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# Effect of heat treatment, final pH of acidification, and homogenization pressure on the texture properties of cream cheese 

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#### Abstract

Cream cheese is a good model for studying the effect of process and formulation changes on the modification of product texture. In this study, the intensity of heat treatment $\left(72{ }^{\circ} \mathrm{C} / 20 \mathrm{~s}\right.$ or $\left.94^{\circ} \mathrm{C} / 40 \mathrm{~s}\right)$, the final pH of acidification ( pH 5.2 or 4.9 ) and the homogenization pressure ( $0,5,20$, or 60 MPa ) were studied. Special attention was paid to the whey protein denaturation, casein micelle dissociation, fat globule size and their relations with structural, rheological, and sensory characteristics of the cream cheese model. Rheological properties of final cream cheese mainly depended on homogenization pressure. Increasing the homogenization pressure led to a decrease in fat globule size and consequently in an increase in the cream cheese firmness. This result was modulated by heat treatment temperature and the subsequent whey protein denaturation. Cream cheese final pH between 5.2 and 4.9 had a low impact on rheological properties but was the most discriminating factor for sensory perception by strongly affecting product appearance especially its brightness and its shade.


Keywords Acid gel • Cream cheese • Heat treatment • Homogenization • Texture

## 1 Introduction

Cream cheese is a dairy oil-in-water emulsion, acidified by lactic bacteria, and textured by heat treatments and homogenization. It is a very popular type of fresh

[^0]cheese especially in North America, Asia, and Oceania. Its process includes classical unit operations of the dairy industry: heat treatment, acidification by lactic acid bacteria, and homogenization. This product does not need any ripening and it may be consumed immediately after production and cooling. It is therefore a good model for the study of the effect of process and formulation changes on the modifications of product texture. Moreover, cream cheese is stable at least 3 months at $4{ }^{\circ} \mathrm{C}$, so it is well fitted for sensory and nutritional studies. Cream cheese classically contains at least $33 \%$ milk fat. Along the simplest process, cream cheese production involves the preparation of standardized milk, which is then acidified and finally treated on a centrifugal separator to obtain the desired dry matter (Phadungath 2005).

Cream cheese contains milk proteins, that is caseins and whey proteins (WP) in variable proportions. Proteins form a network which includes fat droplets and they also act as emulsifiers. Each step of the cream cheese process has a specific impact on the different components of the product.

When the pH changes, as happens during the acidification step of the cream cheese process, casein micelles may undergo several modifications. Casein micelles are supramolecular complexes of caseins stabilized by calcium phosphate and hydrophobic interactions. When the pH is decreased to $\sim 5.1$, most of the calcium phosphate is dissolved which leads to the loss of the original micelle structure. When the pH reaches 4.6, caseins form a network and if their concentration is large enough, a gel (Lucey and Singh 1998).

When milk is heated above $69^{\circ} \mathrm{C}$, whey proteins, mainly $\beta$-lactoglobulin ( $\beta-\mathrm{Lg}$ ) and $\alpha$-lactalbumin ( $\alpha$-Lac), may lose their native structure and can aggregate together or with K-caseins (Donato and Guyomarc'h 2009; Mahmoudi et al. 2010; Singh and Creamer 1992). Aggregation of whey proteins on casein micelles modifies their acid gelling properties and results in firmer gels (Guyomarc'h et al. 2009; Lucey and Singh 1998; Vasbinder et al. 2001).

Homogenization is usually the last step of the cream cheese process. The fat globules are reduced in size, a larger interface is created, and the oil-water interface undergoes a massive rearrangement. The new formed interfaces are colonized by proteins which stabilize the emulsion and may connect fat droplets to constitute a network (Sanchez et al. 1994). Temperature and pH modify the aggregation state of whey proteins and casein micelles. The interactions between those process variables lead to various proteins structures available to colonize the new formed interface. Competition occurs between aggregates and soluble proteins and may strongly affect cream cheese texture by modulation of the connectivity between fat droplets (Raikos 2010; Manoi and Rizvi 2009).

The aim of the present study was to investigate the interactions between intensity of heat treatments, final pH of acidification, and homogenization pressure on the textural properties of a simple, reproducible, and transportable cream cheese model. A complete experiment has been designed to allow the study of each factor at various levels to assess their individual impact but also the interactions between them. Moreover, an analytic strategy has been defined to understand how the interactions between whey proteins aggregation ratio, caseins structure, and interface surface affect the mechanical and sensory properties of the cream cheese model.

## 2 Materials and methods

### 2.1 Cream cheese model composition

The model was based on milk proteins and permeate powders and anhydrous milk fat to achieve a defined composition and skip the separation step. The model contained 4.7\% $(w / w)$ of Promilk 852A, a casein micelle powder (Idi ingredient, Arras, France, containing $80 \%$ of casein micelles), $0.2 \%(w / w)$ of Bipro, a whey protein isolates powder (Davisco, Food International, Le Sueur, USA, containing 95\% WP), $2.1 \%(w / w)$ of milk permeate powder (Armor Proteines, Saint-Brice en Coglès, France), $0.8 \%(w / w)$ of NaCl and $33.4 \%$ $(w / w)$ of BC TREX 32, an anhydrous milk fat with a fusion point at $34^{\circ} \mathrm{C}($ Corman, Goé Limbourg, Belgium). The cream cheese model had a dry matter of $40.4 \%$ ( $w / w$ ), including $33.1 \%(w / w)$ of fat, $4 \%(w / w)$ of proteins $(3.5 \%(w / w)$ of caseins and $0.5 \%(w / w)$ of whey proteins), $2 \%(w / w)$ of lactose, and $0.8 \%(w / w)$ of NaCl .

### 2.2 Cream cheese model process

The cream cheese process is described in Fig. 1.

### 2.2.1 Powders rehydration and milk fat emulsion

Powders ( 1.7 kg ) were dispersed and rehydrated in heated deionized water ( 13.5 L ) during 1 h in a tank continuously stirred with a helix and heated at $50^{\circ} \mathrm{C}$. Then, milk

Fig. 1 Description of the cream cheese process

fat ( 7.5 kg ) previously melted at $50^{\circ} \mathrm{C}$ was added and emulsified at $50^{\circ} \mathrm{C}$ for 30 min . During powder hydration and emulsification steps, the mix was continually circulating between the tank and a rotor/stator device magicLab IKA (Mac Technologie, Fontenay Tresigny, France). During the rehydration step, the rotation speeds of the helix and of the rotor/stator were respectively 350 and $10,000 \mathrm{~min}^{-1}$. During the emulsion step, the rotation speeds were increased respectively to 500 and $14,000 \mathrm{~min}^{-1}$. This emulsion step creates a coarse primary emulsion (fat globule size around $8 \mu \mathrm{~m}$ ).

### 2.2.2 First heat treatment

The coarse emulsion was heat treated in a tubular heat treatment pilot plant (Armfield Ltd, Hampshire, UK). Two different heat treatments were tested to study the impact of whey protein denaturation and aggregation on the texture properties of the final product: a low heat treatment at $72{ }^{\circ} \mathrm{C}$ for 20 s and a high heat treatment at $94^{\circ} \mathrm{C}$ during 40 s .

### 2.2.3 Lactic acidification

After cooling to $37{ }^{\circ} \mathrm{C}$, the heat-treated emulsion was inoculated with a Lactococcus lactis starter culture R603 (Chr. Hansen, Horsholm, Denmark) at $10^{8} \mathrm{cfu}^{\mathrm{g}}{ }^{-1}$ and let to acidify for 4 h to get pH 5.2 or 5 h to get pH 4.9 .

### 2.2.4 Second heat treatment and homogenization

The acidified product was pasteurized in a second heat treatment at $75^{\circ} \mathrm{C}$ for 30 s in the same continuous heat exchanger as described above. The heat treatment pilot instrument was connected in line with a high pressure homogenizer Niro Soavi Panda (GEA, Parma, Italy) so the product was directly homogenized after the heat treatment. Four homogenization pressures were tested: $0,5,20$, and 60 MPa at a constant flow (23 L.h ${ }^{-1}$ ).

### 2.2.5 Packing and cooling

The product was hot filled right after homogenization in plastic containers heat sealed with a transparent plastic film and turned upside down to pasteurize the seal. It was then placed at $4{ }^{\circ} \mathrm{C}$ during 48 h before any analysis to allow complete cooling down and fat crystallization.

### 2.3 Product analyses

At each stage of the process, the product was analyzed by laser light diffraction and rheology to characterize its fat dispersion and texture. For each process condition, repeatability was validated on three different batches at least.

### 2.3.1 Particle size measurement

The particle size analyses were performed by laser light scattering using a particle size analyzer Malvern Mastersizer S (Malvern Instruments, Malvern, UK) with laser
source at 633 nm . The refractive index used was 1.46 for anhydrous milk fat. The product was diluted $(1 \% w / v)$ in water or in an aqueous dissociating solution containing sodium dodecyl sulfate $5 \%(w / v)$ to dissociate fat globule clusters and dithiothreitol $5 \mathrm{mmol} \cdot \mathrm{L}^{-1}$ to break disulphides bridges. Product dissociation lasted 30 min at room temperature under a 150 rpm gentle stirring with a magnetic bar ( 2 cm ) and then analyses were performed at $20^{\circ} \mathrm{C}$ after dilution in deionized water. The efficiency of the dissociating solution (SDS/DTT) and method to solubilize proteins and separate individual fat droplets were checked by optical microscopy. Particles size distributions presented are means of at least nine measurements. The analyses were repeated at least three times for each sample of each batch. A typical commercial cream cheese (Philadelphia from France, Kraft food ${ }^{\circledR}$ ) was also analyzed. This product contained $26 \%$ w/w of fat, $5 \%$ $w / w$ of proteins and its pH was equal to 4.85 .

### 2.3.2 Determination of whey protein aggregation ratio

The amount of native whey protein content was measured on intermediate products, before and after heat treatment, and on the final products. The difference between both quantities revealed the ratio of protein aggregated during the heat treatment. All denatured whey proteins are expected to be insoluble at pH 4.6 . The concentration of native whey proteins was obtained by measuring nitrogen content after precipitation and separation of casein micelles and aggregated whey protein at pH 4.6 . The value was then converted in protein content by a factor 6.38 . The acidification was induced by adding acetic acid 1.75 mol.L ${ }^{-1}$ (Grappin and Ribadeau-Dumas 1992).

Nitrogen content was measured using the Dumas method (AOAC 1999) with a TruMac N Leco nitrogen analyzer (Leco France, Garges-Les-Gonesse, France). Denaturation ratios are means of six measurements. The analyses were reproduced twice per sample of each batch.

### 2.3.3 Confocal scanning laser microscopy

The fluorescent dyes Alexa Fluor 546 (Molecular Probes, Leiden, Netherlands) at $0.5 \mathrm{mg} . \mathrm{mL}^{-1}$ and Nile Red (Sigma Aldrich, France) at $0.17 \mathrm{mg} . \mathrm{mL}^{-1}$ diluted together in dimethylformamide were used respectively to stain the protein matrix and the fat. Ten microliters of the solution containing both dyes was poured on the sample. Samples were observed on a Nikon A1 confocal microscope (Nikon, Champigny sur Marne, France) fitted with a spectral modulus. One green laser at 561 nm was used for excitation. The fluorescence emission was detected between 562 and 642 nm with 32 channels of 2.5 nm . A spectral deconvolution was performed by using regions of interest of pure protein and pure fat selected on 0 MPa cream cheese. The stored images are composed of two channels (fat and protein) where each pixel was encoded in 4,096 grey levels. The size of the images obtained was $1,024 \times 1,024$ pixels (image width $=635 \mu \mathrm{~m}$ ).

### 2.3.4 Transmission electronic microscopy

The protocol was adapted by BIBS Team, INRA Nantes, France from the work of Sanchez et al. (1996a). Small pieces of cream cheese were encapsulated in agar gel at
$1.5 \%(50 / 50 \mathrm{v} / \mathrm{v})$ to avoid disintegration during fixation. Cubes of $1 \mathrm{~mm}^{3}$ were then fixed in a sodium cacodylate 0.1 M pH 4.9 buffer containing $3 \%$ of glutaraldehyde at $4^{\circ} \mathrm{C}$ and postfixed by osmium tetra oxide (1\%). Samples were dehydrated in series of ethanol solution and polymerized in Epon resin. Ultrathin sections ( 60 nm ) were cut using an ultramicrotome Leica EM UC7 (Leica Microsystems, Wetzlar, Germany). Observation of the products by transmission electron microscopy was performed using a JEM-1230 (JEOL Ltd, Croissy sur Seine, France) operating at 80 kV .

### 2.3.5 Rheology measurement

The rheological characterization was divided into two analyses performed using a viscometer Rheolab-QC (Anton Paar, Les Ulis, France) connected to a C-LTD80/QC measuring cell. Viscometry measurements were performed for liquid products (emulsion, heat-treated emulsion, and acidified product) and the vane method was applied for the characterization of final solid products.

Viscometry analyses were performed using a double Couette geometry ( 42 mm in diameter and 0.94 mm of gap) under controlled temperature at $50{ }^{\circ} \mathrm{C}$ for the emulsion, $37{ }^{\circ} \mathrm{C}$ for the acidified product and both temperatures for the heat-treated product. The shear rate applied was increased from 10 to $100 \mathrm{~s}^{-1}$ for the primary emulsion and from 0 to $120 \mathrm{~s}^{-1}$ then decreased from 120 to $0 \mathrm{~s}^{-1}$ for the heat treated and the acidified emulsions. Viscosity curves presented are means of three measurements. The analyses were performed once per sample of each batch.

The rheological analyses of final products and a typical commercial cream cheese (Philadelphia, Kraft Food ${ }^{\circledR}$ ) were performed through the yield stress using the vane geometry. A vane with four perpendicular blades Haake-FL 1000 (Gebruder Haake GmbH , Karlsruhe, Germany; 10 mm in diameter and 8.8 mm in height), immersed in the sample until complete blades penetration, was rotated at 0.6 rpm . The rotation was performed until sample fracture. Shear stress at fracture and the time needed to reach it were recorded (Fig. 2). Plotting rupture stress versus apparent yield strain for each product tested produces a "texture map" where yield strain can estimate the deformability of product and where rupture stress is positively correlated with its firmness and negatively with its spreadability. Vane method results presented are means of 18 measurements. The analyses were reproduced three times per sample and performed on two samples for each batch. Analyses were performed at $4^{\circ} \mathrm{C}$ to characterize product spreadability and firmness at fridge temperature (Breidinger and Steffe 2001; Daubert et al. 1998; Truong and Daubert 2000).

### 2.3.6 Sensory analysis

Six products were characterized by sensory analysis to assess the impact of each variable of the process on product properties. The selected cream cheeses are described in Table 1.

The analysis took place in a sensory analysis room at ambient temperature $\left(\sim 20^{\circ} \mathrm{C}\right)$, boxes were lit with a white light and products serving temperature was $10^{\circ} \mathrm{C}$ which is close to actual temperature of products consumption by consumers. Sensory profiling of cream cheese was performed by a panel of seven trained expert judges. The intensity of specific attributes defined in Table 2 was graded on a continuous scale ranging from 0


Fig. 2 Description of the curve obtained by vane method
(low) to 10 (high). The sensory attributes were selected to described the "texture in mouth", the "appearance and use", and the "taste" of the product.

Analyses were divided in two sessions. During the first session, 30 g of product were presented in small cups for "taste" characterization. During the second session, "texture in mouth" attributes were assessed on products in the same way than for "taste" characterization then "appearance and use" attributes were assessed on 15 g of product set up at an extremity of a slice of bread. The cheese could then be crushed, spread, etc....on the bread. Mineral water was used to wash the mouth between each sample. The cheese samples were presented in individual randomized order.

### 2.4 Statistical analyses

### 2.4.1 Statistical analyses of the rheology results

In order to assess the impact of each parameter on the cream cheese model texture, statistical analyses were performed using StatGraphics ${ }^{\circledR}$ Plus, 5.1 (Statpoint Technologies, Inc., Warrenton, VA, USA). Experimental design results were analyzed using a general linear model (GLM) in order to quantify the effect of temperature, pH , pressure, and their interactions on rupture stress.

Table 1 Codes of cream cheeses analyzed by sensory analysis

|  | pH | Temperature of <br> heat treatment $\left({ }^{\circ} \mathrm{C}\right)$ | Homogenization <br> pressure (MPa) |
| :--- | :--- | :--- | :--- |
| A | 5.2 | 72 | 5 |
| B | 5.2 | 94 | 5 |
| C | 4.9 | 72 | 5 |
| D | 4.9 | 72 | 60 |
| E | 4.9 | 94 | 5 |
| F | 4.9 | 94 | 60 |

Table 2 Definition of the sensory attributes

| Attribute | Definition |
| :---: | :---: |
| Texture in mouth |  |
| Firmness | Evaluate the pressure required for a piece of cheese undergoes an early deformation or fracture. |
| Granulosity | Evaluate the degree of irregularity related to the presence of grains in the mass of chewing sample. |
| Brittleness | Evaluate the quantity of pieces related to the stresses of chewing. |
| Melting | Evaluate the speed at which the sample passes from a solid to a liquid state, without chewing first. |
| Pasty | Evaluate the ease to form a cohesive amalgam (paste) that requires an extra addition of saliva to allow swallowing. |
| Dry | Evaluate the impression of a dry feeling in the mouth during the formation of the bolus. |
| Fat | Evaluate the impression of a fat feeling in the mouth during the formation of the bolus and after swallowing (formation of a thin film covering the oral mucosa). |
| Mealy film | Evaluate the mealy film in the mouth. |
| Appearance and use |  |
| Spreadability | Evaluate the facility to spread the product on the bread using a knife. |
| Roughness | Evaluate the degree of surface asperity. |
| Shade | Evaluate the shade of the spreaded product, from white to yellow. |
| Brightness | Evaluate the trend of the product to reflect the light, from matt to very shiny. |
| Taste |  |
| Global intensity |  |
| Cooked milk |  |
| Cream butter |  |
| Fermented milk |  |
| Rance, oxidized |  |
| Cardboard |  |
| Sour |  |
| Salty |  |

### 2.4.2 Statistical analyses of the sensory analysis results

The results were recorded using R software (2.14.0) ${ }^{\circledR}$ (R Foundation for Statistical Computing, Vienna, Austria) and then analyzed using StatGraphics ${ }^{\circledR}$ Plus, 5.1 (Statpoint Technologies, Inc.). The results were analyzed by an analysis of variance (ANOVA; $p$ value $<0.05$ ). A multiple means comparison test of Student-NewmanKeuls ( $p$ value $<0.01$ ) was then performed on attributes for which significant differences were found by the ANOVA. It allowed to determine the significant differences between means for each attributes and then to build groups of similar products (represented by different letters).

## 3 Results and discussion

Each step of the process has been studied to understand how the formation of the product affects its final texture.

### 3.1 Impact of whey proteins denaturation on emulsion properties

The two different heat treatments induced various denaturation and therefore different aggregation ratios. When the primary emulsion was treated by a low heat treatment ( $72{ }^{\circ} \mathrm{C} / 20 \mathrm{~s}$ ), only $5 \%$ of the whey proteins were aggregated ( $\pm 1 \%$ ) while the high heat treatment $\left(94^{\circ} \mathrm{C} / 40 \mathrm{~s}\right)$ led to an aggregation of around $55 \%( \pm 4 \%)$ of the whey proteins.

The particle size analysis of the primary emulsion led to a distribution with two peaks (Fig. 3a). The small one was in the range between 0.05 and $1 \mu \mathrm{~m}$ with a maximum reach for $0.3 \mu \mathrm{~m}$. It could represent casein micelles which generally measure between 0.05 and $0.5 \mu \mathrm{~m}$ (Dalgleish et al. 1989). The larger peak showed a modal diameter of $8 \mu \mathrm{~m}$ which may be attributed to the fat globules (confirmed by microscopic observation). The particle size distribution of the emulsion was similar in water and in dissociating solution. Neither SDS nor DTT dissociated any large particles. It indicated that there was no covalent aggregation at this step (Fig. 3b).

The size distribution was not altered by heat treatments (Fig. 3c). Regardless of the heat treatment or the dissociating solution, both peaks were kept without significant variation (Fig. 3d). Within this measurement uncertainty, there was no apparent aggregate and no coalescence induced by the first heat treatment.

The emulsion had a reproducible Newtonian behavior with a viscosity of 0.006 Pa.s ( $\pm 0.0003$ Pa.s; Fig. 4). After the low heat treatment, the emulsion kept a viscosity of $0.006 \mathrm{~Pa} . \mathrm{s}( \pm 0.0003 \mathrm{~Pa} . \mathrm{s})$ and kept its Newtonian behavior. However, the high heat treatment increased significantly the emulsion viscosity. The emulsion heat treated at $94{ }^{\circ} \mathrm{C}$ had a viscosity of 0.02 Pa.s ( $\pm 0.001$ Pa.s) at a shear rate of $10 \mathrm{~s}^{-1}$ and its viscosity decreased until 0.01 Pa.s at a shear rate of $100 \mathrm{~s}^{-1}( \pm 0.0004$ Pa.s). The fluid became shear thinning and thixotropic after the first high heat treatment.

The viscosity increase and the measurement of the aggregation ratio indicated a heat sensitivity of the product and some impact of protein denaturation and aggregation. The apparent increase in viscosity could be explained by the aggregation or flocculation of fat globules and by the appearance of small whey proteins aggregates (smaller to be detected by the used particle size analyzer). However, no flocculation was observed through particle size analyses after high dilution in dissociative solution. The overall stacking and transient interactions between particles was likely due to H bounds. These low energy connections were broken during dilution. They could explain both thixotropy and the appearance of shear-thinning properties.

### 3.2 Impact of acidification on emulsion properties

The viscosities of both heat-treated emulsions (mix before acidification) and acidified emulsions were measured at $37^{\circ} \mathrm{C}$. Irrespective of the pH , for both heat treatments, the acidified products did not behave as a gel but only as viscous emulsions. The acidification increased the viscosity of every emulsion but they did never gel. This


Fig. 3 Particle size distribution of the native emulsion dissociated at $1 \%(w / v)$ in water (a) and in $5 \%$ SDS and 5 mM DTT (b); particle size distribution of emulsion after the first heat treatment: $72{ }^{\circ} \mathrm{C} / 20 \mathrm{~s}$ (black square) or $94{ }^{\circ} \mathrm{C} / 40 \mathrm{~s}$ (white square) dissociated at $1 \%(w / v)$ in water (c) and in $5 \%$ SDS and 5 mM DTT (d); particle size distribution of the heat-treated emulsion after acidification: pH 4.9 at $72{ }^{\circ} \mathrm{C}$ (black circle) and at $94^{\circ} \mathrm{C}$ (white circle) or pH 5.2 at $72^{\circ} \mathrm{C}$ (black diamond) and at $94^{\circ} \mathrm{C}$ (white diamond) dissociated at $1 \%(w / v)$ in water (e) and in $5 \%$ SDS and 5 mM DTT (f); particle size distribution of the final products: at $94{ }^{\circ} \mathrm{C} \mathrm{pH} 4.9$ homogenized at 0 MPa (black square), 5 MPa (black circle), 20 MPa (black diamond), or 60 MPa (black triangle) dissociate at $1 \%(w / v)$ in $5 \%$ SDS and 5 mM DTT (g); particle size distribution of the final products: homogenized at 5 MPa at $94^{\circ} \mathrm{C} \mathrm{pH} 4.9$ (white circle) or pH 5.2 (white triangle), at $72^{\circ} \mathrm{C}$ pH 4.9 (white diamond) or pH 5.2 (white square) and homogenized at 60 MPa at $94{ }^{\circ} \mathrm{C} \mathrm{pH} 4.9$ (white circle) or pH 5.2 (black triangle), at $72^{\circ} \mathrm{C} \mathrm{pH}=4.9$ (black diamond) or $\mathrm{pH}=5.2$ (black square) dissociate at $1 \%(w / v)$ in SDS $5 \%$ and DTT 5 mM (h)

Fig. 4 Viscosity curves of the native emulsion (black triangle), the emulsion heat treated at $72{ }^{\circ} \mathrm{C} / 20 \mathrm{~s}$ (black square) and the emulsion heat treated at $94^{\circ} \mathrm{C} / 40 \mathrm{~s}$ (white square), measurement realized at $50^{\circ} \mathrm{C}$

phenomenon could be due to the use of powders which never behave as liquid milk. This viscosity increase kept differences between both 72 and $94^{\circ} \mathrm{C}$ emulsions. For both heat treatments, products acidified at pH 4.9 were more viscous than those acidified at pH 5.2 (Fig. 5). The acidified emulsions presented a shear thinning and thixotropic behavior. At this step, the comparison of particles sizes in water or in SDS/DTT allowed to characterize emulsion stability against flocculation and/or coalescence.

The particle size analyses of the acidified product led to a distribution with two peaks (Fig. 3e, f). All products still had the small peak at $0.3 \mu \mathrm{~m}$ in their distribution. This peak was not affected by the acidification either at pH 5.2 or 4.9. It remained undissociated casein supramolecules until a pH of 4.9 during acidification as shown by previous works (McMahon et al. 2009). They have a size in a range of diameter between 100 and 300 nm which correspond to particles sizes of this peak. The other peaks were centered on larger particles, and their sizes were dependent on process conditions and on dissociating solution. In water, particle sizes of emulsions increased after the acidification and became larger than those of the heat-treated emulsion (Fig. 3c, e) except for the

Fig. 5 Viscosity curves of the emulsions heat treated at $72{ }^{\circ} \mathrm{C} / 20 \mathrm{~s}$ (black square) or at $94{ }^{\circ} \mathrm{C} / 40 \mathrm{~s}$ (white square), the acidified product pH 4.9 at $72{ }^{\circ} \mathrm{C}$ (black circle) and at $94^{\circ} \mathrm{C}$ (white circle) or pH 5.2 at $72{ }^{\circ} \mathrm{C}$ (black diamond) and at $94{ }^{\circ} \mathrm{C}$ (white diamond) measurement realized at $37{ }^{\circ} \mathrm{C}$


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emulsion heat treated at $72{ }^{\circ} \mathrm{C}$ and acidified at pH 5.2 . At this pH , acidification did not affect the particles which kept a size of $8 \mu \mathrm{~m}$.

After dissociation by SDS and DTT (Fig. 3f), the major particle size was back to $8 \mu \mathrm{~m}$ for all conditions of pH . It showed that, for a heat treatment at $94{ }^{\circ} \mathrm{C}$, acidification of the product at both pH 4.9 and 5.2 led to proteins and fat globules aggregation.

After a heat treatment at $72{ }^{\circ} \mathrm{C}$, particles did not aggregate at $\mathrm{pH}=5.2$ (Fig. 3e). At this pH , denatured whey proteins were thus necessary to form proteins/fat globules aggregates. It has been shown that heat treatment reduced gelation time and then increased pH of gelation (Lucey et al. 1997, 1998) from 4.9 for unheated milk to 5.3 for milk heat treated $\left(>80^{\circ} \mathrm{C}\right)$. This is the isoelectric pH of the major whey protein, $\beta$-lactoglobulin. After their heat treatment and denaturation, they aggregate on casein micelles and modify their surface electronegativity. It explains the aggregation at higher pH of the heated milk and the difference of particle size at $\mathrm{pH}=5.2$ between heat-treated emulsion at 72 or at $94{ }^{\circ} \mathrm{C}$ observed in the present study.

Denatured whey proteins, by interacting with the casein network, strengthened it, and then increased the viscosity of acidified products. The aggregated whey protein and caseins at fat globule interface induced fat droplets flocculation leading also to the increase of viscosity (Lucey and Singh 1998; McClements 1999).

### 3.3 Impact of the total process on final product texture

### 3.3.1 Impact of the total process on product physical characteristics

Four different homogenization pressures were applied to the acidified curds to assess their impact on fat globule size and available interface and then on textural properties of the product. Particle size analyses revealed a fat globule size equal to $1 \mu \mathrm{~m}$ for the commercial product (results not shown) and a decrease of fat globule size of the model with the increase of homogenization pressure (from $8 \mu \mathrm{~m}$ at 0 MPa to $0.3 \mu \mathrm{~m}$ at 60 MPa ). These trends were reproducible and kept for both heat treatments and final pH (Fig. 3g). There was no obvious impact of heat treatment or pH on the particle size distributions (Fig. 3h). The increase of homogenization pressure led to specific surface area equal to $2.3 \mathrm{~m}^{2} . \mathrm{g}^{-1}\left( \pm 0.2 \mathrm{~m}^{2} . \mathrm{g}^{-1}\right)$ for 5 MPa cream cheeses, $3.5 \mathrm{~m}^{2} . \mathrm{g}^{-1}\left( \pm 0.5 \mathrm{~m}^{2} . \mathrm{g}^{-1}\right)$ for 20 MPa cream cheeses, and $6.3 \mathrm{~m}^{2} . \mathrm{g}^{-1}\left( \pm 0.5 \mathrm{~m}^{2} . \mathrm{g}^{-1}\right)$ for 60 MPa cream cheeses. Those values were calculated from the mean surface diameters $\left(d_{3,2}\right)$ provided by particle size measurements, the density of fat and the percentage of fat in products (McClements 1999).

Transmission electronic microscopy images presented in Fig. 6 show cream cheeses homogenized at 60 MPa with droplets smaller than 100 nm . These small droplets seemed too small to be characterized by particle size measurements. Moreover, these images show that, at 60 MPa , fat droplets of the cream cheese previously heat treated at $72{ }^{\circ} \mathrm{C}\left(\mathrm{CC} 72^{\circ} \mathrm{C}\right.$; Fig. 6 a) seemed smaller than those from the cream cheese previously heat treated at $94{ }^{\circ} \mathrm{C}\left(\mathrm{CC} 94{ }^{\circ} \mathrm{C}\right.$; Fig. 6b) even if it was not obvious to observe on particle size analyses. These small globules $(<100 \mathrm{~nm})$ modified the $d_{3,2}$ and induced a larger specific area than the $6.3 \mathrm{~m}^{2} . \mathrm{g}^{-1}$ calculated from particle size measurement. The cream

Fig. 6 Transmission electron microscopy of the cream cheese product homogenized at 60 MPa , pH 4.9 , heat treated at $72{ }^{\circ} \mathrm{C}(\mathbf{a})$ or $94{ }^{\circ} \mathrm{C}(\mathbf{b})$

cheese model contained $4 \%$ of proteins and $33 \%$ of fat. For a $d_{3,2}$ decreasing from 0.3 to $0.2 \mu \mathrm{~m}$, the quantity of proteins available to cover the interface decreased from 6 to $4 \mathrm{mg} \cdot \mathrm{m}^{-2}$. The quantity of casein micelles needed to cover $1 \mathrm{~m}^{2}$ of interface varied between 6 and $10 \mathrm{mg} . \mathrm{m}^{-2}$ for a ratio casein micelles/whey proteins equal to $80 / 20$ (Surel et al. 2013). The structure of the different proteins was not perfectly characterized at this step and their various structures (dissociated micelle, aggregated whey proteins) could slightly modify the quantity needed to cover the interface. Despite these possible variations, the quantity of proteins available in cream cheese homogenized at 60 MPa to cover the interface was low and could become limiting to cover the interface.

Microstructures of 60 MPa pH 4.9 cream cheeses, observed by confocal laser microscopy, are shown in Fig. 7. Structures at 5 MPa were very similar irrespective of the heat treatment (results not shown). However, the confocal microscopy pictures show various structures of 60 MPa products depending on the heat treatment temperature. Cream cheeses heat treated at $94^{\circ} \mathrm{C}$ presented a rougher structure than the $\mathrm{CC} 72{ }^{\circ} \mathrm{C}$ with crack zone-containing whey. Cream cheeses heat treated at $72{ }^{\circ} \mathrm{C}$ had a structure more dense with grains containing high amount of proteins.

The results of the vane method analyses performed on final products have been plotted on a texture map (Fig. 8). The developed cream cheese models were reproducible and in the range of the commercial product analyzed for both particle size and rheological measurements (Fig. 8a), so they could be considered as realistic. The results of GLM analysis are presented in Table 3. The interactions between homogenization pressure and pH and between homogenization pressure and temperature on cream cheese rupture stress have been plotted in Fig. 8. Products were not discriminated by yield strain, they had

Fig. 7 Confocal scanning light microscopy of the cream cheese homogenized at $60 \mathrm{MPa}, \mathrm{pH} 4.9$, heat treated at $72{ }^{\circ} \mathrm{C}(\mathbf{a})$ or $94{ }^{\circ} \mathrm{C}$ (b), lipids are presented in red and proteins in green

approximately the same deformability (Fig. 8) but each factor had a significant effect on product rupture stress (Table 2). The raise of homogenization pressure led to an increase of rupture stress so to a decrease in spreadability and an increase in product firmness (Fig. 8). These results are in agreement with previous works on the impact of homogenization on cream cheese (Sanchez et al. 1994, 1996a, b). The authors explained that soluble and aggregated proteins acted as emulsifiers, promoted interactions, and bridges between fat droplets interface and allowed to form a network. As the homogenization pressure increased, the interface to be colonized became larger which increased the number of possible interactions and so the product firmness.

The differences induced in earlier stages by pH were kept regardless of the homogenization pressure, pH 4.9 cream cheeses were always less spreadable than those acidified at pH 5.2 (Fig. 9a). At pH 5.2 , less caseins were involved in the proteins aggregates and network. Non-aggregated caseins competed with casein aggregates to cover interface and reduced bridges formation between droplets which decreased product rupture stress.

However, the impact of temperature on cream cheese texture was also dependent of homogenization pressure (Figs. 8 and 9 b ). At low pressures ( $0-20 \mathrm{MPa}$ ), texture


Fig. 8 Texture map of the cream cheese model at pH 4.9 (a) and pH 5.2 (b), heat treated at $72{ }^{\circ} \mathrm{C}$ homogenized at 0 MPa (black square), 5 MPa (black circle), 20 MPa (black diamond), or 60 MPa (black triangle) and heat treated at $94{ }^{\circ} \mathrm{C}$ homogenized at 0 MPa (white square), 5 MPa (white circle), 20 MPa (white diamond), and 60 MPa (white triangle). Philadelphia ${ }^{\circledR}$ product with a pH of $4.85(X)$ is on map (a). Error bars show the standard deviation calculated from repeated measurements
differences previously observed related to the heat treatment, were canceled and both CC $72{ }^{\circ} \mathrm{C}$ and CC $94{ }^{\circ} \mathrm{C}$ displayed similar rupture stresses. Moreover, for higher pressure ( 60 MPa ) texture differences were reversed and CC $94^{\circ} \mathrm{C}$ was less firm and more spreadable than CC $72{ }^{\circ} \mathrm{C}$. This seemed consistent with confocal microscopy images (Fig. 7) which showed more homogenous structure of CC $72{ }^{\circ} \mathrm{C}$. They had less cracks and could be less fragile, more firm, and less spreadable.

The homogenizer outlet temperature was $75^{\circ} \mathrm{C}$ at 5 MPa but reached $95^{\circ} \mathrm{C}$ at 60 MPa . This undergone heat-treatment denatured the residual native whey proteins. It reduced the differences in aggregation ratios induced by the first heat treatment. The difference of texture between $\mathrm{CC} 72{ }^{\circ} \mathrm{C}$ and $\mathrm{CC} 94^{\circ} \mathrm{C}$ at high homogenization pressure could be related to the increase of temperature in the homogenizer induced by the increase of pressure. Whey proteins denatured at the first heat treatment were parts of some pieces of acidified network since the acidification step. They formed large aggregates with caseins which could be too large and too rigid to cover efficiently the entire interface of the very small fat globules. However, whey proteins newly denatured during homogenization could form aggregates more flexible which would be better emulsifiers and would stabilize big interface, so very small droplets.

Table 3 Sum of square and probabilities table of the general linear model calculated on the impact of process parameters ( pH , homogenization pressure, and heat treatment temperature) and their interactions on product rupture stress

| Source | Sum of square | DF | Mean square | $F$ ratio | Probability |
| :--- | :--- | :--- | :--- | ---: | ---: |
| pH | $1.69 \times 10^{7}$ | 1 | $1.69 \times 10^{7}$ | 71.49 | $<0.0001$ |
| Pressure | $5.49 \times 10^{8}$ | 3 | $1,83 \times 10^{8}$ | 775.47 | $<0.0001$ |
| Temperature | $5.54 \times 10^{6}$ | 1 | $5.54 \times 10^{6}$ | 23.49 | $<0.0001$ |
| $\mathrm{pH} \times$ pressure | $3.35 \times 10^{6}$ | 3 | $1.12 \times 10^{6}$ | 4.73 | 0.0031 |
| $\mathrm{pH} \times$ temperature | $3.75 \times 10^{6}$ | 1 | $3.75 \times 10^{6}$ | 15.92 | 0.0001 |
| Pressure $\times$ temperature | $2.53 \times 10^{7}$ | 3 | $8.44 \times 10^{6}$ | 35.81 | $<0.0001$ |
| Total (corrected) | $6.86 \times 10^{8}$ | 283 |  |  |  |

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Fig. 9 General linear model results of the variation of the cream cheese rupture stress as function of the homogenization pressure at two $\mathrm{pH}(\mathbf{a})$ : pH 4.9 (white square) and $\mathrm{pH}=5.2$ (black square) and at two temperatures (b): $94^{\circ} \mathrm{C}$ (white triangle) and $72^{\circ} \mathrm{C}$ (black triangle). Error bars show the standard deviation calculated by the statistical analysis from repeated measurements

This could explain differences in droplets size between CC $72{ }^{\circ} \mathrm{C}$ and CC $94{ }^{\circ} \mathrm{C}$ observed in electronic microscopy.

The homogenization at 60 MPa of the mix heated at $72^{\circ} \mathrm{C}$ led to the denaturation of $64 \%$ whey proteins. Newly denatured whey proteins could enhance emulsifying properties, lead to the stabilization of a bigger interface, and so increase proteinmediated interactions between fat globules. The network was tighter and so induced firmer and less spreadable cream cheese. Those results showed strong interactions between heat treatments and homogenization pressure affecting both structural and rheological properties of cream cheese.

### 3.3.2 Impact of the total process on product sensory properties

In our study, there was no significant difference in "taste" attributes category between products. The variations in acidity and heat treatment did not induce detectable changes in product taste.

However, the results of the ANOVA and of the Student-Newman-Keuls test showed that the panel could distinguish products according to "texture in mouth" and "appearance and use" attributes categories (Fig. 10). "Brightness", "shade", "granulosity", and "spreadability" were the sensory attributes which most discriminated our cream cheeses. These attributes were mostly influenced by final pH which had a significant impact on the sensory characteristics of cream cheeses, especially on product appearance. Products at pH 5.2 (products A and B) were brighter, lighter, more spreadable, less granular, less mealy, and less dry than product at pH 4.9 (C, D, E, and F). Products at pH 5.2 were softer, so they were subjected to a slight subsidence.


Fig. 10 Results of ANOVA and Student-Newman-Keuls test for sensory attributes. Mean value of each product are presented for each sensory attribute which significantly differed between treatments. Groups of similar products are built. They are represented by the same letter

Consequently, the surfaces of products were smoother and less granular which decreased light dispersion. It could explain these differences of brightness between products at pH 5.2 and 4.9.

To a lesser extent, products at pH 4.9 could be divided in two groups by the homogenization pressure. The increase of homogenization pressure induced products less spreadable, drier and slightly pastier for both 72 and $94^{\circ} \mathrm{C}$ heat-treated products.

Homogenization pressure significantly affected rupture stress ( $p$ value $<0.0001$ ). Rupture stress was shown as correlated with "spreadability". This is in agreement
with the decrease of spreadability when the homogenization pressure increased (Figs. 7 and 8). pH had also an influence on products rupture stress (Fig. 8a) although to a lesser extent than homogenization (Fig. 8). While instrumental characterization showed homogenization as the major factor influencing the cream cheese texture, sensory analysis appeared more sensitive to pH . Indeed, for sensory characterization, the impact of homogenization pressure on "spreadability" attribute was less significant than the influence of the pH factor. The increase of pH decreased the score of the attribute "spreadability" of two points whereas the increase of homogenization pressure from 5 to 60 MPa decreased the score of only one point. It pointed out some differences between rheological and sensory characterizations.

Moreover, as for vane method measurements (Fig. 8b), the modifications of sensory properties induced by the increase of homogenization pressure, tended to be slightly greater for products heat treated at $72{ }^{\circ} \mathrm{C}$ than for products heat treated at $94^{\circ} \mathrm{C}$ (Fig. 10). However, these modifications were most of the time not significant as for the attributes "granulosity", "dry", and "pasty". Nevertheless, the product heat treated at $72{ }^{\circ} \mathrm{C}$ and homogenized at $60 \mathrm{MPa}(\mathrm{D})$ was significantly mealier than the others. The combination of low heat treatment and high homogenization pressure which led to the formation of the small and flexible aggregates could induce this mealy feeling in the mouth.

Besides, the products seemed to be better discriminated by "appearance and use" attributes (especially "brightness", "shade", and "spreadability") than by "texture in mouth" attributes. "Firmness" attributes could be easily negatively correlated with "spreadability"; however, this attribute was not significant in sensory characterization. This could be due to product temperature during the measurements. Indeed, rheological analyses occurred at $4^{\circ} \mathrm{C}$, "appearance and use" characterization was carried out at $10^{\circ} \mathrm{C}$ and the sensory study of texture happened at mouth temperature. As cream cheese products contained $33 \%$ of fat, they were very sensitive to heat, melted at $37^{\circ} \mathrm{C}$, and lost their structure. It was therefore difficult to assess some of their texture attributes in mouth, in particular their firmness. Firmness differences mainly induced by homogenization are then attenuated in favor to "appearance and use" characteristics or attributes describing products after their destruction in mouth (granulosity, mealy film, pasty, etc....).

## 4 Conclusion

This work led to the development of a cream cheese model easy to carry out with a lab setup. The cream cheese model was reproducible, stable, and similar to commercial products in terms of structural and rheological properties. The modification of the major process parameters allowed to obtain a large range of textures from a liquid state to a very firm product without addition of texturing agents. The interaction between heat treatment and homogenization had a strong impact on textural properties of the model most likely through the structure of resulting proteins aggregates. While homogenization pressure is the most important factor for the rheological behavior of the product, a higher level of the initial heat treatment temperature and a lower pH value both significantly increased the cream cheese rupture stress.

Notwithstanding, sensory analysis revealed a higher impact of final pH on cream cheese properties. An increase in cream cheese pH made it brighter, lighter, smoother,
more spreadable, and less pasty. For our cream cheeses, "texture in mouth" sensory attributes were poorly related to rupture stress but "appearance and use" attributes completed the description and discrimination of the products.

This cream cheese model was easy to produce at lab scale, thoroughly characterized, and thus can be used for future sensory and nutritional studies.

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