Native vs. Damaged Milk Fat Globules: Membrane Properties Affect the Viscoelasticity of Milk Gels

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ABSTRACT

The storage modulus G' of rennet and acid milk gels filled with milk fat globules was measured as a function of the fat globule surface composition (native milk fat globule membrane, caseins and whey proteins, or a mixture of the three due to mechanical treatments) and surface area (i.e., the fat globule size). By different technological procedures, it was possible to obtain fat globules of constant surface composition but various sizes, and vice-versa, which had never been done. For both rennet and acid gels, a critical fraction of the fat globule surface covered by caseins and whey proteins was identified (~40%), beyond which G' increased. Below this threshold, the gel viscoelasticity was unaffected by mechanical treatments. When the diameter of native milk fat globules decreased, the G' of rennet gels increased slightly, whereas that of acid gels decreased sharply. For both types of gels, G' increased when the diameter of partially disrupted fat globules decreased. For recombined globules completely covered with caseins and few whey proteins, G' increased with fat globule surface area for rennet gels whereas it decreased for acid gels. With the help of confocal microscopy and in the light of general structural differences between rennet and acid gels, a mechanism is proposed for the effect of fat globule damage and diameter on G' , depending on the interactions the globules can undergo with the casein network.

(Key words: milk fat globule membrane, gel, viscoelasticity, mechanical treatment)

Abbreviation key: **A** = total surface area of the fat globules per unit mass of a milk sample ($\text{m}^2 \cdot \text{kg}^{-1}$ milk), **AMF** = anhydrous milk fat, **CLSM** = confocal laser scanning microscopy, **d_{32}** = Sauter average diameter (μm), **F** = fat content ($\text{g} \cdot \text{kg}^{-1}$), **G'** = storage modulus of a milk gel (Pa), **MFGM** = milk fat globule membrane,

NCN = noncasein nitrogen, **S_0** = initial specific surface area of the native milk fat globules ($\text{m}^2 \cdot \text{g}^{-1}$ fat), **S_d** = specific surface area of the milk fat globules that are mechanically damaged ($\text{m}^2 \cdot \text{g}^{-1}$ fat), **v_i** = volume of particles in a size class of diameter d_i (μm^3), **Φ** = fraction of the fat globule surface that is new and covered by proteins (mainly caseins, plus whey proteins) due to mechanical treatments (%), **TN** = total nitrogen, **ULH-SMP** = ultra-low-heat skim milk powder, **ζ** = particle zeta-potential (mV).

INTRODUCTION

Fat is known to contribute greatly to the textural characteristics of many dairy products, such as yogurts and cheeses. Milk fat is present in milk and dairy gels in the form of small droplets, from 0.1 to 15 μm diameter: the milk fat globules (Mulder and Walstra, 1974). There are numerous small globules representing a small fraction of the fat, and very few large ones comprising a larger fat percentage. These globules are naturally covered by the native milk fat globule membrane (**MFGM**), composed mainly of phospholipids, proteins and enzymes (McPherson and Kitchen, 1983). If milk is subjected to inappropriate mechanical treatments (e.g., shear in pumps), the milk fat globule diameter decreases. This results in the adsorption of proteins (mainly caseins, plus whey proteins) onto the fat globule surface: the MFGM is damaged. These composition changes of the fat globule interface, highest when milk is homogenized, modify the electrokinetic potential of the milk fat globules and the interactions they undergo with proteins in dairy gels (Mulder and Walstra, 1974; Michalski et al., 2002a).

In acid dairy gels filled with fat globules, the fat composition has little influence on the rheological properties of the gel comparing to the composition of the fat globule surface, between 15 and 55°C (Xiong and Kinsella, 1991). If recombined fat globules are surrounded by sodium caseinate, the resulting gel is firmer than with skim milk powder but less firm than with denatured whey proteins (Xiong and Kinsella, 1991;

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Lucey et al., 1998a). Moreover, when the volume fraction of fat globules stabilized by denatured whey proteins increases, the storage modulus G' of the acid gel increases as a result of denatured whey proteins interaction with caseins (Lucey et al., 1998b). Typically, if the milk fat globule surface is composed of noninteracting, inactive material (such as natural whey proteins or the native MFGM), the structure of the gel is unchanged if fat globules are smaller than the network pores (inert filler). Conversely, if the interfacial material interacts with the casein network, G' increases all the more with the volume fraction (Cho et al., 1999) and the smallness of fat globules (van Vliet and Dentener-Kikkert, 1982; van Vliet, 1988; Xiong et al., 1991). The effect of milk fat globules on the viscoelasticity of rennet gels, however, has little been studied, and results are somehow conflicting (Walstra, 1995). Fat globules covered by caseins due to homogenization would increase the modulus of filled rennet gels but decrease their firmness, calculated as a yield stress (Walstra, 1995).

Up to now, studies on the viscoelasticity of filled milk gels concerned either fully homogenized milk fat globules, regular native ones, or globules covered by an artificial membrane. The effect of the gradual increase of the casein and whey protein content of the fat globule surface, due to mechanical treatments, on the gel storage modulus had not yet been studied. Moreover, the effect of fat globule size had only been studied for fat globules completely covered with an artificial membrane, and the influence of native milk fat globule diameter on the rheological properties of the gels was not known.

This study aims at elucidating independently the role of the milk fat globule surface composition and surface area on the viscoelasticity of rennet and acid milk gels. The globule surface composition was varied by different homogenization pressures. The native milk fat globule diameter and surface area were varied using an original microfiltration process designed in the laboratory (Goudedranche et al., 1998), and fat globules of various sizes covered by caseins and whey proteins (so-called recombined globules) were produced by milk fat emulsification in skim milk and homogenization. This new approach yielded milk fat globules of equal size but with different surface composition, and milk fat globules of constant membrane composition (inert or interacting with caseins) but different sizes.

MATERIALS AND METHODS

Materials

Raw whole milk was purchased from a dairy plant (Compagnie Laitière Européenne, Montauban-de-Bretagne, France), from April to July. The milk was col-

Figure 1. Technological scheme describing the method used to obtain milk fat globules with various sizes and surface compositions. MFGM = native milk fat globule membrane. See text for further explanation.

lected directly from the refrigerated truck in order to avoid damage of the native milk fat globules. Ultra-low-heat skim milk powder (ULH-SMP) was produced in the laboratory (LRTL, INRA, Rennes, France). Its composition (% of total solids) was: total nitrogen, 37.3%; noncasein nitrogen, 7.4%; nonprotein nitrogen, 2.0%; lactose, 54.5%; ash 8.2%. Anhydrous milk fat (AMF) was from Lactalis (Bourgbarré, France). Water was purified by reverse osmosis (MilliPore) in the laboratory. The calf rennet extract (Gand Gassiot 140 IMCU/mL) was purchased from Chris Hansen (Arpajon, France). EDTA (99%), imidazole (99%), SDS, NaCl, CaCl_2 , and thiomersal were from Merck (Darmstadt, Germany). A laboratory cream separator (Elecram, Vanves, France) and a two-stage pilot homogenizer (Rannie Machine Works, Copenhagen, Denmark) were used.

Sample Preparation

Each type of milk fat globules in the experimental scheme corresponded to a specific technological procedure including one or more of the following steps (Figure 1): (i) a cross-flow microfiltration to obtain native milk fat globules of different sizes (Goudedranche et al., 1998); (ii) an homogenization at 50°C from 2 to 10 MPa; (iii) the use of a premix of 74 g AMF per kg of skim milk. The original milk and milk obtained by steps (i), (ii), or a combination of steps (i) and (ii) or (iii) and (ii), were creamed at 40°C and diluted in reconstituted skim milk, to maintain a constant composition of the continuous phase for each sample. Final samples will be called standardized milks. Reconstituted skim milk was prepared by mixing 111.87 g of ULH-SMP with

3.33 g of thiomersal in 1 L of demineralized water. The final fat content of standardized milk was $150.6 \text{ g} \cdot \text{kg}^{-1}$ for rennet gels or $159 \text{ g} \cdot \text{kg}^{-1}$ for acid gels (error always <4%). The milk fat content was measured with an infrared analyzer (DairyLab 2, Multispec, York, UK), and complementary analyses were performed with the Gerber method. Total nitrogen (TN) and noncasein nitrogen (NCN) were measured by reference methods of analysis (International Dairy Federation, 1964; International Dairy Federation, 1986).

Particle Size and ζ -Potential Measurements

The particle size distribution of standardized milk was measured by Laser Light Scattering with a Mastersizer 2000 (Malvern Instruments, Malvern, UK). The refractive index of milk fat was taken to be 1.460 at 466 nm and 1.458 at 633 nm; that of the aqueous phase was 1.33 (Michalski et al., 2001). The milk sample was diluted (1:1) in EDTA 35 mM pH 7 to dissociate casein micelles, before drops were introduced in the circulating cell containing water with 0.05% SDS to dissociate clusters. The Sauter average diameter $d_{32} = \sum v_i / \sum (v_i / d_i)$ was calculated by the software (where, v_i is the volume of globules in a size class of diameter d_i), as well as the specific surface area of fat globules, S ($\text{m}^2 \cdot \text{g}^{-1} \text{ fat}$). The fraction of the fat globule surface covered by caseins and whey proteins due to disruption, Φ , was subsequently estimated as (Michalski et al., 2002): $\Phi = (S_d - S_0) / S_d$, where, S_0 is the initial specific surface area of the native milk fat globules, and S_d is their specific surface area after damage by homogenization. There we suppose that the fraction of newly created surface in the damaged globules corresponds to the fraction of their surface covered by caseins and whey proteins, since these proteins adsorb on the new interface. In a milk of fat content F , the total surface area of milk fat globules can be calculated as $A = FS_{(0 \text{ or } d)}$, and the damaged surface area is $A\Phi$. The interglobular distance was calculated as $0.225d_{32}[(0.74/F)-1]$ (Walstra, 1969b).

The ζ -potential of milk fat globules was found to be a good complementary indicator of the degree of damage of the MFGM (Michalski et al., 2002b). ζ was measured by Laser Doppler Electrophoresis, using the ZetaSizer 3000 HS (Malvern, UK), after diluting the sample in 20 mM imidazole, 50 mM of NaCl, 5 mM of CaCl_2 , and a pH 7 buffer. This results in the same values than after washing the globules to remove casein micelles (Michalski et al., 2002b).

Rheological Measurements

Rheological measurements were performed the same day after globule and milk preparation to prevent the

modification of globular structure by crystallization or possible changes in the surface composition of the globules (when the sample was kept overnight, rheological measurements were no longer reproducible). Doing so, measurements could be performed in duplicate only and did not allow statistical analyses, but were more accurate.

Rennet milk gels. Standardized milk samples were let to equilibrate 1 h in a water bath at 32°C . Then, 200 μl of rennet diluted 10-fold was added to 50 g of milk, at time $t = 0$. The final fat content of the gels was $150 \text{ g} \cdot \text{kg}^{-1}$ ($\pm 2.5\%$), and the casein content of the aqueous phase was $27.8 \text{ g} \cdot \text{kg}^{-1}$ ($\pm 2.5\%$). A precise volume of renneted milk was introduced in the dynamic controlled-stress rheometer (Carrimed CSRH50, TA Instruments, Guyancourt, France) with a cone-plate device. The acrylic cone was 6 cm in diameter, the angle was 3 degrees 59, and the gap was 127 μm ; it was surrounded by paraffin oil to prevent evaporation. Oscillatory measurements were performed by following the change of the storage modulus G' as a function of time during 90 min after 14 min of rest, at a fixed frequency of 0.1 Hz and 0.4% of strain amplitude (lowest possible value, checked to ensure the linear viscoelastic region).

Acid milk gels. Standardized milk samples were equilibrated 1 h at 4°C . Acidification was performed with a Metrohm titrator (Heriseau, Switzerland): HCl 1 N was delivered to 50 g of milk at $0.2 \text{ ml} \cdot \text{min}^{-1}$ until a final pH of 4.6 was reached (typically, after 12 min). The volume of acid added to milk was completed with distilled water, so that all the gels had a final fat content of $150 \text{ g} \cdot \text{kg}^{-1}$. Viscoelastic measurements were performed with an AR1000 rheometer (TA Instruments) equipped with DIN coaxial cylinders ($R_1 = 23.05 \text{ mm}$, $R_2 = 25 \text{ mm}$, immersed height = 30 mm). Acidified milk in the cold (45 ml) was placed between cylinders at 2°C (gap at the bottom of cylinders = 4 mm) and covered with paraffin oil. After equilibrating during 1 min, storage modulus change was followed at a frequency of 0.1 Hz at 0.5% strain during the temperature increase, up to 40°C at a rate of $1^\circ\text{C} \cdot \text{min}^{-1}$. Once this temperature was reached, the change of G' with time was followed for 25 min.

Confocal Laser Scanning Microscopy (CLSM) of Rennet Gels

The CLSM (LSM 410 Axiovert, Zeiss, Le Pecq, France) was performed in fluorescence mode (Herbert et al., 1999). The protein matrix of renneted milks was stained by the fluorescent dye FITC (Sigma, St-Quentin-Fallavier, France; 2 to 3 mg per 5 ml of milk), and the fat globules were stained by Nile red (Aldrich, St-

Figure 2. Experimental design performed in this study: diameter (d_{32}) and fraction of surface covered by caseins and whey proteins (Φ) of the different milk fat globules. (●) native milk fat globules covered by their natural membrane; (■) homogenized globules partially covered by caseins and whey proteins from native globules of raw milk; (◆) homogenized globules from small native globules obtained by microfiltration; (▲) recombined fat globules from emulsified anhydrous milk fat, covered by caseins and whey proteins. White triangle zones represent d_{32} - Φ combinations that cannot presently be obtained by technological means in sufficient quantity.

Quentin-Fallavier, France; 1 mg per 5 ml of milk before any treatments; (Blonk and van Aalst, 1993). After renneting, a few drops of the labelled renneted milk was transferred to microscope slides with concave cavities, covered with a cover slip, sealed to prevent evaporation and incubated 30 min in an oven at 30°C. Observations of the gels with the CSLM were performed on the same field with a $\times 63$ oil immersion objective at wavelengths of 543 and 488 nm, which are close to the excitation maximum of Nile red and FITC, respectively. Nile red fluorescence emission was recorded between 575 and 640 nm, whereas the emission of FITC was recorded between 510 and 525 nm, which allows a good spectral discrimination between the two components. This procedure allows a colocalization of fat and proteins in the same field of observation. Multiple observations were performed for each sample at zooms 2 and 4, and typical pictures were chosen. Five different milk samples were used: milk with native fat globules with diameters of 6, 4, and 2.5 μm , and milk homogenized at 3 and 10 MPa.

RESULTS

Experimental Design

The experimental scheme performed in this study (combinations of milk fat globule d_{32} and membrane damage) is presented Figure 2. No experimental design

in the strict sense could be performed since the properties of milk fat globules depended on the technological processes used. Still, we obtained fat globules with equal diameter but various surface composition and vice versa. The properties of each type of globules are summarized in Table 1 and examples of globule size distributions are given Figure 3. The range of interglobular distance was from 1.2 μm to 3.6 μm in this study. Usually, native milk fat globules have a ζ -potential ~ 13.5 mV and casein micelles ~ 20 mV. The increase of ζ -potential for damaged milk fat globules is, thus, due to the adsorption of caseins and whey proteins at their surface (Michalski et al., 2002b). This way, we verified that small and large milk fat globules remained native after the microfiltration process, since their ζ -potential was the same as that from the initial milk. We could not analyze the exact composition of the surface of fat globules in this study for practical reasons, but the degree of damage was estimated from the increase in ζ -potential and surface area data obtained by laser light scattering.

Gelation Curves

Typical curves of G' increase during coagulation are shown Figure 4. After a lag time, G' increased strongly (which defines the setting time) and tended to level off. For rennet gels (Figure 4A), setting times were similar and consistent with Zoon et al. (1988b), although shorter for homogenized milks, which is consistent with

Figure 3. Point examples of the size distribution of fat globules used in this study: (—○—) small native with $d_{32} = 2.9$ μm , (—●—) regular native with $d_{32} = 3.8$ μm , (—●—) large native with $d_{32} = 4.6$ μm , (—□—) regular homogenized at 3 MPa with $d_{32} = 1.9$ μm , (—■—) regular homogenized at 8 MPa with $d_{32} = 1.5$ μm , (—□—) small homogenized at 8 MPa with $d_{32} = 1.6$ μm , (—△—) recombined from anhydrous milk fat at 3 MPa with $d_{32} = 3.0$ μm , (—▲—) recombined from anhydrous milk fat at 8 MPa with $d_{32} = 1.9$ μm .

Table 1. Physico-chemical properties of milk fat globules (d_{32} , ζ , fraction of surface damaged Φ) and milks with 150 g·kg⁻¹ fat (interglobular distance).

Milk fat globule type	Milk fat globules			Milks (150 g·kg ⁻¹)
	d_{32} (μm)	ζ (mV)	Φ (%)	Interglobular distance (μm)
Standard native ¹	3.83	-14.5	0	3.11
	3.69	-15.0	0	2.92
	3.76	-12.9	0	2.94
Small native ¹	2.63	n.d. ²	0	2.06
	2.86	-12.5	0	2.26
	4.6	-12.8	0	3.61
Large native Homogenized 2 MPa	2.82	-15.3	25	2.35
	1.89	-17.4	44	1.42
	2.16	-16.2	38	1.61
	1.46	-17.2	60	1.21
	1.48	-17.5	59	1.18
	2.07	-16.4	29	1.63
Small homog. 3 MPa	1.60	-14.8	44	1.24
	1.58	n.d.	42	1.25
	1.58	n.d.	42	1.25
Reconstituted from AMF ¹	3.88	-20.5	100	2.86
	2.99	-21.3	100	2.35
	1.91	-18.7	100	1.48

¹Each line corresponds to a different sample obtained by the same technology.²Not determined.

Robson and Dalgleish (1984). Gels often exhibited syneresis after 3000 to 6000 s (consistent with 10^3 to 10^4 s indicated by van Vliet et al., 1991), which caused the measured G' to decrease due to slipping of the cone (not shown, consistent with Zoon et al. (1988a)). Therefore, the rennet coagulation curves were modeled using the Scott-Blair model, which is the most suitable for rennet gels (O'Callaghan and Guinee, 1995; Guinee et al., 1997): $G' = G'_{\max} \exp[-b/(t-\tau)]$ for $t > \tau$, where b is a constant and τ is a characteristic time close to the setting time. The equilibrium G' value ($G'_{\max}$) and b were obtained by fitting for each gel (using TableCurve software, AISN Software, Inc. and choosing the τ value providing the best R^2 ; O'Callaghan and Guinee, 1995). For acid gels, coagulation curves were less regular (Figure 4B), which can be due to the formation of a gel at the initial part of the heating up phase due to the heating rate (Roefs, 1985) and is consistent with Xiong and Kinsella (1991). Moreover, most of the milk fat is solid at 2°C, which imparts rigidity to the product (Zhou and Mulvaney, 1998; Jaros et al., 2001), and subsequent local decreases of G' up to 40°C can correspond to the gradual fusion of the fat. Therefore, G' values were compared after 1 h, i.e. when the relative viscoelastic changes of all gels tended to level off.

Effect of Surface Damage and Surface Area on G'

Figure 5 shows the storage modulus of rennet and acid gels versus the fraction of fat globule surface covered by caseins and whey proteins (Φ). The discrepancy

along the y-axis for a given Φ is due to the different diameters of fat globules. For both types of gels, G' was not a linear function of fat globule damage: It began to increase significantly ($P < 0.05$) above a critical value of $\Phi \sim 40\%$ only. For rennet gels, the increase seemed then linear from 50 to 200 Pa up to $\Phi = 100\%$. For acid gels, however, G' was not higher at $\Phi = 100\%$ than at $\Phi = 60\%$.

Figure 6 shows the variations of G' versus the total surface area of fat globules in the sample, apart from their surface composition. For rennet gels, G' increased with the fat globule surface area (i.e., it increased for decreasing fat globule diameter at constant fat content), whatever the globule surface composition. The increase was sharper for recombined fat globules. For homogenized fat globules, the lowest point corresponded to the least damaged surface. For acid gels, conversely, the G' of native and recombined fat globules decreased significantly when the fat globule surface area S increased, which does not support results by Xiong et al. (1991) on gels with 10% recombined fat globules. For homogenized globules, G' usually increased with S , except for a few samples that were less damaged. For native and homogenized milk fat globules, the influence of fat globule size on the storage modulus was higher in acid gels than in rennet gels, within the diameter range studied.

Rennet Gel Confocal Micrographs

Confocal micrographs of rennet gels with native globules of various sizes and homogenized globules are

Figure 4. Typical coagulation curves: storage modulus changes with time as a function of the type of milk fat globules: (●) regular native, (□) homogenized at 3 MPa, (■) homogenized at 8 MPa, (▲) recombined from anhydrous milk fat, (—) temperature for acid gels. A: Rennet gels (32°C), curves modeled by the Scott-Blair model; B: acid gels (up to 40°C).

shown Figure 7. Fluorescence of Nile red, revealing lipid localization, was coded in red, while FITC fluorescence, staining proteins, was coded in blue. The micrographs reveal the porous structure of the casein network in rennet gels, with pores in the order of 10 μm . Native milk fat globules (Figure 7A) are mainly located in the serum pores of the gels, whatever their size. In contrast, strongly homogenized milk fat globules (Figure 7B) look embedded in the proteinaceous matrix. The effect of globule structure on the casein matrix could, however, hardly be observed. Each type of fat globule retained its spherical globular structure within the rennet gel.

DISCUSSION

The milk gel properties are different whether induced by rennet addition or by acidification (Roefs, 1985; Zoon et al., 1988a). Rennet gels have a high cohesion, high elasticity and large pores, especially at high tempera-

Figure 5. Storage modulus of milk gels as a function of the fraction of milk fat globule surface covered by caseins and whey proteins due to damage, Φ . (●) native milk fat globules covered by their natural membrane; (■, ◆) homogenized milk fat globules partially covered by caseins and whey proteins (◆: homogenized from small native globules); (▲) recombined fat globules from emulsified anhydrous milk fat, covered by caseins and whey proteins. A: G'_{max} of rennet gels from the Scott-Blair model; B: G'_{1h} of acid gels. Dotted lines are to guide the eye (not a linear fitting).

ture, and exhibit syneresis. As casein particles flocculate into irregular strands, the resulting network is heterogeneous and has openings up to about 10 μm in diameter, varying with gel forming conditions (Kalab and Harwalkar, 1973; Green et al., 1978; Zoon et al., 1988a; van Vliet et al., 1991). Acid gels are brittle, their elasticity and plasticity are very low because the network is poorly structured, and their pores are smaller. The main links are hydrophobic, hydrogen, and electrostatic bonds, and they have a low resistance to mechanical treatments. These structural differences should be considered when analyzing the effect of milk fat globule properties on both types of gels.

Figure 6. Storage modulus of milk gels as a function of the total surface area of milk fat globule in the sample. (●) native milk fat globules covered by their natural membrane; (■, ◆) homogenized milk fat globules partially covered by caseins and whey proteins (◆: homogenized from small native globules); (▲) recombined fat globules from emulsified anhydrous milk fat, covered by caseins and whey proteins. A: $G'_{\max}$ of rennet gels from the Scott-Blair model; B: G'_{1h} of acid gels. Lines are to guide the eye (not a linear fitting).

Critical Fraction of Membrane Damaged Φ for G' to Increase

The surface damage of milk fat globules only had a significant effect on G' above $\Phi \sim 40\%$ (Figure 5). This cannot be due to a lack of caseins at the globule surface at lower damage: even at low homogenization pressure, casein micelles adsorb faster on fat globules than whey proteins (Oortwijn and Walstra, 1979). Moreover, the rapid increase of fat globule ζ -potential at very low surface coverage with caseins and whey proteins supports the concept that casein micelles first adsorb and protrude outside the MFGM (Michalski et al., 2002b; Table 1). We should highlight that at low degrees of damage of milk fat globules, the size distribution is not

Figure 7. Confocal micrographs of rennet milk gels with different types of milk fat globules: (A) native of various sizes, (B) homogenized at 3 or 10 MPa. (Bars indicate a length of $10 \mu\text{m}$.)

Figure 8. Influence of the milk fat globule properties on the structure of milk gels. (●) casein micelles; (○) fat globules. To be compared with the works by Cho et al. (1999) and Xiong and Kinsella (1991). See text for detailed explanations.

as narrow as that of strongly homogenized globules. For example, globules homogenized at 3 MPa, with $\Phi \sim 40\%$ and $d_{32} = 1.9 \mu\text{m}$, present a “tail” of larger globules up to $6 \mu\text{m}$, that does not exist for globules homogenized at 8 MPa (Figure 3). This corresponds to two subpopulations (Walstra, 1969a), and is consistent with CLSM micrographs (Figure 7B, 3 MPa), in which a population of larger globules can be seen among apparently smaller globules of narrower size range. The calculated Φ is, thus, an average value: assuming that the largest globules remain mainly native, some smaller globules have thus $\Phi > 40\%$. Conversely, these damaged globules are likely to interact with the casein matrix (van Vliet, 1988; Guinee et al., 1997) in which they are evenly embedded (Figure 7B). However, due to their small size, they represent a lesser proportion of the fat volume fraction F in the gel. The fat volume fraction of homogenized globules has a great influence on their ability to increase gel modulus (van Vliet, 1988; Guinee et al., 1997). Therefore, for milk fat globules submitted to moderate mechanical treatments, the low volume fraction of interacting fat globules would explain the existence of a critical value of Φ below which G' was not affected (Figure 8). However, among the moderately damaged globules, there are also globules with few caseins and whey proteins adsorbed on their surface. We can suppose that these globules did not increase G' because adsorbed caseins were not numerous enough to make the fat globules interact with each other and to increase the interconnectivity between casein strands. Slightly damaged globules remained, thus, as inert fill-

lers in acid and rennet gels (Figure 8). It should be checked by other techniques, however, that the lack of effect at low to moderate treatments was not due to a lack of sensitivity in the technique used for G' measurements.

Above the critical Φ , the fat globule surface is sufficiently covered by caseins for the globules to interact with each other and with the casein network (according to Sharma et al., 1996, caseins make up to $\sim 75\%$ of the surface of a recombined globule membrane). Strongly damaged fat globules acted as structure promoters and increased G' (Figure 8). These results are consistent with other studies on the effect of homogenized or recombined globules on rennet gels (Guinee et al., 1997) and acid gels (Xiong et al., 1991; Lucey et al., 1998a). Zhou and Mulvaney (1998), however, found that this effect on rennet gels occurred mainly at lower temperatures.

Influence of Native Fat Globule Size for Both Types of Gels

In rennet gels, G' tended to increase when the size of native fat globules decreased, i.e., when S increased (Figure 6A). Conversely, for acid gels, G' decreased significantly with decreasing fat globule size, and sharper than for rennet gels (Figure 6B). Since the native MFGM does not interact with the casein matrix, these differences can be examined in the light of acid and rennet gel properties described above. The serum pores in rennet gels are likely to be larger than in acid gels,

which also depends on the time point that the rennet gel is examined and the method used to make the acid gel system. In rennet gels (Figure 7), only the biggest fat globules were likely to be larger than the pores. They acted as structure breakers (Figure 8) and weakened the gel modulus, since they impaired locally the normal casein strand formation. Guinee et al. (1997) suggest that gel strands are thinner and weaker around large fat globules. Conversely, regular and small milk fat globules were smaller than the network pores in which they lie (Figure 7): They would then act as inert fillers (Figure 8). In acid gels, results can be explained assuming that native fat globules of all sizes tested are likely to be larger than the gel pores. For smaller fat globules, at equal fat content, the number of fat globules is greater. This induced more weak points in the network: Fat globules act even more as structure breakers in acid gels as they are smaller (within the size range tested).

Difference between Rennet and Acid Gels with Homogenized and Recombined Globules

For homogenized globules, the increase of G' with $\Phi > 40\%$ (Figure 5) and with globule surface area (Figure 6) was sharper in acid than in rennet gels. This can be explained by the larger pores of rennet gels (Lefebvre-Cases et al., 1998): Small homogenized globules can certainly not interconnect strands as efficiently as in acid gels. For homogenized globules with $\Phi > 40\%$, the structure-promoting effect is enhanced when the surface area of fat globule increases. This can be due to the increased number of connections between fat globules and casein strands as S increases, and is consistent with the literature (Guinee et al., 1997). The G' of gels with small globules that were homogenized was below the one of regular homogenized globules for the same surface area because the former have a lower Φ .

The G' of recombined globules with $\Phi = 100\%$ was higher in rennet gels (~150 to 250 Pa) than in acid gels (~100 to 200 Pa; Figure 5). Moreover, G' increased with recombined fat globule surface area in rennet gels, whereas it decreased for acid gels (Figure 6). In rennet gels, the increase of G' with S is certainly due to the increased interconnectivity between globules and caseins. In acid gels, the decrease of G' when S increases is harder to explain. However, the literature shows some discrepancies. Xiong et al. (1991) found that smaller recombined globules had a larger effect on acid gels. Conversely, Cobos et al. (1995) found no significant effect of the homogenizing pressure, and, therefore, recombined globule size on gel modulus. The formation of some kind of aggregates (van Vliet, 1988) by the

smallest recombined globules would also reduce interactions with the casein network.

An effect of milk fat crystallization properties in globules can also be suggested. In rennet gels, fat was kept above 30°C, so that no significant fat crystallization could occur and fat globules can act as a plasticizer (Zhou and Mulvaney, 1998). The G' of acid gels with homogenized globules can increase faster than in rennet gels because part of the fat is still crystallized due to tempering at 4°C. In recombined globules, there would be less solid fat at high temperature in the smallest ones (Mulder and Walstra, 1974). This can also partly explain why, in acid gels, G' decreased when recombined globule size decreased.

Difference between Homogenized and Recombined Globules in Acid Gels

In acid gels, the G' with recombined globules ($\Phi = 100\%$) decreased with S , reaching lower values than the G' with homogenized globules at $\Phi > 40\%$, that increased with S (Figure 6B). This difference is first surprising, since recombined fat globules would be expected to be similar to strongly homogenized ones. However, Xiong and Kinsella (1991), with acid gels with 10% fat and $d_{32} \sim 1 \mu\text{m}$, also found that G' was higher with homogenized globules than with globules recombined in skim milk. A difference in surface composition between recombined and homogenized globules can be suggested. Sharma et al. (1996) have shown that 33% of the surface of recombined globules from AMF in skim milk is covered by whey proteins that do not interact with the casein network (Lucey et al., 1998a). Only 67% of the recombined globule surface could, thus, be a structure promoter. Regarding the surface composition of homogenized globules, Cano-Ruiz and Richter (1997) found that they also contain at least 10% w/w of whey proteins. However, their samples were subjected to heat treatments, which cause the absorption of whey proteins onto the fat globule surface. Sharma and Dalgleish (1993), conversely, have shown that raw homogenized globules contain no whey proteins. Now, in this study, for $\Phi \sim 60\%$, homogenized fat globules are smaller ($d_{32} \sim 1.5 \mu\text{m}$, Table 1) than recombined ones ($d_{32} \sim 1.9 \mu\text{m}$). This could explain why some gels with recombined globules are weaker. However, a precise analysis of the surface composition of the different fat globules is needed to understand these differences.

CONCLUSION

This work has shown that damaging milk fat globules by mechanical treatments alone had a significant effect on the modulus of filled dairy gels beyond a critical

value of the globule surface coverage with caseins and whey proteins (about half of the surface). Below this surface coverage, damaged fat globules are not numerous enough, nor they do not contain enough casein, to interconnect casein strands efficiently. Critically damaged milk fat globules acted as structure promoters in both acid and rennet gels, which usually increased with fat globule surface area at a constant fat volume fraction. We have shown that native milk fat globules could act either as inert fillers or as structure breakers, depending on their size and gel structure: The modulus of rennet gels was slightly higher for smaller globules, whereas the modulus of acid gels increased with the fat globule diameter.

Investigating more precisely the compositional and structural differences between the membranes of homogenized and recombined fat globules would help in understanding their impact in gel structure. Since the physical state of fat is likely to affect gel modulus, and dairy products are subjected to refrigeration, the effect of fat globule properties on the viscoelasticity of gels at lower temperature should also be studied. Image analysis techniques to study the texture of confocal images could help in understanding the effect of milk fat globules on the organization of the protein network. Moreover, measurements of gel strength at large deformation, that does not necessarily vary the same way that G' (van Vliet, 1988), would provide insights on the impact of fat globule properties on gel texture when fractured in the mouth.

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