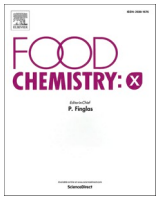
Food Chemistry: X 21 (2024) 101187

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Utilization of low-temperature heating method to improve skim milk production: Microstructure, stability, and constituents of milk fat globule membrane

Juan Wang [a], Yanmei Xi [a], Baoguo Sun [a], Jianjun Deng [b,*], Nasi Ai [a,*]

[a] Key Laboratory of Geriatric Nutrition and Health (Beijing Technology and Business University), Ministry of Education, Beijing 100048, China
[b] Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China

ARTICLE INFO

Keywords:
Heat treatment
Evaporated milk
Membrane structure of milk fat globule
Milk fat globule membrane protein
Flavor quality

ABSTRACT

In the process of defatting milk, preheating treatment is an important factor affecting the flavor of skim milk. Here, raw milk was preheated at different times and temperatures. Then laser confocal microscopy, multiple-light scattering instrument, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were used to analyze the microstructure of milk fat globule membrane (MFGM), milk stability, and MFGM protein (MFGMP) components. Results showed that phospholipid domain of MFGM changed from an ordered state (Lo) to a disordered state (Ld) with increase in treatment temperature and time, leading to an increase in MFGMP content in skim milk. During the stability test, the stability of raw milk decreased significantly with increase in preheating temperature, while the opposite was true for skim milk. Finally, the results of MFGMP differentiation analysis showed that, the content of ten taste-related MFGMPs in the control group samples was significantly lower compared to the optimal group ($P < 0.05$).

1. Introduction

Milk is considered to be an ideal drink that is rich in nutrients and easily absorbed by the body. With its unique natural, green and nutritional balance characteristics, milk become a favorite food of consumers (Du et al., 2022). Research has shown that a higher total intake of dairy products is significantly correlated with a decrease in global abdominal obesity rates, with a reduction of up to 50 % (Crichton & Alkerwi, 2014). Fat is the main factor affecting the sensory attributes of milk and plays a vital role in its appearance, texture, flavor and palatability (Richardson-Harman et al., 2000). Fat creates a pleasant mouthfeel on the palate (Lopez, Camier, & Gassi, 2007). According to the milk fat content, milk is divided into skim milk, low-fat milk and whole milk (Ai et al., 2015). Studies have demonstrated that the intake of high-fat meals can lead to impaired intestinal function, cardiovascular disease and obesity (Norris, Jiang, Ryan, Porter, & Blesso, 2016). Consequently, doctors, the federal government and the mass health media all advocate low-fat diets (Briefel & Johnson, 2004). Skim milk, obtained after removing the fat in milk, retains other nutrients of milk, and has higher protein and lower cholesterol. It is suitable for people with weak constitutions and low digestive ability (Bimbo, Bonanno, Xuan, & Viscecchia, 2016). However, due to the absence of fat, skim milk has poor flavor, so it is particularly crucial to improve its flavor characteristics.

To improve the taste of skim milk, optimizing the preheating program is a good method, as heat treatment is an extremely crucial procedure in the processing of dairy products. In particular, the combination of heating time and temperature has a significant impact on milk (Ye, Singh, Taylor, & Anema, 2004). Therefore, heat treatment of milk before skimming has a positive effect on the flavor of skim milk.

Different heat treatment intensities led to changes in fat globules, fat globule membranes and fat globule membrane proteins. Thus, heat treatment alters flavor quality of skim milk. Milk is a complex suspension, and fat is one of its most critical constituents, accounting for 3 % to 5 %. Milk fat is present in the form of small droplets in milk; the surface is covered with a layer of 10–20 nm thin film, spherical, with a diameter of about 0.2–15 μm, called milk fat globule membrane (MFGM) (Alessandro, Scaloni, & Zolla, 2010). It consists chiefly of polar lipids, such as phospholipids, sphingolipids, cholesterol, proteins, glycoproteins and enzymes (Keenan, 2001). Among them, phospholipids and proteins account for more than 90 % of the dry weight of MFGM (Dewettinck et al., 2008; Lopez, Madec, & Jimenez-Flores, 2010). Changes in MFGM phospholipids after milk processing influence the physicochemical

* Corresponding authors.
E-mail addresses: dengjianjun@nwu.edu.cn (J. Deng), ainasi@btbu.edu.cn (N. Ai).

https://doi.org/10.1016/j.fochx.2024.101187
Received 7 November 2023; Received in revised form 29 January 2024; Accepted 2 February 2024
Available online 5 February 2024

properties of milk such as emulsification and stability, and may alter the quality of milk. MFGM can primarily ensure the stability of milk fat globules and prevent them from aggregation and decomposition (Singh, 2006). MFGM has a three-layer membrane structure. The inner membrane is derived from the endoplasmic reticulum while the outer phospholipid bilayer is derived from the apical plasma membrane of mammary epithelial cells (Heid & Keenan, 2005). Milk fat globule membrane protein (MFGMP) is randomly distributed in MFGM, accounting for 25 % ~ 60 % of the total MFGM (Fong, Norris, & MacGibbon, 2007). Changes in the structure and composition of fat globules, MFGM, and MFGMP will alter the flavor properties and function of milk.

MFGM with many bioactive compounds has diverse biological functions and health benefits, such as intestinal development, neonatal maturation etc. (Nguyen et al., 2015). MFGM has a large content of proteins and phospholipids, which have outstanding latent capacity in preventing or ameliorating chronic diseases such as cancer, obesity, diabetes, and cardiovascular disease (Jiménez-Flores, Higuera-Ciapara, & Pouliot, 2009).

Heat treatment affects the microstructure and composition of MFGM during skim milk production, and MFGM has a vital impact on the physical stability of fat globules in milk (Nguyen et al., 2016). The stability of heat-treated milk will affect the emulsification of milk under different conditions. This may cause the MFGM to break more easily during centrifugation, resulting in loss of MFGMP in skim milk, thus affecting the flavor of skim milk. Studies have indicated that differentiation antigen protein (CD36) and phosphorylated glycoprotein (PP3) in MFGMP are associated with taste. Acting as a transporter for basic fatty acids, CD36 exerts a marked effect on in improving taste (Asch, 1996; Greenwalt et al., 1992). PP3 has very stable emulsifying properties, which affects the flavor quality of skim milk (Marshall et al., 2004). Therefore, in this work, the effects of heat treatment on the microstructure, composition and flavor quality of skim milk were investigated, focusing on MFGM.

2. Material and methods

2.1. Sample collection

A total of 10 milk samples were analyzed. All the milk samples were purchased from Sanyuan Company in Beijing, and the collected raw milk was kept at 4 °C during the transportation process from the farm to the laboratory. About 300 mL of raw milk was taken for heat treatment at different times (10 min, 20 min, 30 min, 40 min, 50 min) and different temperatures (4 °C, 30 °C, 40 °C, 50 °C, 60 °C). The samples were centrifuged (Multifuge X1R, Thermo Scientific, Massachusetts, America) at 4 °C, 4000 rpm for 30 min. The upper fat was removed and the lower skim milk was collected. The sample was titrated with a pH meter (STARTER2100, OHAUS, New Jersey, America) to pH = 4.6 to separate whey and casein. The above sample was then centrifuged at 4 °C, 6000 rpm for 15 min, and the upper layer of whey solution was collected. Then 25 μL of the upper liquid was placed into a 2.5 mL centrifuge tube, 5 × SDS protein loading buffer (Dingguo Changsheng Biotechnology Co., Ltd., Beijing, China) was added, and the mixture was boiled for 10 min. Skim milk samples were then stored at −20 °C for further analysis.

2.2. Microstructural analysis

The microstructure of MFGM was analyzed by double fluorescent labeling (i.e., RhDOPE and lectin WGA-488) (Nguyen et al., 2016). Nile red fluorescent probe (100 μL 42 μg/mL; Sigma-Aldrich, Missouri, America) was used to stain triglycerides in different samples: Rh-DOPE (40 μL,1 mg/mL; Sigma-Aldrich, Missouri, America) and WGA-488 (10 μL, 1 mg/mL; Beijing Yili Fine Chemicals Co., Ltd, Beijing, China) fluorescent dyes were respectively used to stain phospholipids and glycosylated molecules (glycoproteins and glycolipids) of MFGM in samples (Nguyen et al., 2015).

First, low melting point agarose (5 g/L) was prepared and stored at 45 °C before use. Then, 5 μL of the sample stained with fluorescent dye was dropped onto a slide, and 20 μL of low-melting agarose was quickly mixed with it and covered with a coverslip. Finally, the microstructures of the prepared samples were observed and analyzed by laser confocal microscopy (CLSM) (LSM 700, Carl Zeiss AG, Oberkochen, Germany) (Nguyen et al., 2016).

The microscope had three lasers. The Nile red fluorescent probe was excited at 543 nm with a He-Ne laser, and the excitation light was captured after passing through a 565–615 nm filter. The WGA-488 fluorescent probe was excited at 488 nm using an argon laser and capture the excitation light after passing through a 500–530 nm filter. The Rh-DOPE fluorescent probe was excited at 633 nm using a diode laser, and the excitation light was captured after passing through a > 650 nm filter (Matignon et al., 2014).

2.3. Effect of heat treatment on emulsion stability

Skim milk and raw milk samples that were not skimmed after different heat treatment treatments were placed into a 20 mL measuring cell and measured by astability analysis tester (AGS, Landison Technology Co., Ltd, Beijing, China). The measuring probe measured every 40 μm from the bottom of the sample cell to the top of the sample cell. The temperature was 25 °C and the scan was performed every 30 min for 12 h (Krieg, Dong, Schwamborn, & Knuechel, 2005).

2.4. MGMP extraction

To fresh raw milk, add protease inhibitor (Sigma-Aldrich, Missouri, America) was added and mixed evenly at 4 °C, The mixture was centrifuged at 5000 g for 15 min, and the separated upper fat was collected.

Phosphate-buffered saline (PBS, 0.1 M, pH 6.8) (Sigma-Aldrich, Missouri, America) solution was prepared and added to the collected upper layer of fat. The mixture was shaken in a water bath shaker at 37 °C for 20 min, followed by refrigerated centrifugation at 4 °C and 5000 g for 15 min. The above process was repeated at least 3 times until there was no soluble precipitate in milk fat solution.

Next, the membrane was ruptured. SDS (4 % vol: vol) at pH 6.8 and Tris-HC lysis solution were added to the washed milk fat at a volume of 1:2. The mixture was kept in a water bath at 60 °C in a constant temperature shaker for 20 min, followed by refrigerated centrifugation at 4 °C, 5000 g r for 20 min. The milk fat separated from the upper layer was discarded, and the lower liquid layer was containing MFGMP was collected. The protein concentration was determined by bicinchoninic acid assay (BCA) (Krieg et al., 2005).

2.5. SDS-PAGE

In this experiment, 12 % separating gel and 5 % stacking gel were prepared as prefabricated strips to separate MFGMP.

Measured MFGMP was normalized for different samples. The loading volume extracted from the samples was all 5 μg. Equal volumes of different MFGMPs were mixed with 5 × SDS-PAGE loading buffer (with DTT) (10 mL, P1040, Solarbio, Beijing, China) at a volume of 4:1. The mixture was heated at 100 °C for 5 min and cooled for later use.

The initial voltage was 80 V, and the voltage was adjusted to 120 V about 30 min after the bromophenol blue indicator entered the separating gel.. When the bromophenol blue indicator was 1–2 cm from the bottom of the separating gel, the power was turned off and electrophoresis was stopped. After electrophoresis, staining required 1–2 h. Then decontamination was performed by adding a destaining solution, generally until the gel had no background color (Jukkola, Partanen, Xiang, Heino, & Rojas, 2019).

2.6. Differential protein spot analysis

The gel was scanned by the Molecular Imager® Gel DoctM XR + System gel imaging system (Universal Hood II, Bio-Rad Laboratories, California, America). The MFGMP content of different samples was analyzed by differential point analysis and semi-quantitative analysis using image gray analysis software.

First, an image was used to reduce the background of the obtained gel map so that the gel background and gel band colors were interchanged. Then, performed a point of difference analysis was performed on the gel bands. Finally, the differential bands were manually selected for semi-quantitative analysis based on the fluorescence intensity of each gel band.

3. Results and discussion

3.1. Analysis of MFGM after heat treatment

3.1.1. Status of milk fat globules (MFG) and protein (PR) in raw milk

Milk fat globules (MFG) and proteins (PR) in raw milk treated under different conditions were stained with Nile red fluorescent dye and Fast Green FCF fluorescent dyes to explore the effect of heat treatment. The fat globules in milk dyed with Nile and FCF fluorescent dyes after processing whole milk samples at different temperatures and at different times are shown in Fig. 1.

MFG in milk aggregated with the increase in temperature, which made MFG in milk larger (Fig. 1A–E). Fat globule aggregation was not apparent at 50 °C (Fig. 1D). The MFG aggregation phenomenon was also not evident in Fig. 1H, when the temperature was 50 °C and heating time was 30 min. The changes in milk protein (PR) of different samples in Fig. 1 were insignificant. Thus, it can be inferred that t the flavor of skim milk may be affected by the following reasons: MFG becomes unstable with the increase in heat treatment temperature and time. This causes MFGM to break more easily during subsequent centrifugation and skim and MFGMP easily falls off in skim milk. Therefore, preheating at 50 °C for 30 min was the optimal treatment condition (Fig. 1D and H). The results displayed that heat treatment had a certain effect on the structure of MFGM.

3.1.2. Status of polar phospholipid domains of MFGM in raw milk

The Rh-DOPE fluorescent dye was used to stain the polar phospholipids of MFGM in raw milk treated under different conditions, and the effect of heat treatment on the phospholipid domain was explored. The changes in phospholipid domains in MFGM for whole milk samples treated at different temperatures and different times are shown in Fig. 2Aa–Aj.

The fluorescent area on the MFGM also expanded with increase in heat treatment temperature (Fig. 2Aa–Ae). The results showed that these regions on MFGM were closely related to temperature changes. Most MFGMs consisted of phospholipids, which were mixed with a small amount of MFGM protein. Changes in Rh-DOPE-labeled phospholipids indicated that the phospholipid domains in MFGM changed from an ordered state (Lo) to a disordered state (Ld) with increase in temperature. This may make the MFGM more likely to break. When the heat treatment temperature was 50 °C, the phospholipid domains in MFGM did not show an increasing trend with the increase in heat treatment time, and the fluorescence area instead showed an irregular change (Fig. 2Af–Aj). The indicated that the change in heating time had no effect on the phospholipid domain of MFGM.

It can be inferred from Fig. 2A that the state of phospholipid structural domains of MFGM changed with increase in temperature, while change in heating time did not affect the phospholipid domains of MFGM. The results in Tables 1 and 2 demonstrated that heat treatment had a certain effect on the microstructure of MFGM. The phospholipid threshold of the lactolipid globule membrane of the samples increased significantly with increase in heat treatment temperature ($P < 0.05$). It

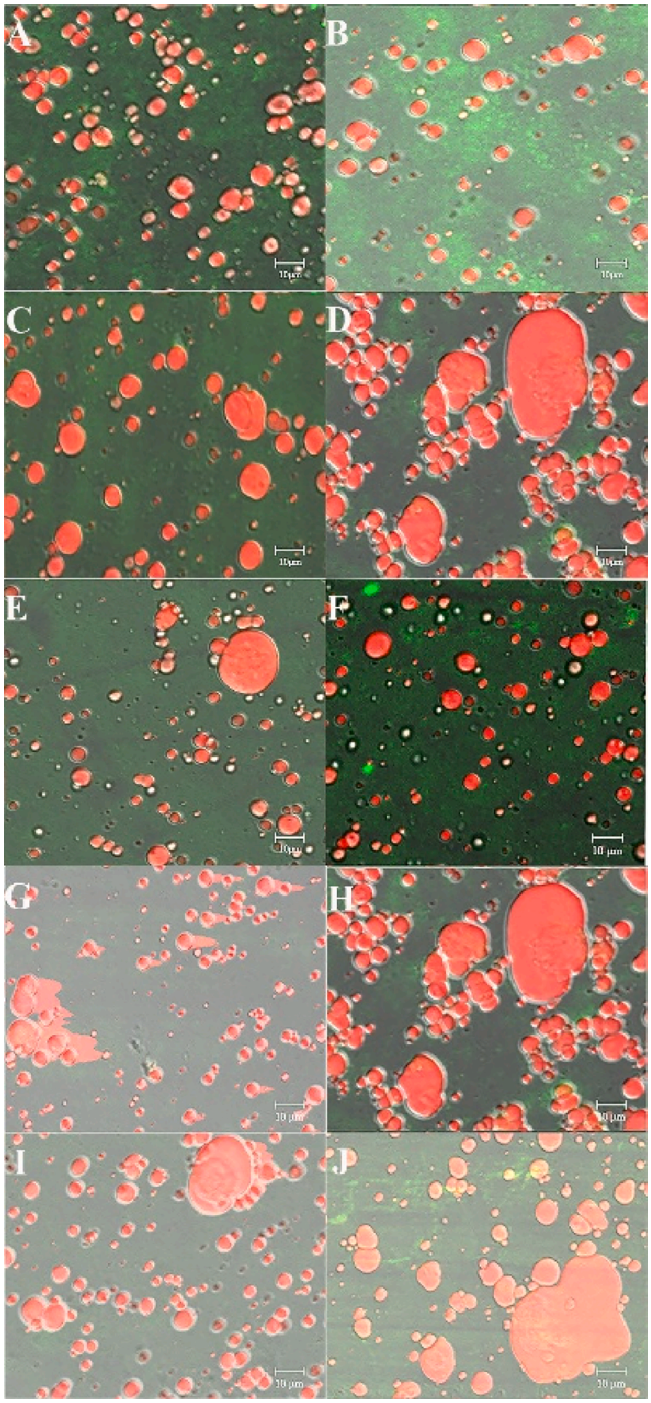


Fig. 1. State of fat globules and milk proteins of whole milk samples treated with Nile red and FCF fluorescent dyes after treatment at different temperatures and different times (A: control group; B: 30 °C; C: 40 °C; D: 50 °C; E: 60 °C; F: 10 min; G: 20 min; H: 30 min; I: 40 min; J: 50 min) All scale bars are in 10 μm.

was previously demonstrated that heat treatment reduces the shielding effect of MFGM proteins when heated above 80 °C, leading to the accumulation of fat particles. This affects the distribution of milk fat and increase the formation of oxidized flavor compounds (Li, Zhang, Shao, Guo, & Wang, 2019). The lipid fraction of MFGM has been reported to be temperature sensitive, and the number, size and shape of the phospholipid structural domains are altered by temperature (Vegarud, Langsrud, & Svenning, 2000). Heating has different effects on the rheological properties and microstructure of gels in skim milk (Theo, Jeurnink, Kees, & Kruif, 1993). Therefore, it may affect the emulsification of milk. PP3

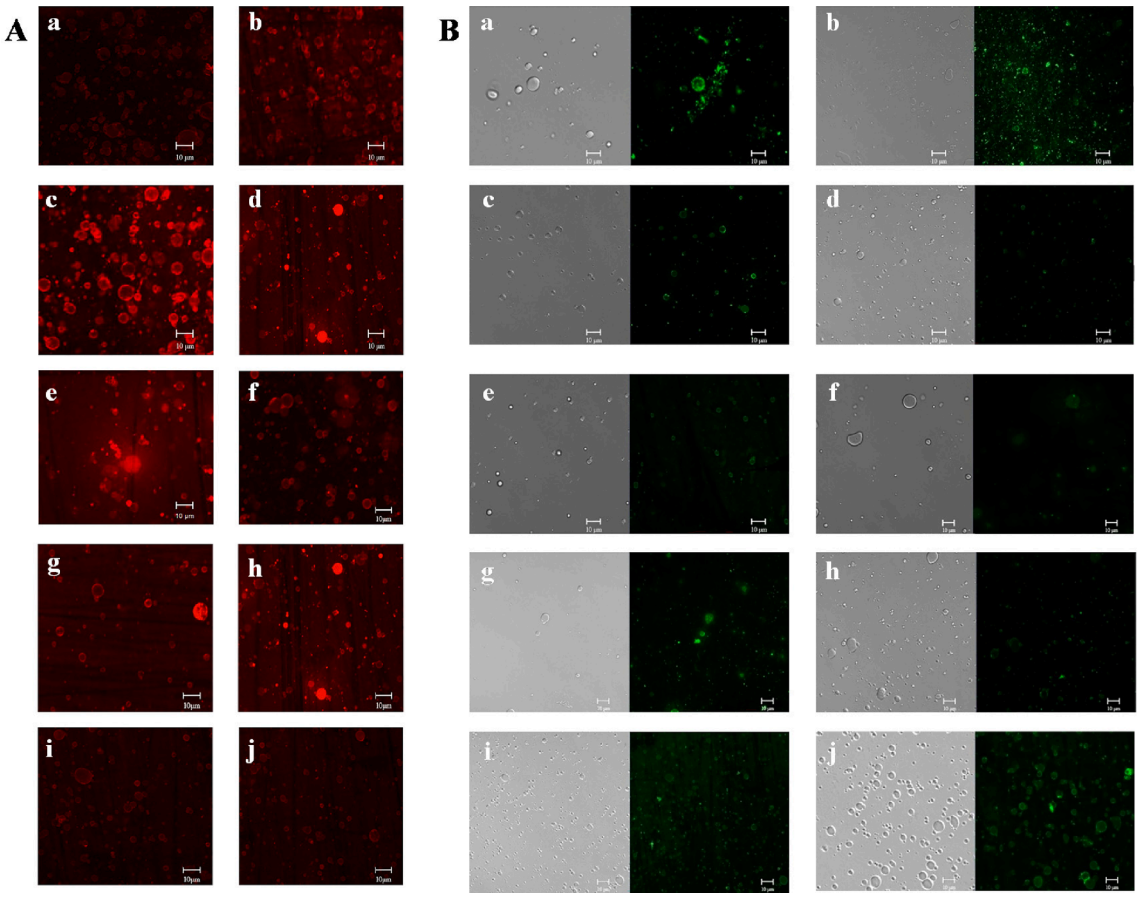


Fig. 2. State of MFGM phospholipid domains in whole milk samples and changes in MFGM proteins in skim milk samples. (A) State of phospholipid domains in fat globule membranes of whole milk samples treated with RH-DOPE fluorescent dye after treatment at different temperatures and different times (a: control group; b: 30 °C; c: 40 °C; d: 50 °C; e: 60 °C; f: 10 min; g: 20 min; h: 30 min; i: 40 min; j: 50 min). (B) Changes of MFGM proteins in fat after treatment with WGA-488 fluorescent dye (a: control group; b: 30 °C; c: 40 °C; d: 50 °C; e: 60 °C; f:10 min; g: 20 min; h: 30 min; i: 40 min; j: 50 min). All scale bars are in 10 μm.

Table 1
Average size of phospholipid domains in MFGM of whole milk samples at different temperatures.

Groups	Average area
A	20.86 ± 3.66^{a}
B	35.06 ± 2.61^{d}
C	46.48 ± 6.18^{c}
D	58.03 ± 5.0^{b}
E	129.36 ± 1.45^{a}

A: control group; B: 30 °C; C: 40 °C; D: 50 °C; E: 60 °C.
Mean ± standard deviation (n = 3).
Different superscript letters indicate significant differences between preheating treatment temperatures ($p < 0.05$).

Table 2
Average size of phospholipid domains in MFGM of whole milk samples at different times.

Groups	Average area
F	55.56 ± 7.80^{a}
G	23.37 ± 0.63^{c}
H	37.54 ± 2.46^{b}
I	22.27 ± 2.71^{c}
J	20.60 ± 1.69^{c}

F: 10 min; G: 20 min; H: 30 min; I: 40 min; J: 50 min.
Mean ± standard deviation (n = 3).
Different superscript letters indicate significant differences between preheating treatment times ($p < 0.05$).

in MFGMP has very stable emulsifying properties and plays an important role in milk processing. Proteins in MFGM may be shed in skim milk due to the breakdown of MFGM, affecting the flavor of skim milk. CD36 and PP3 in MFGMP have been reported to be associated with flavor (Lynes, Narisawa, Millán, & Widmaier, 2011).

3.1.3. Analysis of the amount of MFGMP

To verify whether more the MFGMP was shed in the skim milk after preheating. Therefore, WGA488 (Wheat germ agglutinin Alexa FluorTM 488 conjugate) fluorescent dye was used to stain MFGMP in fat obtained by centrifugation after preheating. The change in MFGMP in the removing fat can indicate the amount of MFGMP in the skim milk.

The content of lactolipoglobulin in fat is was found to be strongly correlated with change in heat treatment temperature (Fig. 2Ba–Be). As the temperature increased, the MFGMP content in the centrifuged fat decreased. Therefore, MFGMP content in skim milk increased due to the rise in temperature. When the heat treatment temperature was 50 °C, the MFGMP content in fat did not increase with heat treatment time, and the fluorescence area changed irregularly (Fig. 2Bf–Bj). The content of MFGMP in fat was lower after 10 min and 30 min treatments (Fig. 2Bf and Bh). In contrast, skim milk had a higher MFGMP content when heated at 50 °C for 30 min. The heating time should not be too long, preferably 30 min. From this point of view, heating at 50 °C for 30 min was the optimal processing condition (Fig. 2Bd and Bh).

It can be inferred from Fig. 2B that heat treatment had a certain effect on the microstructure and composition of MFGM as well as flavor quality. The addition of MFGMP allows for a more stable MFGM mechanism, and MFGM prevents unwanted rancid odors as it adsorbs at the interface and protects the lipid core from excessive lipolysis (Vanderghem et al., 2010). Our previous study showed that preheating treatment at higher temperatures could alter the flavor quality of skim milk. Specifically, the intensity of sensory attributes such as milky, caramelized, and sweetened flavors of skim milk obtained by centrifugal separation of the raw milk was increased after preheating treatment at 137–141 °C (Tong et al., 2019). MFGMP content in the fat obtained by centrifugation gradually decreased with the increase in temperature. Conversely, MFGMP content gradually increased in skim milk. At the same processing temperature, time change had no significant effect on MFGMP content in skim milk. This phenomenon indicated that the increase in MFGMP content in skim milk might have a certain impact on improving its flavor.

3.2. Stability analysis

Milk stability analysis mainly was performed to evaluate both the stability of raw milk after heat treatment and the stability of skim milk obtained by centrifugation after heat treatment. Fig. 3A presents the graph of stability analysis of raw milk treated at different temperatures and times. Fig. 3B shows the graph of stability analysis of skim milk treated at different temperatures and times.

The stability of raw milk decreased with increase in heat treatment temperature (Fig. 3Aa–Ae). The treatments at 40 °C (Fig. 3Ac), 50 °C (Fig. 3Ad) and 60 °C (Fig. 3Ae) showed no significant difference in raw milk stability, and all were inferior to the control and 30 °C treatment of raw milk (Fig. 3Aa and Ab). Different heating times did not noticeably influence the stability of raw milk at 50 °C (Fig. 3Af–Aj). As seen from Fig. 3A, different heating temperature affected the stability of raw milk, and may affect the emulsification of MFGM in the subsequent centrifugal defatting process. This may cause MFGM to break more easily during centrifugation. Thus, more MFGMP residues would be left in the skim milk, affecting the taste and flavor of the skim milk.

The stability of skim milk increased with preheating temperature (Fig. 3Ba–Be). However, the stability fluctuated when the treatment temperature was 60 °C (Fig. 3Be). It has been reported that treatment lower than 50 °Chas no significant effect on the viscosity of milk. When the temperature exceeds 60 °C, the viscosity of milk increases (Theo et al., 1993). As seen from Fig. 3Bf–Bj, the stability of the obtained skim milk was good when it was heated at 50 °C, and the change in heating

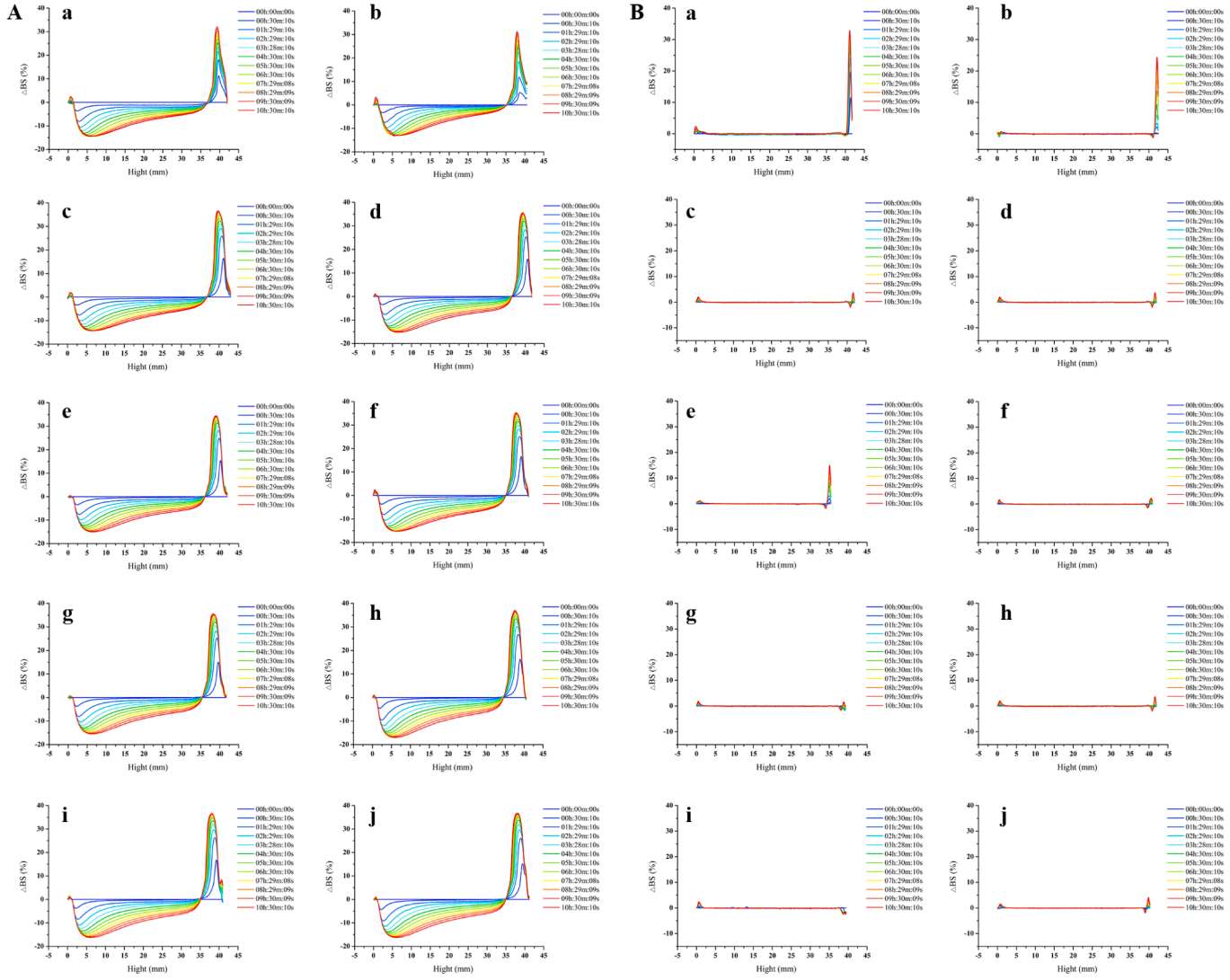


Fig. 3. Stability analysis of samples with different preheating treatment temperatures and times (A) Whole milk (a: control group; b: 30 °C; c: 40 °C; d: 50 °C; e: 60 °C; f: 10 min; g: 20 min; h: 30 min; i: 40 min; j: 50 min). (B) Skim milk (a: control group; b: 30 °C; c: 40 °C; d: 50 °C; e: 60 °C; f: 10 min; g: 20 min; h: 30 min; i: 40 min; j: 50 min).

time had no significant effect on the stability of the obtained skim milk. In conclusion, skim milk has the best stability when heated to 50 °C (Fig. 3Bd).

3.3. SDS-PAGE analysis

3.3.1. SDS-PAGE analysis of milk-like MFGM protein

In order to accurately identify the specific MFGM proteins in the two samples, and preliminarily assess the influence of the difference in MFGM protein on the flavor quality of skim milk, SDS-PAGE was used to analyze t MFGM proteins in the untreated samples and the samples were treated under the optimal conditions (50 °C, 30 min treatment).

In this experiment, organic reagents were used to separate the raw milk after different treatments by centrifugation, and MFGM proteins were extracted from the separated fat. The extracted MFGM proteins were analyzed by SDS-PAGE and Image gel analysis software. Differences in MFGM proteins in skim milk were analyzed from the opposite perspective.

The SDS-PAGE electrophoresis patterns of the samples in the control group and the treatment group after being separated by SDS-PAGE gel are shown in Fig. 4A. As seen from the electropherogram, SDS-PAGE had a good separation effect on the MFGM proteins in the sample, and could distinguish each protein with different molecular weights (1, 3, 5, 7, and 9 are the control groups; 2, 4, 6, 8, and 10 are the treatment groups). The content of the same MFGM protein differed significantly between the control and treatment groups.

Semi-quantitative analysis of MFGM proteins in samples under different treatment conditions was performed using Image gel analysis software. As seen from Fig. 4B, a total of 10 MFGM proteins were significantly different between control and treated samples, namely, XDH/XO (xanthine dehydrogenase/oxidase), Lane3, PASIII (periodic acid-Schiff stain), CD36 (differentiation antigen), BTN (butyric acid), ADPH (adipose differentiation-associated protein), Lane11, Lane13, Lane14, and PP3. Under different treatment conditions, skim milk produced had significantly higher levels of these 10 MFGM proteins than controls, including flavor-related proteins (CD36 and PP3).

The protein content in the treatment group (50 °C for 30 min) was markedly higher than that in the control group, including CD36 and PP3. Previous studies have pointed out that CD36 and PP3 are related to taste. As a transporter of essential fatty acids, CD36 has a certain effect on the improvement of taste, while PP3 has very stable emulsifying properties and affects the flavor of skim milk (Marshall et al., 2010). SDS-PAGE analysis showed that XO, CD36, BTN and ADPH existed in the MFGM through disulfide bonds, and heat treatment resulted in the weakening of disulfide bonds between some XO, BTN and ADPH proteins (Corredig & Dalgleish, 1999). The influence of these components led to the rupture of MFGM, causing more MFGMP to fall off in the skim milk, which changed the flavor change of the skim milk.

The contents of 10 MFGM proteins in skimmilk were significantly higher than those in the control group under the optimal treatment conditions of heating at 50 °C for 30 min. MFGM contains many other kinds of proteins and enzymes including approximately 554 proteins, most of which have unknown functional properties (Cartier & Chilliard, 1986). As seen from Fig. 4A and B, heat treatment had a certain impact on the microstructure and composition of the MFGM of skimmilk. Different heat treatment conditions may lead to MFGM being more easily broken in the subsequent centrifugal defatting process, resulting in more falling off of MFGMP in skim milk, thus affecting the flavor quality of skim milk. This result showed that heat treatment was effective in enhancing the taste of skim milk.

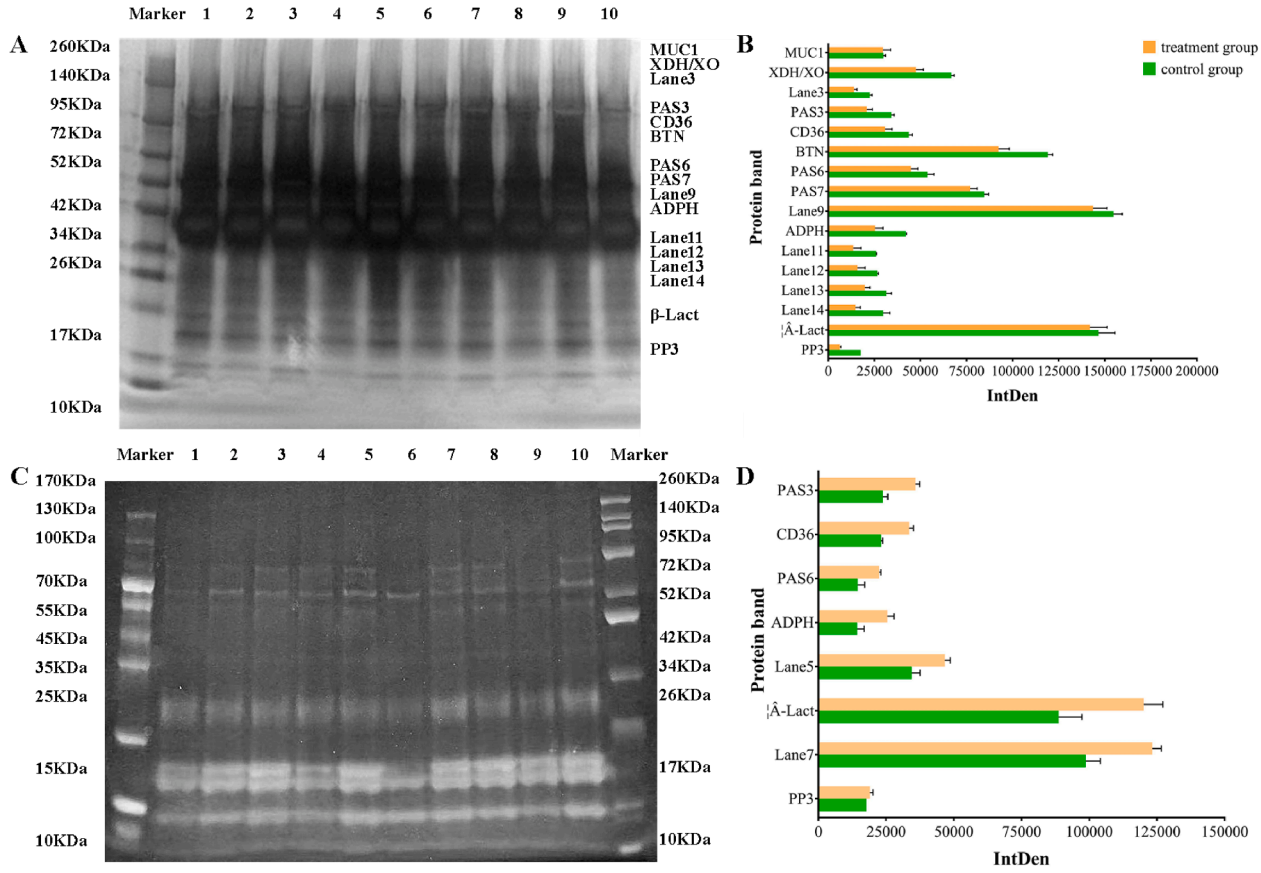


Fig. 4. SDS-PAGE and Grayscale analysis of MFGM. (A) SDS-PAGE of MFGM proteins in fat. Control group (1, 3, 5, 7, 9), treatment group (2, 4, 6, 8, 10). (B) Grayscale analysis of MFGM proteins in fat. (C) SDS-PAGE of MFGM proteins in whey. (D) Grayscale analysis of MFGM proteins in whey.

3.3.2. SDS-PAGE and grayscale analysis of whey samples

Ten whey samples (control and treatment groups) were subjected to SDS-PAGE gel electrophoresis and subjected to differential analysis of MFGM proteins. Fig. 4C shows the electropherograms in whey of SDS-PAGE gel separation of control samples and 9 groups of samples with different heat treatment temperatures and times (control group: 1; treatment groups: 2, 3, 4, 5, 6, 7, 8, and 9). The samples of groups 1–10 with different heat treatment temperatures and times were separated by SDS-PAGE gel as follows: 4 °C (control group), 50 °C for 10 min, 50 °C for 20 min, 50 °C for 30 min, 50 °C for 40 min, 50 °C for 50 min, 30 °C for 30 min, 40 °C for 30 min, 50 °C for 30 min, and 60 °C for 30 min. It can be seen from the electropherogram that SDS-PAGE had a good separation effect on the MFGM proteins in the sample, and distinguished proteins of different molecular weights well.

Fig. 4D represents the grayscale analysis results of MFGMP in the blank group and the optimal treatment group (50 °C, 30 min) of skim milk samples. The MFGM found in whey had 8 proteins, the total content of which was lower than that of MFGM in fat, indicating that a small amount of MFGM was shed into milk. Among them, CD36, PP3 and other taste substances will have a certain impact on the flavor of skim milk. This result may affect the flavor of the milk.

According to literature, CD36 has various functions, such as removal of apoptotic cells and hematopoiesis (Asch, 1996; Greenwalt et al., 1992). It has also been reported in the literature that CD36 can carry long-chain fatty acids as an essential fatty acid transporter (Abumrad, Harmon, & Ibrahimi, 1998). Therefore, it may influence a certain effect on the improvement of taste. Studies have shown that PP3 has the ability to inhibit the activity of lipoprotein lipase, and has an inhibitory effect on the spontaneous lipolysis of fat in milk (Cartier, Chilliard, & Paquet, 1990; Girardet, Linden, Loye, Courthaudon, & Lorient, 1993). Another study indicated that PP3 plays a vital role in milk processing because of its stable emulsification properties (Anderson, 1981). In view of the above functional properties of PP3, it may have a certain impact on the flavor of milk.

4. Conclusion

With the development of society and the improvement of people's living standards, in terms of diet, more and more people are inclined to choose foods that are both healthy and nutritious, especially for obese and elderly people. Due to of its lower fat content, skim milk is considered more nutritious and healthier. However, precisely because skim milk lacks fat, its flavor is worse than that of regular milk and cannot be accepted by consumers. Therefore, improving the flavor of skim milk has become particularly important. As the flavor of skim milk varies with different preheating conditions, the present study was conducted to investigate the reasons for the improvement of the flavor of skim milk by physicochemical analysis methods. The microstructure of MFGM, the stability of skim milk and the difference of MFGMP were analyzed by LSM, multiple light scattering instrument and SDS-PAGE. The results showed that: the preheating treatment not only led to the aggregation of MFG, but also changed the phospholipid structural domains in MFGM from an ordered state to a disordered state. This affected the degree of rupture of the MFGM during centrifugation and led to an increase in the content of lactoglobulin in skim milk. In the stability test, the stability of raw milk decreased significantly with the increase in preheating temperature, while the opposite was true for skim milk. However, different times of heat treatment at 50 °C had no significant effect on the stability of raw and skim milk. Finally, the MFGM proteins of the control and optimal groups were compared by SDS-PAGE, and it was found that 10 MFGM proteins in the optimal group were significantly higher than those in the control group. These included CD36 and PP3, which are known to be associated with flavor quality.

Funding

This work was supported by a grant from the National Natural Science Foundation of China (32272460).

CRediT authorship contribution statement

Juan Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yanmei Xi:** Investigation, Conceptualization. **Baoguo Sun:** Project administration. **Jianjun Deng:** Writing – review & editing, Supervision. **Nasi Ai:** Writing – review & editing, Validation, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author's.

Acknowledgement

We would like to acknowledge the reviewers for their helpful comments on this paper.

Ethical statement

Not applicable.

Consent for publication

Not applicable.

References

Abumrad, N., Harmon, C., & Ibrahimi, A. (1998). Membrane transport of long-chain fatty acids: Evidence for a facilitated process. *Journal of Lipid Research, 39*(12), 2309–2318.

Ai, N. S., Liu, H. L., Wang, J., Zhang, X. M., Zhang, H. J., Chen, H. T., & Sun, B. G. (2015). Triple-channel comparative analysis of volatile flavour composition in raw whole and skim milk via electronic nose, GC-MS and GC-O. *Analytical Methods, 7*(10), 4278–4284.

Alessandro, A. D., Scaloni, A., & Zolla, L. (2010). Human milk proteins: An interactomics and updated functional overview. *Journal of proteome research, 9*(7), 3339–3373.

Anderson, M. (1981). Inhibition of lipolysis in bovine milk by proteose peptone. *Journal of Dairy Research, 48*(02), 247–252.

Asch, A. (1996). To tell the truth: Will the real cd36 please stand up? *Journal of Laboratory and Clinical Medicine, 127*(4), 321–325.

Bimbo, F., Bonanno, A., Xuan, L., & Viscecchia, R. (2016). Hedonic analysis of the price of uht-treated milk in Italy. *Journal of Dairy Science, 99*(2), 1095–1102.

Briefel, R. R., Johnson, C. L. (2004). Secular trends in dietary intake in the united states. Annual Review of Nutrition, 24(1), 401-431.

Cartier, P., Chillard, Y., & Paquet, D. (1990). Inhibiting and activating effects of skim milks and proteose-peptone fractions on spontaneous lipolysis and purified lipoprotein lipase activity in bovine milk. *Journal of Dairy Science, 73*(5), 1173–1177.

Cartier, P., & Chilliard, Y. (1986). Effects of different skim milk fractions on activity of cow milk purified lipoprotein lipase. *Journal of Dairy Science, 69*(4), 951–955.

Corredig, M., & Dalgleish, D. G. (1999). The mechanisms of the heat-induced interaction of whey proteins with casein micelles in milk. *International Dairy Journal, 9*(3), 233–236.

Crichton, G. E., & Alkerwi, A. (2014). Whole-fat dairy food intake is inversely associated with obesity prevalence: Findings from the Observation of Cardiovascular Risk Factors in Luxembourg study. *Nutrition Research, 34*(11), 936–943.

Dewettinck, K., Rombaut, R., Thienpont, N., Le, T. T., Messens, K., & Camp, J. V. (2008). Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal, 18*(5), 436–457.

Du, B., Meng, L., Liu, H., Zheng, N., Zhang, Y., Zhao, S., & Wang, J. (2022). Diversity and proteolytic activity of Pseudomonas species isolated from raw cow milk samples across China. *The Science of the total environment, 838*(Pt 3), Article 156382.

Fong, B. Y., Norris, C. S., & MacGibbon, A. K. H. (2007). Protein and lipid composition of bovine milk-fat-globule membrane. *International Dairy Journal, 17*(4), 275–288.

Girardet, J. M., Linden, G., Loye, S., Courthaudon, J. L., & Lorient, D. (1993). Study of mechanism of lipolysis inhibition by bovine milk proteose-peptone component 3. *Journal of Dairy Science, 76*(8), 2156–2163.

Greenwalt, D. E., Lipsky, R. H., Ockenhouse, C. F., Ikeda, H., Tandon, N. N., & Jamieson, G. A. (1992). Membrane glycoprotein cd36: A review of its roles in adherence, signal transduction, and transfusion medicine. *Blood, 80*(5), 1105–1115.

Heid, H. W., & Keenan, T. W. (2005). Intracellular origin and secretion of milk fat globules. *European Journal of Cell Biology, 84*(2–3), 245–258.

Jiménez-Flores, R., Higuera-Ciapara, I., & Pouliot, Y. (2009). 11–beverages based on milk fat globule membrane (MFGM) and other novel concepts for dairy-based functional beverages. *Functional and Speciality Beverage Technology*, 281–296.

Jukkola, A., Partanen, R., Xiang, W., Heino, A., & Rojas, O. J. (2019). Food emulsifiers based on milk fat globule membranes and their interactions with calcium and casein phosphoproteins. *Food Hydrocolloid, 94*(SEP.), 30–37.

Keenan, T. W. (2001). Milk lipid globules and their surrounding membrane: A brief history and perspectives for future research. *Journal of Mammary Gland Biology and Neoplasia, 6*(3), 365–371.

Krieg, R. C., Dong, Y., Schwamborn, K., & Knuechel, R. (2005). Protein quantification and its tolerance for different interfering reagents using the BCA-method with regard to 2D SDS PAGE. *Journal of Biochemical and Biophysical Methods, 65*(1), 13–19.

Li, Y. H., Zhang, F., Shao, Z. P., Guo, L., & Wang, W. J. (2019). Formation of the oxidized flavor compounds at different heat treatment and changes in the oxidation stability of milk. *Food Science & Nutrition, 7*(1), 238–246.

Lopez, C., Camier, B., & Gassi, J. Y. (2007). Development of the milk fat microstructure during the manufacture and ripening of emmental cheese observed by confocal laser scanning microscopy. *International Dairy Journal, 17*(3), 235–247.

Lopez, C., Madec, M. N., & Jimenez-Flores, R. (2010). Lipid rafts in the bovine milk fat globule membrane revealed by the lateral segregation of phospholipids and heterogeneous distribution of glycoproteins. *Food Chemistry, 120*(1), 22–33.

Lynes, M., Narisawa, S., Millán, J. L., & Widmaier, E. P. (2011). Interactions between CD36 and global intestinal alkaline phosphatase in mouse small intestine and effects of high-fat diet. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 301*(6), R1738–R1747.

Matignon, A., Moulin, G., Barey, P., Desprairies, M., Mauduit, S., Sieffermann, J. M., & Michon, C. (2014). Starch/carrageenan/milk proteins interactions studied using multiple staining and confocal laser scanning microscopy. *Carbohydrate Polymers, 99*, 345–355.

Nguyen, H. T. H., Madec, M. N., Ong, L., Kentish, S. E., Gras, S. L., & Lopez, C. (2016). The dynamics of the biological membrane surrounding the buffalo milk fat globule investigated as a function of temperature. *Food Chemistry, 204*(aug.1), 343–351.

Nguyen, H. T., Ong, L., Beaucher, E., Madec, M. N., Kentish, S. E., Gras, S. L., & Lopez, C. (2015). Buffalo milk fat globules and their biological membrane: In situ structural investigations. *Food Research International, 67*, 35–43.

Norris, G. H., Jiang, C., Ryan, J., Porter, C. M., & Blesso, C. N. (2016). Milk sphingomyelin improves lipid metabolism and alters gut microbiota in high fat diet-fed mice. *Journal of Nutritional Biochemistry, 30*, 93–101.

Richardson-Harman, N. J., Stevens, R., Walker, S., Gamble, J., Miller, M., Wong, M., & McPherson, A. (2000). Mapping consumer perceptions of creaminess and liking for liquid dairy products. *Food Quality and Preference, 11*(3), 239–246.

Singh, H. (2006). The milk fat globule membrane: A biophysical system for food applications. *Current Opinion in Colloid and Interface Science, 11*(2–3), 154–163. https://doi.org/10.1016/j.cocis.2005.11.002

Theo, J., Jeurnink, M., Kees, G., & Kruif, D.e. (1993). Change in milk on heating: Viscosity measurements. *Journal of Dairy Research, 60*, 139–150.

Tong, L., Yi, H., Wang, J., Pan, M., Chi, X., Hao, H., & Ai, N. (2019). Effect of Preheating Treatment before Defatting on the Flavor Quality of Skim Milk. *Molecules, 24*(15), 2824.

Vanderghem, C., Bodson, P., Danthine, S., Paquot, M., Deroanne, C., & Blecker, C. S. (2010). Milk fat globule membrane and buttermilks: From composition to valorization. *Biotechnologie, Agronomie, Société et Environnement, 14*, 485–500.

Vegarud, G. E., Langsrud, T., & Svenning, C. (2000). Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. *British Journal of Nutrition, 84*(S1), 91–98.

Ye, A., Singh, H., Taylor, M. W., & Anema, S. G. (2004). Interactions of fat globule surface proteins during concentration of whole milk in a pilot-scale multiple-effect evaporator. *Journal of Dairy Research, 71*(4), 471–479.