



Effects of heat treatment and homogenization on milk fat globules and proteins in whipping creams



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ABSTRACT

The stability against solidification during storage and whippability of commercial cream products vary depending on the repetition of heat treatment and homogenization necessary for extended shelf-life. First, the two commercial creams subjected to different times of heat treatment and homogenization were investigated. Among several factors such as the colloidal properties, melting profiles of fat globules and protein content and composition, the amount of proteins present at the fat globule surfaces should play a major role in determining the cream quality probably in relation to the degree of expected partial coalescence of fat globules during transportation and whipping, while the composition of proteins adsorbed to the oil–water interface was not the critical factor affecting the quality of creams. A further step-wise preparation of cream samples in a laboratory scale revealed that the second homogenization significantly reduced the amount of proteins adsorbed to the surface of fat globules. There is a possibility that physical and colloidal macro-ordered properties of whipping creams can be controlled by appropriately managing the homogenization process.

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1. Introduction

Cream is originally the concentrated fat globules that rise to the top of non-homogenized milk during the butter-making process and generally contain about 35% fat in the USA and UK (D'Amico, 1999; Davidson, 2014). It is nowadays produced throughout centrifugation process followed by heat treatment like UHT/HTST, homogenization, etc. to be used for food products such as coffee/tea, breads and cakes, often in the whipped state.

Cream is a naturally-derived oil-in-water emulsion where milk fat is dispersed in another immiscible liquid, water. An emulsion is often kinetically stable during a required term until consumption but thermodynamically unstable to undergo various types of destabilization including creaming, aggregation and coalescence (Dickinson, 1992). Among the destabilization phenomena, a kind of

coalescence, partial coalescence particularly observed for emulsions produced and consumed at low-temperature like ice creams plays an important role for instability including solidification/thickening in liquid state during transportation/storage and for formation of the aerated unique structure of whipping creams during agitation (Mutoh, Nakagawa, & Noda, 2001; Rousseau, 2000).

Partial coalescence refers to the phenomenon generally occurring within a mechanical shear, in which fat crystals in one droplet induce instability of emulsions by penetrating the thin film between two approaching droplets and allowing liquid oils to flow out around the interactive site (Vanapalli and Coupland, 2004). Factors affecting partial coalescence are shear stress related to contact time and collision frequency, colloidal interactions, crystallization of fat globules and interfacial thickness related to membrane strength (McClements, 2004a). Goff (1997) pointed out that membrane composition of the fat globules seemed to be vital to partial coalescence and foam stability of the protein-based emulsions. Smith, Kakuda, and Goff (2000) emphasized the importance of proteins in the serum layer of whipped creams on the foam. There is an agreement based on lots of previous research that emulsions stabilized by caseins are more stable

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against partial coalescence than those by whey proteins (Fredrick, Walstra, & Dewettinck, 2010).

In Japan, since producing area and consuming area are not necessarily close to each other, manufacturers of dairy products are often required to carry out an additional heat treatment coupled with an additional homogenization on commercial creams already subjected to a heat treatment and homogenization in order to achieve extended shelf-life of the creams on the biological basis. On the other hand, such additional treatments are empirically known to result in substantial changes in emulsion stability and foaming properties of the creams according to manufacturers (Horiguchi et al., 2011). The creams manufactured through a single heat treatment and homogenization process are relatively unstable in the liquid state against solidification or thickening, easy to control whipped state and less off-flavored, whereas that through double processes is stable in a liquid state, difficult to control whipped state and more off-flavored (Horiguchi et al., 2011). However, mechanisms of the different characteristics between the two kinds of creams are unclear.

In the current study, in order to clarify the mechanisms of different characteristics of the creams to stability in the liquid state and different whippability, we used two commercial cream products subjected to single and double heating-homogenization processing to analyze colloidal properties and melting profiles of fat globules and proteins at the oil–water interface and in the bulk aqueous phase. As significant differences were found for the proteins particularly at the fat globule surfaces between the two commercial creams, we subsequently prepared more precisely controlled samples in a laboratory scale to elucidate which process of the treatments had a critical impact on the adsorbed proteins.

2. Materials and methods

2.1. Materials

Two kinds of large-scale whipping cream commercial products subjected to single thermal treatment (T) in a UHT range (defined as 120–150 °C for <5 s) combined with high-pressure homogenization (H) or double one with the repeated processing, namely 1-TH or 2-TH creams were provided by Megmilk Snow Brand Co., Ltd. The commercial products were different in instability in the liquid state and whippability during agitation as described in the introduction part. Laboratory samples, i.e., small-scale whipping creams were produced from a raw cream via different times of the thermal treatment (T) at 120 °C for 3 s and the homogenization (H) at 1.0 MPa in a step-wise way to make the T0H0, T1H0, T1H1, T2H1 and T2H2 creams, where the number represents that of each processing. The thermal treatment and homogenization for laboratory samples were conducted by a plate heat exchanger (FT74, Armfield Ltd., UK) and a high-pressure homogenizer (APV LAB1000, SMT Co., Ltd., Japan), respectively. The fat content of the creams for commercial and laboratory samples was of 47.0 wt%. All other chemicals used were of analytical grade. Deionized water was used for the preparation of samples and chemical reagents.

2.2. Experiments

2.2.1. Particle size analysis

The particle size distribution of cream samples was analyzed by a laser-diffraction particle size analyzer (SALD-2200, Shimadzu, Kyoto, Japan). Samples were diluted with appropriate amount of deionized water to avoid multiple scattering. A refractive index of 1.45 – 0.01i was used to calculate the particle size distribution based on the Mie theory. The particle size was reported as the volume-weighted mean diameter, $d_{4,3}$.

2.2.2. ζ -Potential measurement

ζ -Potential of fat globules in cream samples was measured using a particle electrophoresis instrument (ELS-Z1, Photal, Otsuka Electronics Co., Ltd, Japan). Cream samples were 2000-times diluted with deionized water prior to the measurements.

2.2.3. Cryo-scanning electron microscopy (Cryo-SEM)

The cream samples were rapidly frozen in liquid nitrogen and quickly transferred into the specimen chamber of a scanning electron microscope equipped with a cryo-unit (S4100, Hitachi, Ltd., Tokyo, Japan). They were etched at –70 °C for approximately 45 min to remove water molecules on the surface of frozen samples and then sputter coated with gold. The samples were observed at the same temperature with an acceleration voltage of 5.0 kV.

2.2.4. Fractionation of cream

Cream samples were 5-times diluted with deionized water and subsequently centrifuged at 4 °C at 72,400 × g for 15 min using an ultracentrifuge (CP-75 β , Hitachi Koki Co., Ltd, Japan) to separate them into cream layer at the top, transparent aqueous phase at the middle and precipitate at the bottom in a tube. Significant coalescence of the droplets was not observed after centrifugation probably due to the dilution of the cream. The aqueous phase was collected with a syringe and then passed to a syringe-driven 0.22 μ m-pore filter to remove other insoluble components. The precipitate was collected from the tube with a spatula. The cream layer left was again diluted and centrifuged in the same manner described above to remove proteins and other materials involved in the layer to obtain isolated cream layers. The isolated cream layer for protein analysis was freeze-dried to remove water.

2.2.5. Differential scanning calorimetry (DSC)

DSC was carried out for whole cream samples and the isolated cream layers to obtain melting profiles of the fat globules using a differential scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan) in the temperature range from –20.0 °C to 40.0 °C for the whole samples and that from 5.0 °C to 40.0 °C for the isolated cream layers with heating and cooling rates of 2.0 °C/min. Approximately 5.0 mg of the samples was placed in a silver pan.

2.2.6. Quantification of proteins and lipids

Protein content of whole cream samples, the cream layers and the precipitates was determined by the Kjeldahl method (Kjeldahl, 1883) using 6.38 as the N factor, while soluble proteins in the aqueous phases were measured by Lowry's method using OVA as a standard (Lowry, Rosebrough, Farr, & Randall, 1951). The amount of proteins adsorbed to the surface of fat globules was expressed as surface concentration (mg/m²), which was calculated from the protein amount in the cream layer and the specific surface area that was estimated from the measured particle size, $d_{3,2}$ (McClements, 2004b). Lipids involved in the precipitates were determined by the Soxhlet method (Soxhlet, 1879).

2.2.7. Compositional analysis of proteins

Proteins adsorbed at the fat globule surfaces were collected by removing the fat with a chloroform–methanol (2:1) solution three times and then dissolved in a 125 mM Tris–HCl buffer solution including 4% of SDS and 20% of glycerol (pH 6.8) to make a sample solution. The other sample solutions were prepared by mixing the buffer solution with the fractionated aqueous phase and precipitate. The composition of proteins present in the aqueous phase, precipitates and adsorbed layer was analyzed by SDS-PAGE using a 15% polyacrylamide gel plate with/without 2-mercaptoethanol (Laemmli, 1970). The weight of proteins applied to the gel plate was adjusted to 10 μ g.

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