Research Article
Effect of Cumin Essential Oil (*Cuminum cyminum* L.) on Milk Fat Globule Membrane Stability and Micro Structure Properties After Heat Treatment

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Abstract

Background and Objective: Milk Fat Globule Membrane (MFGM) damage represents a huge problem in dairy industry. The effect of cumin essential oil (CEO) against the heat treatment of MFGM was evaluated in the present study. **Materials and Methods:** Particle size distribution of emulsion, SDS-PAGE, stability, dynamic surface tension and bright field light microscopy were used for MFGM examination. **Results:** Briefly, particle sizes for heated milk with CEO (CM) was significantly different from heated milk without CEO (HM) as a result of CEO homogenization with milk fat. Casein ratio in HM was totally higher than CM. The CEO played the same role as sodium citrate to dissociate casein micelles in CM sample as observed from particle size data. The HM was affected by the high temperature and only casein and whey protein bands were observed. The stability changed for HM, but did not change for CM according to current findings of particle size. Bright field light microscopy showed presence of individual droplets with some aggregated droplets without any damage to the membrane surface for CM sample. **Conclusion:** The CEO had a weak polarization effect on MFGM and resulted in dissociation of aggregation. The addition of CEO proved many changes in the physiochemical and microstructure properties of MFGM in high temperature.

INTRODUCTION

In the last few years there has been a growing interest in milk fat globule membrane (MFGM). The MFGM is a protective layer of fat globules present in milk, consisting of proteins, phospholipids, glycoproteins, triglycerides, cholesterol, di-monoglycerides, free fatty acids and enzymes. MFGM is affected by various treatments and processing steps such as heating, cooling, agitation and homogenization[1,2]. The most important adverse effect on the nutritional value of the MFGM is thermal processing, but at the same time, it is used to achieve the safety of dairy products and increase their shelf life[3].

Creaming is a common phenomenon of instability for emulsions or suspensions that occurs when the dispersed phase has a lower density than the continuous phase. It can be coupled with coalescence or flocculation and will finally lead to phase separation. This phenomenon will affect the volume fraction of the particles in the samples[4]. Although cream stability is affected by various factors, such as heating, cooling, pressure and pH, heating is the most influential aspect. Heat treatment influences whey proteins and the interactions between the MFGM and β-lactoglobulin (β-LG). Despite the knowledge of the impact of heating, differences in emulsifying properties depend on the degree of temperature and kind of emulsifier, either just buttermilk or the MFGM. Heat treatment above 70°C up to 85°C does not influence the particle size or stabilization of buttermilk emulsion, in contrast to those of the MFGM[5,6]. It is well-established that heating cream affects the emulsifying properties of the MFGM.

Cumin has been used since ancient times and has also been reported from several ancient Egyptian archaeological sites. In the ancient Egyptian civilization, cumin seeds were used as a spice and as a preservative in mummifications[7]. Seeds of cumin were used as a spice for its distinctive taste and aroma. Therefore, cumin has been used in some cheeses, it helped to add a warming feeling to food, enhancing appetite, taste perception, digestion, vision, strength and lactation. Many symptoms have been treated using cumin such as fever, loss of appetite, diarrhea, vomiting, abdominal distension, edema and other disorders[8]. Cuminaldehyde, cymene and terpenoids are the major volatile components of cumin. Vitamin A, beta-carotene, vitamin B1, riboflavin B2, niacin B3, vitamin B6, folate B9, choline, vitamin C, vitamin E and vitamin K have been found in cumin[9]. According to United State Department of Agriculture (USDA), one tablespoon of cumin contains: 22 kcal of food energy, 1.34 g fat, 2.63 g carbohydrates, 0.6 g fiber and 1.07 g protein. Cumin could positively contribute to the sensory scores, cheese proteolysis and also has enhanced the volatiles in cheese both qualitatively and quantitatively[10].

The addition of black cumin has caused an increase in total protein, ash, total nitrogen contents and water-soluble nitrogen concentrations. Black cumin seed oil contains fatty acids very high level of tocochromanols especially γ-tocopherol and fat-soluble bioactive compounds which responsible for good blending with other oils. The blending of rapeseed oil with black cumin oil shown better oxidative stability than parent oil, also increasing tocotrienols and phytosterols ratios. Tocopherol is a fat soluble vitamin with potent antioxidant properties in protecting cells from oxidative stress and stabilization of biological membranes (with high ratios of polyunsaturated fatty acids). Tocopherols and phytosterols presence as fat soluble bioactive compounds in cumin was main reason for good blending with other oils to achieve more stability for oil[11,12].

Cumin essential oil has broad applications in food industry and marked effect on fatty acids but the effects of cumin on MFGM stabilization after industrial processes, like homogenization and heat treatment are not yet clear. Therefore, this study aimed to evaluate the impact of cumin essential oil (*Cuminum cyminum* L.) on physiochemical and microstructure properties of MFGM after heating. It could provide a new material to the manufacture processing from natural herbals and increasing the nutritional value as well.

MATERIALS AND METHODS

Raw materials: Raw cream (40% fat) was collected from a local dairy (TEDA, Tianjin, China) and used in all experiments. Cumin powder was purchased from Cheng Peng Company, Henan province, China. Trichloromethane (98%), Methanol (99%) and Boron trifluoride-methanol (97%) were purchased from Sigma Chemical, St Louis, MO, USA.

Cumin preparation and extraction: Cumin seeds were ground in a domestic grinder and sieved to obtain a particle size under 420 microns. The ground cumin was stored in polyethylene bags for further use. According to Milan *et al.*[13], cumin had been extracted.

Oil extraction: As previously described[14], oil was prepared by hydro-distillation of ground cumin for 3 h using a Clevenger distillation method. Anhydrous sodium sulphate was used for decanting and drying the oil and then the mild yellowish colored oil was stored at 4°C for further experiments.

MFGM isolation: Raw cream (40% fat) was used to isolate the MFGM according to Holzmuller *et al.*[15]. Raw cream was diluted using a pilot scale cream separator (GEA Ahlborn GmbH and Co., KG, Sarstedt, Germany). The fat content was re-dispersed in simulated milk ultrafiltrate using a benchtop homogenizer and then washed[16]. The cream was churned using a domestic hand blender (ZIP 421, Spillane Corporation, Auckland, New Zealand). Centrifugation has been done by using H1850R high-speed refrigerated centrifuge; Cence Company, China to remove all residual fat. All MFGM content was collected and stored at -20°C for further experiments.

Emulsions preparation and pasteurization: Oil-in-water emulsions were prepared by homogenizing 10 wt% with 1% CEO (v/v) using a Polytron PT, Shanghai Specimen and Model Factory, China at 8000 g for 4 min, followed by homogenization using an ultrasonic processor (Bioblock Scientific, Illkirch, France). An ice bath was used to preserve the temperature below 25°C. All emulsions were stored overnight at 4°C, to ensure full hydration. Samples were pasteurized at 72°C for 20 sec, except for the control sample (C0).

Gas chromatography-mass spectroscopy (GC/MS) analysis: The GC/MS was used to profile the essential oil. According to Sharma *et al.*[17], the oil was injected to a HP 5 MS column (Agilent, USA, 30 m×0.250 mm film thickness 0.25 μm) using auto sampler (Agilent 7693). The data were taken under following conditions: the temperature was designed at 50°C for 3 min, then raised to 10°C min^{-1} to 180°C and 45°C min^{-1} to 280°C.

Particle size distribution: The size distribution for samples were determined with integrated light scattering using better size (Bt-9300S, China). The ratio of the refractive index of the emulsion droplets 1.46, a buffer refractive index of 1.33 and an absorbance of 0.00 were used. The droplet size measurements are reported as average diameters, d_{32}, with d_{32} being defined as:

$$\frac{\Sigma n_i d_{i3}}{\Sigma n_i d_{i2}}$$

where, n_i is the number of particles with diameter d_i. All the measurements were done in triplicate.

SDS-PAGE for protein profile: The relative protein contents of casein, whey protein and MFGM protein in HM and CM were analyzed by gel electrophoresis under reducing conditions using separating and stacking gels containing 12% acrylamide. Gels were run at 200 V and stained using 0.1 (w/v) Coomassie Brilliant Blue G 250.

Emulsion stability: The two samples emulsions were filled in flat-bottomed glass tubes (20 mL, which corresponds to approx. 42 mm) and analyzed by a TurbiScan LAB (Formulaction smart scientific analysis, Toulouse, France) in backscattering mode of a pulsed near-infrared light source as a function of storage time. After the centrifuge, 20 mL emulsions were transferred into graduated tubes of 20 mm diameter and stored at 4°C for 7 days. The measurements on emulsions were performed after 0, 3 and 7 days at room temperature.

Dynamic surface tension: An automated drop volume tensiometer (TVT2, Baite Co. Ltd., Dandong, China) was used in dynamic mode for the measurement of surface tension as a function of time at room temperature. Samples were stored at 4°C overnight before measurement. The HM and CM were pumped down from a 5 mL syringe at a flow rate ranging from 0.03125 up to 0.5 mL min^{-1} and the lifetime of the drop could vary between 2 sec and 2 min. The measurements were performed triplicate.

Microscopy analysis: Samples were stored for 24 h and then all the observations were carried out at room temperature using bright field light microscopy (XSP-2GA, Shanghai Optical Instrument Factory, China) with a 10×objective magnification. The Pictures were taken using an in-built color view II camera (Olympus, Aartselaar, Belgium).

Experimental design: All experiments were performed in triplicate to obtain parallel data as shown in graphical form and GC data were presented as the Mean±Standard Deviation (SD) from three replicates. This study had been done in Tianjin University of Science and Technology, Tianjin, China and 6 months has been spent to finish all the experiments. The graphs were prepared using Origin 9.

RESULTS AND DISCUSSION

Chemical and physicochemical properties of emulsions Identify and quantify the essential oil profiling of cumin seeds: Gas chromatography-mass spectrometry (GC/MS) considered the ideal method to separate, identify and quantify the low molecular weight compounds. The GC/MS was used
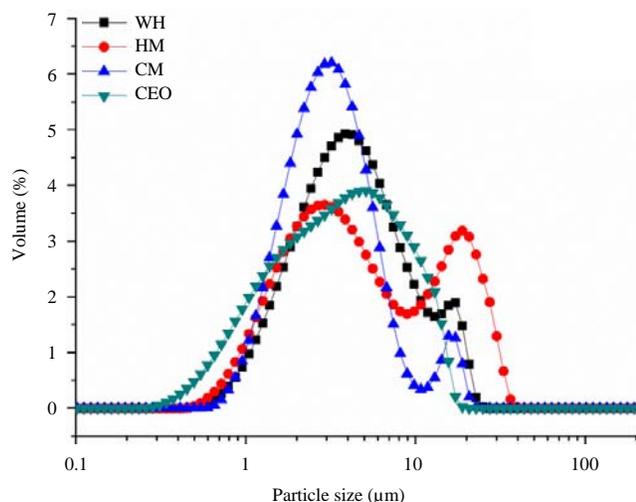
Fig. 1: Particle size distribution of milk fat globules obtained from light scattering measurements of cream. The samples heated at 100°C for 10 min. Samples stored at 4°C over night

WH: Control sample, HM: Heated milk, CEO: Cumin essential oil, CM: Heated milk with CEO

Table 1: Composition of volatile constituents of cumin essential oil (CEO) after extraction

Compounds	RI[a]	Ratio
α-thujene	902	0.10±0.0011
P-mentha-1(7),3-diene	993	-
Camphene	943	0.05±0.01
B-pinene	943	15.79±0.277
α-pinene	948	1.20±0.059
Myrcene	958	0.31±0.018
γ-terpinen	998	2.99±0.003
Terpinolene	1023	0.40±0.018
P-cymene	1042	14.33±0.049
4-Isopropylanisole	1117	0.19±0.788
2-caren-10-al	1136	-
O-cuminol	1149	2.28±0.009
m-cuminol	1149	0.11±0.016
4-Allyl anisole	1172	2.00±0.004
Anethol+estyragol	1190	7.71±0.007
Cuminaldehyde	1230	48.12±0.60
P-cuminol	1254	3.56±0.108
Carvacrol	1262	-
Geranyl acetate	1352	0.19±0.001
Total		99.33

n = 3 replicates is standard deviation

to identify and quantify the cumin essential oil (CEO) after the extraction as given in Table 1. Sixteen major compounds were identified and quantified in CEO. Cuminaldehyde was significantly the major volatile compound in CEO, followed by β-Pinene, P-Cymene and Anethol+Estyragol. This result was similar to Sharma *et al.*[17] study, who evaluated the effect of cryogenic grinding on volatile constituents of cumin from different genotypes and reported that cumin aldehyde was the main compound in all the genotypes then monoterpenes ratio and to Ehsani *et al.*[18] study which reported that cuminaldehyde was the most abundant compound in *Bunium persicum* essential oil.

Effect of CEO on particle size distribution of emulsion: The droplet size of HM and CM after heating at 72°C for 20 sec and overnight storage at 4°C were shown in Fig. 1. The CEO particle sizes were almost the same as MFGM (less than 4 μm) and no other peak for any component and this result was in agreement with previous studies[19-21]. MFGM and casein ratios before and after heating showed the effect of heating on MFGM and the ambit of the interactions between β-lactoglobulin, α-lactalbumin and MFGM. The addition of CEO decreased the interactions after heating, so casein ratio decreased after washing.

In spite of the high temperature, CEO organized the particles size inside the emulsion and distributed the particles with almost equal manner. The regular distribution of MFGM particle size was reason for more emulsion stability in contrast to raw milk. Corredig and Dalgleish[22] reported that droplet size distribution of MFGM was strongly influenced by isolated MFGM concentrations. Which mean that, CM sample isolated more MFGM than HM sample. The CEO could not isolate MFGM by a 100% but definitely reduced the effect of heating which appeared in high ratio of MFGM and small ratio of casein. CEO homogenization with milk fat during the blending for one day could be the reason for improving the properties of MFGM surface from damaging.

Protein pattern of emulsions after addition of cumin: The effect of heat treatment on MFGM proteins through the SDS-PAGE electrophoresis was given in Fig. 2. Figure 2 shown that the high temperature affected MFGM proteins in HM without CEO and only casein, β-LG and α-LA were present. Previous studies revealed that β-LG was associating with MFGM after heating between 60-65°C[23,24]. Two mechanisms were the main reason for this association, a disulfide bonds (the same mechanism for α-LA but in a lesser level) or a displacement of original MFGM. High temperature more than pasteurization temperature may detented β-LG on the MFGM by interacting with casein micelles which adsorbed onto the surface, in addition to an increase of casein micelle size[25,26]. Many studies reported that α-LA increased with increasing the time[23] at 80°C and the current results were similar.

The high temperature strongly affected the MFGM in CM sample, but still some bands clear and others fainting. From
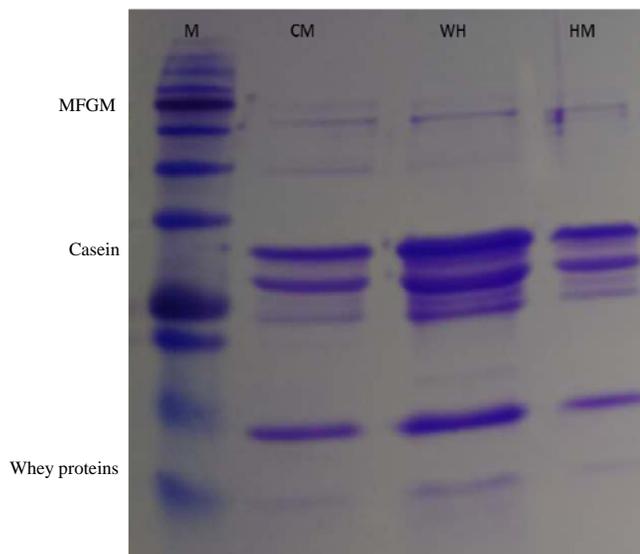
Fig. 2: SDS-page patterns for HM and CM after heating at 72°C for 20 sec. M: Marker, CM: Heated milk after CEO addition, WH: Control sample and HM: Heated milk

current data, the most important factor was the retention time with high temperature, retained the samples on high temperature for 10 min did not let us observe clear bands for MFGM in HM sample but CM sample was not affect by the time.

Emulsion stability: The creaming stability was useful to characterize the thickness of the separated phases and it could be computed for the top and the bottom of the samples if there was migration of the particles. As it was cleared from Fig. 3, CEO achieved more stabilization with storage from the beginning of the experiment comparing to HM. Might be the temperature defected in the emulsion, but CEO attained stabilization in the emulsion from the beginning till the end due to the uniform distribution between the droplets. Smaller particles in the emulsion had a better emulsifying ability of the milk proteins[27,28]. Saffon *et al.*[21] reported that the reason of better stability in their samples was due to the possible combination of whey proteins denaturation together with some modifications in casein micelles or the MFGM surface during the heating. The present results were compatible with Corredig and Dalgleish[5], who studied the effect of the heat treatment of cream and displayed that temperatures higher than 85°C was decisive temperature.

Previous studies investigated the effect of fat soluble bioactive components inside CEO on the blending efficiency with other oils[29]. Tocopherols and phytosterols were
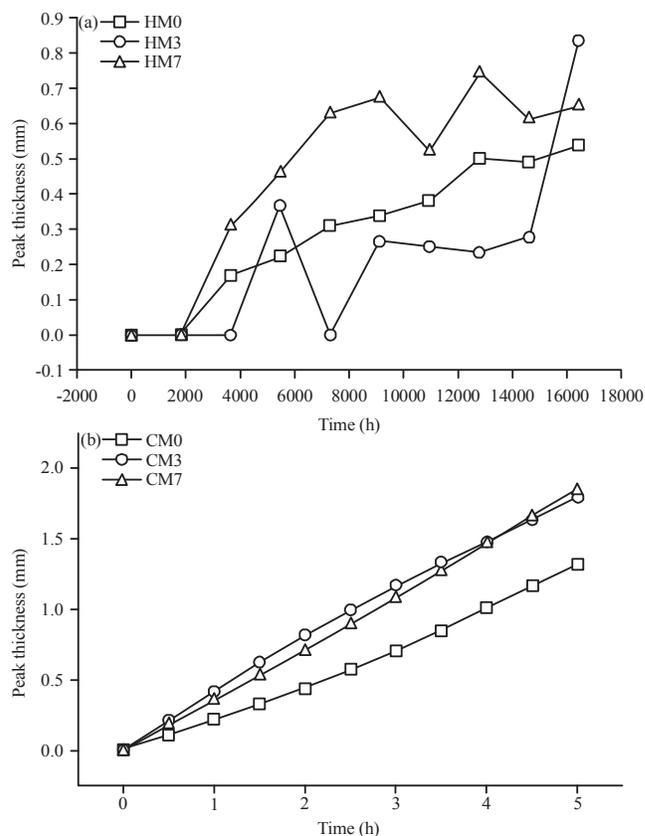

Fig. 3(a-b): Stability of the emulsions as a function of storage time, (a) HM0, HM3 and HM7 (heated milk at 72°C/20 sec and measured after 0, 3 and 7 days) and (b) CM0, CM3 and CM7 (heated milk with CEO at 72°C/20 sec and measured after 0, 3 and 7 days)

responsible for CM stabilization after heating due to an increase in their amount in MFGM after blending. The Tocopherol covered the polyunsaturated fatty acids and achieved a stabilization of MFGM membrane.

Analysis of dynamic surface tension of emulsions: The dynamic surface tension (DST) technique was used to study the physiochemical behavior of HM and CM. The DST could identify the propagation rates at interfaces and also assessed the ratio of the magnitude of the surfactant which used to reduce the surface tension. As Fig. 4 showed the dynamic surface tension of the air-water between the water, CM and HM. The value of HM decreased with increasing the drop formation time, which because of the high temperature adapted the molecules to position themselves regularly at the interface. In contrast to CM which reduced the surface tension at a continuously expanding surface.

Low critical aggregation was responsible for reducing the dynamic surface tension in any material. Both of hydroxylated and highly hydrolysed lecithins significantly reduced the dynamic surface tension[30]. Previous studies have shown that cumin influenced on milk fatty acid composition and the changes were reflected by an increase in C4:0, C18:1n9c, C18:1t11, C18:2n-6, C18:3n-3 and cis-9 trans-11 CLA. Additionally, cumin reduced the concentration of saturated fatty acids in milk and increased poly-unsaturated fatty acids, mono-unsaturated fatty acids and total unsaturated fatty acids[31]. From the same principle, it can say that the phospholipid monomers in CEO adsorbed much faster at the air-water interface because CEO increased the low critical aggregation in the raw milk.

Micro structure of emulsions

Bright field light microscopy of samples: Microscopy images of CM and HM after heating at 72°C for 20 sec to obtain MFGM were shown in Fig. 5. This technique allowed to characterize the aggregation behavior of the complex particles that were formed in the emulsions. Big differences between CM and HM under the microscope were clear. By deep look at A images, adamages of MFGM membrane and a lack of regular distributions for the particles inside the emulsion after the high temperature were quite clear, in contrast to B images. CM showed presence of individual droplets with some aggregated droplets without any damage for the membrane and the surface. The CEO proved through these

images its ability to protect the MFGM material from the high temperature and also helped to made uniform distribution of the emulsion.

Morphological changes were depending on two parameters: Hydrophobic forces and weak polarization effects[32]. Hydrophobic forces led to aggregation of the particles but the weak polarization resulting in dissociation of aggregation. The CEO had a weak polarization effect on MFGM and casein micelles and resulted in dissociation of aggregation.
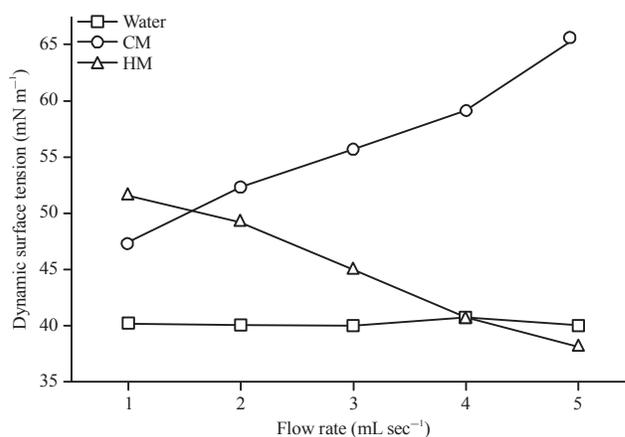
Fig. 4: Dynamic surface tension of water and MFGM samples after heating at 72°C/20 sec as a function of the flow rate at which the droplets were formed at the tip of a 5 mm glass capillary at room temperature
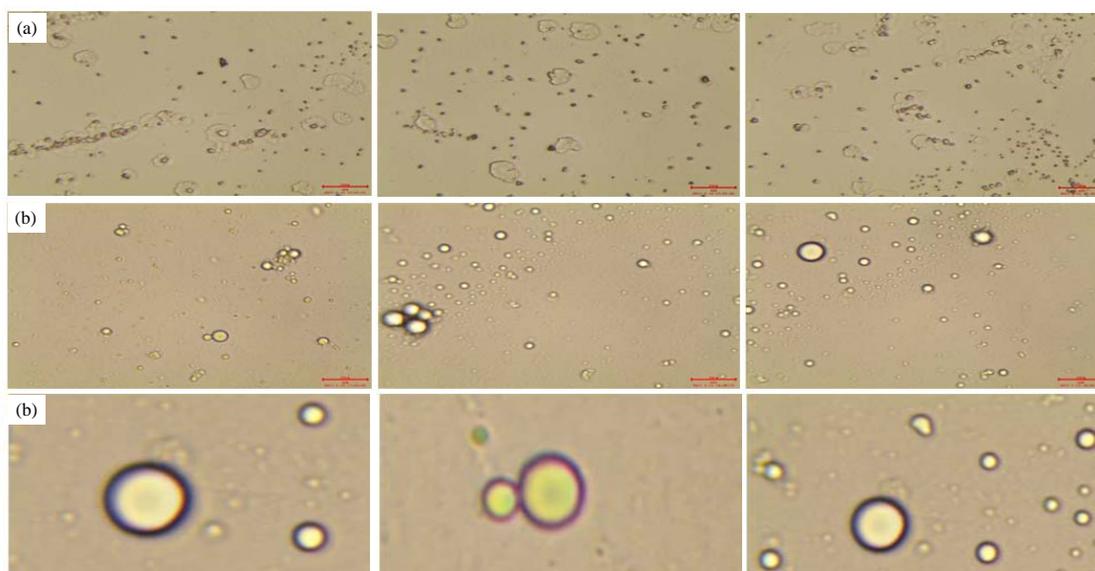HM: Heated milk and CM: Heated milk with CEO

Fig. 5(a-b): Microscopy images of emulsions prepared from heated milk and heated milk with CEO at 72°C/20 sec, (a) HM and (b) CM
Bars indicate 20 µm

The effect of CEO might be depending on the principle of blending with milk fat which cover the MFGM surface to build new outer membrane which could protect MFGM from the effect of heating.

CEO played two roles in provided sample; the first was MFGM membrane protection from high temperature. The second role was the same function of sodium citrate to dissociate maximum casein micelles on our sample.

CONCLUSION

The physiochemical and microstructure properties of MFGM with and without addition of cumin essential oil (CEO) was studied after heating at $100\,^{\circ}$C for 10 min. Particle size distribution, protein profiles, emulsions stabilize, dynamin surface tension and finally morphological changes were investigated. The results showed that CEO homogenization with milk fat during the blending for one day could be the reason for improving the properties of MFGM surface from damaging. Tocopherol covered the poly-unsaturated fatty acids MFGM membrane stabilization. Phospholipid monomers in CEO adsorbed much faster at the air-water interface because CEO increased the low critical aggregation in the heated milk.

SIGNIFICANCE STATEMENT

This study confirmed that CEO homogenization with milk fat during the blending for one day could be the reason for improving the properties of MFGM surface from damaging. Tocopherol covered the poly-unsaturated fatty acids MFGM membrane stabilization. Phospholipid monomers in CEO adsorbed much faster at the air-water interface because CEO increased the low critical aggregation in the heated milk.

ACKNOWLEDGMENT

REFERENCES

1. McPherson, A.V. and B.J. Kitchen, 1983. Reviews of the progress of dairy science: the bovine milk fat globule membrane-its formation, composition, structure and behaviour in milk and dairy products. J. Dairy Res., 50: 107-133.

2. Keenan, T.W. and I.H. Mather, 2006. Intracellular Origin of Milk Fat Globules and the Nature of the Milk Fat Globule Membrane. In: Advanced Dairy Chemistry Volume-2- Lipids, Fox, P.F. and P.L.H. McSweeney (Eds.). 3rd Edn., Springer, New York, pp: 137-171.

3. Ye, A., H. Singh, M.W. Taylor and S. Anema, 2004. Interactions of whey proteins with milk fat globule membrane proteins during heat treatment of whole milk. Le Lait, 84: 269-283.

4. Robins, M.M., 2000. Emulsions-creaming phenomena. Curr. Opin. Colloid Interface Sci., 5: 265-272.

5. Corredig, M. and D.G. Dalgleish, 1998. Effect of heating of cream on the properties of milk fat globule membrane isolates. J. Agric. Food Chem., 46: 2533-2540.

6. Ye, A., 2010. Surface protein composition and concentration of whey protein isolate-stabilized oil-in-water emulsions: Effect of heat treatment. Colloids Surfaces B: Biointerfaces, 78: 24-29.

7. Zohary, D. and M. Hopf, 2000. Domestication of Plants in the Old World. 3rd Edn., University Press, Oxford, Pages: 206.

8. Rathore, S.S., S.N. Saxena and B. Singh, 2013. Potential health benefits of major seed spices. Int. J. Seed Spices, 3: 1-12.

9. Bettaieb, I., S. Bourgou, J. Sriti, K. Msaada, F. Limam and B. Marzouk, 2011. Essential oils and fatty acids composition of Tunisian and Indian cumin (*Cuminum cyminum* L.) seeds: A comparative study. J. Sci. Food Agric., 91: 2100-2107.

10. Cakir, Y., S. Cakmakci and A.A. Hayaloglu, 2016. The effect of addition of black cumin (*Nigella sativa* L.) and ripening period on proteolysis, sensory properties and volatile profiles of Erzincan Tulum (Şavak) cheese made from raw Akkaraman sheep's milk. Small Ruminant Res., 134: 65-73.

11. Hassanien, M.M.M., A.G. Abdel-Razek, M. Rudzinska, A. Siger, K. Ratusz and R. Przybylski, 2014. Phytochemical contents and oxidative stability of oils from non-traditional sources. Eur. J. Lipid Sci.Technol., 116: 1563-1571.

12. Karupaiah, T. and K. Sundram, 2013. Modulation of human postprandial lipemia by changing ratios of polyunsaturated to saturated (P/S) fatty acid content of blended dietary fats: A cross-over design with repeated measures. Nutr. J., Vol. 12. 10.1186/1475-2891-12-122.

13. Milan, K.S.M., H. Dholakia, P.K. Tiku and P. Vishveshwaraiah, 2008. Enhancement of digestive enzymatic activity by cumin (*Cuminum cyminum* L.) and role of spent cumin as a bionutrient. Food Chem., 110: 678-683.

14. AOAC., 2006. Official Methods of Analysis of AOAC International. 17th Edn., Association of Official Analytical Chemists, Arlington, VA.

15. Holzmuller, W., O. Gmach, A. Griebel and U. Kulozik, 2016. Casein precipitation by acid and rennet coagulation of buttermilk: Impact of pH and temperature on the isolation of milk fat globule membrane proteins. Int. Dairy J., 63: 115-123.

16. Jenness, R. and J. Koops, 1962. Preparation and properties of a salt solution which simulates milk ultrafiltrate. Netherlands Milk Dairy J., 16: 153-164.

17. Sharma, L.K., D. Agarwal, S.S. Rathore, S.K. Malhotra and S.N. Saxena, 2016. Effect of cryogenic grinding on volatile and fatty oil constituents of cumin (*Cuminum cyminum* L.) genotypes. J. Food Sci. Technol., 53: 2827-2834.

18. Ehsani, A., M. Hashemi, S.S. Naghibi, S. Mohammadi and S. Khalili Sadaghiani, 2016. Properties of *Bunium persicum* essential oil and its application in Iranian white cheese against *Listeria monocytogenes* and *Escherichia coli O157: H7*. J. Food Safety, 36: 563-570.

19. Morin, P., M. Britten, R. Jimenez-Flores and Y. Pouliot, 2007. Microfiltration of buttermilk and washed cream buttermilk for concentration of milk fat globule membrane components. J. Dairy Sci., 90: 2132-2140.

20. Fox, P.F. and A. Brodkorb, 2008. The casein micelle: Historical aspects, current concepts and significance. Int. Dairy J., 18: 677-684.

21. Saffon, M., R. Jimenez-Flores, M. Britten and Y. Pouliot, 2015. Effect of heating whey proteins in the presence of milk fat globule membrane extract or phospholipids from buttermilk. Int. Dairy J., 48: 60-65.

22. Corredig, M. and D.G. Dalgleish, 1999. The mechanisms of the heat-induced interaction of whey proteins with casein micelles in milk. Int. Dairy J., 9: 233-236.

23. Dalgleish, D.G. and J.M. Banks, 1991. The formation of complexes between serum proteins and fat globules during heating of whole milk. Milchwissenschaft, 46: 75-78.

24. Ye, A., H. Singh, M.W. Taylor and S. Anema, 2002. Characterization of protein components of natural and heat-treated milk fat globule membranes. Int. Dairy J., 12: 393-402.

25. Anema, S.G. and Y. Li, 2003. Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. J. Dairy Res., 70: 73-83.

26. Zamora, A., V. Ferragut, B. Guamis and A.J. Trujillo, 2012. Changes in the surface protein of the fat globules during ultra-high pressure homogenisation and conventional treatments of milk. Food Hydrocolloids, 29: 135-143.

27. McClements, D.J., 2004. Food Emulsions: Principles, Practices and Techniques. 2nd Edn., CRC Press, Boca Raton, ISBN: 9781420039436, Pages: 632.

28. Ye, A., 2011. Functional properties of milk protein concentrates: Emulsifying properties, adsorption and stability of emulsions. Int. Dairy J., 21: 14-20.

29. Rudzinska, M., M.M.M. Hassanein, A.G. Abdel-Razek, K. Ratusz and A. Siger, 2016. Blends of rapeseed oil with black cumin and rice bran oils for increasing the oxidative stability. J. Food Sci. Technol., 53: 1055-1062.

30. Le, T.T., M. El-Bakry, N. Neirynck, M. Bogus, H. Dinh Hoa and P. van der Meeren, 2007. Hydrophilic lecithins protect milk proteins against heat-induced aggregation. Colloids Surf. B., 60: 167-173.

31. Miri, V.H., A.K. Tyagi, S.H. Ebrahimi and M. Mohini, 2013. Effect of cumin (*Cuminum cyminum*) seed extract on milk fatty acid profile and methane emission in lactating goat. Small Ruminant Res., 113: 66-72.

32. Antonov, Y.A., P. Moldenaers and R. Cardinaels, 2017. Complexation of lysozyme with sodium caseinate and micellar casein in aqueous buffered solutions. Food Hydrocolloids, 62: 102-118.