant as shown in Table 3) allows one to achieve MFG fractions of extreme diameters (d_{43} ~1 and ~7 μm) whose size distributions do not overlap (Figure 6). This performance of MFG separation is a unique feature of the present process. The largest fat globule fraction can be used in dairy applications because the fat content of the

Table 2. Volume mean diameter (d_{43}), specific surface area (S), and apparent ζ -potential of regular milk fat globules in raw whole milk, of small globules in the permeate obtained by microfiltration on a 5- μm membrane (tangential shear stress, $\tau_w = 80$ Pa, permeate flux, $J_p = 1,042$ L/h per m^2 , volume reduction factor, $\text{VRF} \leq 4$) and of large globules in the retentate ($\tau_w = 80$ Pa, $J_p = 1,458$ L/h per m^2 , $\text{VRF} \geq 15$), and corresponding fat content and total nitrogen (TN) of the milk fraction from which they originate¹

	d_{43} (μm)	S (m^2/g)	ζ (mV)	Fat (g/kg)	TN (g/kg)
Regular milk fat globules	$4.2^a \pm 0.0$	$1.8^a \pm 0.0$	$-12.5^a \pm 0.2$	$42.7^a \pm 0.6$	$33.6^a \pm 0.3$
Small globules (permeate)	$3.3^b \pm 0.0$	$2.3^b \pm 0.0$	$-12.8^a \pm 0.2$	$16.5^b \pm 0.3$	$33.8^a \pm 0.5$
Large globules (retentate)	$6.0^c \pm 0.1$	$1.5^c \pm 0.0$	$-12.9^a \pm 0.2$	$190.4^c \pm 4.6$	$27.8^b \pm 0.6$

^{a-c}Means in a column with different superscripts are significantly different ($P < 0.001$ for d_{43} and Fat; $P < 0.01$ for S and TN).

¹Results are mean $\pm$ SEM ($n = 24$).

retentate is high enough. The butter-making industry could take advantage of these large globules that are easier to churn because they are thermodynamically less stable. The smallest MFG fraction ($\leq 1 \mu\text{m}$), however, do not contain more than 1 g/kg of fat; and thus, would be mainly interesting for scientific investigations aiming to characterize MFG of different sizes. However, separation of small fat globules of $d_{43} \sim 2.5 \mu\text{m}$ (40% smaller than the entire MFG population, using a 3- μm membrane), allows the attainment of milk fractions with ~ 10 to 15 g/kg of fat that can easily be used in dairy industrial applications for the development of novel products.

Performance of the Microfiltration Process Compared with other MFG Separation Methods

Variations in MFG size can be achieved naturally through breed selection and feeding; values such as d_{43} in the range from 3.5 (Holstein cows fed a diet with 3.7% unsaturated fat, or Friesian cows) to 5.9 μm (high-fat-yield Holstein cows) were reported (Mulder and Walstra, 1974; Wiking et al., 2003, 2004, 2005). However, MFG size cannot be decreased as much using natural methods as with MiFi (0.3- μm decrease with 30% fresh grass in the Holstein-cow diet vs. our results in Table 3; Couvreur et al., 2006). O'Mahony et al.

Table 3. Characteristics of the milk fractions containing milk fat globules of different sizes obtained with the microfiltration process operated at 50°C using various nominal membrane pore sizes and hydrodynamic conditions

Fraction and related hydrodynamic parameters ¹	Characteristics ²						
	d_{mod} (μm)	d_{43} (μm)	S (m^2/g)	Yield (%)	Fat (g/kg)	TN (g/kg)	TS (g/kg)
Small fat globule fraction (permeate)							
2- μm mb, VRF = 2.5, $\tau_w = 45$ Pa, $J_p = 417$ L/h per m^2	0.75	0.93	17.20	<1	<1	— ³	—
2- μm mb, VRF = 2.5, $\tau_w = 45$ Pa, $J_p = 417$ L/h per m^2	0.94	0.96	8.27	<1	<1	34.4	91.2
2- μm mb, VRF = 2.5, $\tau_w = 45$ Pa, $J_p = 417$ L/h per m^2	1.51	1.50	12.10	<1	<1	34.0	90.6
2- μm mb, VRF = 3, $\tau_w = 45$ Pa, $J_p = 417$ L/h per m^2	1.47	1.75	4.44	<1	<1	35.0	92.3
3- μm mb, VRF = 2, $\tau_w = 80$ Pa, $J_p = 1,042$ L/h per m^2	2.02	2.13	3.46	4.7	4.0	35.4	97.0
3- μm mb, VRF = 4, $\tau_w = 80$ Pa, $J_p = 1,042$ L/h per m^2	2.59	2.78	2.66	16.8	9.5	34.8	101.3
3- μm mb, VRF = 6, $\tau_w = 80$ Pa, $J_p = 1,042$ L/h per m^2	2.85	3.09	2.39	24.7	12.6	32.9	99.5
5- μm mb, VRF = 4, $\tau_w = 80$ Pa, $J_p = 1,042$ L/h per m^2	3.01	3.26	2.27	26.2	15.5	34.2	103.8
Large fat globule fraction (retentate)							
2- μm mb, VRF = 3, $\tau_w = 45$ Pa, $J_p = 417$ L/h per m^2	4.89	5.41	1.38	94.8	113.8	—	—
12- μm mb, VRF = 25, $\tau_w = 80$ Pa, $J_p = 1,458$ L/h per m^2	6.09	6.01	1.10	29.4	196.0	30.0	290.0
5- μm mb, VRF = 15, $\tau_w = 80$ Pa, $J_p = 1,458$ L/h per m^2	6.32	6.84	1.06	32.4	215.8	27.8	280.0
12- μm mb, VRF = 25, $\tau_w = 80$ Pa, $J_p = 1,458$ L/h per m^2	6.06	6.20	1.08	16.7	185.7	—	—
12- μm mb, VRF = 25, $\tau_w = 80$ Pa, $J_p = 1,458$ L/h per m^2 ⁴	7.14	7.15	1.00	10.3	114.2	—	—
12- μm mb, VRF = 25, $\tau_w = 80$ Pa, $J_p = 1,458$ L/h per m^2	6.24	6.40	1.09	18.4	206.8	—	—
12- μm mb, VRF = 25, $\tau_w = 80$ Pa, $J_p = 1,458$ L/h per m^2 ⁴	7.12	7.34	0.97	11.6	130.7	—	—

¹mb = microfiltration membrane (with nominal pore size indicated); VRF = volume reduction factor; τ_w = tangential shear stress at the wall; J_p = permeate flux.

²Characteristics: d_{mod} = modal diameter of the fat globules; d_{43} = volume mean diameter of the fat globules; S = specific surface area of the fat globules; Yield = recovery of fat in the fraction compared with unseparated milk; Fat = milk fat content of the fraction; TN = total nitrogen in the fraction; TS = total solids in the fraction.

³Not determined.

⁴Followed by a diafiltration with 15 volumes of skimmed milk to enhance milk fat globule fractionation.

Figure 6. Particle size distributions of milk fractions with different sizes of fat globules obtained by the microfiltration process operated at 50°C using various nominal membrane pore sizes and different hydrodynamic conditions. (—) Raw whole milk; (---○---) permeate obtained with nominal membrane pore size = 2 μm , volume reduction factor (VRF) = 3, shear stress at the wall (τ_w) = 45 Pa, permeation flux (J_p) = 417 L/h per m^2 ; (---○---) permeate obtained with nominal membrane pore size = 2 μm , VRF = 2.5, τ_w = 45 Pa, J_p = 417 L/h per m^2 ; (—●—) retentate obtained with nominal membrane pore size = 2 μm , VRF = 3, τ_w = 45 Pa, J_p = 417 L/h per m^2 ; (—●—) retentate obtained with nominal membrane pore size = 12 μm , VRF = 25, τ_w = 80 Pa, J_p = 1,458 L/h per m^2 followed by a 13-volume diafiltration with skimmed milk for 10 min at 350 L/h to enhance milk fat globule fractionation while maintaining the composition of the continuous phase. Inserts show polarized light micrographs of creams obtained from the milk fractions (scale bar represents 10 μm).

(2005) used a 2-stage gravity separation method to produce mini-Cheddar cheeses with so-called small (3.4 μm), regular (3.6 μm), or large (4.7 μm) native MFG. However, that separation method is not efficient, because the particle size distributions of the small and regular globules were almost superimposed and not significantly different. Besides, the average diameter of the small globules was only slightly smaller (0.2 μm) than the regular ones, lying within natural variations of MFG size (Wiking et al., 2004). Finally, the size distribution and diameter of their large globules were in the range of regular unseparated MFG (Lopez, 2005).

Ma and Barbano (2000) proposed a gravity separation method as a simpler and more efficient method than centrifugation in obtaining native MFG of smaller size. However, no pilot-scale results of this gravity separation procedure for industrial use have been described, and the presented d_{43} values of MFG seem to be slightly underestimated due to the presence of the casein micelle population in their MFG size distribution curves (Ma and Barbano, 2000). We noted, however, that compared with their regular MFG (d_{43} = 3.2 μm), slightly

larger fat globules of 3.6 μm could be obtained by gravity separation after 2 h at 15°C. On the other hand, a fraction with fat globules of d_{43} = 1.2 μm and with 2 g/kg of fat could be obtained after aging for 48 h at 4°C. Using this technique, the milk was aged at least 24 h. Table 4 compares the MFG fractions obtained with the present MiFi process and with gravity separation according to Ma and Barbano (2000). The fraction with globules of ~1 μm obtained by MiFi presented a greater diameter decrease than by gravity separation (–79 vs. –63%) and allowed recovery of a much larger volume (50 vs. 8%). Regarding fractions of industrial interest, the 2.8- μm globule fraction obtained by MiFi represented a 38% decrease in fat globule size, whereas the 2.8- μm globule fraction obtained by gravity separation represents a lower decrease in fat globule size (–13%). If we compare fractions with similar decrease in d_{43} obtained with both processes, the 3.1- μm globule fraction obtained by MiFi (–33% in size) comprised 25% of initial fat content and 83% of initial milk volume, whereas the 2.3- μm fraction obtained by gravity separation (–28% in size) represent a lower fat and volume

Table 4. Comparison of the performance of the present microfiltration process and gravity separation to obtain small and large native milk fat globule fractions from raw whole milk

Technique ¹	Characteristic ²				
	d ₄₃ (μm)	d ₄₃ -change (%)	Fat (g·kg ⁻¹)	Yield (%)	Volume (%)
Microfiltration (present study)					
SFG: 2-μm mb, VRF = 2.5, τ _w = 45 Pa, J _p = 417 L/h per m ²	0.9	-79	<1	<1	50
SFG: 3-μm mb, VRF = 2, τ _w = 80 Pa, J _p = 1,042 L/h per m ²	2.1	-52	4	5	50
SFG: 3-μm mb, VRF = 4, τ _w = 80 Pa, J _p = 1,042 L/h per m ²	2.8	-38	10	17	75
SFG: 3-μm mb, VRF = 6, τ _w = 80 Pa, J _p = 1,042 L/h per m ²	3.1	-33	13	25	83
SFG: 5-μm mb, VRF = 4, τ _w = 80 Pa, J _p = 1,042 L/h per m ²	3.3	-21	15	26	75
LFG: 5-μm mb, VRF = 15, τ _w = 80 Pa, J _p = 1,458 L/h per m ²	6.8	+62	216	32	7
LFG: 12-μm mb, VRF = 25, τ _w = 80 Pa, J _p = 1,458 L/h per m ²	6.2	+63	186	17	4
LFG: 12-μm mb, VRF = 25, τ _w = 80 Pa, J _p = 1,458 L/h per m ² ³	7.1	+69	114	10	4
Gravity separation (Ma and Barbano, 2000)					
SFG: 48 h, 4°C, bottom fraction	1.2 ⁴	-63	2	<1	8
SFG: 48 h, 4°C, middle fractions 2 to 4	2.3 ⁴	-28	10	14	50
SFG: 24 h, 4°C, bottom fractions 1 to 6	2.8 ⁴	-13	22	55	92
LFG: 48 h, 4°C, top fraction	3.6 ⁴	+13	266	62	8
LFG: 2 h, 15°C, top fraction	3.6 ⁴	+13	— ⁵	—	—

¹SFG = Small fat globules; mb, microfiltration membrane (with nominal pore size indicated); VRF = volume reduction factor; τ_w = tangential shear stress at the wall; J_p = permeate flux; LFG = large fat globules.

²d₄₃ = Volume mean diameter of the fat globules; d₄₃-change = relative increase or decrease of d₄₃ (expressed as a percentage) compared with the milk fat globules in the original unseparated milk (for gravity separation, some values were calculated from Ma and Barbano, 2000); Fat = milk fat content of the fraction; Yield = recovery of fat in the fraction compared with unseparated milk; Volume = volume percentage of the fraction compared with the unseparated milk volume (for gravity separation, some values were calculated from Ma and Barbano).

³Followed by a diafiltration with 15 volumes of skimmed milk to enhance milk fat globule fractionation.

⁴It seems that fat globule size distributions of Ma and Barbano (2000) include casein micelles: their values of d₄₃ must be underestimated.

⁵Not specified by Ma and Barbano (2000).

recovery (14 and 50%, respectively). Moreover, the latter fraction was obtained by collecting the middle part of the milk column, which is not a straightforward technique. By gravity separation, the cream layer can be enriched in large fat globules but the latter cannot be truly separated because the smallest fat globules originally present at the top of the cylinder remain. On the contrary, the MiFi process truly separated out the small fat globules from the large ones.

In short, using whole milk with MFG of d₄₃ ~4.2 μm and appropriate membrane pore size and hydrodynamic conditions, we could collect the 2 extremes of the initial MFG distribution consisting of 1) a retentate containing large MFG of d₄₃ in the range 5 to 7.5 μm (with up to 250 g/kg of fat, up to 35% of initial milk fat, and up to 10% of initial milk volume), and 2) a permeate containing small MFG of 0.9 to 3.3 μm (with up to 20 g/kg of fat, up to 30% of initial milk fat and up to 85% of initial milk volume). Therefore, this process avoided the need for milk aging, provided sufficient fraction volume for industrial use, and appears to be more efficient than gravity separation in selecting native fat globules of different sizes, at least 1-μm smaller or larger than in raw whole milk.

Review of the Physicochemical and Technological Properties of MFG of Different Sizes

To highlight the functional properties of the different MFG fractions obtained with the MiFi process, our previously published characterizations are summarized below. First, as summarized in Table 5, MFG of different sizes slightly differ in fatty acid composition, which is mainly due to their triglyceride composition (Fauquant et al., 2005). Moreover, the crystallization temperature increases logarithmically with d₄₃ (Michalski et al., 2004a). These results are of importance in the manufacture of dairy products such as cheese and butter, where tempering periods are involved in the technological process. In this respect, using native MFG of different sizes can lead to new functionalities.

The MFG size appears to affect the rheological properties of milk gels (Michalski et al., 2002, highlighted by van Vliet et al., 2004). Using the present MiFi process, we have shown that in rennet gels filled with 150 g/kg of fat, the storage modulus (G') decreased (70 to 35 Pa) when the native MFG diameter increased in the range 3 to 5.5 μm. On the contrary, for acid gels, G' increased sharply (20 to 100 Pa) with MFG size. The difference would be due to the size of the serum pores

Table 5. Fatty acid composition (g per 100 g of fatty acid) of milk fat globules of different sizes obtained using the present microfiltration process (adapted from Briard et al., 2003; Fauquant et al., 2005; Michalski et al., 2005)¹

	Total fat globule (February)		Total fat globule (April)		Fat globule core (September)	
	SFG _{Febr.}	LFG _{Febr.}	SFG _{April}	LFG _{April}	SFG _{Sept.}	LFG _{Sept.}
d ₄₃ (μm)	1.5 ^{a,†}	7.3 ^{b,†}	3.2 ^{a,†} ± 0.1	6.4 ^{b,†} ± 0.5	3.4 ^{a,†} ± 0.1	6.3 ^{b,†} ± 0.3
Fatty acid						
4:0	7.6 ^a ± 0.2	7.1 ^a ± 0.4	5.3 ^a ± 0.6	6.2 ^a ± 0.3		
6:0	2.5 ^a ± 0.1	2.4 ^a ± 0.2	2.7 ^a ± 0.1	2.6 ^a ± 0.1		
8:0	1.5 ^a ± 0.0	1.5 ^a ± 0.0	1.6 ^a ± 0.1	1.6 ^a ± 0.1		
10:0	3.3 ^a ± 0.1	3.2 ^a ± 0.1	3.8 ^a ± 0.2	3.7 ^a ± 0.1	2.5 ^a ± 0.1	2.5 ^a ± 0.1
11:0					0.1 ^a ± 0.0	0.0 ^a ± 0.0
12:0	3.9 ^a ± 0.1	3.6 ^b ± 0.1	4.4 ^a ± 0.2	4.2 ^b ± 0.2	3.7 ^{a,‡} ± 0.2	3.5 ^{b,‡} ± 0.1
13:0	0.1 ^a ± 0.1	0.1 ^a ± 0.1			0.1 ^a ± 0.0	0.1 ^a ± 0.0
14:0	12.5 ^a ± 0.2	11.4 ^b ± 0.1	12.8 ^a ± 0.2	12.2 ^b ± 0.3	13.2 ^a ± 0.2	12.5 ^b ± 0.1
14:1					2.0 ^a ± 0.1	1.5 ^b ± 0.0
16:0	35.5 ^a ± 0.3	34.1 ^b ± 0.4	31.4 ^a ± 0.6	32.0 ^a ± 0.6	39.2 ^{a,‡} ± 0.0	37.8 ^{b,‡} ± 0.6
16:1n-9					0.5 ^a ± 0.0	0.6 ^a ± 0.0
16:1n-7 <i>cis</i>	2.1 ^a ± 0.0	1.9 ^b ± 0.1	1.7 ^a ± 0.1	1.5 ^b ± 0.1	2.2 ^{a,‡} ± 0.1	2.0 ^{b,‡} ± 0.1
17:0					0.6 ^a ± 0.0	0.7 ^a ± 0.0
18:0	8.9 ^a ± 0.1	10.3 ^b ± 0.1	8.8 ^a ± 0.5	10.2 ^b ± 0.3	9.0 ^a ± 0.3	11.5 ^b ± 0.3
18:1n-9 <i>trans</i>					1.4 ^a ± 0.1	1.4 ^a ± 0.1
18:1n-9 <i>cis</i>	20.9 ^a ± 0.1	22.7 ^b ± 0.6	23.4 ^a ± 0.6	22.8 ^a ± 0.5	21.0 ^a ± 0.2	21.3 ^a ± 0.5
18:1n-7 <i>cis</i>					0.9 ^a ± 0.1	0.9 ^a ± 0.1
Conjugated linoleic acid total			1.8 ^{a,‡} ± 0.5	1.1 ^{b,‡} ± 0.1	0.9 ^a ± 0.1	0.9 ^a ± 0.1
(<i>cis</i> -9, <i>trans</i> -11)			(1.6 ± 0.4)	(0.9 ± 0.2)		
18:2n-6 <i>cis</i>	1.4 ^a ± 0.0	1.7 ^b ± 0.0	1.4 ^a ± 0.2	1.4 ^a ± 0.2	1.5 ^a ± 0.1	1.5 ^a ± 0.1
20:0			0.7 ^a ± 0.2	1.1 ^a ± 0.1	0.1 ^a ± 0.0	0.1 ^a ± 0.0
18:3n-3					0.3 ^a ± 0.1	0.3 ^a ± 0.1
20:1n-9					0.1 ^a ± 0.0	0.1 ^a ± 0.0
21:0					0.3 ^a ± 0.0	0.3 ^a ± 0.0
20:2n-6					0.3 ^a ± 0.0	0.3 ^a ± 0.0
20:3n-6					0.1 ^a ± 0.0	0.1 ^a ± 0.0
20:4n-6					0.1 ^a ± 0.0	0.1 ^a ± 0.0
22:5n-3					0.1 ^a ± 0.0	0.1 ^a ± 0.0

^{a,b}Pairwise comparisons were performed between SFG and its corresponding LFG obtained from the same unseparated milk: means in 2 such cells of a row with different superscripts are significantly different ($P < 0.05$; except $†P < 0.001$ and $‡P < 0.1$).

¹Results are mean ± SEM ($n \geq 3$). SFG = small fat globules, LFG = large fat globules. Fractions were separated from raw whole milk collected in February, April, or September.

vs. the MFG size (Michalski et al., 2002). We have also characterized the strength of rennet gels, filled with MFG of different sizes, at large deformation. Figure 7 shows that the shear stress (measured at 13 mm depth during the penetration of a cone, simulating in-mouth behavior; Walstra, 1995) increases with MFG diameter, suggesting a softer texture of native SFG-filled gels. It seems that during gel destruction, the larger number of small fat globules enhance structure collapse by providing numerous weak points.

Finally, the technological functionality of the native MFG of different sizes obtained by the present MiFi process was investigated in various model dairy matrices. We have recently produced whipped creams with SFG that were found more stable than regular whipped cream. Only 12% had collapsed after 7 d of storage vs. 35% for regular cream and 29% for LFG cream (our unpublished data); the possible improvement of stability and texture in whipped dairy products using SFG should be explored further. We demonstrated that using SFG vs. LFG results in different physicochemical

and sensory properties of Camembert cheese (Michalski et al., 2003), in which fat remains essentially in its globular form, and in different physicochemical and functional properties of Emmental cheese (Michalski et al., 2004b), in which the globular structure of fat is destroyed during the process (Lopez, 2005; Lopez et al., 2006). When produced using the same technology, Camembert cheeses with SFG contained 0.4% more moisture than regular Camembert cheeses, whereas Emmental cheeses with SFG contained 4.1% more moisture than regular Emmental cheeses (our unpublished data). Saint-Gelais et al. (1997) showed that large MFG obtained by microfiltration, combined with adapted mineral content, improve the sensory characteristics of low-fat Cheddar cheese. Due to their greater moisture, SFG Camembert curds are less rigid and less firm than LFG and regular ones and undergo greater proteolysis, resulting in a higher melting and elastic texture and a higher flowing aspect (Michalski et al., 2003). Ripened SFG Emmental cheeses undergo greater proteolysis and present increased stretching,

Figure 7. Firmness of rennet milk gels containing 150 g/kg of fat in the form of native milk fat globules of various sizes (d_{mod} = modal diameter of the native milk fat globules), measured as the penetration shear stress at 13 mm depth using a universal testing machine equipped with a 90° cone moving down at 60 mm/min.

elastic increase, and melting, and lower extrusion force (Michalski et al., 2004b). When manufactured with adapted technologies to ensure a similar humidity of the ripened cheeses, the yellow index is greater for SFG Emmental cheeses compared with regular Emmental cheeses and some sensory properties such as the fruity taste are also improved (our unpublished data, 2006). Regardless of moisture, the stretching and elasticity increase are greater for SFG Emmental cheeses, which also present smaller eyes. Using the MiFi process analyzed above, we were able to demonstrate that the ultrastructure of milk fat plays a key role in the sensory and functional properties of cheeses. The use of SFG of 2.5 to 3 μm is industrially possible and appears to be advantageous regarding the quality of final dairy products and can lead to new products with adapted properties.

Future Prospects

Experiments are being carried out in our laboratory to optimize the MiFi process for goat milk, which may not behave similarly to cow milk. The microfiltration of pasteurized milk or cream should also be investigated. Moreover, it may be interesting to add a diafiltration at the end of the regular MiFi run, to further increase the MFG size, as shown in Table 3. The small differences in fatty acid profile (Table 5) and digestion kinetics (Michalski et al., 2006) of MFG of different sizes encourage further research regarding their nutritional properties.

CONCLUSIONS

We have optimized and analyzed the microfiltration process that accurately separates different fractions of

the native milk fat globule population according to size. Using whole milk and appropriate membrane pore size and hydrodynamic conditions, we collected the 2 extremes of the initial MFG distribution consisting of a retentate containing large milk fat globules of 5 to 7.5 μm (up to 35% of initial milk fat), and a permeate containing small milk fat globules of 0.9 to 3.3 μm (up to 30% of initial milk fat). Therefore, this microfiltration process, which obviates the need for milk aging, appears to be more efficient than gravity separation in selecting native fat globules of different sizes, at least 1- μm smaller or larger than in raw whole milk. Moreover, using a membrane with pore size of 5 μm allows, by varying hydrodynamic conditions, the attainment of small and large fat globule fractions with sufficient fat content ($>15 \text{ g}\cdot\text{kg}^{-1}$) for industrial applications; 3- μm membranes are also promising. The use of milk fat globules of different sizes obtained as described in the present study can lead to the development of new dairy products and contribute to improve quality (sensory, functional, and perhaps nutritional).

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