

## The size of native milk fat globules affects physico-chemical and sensory properties of Camembert cheese

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(Received 28 February 2002; accepted 18 July 2002)

**Abstract** – Camembert cheeses were produced using either small (~3  $\mu\text{m}$ , SFG) or large (~6  $\mu\text{m}$ , LFG) native milk fat globules obtained by a patented microfiltration process. The composition of the cheese milk did not depend on the fat globule size. Less whey was extracted from the SFG cheeses, that subsequently contained more moisture than LFG cheeses throughout the ripening period. The SFG curds were less rigid and less firm than the LFG ones and underwent greater proteolysis during ripening. Camembert cheeses with small fat globules had a higher melting and elastic texture, a higher flowing aspect and were less yellow. The results were explained (i) by the greater surface area of native milk fat globule membrane for SFG vs. LFG, at a given fat content, and (ii) by the thinner casein strands in SFG cheeses due to the smaller interglobular distance. The use of native milk fat globules with different sizes can thus lead to new products with different technological and sensory properties.

**Milk fat / fat globule / Camembert cheese / cheese yield / rheology / particle size / microfiltration / sensory analysis**

**Résumé** – Des propriétés physico-chimiques et sensorielles du Camembert sont affectées par la taille des globules gras natifs du lait. Des camemberts ont été fabriqués en utilisant des globules gras natifs du lait de petit diamètre (~3  $\mu\text{m}$ ) ou de grand diamètre (~6  $\mu\text{m}$ ), obtenus par un procédé de microfiltration breveté. La composition des laits de fromagerie ne dépend pas de la taille des globules gras. Il s'égoutte moins de sérum des camemberts à petits globules, qui sont ensuite plus humides que les fromages à gros globules au cours de l'affinage. Les caillés à petits globules sont moins fermes et moins rigides que ceux à gros globules et sont plus protéolysés durant l'affinage. Les camemberts à petits globules ont une texture plus fondante et plus élastique, sont plus coulants et moins jaunes. Ces résultats peuvent être expliqués (i) par la plus grande surface de membrane native pour les petits globules par rapport aux gros globules à une teneur donnée en matière grasse et (ii) par la moindre épaisseur des brins de caséine dans les camemberts à petits globules, à cause de la plus faible distance moyenne entre globules. L'utilisation de globules gras natifs du lait de différentes tailles peut donc aboutir à de nouveaux produits présentant des propriétés technologiques et sensorielles différentes.

**Matière grasse du lait / globule gras / Camembert / rendement fromager / granulométrie / rhéologie / analyse sensorielle / microfiltration**

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## 1. INTRODUCTION

Milk fat is recognized to contribute greatly to the texture, flavor and physico-chemical properties of many dairy products, especially cheese. Particularly, depending on its solid fat content, it acts more or less as a plasticizer [12]. More than 95% of the mass of lipids in cow's milk is present in the form of spherical milk fat globules (MFG) [13]. The diameter of MFG ranges from 0.1  $\mu\text{m}$  to 15  $\mu\text{m}$ , with a volumetric average around 4  $\mu\text{m}$ , depending e.g. on cow breed, feed and season [28]. They are surrounded by a native stabilizing membrane, allowing them to be compatible with the aqueous environment: the milk fat globule membrane (MFGM). This complex MFGM, around 15 nm thick, originates from a primary membrane in the secretory cell, additioned with the membrane of the apical cell after secretion. The MFGM is composed mainly of phospholipids, glycolipids, proteins (25–60% of MFGM mass), lipoproteins and enzymes such as butyrophilin and xanthine oxidase [3, 13]. This composition results in an average  $\zeta$ -potential of  $-13.5$  mV [21] and in uncommon surface active properties, with the MFG interfacial tension being as low as  $1\text{--}1.5$  mN $\cdot\text{m}^{-1}$  [22]. Moreover, at least 25 different enzyme activities have been found to be associated with the MFGM, which also presents a high water-binding ability [8, 13]. Considering the average milk fat globule size distribution, the MFGM surface area is  $\sim 1.85$  m $^2\cdot\text{g}^{-1}$  fat, i.e.,  $\sim 75$  m $^2\cdot\text{kg}^{-1}$  milk [20]. The smallest globules ( $<1$   $\mu\text{m}$ ) represent more than 80% of the total number of globules, although a few percent of the fat volume, and have a much higher specific surface area. A few large globules ( $>10$   $\mu\text{m}$ ) also exist, comprising about 2% of the fat, with a lower specific surface area [28]. It has been suggested that small and large fat globules would differ slightly in composition [24], but this is still not fully elucidated [28].

The structure and texture of dairy gels and cheeses are affected by the interactions between the surface of milk fat globules and the casein matrix. In this respect, native milk fat globules do not interact with the protein network in dairy gels and act mainly as inert fillers or structure breakers, depending on their size and number [19, 25]. For a given fat content, if native MFG were smaller, they would represent a greater number of weak points in the matrix and a larger surface area of MFGM, with a higher water-binding ability and enzymatic content. Conversely, large fat globules can be expected to provide larger weak points, although less numerous. The use of small or large native MFG can be expected to result in different cheese texture and aroma, as already observed for various dairy products in a preliminary study [8] and in low-fat Cheddar cheese [23]. We should point out that the use of small globules produced by homogenization, where the native MFGM has been disrupted and replaced by casein micelle fragments, results in structure enforcement by creating links with the casein network [2]. Therefore, this type of globule does not possess the genuine characteristics of native globules and could not be compared with products made with small native MFG.

Until recently, it was not possible to select milk fat globules efficiently according to their size, since centrifugation techniques were not selective enough. However, it is now possible to obtain native milk fat globules of various sizes from whole milk by using a new microfiltration process developed in our laboratory [7], that can lead to new manufacture processes. Using this technology, milk is filtered on tubular microporous ceramic membranes with a uniform transmembrane pressure, which allows the collection of the smallest fat globules in the permeate or the largest ones in the retentate. The small globules obtained in this way are native, conversely to globules

formerly obtained by homogenization, that are disrupted and covered by caseins.

The aim of this work was to investigate the influence of the size of the native milk fat globules on the physico-chemical and sensory characteristics of Camembert cheese, using milk fractions with small ( $\sim 3 \mu\text{m}$ ) or large ( $\sim 6 \mu\text{m}$ ) fat globules obtained by microfiltration.

## 2. MATERIALS AND METHODS

### 2.1. Camembert cheese production

Three series of Camembert cheese manufactures were performed according to the following process. The results presented are the average of the three manufactures for each type of Camembert cheese (small or large fat globules).

*Day D-1.* Raw whole milk purchased from a local dairy plant (Entremont, Montauban-de-Bretagne, France) was collected and stored at  $4^\circ\text{C}$  the day before Camembert production (D-1). Milk was microfiltered with a uniform transmembrane pressure according to a process patented in the laboratory [7]. Appropriate membrane pore sizes and hydrodynamic conditions were used, so as to obtain two milk fractions differing in the diameter of fat globules, namely, around 3 and  $6 \mu\text{m}$ , respectively. The corresponding fractions will be called small fat globule (SFG) and large fat globule (LFG) fractions. The SFG fraction was concentrated using a cream separator (Elecrem, Vanves, France). It was checked that the milk fat globules remained native and were not disrupted by the microfiltration process by measuring the globule  $\zeta$ -potential, with the procedure and apparatus described by Michalski et al. [21]. Milk samples were adjusted to a fat content of  $28 \text{ g}\cdot\text{kg}^{-1}$  using skimmed milk, before being pasteurized at  $72^\circ\text{C}$  for 20 s (Actini, France). Cooling was performed in a milk vat at  $11^\circ\text{C}$ . The milk was inoculated ( $2 \times 10^{11}$  per 100 kg) with mesophilic starters MM100 (Rhodia,

Tours, France) – *Lactococcus lactis* subsp. *lactis*, *cremoris*, and *lactis* biovar *diacetyllactis* – and ripening flora (Rhodia): *Geotrichum candidum* GEO 17 (2 doses per 1000 kg), *Penicillium camemberti* LV2 (5 doses per 1000 kg), and *Kluyveromyces lactis* 71 (2 doses per 1000 kg). 14 mL of a solution of  $\text{CaCl}_2$  at  $510 \text{ g}\cdot\text{L}^{-1}$  was added per 100 kg of milk. The milk was then matured at  $11^\circ\text{C}$  for 18 h.

*Day D.* The milk was heated at  $34^\circ\text{C}$  in the milk vat and left to ripen until it reached a pH of 6.35. After transfer into a cheese vat, renneting was carried out at  $33^\circ\text{C}$  using  $0.22 \text{ mL}\cdot\text{L}^{-1}$  of rennet (at  $520 \text{ mg}\cdot\text{L}^{-1}$  of chymosin, Gand Gassiot, purchased from Chris Hansen, Arpajon, France). Setting time was in the range 8–10 min in a room at  $28^\circ\text{C}$ . The curd was cut after four setting times into  $1.5 \times 1.7 \times 1.7 \text{ cm}$  cubes. Two stirrings were operated, at 10 and 25 min after cutting, before moulding into 20 cm diameter moulds at  $26^\circ\text{C}$ . The cheeses were turned at the following times after moulding and temperature: 45 min ( $23^\circ\text{C}$ ), 2 h ( $21^\circ\text{C}$ ) and 5 h ( $18^\circ\text{C}$ ). Whey was extracted continuously from stirring at day D to taking the cheeses out of the moulds at day D+1.

*Day D+1.* The cheeses were taken out of the moulds and salted in brine (350 g NaCl per L water, no calcium added) at  $12^\circ\text{C}$  for 25 min.

Ripening was performed at  $12^\circ\text{C}$ , 98% relative humidity for 10 d, with one turning after 6 d. After 10 d, the cheeses were cooled to  $4^\circ\text{C}$  and packed in laminated paper and poplar boxes for Camembert.

### 2.2. Biochemical and physico-chemical analyses

Cheese samples were analyzed after 1, 10, 20, 30 and 40 d (D+1, ... D+40). The pH was measured at D+1. Total solids (TS) were estimated by drying 2 g of cheese mixed with sand at  $102^\circ\text{C}$  ( $\pm 2^\circ\text{C}$ ) for 7 h [4]. Fat content was determined using

the acid butyrometric method of van Gulick [6]. Total nitrogen (TN) in cheeses was obtained from nitrogen analysis using the Kjeldahl method [5]. For all samples, the protein breakdown during ripening was measured by the evolution of soluble nitrogen at pH 4.6 (NCN, non-casein nitrogen), and 12% TCA-soluble nitrogen (NPN, non-protein nitrogen), according to the method described by Gripon et al. [9]. To quantify the evolution of proteolysis, the following ratios were calculated: NPN/TN and (NCN-NPN)/TN, expressed as a percentage of the cheese TN content. Analyses were performed in triplicate.

Corrected cheese yield ( $Y$ , kg per 100 kg milk) on a  $440 \text{ g}\cdot\text{kg}^{-1}$  TS basis was calculated as follows [16]:  $Y = (\text{TS}_{\text{milk}} - \text{TS}_{\text{whey}}) / (440 - \text{TS}_{\text{whey}}) \times 100$ . Moisture on a fat-free basis (MFFB, %) was calculated as follows:  $\text{MFFB} = (1000 - \text{TS}) / (1000 - \text{Fat content}) \times 100$ . Fat in dry matter (FDM, %) was calculated as follows:  $\text{FDM} = \text{Fat} / \text{TS} \times 100$ .

### 2.3. Size distribution measurements

The particle size distribution of calibrated milk was measured by laser light scattering (LLS) using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) with two laser sources. The refractive index of milk fat was taken to be 1.460 at 466 nm and 1.458 at 633 nm [18]. The milk sample was diluted (1:1) in EDTA (99%, Merck, Darmstadt, Germany)  $35 \text{ mmol}\cdot\text{L}^{-1}$  pH 7, before a few drops were introduced into the apparatus circulating cell containing 100 mL of distilled water with 0.05% SDS (sodium dodecyl sulfate, Merck). EDTA is used to dissociate casein micelles in order to detect the fat globules only, and the slight quantity of SDS dissociates clusters. The refractive index of the aqueous phase was 1.33. From the size distribution, the average diameter  $d_{43} = \Sigma(v_i \times d_i) / \Sigma v_i$  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. In order to estimate the fat globule

size distribution within the cheese curd, the casein matrix was dissociated with  $6 \text{ mol}\cdot\text{L}^{-1}$  urea,  $100 \text{ mmol}\cdot\text{L}^{-1}$  EDTA,  $20 \text{ mmol}\cdot\text{L}^{-1}$  imidazole buffer pH 7 and shaken gently for 30 min, prior to measurement as described above.

### 2.4. Rheological measurements on Camembert curds

Rheological properties of Camembert curd at the end of drainage were measured with a universal testing machine (Instron, model 4501) using the series IX software, equipped with a 100 N load cell and a plate of 60 mm. Sampling was performed on the drained curds at  $18 \text{ }^\circ\text{C}$  and involved the cutting of  $\approx 10$  plugs (20 mm in diameter and 20 mm height). Plugs were stored at  $20 \text{ }^\circ\text{C}$  for at least 1 h in an aluminium foil prior to compression. The crosshead speed was  $30 \text{ mm}\cdot\text{min}^{-1}$ . The rheological parameters were the fracture stress (Pa), the fracture Hencky's strain and the Young's modulus (Pa) [26]. Stress data were corrected for the increase in the plug surface during compression, assuming a constant sample volume.

### 2.5. Scanning electron microscopy (SEM)

Slices ( $1 \times 1 \times 3 \text{ mm}$ ) of SFG and LFG Camembert cheeses at day D+10 were left overnight at  $4 \text{ }^\circ\text{C}$  in  $0.1 \text{ mol}\cdot\text{L}^{-1}$  cacodylate buffer, pH 7.0, with 2.5% glutaraldehyde (Sigma, Saint-Louis, USA). They were rinsed thoroughly with osmosed water and dehydrated in successive 20 min-baths of ethanol (10, 25, 50, 75, 95) at  $4 \text{ }^\circ\text{C}$ , followed by two baths in absolute ethanol (Merck, Darmstadt, Germany). Samples were dried by the critical point technique in liquid  $\text{CO}_2$ . After fracture and glueing onto sample holders, they were stained with gold by sputtering. Observations were performed with a SEM microscope Philips XL 20 (acceleration tension: 8 or 10 kV).

## 2.6. Confocal laser scanning microscopy (CLSM)

The CLSM (LSM 410 Axiovert, Le Pecq, France) was performed in fluorescence mode [10]. Cheese pieces cut from the Camembert center were frozen at  $-150\text{ }^{\circ}\text{C}$  by plunging into isopentane maintained into liquid nitrogen. Samples were transferred in a cryotome chamber at  $-20\text{ }^{\circ}\text{C}$ . Slices ( $20\text{ }\mu\text{m}$  thick) were cut from the cheese pieces and left to dry. The protein matrix of SFG and LFG Camembert cheeses was stained by the fluorescent dye FITC (Sigma, St-Quentin-Fallavier, France) and the fat globules were stained by Nile red (Aldrich, St-Quentin-Fallavier, France) [1]. The dyes were dissolved in an ethylene glycol/water mixture and one drop was left in contact with the cheese slice for 15 min, before rinsing with distilled water. Labeled cryotomed slices were transferred to microscope slides with concave cavities, covered with a sealed cover slip. Observations of the cheeses 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 that 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. Observations were performed for each sample at zooms 2 and 4, and typical pictures were chosen.

## 2.7. Sensory analysis

Sensory analyses were performed by a specialized company (Les Maisons du Goût, Rennes, France). The sensorial profile of the Camembert cheeses (3rd manufacture) at D+25 was described in duplicate by a panel of 12 individuals. The panel was specially trained to test soft

ripened cheeses. The descriptive approach allowed the identification of organoleptic differences between the two types of Camembert cheese (SFG vs. LFG). Marking for each descriptor was performed on an intensity scale ranging from 0 to 10. Sensory descriptors were: homogeneous aspect of the curd, flowing aspect of the curd, color of the curd, odor intensity, elastic texture, firm texture, crust perception, chalky texture of the curd, sticky texture, melting texture, flavor intensity, richness of aromas, salt savor, acid savor, bitter savor, picking taste, ammoniac flavor. Samples were kept at  $15\text{ }^{\circ}\text{C}$  for 2 h before being served at  $13\text{ }^{\circ}\text{C}$ . Sensory analyses were performed with  $\frac{1}{4}$  of SFG and LFG cheeses for each consumer, in air-conditioned individual boxes, with natural light. Samples were encoded to remain anonymous to the panel, and the consumers were asked to consume them in a specified order, so that half of the panel tasted the SFG cheese first, and vice versa.

## 2.8. Statistical analysis

The StatGraphics Plus software (Manugistics, Rockville, USA) was used to perform analysis of variance. A Fisher test was followed by a least square difference procedure by size, to test the significance of result differences. Below, a significantly different result means  $P < 0.05$  and a very significantly different result means  $P < 0.01$ . Concerning sensory analysis, analysis of variance with two factors (product and consumer, Newman-Keuls test) identified significantly different characteristics between both cheeses (SFG and LFG).

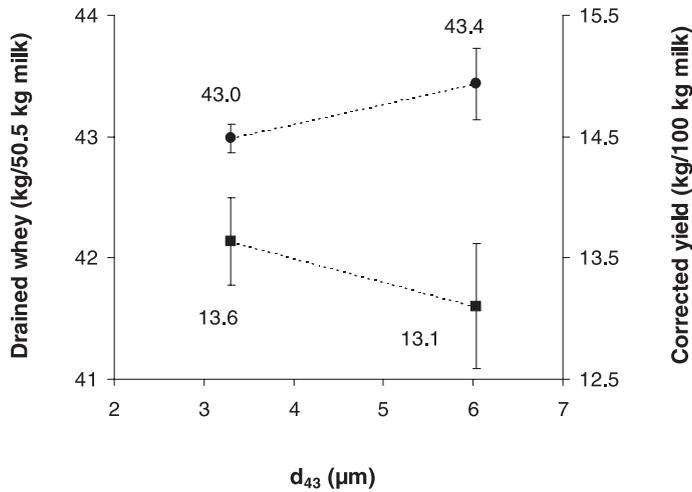
## 3. RESULTS AND DISCUSSION

The  $\zeta$ -potential of SFG was  $-13.5 \pm 0.6\text{ mV}$  and that of LFG  $-13.4 \pm 0.4\text{ mV}$ , which is the same value as that of the original globules from whole milk and shows that the fat globules remained native throughout the microfiltration process [21].

**Table I.** Physico-chemical properties and composition of the milk and fat globule used to produce Camembert, and of the whey extracted from the corresponding Camembert curd (average of the three different cheese productions).

		$d_{43}$ ( $\mu\text{m}$ )	Fat ( $\text{g}\cdot\text{kg}^{-1}$ )	TN ( $\text{g}\cdot\text{kg}^{-1}$ )	NCN ( $\text{g}\cdot\text{kg}^{-1}$ )	NPN ( $\text{g}\cdot\text{kg}^{-1}$ )	TS ( $\text{g}\cdot\text{kg}^{-1}$ )
Milk:	SFG	$3.30 \pm 0.03$	$28.6 \pm 0.6$	$33.5 \pm 0.3$	$7.7 \pm 0.1$	$1.7 \pm 0.0$	$115.9 \pm 0.8$
	LFG	$6.03 \pm 0.04$	$28.8 \pm 0.9$	$33.4 \pm 0.1$	$7.9 \pm 0.2$	$1.7 \pm 0.0$	$114.8 \pm 1.3$
Whey:	SFG		$1 \pm 0$	$9.6 \pm 0.2$			$65.3 \pm 0.5$
	LFG		$1 \pm 0$	$9.7 \pm 0.0$			$65.3 \pm 0.6$

TN: total nitrogen; NCN: non casein nitrogen; NPN: non protein nitrogen; TS: total solids (dry matter). SFG: small fat globule milk; LFG: large fat globule milk.



**Figure 1.** Weight of drained whey (●) and corrected yield accounting for a dry matter of  $440 \text{ g}\cdot\text{kg}^{-1}$  from Camembert cheeses with different diameter of native fat globules ( $d_{43}$ ). Bars represent the standard deviation.

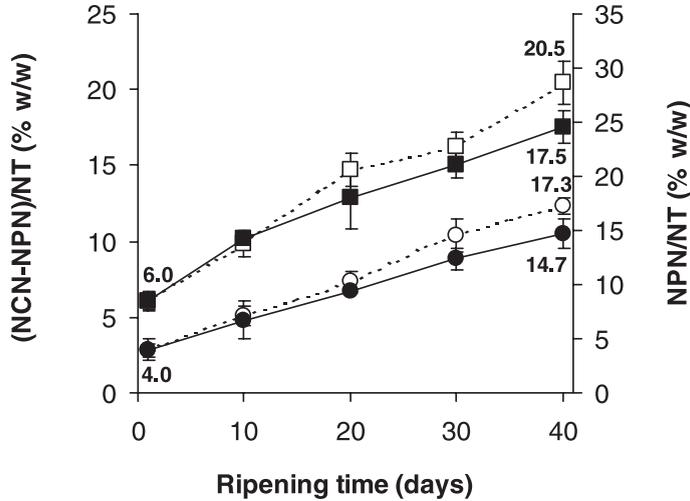
As shown in Table I, the composition of SFG and LFG milks used to prepare cheeses was not significantly different. Figure 1 shows that less whey (0.93%) was collected from the SFG fraction, with similar composition (Tab. I). This difference was significant even at the first turn. Consequently, the corrected yield was thus 3.7% higher for SFG cheeses (Fig. 1). Moreover, fat recovery was very significantly different between both types of cheeses:  $93.4 \pm 1.8\%$  for LFG vs.  $99.9 \pm 0.1\%$  for SFG. However, the fat globule size did not affect other technological parameters such as manufacture time and pH decrease. Throughout the entire

ripening period, the FDM and total nitrogen were not different according to the fat globule size of the cheeses. However, Table II shows that the MFFB was

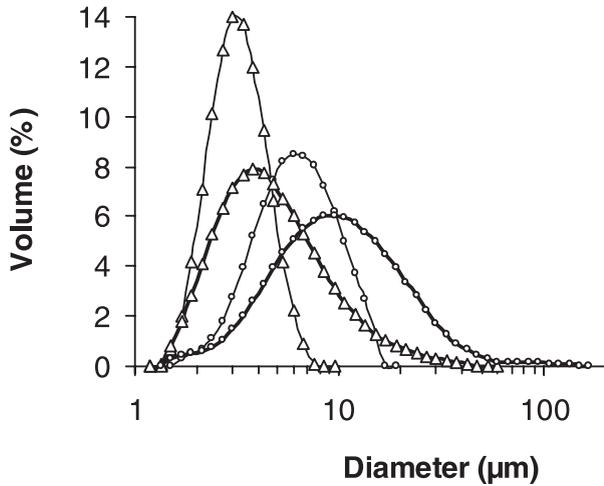
**Table II.** Composition of Camembert cheeses, with different sizes of fat globules, after 40 days of ripening.

	SFG	LFG
FDM (% w/w)	$51.1 \pm 0.6$	$51.2 \pm 0.6$
MFFB (% w/w)	$71.5 \pm 0.6$	$69.3 \pm 0.2$

FDM: fat on dry matter; MFFB: moisture on a fat-free basis. SFG: small fat globule milk; LFG: large fat globule milk.



**Figure 2.** Evolution of soluble nitrogen of cheeses during ripening, as a function of native fat globule size. Peptides and amino-acids (NPN/TN): (○) SFG, (●) LFG. Soluble proteins ([NCN-NPN]/TN): (□) SFG, (■) LFG. Bars represent the standard deviation.

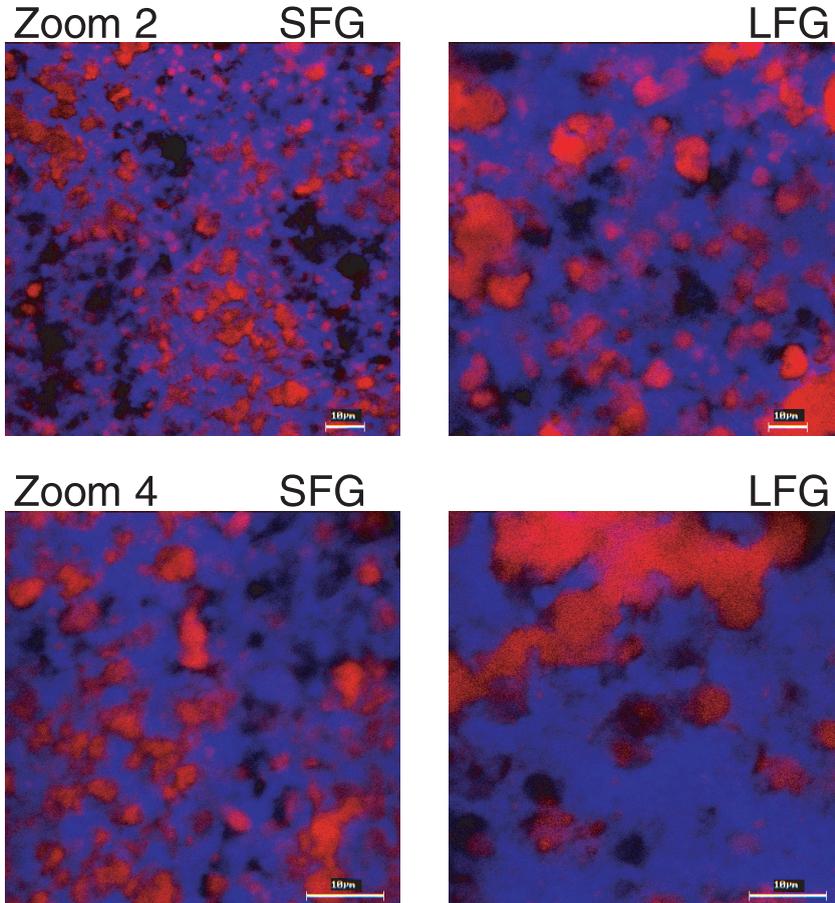


**Figure 3.** Particle size distribution of small ( $\Delta$ ) and large ( $\circ$ ) fat globules in cheese milk (thin line) and in cheese curd (thick line) the day after manufacture, after dissociation of the casein matrix by EDTA, urea and SDS (typical example).

significantly different between SFG and LFG cheeses, SFG cheeses having 2.2% more moisture (MFFB) than LFG 40 d after manufacture. As presented in Figure 2, peptide and amino acid content (NPN/TN) was significantly higher in SFG cheeses, as well as soluble proteins

([NCN-NPN]/TN), throughout the entire ripening period.

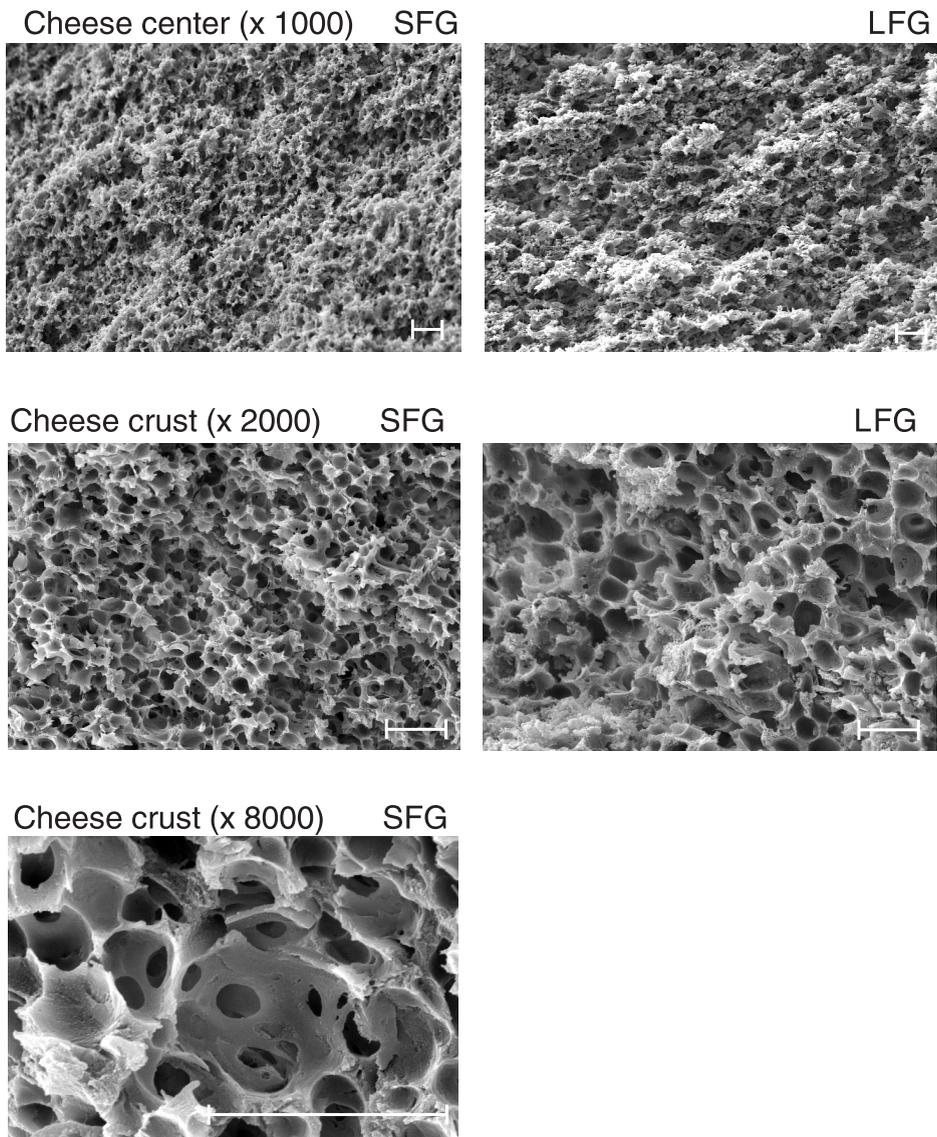
In Figure 3, we can see that larger particles than the original fat globules remained in the Camembert cheese when the casein matrix had been dissociated. As observed by optical microscopy (result not



**Figure 4.** Confocal laser scanning micrographs of small (SFG) and large (LFG) native fat globules at the center of Camembert cheeses, at two different magnifications. Fat is coded in red and proteins in blue. Scale bar represents 10  $\mu\text{m}$ .

shown), these particles corresponded to fat globule aggregates. The latter represent the way fat globules were arranged within the cheese structure. Figure 4 shows confocal micrographs of SFG and LFG cheese centres. The difference in fat globule size can be readily observed, as well as the presence of globule aggregates, especially for LFG. It is clear that milk fat globules are embedded within the casein matrix. Scanning electron micrographs, taken in the cheese center or in the crust (below

*Penicillium*), are shown in Figure 5. Voids correspond to the fat globules, only the casein matrix structure is observed. It can be seen from the highest magnification (Fig. 5 – bottom) that some fat globules may be interconnected, which is consistent with the aggregates observed by CLSM and particle size measurements. The distribution of fat globules throughout the matrix seems to be rather homogeneous. It was observed that bacteria colonies were localized around milk fat globules (results



**Figure 5.** Scanning electron micrographs of the center and the crust of Camembert cheeses with small (SFG) and large (LFG) native fat globules, at different magnifications. Scale bar represents 10  $\mu\text{m}$ .

**Table III.** Rheological characteristics of Camembert curd made with small (SFG) or large (LFG) fat globules.

	SFG	LFG
Number of repetitions	50	47
Young's modulus (Pa)	42581 <sup>a</sup> ± 8108	53639 <sup>b</sup> ± 8338
Fracture strain	0.45 <sup>a</sup> ± 0.04	0.46 <sup>a</sup> ± 0.04
Fracture stress (Pa)	16343 <sup>a</sup> ± 2491	20613 <sup>b</sup> ± 2958

Significant differences exist between values followed by different superscripts.

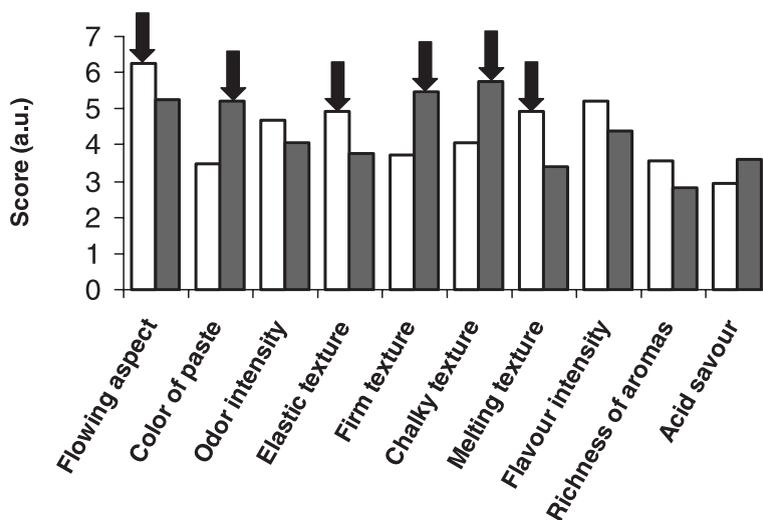
not shown); however, it is not known whether this is significant, considering the number of micrographs.

Fat is known to be important in allowing moisture to be retained in cheese [17], which is linked to the ability of the native milk fat globule membrane to bind water. This water-binding ability can explain the lower whey to milk ratio of SFG cheeses compared with LFG. Indeed, for a given fat content, SFG have a greater total surface area of MFGM:  $\sim 2.2 \text{ m}^2 \cdot \text{g}^{-1}$  for SFL vs.  $\sim 1.2 \text{ m}^2 \cdot \text{g}^{-1}$  for LFG in this study, i.e., the surface is two-fold when the diameter is halved. Consequently, the 0.4 kg difference in the drained whey ratio between both fractions (Fig. 1) corresponded to about 250 mg of water per  $\text{m}^2$  of MFGM. Moreover, considering Figure 3 and Figure 4, it seems that SFG formed less aggregates than LFG, which would also contribute to their greater water-binding ability. Finally, the cavities formed by the fat globules in the casein matrix are smaller for SFG (Fig. 5), and these smaller pores are more likely to retain the serum than large pores. The better fat recovery for SFG suggests that small globules are better entrapped in the casein matrix, even though they do not interact positively with caseins. These results are consistent with the higher MFFB observed for SFG. This increase in available water is also likely to explain the greater proteolysis of SFG compared with LFG, as enzymatic activities are enhanced.

The rheological properties of the curd are presented Table III. The compression

curves of the Camembert cheese showed quite a linear part until a Hencky's strain of 0.5, followed by a marked fracture. The rigidity (Young's modulus) and the firmness (fracture stress) of SFG cheese curd at D+1 were significantly lower than those of LFG cheese. No difference was observed regarding flexibility (fracture strain) the day after manufacture. The lower rigidity and firmness of SFG curds can be related to the finer dispersion of small fat globules within the matrix, corresponding to more numerous weak points in the casein network. Roughly, the SFG average diameter being about half the LFG diameter, this would correspond to eight times more globules in the SFG curd. The interglobular distance (or mean free distance between fat globules) can be estimated from the fat globule diameter and fat volume fraction according to Walstra [27]:  $\sim 1.8 \text{ }\mu\text{m}$  for SFG cheese vs.  $\sim 3.3 \text{ }\mu\text{m}$  for LFG cheese. Thinner casein strands are thus formed between the globule pores in SFG (Figs. 4 and 5), resulting in a more fragile structure.

Sensorial profiles of SFG and LFG cheeses are presented Figure 6. Significant differences concerned the greater flowing aspect of SFG, together with its more elastic and melting texture, and the yellower color of LFG, together with its firmer and chalkier texture. Overall, the SFG cheeses can be defined as smoother, which is consistent with their higher MFFB (Tab. II), the more advanced proteolysis (Fig. 2) and rheological properties of curds (lower rigidity and firmness, Tab. III). The cheese meltability is known to be due to the



**Figure 6.** Sensorial profile of Camembert cheeses with small (□) and large (■) native milk fat globules. Arrows show significantly different characteristics ( $P < 0.05$ , Newman-Keuls test).

melting of milk fat, followed by collapsing of the cheese matrix due to reduced support by the fat [14]. This collapse can occur faster in SFG cheeses, because the inter-globular distance is smaller and thus casein strands are thinner. The overall smoothness can also be due to the in-mouth perception of milk fat globules. The SFG surface area being larger than that of LFG, it provides a greater fat interface in contact with the mouth. Moreover, SFG are closer to each other, which we suppose may lead to a better fat perception. The firmer texture of LFG cheeses is also consistent with rheological measurements on curds (fracture stress, Tab. III). Their chalkier texture can be related to their lower MFFB and to their thicker (almost twice) inter-globule casein strands. The yellower color of LFG can be related to the light diffraction phenomenon, that is lower in LFG and results in a more intense color perception. Compositional differences between globules that would lead to color differences would have to be investigated.

Other sensory parameters of importance, even though not significantly

different, were the richer and more intense flavor of SFG, and the higher acidity of LFG. This difference in flavor is consistent with the higher proteolysis of SFG, especially concerning the presence of free peptides and amino acids (NPN/TN, Fig. 2). Moreover, for a given fat content, SFG are more numerous than LFG, which results in a larger surface area of MFGM. This interface being the specific site for lipolytic activity, flavor-producing reactions are thus likely to be enhanced in the SFG cheese. Moreover, the MFGM contains more than 25 enzymes, half of which being members of the hydrolase class (including lipases), that are involved in the development of cheese flavor [13]. Consequently, the richer and more intense flavor of SFG cheese may be due to its greater MFGM content, too. This larger MFGM surface area also provides a greater contact surface of fat in the mouth, that is likely to enhance aroma perception. Wijesundera [30] indicates that the sphericity and composition of model fat globules affect Cheddar cheese flavor. However, the process for this type of cheese induces fat globule distortion and

coalescence, which cannot be compared with Camembert cheese. Saint-Gelais et al. [23] found that low-fat Cheddar cheeses with native globules of 2.4  $\mu\text{m}$  diameter had improved sensory characteristics compared with globules of 1.6  $\mu\text{m}$  diameter. However, we can hardly compare these results with ours since both the process and the fat globule diameter are different.

We should discuss that part of the results can be due to physico-chemical and biochemical differences between milk fat globules according to their size. Indeed, when artificial recombined fat globules are created by emulsifying milk fat in skim milk, whatever their size, they have the same composition and their membrane is composed of caseins. Conversely, native milk fat globules are likely to differ according to their size, even though this has still to be fully elucidated [28]. It has been found, using recombined fat globules of various sizes with milk fat or model fat, that a deeper supercooling is needed to crystallize smaller fat globules, that also have a lower crystallization rate [15, 29]. We can suppose that these results would also hold for native milk fat globule of various sizes, since they are explained by the lesser number of catalytic impurities per globule for smaller ones [29]. Moreover, the composition of the interface, using various proteins, has been found to affect the crystallization properties of model fat globules [15]. Therefore, the solid fat content in cheese fat globules at a given temperature is likely to be lower for SFG. This could contribute to the greater flowing aspect and melting texture of SFG cheeses [11, 12]. Finally, possible compositional differences according to fat globule size [24] could contribute to the differences in cheese flavor, as different aroma precursors would exist in the two fractions.

#### 4. CONCLUSION

The size of native milk fat globules was found to affect the physico-chemical and

sensory properties of Camembert cheese. The use of smaller milk fat globules resulted in cheese with more moisture and a more melting texture, which suggests the possibility of developing products with new technological and sensory properties. These results can be explained by the larger surface area of the milk fat globule membrane and thinner casein strands of small fat globule cheese. Experiments are being carried out in our laboratory to further characterize the possible differences of composition, crystallization and fusion properties of native milk fat globules according to their size. This current work would provide a better knowledge concerning the role of these fat globules in cheese. Moreover, the influence of the size of native milk fat globules on the properties of other dairy products should be investigated.

#### ACKNOWLEDGEMENTS

J. Berrier and C. Garnier are acknowledged for performing SEM and CLSM observations, respectively. We thank E. Le Put, M.N. Madec and H. Goudéranche for technical assistance.

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