The effect of sonication and high pressure homogenisation on the properties of pure cream

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ABSTRACT

The homogenisation of milk and cream has been widely studied, but the effect of sonication on the structural and functional properties of cream is not well known. In this study, raw milk, ultrafiltration retentate and cream samples were sonicated at 20 kHz and the rennet and acid gelation properties of these sonicated samples investigated. High pressure homogenisation at 80 bar was also performed for comparison. Sonication of raw milk and retentate samples led to a decrease in the fat globule size. Conversely, the fat globules in cream samples sonicated at <10˚C flocculated to form grapelike structures, whereas the cream samples sonicated at 50˚C did not form such aggregates. High pressure homogenisation at 50˚C led to similar flocculated structures, but these were not observed at low temperatures. This suggests a potential benefit of sonication technology in allowing low temperatures to be utilised for cream homogenisation, reducing energy demand. However, a gel made using cheese-milk with sonicated cream resulted in separation of a fat layer rather than the incorporation of the fat globules into the gel matrix. Rennet gelation properties of both the sonicated or homogenised samples were significantly superior to a native control sample where the resultant gels had shorter coagulation times and decreased syneresis.

Keywords: Cheese milk; homogenisation; sonication; rennet gels; acid gels

1. Introduction

Milk fat is present in milk as droplets of diameter in the range of 1–10 μm. These globules are covered with a natural milk fat globule membrane (MFGM) composed mainly of phospholipids and enzymes. The sensorial and rheological properties of many dairy products depend greatly on the size distribution of the fat globules and on the composition of the membrane (Cho, Lucey, & Singh, 1999; Lopez & Dufour, 2001). Reduction of the fat globule size and the consequent disruption of the fat globule membrane through ultrasonication alone or in combination with conventional homogenisation may lead to a range of new dairy products with different physico-chemical and functional properties. Although, such fat globule size reduction is not desirable for Cheddar cheese manufacture, it has many benefits in the manufacture of soft cheeses and dairy gels where the resulting high moisture content, creamier, smoother and softer textures are desirable. Further, the smaller fat globules are more sensitive to the influence of the lipolytic enzymes in making specialised products, such as blue cheese.

The milk fat globule membrane (MFGM) does not interact with the protein network in native dairy gels and so the fat globules act mainly as an inert filler or structure breaker (Milchaski et al., 2004). However, when such dairy systems are subjected to shear, the fat globules are disrupted and their average diameter decreases significantly (Bernudez-Aguirre & Barbosa-Canovas, 2010). Milk homogenization also disrupts the fat globule membrane, which is replaced by membrane fragments complexed with casein (Tunick, Van Hekken, Cooke, Smith & Malin, 2000). These homogenized fat globules are then able to form cross links with the casein network, and this effect is enhanced by their large surface area (Metzger & Mistry, 1995). Michalski et al., (2002a) found that homogenized milk contained three types of fat particles: (i) regular milk fat globules with a fraction of the surface covered by casein micelles, (ii) tiny native milk fat globules around 100 nm in diameter that are not affected by

homogenization due to their size and (iii) small newly formed lipid-protein complexes with new membrane composed mainly of casein. Dalgleish, Tosh & West, (1996) concluded that casein micelle fragments, rather than intact micelles, are adsorbed on the globules during micro fluidization of milk. They attributed the disruption of the casein micelles to the forces encountered during the shearing process, which are experienced by micelles adsorbing at the fat/serum interface. Hayes, Fox, & Kelly, (2005) also found that fat globules in high pressure (HP) homogenised milk are surrounded by a layer of casein micelle fragments rather than intact casein micelles. In contrast, Tosh & Dalgelish (1998) stated that disrupted fat globules are mainly bound by intact casein micelles.

Several studies have found that such changes during homogenisation (Michalski et al., 2002a,b, 2004; Sandra & Dalgleish 2007; Shaker, Abu-Jdayl, Jumah, & Ibrahim, 2002; Titapiccolo, Alexander, & Corredig, 2010; Yiran, Lee, & Anema, 2011; Zamora, Ferragut, Jaramillo, Guamis, & Trujillo, 2006) and micro fluidization (Ciron, Gee, Kelly, & Auty, 2012; Lemay, Paquin, & Lacroix, 1994; Path, Gellman, Schimdt, & Herforth-Kennedy, 1989; Tunick et al., 2000; van Hekken, Tunick, Marlin, & Holsinger, 2007) influence milk gelation kinetics and the resulting milk gel properties. The reduction of fat globule size implies a dispersion of fat into an increased number of smaller globules. The newly built surfaces are modified by the presence of adhering casein particles and become part of the para-casein network, hindering shrinkage of the network and thus lowering the syneresis and fat loss (Lemay et al., 1994). Green, Marshal, & Glover, (1983) observed that curds from conventionally homogenized milk had a less coarse protein network, which retained moisture more effectively than curds from non homogenized milks. However, the formation of complexes between casein and MFGM decreases the amount of casein available to form stronger casein-casein bonds (Lemay et al., 1994). In turn, this affects cheese body and

texture by a reduction of curd firming (Emmons, Kalab, & Larmond, 1980; Green et al., 1983). The weaker texture is also due to the new milk fat globules participating directly in the network instead of remaining trapped within the casein matrix.

Similar fat globule – protein complexes have also been observed when milk is subjected to ultrasonication (Michalski et al., 2002a). During exposure to an acoustic field, microbubbles are generated within the dairy fluid. The collapse of these microbubbles induces localised shear forces that are readily capable of disrupting fat globules. Bernudez-Aguirre, Mawson, & Barbosa-Canovas, (2008) found that the sheared fat globules had a roughened granular surface due to the interaction between the disrupted MFGM and nearby casein micelles. Such changes induced noticeable improvements in the quality of Hispanic Cheese (handmade cheese consumed in Latin America) when the cheese milk was sonicated at 63˚C for 30 min (Bermudez-Aguirre & Barbosa-Canovas, 2010). The cheese had a whiter colour, higher cheese yield and better textural and micro-structural properties with only a minor degree of syneresis. Increased water holding capacities for Emmental cheese and high lipolytic enzyme activity for blue cheeses has also been achieved through a reduction in fat globule size using sonication of the feed milk (Milchalski et al., 2004).

While there is much work on the use of both homogenisation and sonication of cheese milk prior to gel formation, there is no work available on the sonication of the raw cream alone; prior to addition to cheese milk. Cream is commonly used to increase the fat concentration of milk for the production of soft, cream or high fat hard cheeses. It is a common practice to homogenize this cream before addition, to improve texture (Madadlou, Mousavi, Asl, Emam-Djome, & Zargaran, 2007; Sanchez, Beauregard, Chassagne, Bimbenet, & Hardy, 1996). In this study, we have looked at the effect of sonication on cream as a comparison to the effect

observed during homogenisation. Separate sonication of cream prior to addition to the cheese milk could avoid the casein-MFGM interactions that reduce the capacity for these proteins to participate in gel formation. The present study uses cream systems containing ~40% fat which were subjected to sonication (50 W for 1 min) or homogenisation (80 bar) prior to addition to standardised cheese milk. The acid and rennet gelation properties were then investigated using these cheese milk systems.

2. Materials and methods

2.1. Materials

Raw milk, ultrafiltrate (UF) retentate, skim milk (SM), skim milk concentrate (SMC) and cream were obtained from a local Victorian dairy manufacturer. The composition of these samples, as supplied by the manufacturer, is given in Table 1.

Table 1 here

Cheese-milk is defined as the milk standardised for the manufacture of Cheddar cheese, obtained by blending skim milk with skim milk concentrate and cream to obtain the desired protein and fat content of 3.8% w/w and 4.6% w/w, respectively. Three types of cheese-milk were investigated in this study:

1. A blend of SM, SMC and cream with no treatment (native);
2. A blend of SM, SMC and sonicated cream
3. A blend of SM, SMC and homogenised cream

The milk was blended by hand stirring for 1 minute and each mixture was pasteurized at 85˚C for 30 min.

2.2.Sonication and homogenisation conditions

Batch solutions of milk and cream were sonicated in a glass vessel equipped with a cooling jacket using a 20 kHz, 450 W Ultrasonic horn (19 mm diameter, Branson Sonifier 450, Danbury, CT). To maintain a constant energy density, samples of 40 ml were sonicated at an amplitude of 60% and samples of 60 ml were sonicated at an amplitude of 40%. The power draw, as determined from a single-phase energy cost meter (Arlec, Victoria, Australia), was measured as 101 and 189 W under these conditions, giving an input energy density of 152 ± 3 J/ml for one minute sonication in both cases. The settings equated to delivered power levels of 31±2 W and 50±2 W as determined by calorimetry (Contamine, Wilhelm, Berlan & Delmas, 1995). During sonication, water was continuously circulated through the cooling jacket to maintain the desired sample temperature.

The homogeniser used was a GEA Panda PLUS 1000 (GEA Nitro Savi, Parma, Italy) equipped with a cell disruption valve. Single stage and single pass homogenisation was performed on 500 ml of solution at an operating pressure of 80 bar. In this case, the power drawn was 570 W. The flow rate of the solution was set at 3.73 ml/s to provide an identical input energy density of 153±3 J/ml.

2.3. Particle Size Distribution

The particle size distribution of the fat globules in samples was measured using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) using a refractive index for milk fat of 1.460 (Yiran et al., 2011). The milk sample was diluted (1:1) in ethylenediamine tetraacetic acid (EDTA; 50 mM, pH 7). The milk samples were then added into the circulating cell with/without 0.05% sodium dodecyl sulphate (SDS). EDTA was used to dissociate casein micelles from the fat globules and SDS added to dissociate any aggregates containing fat

globules. The volume weighted mean diameter D[4,3] was calculated by the Mastersizer 2000 software. Triplicate measurements were carried out for each of the samples.

2.4. Rennet- or acid- induced gel preparation

Rennet-induced gels were prepared from the blended and pasteurised milk samples, to replicate cheddar cheese gels. The milk was first tempered to 33˚C before inoculation with the starter culture. A freeze-dried mixed strain direct vat set (DVS) mesophilic starter culture containing *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Chr. Hansen, Bayswater, Victoria, Australia) was added at a concentration of 0.05g/l of milk. When the pH of the milk reached 6.50, rennet (Hannilase, 690 IMCU/ml; Chr. Hansen) was added (0.1 ml/l of milk). The milk was allowed to coagulate for a period of 45 min at 33˚C following the standard protocol for Cheddar cheese production (Ong, Dagastine, Kentish, & Gras, 2010). The acid-induced gel was prepared by adding 2.5% (w/w) Glucono Delta Lactone (Sigma-Aldrich, Australia) to the pasteurised milk samples, which were then incubated at 33˚C to form an acid gel for 2 hours.

2.5. Sample Analysis

The viscoelastic properties of the acid and rennet gels were analysed using an Advanced Rheometric Expansion System (ARES) rheometer (TA Instruments, New Castle, USA) equipped with a cup (34 mm diameter) and bob (32 mm diameter, 33 mm length) accessory. A sample of cheese-milk (15 ml) that had been inoculated with starter culture (0.05 g/l) and ripened to pH of 6.5 was added to the cup immediately after the addition of rennet (0.1 ml/l). The temperature of the milk was maintained at 33°C. A dynamic time sweep (9000 s) analysis at angular frequency of 5 rad/s and 1% strain was used to analyse the changes in storage modulus (G') as the milk coagulated following a published protocol (Ong, Dagastine,

Kentish, & Gras, 2012). The gelation time was defined as the time taken for each sample to reach G'=5Pa.

Gel syneresis was defined as the ratio of the liquid mass expelled during centrifugation (Heraeus Biofuge Primo, Germany) at 1449 g for 20 min and the weight of the original sample (Zisu et al., 2011).

The gel strength of the samples was determined using a TAXT2 texture analyzer (Stable Micro Systems, Godalming, England) equipped with 2 kg load cell and a cylindrical acrylic probe (2 cm in diameter and 35 mm in height) as described by Ong, Dagastine, Kentish, & Gras, (2011). All samples were analysed 4 h after the gel had set. For the rennet gels, this was 4 h and 45 minutes after rennet addition, while for the acid gels this was a total of six hours after acid addition. A test speed of 1 mm/s was used to compress the sample to 50% of the original height (30 mm). The yield stress was defined as the force required to deform or fracture the sample. The maximum force measured was used as a measure of gel strength. A total of three gel samples were analysed for each processed milk preparation.

The gel samples formed from the process milk were prepared for observation by Confocal Laser Scanning Microscopy (Leica Microsystems, Heidelberg, Germany), as previously reported in Ong et al., (2011). Fat globules were stained with Nile red and protein stained with FCF fast green. The MFGM was stained with lectin wheat germ agglutinin WGA488.

A one way ANOVA with 95% confidence interval was used to determine statistical significance, where $p<0.05$ were considered statistically significant.

3. Results and discussion

3.1.Effects of ultrasound and homogenisation on the raw ingredients

3.1.1. Raw Milk (~4% fat) & UF-Retentate (~8% fat)

The D[4,3] of fat globules in raw and sonicated milk are presented in Table 2. Native raw milk had a D[4,3] of ~3.8 μm . With prolonged sonication at 31 W for 30 min, the value decreased to 1.5 μm, reflecting smaller fat globules. Increasing the power level at the same time interval further decreased the size of fat globules, giving a D[4,3] of 160 nm. Most importantly when the energy densities were kept constant at ~153 J/ml during sonication at 31 W and 50 W for 1 min, the milk solutions contained fat globules with similar D[4,3] of ~3.7 μm and ~3.6 μm, respectively. These data are consistent with previous observations using micro fluidisation or homogenisation, where particle size ranges from 2 μm to 0.5 μm were reported depending on the intensity of the process (Hayes et al., 2005; Lemay et al., 1994; Michalski et al., 2002a, 2004; Thiebaud, Dumay, Picart, Guirand, & Cheftel, 2003; van Hekken et al., 2007). Sonication induced similar changes in the size of fat globules in UF retentate (Table 2). The fat globules present in the UF solution were a little larger than the fat globules present in raw milk. This may be due to the incorporation of some whey proteins onto the MFGM surface under the shear forces involved in membrane processing (Ye, Anema, & Singh, 2004). Alternatively, the higher viscosity of the UF retentates as compared to raw milk may have meant that reduced shear forces acted on the fat globules. Increasing sonication time or power decreased the average fat globule size.

Table 2 here

Figure 1 shows CLSM images of the fat globules present in (A) raw milk & (B) UF retentate before and after sonication. These images confirm the presence of the disrupted fat globules and smaller fat globules following sonication. The CLSM images also show that the disrupted MFGM is coated by a thick layer of casein/casein micelles (Fig 1Aii and Bii). Increasing the

treatment time and power level leads to the complete disruption of the MFGM, leading to the fat globules being fully coated by proteins. This was especially notable at 50 W/ 30 min (4620 J/ml). The CSLM data are consistent with the particle size data presented in Table 2. Acoustic cavitation generated through sonication results in physical effects such as shockwave formation and high turbulence (Chandrapala et al., 2011). The resultant large shear forces break down the fat globules.

3.1.2.Cream

Table 2 shows the D[4,3] of fat globules present in cream samples sonicated at 50 W for different times below 10 ^{o}C and at 50 ^{o}C. The native cream sample showed a D[4,3] of ~4.0 µm. Sonication for 30 s below 10 ^{o}C led to an increase in D[4,3] suggesting flocculation of the fat globules. However, prolonged sonication or with the addition of SDS led to decreased D[4,3] values (Table 2). These results suggest that sonication at shorter times result in flocculation of fat globules; but that these aggregates are readily dispersed either by prolonged sonication or the use of SDS. These results can be confirmed by CSLM images (Fig 2A). CSLM images showed flocculated aggregates 30 s of sonication but these disappeared at longer times. Images of fat stained with WGA 488 for the MFGM also show that these flocculated fat globules have lost their MFGM and are coated by milk proteins (Fig 2B). At this stage, it is not fully understood as to why the fat globules flocculate at shorter sonication times but it can be suggested that shorter sonication times break down the MFGM so that the fat globules lose their stability. These disrupted fat globules can attach to proteins and form protein-protein interactions that cause clumping. However, prolonged sonication can break down these flocculated fat globules through the strong shear forces generated through acoustic cavitation.

Figure 2 here

Figure 3 shows CLSM images of cream samples sonicated under the two temperature conditions (<10 ˚C and 50 ˚C). The flocculated grapelike structures were observed in samples sonicated at low temperature, whereas sonication at high temperature resulted in smaller globule sizes. These differences are also clear from the size distributions of the fat globules shown in Figure 4. Tunick et al., (2000) also found a greater reduction in fat globule size with increasing temperature during microfluidisation and attributed this to the physical state of the fat. Much of the fat is in the solid state when sheared at lower temperatures whereas at high temperatures the fat is in the liquid state and thus can be easily fragmented into tiny droplets. Furthermore, at higher temperatures, an increase in the number of cavitation events per unit time is usually observed, although the collapse is less violent (Kentish and Feng, 2014).

Figure 3 here

Figure 4 here

Cream samples conventionally homogenised at similar temperatures and energy densities were also analysed (Fig 5). In this case, homogenisation at high temperature resulted in the formation of the flocculated fat globule structures, whereas these were absent at the lower temperature. These results are again reflected in the particle size distribution (Fig 4). Native cream samples at 50˚C showed a peak at ~ 5 μm with a small shoulder at 1 μm. High temperature homogenisation led to an increased particle size of ~50 μm. In contrast, low temperature homogenisation led to only a slight broadening of the central peak at 5 μm and an increase in the shoulder at 1 μm. A broadening of the size distribution was also observed for single stage ultrahigh pressure homogenisation of warmed milk at 300 MPa (Thiebaud et al., 2003). The authors of this prior study suspected that the formation of larger particles was due to unfolding and aggregation of whey proteins at the surface of the newly created

droplets. Cream samples conventionally homogenised at 50° C contained a large amount of 10-100 µm particles (Fig 4). It should be noted that Koh et al., (2014) showed that cavitation did not occur during conventional homogenisation with the conditions used here.

Figure 5 here

3.2.Effects of ultrasound and homogenisation on rennet- and acid- induced gelation properties

The addition of homogenised or sonicated cream into cheese milk reduced the rennet gelation time by more than half, relative to the use of native cream ($P < 0.05$) (Table 3). The rennet-induced gelation time of the control batch was ~40 min whereas homogenisation and sonication gave gelation times of 12 and 15 min, respectively. In contrast, there was no statistically significant change for acid gelation. Zamora et al. (2006) also found that the use of a homogenised cheese milk results in a significantly lower rennet clotting time (RCT) relative to raw milk. The lower RCT of homogenized milks could be explained by the fact that most κ-casein is located on the micelle surface. As the casein enrobes the fat globules, the κ- casein level is effectively diluted and a smaller critical level of κ-casein hydrolysis is required to start coagulation (Guinee et al., 1997). Furthermore, homogenization increases the surface area of available casein, making the κ-casein more available for chymosin action and thus, reducing the RCT (Ghosh, Steffl, Hinrichs, & Kessler, 1994).

Table 3 here

Gel strength and yield stress did not change, within experimental error (Table 3), when homogenised or sonicated cream was used. This is in contrast to other work such as that by Ghosh et al., (1994), Humbert. Drion, Guerrin, & Alais, (1980), Lemay et al., (1994), Robson & Dalgleish, (1984) & Zamora et al. (2006). These workers observed a loss in curd firmness when homogenisation or microfluidisation was used. The loss of gel strength was attributed to a greater dispersion of fat in the curd, to a reduced number of casein particles available to

form a strong network, or to the small fat globules that are entrapped in the gel disrupting the continuity of gel structure and acting as weak centres in the gel.

Syneresis was lowered by more than half with conventional homogenisation in comparison to the control sample for the rennet gel. Sonication also led to a significant decrease in syneresis as compared to the untreated control. This decrease in syneresis is consistent with the literature. Bermudez-Aguirre & Babosa-Canovas (2010) found less syneresis for *queso fresco* cheese made using sonicated milk compared to cheese made from thermally treated milk and syneresis decreased further with increase in sonication time. The decrease was attributed to disrupted fat particles and a reorganization of the proteins to form protein-fat complexes within the cheese matrix.

Figure 6 shows a typical G' vs time plots for rennet and acid gels prepared using native, homogenised or sonicated cream. Sonication and homogenisation both led to increased G' compared to the control for rennet gels, with the highest G' measured with homogenisation. Van Hekken et al. (2007) found that micro fluidization of cheese milk resulted in Mozzarella cheeses that showed more elastic than viscous properties, indicating that their internal structure stretched more and flowed less when subjected to a low strain. This is in agreement with our own results. Others find that if the interfacial material on the fat globule interacts with the casein network, G′ increases with the volume fraction (Cho et al., 1999) of these globules and as their size decreases (Xiong, Aguilera, & Kinsella, 1991; Zhou & Mulvaney, 1998). Thus, fat globules covered by caseins increase the modulus of rennet gels but the yield stress is not significantly different.

Conversely, changes in G' were insignificant for the acid induced gels. During acidification, the net negative charge of the casein micelles is neutralized, causing a reduction in the

amphiphilic character of the β and κ caseins and increasing the solubility of calcium phosphate. Electrostatic repulsion between the micelles is weakened and α_s caseins depolymerise leading to aggregation and formation of chains and clusters linked together as a three dimensional network. In contrast, rennet coagulation is primarily through enzymatic hydrolysis, where κ-casein is cleaved by rennet at the Phe_{105}-Met_{106} bond, resulting in altered casein micelles that are susceptible to aggregation. Rennet gelation occurs earlier in Figure 6, as it is less dependent on pH and the three dimensional network forms faster compared to acid gelation. The slower acid gelation time (~1500 s compared to ~900 s) possibly allows casein particles and fat globules to re-arrange within the acid gel, increasing the contact between casein particles and leading to a gel with a higher storage modulus, regardless of fat globule size.

Figure 6 here

Figure 7 here

Figure 7 shows the CSLM images of the (A) rennet and (B) acid gels. Each fat globule retained its spherical globular structure within the rennet gel. The casein networks appear as strands of aggregated casein micelles with entrapped lipid droplets. The rennet gels appear more cohesive than the acid gels, with fewer and larger pores, consistent with observations in the literature (Green, Hobbs, Marant, & Hill, 1978).

Interestingly, some of the grapelike structures observed initially within the sonicated cream samples persist within the gel network. However, some of the larger fat globule aggregates do not appear to interact with the casein network (Fig 7Aiii and Biii). Conversely, the fat globule aggregates in the homogenised sample appear better integrated (Fig 7Aii and Bii). Visually, the sonicated gels also showed a fat layer on the surface indicating the separation of coalesced fat globules. A lower sonication power may have the potential of preventing this

fat separation while still achieving an improved syneresis and shorter processing time, consistent with the homogenised sample.

The localised high temperature conditions generated during acoustic cavitation can lead to the formation of radicals in some conditions (Ashokkumar et al., 2008). There is a possibility that these radicals may oxidise lipids in the system. However, Ashokkumar et al. (2008) have reported that generation of such radicals at 20 kHz is insignificant due to the low number of active cavitation bubbles. Similarly, Juliano et al., (2014) have studied the generation of volatile compounds by lipid oxidation in raw and heat treated milk samples subjected to sonication across a wide range of frequencies from 20 kHz to 2000 kHz. They found no oxidative volatile compounds below 230 J/ml in batch systems using 20 kHz. The present study used an energy density of ~153 J/mL. It thus can be assumed that oxidation of lipids did not occur under these experimental conditions.

4. Conclusion

This work has shown that the use of sonication for the reduction in milk fat globule size has comparable effects to homogenisation. An increase in the elastic modulus and a decrease in syneresis are observed for cheddar cheese gels prepared using this approach. Interestingly, our results show that low temperature sonication gives similar structural changes to the fat globules to that obtained using high temperature homogenization under the same energy density conditions (~153 J/ml). Homogenisation is currently conducted at 50˚C as a common practice, as the higher temperature liquifies the fat globules, resulting in greater reduction of fat globule size. The use of sonication at low temperature may offer major benefits, as it permits manufacturers to use low temperature sonication in place of high temperature

388 homogenisation to achieve similar size reductions; as the total energy demand would
389 decrease. However, in the present case, the use of such sonication led to fat separation from
390 the gel structure rather than an effective incorporation. Hence, careful consideration of
391 processing parameters is needed.
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Table Captions

Table 1: Composition of milk, retentate, concentrate and cream

Table 2: D[4,3] of fat globules present in untreated/treated raw milk, UF retentate and cream samples

Table 3: Gel properties of rennet and acid gels made using three different cheese milks

Figure Captions

Fig. 1. CLSM of native and sonicated (A) raw milk & (B) UF retentate under two different power levels as a function of treatment time. (i) The Nile red stained fat globules appear red and the FCF fast green stained protein appears green. (ii) The WGA488 stained MFGM appears red and the FCF fast green stained protein appears green.

Fig. 2. CSLM of sonicated (50 W) cream under different time intervals. (A) Nile red stained fat globules appear red and the FCF fast green stained protein appears green. (B) The WGA488 stained MFGM appears red and the FCF stained protein appears green. The images were taken using 100x objective lens with 2x (A-bottom & B-top row) and 4x (A-top & B-bottom row) digital magnifications. Thick arrows indicate grape like fat globule structure. Thin arrows indicate native MFGM.

Fig. 3. CLSM of cream sonicated at different times under two temperature conditions <10˚C and 50˚C. The Nile red stained fat appears red and the FCF fast green stained protein appears green.

Fig. 4. Particle size distribution of the fat globules present in native cream (▲), cream which is homogenised at 10°C (■) and at 50°C (□) and cream samples which are sonicated at 10°C (•) and at 50°C (o) under the same energy density conditions (153 J/ml).

554 **Fig. 5**. CLSM of cream homogenised at (i) <10˚C & (ii) 50˚C for (A) the Nile red stained fat
555 globules appear red and the FCF fast green stained protein appears green and (B) WGA488
556 stained MFGM appears red and the FCF stained protein appears green. The images within
557 (A) and (B) were taken using 100x objective lens with 2x and 4 x digital magnifications for
558 top and bottom images respectively.

559 **Fig. 6**. Storage module (G') of different cheese-milk preparations during gelation for (A)
560 rennet-induced gel and (B) acid-induced gel. Black line: SM, SMC and native cream
561 (control); Light grey line: SM, SMC with sonicated cream (50W/1 min at <10˚C); and Dark
562 Grey line: SM, SMC with homogenised cream (80 bar at 50˚C). The result presented is a
563 representative graph of three trials.

564 **Fig. 7**: CSLM of (A) rennet-induced & (B) acid-induced gels formed using 3 different
565 cheese-milk systems. (i) SM, SMC and native cream (control), (ii) SM, SMC with
566 homogenised cream (80 bar/ at 50˚C) and (iii) SM, SMC with sonicated cream (50W/1 min at
567 <10˚C). The Nile red stained fat appears red and the FCF fast green stained protein appears
568 green. The images within (A) and (B) were taken using 100x objective lens with 2x and 4 x
569 digital magnifications for top and bottom images, respectively.

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Figure 1a
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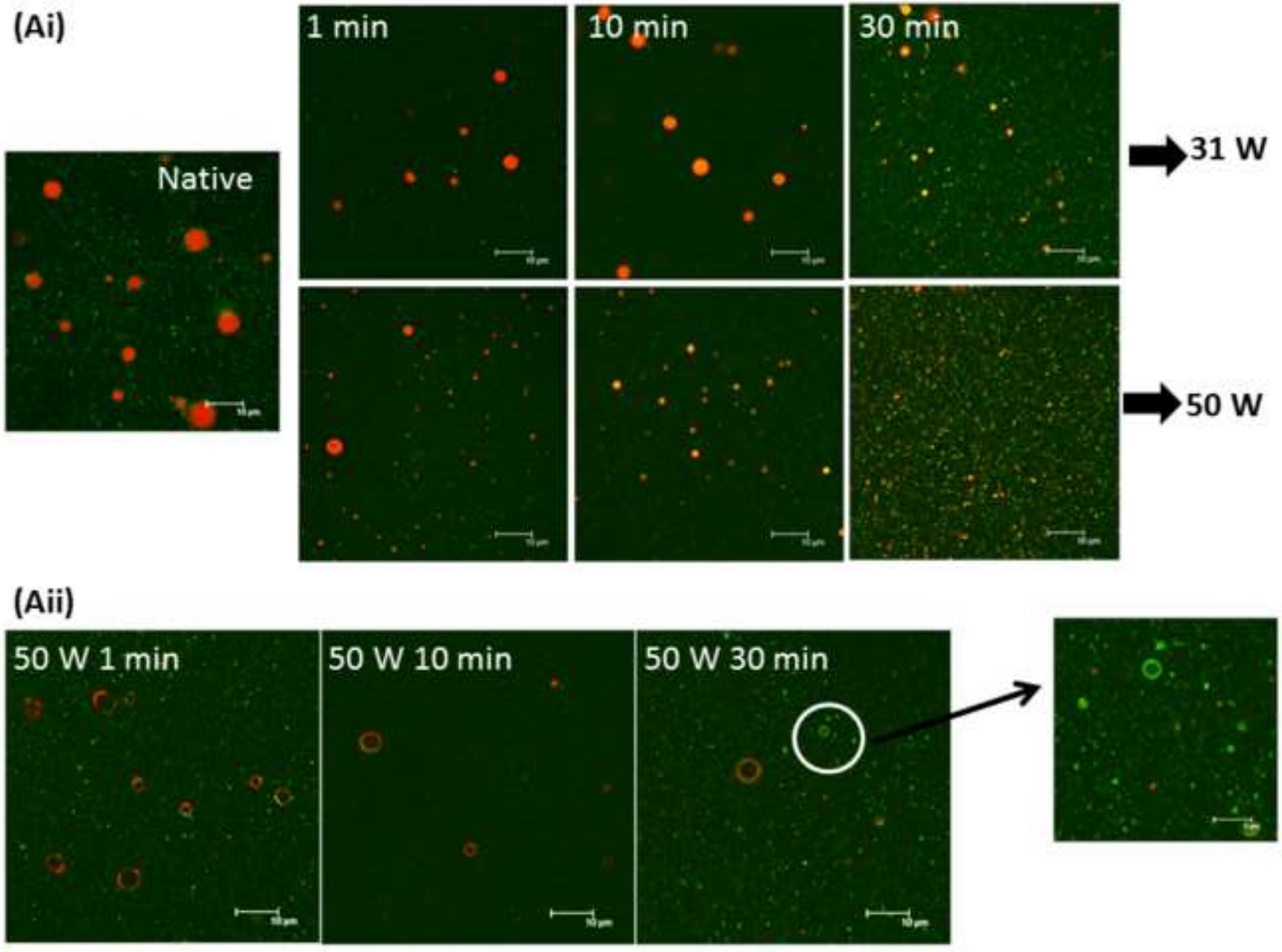
Figure 1



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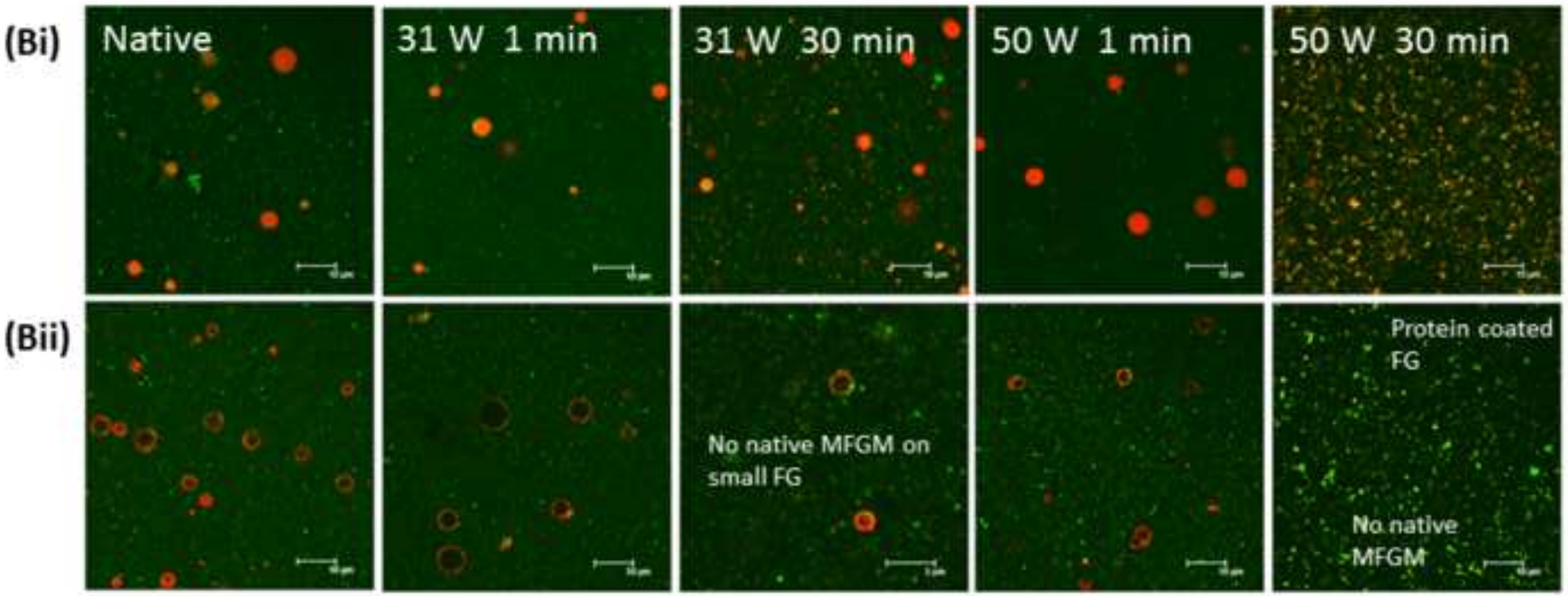

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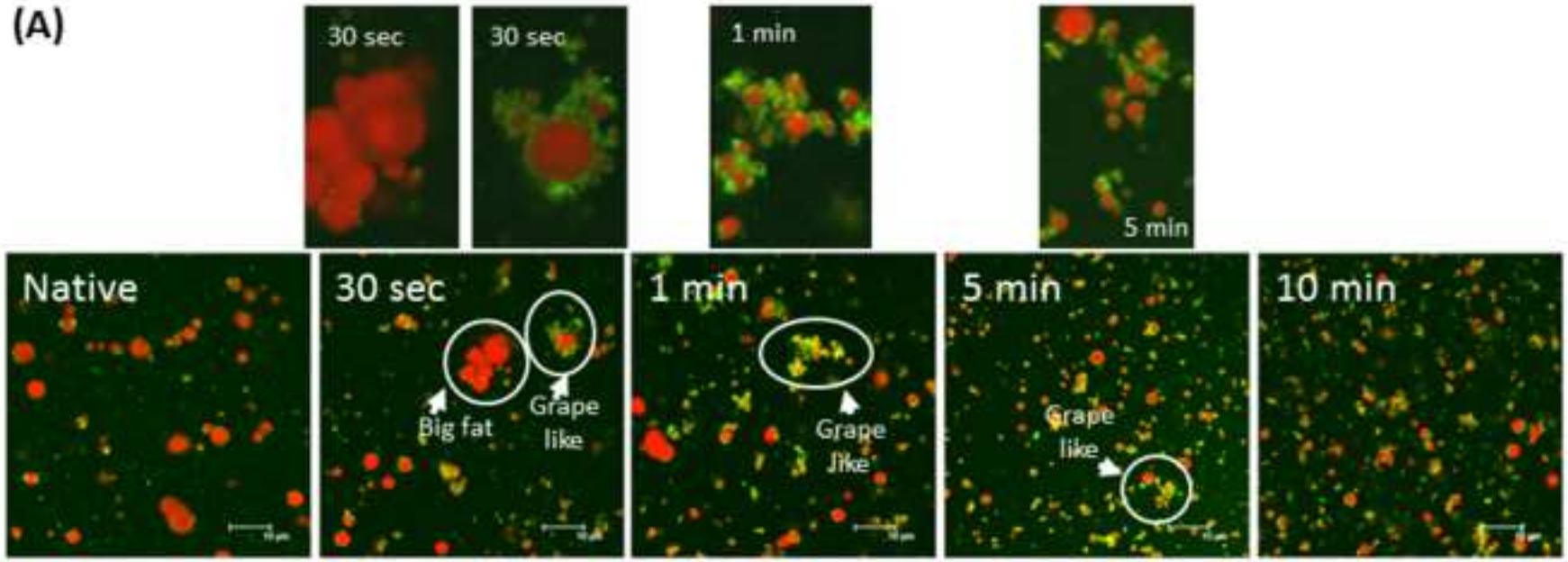

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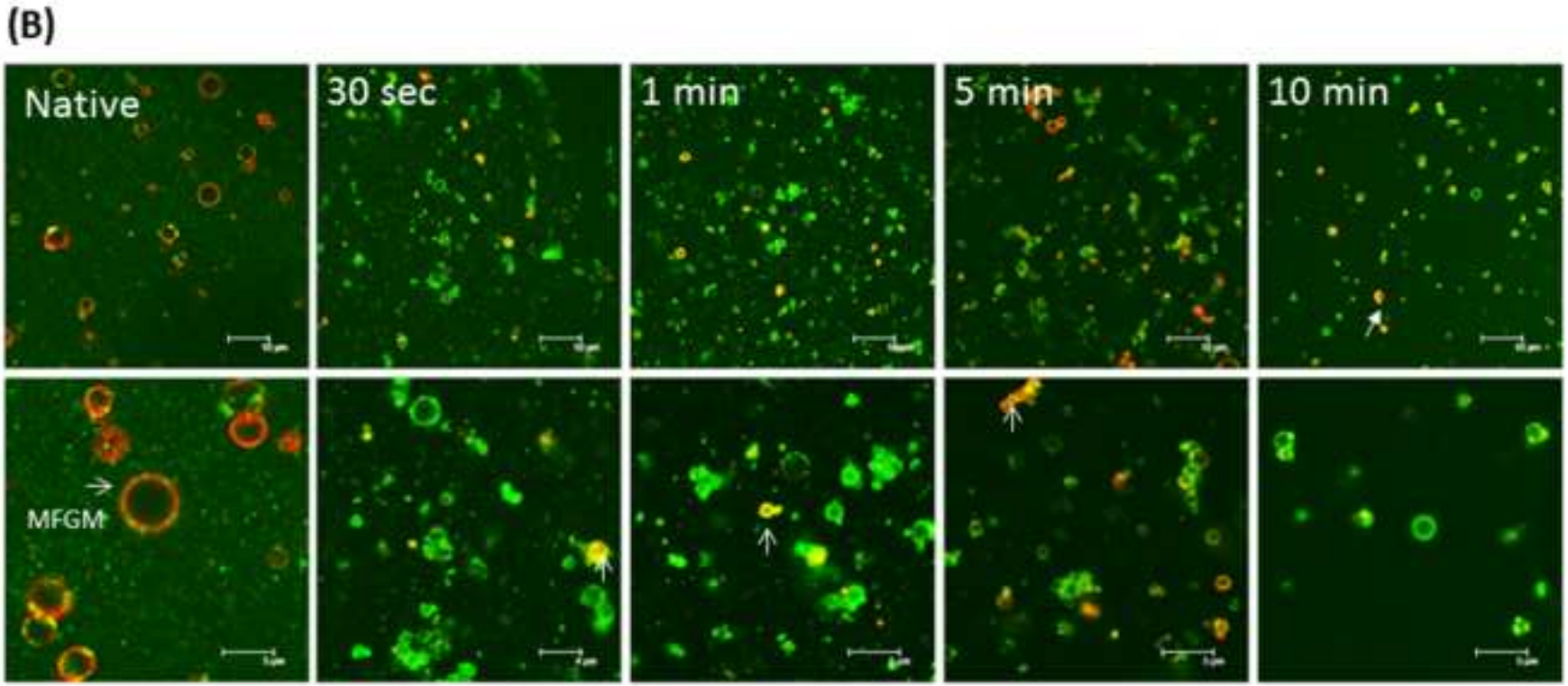

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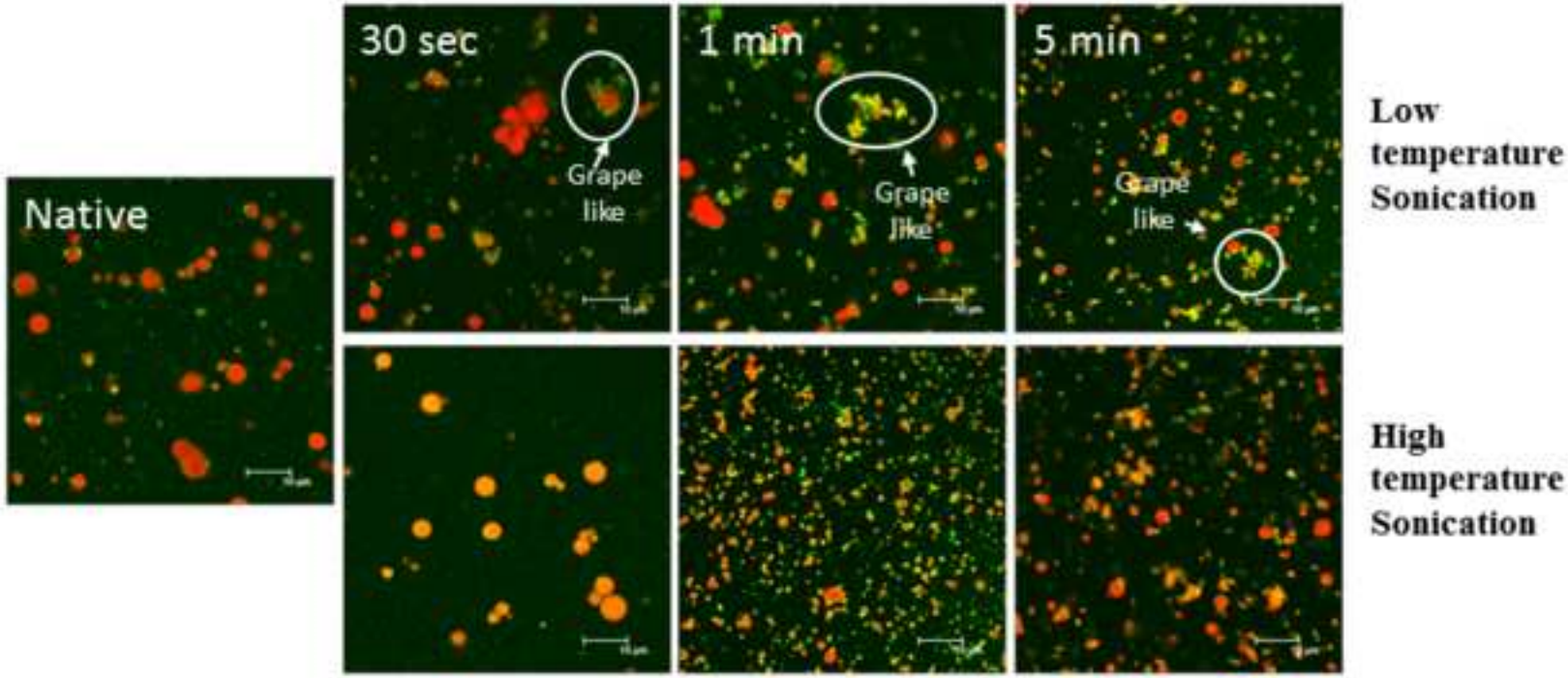

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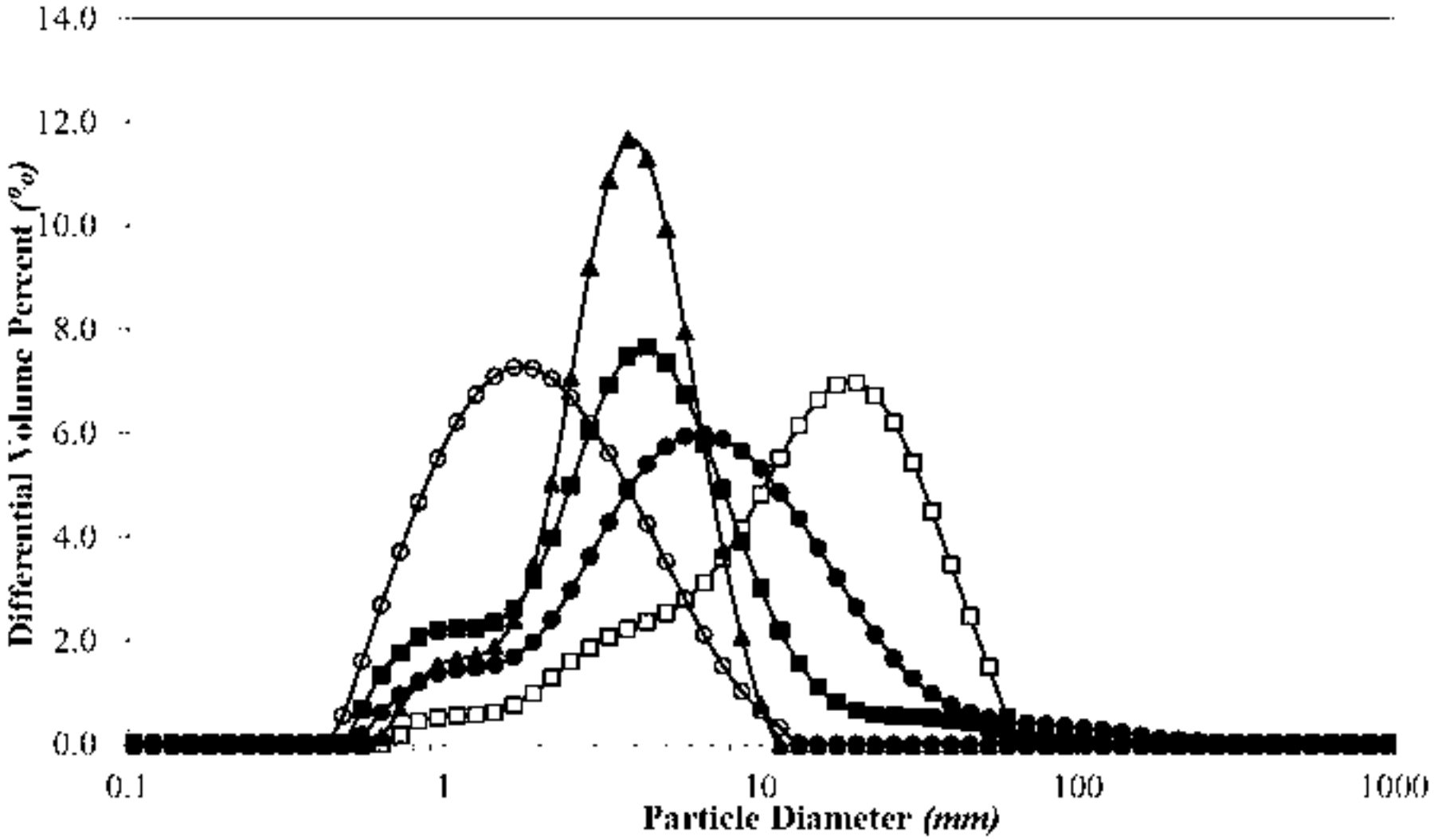

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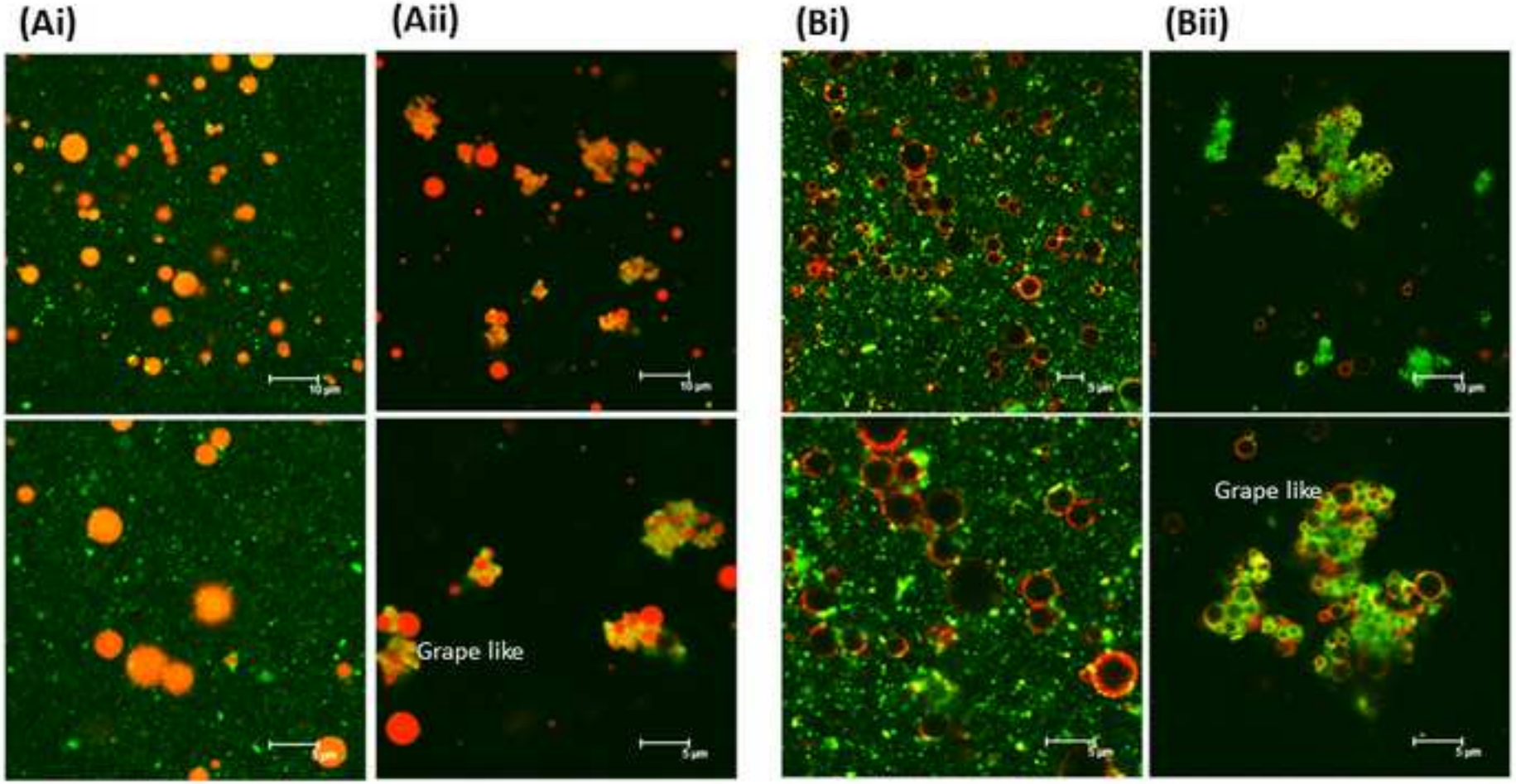

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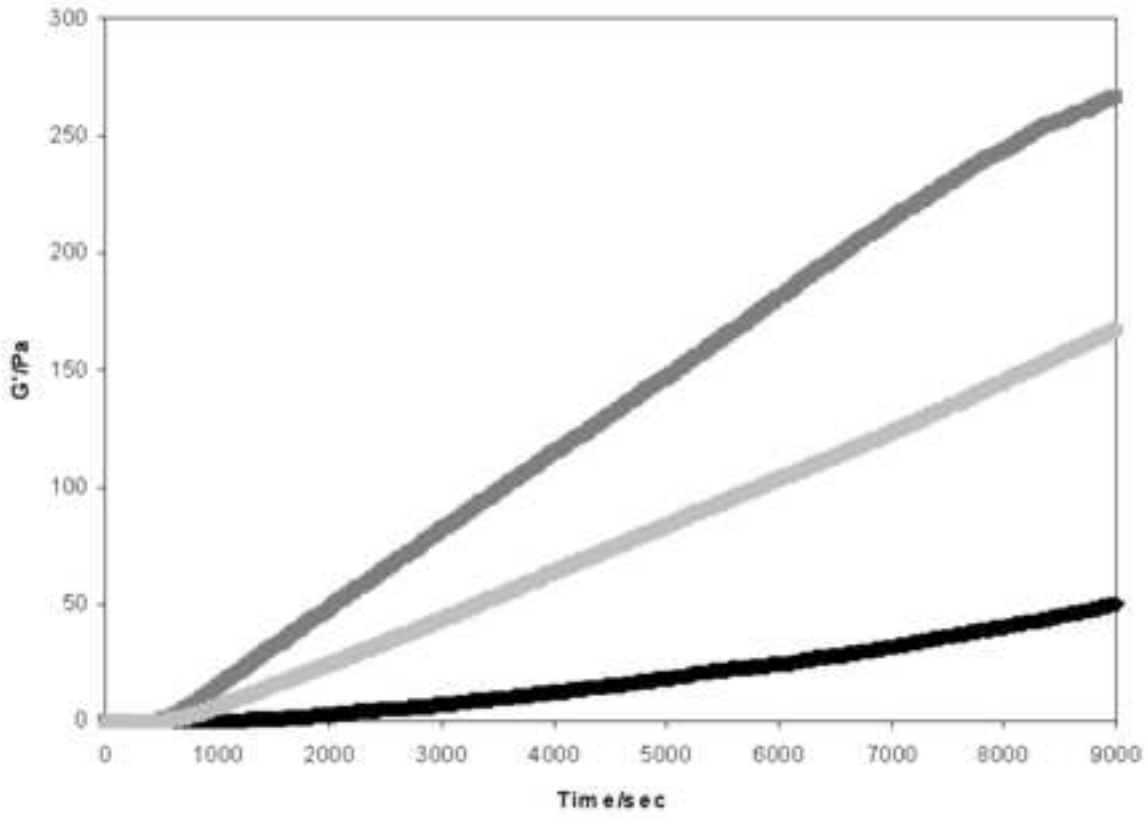

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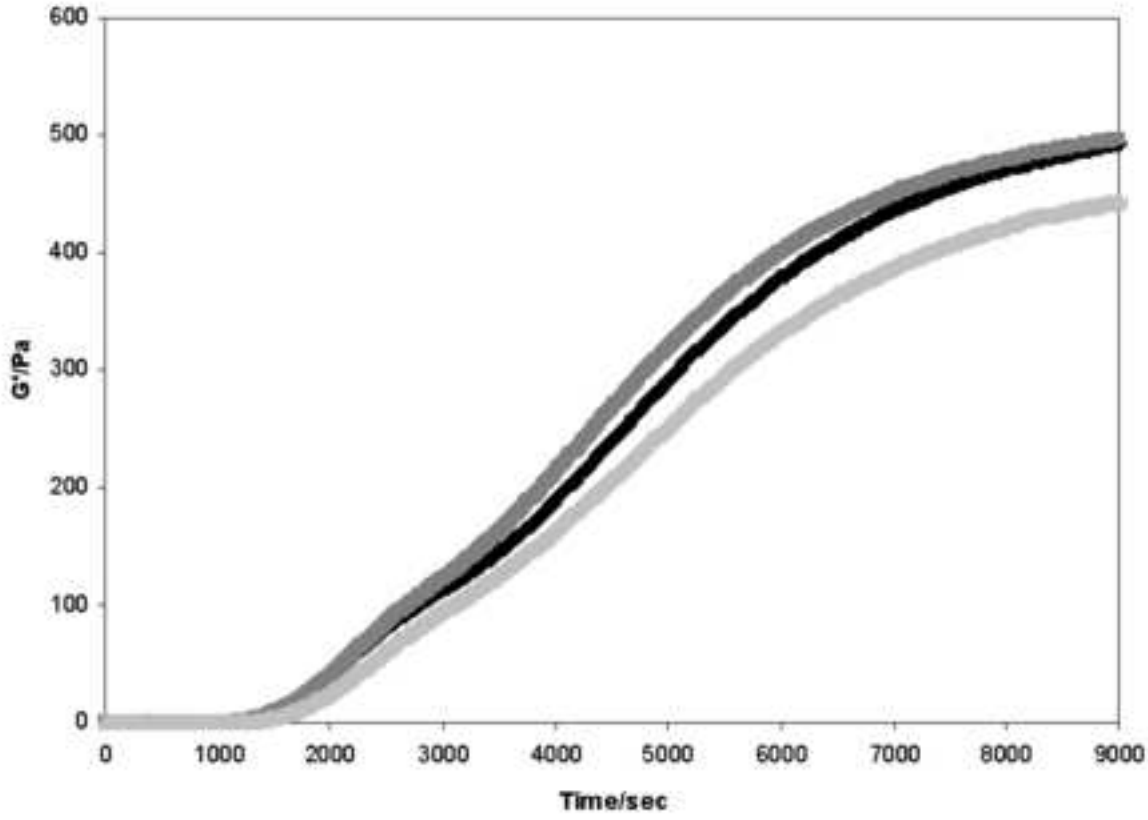

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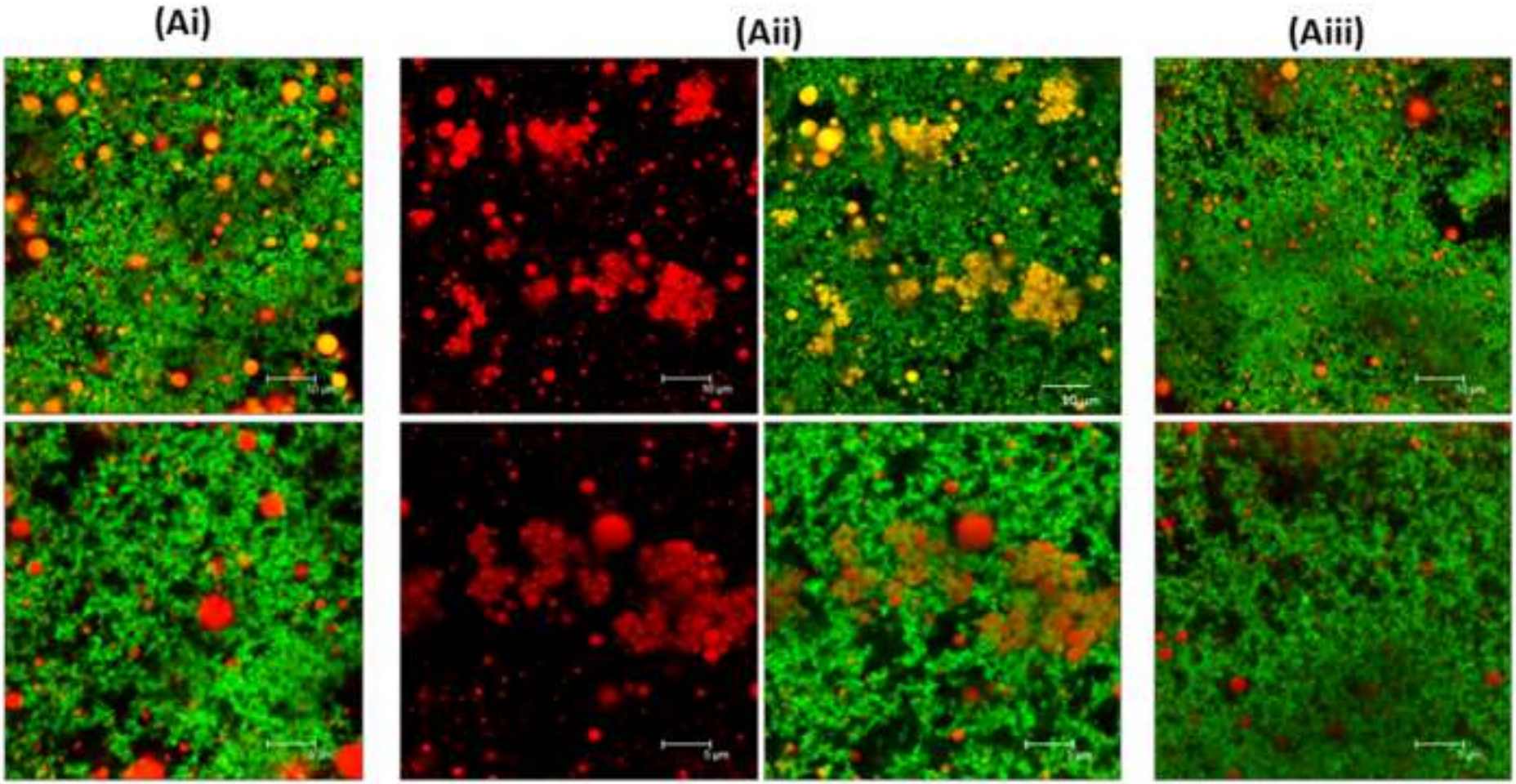

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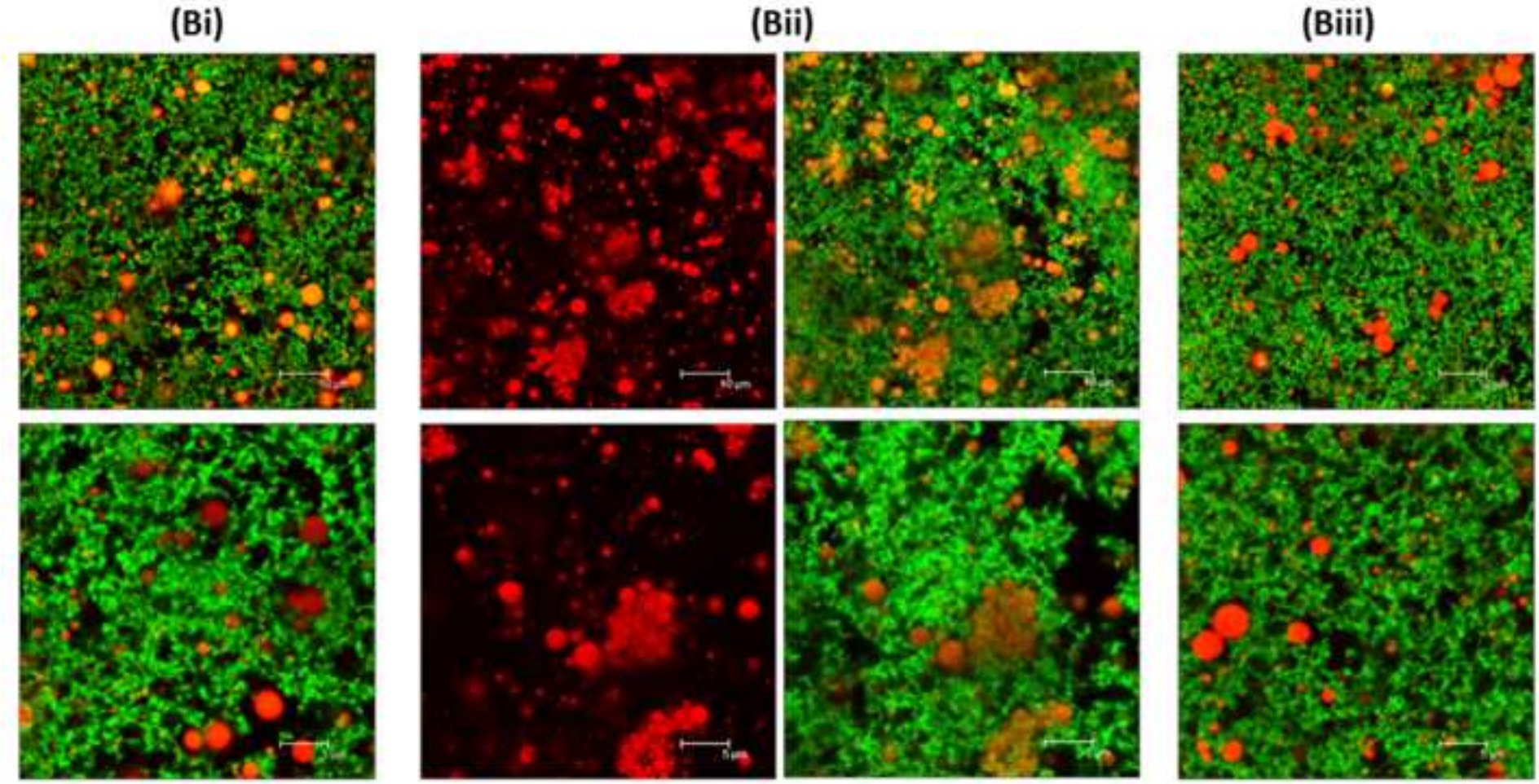

Table

Table 1: Composition of milk, retentate, concentrate and cream

Composition	Raw milk	UF retentate	SMC	Skim milk	Cream
Protein	3.6 ± 0.2	7.1 ± 0.4	13.8 ± 0.1	3.68 ± 0.11	2.1 ± 0.2
Fat	4.2 ± 0.2	8.2 ± 0.6	0.22 ± 0.03	0.08 ± 0.01	42.0 ± 1.0

Data are mean ± standard deviation of mean (n = 3). SMC = Skim Milk Concentrate

Table 2: D[4,3] of fat globules present in untreated/treated raw milk, UF milk and cream samples

Treatment			Raw Milk	UF Retentate	Cream Without SDS	Cream With SDS
Native			3.76 ± 0.04	4.19 ± 0.05	4.03 ± 0.08	
31 W	**1min**	**50°C**	3.68 ± 0.03	4.05 ± 0.04		
	10 min	**50°C**	3.69 ± 0.04	3.88 ± 0.04		
	30 min	**50°C**	1.49 ± 0.02	2.96 ± 0.03		
50 W	**30 sec**	**50°C**			2.4 ± 0.4	
	1 min	**50°C**	3.63 ± 0.03	4.11 ± 0.03	2.3 ± 0.2	
	10 min	**50°C**	1.30 ± 0.01	0.26 ± 0.01	1.63 ± 0.09	
	30 min	**50°C**	0.16 ± 0.002	0.16 ± 0.003		
50 W	**30 sec**	**<10°C**			24.4 ± 1.1	9.2 ± 0.9
	1 min	**<10°C**			10.9 ± 0.9	5.25 ± 0.13
	5 min	**<10°C**			5.50 ± 0.06	2.16 ± 0.05
	10 min	**<10°C**			3.35 ± 0.04	1.58 ± 0.04

Data are mean ± standard deviation of mean ($n \geq 3$).

Table 3: Gel properties of rennet and acid gels made using three different cheese milks

Measurement	Control	Homogenisation	Sonication
Rennet			
Yield Stress/g	4.4 ± 0.2	4.1 ± 0.4	3.8 ± 0.5
Gel Strength/g	5.5 ± 0.2	5.1 ± 0.3	4.9 ± 0.4
Gelation times/min	41 ± 1^{a}	12.1 ± 0.8^{b}	15.1 ± 1.1^{c}
Syneresis/% w/w	60 ± 3^{a}	21 ± 3^{b}	37 ± 7^{c}
Final G'/Pa	50 ± 3^{a}	267 ± 10^{b}	168 ± 7^{c}
Acid			
Yield Stress/g	16.4 ± 1.3	17.1 ± 1.1	16.8 ± 0.9
Gel Strength/g	19 ± 2	21 ± 1	17 ± 1
Gelation times/min	22 ± 2	22 ± 1	24 ± 1
Syneresis/%	24 ± 2	20 ± 2	18 ± 2
Final G'/Pa	493 ± 13	497 ± 15	443 ± 15

^{abc} Results with different superscripts are significantly different ($P < 0.05$)