

# Microstructure of Fat Globules in Whole Milk after Thermosonication Treatment

D. BERMÚDEZ-AGUIRRE, R. MAWSON, AND G.V. BARBOSA-CÁNOVAS

**ABSTRACT:** The structure of fat globules in whole milk was studied after heat and thermosonication treatments to observe what happens during these processes at the microscopic level using scanning electron microscopy. Raw whole milk was thermosonicated in an ultrasonic processor—Hielscher® UP400S (400 W, 24 kHz, 120  $\mu\text{m}$  amplitude), using a 22-mm probe at 63 °C for 30 min. Heat treatment involved heating the milk at 63 °C for 30 min. Color and fat content were measured to correlate the images with analytical measurements. The results showed that the surface of the fat globule was completely roughened after thermosonication. Ultrasound waves were responsible for disintegrating the milk fat globule membrane (MFGM) by releasing the triacylglycerols. Furthermore, the overall structure of milk after sonication showed smaller fat globules (smaller than 1  $\mu\text{m}$ ) and a granular surface. This was due to the interaction between the disrupted MFGM and some casein micelles. Minor changes in the aspect of the globules between thermal and raw milks were detected. Color measurements showed higher  $L^*$  values for sonicated samples. Sonicated milk was whiter ( $92.37 \pm 0.20$ ) and generally showed a better degree of luminosity and homogenization compared to thermal treated milk ( $88.25 \pm 0.67$ ) and raw milk ( $87.82 \pm 0.18$ ). Fat content analysis yielded a higher value after sonication (4.24%) compared to untreated raw milk (4.04%) because fat extraction is more efficient after sonication. The advantages of thermosonicated milk are that it can be pasteurized and homogenized in just 1 step, it can be produced with important cost savings, and it has better characteristics, making thermosonication a potential processing method for milk and most other dairy products.

**Keywords:** fat globule, MFGM, microstructure, milk, scanning electron microscopy, ultrasound

## Introduction

Milk fat globule membrane (MFGM) is related to the membrane and the material associated with it that surrounds the milk fat globules. The core of these micro droplets contains triacylglycerols. The surface coating material contains cholesterol and phospholipids such as sphingomyelin and phosphoglycerides of choline, ethanolamine, inositol, and serine (Keenan and Patton 1995), along with lipoproteins, glycoproteins, and enzymes (Michalski and others 2002a) such as xanthine oxidase (Wiking and others 2003), butyrophillin, and  $\gamma$ -glutamyl transpeptidase, among others (Wiking and others 2006). The MFGM and lipid droplets represent 80% of the cholesterol supplied by milk (Mather and Keenan 1998). The triacylglycerol content of the fat globule's core is mainly composed of lauric, myristic, oleic, stearic, and linoleic acids (Michalski and others 2005). In cow's milk, the size of a fat globule is between 0.2 to 10  $\mu\text{m}$  in diameter. Almost 90% of the fat content in milk corresponds to fat globules that are between 1 and 8  $\mu\text{m}$  (Keenan and Patton 1995), with an average diameter of 4  $\mu\text{m}$  (Briard and others 2003; Michalski and others 2005). However, small and large fat globules differ a little in composition (Michalski and others 2004); smaller globules (1.5  $\mu\text{m}$ ) have fewer short chain fatty acids, less stearic acid, and more oleic acid (Briard and others 2003). In fat globules smaller than 0.8  $\mu\text{m}$ , the creaming effect of milk during storage is very slow (Villamiel and de Jong 2000). The experimental results show that during homog-

enization of milk the mechanical alteration of fat globules is generated by pressure differences due to shear, turbulence, and cavitation; this last phenomenon is sometimes present in the valves (Walstra 1969; Thiebaud and others 2003). Some operations in milk processing include pumping, agitation, pooling, cooling, clarification, pasteurization, and homogenization (Jensen and others 1995). Stabilization of fat, creamy texture, and resistance of milk to oxidized flavor are some of the advantages of the homogenization process, which consists of pumping the milk at high pressures through small eddies at pasteurization temperatures (Keenan and Patton 1995). This operation requires a high volume of milk for processing (Carcel and others 1998) and uses pressures from 20 to 50 MPa to reduce the size of fat globules from 0.3 to 0.8  $\mu\text{m}$  (Thiebaud and others 2003), thus changing the structure of the globules (Corredig and Dalgleish 1996) and increasing the surface area of fat more than 10-fold (Sharma and Dalgleish 1993). There are 3 constituent parts in homogenized milk, regular milk fat globules, tiny and native fat globules (100 nm), and small and new lipid-protein complexes (<500 nm) (Michalski and others 2002b; Michalski and Januel 2006). The size of fat globules and the composition of the membrane are of tremendous importance in the technical and sensory properties of dairy products (Michalski and others 2002a, 2004; Fauquant and others 2005). Novel nonthermal technologies such as high pressure, pulsed electric fields, or ultrasound are of great interest in the research and development of food processing, not only because of the advantages they present compared with thermal processing (less thermal damage and higher overall characteristics), but also because of the potential generation of new ingredients and products with specific features due to action in the food matrix (Knorr and others 2002). Ultrasound is responsible for the breakage of fat globules in milk, which releases liposoluble components such as phenilureas; sometimes sonication is used

MS 20070492 Submitted 6/27/2007, Accepted 6/15/2008. Authors Bermúdez-Aguirre and Barbosa-Cánovas are with Center for Nonthermal Processing of Food, Washington State Univ., Pullman, WA 99164, U.S.A. Author Mawson is with CSIRO Food Science Australia, 671 Sneydes Rd., Werribee, Victoria 3030, Australia. Direct inquiries to author Barbosa-Cánovas (E-mail: barbosa@mail.wsu.edu).

with the objective of extraction in the dairy industry (Carcel and others 1998). The main mechanism of action in ultrasound technology is called cavitation, a phenomenon that can be either stable or transient. Stable cavitation is associated with the small bubbles dissolved in a liquid, while transient cavitation occurs when the bubble size changes quickly and collapses, and as a result produces very high pressure (100 MPa) and high temperature (5000 K) (Earnshaw and others 1995). One of the current applications of ultrasound is human milk homogenization in hospitals; some nutrients such as liposoluble vitamins, proteins, and minerals are usually bound to fat globules; if milk is not homogenized these nutrients are lost when the fluid circulates through the feeding system designed for newborn infants (Carcel and others 1998). The nanometer resolution of scanning electron microscopy (SEM) enables detailed analysis of casein micelles, fat globule membranes, bacteriophages, very small particles, and the changes produced during processing (Kaláb 1981, 1993).

The objective of this research was to study the changes in microstructure of the fat globules after heat and thermosonication treatments by using SEM to understand these changes at the microscopic level. Also, change in color was studied and quantification of fat content performed to correlate these parameters with microscopy images.

## Materials and Methods

### Milk samples

Raw and whole cow's milk was obtained from the Washington State Univ. Creamery. Milking process was performed previously using automatic milkers for different cows. Milk was kept in refrigeration at 4 °C until used. The raw milk (15 mL) was then transferred to a disposable 15 mL PET conical and sterile test tube (Corning®, Scientific Commodities Inc., Lake Havasu City, AZ, U.S.A.) and used as a control sample during microscopy sample preparation.

### Thermal and thermosonication treatments

**Ultrasound equipment.** An ultrasonic processor—Hielscher® USA Inc. (Ringwood, N.J., U.S.A.), model UP400S (400 W, 24 kHz, 120  $\mu$ m amplitude), was used with a 22-mm-dia probe. Raw milk (500 mL) was placed in the treatment chamber, a double-walled vessel (500 mL) with an internal diameter of 8 cm and a depth of 13.5 cm. A magnetic stirrer was used inside the treatment chamber to assure the homogeneity of the treatment. Ultrasonication was carried out at 100% (120  $\mu$ m amplitude) and 63  $\pm$  0.5 °C temperature. Temperature was kept constant with a refrigerated bath (VWR Scientific Model 1166, Niles, Ill., U.S.A.). A thermocouple was used in the treatment chamber to monitor the temperature (63  $\pm$  0.5 °C) throughout the experiments. Treatment times were 10 and 30 min. Samples were collected and transferred to disposable 15 mL PET conical and sterile test tubes and kept at 4 °C.

**Heat treatment.** Heat treatment was carried out in the same 500 mL double-walled vessel. The same volume of milk was used, and temperature of the medium was kept at 63  $\pm$  0.5 °C by using a heating bath maintained at 65 °C. Temperature was monitored with a thermocouple. After 30 min of treatment, samples were transferred to disposable 15 mL PET conical, sterile test tubes.

### Scanning electron microscopy

**Sample preparation.** Disposable 15 mL PET conical, sterile test tubes containing raw milk samples (thermosonicated and heated) were centrifuged at 1500 rpm for 5 min at 10 °C. The supernatant fat layer of the sample was transferred to disposable 1.5 mL sterile plastic microcentrifuge tubes (Fisherbrand®). A 0.5 mL solution

of glutaraldehyde (2%) paraformaldehyde (2%) in 0.1 M phosphate buffer (pH 7.2) was added to each microtube; the fixation process was allowed to proceed for 24 h at 4 °C. After fixation, the fat was washed for 10 min with phosphate buffer (0.1 M), followed by 2 consecutive 10 min washes with cacodylate buffer (0.1 M). The post-fixing procedure consisted of adding 2% osmium tetroxide in cacodylate buffer (0.1 M) at 4 °C for 24 h. Samples were then washed 3 times with cacodylate buffer (0.1M) for 10 min each time.

Dehydration of samples was achieved with serial dilution solutions of ethanol (30%, 50%, 60%, 70%, 95%, and 100%). Each solution was maintained in contact with the sample for 10 min; the last solution (100% ethanol) was repeated 3 times.

After dehydrating the samples with ethanol, a 2nd dehydration procedure with hexamethyldisilazane (HMDS) was carried out on the samples. Consecutive contact (15 min each time) was used between the samples and ethanol/acetone/HMDS solutions at different ratios (1:0:0, 1:1:0, 0:1:0, 0:1:1, 0:0:1, 0:0:1). Air drying was used as a final step, leaving the microcentrifuge tubes with an open lid for at least 24 h. Samples were mounted on aluminum stubs, and gold plating was used as a final step to view the samples with a Hitachi S-570 (Tokyo, Japan) scanning electron microscope (SEM) operating at 20 kV.

**Color.** Lightness to darkness ( $L^*$ ) (100 to 0), redness(+)-greenness(-) ( $a^*$ ), and yellowness(+)-blueness(-) ( $b^*$ ) color parameters were determined using a Minolta CM-2002 spectrophotometer (Minolta Camera Co., Osaka, Japan) in reflection mode. Samples (20 mL) of raw and thermo-ultrasonicated milk were poured into sterile plastic bags. A white ceramic plate was used for standardizing the instrument ( $L^* = 93.4$ ,  $a^* = -0.67$ ,  $b^* = 0.78$ ). The net color difference ( $\Delta E^*$ ) was calculated as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

**Fat content.** Fat content was determined using a LactiCheck(tm) milk analyzer (Page & Pedersen Intl. Ltd., Hopkinton, Mass., U.S.A.). The performance of this equipment was based on high frequency ultrasound. The milk analyzer was validated to determine butter fat content (Gerber) according to the Assn. of Analytical Chemists (AOAC 1986) methodology. The equipment was previously calibrated with raw cow's milk, and samples were adjusted to room temperature (20 °C). Each sample was analyzed in triplicate. No previous preparation was required for heat and thermo-ultrasonicated samples.

### Statistical analysis

All treatments were performed at least in duplicate, and the physicochemical and composition characteristics were evaluated in triplicate for each sample. Statistical analysis of the data was performed using a Microsoft Excel program. Analysis of variance (ANOVA) was calculated with the SAS program (SAS Inst. Inc., Cary, N.C., U.S.A. 1999) and a confidence level  $\alpha$  of 0.05 was used to evaluate significant differences.

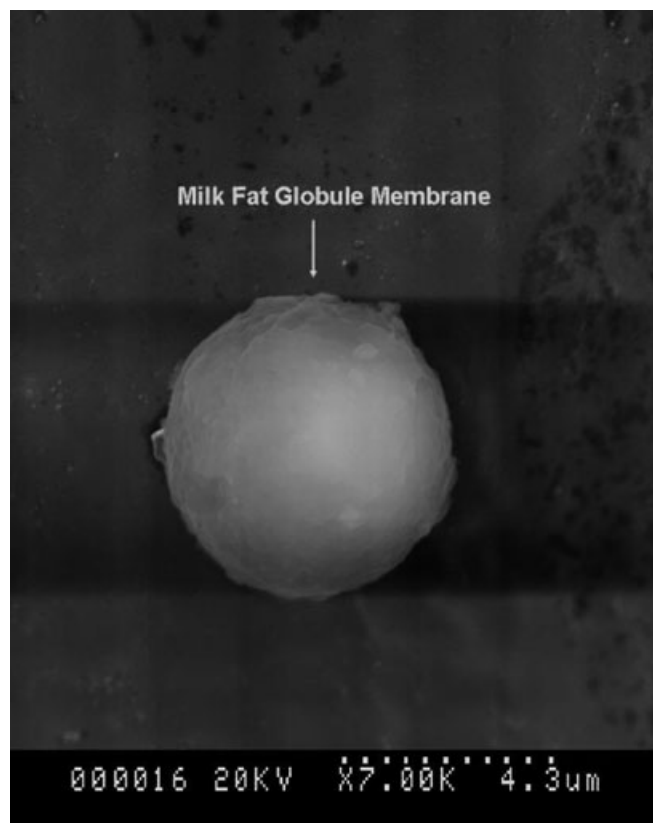
## Results and Discussion

### Microstructure of milk: fat globules

Fat globules are delimited with a membrane, derived from the apical plasma membrane (Corredig and Dalgleish 1998). In Figure 1, the structural appearance of a fat globule in raw and whole cow's milk is shown. This structure represents the control sample and shows the native form of the globule structure. Despite the fact that the MFGM shows no important changes or disruption, little

damage can be present in the globule because of the milk handling. Changes in composition or structure of the MFGM occur as a result of milk handling and treatment during and after milking; these changes could be because of the induced motion of fat globules, changes in temperature, microbial growth, or biochemical reactions (Evers 2004). The scale of the image in Figure 1 is  $4.3 \mu\text{m}$ ; the fat globule has a diameter slightly bigger than the average size generally recorded in the literature, although it is inside the range reported by Keenan and Patton (1995) for most of the fat globules present in milk. According to Wiking and others (2004), large fat globules are more predisposed to coalescence and lipolysis during some milking operations, such as pumping, so the size of the globules is of high importance in the stability of milk. In cheese-making, smaller fat globules are preferred because this physical characteristic can reduce the rennet coagulation time as well as the curd firming rate (Tosh and Dalglish 1998).

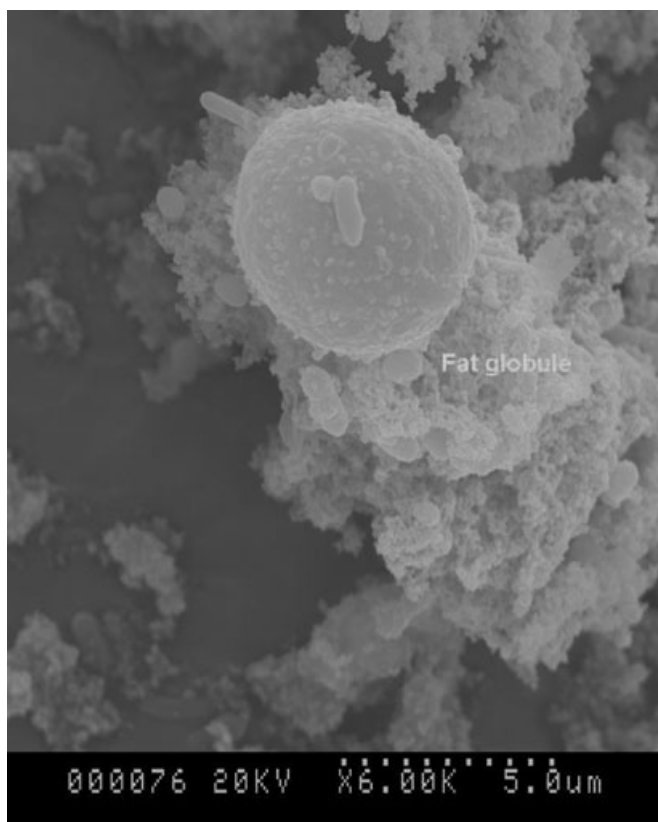
Figure 2 shows the microstructure of another fat globule after heat treatment at  $63^\circ\text{C}$  for 30 min. The general appearance of the fat globule shows a surface with no major changes compared to Figure 1. According to Corredig and Dalglish (1998), there are no important changes in the size of the fat globules taking place during heating. However, a more resistant membrane is developed outside the fat globules, which is probably due to the interaction with proteins ( $\beta$ -lactoglobulin and MFGM proteins) generating a polymerized new surface that is more resistant to coalescence; these complexes are formed by disulphide bridges. The appearance of heated fat globules is characterized by fairly thick deposits. These deposits could be protein in the membrane (Corredig and Dalglish 1996), which coincides with the appearance of the globule shown in Figure 2.



**Figure 1** – Fat globule in raw whole milk used as a control sample. The globule shows its integrity; no important changes on the surface or membrane are detected. Magnification  $7000\times$ .

In Figure 3, some examples of fat globules after thermosonication are shown; treatment time was 30 min. A visible orifice on the surface of each globule is shown, allowing observation of the disruption and cracking of the fat globule membrane, which is composed of cholesterol, phospholipids, proteins, and enzymes. Despite the damage shown in these fat globules, they can be considered as not totally disrupted by cavitation. Heertje and others (1987) showed the structure of fat globules in butter after a de-oiling process as being hollow spheres, which is similar to the globule shown in Figure 3 (bottom right). However, MFGM protects the globule's fat content from lipolysis and oxidation; when the membrane is damaged and disrupted due to physical stress, coalescence of the milk fat globules occurs and the presence of casein is observed in the new interface (Wiking and others 2003). When a fat globule has lost part of its membrane, a granular material can be seen covering the surface.

During conventional homogenization, there is turbulence in the medium, and cavitation forces are generated by the high pressures. These forces disrupt the fat globules, disintegrating them and reducing their size to less than  $1 \mu\text{m}$  (Keenan and Patton 1995). In Figure 4, a graphic representation of the changes occurring in the fat globule and milk after homogenization is presented. First, it can be seen that after the disruption of the fat globule membrane, a number of casein micelles are added to the new surface as well as some fragments of this protein. Some whey proteins can be present as well in forming the new structure of the homogenized fat globules. In the same figure (see bottom) a possible depiction of how the new microstructure of milk would look can be seen; showing: the lipoprotein complexes ( $< 500 \text{ nm}$ ) with membranes composed mainly of caseins; the tiny native fat globules ( $100 \text{ nm}$ ), which are not affected during homogenization because of their size; the homogenized fat globules (disrupted and covered



**Figure 2** – Microstructure of thermally treated fat globule after 30 min at  $63^\circ\text{C}$  in whole milk. Magnification  $6000\times$ .

by casein micelles); and some fragments of MFGM (Michalski and others 2002b; Michalski and Januel 2006).

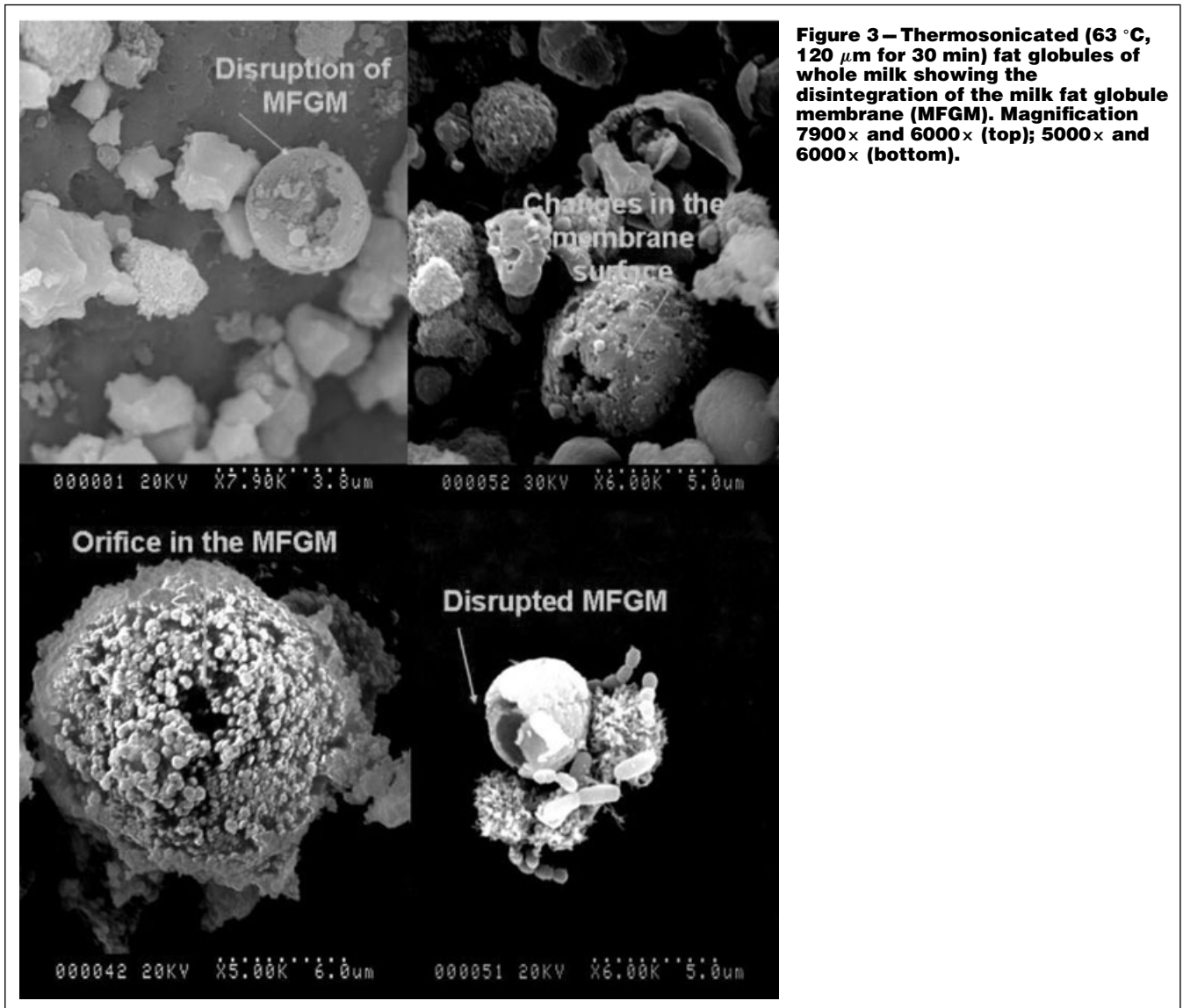
In Figure 5, two examples of fat globules after thermosonication (basically a homogenization process) can be seen. Both globules were thermosonicated for 30 min; the effect of high pressurization generated through cavitation totally disintegrated the MFGM, resulting in a granular interface. This effect is a result of the increase in pressure caused by the violent collapse of bubbles (Villamiel and de Jong 2000). In Figure 5 (left top), some free casein micelles are also shown; according to Dalgleish (1998) these are colloidal particles and are considered to be solid spheres with a protein coating. SEM images of untreated, heated, and homogenized milk with ultra-high pressure (Dalgleish and others 2004; Sandra and Dalgleish 2005; Martin and others 2006) show similarity with the casein micelles observed in Figure 5. In the same figure (right side), the fat globule can be seen after thermosonication, showing a more granular surface compared to the previous one. This could be due to the adhesion of casein micelles present in the environment after sonication; but also a stronger effect of cavitation could be the reduction in size of the fat globule, giving off smaller structures composed mainly of triacylglycerols micro-droplets. When a fat globule

is disrupted, the new area is covered with the hydrophobic part of casein particles, generating a new membrane for the fat globule with a different composition (Meyer and others 2006) and binding mainly casein micelles with fat globules (Tosh and Dalgleish 1998). Part of the original MFGM remains on the globule but it is insufficient to cover the new surface. For that reason casein semi-intact micelles and micellar fragments completely wrap the new surface and avoid the coalescence of fat globules (Sharma and Dalgleish 1993), although the presence of some whey proteins is also possible in minor proportion (Michalski and others 2002b).

The effect on size reduction of fat globules can be observed in Figure 6. Two main fat globules can be seen at the top of the image. Their diameters are approximately 2  $\mu\text{m}$  each, showing a reduction in dimension from the native fat globule (4.3  $\mu\text{m}$ ). Changes in MFGM are generated by disruption of the fat globules (Ye and others 2004). Further, in Figure 6 there are thousands of casein micelles with sizes below 0.5  $\mu\text{m}$ .

Ultrasound homogenization showed that the fat globule's size can be reduced below 1  $\mu\text{m}$  when the treatment is carried out at 60 °C. At the same time, the globules have more binding sites on the membrane, favoring the amalgamation of casein and serum

E: Food engineering & physical properties



**Figure 3 – Thermosonicated (63 °C, 120  $\mu\text{m}$  for 30 min) fat globules of whole milk showing the disintegration of the milk fat globule membrane (MFGM). Magnification 7900 $\times$  and 6000 $\times$  (top); 5000 $\times$  and 6000 $\times$  (bottom).**

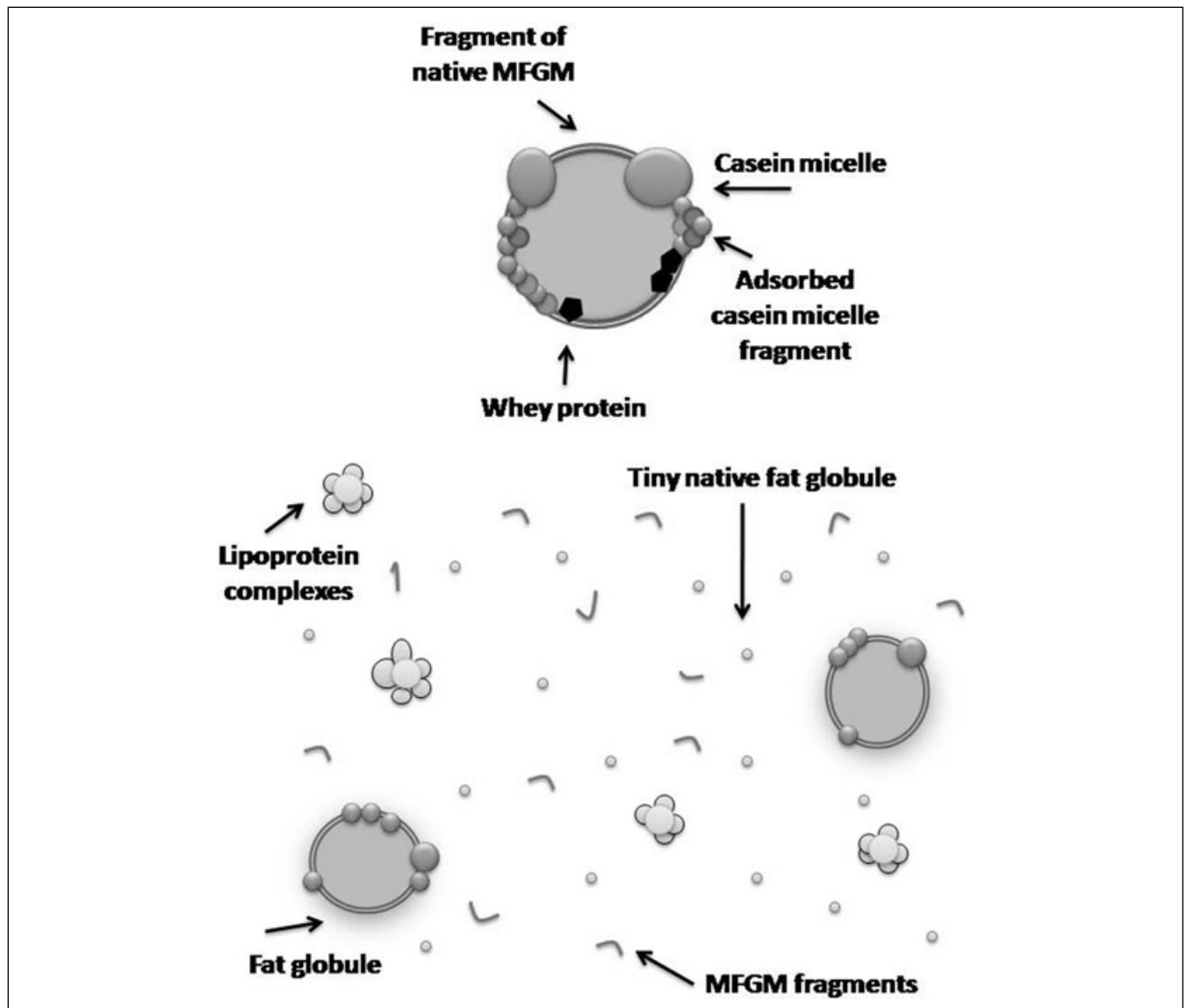


Figure 4 – Graphic representation of the changes in the MFGM in a fat globule after homogenization (top) and general microstructure of milk after homogenization (bottom) (adapted from Michalski and Januel 2006).

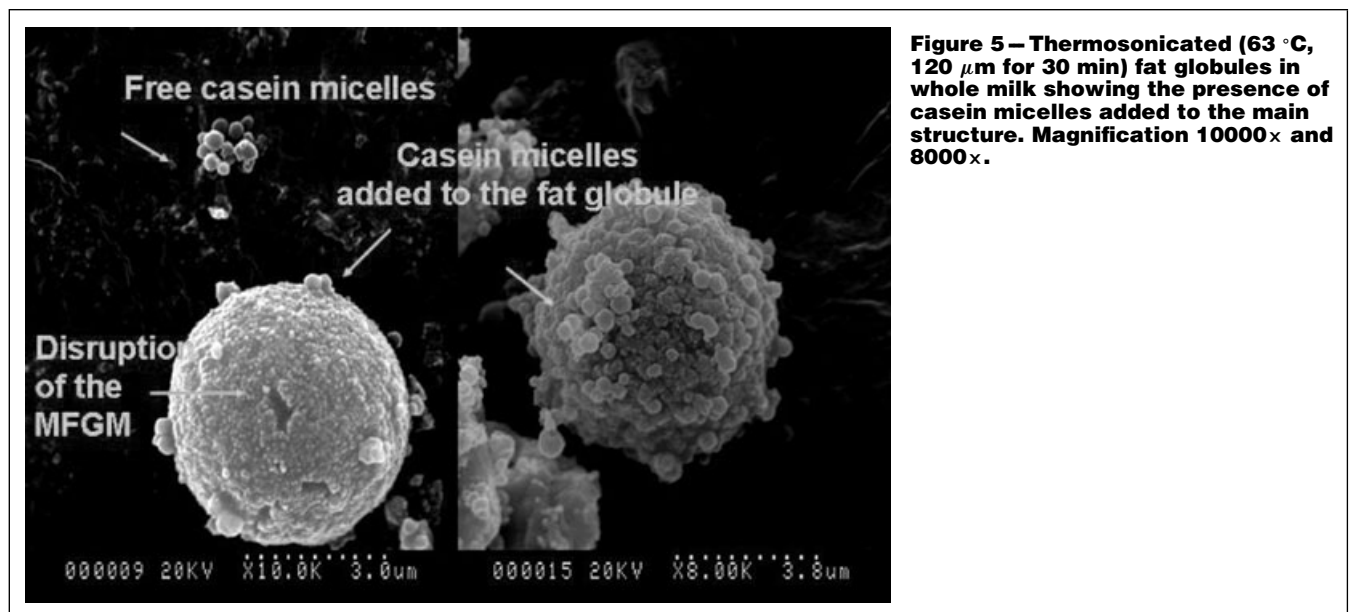


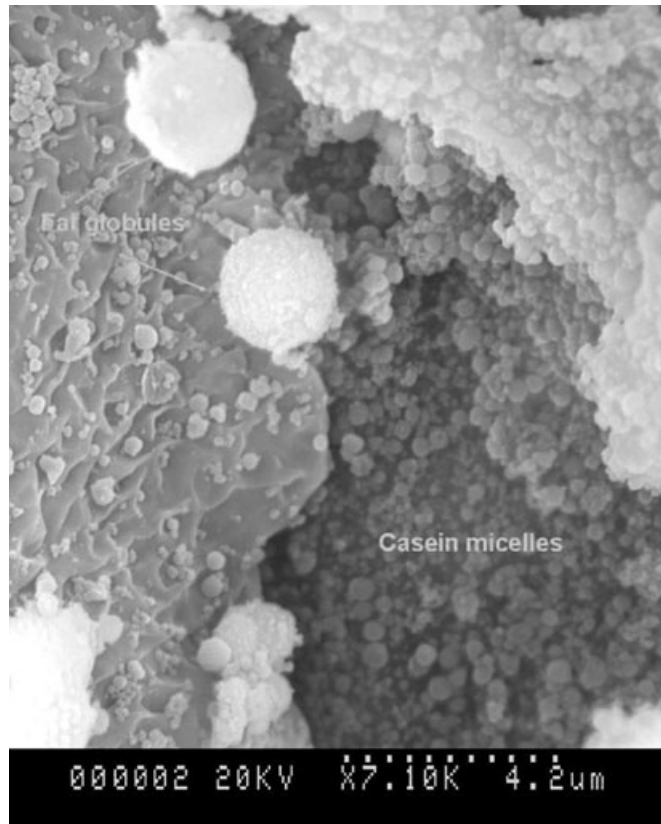
Figure 5 – Thermosonicated (63 °C, 120 μm for 30 min) fat globules in whole milk showing the presence of casein micelles added to the main structure. Magnification 1000× and 800×.

proteins, and thus producing an ideal ingredient for cheese-making (Villamiel and others 1999) because of the enhancement of the large surface area (fat globules-casein network) (Michalski and oth-

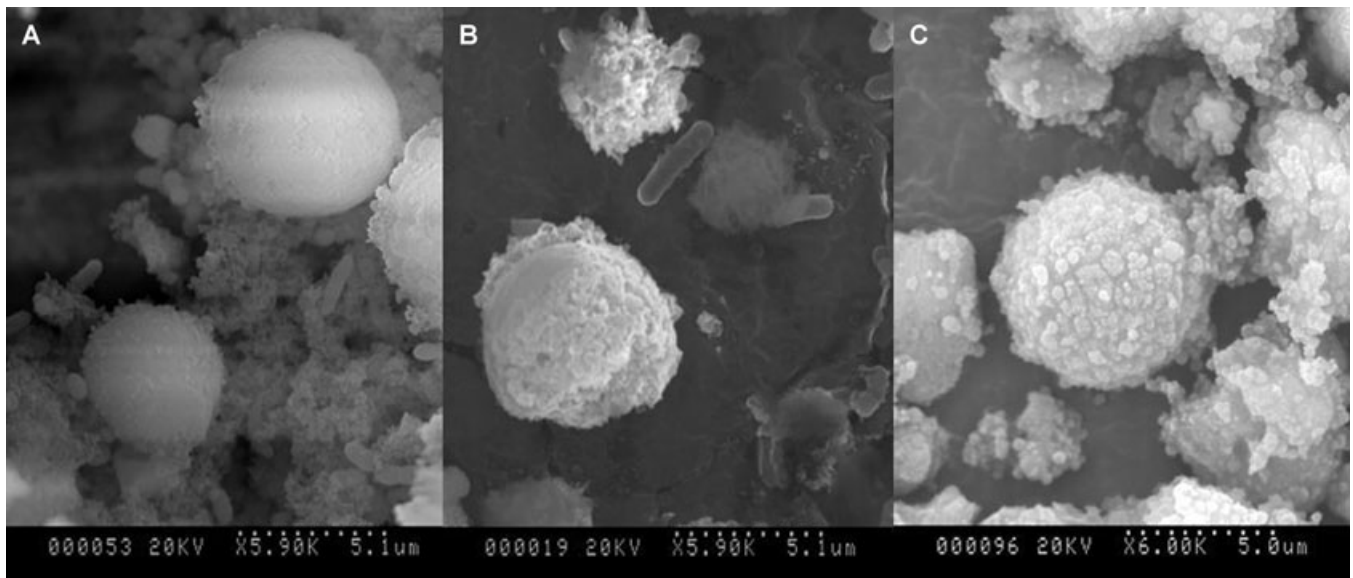
ers 2002b). Heating the milk at the same time as sonication reduces viscosity, enhancing the action of ultrasound in the size reduction of globules. With temperatures between 55 and 75.5 °C plus continuous flow sonication, a reduction of up to 81.5% in fat globule size was achieved by Villamiel and de Jong (2000).

The localized transient pressure that could be achieved with ultrasound (up to 100 MPa) is substantially higher than the conventional lower duration pressures used in homogenization (20 to 50 MPa). Thiebaud and others (2003) found that pressures above 200 MPa demonstrated certain benefits: in the stability of milk, size of fat droplets, microbial and enzymatic inactivation, and modification of rheological properties in some emulsions. However, pressures up to or above 300 MPa were responsible for protein denaturation and poor emulsification characteristics. Emulsification at 150 MPa generated oil-in-water emulsions with a droplet size below 1 μm (Thiebaud and others 2003). The beneficial effects observed in milk after sonication could be a potential tool in future research, development, and improvement of dairy products; thermosonicated milk could also be a viable option for milk beverages. With sonication, the volume of fat globules is reduced and a larger contact surface for lipolytic enzymes is generated, making the digestion process easier (Paci 1953) for consumers, as pointed out by Michalski and Januel (2006) in comparing homogenized milk with untreated milk.

Finally, in Figure 7, a comparison between heat-treated and thermosonicated fat globules is shown. Figure 7A shows the appearance of MFGM, followed by initial disruption of the surface of the fat globule after 10 min of thermosonation (Figure 7B); the final microstructure of the fat globule, subjected to 30 min of ultrasound technology, shows a rough surface and loss of MFGM with subsequent adhesion of casein micelles (Figure 7C). In this comparison, the effects of ultrasound clearly allow the possibility of research and development of new products based on this technology. Power ultrasound has the ability to disrupt cells and membranes, and to promote chemical reactions. Depending on the intensity of treatment, the effects may or may not be more noticeable. Cavitation is the main mechanism behind globule disruption in an ultrasound homogenizer (Walstra 1969). In Figure 7B, a fat globule of milk



**Figure 6—Microstructure of fat content in thermosonicated milk (63 °C, 120 μm for 30 min), showing size reduction in fat globules and the presence of thousands of casein micelles. Magnification 7100x.**



**Figure 7—A comparison of the microstructure of fat globules after 3 different treatments using scanning electron microscopy (SEM): (A) thermally treated (63 °C) fat globule structure, (B) thermosonicated (63 °C, 120 μm) fat globule structure after 10 min of treatment, and (C) thermosonicated (63 °C, 120 μm) fat globule after 30 min of treatment in whole milk. Magnification (A) 5900x, (B) 5900x, (C) 6000x.**

E: Food engineering & physical properties

**Table 1—Color parameters, net change in color, and fat content of raw whole milk, heat treated milk, and thermosonicated milk.**

Sample	$L^*$	$a^*$	$b^*$	$\Delta E$	Fat content <sup>a</sup> (%)
Raw milk	87.82 ± 0.18	-1.70 ± 0.13	5.91 ± 0.25		4.04 ± 0.05
Heat treatment	88.25 <sup>b</sup> ± 0.67	-1.97 <sup>b</sup> ± 0.07	5.61 ± 0.06	0.87 ± 0.38	4.22 <sup>b</sup> ± 0.02
Thermosonication	92.37 <sup>b</sup> ± 0.20	-1.55 <sup>b</sup> ± 0.02	5.64 ± 0.09	4.56 ± 0.03	4.24 <sup>b</sup> ± 0.02

<sup>a</sup>Accuracy ± 0.1%.

<sup>b</sup>Significantly different results ( $P < 0.05$ ) compared to raw milk samples. All experiments were performed at least by triplicate.

after 10 min of thermosonication is shown. The MFGM shows a high degree of disruption; the smooth surfaces shown in the native and heated globules have almost disappeared, generating a new structure with a rough surface, and releasing the internal triacylglycerol content of the fat globules. Cavitation is not only responsible for disruption of fat globules with subsequent adhesion of casein micelles; other small particles can be observed after ultrasonication because of the mechanical stress and strong forces involved (Michalski and others 2002b).

The strong results of power ultrasound in microbial inactivation, which is still under research (Pagán and others 1999; Bermúdez-Aguirre and others 2005; Ugarte-Romero and others 2006), could make thermosonication a viable option for pasteurization-homogenization of milk in the near future, because of the positive effects of cavitation on the microstructure of milk.

### Net color change and fat content

Because of the new and improved physical characteristics (for example, appearance, color, homogenization, and stability) of thermo-sonicated milk, the color and fat content were quantified to analyze how these parameters change in the product with heat and thermosonication, and how they can be correlated with changes in the microstructure of milk.

In Table 1 the color parameters  $L^*$ ,  $a^*$ , and  $b^*$  are shown for the 3 different samples: raw milk, heated milk, and thermo-sonicated milk. Raw and heated milk have similar values for Hunter's parameters, as can also be seen in the net change of color  $\Delta E$  (0.87), confirming that heat treatments do not change milk characteristics to a high degree. The color parameters for thermo-sonicated milk show an important variation with respect to the raw milk values, especially for the  $L^*$  value. The whiter color of milk observed at first sight in the samples after thermosonication is confirmed with a higher  $L^*$  value, meaning a greater luminosity of the sample. This value could be related to the better homogenization observed in the samples after sonication. Smaller fat globules are produced by cavitation, and apparently create a more uniform sample. The net change in color ( $\Delta E$ ) for the thermo-sonicated sample compared to raw milk also showed a high value (4.56). According to these studies on whole milk, the homogenization by cavitation could be achieved by eliminating or destroying smaller size clumps of fat that are sometimes present in raw milk.

The release of triacylglycerols from the core of the fat globules is confirmed by proximal analysis, as shown in Table 1. Measured fat content was 4.04% for raw whole milk; after 30 min of thermosonication the final result for lipids content was increased to 4.24%. This value demonstrates that the triacylglycerols content encapsulated inside the MFGM of the fat globules is released to the environment, making their extraction and further quantification easier after cavitation. Other lipids, such as cholesterol and phospholipids, which usually wrap the core of the fat globules, are also quantified after sonication because of the release of this membrane and later emulsification in the medium. Ultrasound generates the transfer of particles inside the medium. Villamiel and others (1999) mention

that the mechanical effects generated by cavitation enhance mass transfer. For this reason thermo-sonicated milk has better homogenization and stability.

### Conclusions

Ultrasound was responsible for the disruption of MFGM in the fat globules, generating a roughened surface and smaller size of droplets, as well as the adhesion of casein micelles to the main globule structure. Better homogenization, stability, and appearance than in heat-treated milk were observed in thermo-sonicated milk. The color was improved and the fat content was available with ease because of the cavitation effect. These new properties of milk following sonication treatment can be used in developing and improving the sensorial and quality characteristics of commercial and new dairy products.

### References

- [AOAC]. Assn. of Official Analytical Chemists. 1986. Official methods of analysis. Washington, D.C.: AOAC.
- Bermúdez-Aguirre D, Mobbs T, Versteeg K, Barbosa-Cánovas GV. 2005. Inactivation of *Listeria innocua* by ultrasound of low frequency in raw whole milk. IFT Annual Meeting, Book of Abstracts. 54-F-1. New Orleans, La.
- Briard V, Leconte N, Michel F, Michalski MC. 2003. The fatty acid composition of small and large naturally occurring milk fat globules. *Eur J Lipid Sci Technol* 105:677-82.
- Carcel JA, Benedito J, Sanjuán N, Sánchez E. 1998. Aplicación de los ultrasonidos en las industrias de productos lácteos y derivados. *Alimentación Equipos y Tecnología* 135-41.
- Corredig M, Dalgleish DG. 1996. Effect of different heat treatments on the strong binding interactions between whey proteins and milk fat globules in whole milk. *J Dairy Res* 63:441-9.
- Corredig M, Dalgleish DG. 1998. Effect of heating of cream on the properties of milk fat globule membrane isolates. *J Agric Food Chem* 46:2533-40.
- Dalgleish DG. 1998. Casein micelles as colloids: surface structures and stabilities. *J Dairy Sci* 81:3013-8.
- Dalgleish DG, Spagnuolo PA, Goff HD. 2004. A possible structure of the casein micelle based on high-resolution field-emission scanning electron microscopy. *Int Dairy J* 14:1025-31.
- Earnshaw RG, Appleyard J, Hurst RM. 1995. Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure. *Int J Food Microbiol* 23:197-219.
- Evers JM. 2004. The milkfat globule membrane—methodologies for measuring milkfat globule (membrane) damage. *Rev Int Dairy J* 14:747-60.
- Fauquant C, Briard V, Leconte N, Michalski MC. 2005. Differently sized native milk fat globules separated by microfiltration: fatty acid composition of the milk fat globule membrane and triglyceride core. *Eur J Lipid Sci Technol* 107:80-6.
- Heertje I, Leunis M, van Zeyl WJM, Berends E. 1987. Product morphology of fatty products. *Food Microstruct* 6:1-8.
- Jensen RG, Blanc B, Patton S. 1995. Particulate constituents in human and bovine milk. In: Jensen RG, editor. *Handbook of milk composition*. San Diego, Calif.: Academic Press. p 50-80.
- Kaláb M. 1981. Electron microscopy of milk products: a review of techniques. *Scan Electron Microsc* 3:453-72.
- Kaláb M. 1993. Practical aspects of electron microscopy in dairy research. *Food Struct* 12:95-114.
- Keenan TW, Patton S. 1995. The structure of milk: implications for sampling and storage. In: Jensen RG, editor. *Handbook of milk composition*. San Diego, Calif.: Academic Press. p 5-50.
- Knorr D, Ade-Omowaye BIO, Heinz V. 2002. Nutritional improvement of plant foods by non-thermal processing. *P Nutr Soc* 61:311-8.
- Martin AH, Goff HD, Smith A, Dalgleish DG. 2006. Immobilization of casein micelles for probing their structure and interactions with polysaccharides using scanning electron microscopy (SEM). *Food Hydrocolloid* 20:817-24.
- Mather IH, Keenan TW. 1998. Origin and secretion of milk lipids. *J Mammary Gland Biol* 3(3):259-73.
- Meyer S, Berrut S, Goodenough TJJ, Rajendram VS, Pinfield VJ, Povey MJW. 2006. A comparative study of ultrasound and laser light diffraction techniques for particle size determination in dairy beverages. *Meas Sci Technol* 17:289-97.
- Michalski MC, Manuel C. 2006. Does homogenization affect the human health properties of cow's milk? *Trends Food Sci Tech* 17:423-37.

- Michalski MC, Cariou R, Michel F, Garnier C. 2002a. Native vs. damaged milk fat globules: membrane properties affect the viscoelasticity of milk gels. *J Dairy Sci* 85:2451–61.
- Michalski MC, Michel F, Geneste C. 2002b. Appearance and submicromic particles in the milk fat globule size distribution upon mechanical treatments. *Lait* 82:193–208.
- Michalski MC, Camier B, Briard V, Leconte N, Gassi JY, Goudéranche H, Michel F, Fauquant J. 2004. The size of native milk fat globules affects physico-chemical and functional properties of Emmental cheese. *Lait* 84:343–58.
- Michalski MC, Briard V, Juaneda P. 2005. CLA profile in native fat globules of different sizes selected from raw milk. *Int Dairy J* 15:1089–94.
- Paci C. 1953. L'emploi des ultra-sons pour l'assainissement du lait. *Lait* 33:610–5.
- Pagán R, Mañas P, Álvarez I, Condón S. 1999. Resistance of *Listeria monocytogenes* to ultrasonic waves under pressure at sublethal (manosonication) and lethal (manothermosonication) temperatures. *Food Microbiol* 16:139–48.
- Sandra S, Dalgleish DG. 2005. Effects of ultra-high-pressure homogenization and heating on structural properties of casein micelles in reconstituted skim milk powder. *Int Dairy J* 15:1095–104.
- Sharma SK, Dalgleish DG. 1993. Interactions between milk serum proteins and synthetic fat globule membrane during heating of homogenized whole milk. *J Agric Food Chem* 41:1407–12.
- Thiebaud M, Dumay E, Picart L, Guiraud JP, Cheftel JC. 2003. High-pressure homogenization of raw bovine milk. Effects on fat globule size distribution and microbial inactivation. *Int Dairy J* 13:427–39.
- Tosh SM, Dalgleish DG. 1998. The physical properties and renneting characteristics of the synthetic membrane on the fat globules of microfluidized milk. *J Dairy Sci* 81:1840–7.
- Ugarte-Romero E, Feng H, Martin SE, Cadwallader KR, Robinson SJ. 2006. Inactivation of *Escherichia coli* with power ultrasound in apple cider. *J Food Sci* 71(2):E102–8.
- Villamiel M, de Jong P. 2000. Influence of high-intensity ultrasound and heat treatments in continuous flow on fat, proteins, and native enzymes of milk. *J Agric Food Chem* 48:472–8.
- Villamiel M, van Hamersveld EH, de Jong P. 1999. Review: effect of ultrasound processing on the quality of dairy products. *Milchwissenschaft* 54(2):69–73.
- Walstra P. 1969. Preliminary note on the mechanism of homogenization. *Neth Milk Dairy J* 23:290–2.
- Wiking L, Björck L, Nielsen JH. 2003. Influence of feed composition on stability of fat globules during pumping of raw milk. *Int Dairy J* 13:797–803.
- Wiking L, Stagsted J, Björck L, Nielsen JH. 2004. Milk fat globule size is affected by fat production in dairy cows. *Int Dairy J* 14:909–13.
- Wiking L, Nielsen JH, Båvius AK, Edvardsson A, Svennersten-Sjaunja K. 2006. Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition. *J Dairy Sci* 89:1004–9.
- Ye A, Singh H, Taylor MW, Anema SG. 2004. Interactions of fat globule surface proteins during concentration of whole milk in a pilot-scale multiple-effect evaporator. *J Dairy Res* 71:471–9.