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# Ultrasound homogenization of milk cream at low temperature 

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## KEYWORDS

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#### Abstract

Ultrasonication has been identified as a particularly promising technology for homogenization of dairy products. Homogenization of cream, by reducing fat globule size, can be utilized to inhibit creaming. The homogenization of cream usually leads to increased viscosity. Cream with fat level greater than $17 \%$ cannot be homogenized with satisfactory results since conventional homogenization methods cause coalescence and mostly agglomeration. The aim of this study was to investigate the influence of ultrasonication on milk cream (5-30\% fat) and to study the phenomenon of formation of fat clusters during sonication ( $0.5-15 \mathrm{mins}$ ) at low temperature $\left(2^{\circ} \mathrm{C}\right)$. The results showed that ultrasonication can reduce the fat globule size, although it resulted in the formation of fat clusters at short time ( $<1 \mathrm{~min}$ ), but at longer time, fat clusters can be broken. On the other hand, ultrasound homogenization tends to increase the viscosity of cream at various fat contents. Microstructure of solid phase showed that there was formation of double emulsions and partial fat coalescence. Ultrasound homogenization with the addition of SDS as small-molecule surfactant can prevent the formation of fat clusters. Fatty acid composition in solid phase shows that it consists of long-chain fatty acids in higher amount compared to that present in the liquid fraction. Whereas the concentration of short and medium chain fatty acids in the liquid phase was higher compared to that in solid phase. The utilization and optimization of ultrasound for cream homogenization has a potency to solve the limitation of conventional method (pressure homogenizer) which commonly used in dairy industry.


## Introduction

Dairy cream is an emulsion of a concentrated (30$40 \%$ ) dispersion of fat globules. The average size of the native fat globules in bovine milk is around 4 $\mu \mathrm{m}$ with the range of $\sim 0.2 \mathrm{~mm}$ to 20 mm (Briard et al., 2003) and the size should be reduced to prevent creaming. The size of the fat globules is very important in the stability of milk. In addition, Wiking et al. (2004) pointed out that large fat globules are more susceptible to coalescence and lipolysis during some dairy processes. However, the homogenization of cream usually leads to increased viscosity. In homogenized cream, large agglomerates of fat globules, called homogenization clusters containing many fat globules, up to $10^{5}$, can be resulted (Walstra,

1999a). Casein micelles connect the fat globules in the cluster, and casein micelle-dissolving agents have been shown to disperse the clusters. Some conditions which promote the formation of homogenization clusters are high fat content, low protein content, high homogenizing pressure, a relatively high surface excess of protein which is stimulated by low homogenization temperature and intense preheating (Walstra, 1999b).

There are some limitations in conventional homogenization process used to improve dairy product properties; limits due to fat content (only less than $17 \%$ fat content by volume can be satisfactory processed) and fat globule size reduction (Köhler et al., 2008). Ultrasonication has been identified as a particularly promising
technology for processing specific food materials, including dairy products (Ashokkumar et al., 2010). The use of ultrasound is of great interest for milk homogenization to improve the product stability against creaming by reducing fat globule size (Bermúdez-Aguirre et al., 2008; Chandrapala \& Leong, 2015; Chandrapala et al., 2012; Ertugay et al., 2004; Gao et al., 2014; Nguyen and Anema, 2017; Riener et al., 2009; Sfakianakis et al., 2015; Shanmugam et al., 2012; Vercet et al., 2002; Vijayakumar et al., 2015; Villamiel and de Jong, 2000; Wu et al., 2000). Recently, two studies were reported on ultrasound homogenization of cream. Zisu and Chandrapala (2015) conducted research on ultrasound homogenization of high fat dairy systems (cream containing $43 \%$ fat) at 50 W under cold condition $\left(<10^{\circ} \mathrm{C}\right)$ and a frequency of 20 kHz and found that ultrasonication can be utilized as a homogenization process to reduce the fat globule in cream. However, problems occur during ultrasonication process at 30 seconds due to the formation of coalesced fat globules resulting in large fat clusters. The disruption of the large fat clusters to form smaller agglomerates starts after 1 minute and results in separated and homogenized fat globules into small individual ones within 5 min sonication. Large fat clusters formed during sonication in cold condition can increase the viscosity of the cream and may even produce highly viscous and slow flowing cream that covers the sonotrode. If this condition is uncontrolled, it may cause inefficiencies in the equipment operation and reduce the performance of the homogenization process. Chandrapala et al. (2016) compared homogenization using high-pressure and ultrasonication of milk products. Sonication of cream at $<10^{\circ} \mathrm{C}$ led to flocculation of the fat globules and formation of grapelike structures. This suggests a potential benefit to utilize sonication technology in allowing low temperatures for cream homogenization and reducing energy demand. Temperature and surfactant addition play important role in the final fat droplet size during homogenization. Temperature is connected to fat crystallization, and to the ability of casein micelles to spread at the oilwater interface (Walstra, 1999b) and influences the viscosity of the emulsions (Earnshaw et al., 1995). The ultimate size of a homogenized emulsion is driven by the balance between two opposing processes; droplet break-up and droplet recoalescence, and the surfactant plays a critical role in both processes. The small molecule surfactants have greater capability to adsorb rapidly to
interfaces and lower greatly the interfacial tension (Leong et al., 2009).

The aim of this work was to investigate the influence of ultrasonication on milk cream ( 5,10 , 20 , and $30 \%$ fat) and to study the phenomenon of formation of fat clusters during sonication ( $0.5,1$, $2.5,5,10$, and 15 mins ) at low temperature $\left(2^{\circ} \mathrm{C}\right)$. The effect of the addition of small-molecule surfactant were also examined. Particle size was measured using Laser Light Scattering (Mastersizer 2000) and viscosity was measured using Rheometer (MCR-301, Anton Paar), the microstructures were observed using Confocal Laser Scanning Microscopy (Olympus FV1000), and the fatty acid compositions were obtained using a GC-MS (Shimadzu GCMS-QP2010).

## Research Methods <br> Materials and sample preparation

Cream (Anchor Pure Cream, Fonterra New Zealand) was purchased from a local supermarket and immediately stored at $4^{\circ} \mathrm{C}$. Each 100 mL of cream consists of 2.4 g protein, 37.3 g fat, 3.0 g carbohydrate, and 25 mg sodium as provided by the manufacturer. Ultrapure (Double destilated) water was used at all times. Samples were original cream which diluted with ultrapure water to find desired fat contents of 5, 10, 20, and $30 \%$. Sodium azide (ECP, New Zealand) of $0.02 \%$ was added to prevent bacterial growth and the creams were stirred using magnetic stirrer for 5 mins.

## Ultrasonication

An ultrasonic processor VCX 750 (Sonics \& Materials, Inc., CT, USA) which equipped with a 13 mm diameter tip and working at a constant frequency of 20 kHz and amplitude of $50 \%$ was used for conducting ultrasonication. Samples (25 mL ) were placed in 40 mL glass vials ( 26 mm diameter and 94 mm height) and the ultrasound probe was immersed into the samples at the depth of approximately 1 cm . The vials were placed in a double walled glass vessel, which was connected to a circulating water bath (SD07R-20-A12E, PolyScience, USA) to control the temperature of the sample.

Samples were unsonicated and sonicated at constant temperatures of $2^{\circ} \mathrm{C}$ for different times of $0.5,1,2.5,5,10$, and 15 mins . The sonication was performed at $50 \%$ of the total power ( 750 W ) corresponding to a power drawn of 24.50 W which calculated using a calorimetric method (Contamine et al., 1995). Each sonication time resulted in different energy density. The energy density was calculated as follows.

Power Drawn $=\mathrm{m} \times \mathrm{c} \times \mathrm{dT} / \mathrm{dt}$
Energy density $\left(\frac{\mathrm{J}}{\mathrm{mL}}\right)=\frac{\text { Power drawn }(\mathrm{W}) \times \text { Time }(\mathrm{s})}{\text { Volume }(\mathrm{mL})}$.
Where $m$ is the mass of water $(\mathrm{g}), c$ is the specific heat of water ( $4.18 \mathrm{~J} / \mathrm{g} . \mathrm{C}$ ) and $d T / d t$ is the rate of temperature change with time. Using Eq (2), the energy density is $29.40,58.80,147.01,294.02$, 588.04, and $882.06(\mathrm{~J} / \mathrm{mL})$ for $0.5,1,2.5,5,10$, and 15 min sonication, respectively.

## Fat globule size measurement

The milk fat globule size distribution was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK), which uses $\mathrm{He} / \mathrm{Ne}$ laser ( 633 nm ) and an electroluminescent diode ( 466 nm ), fitted with a dispersing unit Hydro 2000 SM. The refractive index for fat globules is 1.456 (Ye et al., 2004) and for water is 1.33 and the particle absorption index was set at 0.01 . From the size distribution obtained with the Malvern software, the Sauter volume/surface diameter $\mathrm{D}[3,2]$ (also called surface-weighted diameter) was calculated (Michalski et al., 2002). All measurements were performed at least in duplicates and carried out at room temperature, and each sample was measured 5 times. The cream sample of 0.05 to 0.5 mL was diluted in 75 mL of milli-Q water in a dispersing unit until the signal showed that the quantity of the sample reached the required amount which depends on the fat content of each cream sample.

## Confocal Laser Scanning Microscopy (CLSM) observation

CLSM was performed on an Olympus FV1000 (Japan). Samples ( 1 g of solid phase or 1 mL of cream) were mixed with 100 mL Nile Blue ( $0.33 \%$ w/w solution in water), specific dye which stains fat, and 10 mL Fast Green FCF ( $10 \mathrm{mg} / \mathrm{mL}$ water) which stains protein. The stained samples were placed immediately on a glass microscope slide and a cover glass slip was placed on top of the sample. The sample was examined with a 60 x and 100x objective lens. Nile Blue was excited at a wavelength of 473 nm and the emission wavelength was 520 nm . Fast Green FCF was excited at a wavelength of 635 nm and the emission wavelength was 647 nm . The micrographs obtained show fat in a red color and protein in green.

## Fatty acid composition

The fatty acid analysis was based on the method of Sun and Zhao (2014), the standard solution is Supelco 37 Component FAME Mix (Sigma Aldrich, New Zealand) which is a mixture of 37 Fatty Acid Methyl Ester (FAME) with varied concentration (around $200-600 \mu \mathrm{~g} / \mathrm{mL}$ ) in dichloromethane. This standard was diluted 5, 10, 20,50 , and 100 times in hexane and spiked with $10 \%$ of biphenyl ( 200 ppm ) as internal standard. Samples were unsonicated cream and cream sonicated at $2^{\circ} \mathrm{C}$ for 1 min which separated into solid phase and liquid phase. Sample aliquots ( 0.25 g of unsonicated cream, 0.1 g of solid phase, and 0.5 g of liquid phase) was placed in a $15-\mathrm{mL}$ centrifuge tube with 10 mL of hexane and 1 mL of sodium methoxide ( 5.4 M ) in methanol solution. After strongly vortexing for 1 min , the samples were centrifuged at 3500 rpm for 10 mins to obtain clear layer of hexane. This supernatant was diluted 20 times in hexane and spiked with $10 \%$ biphenyl 200 ppm and loaded onto GC-MS. (Shimadzu GCMS-QP2010, Japan) on a DB-Wax column. The temperature program of the oven was as follows: $45^{\circ} \mathrm{C}$ maintained for 5 min , then raised to $200^{\circ} \mathrm{C}$ at $10^{\circ} \mathrm{C} / \mathrm{min}$, finally raised to $230^{\circ} \mathrm{C}$ at $3^{\circ} \mathrm{C} / \mathrm{min}$ and maintained for 23 mins . Running time was 53.5 mins and equilibration time was 0.5 mins. Sample ( $1 \mu \mathrm{l}$ ) was injected in split-less mode. The injector temperature was set at temperature of $250^{\circ} \mathrm{C}$. The flow of helium was constant at 1 $\mathrm{mL} / \mathrm{min}$. The temperature of transfer line and the ion source was set at $250^{\circ} \mathrm{C}$ and $230^{\circ} \mathrm{C}$, respectively. The scan range covered $\mathrm{m} / \mathrm{z}$ of 46-500 u.

## Results and Discussion <br> Average size of fat globule

The fat globule size of creams with different fat content as a result of sonication at $2^{\circ} \mathrm{C}$ is shown in Figure 1. The fat globule size of unsonicated cream was $3.93 \pm 0.10 \mu \mathrm{~m}$. The size of a fat globule in cow milk is in the range of under 0.2 to over $10 \mu \mathrm{~m}$ in diameter, with almost $90 \%$ of the total volume of milk fat having globule size between 1 and $8 \mu \mathrm{~m}$ (Keenan and Patton, 1995), with an average diameter of $4 \mu \mathrm{~m}$ (Briard et al., 2003).

The fat globule size of cream decreased during sonication. The fat globule size decreased sharply until 5 mins of sonication and then remained constant (Figure 1). For example, the fat globule sizes of $30 \%$ cream were $3.84 \pm 0.01,0.24 \pm 0.01$, and $0.23 \pm 0.01 \mathrm{~mm}$ for unsonicated cream, cream sonicated for 5 and 15 mins , respectively.


Figure 1. Average size of fat globule, D[3,2] of cream with various fat contents. Symbols are: 5\% fat cream $\bullet, 10 \%$ fat cream •, 20\% fat cream • and $30 \%$ fat cream $\bullet$. For 20 and $30 \%$ fat cream sonicated for 0.5 and 1 min , it was measured from liquid phase. Error bars correspond to standard deviations from duplicates.

Cavitation generated during sonication in the fluid is one of the reasons for the homogenizing effects of ultrasound (Ertugay et al., 2004). The homogenizing effect increased with sonication time, as previously reported in the case of milk fat (Chandrapala et al., 2012; Ertugay et al., 2004; Gao et al., 2014; Nguyen and Anema, 2017; Shanmugam et al., 2012; Wu et al., 2000).

Sonication tends to result in smaller fat globule size for cream with less fat content at short time ( 2.5 min ). The viscosity of the liquid plays an important role as it affects ultrasound cavitation. In highly viscous products, the disruption of ultrasound diffusion is easily occurred and it decreases the level of cavitation (Earnshaw et al., 1995). Cream with higher fat content has higher viscosity compared to those with lower fat content. At longer sonication time, it leads to the same result for all fat content which can reach the size around $0.3 \mu \mathrm{~m}$. Zisu and Chandrapala (2015) found that sonication of milk containing $3.5 \%$ fat at a frequency of 20 kHz decreases fat globule size in response to time and changes its distribution. Comparable effects are detected when sonicating milk-based solutions comprising up to $8 \%$ fat.

Sonication at low temperature and short time $\left(2^{\circ} \mathrm{C}\right.$ for 0.5 and 1 min$)$ leads to the formation of large fat clusters. The fat clusters formed at low temperature can be disrupted at longer sonication time, leading to smaller fat globule size at $\geq 5 \mathrm{mins}$. These results are similar to those reported by Zisu and Chandrapala (2015) which found that sonication of cream containing $43 \%$ fat at 50 W $\left(<10^{\circ} \mathrm{C}\right)$ and a frequency of 20 kHz for 30 seconds results in the formation of large fat clusters. The
beginning of disruption of the large fat clusters occurs at sonication for longer times ( 1 min ) creating smaller agglomerates until the fat globules were ultimately separated and reduced to small individual fat globules at 5 min . These data can be explained by the role of temperature in determining fat globule size. If the temperature is very low, homogenization is poor since part of the fat is crystalline, which results in partial coalescence of the fat droplets. Temperature also related to surface layers which cover fat. The caseins, likely present in the cream, would spread more rapidly over the fat-water interface at higher temperature (Walstra, 1999b).

## Relative Apparent Viscosity

Figure 2 shows the relative apparent viscosity of cream at various fat contents and sonicated at $2^{\circ} \mathrm{C}$. When sonicated for short time ( 0.5 and 1 min ), the viscosity of creams increased markedly. For example, for $30 \%$ fat cream sonicated for 1 min , the viscosity was $6.635 \pm 0.233$ Pa.s. At longer sonication time ( 5 min ), the viscosity decreases from the high value reached when sonicated for short times. However, the final viscosity at the longest sonication time ( 15 min ) remained higher than those of unsonicated cream. Before sonication, the viscosity was $0.018 \pm 0.0004,0.019 \pm 0.0004$, $0.016 \pm 0.003$, and $0.028 \pm 0.010$ Pa.s for $5,10,20$, and $30 \%$ fat creams, respectively; and after 15 min sonication the viscosity was $0.025 \pm 0.009$, $0.019 \pm 0.004,0.037 \pm 0.007$, and $0.059 \pm 0.023$ Pa.s for 5, 10, 20, and $30 \%$ fat creams, respectively. The homogenization of cream usually leads to increased viscosity.


Figure 2. Relative apparent viscosity of sonicated cream with various fat contents. Symbols are: 5\% fat cream $\bullet, 10 \%$ fat cream $\bullet, 20 \%$ fat cream $\bullet$, and $30 \%$ fat cream $\bullet$. For $20 \%$ and $30 \%$ fat cream sonicated for 0.5 min and 1 min , it was measured from solid phase. Error bars correspond to standard deviations from duplicates.

In homogenized cream, large agglomerates of fat globules, called homogenization clusters contain many fat globules, up to $10^{5}$. It was suggested that the fat globules in the cluster are interconnected by casein micelles (Walstra, 1999b).

Sonication at low temperature and short times $\left(2^{\circ} \mathrm{C}\right.$ for 0.5 and 1 min$)$ results in high viscosities. The fat globule in milk comprises of a triacylglycerols with various melting points. Due to partial coalescence that results in large fat droplets clusters, which will represent a larger effective volume fraction of the fat, compared to independent fat droplets at the same fat concentration. Since the viscosity is expected to depend on the volume fraction occupied by the fat droplets, thus the viscosity is expected to increase markedly when the droplets are partially coalesced.

This result was consistent with that reported by Zisu and Chandrapala (2015) which found that sonication of high fat dairy systems under cold condition $\left(<10^{\circ} \mathrm{C}\right)$ for 30 seconds leads to the formation of large fat clusters. They also reported that the large fat clusters can increase the viscosity of the fluid phase and may even result in highly viscous and slow flowing emulsions.

Large fat clusters with increased viscosity as a result of sonication of cream was formed at $2^{\circ} \mathrm{C}$ for 0.5 min and 1 min . Irregular clumps of the partially coalesced globules lead to an increase of the hydrodynamic volume fraction of the dispersed phase and thus the viscosity. Actually, if the process occurs until a continuous space-filling network is formed throughout the whole volume
of the system, the fat clumps can immobilize the aqueous phase. Therefore, the emulsion is converted to a solid-like system. In 'true' coalescence, two droplets completely merge into a bigger spherical globule, the increase of viscosity cannot be detected (Fredrick, Walstra, \& Dewettinck, 2010).

## Microstructure observation using Confocal Laser Scanning Microscopy (CLSM)

Figure 3 presents the microstructure of cream before and after sonication at $2^{\circ} \mathrm{C}$ and also the effect of SDS addition. The fat globules in unsonicated cream are in red, however droplets in green are also seen and this could be the result of fat droplets covered by proteins and milk fat globule membrane (MFGM) (Figure 3a). The microstructure of cream without SDS sonicated at $2^{\circ} \mathrm{C}$ for 1 min (Figure 3b) showed that relatively large water droplets coloured dark green are dispersed in grey-black zones containing fat crystals. After sonication for 5 min (Figure 3c), it can be seen some fat globules coloured red and others are dominated by green coloured ones, which could be fat globule milk was covered by protein. Fat globule in milk naturally is covered by milk fat globule membrane (MFGM).

The addition of SDS influenced the microstructure of cream sonicated at $2^{\circ} \mathrm{C}$. Fat globules can be seen clearly which can be considered that there is no partial fat coalescence under this condition (Figure 3d). The formation of fat clumps which take place at 1 min are inhibited by the addition of SDS.


Figure 3. CLSM micrograph of: (A) unsonicated cream, (B) solid phase of cream sonicated at $2^{\circ} \mathrm{C}$ for 1 min without SDS addition, (C) cream sonicated at $2^{\circ} \mathrm{C}$ for 5 min without SDS addition, (D) cream sonicated at $2^{\circ} \mathrm{C}$ for 1 min with SDS addition, and (E) cream sonicated at $2^{\circ} \mathrm{C}$ for 5 min with SDS addition. Fat appears as red and protein and aqueous solution appears green. Scale corresponds to $20 \mu \mathrm{~m}$.

SDS as a small-molecule surfactant can cover fat globules faster than protein thus it can inhibit partial fat coalescence. After sonication for 5 min (Figure 3e), the fat globule size was reduced extensively. This experiment showed clearly that the partial coalescence is a result of fat droplet crystalline and the lack of surface-active agent.

## Fatty acid composition

Table 1 shows the fatty acid composition in unsonicated cream, liquid phase, and solid phase from cream sonicated at $2^{\circ} \mathrm{C}$ for 1 min . The composition of fatty acid in cream and solid phase (butter) in this work are roughly comparable to other reported values (Buldo et al., 2013; Couvreur et al., 2006; Ferrand-Calmels et al., 2014; Kliem et al., 2013). The concentration of C 8 and C 18 in this research were $1.49 \pm 0.04 \%$ and $11.38 \pm 0.12 \%$ for cream and $1.42 \pm 0.02 \%$ and $11.82 \pm 0.04 \%$ in solid phase. In previous research, the concentration of C8 and C18 were $1.44 \%$ and $11.06 \%$ for cream (Buldo et al., 2013) and 1.4 \% and $9.3 \%$ for butter (Rutkowska and Adamska, 2011). Concentration of short and medium chain
fatty acid (C4-C12) in the liquid phase was higher than that in unsonicated cream and solid phase. For example, the concentration of C 4 (butyric acid) were $2.54 \pm 0.14 \%, 3.29 \pm 0.27 \%$, and $6.90 \pm 0.18 \%$ in solid phase, unsonicated cream, and liquid phase, respectively. In contrast, the concentration of long chain fatty acid (C14C18) in liquid phase was lower compared to that in cream and solid phase. As an example, the concentration of C16 (palmitic acid) were $28.93 \pm 0.18 \%, 29.05 \pm 0.18 \%$, and $24.23 \pm 0.26 \%$ in solid phase, unsonicated cream, and liquid phase, respectively. It may be due to short, medium, and unsaturated fatty acids having lower melting points compared to long saturated fatty acid. The melting points of fatty acids are $-5,-3$, and $16^{\circ} \mathrm{C}$ for $\mathrm{C} 4: 0, \mathrm{C} 6: 0$, and $\mathrm{C} 8: 0$, respectively and mostly above $10^{\circ} \mathrm{C}$ for other fatty acids (Liang et al., 2013). Timms (1980) reported that milk fat at $5^{\circ} \mathrm{C}$, crystallization of the high-melting fraction (HMF) and the middle-melting fraction (MMF) will occur and establish the solid phase while the low-melting fraction (LMF) will mainly occupy the liquid phase.

Table 1. Fatty acid composition of unsonicated cream, liquid phase and solid phase of cream sonicated at $2^{\circ} \mathrm{C}$ for 1 min .

| No | Fatty acid |  | Concentration (\% fatty acid / total fatty acid) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | Cream |  | Liquid phase | Solid | phase | Significance |
| 1 | C4:0 | Butyric | 3.29 | ${ }^{\text {a }}$ | 6.90 | 2.54 | a |  |
| 2 | C6:0 | Caproic | 2.06 | b | 3.49 | 1.83 | a | * |
| 3 | C8:0 | Caprylic | 1.49 | a | 2.35 | 1.42 | a | * |
| 4 | C10:0 | Capric | 3.17 | a | 4.57 | 3.23 | a | * |
| 5 | C12:0 | Lauric | 6.65 | a | 7.65 | 6.80 | a | * |
| 6 | C14:0 | Myristic | 13.51 | b | 12.45 | 13.33 | b | * |
| 7 | C16:0 | Palmitic | 29.05 | b | 24.23 | 28.93 | b | * |
| 8 | C16:1 | Palmitoleic | 2.71 | a | 3.00 | 2.70 | a | n.s. |
| 9 | C17:0 | Heptadecanoic | 0.76 | ${ }^{\text {a }}$ | 0.72 | 0.81 | a | n.s. |
| 10 | C18:0 | Stearic | 11.38 | b | 9.49 | 11.82 | b | * |
| 11 | C18:1 (c and t) | Elaidic, Oleic | 20.94 | b | 18.92 | 21.27 | b | * |
| 12 | C18:2 (c and t) | Linoleaidic, Linoleic | 0.93 | a | 1.42 | 1.14 | a | * |
| 13 | C18:3 n-3 | Linolenic | 0.63 | a | 0.77 | 0.65 | a | * |

Note: Values with different letter in the same row are significantly different

Fredrick et al. (2010) pointed out that the solid phase will mostly comprise of TAGs containing three long-chain saturated fatty acids and TAGs containing two long-chain saturated fatty acids and a short-chain saturated or a longchain unsaturated fatty acid.

## Conclusion

Low-frequency ultrasound can reduce the fat globule size of cream at low temperature and various fat contents. Initially, the fat globule size decreased sharply followed by the slight decrease at longer sonication time. On the other hand, ultrasound homogenization tends to increase the viscosity of cream at various fat contents.

Sonication on high fat creams ( $20 \%$ and $30 \%$ fat) at low temperatures $\left(2^{\circ} \mathrm{C}\right)$ for short times ( 0.5 and 1 min ) resulted in the formation of fat clusters which changed liquid cream into solid phase with sharply increased viscosity, but at longer sonication time, fat clusters can be disrupted and lead to decreased viscosity. The addition of SDS as small-molecule surfactant can prevent the formation of fat clusters. Fat globules can be seen clearly, which confirmed by CLSM, supposed that there is no partial fat coalescence in this condition.

Comparing fatty acid composition in unsonicated cream, liquid phase, and solid phase of cream sonicated at $2^{\circ} \mathrm{C}$ for 1 min showed that concentration of short and medium chain fatty acids ( $\mathrm{C} 4-\mathrm{C} 12$ ) in the liquid phase was higher compared to that in unsonicated cream and solid
phase. Whereas the concentration of long chain fatty acid (C14-C18) in liquid phase was lower than that in cream and solid phase. It may be due to short, medium, and unsaturated fatty acids having lower melting point compared to saturated fatty acid, so they tend to occupy the liquid phase.

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