Effect of shearing-induced lipolysis on foaming properties of milk

Ho, Minh Thao

2023-04-04

Effect of shearing-induced lipolysis on foaming properties of milk

Thao M Ho,[a,b,c] Bhesh R Bhandari[b,c] and Nidhi Bansal[b,c*]

Abstract

BACKGROUND: The attraction of cappuccino-style beverages is attributed to the foam layer, as it greatly improves the texture, appearance, and taste of these products. Typical milk has a low concentration of free fatty acids (FFAs), but their concentration can increase due to lipolysis during processing and storage, which is detrimental to the foamability and foam stability of milk. There are contradictory results in reported studies concerning the effects of FFAs on the foaming properties of milk due to differences in milk sources, methods inducing lipolysis, and methods of creating foam. In this study, the foaming properties and foam structure of milk samples whose lipolysis was induced by ultra-turraxing, homogenisation, and microfluidisation (1.5–3.5 μ-equiv. mL^{-1} FFAs) were investigated.

Results: Compared with others, microfluidised milk samples had the smallest particle size, lowest absolute zeta potential, and highest surface tension; thus exhibited high foamability and foam stability, and very small and homogeneous air bubbles in foam structure. For all shearing methods, increasing FFA content from 1.5 to 3.5 μ-equiv. mL^{-1} markedly decreased the surface tension, foamability, and foam stability of milk samples. The FFA level that led to undesirable foam structure was 1.5 μ-equiv. mL^{-1} for ultra-turraxed milk samples and 2.5 μ-equiv. mL^{-1} for homogenised and microfluidised ones.

Conclusion: Shearing-induced lipolysis greatly affected the physical properties of milk samples and subsequently their foaming properties and foam structure. At the same FFA level, lipolysis induced by microfluidisation was much less detrimental to the foaming properties of milk than lipolysis induced by ultra-turraxing and homogenisation.
© 2023 The Authors. *Journal of The Science of Food and Agriculture* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

Keywords: steam injection; foamability; foam stability; microfluidisation; ultra-turraxing; homogenisation

INTRODUCTION

Bovine milk fat exists in the form of emulsified globules (2–4 μm in diameter) coated with a membrane, known as milk fat globule membrane (MFGM) primarily made of phospholipids, cholesterol, lipoproteins, glycoproteins, and proteins.[1] In its native form, milk fat is protected by the MFGM, and the rupture of the membrane allows lipase enzymes to access fat substrates by which lipolysis is initiated. There are two types of lipolysis: spontaneous and induced lipolysis. Spontaneous lipolysis is a result of the cooling of milk after secretion (~24 h) and occurs only in certain individual cows due to late lactation, poor-quality feed, and mastitis. Meanwhile, induced lipolysis is caused by physical shearing actions during milk processing (such as agitation, mixing, pumping, homogenisation, and freezing/thawing), which are damaging to the MFGM.[2] Under the action of lipase enzymes, triglycerides – the main component in milk fat – are broken down into diglycerides, monoglycerides, and free fatty acids (FFAs). The consequences of lipolysis in milk are off-flavour production and altered functionality.[2,3] It has been reported that products of milk lipolysis, especially FFAs, caused poor foaming of milk, which is a main concern for cappuccino-style drinks due to the essential contribution of a foam layer to appearance, volume, texture, and mouthfeel of these products.[4-7]

In the foaming of milk, proteins stabilise air bubbles in foam via their intermolecular interactions to form a highly viscoelastic film at the interface. However, the lipolysed products (e.g., diglycerides, monoglycerides, and FFAs) stabilise foam through the 'Gibbs–Marangoni effect' in which they restore the thickness and equilibrium surface tension of thinning films, against the destabilisation of air bubbles, by their migration to the thinnest region of the film. The incompatibility in the

* Correspondence to: N Bansal, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, QLD 4072, Australia, E-mail: n.bansal@uq.edu.au

a Department of Food and Nutrition, University of Helsinki, Helsinki, Finland

b School of Agriculture and Food Sciences, The University of Queensland, Brisbane, Australia

c ARC Dairy Innovation Hub, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, Australia

adsorption mechanism of the lipolysed products and proteins at the interfacial region explains the unfavourable effects of the lipolysed products on the production and stabilisation of foam.[8] The effects of the lipolysed products on the foamability of milk were first reported by Buchanan,[4] who stated that diglycerides and monoglycerides, rather than FFAs, had a detrimental effect on milk foaming. However, in subsequent studies by Deeth and Smith[5] and Kamath et al.,[7] it was found that the formation and stabilisation of foam are substantially reduced with an increase in FFA content.

Deeth and Smith[5] reported that as milk was lipolysed by agitation in a Waring blender for 15 s, the effect of FFAs on foamability was insignificant until the FFAs reached 1.6–2.0 μ-equiv. mL^{-1}. At concentrations higher than 2.2–3.0 μ-equiv. mL^{-1} FFAs, the foamability of milk lipolysed by agitation was negligible. For milk lipolysed by *Candida cylindracea*, the effect of FFAs on foamability was much less profound than that of milk lipolysed by agitation as the poor foamability of enzyme-induced lipolysed milk was only observed at FFA concentrations higher than 4.0 μ-equiv. mL^{-1}. Nevertheless, this study is only dedicated to the foamability of milk without investigating foam stability and structure. Similar negative effects of FFAs on the foamability of milk were also reported by Kamath et al.[7] However, the FFA level at which the negative effects on the foamability of milk were induced reported by Kamath et al.[7] was very different from that reported by Deeth and Smith.[5] As lipolysis of milk was induced by microfluidisation at 150 MPa in the later study, a high volume of stable foam was still obtained at FFA levels higher than 4.0 μ-equiv. mL^{-1}. Moreover, Kamath et al.[7] also found that foam stability dramatically decreased with an increase in the FFA content and that FFA content also affected foam structure. Foam with a smooth and creamy texture was only obtained at low FFA content (< 1.0 μ-equiv. mL^{-1}) and an increase in FFA concentration (1.5–5.0 μ-equiv. mL^{-1}) increased foam coarseness. In these studies, although the foaming method was similar (e.g., steam injection), the type of milk samples, heat treatment, and homogenisation conditions of milk before foaming were different. Therefore, it is difficult to determine whether methods inducing lipolysis affect the foaming properties of milk, and this required further investigation. In this study, the effects of varied FFA concentrations (1.5, 2.5, and 3.5 μ-equiv. mL^{-1}), generated by lipolysis of milk samples subjected to different shearing methods (ultra-turraxing, homogenisation, and microfluidisation), on the foaming properties and foam structure of milk were investigated. The two most widely-used foaming methods with different foaming mechanisms, mechanical mixing and steam injection, were employed to evaluate the foamability and foam stability of lipolysed milk samples. The dependence of foaming properties on foaming methods was well reported.[9-11]

MATERIALS AND METHODS

Materials

Fresh raw milk was obtained from a dairy farm in Queensland, Australia, and kept at 4 °C during transportation. Raw milk samples were subjected to treatment within 4 h of receipt. The chemicals used for the determination of FFA content were of analytical reagent grade and were obtained from Sigma-Aldrich (Queensland, Australia). Milli-Q water was used to prepare all solutions.

Preparation of milk samples with varied free fatty acid levels created by different shearing techniques

Three shearing methods – ultra-turraxing, homogenisation, and microfluidisation – were investigated. Lipolysed milk samples were prepared by gently warming the raw milk in a water bath (Thermoline Scientific, Instrument Choice, Adelaide, Australia) to 40 °C and then subjecting the samples to shearing treatment using different approaches. For ultra-turraxing, milk samples were sheared at about $27\,000 \times g$ for 30 min using a digital high-speed ultra-turraxing system (IKA T-25; Cole-Parmer, Vernon Hills, IL, USA). Due to the foaming of milk during mixing, ultra-turraxing was stopped for 5 min after 15 min of mixing. Ultra-turraxed milk samples were incubated in a water bath (Thermoline Scientific, Instrument Choice) at 40 °C for 4–5 h to induce lipolysis. For homogenisation, milk samples (40 °C) were homogenised using a two-stage Twin Panda homogeniser (Twin Panda 400, NS2002H; GEA Niro Soavi, Parma, Italy) with a homogenisation pressure in the first and second stages of 15 and 3 MPa, respectively. For microfluidisation, milk samples were microfluidised at 150 MPa for two passes (M-110 L; Microfluidics, Westwood, MA, USA). The outlet temperature of milk at each pass was controlled at approximately 40 °C by immersing the outlet tube of the microfluidiser in an ice bath. Both homogenised and microfluidised milk samples were incubated in a water bath at 40 °C for 1 h to induce lipolysis. It is noticed that lipolysis time for homogenised and microfluidised milk samples was much shorter than that for ultra-turraxed ones due to the differences in the lipolysis rate. From preliminary testing, the chosen lipolysis times enabled all lipolysed milk samples to have about 7 μ-equiv. mL^{-1} FFAs. After lipolysis for a certain time, all milk samples were heated in the water bath (Thermoline Scientific, Instrument Choice) to 75 °C for 30 s to stop the lipolysis process. Water in the water bath was preheated to 90 °C before the container of milk samples (1.0 L for each) was placed into it, by which milk samples were heated to 75 °C within 2–3 min. Then, the milk samples were held at 75 °C for 30 s before quickly cooling in an ice bath and kept at 4 °C. During heating and cooling, all samples were gently stirred for even heat distribution.

Unlipolysed milk samples were prepared by following a procedure similar to that used for lipolysed milk samples. However, raw milk samples were initially heated to 75 °C for 30 s to inactivate the lipase enzymes and then cooled to 40 °C in an ice bath before being subjected to different shearing approaches with conditions similar to those used to prepare lipolysed milk samples. All unlipolysed milk samples were cooled and kept at 4 °C. These samples were referred to as the control samples.

For each shearing approach, FFA content in lipolysed and unlipolysed milk samples was determined. Then, they were mixed in different ratios to produce milk samples with 1.5, 2.5 and 3.5 μ-equiv. mL^{-1} FFAs. The FFA content in mixed milk samples was rechecked and, as expected, results showed that the determined FFA levels were similar to the calculated ones and that raw and control milk samples had a similar FFA content (Supporting Information, Fig. S1).

Determination of milk properties
Free fatty acids
FFA content in milk samples was determined using the method reported by Deeth and Smith.[5] In this method, a mixture of isopropanol, hexane, and 4 N sulphuric acid (H_2SO_4) (40:10:1, by volume) was used to extract FFAs from 3 mL of milk. The extracted solution was then titrated with 0.02 N methanolic potassium hydroxide

(KOH) with a few drops of 1% methanolic phenolphthalein as an indicator to determine the amount of FFAs (in μ-equiv. mL^{-1}).

The pH
The pH of the milk samples was determined using an Aqua-pH meter (TPS, Queensland, Australia). The meter was routinely calibrated with buffers before measurements.

Particle size
The particle size of the milk samples was determined using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) by following our previously reported method.[12] The milk sample was directly added to water which was used as a dispersant until an obscuration rate of 15% was reached in the particle size analyser. The particle size of the samples was calculated based on Mie theory with refractive indices of milk fat and water being 1.462 and 1.330, respectively, and with an assumption of spherical particles.

Surface tension
The surface tension of milk samples was measured using a tensiometer (ST9000; Nima Technology, Coventry, UK). All the glassware for surface tension measurement was soaked in 5% Decon 90 detergent overnight and then rinsed with ultrapure Millipore water thoroughly before drying in a hot oven. The tensiometer was calibrated and the accuracy of the tensiometer was checked by measuring the surface tension of the ultrapure Millipore water (72 mN m^{-1}). For the first measurement, a platinum Wilhelmy plate (10.25 mm × 0.16 mm) supplied by Nima Technology was dipped in an absolute ethanol solution and then flame treated with a strong flame source to remove any residue. Between measurements, the plate was rinsed with absolute ethanol solution and ultrapure Millipore water. The surface tension of the samples was a maximum force acting on the plate as it was raised out of the samples.

Zeta potential
A Zetasizer Nano (Malvern Instruments) was used to determine the zeta potential of the milk samples. The samples were diluted with Milli-Q water at a ratio of 1:100, and the mixture was then placed in a disposable polycarbonate cuvette (DTS1061; ATA Scientific Pty Ltd, Caringbah, Australia). Measurements were repeated a minimum of ten times per run with a minimum of three runs.

Viscosity
The viscosity of milk samples was measured with a AR 1500 Rheometer (TA Instruments, Wilmslow, UK) using a cone and plate geometry (cone diameter 40 mm, angle 0, gap 0.2 mm) following a reported method.[12] For each measurement, a 2.0 mL sample was carefully deposited over the plate of the rheometer. Steady-state flow measurements were carried out at 25 ± 0.1 °C in the range of 0–100 s^{-1}. The rheological parameters (shear stress, shear rate, and apparent viscosity) were obtained from the software of TA Instruments (TRIOS, version 5.1.1.46572).

Foaming methods
Two foaming methods – mechanical mixing and steam injection – were used to evaluate foaming properties.[12] For the mechanical approach, a Breville Milk Cafe frother (BMF600; Breville Group, Sydney, Australia) integrated with a 'cappuccino cap' foaming disc was used and the foaming procedure was carried out by following the instructions in the Breville Milk Cafe

booklet in which 250 mL of milk was used for foaming.[13] Both the milk and the foaming jug were cooled to 5 °C in an ice bath. The foaming process was stopped when the milk temperature was 65 °C (after ~2 min). The foam was then poured into a 500 mL measuring plastic cylinder to evaluate foamability and foam stability.

For the steam injection approach, a Café Series EM6910 (Sunbeam, Australia) was used. About 100 mL of milk was poured into a 250 mL graduated plastic container and then cooled to 5 °C in an ice bath. The wand of steam (steam pressure 4 bars) was positioned at an angle of 45° relative to the milk surface and the tip of the steam wand was located about 2 mm under the milk surface. During foaming, an adjustable stage, which had a round holder to firmly hold the milk jug, was used to move the milk jug down steadily to maintain an appropriate point of contact between the steam wand tip and the milk surface (due to a rapid change in the boundary between foam and liquid) until the milk temperature reached 45 °C. Then, the steam wand tip was lowered into the milk to texturise and heat the milk to 65 °C. The milk temperature was continuously measured using a digital thermometer. The foam volume used for evaluating foamability and foam stability was directly measured from the graduated plastic container used for foaming.

Determination of foaming properties
Foamability and foam stability
Foamability was expressed as the volume of foam (in millilitres) produced from 100 mL of milk samples. For both foaming methods, foam volume was measured immediately after foaming as the interfacial layer between liquid and foam was observed.[14] It is noticed that for steam injection, as 100 mL of milk samples was used for foaming, thus the measured foam volume was foamability. Meanwhile, for mechanical mixing, 250 mL of milk samples was used for foaming, thus foamability was converted to 100 mL of milk samples.

Foam stability at room temperature (25 °C) was measured by the change in foam volume with time. Typically, cappuccino-style drinks are consumed within 10 min of the processing; thus foam stability was calculated as the percentage of foam volume reduction after 10 min of foam destabilisation (percentage reduction in V_F after 10 min) using Eqn (1) with V_F and V_{F10} representing foam volume (in millilitres) measured immediately after foaming ($t = 0$) and after 10 min of the destabilisation process, respectively.

$$\text{Percentage reduction in } V_F \text{ after 10 min} = \frac{(V_F - V_{F10})}{V_F} \times 100 \quad (1)$$

Foam structure
Due to technical limitations, mechanical mixing foaming method required pouring the foam from the foaming system to another suitable container (i.e., cylinder). However, it was observed that the foam pouring process was detrimental to the foam structure. Therefore, the foam structure evaluation was carried out only for foam produced from the steam injection. An image of the foam surface was taken using a microscope (Prism Optical, Eagle Farm, Australia) fitted with a 5.0 MP camera system using TSView7 software (Fuzhou Tucsen Image Technology Co., Fuzhou, China) and connected to the video port of a computer. An Olympus LG-PS2 lamp (Prism Optical) was used to illuminate the foam. Images captured at 0 and 10 min of foaming were analysed in terms of the diameter of air bubbles using Image-Pro Plus 6.0 software

J Sci Food Agric 2023
© 2023 The Authors.
wileyonlinelibrary.com/jsfa

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

(Media Cybernetics, Bethesda, MD, USA). Because all air bubbles are not spherical, the longest length was considered as the diameter. For each foaming condition, the diameter of at least 1500 air bubbles chosen from three foam images was determined. The size distribution of the measured air bubbles was summarised in terms of the smoothed distributions (histogram with fit and group function) of the log-diameter of air bubbles using Minitab 17® software (Minitab Inc., State College, PA, USA), following the previously reported method.[15,16]

Design of experiment and statistical analysis

All samples were prepared in triplicate and at least two replicate analyses were performed for each sample, if not stated otherwise, resulting in a total of six measurements per sample and the results are expressed as mean values (± standard deviation). One-way analysis of variance (ANOVA) followed by *post hoc* Tukey's tests were performed to differentiate the mean values. Statistically significant differences were assessed for $P < 0.05$ at a confidence level of 95% using the Minitab 16.0 statistical software (Minitab Inc.).

RESULTS AND DISCUSSION

Properties of milk samples with varied free fatty acid levels created by lipolysis with different shearing techniques

Particle size

Ultra-turraxing, homogenisation, and microfluidisation have been used not only to induce lipolysis but also to reduce the particle size of milk samples. As indicated in Fig. 1(a) and Table 1, raw milk had an average particle size of 4.16 and 3.17 µm for D[3,4] and D[2,3], respectively, and a single-peak particle size distribution (PSD) curve (ranging from 1 to 10 µm), which represents the size of milk fat globules. Shearing actions under ultra-turraxing, homogenisation, and microfluidisation markedly reduced the particle size of raw milk to 1.49, 0.48, and 0.15 µm for D[3,4] and 0.25, 0.18, and 0.11 µm for D[2,3], respectively. The PSD curves of ultra-turraxed and homogenised milk samples had two peaks representing the size of casein micelles (0.03–0.8 µm) and the size of homogenised fat globules (0.8–10 µm). Meanwhile, the PSD curve of microfluidised milk samples showed only one peak, which could be the mixture of fat globules and casein micelles. It was reported that in cow milk, the size of fat globules in their native forms was 4.12 µm for D[3,4] and 3.10 µm for D[2,3],[17] while that of casein micelles was 0.13–0.16 µm,[18] which were similar to those found in this study. These results possibly indicate that the fat globules and casein micelles in microfluidised milk samples shared a similar size. Compared with homogenisation and ultra-turraxing, a much higher pressure level or shearing force was utilised in microfluidisation so the latter yielded significantly smaller fat globules. A similar observation was also reported by Hayes and Kelly.[19] Regardless of the shearing method, FFAs did not affect the particle size of milk as milk samples with varied FFA content had almost identical particle size and PSD curves.

The pH

As indicated in Fig. 1(b), ultra-turraxing, homogenisation, or microfluidisation alone did not affect pH, while increasing the FFA content from 1.5 to 3.5 µ-equiv. mL^{-1} slightly decreased pH. It has been reported that during the storage (~4 °C) of raw and pasteurised whole milk, FFA content increased by 6.0 and 2.0 µ-equiv. mL^{-1}, respectively, but pH values remained constant.[20] Similar observations were also reported by Ho *et al.*[12] for

raw whole, raw skimmed, pasteurised whole, and pasteurised skimmed milk during storage at 4 °C. It could be explained that the acidity of milk caused by an increase in FFA content can be neutralised by buffer systems naturally present in milk, and therefore pH was almost unchanged.[20] In this study, the pH level of all milk samples fluctuated between 6.6 and 6.8, which was in a 'neutral region' of milk and thus could not affect foaming properties, as reported by Huppertz[9] and Borcherding *et al.*[21]
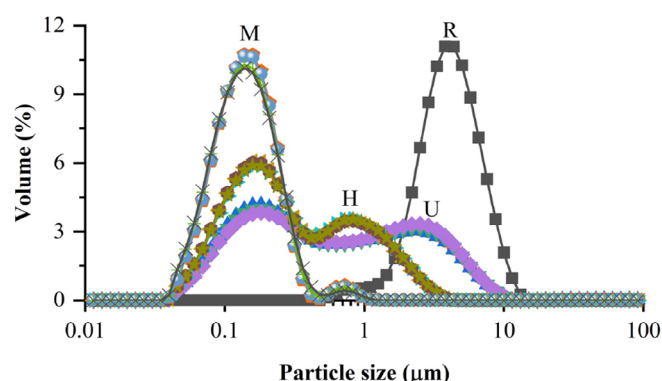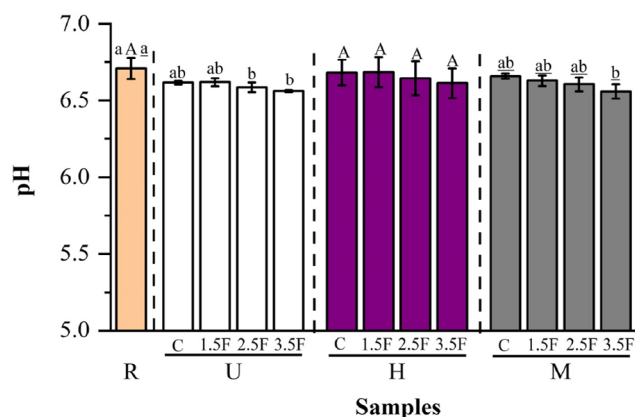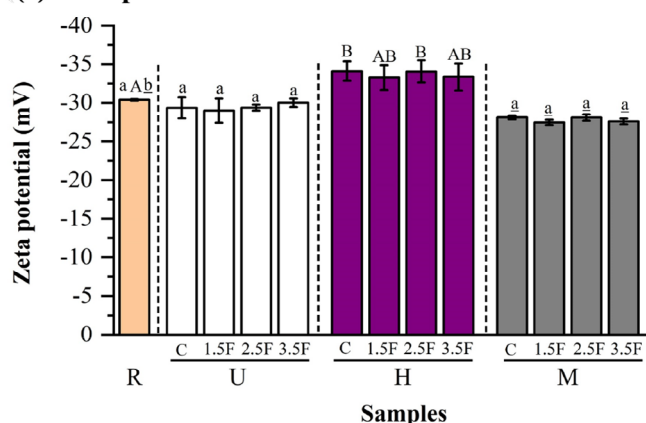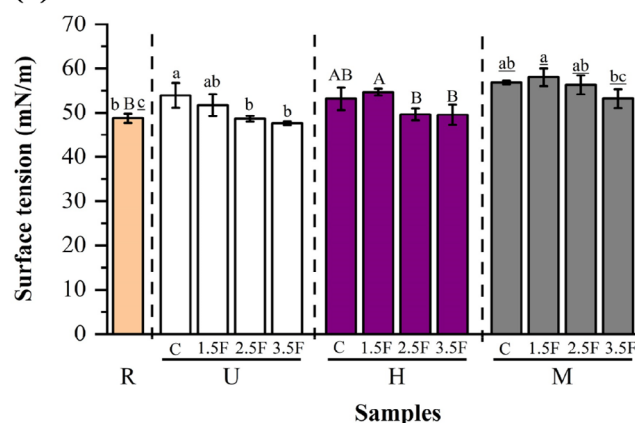
Zeta potential

From Fig. 1(c), it can be seen that unlike ultra-turraxing, which did not affect the zeta potential of milk, homogenisation increased in this value, while microfluidisation led to a decrease. Differences in shearing forces and mechanisms in the breaking of fat globules among these treatment approaches induce dissimilar interactions of proteins and lipids on the surface of milk fat globules, and consequently their surface charge.[22] Moreover, for each shearing method, increasing FFAs up to 3.5 µ-equiv. mL^{-1} did not cause any alterations in zeta potential values ($P > 0.05$). Gallier *et al.*[23] reported that negative zeta potential values of milk fat globules increased with the presence of FFAs due to their accumulation at the interface, and this formation of FFAs was accompanied by a decrease in pH value. It was found by Yang *et al.*[24] that the zeta potential of milk was highly dependent on pH. However, in this study, changes in pH and zeta potential values with an increase in FFA content were marginal. This is possible because a high positive charge of milk fat globule membrane proteins produced in shearing treatment steps can compensate for an increase in negative zeta potential values caused by the FFAs increase.

Surface tension

The surface tension of all investigated milk samples is shown in Fig. 1(d). All shearing treatment methods resulted in a significant increase in surface tension. Fox[25] reported that the surface tension of homogenised milk samples was higher than that of unhomogenised milk samples if the milk samples were pasteurised before homogenisation to inactivate the lipolysis process. Similarly, Michalski and Briard-Bion[26] found that homogenisation of milk at higher than 10 MPa led to increased surface tension due to the deposition of caseins onto the increased, newly formed fat globule surfaces, resulting in a lack of caseins in the continuous phase. Due to the smallest size of microfluidised milk samples (Table 1), which had the highest surface area and the number of fat globules, the surface tension of microfluidised milk samples was much higher than that of ultra-turraxed and homogenised milk samples at the same FFA level. Kamath[14] also reported that the surface tension of pasteurised homogenised whole milk and ultra-heat-treated homogenised whole milk was higher than that of raw whole milk. Regarding FFAs, as expected, for all shearing methods there was a decline in surface tension with increased FFA content. It was reported by Kamath *et al.*[7] that there was a good negative correlation between FFA content and the surface tension of milk samples (R2 ≈ 0.92). However, in this study, correlation between the surface tension and FFA content was low as Pearson correlation coefficient (*r*) was 0.44. Due to the highly active surface of FFA molecules, the surface tension of milk decreases once they are adsorbed at the interface.[7]

Viscosity

The viscosity of all milk samples is shown in Fig. S2. The results indicate that all shearing treatments led to increased viscosity of milk samples. This could be the result of a reduction in fat globule

(a) Particle size distribution

(b) pH

(c) Zeta potential

(d) Surface tension

Figure 1. Particle size distribution (a), pH (b), zeta potential (c) and surface tension (d) of raw and control samples and those with different free fatty acids (FFAs) contents which were produced by lipolysis induced by ultra-turraxing (U), homogenisation (H) and microfluidisation (M). In all figures, R, C, 1.5F, 2.5F and 3.5F represent raw and control milk samples, and those containing 1.5, 2.5 and 3.5 μ-equiv. mL^{-1} FFAs, respectively. In (a), for each shearing method, particle size distribution curves of milk samples at different FFA content (control, and 1.5–3.5 μ-equiv. mL^{-1} FFAs milk samples) overlapped. The data in (b)–(d) are presented as mean values ± standard deviation ($n = 6$). For each property and each shearing method (including raw milk samples), different letters indicate significant difference among samples ($P < 0.05$).

Table 1. Particle size of raw, control milk samples and those containing varied free fatty acids (FFAs) contents due to lipolysis induced by various shearing methods

Samples		Particle size (μm)[†]	
		D[3,4]	D[2,3]
Raw		4.16 ± 0.11[a]	3.17 ± 0.14[a]
Ultra-turraxed	Control	1.49 ± 0.44[b]	0.25 ± 0.01[b]
	1.5 FFA	1.32 ± 0.18[b]	0.24 ± 0.02[b]
	2.5 FFA	1.44 ± 0.23[b]	0.25 ± 0.02[b]
	3.5 FFA	1.39 ± 0.23[b]	0.26 ± 0.04[b]
Homogenised	Control	0.48 ± 0.06[c]	0.18 ± 0.01[c]
	1.5 FFA	0.50 ± 0.09[c]	0.18 ± 0.01[c]
	2.5 FFA	0.49 ± 0.08[c]	0.18 ± 0.01[c]
	3.5 FFA	0.50 ± 0.08[c]	0.18 ± 0.01[c]
Microfluidised	Control	0.15 ± 0.00[d]	0.11 ± 0.01[d]
	1.5 FFA	0.15 ± 0.00[d]	0.11 ± 0.01[d]
	2.5 FFA	0.15 ± 0.00[d]	0.11 ± 0.00[d]
	3.5 FFA	0.15 ± 0.00[d]	0.11 ± 0.00[d]

[†] D[3,4], volume weighted means; D[2,3], surface weighted means. [a] The data are presented in mean values ± standard deviation ($n = 3$). Superscript letters indicate significant difference among samples ($P < 0.05$).

size, and the aggregation and interaction of proteins (e.g., casein micelles) and fat on the newly formed fat globules produced during shearing.[27] Compared with homogenised and microfluidised milk samples, ultra-turraxed counterparts showed the highest extent of a viscosity increase. The presence of large particles and/or aggregates of milk fat globules, which are formed as a result of coalescence and/or bridging flocculation, contributes to increased viscosity.[28] These phenomena likely happened during ultra-turraxing of milk at high speed for a long time ($27\,000 \times g$ for 30 min in this study), while in homogenisation and microfluidisation, clusters and/or aggregates of milk fat globules were broken up into individual fat globules in the second stage of homogenisation or the second pass of microfluidisation. This is supported by the PSD results shown in Fig. 1(a) in which ultra-turraxed milk samples contained quite a high proportion of large fat globules. Moreover, due to the inherent nature of the process, it is difficult to achieve homogenous milk samples with ultra-turraxing, especially ultra-turraxing of a high volume of milk (2 L in this study); viscosity data of ultra-turraxed milk samples exhibited high fluctuation (data not shown). For all shearing methods, the viscosity of milk samples remained unchanged with increased FFA content. An increase in the viscosity of milk samples slows the diffusion rate of surfactants, which markedly decreases foamability, and minimises the rate of liquid drainage in foam, which increases foamability.[29,30]

Foaming properties of milk samples with varied free fatty acid levels created by lipolysis with different shearing techniques

Foamability and foam stability

As shown in Fig. 2, although both foaming methods exhibited a similar trend in foamability and foam stability for all milk samples, steam injection showed a lesser effect than mechanical mixing, especially for milk samples containing high FFA content. The foaming properties of milk were highly dependent on foaming methods due to differences in the manner in which air was incorporated into the bulk liquid of milk and the foaming conditions (e.g., milk volume used to foam, foaming temperature, and time), which greatly affected the physical properties of milk and the functionality of milk components (e.g., physical state of milk fat, adsorption kinetics of surface-active substances and denaturation of proteins). However, a comparison between the two foaming methods was not in the scope of this study but has been reported in several such studies.[11,12,31,32]

Compared with raw milk samples, all shearing methods enhanced foamability and foam stability. The microfluidised milk samples showed the highest foamability and foam stability, followed by the homogenised milk samples. It is noticed that the higher values of percentage reduction in V_F after 10 min indicated lower foam stability. As discussed earlier, all control ultra-turraxed, homogenised, and microfluidised milk samples had similar FFAs and pH values but were different in particle size, surface tension, zeta potential, and viscosity. The reduction in the particle size of fat globules and the changes in milk fat globule membrane compositions, which were induced during ultra-turraxing, homogenisation, and microfluidisation, helped to improve the foamability and foam stability of milk.[14] Fat globule size controlled foamability while alterations in the fat globule membrane composition governed the foam stability.[15] The extensive rupture of milk fat globules under ultra-turraxing, homogenisation, and microfluidisation resulted in a substantial increase in the surface area and the number of fat globules, which led to a deficiency of membrane materials available to stabilise the newly generated surface of the fat globules. In this case, the newly formed surface is reinforced by casein micelles and whey proteins. The dissociation of casein micelles into casein monomers or primary casein particles, and the formation of casein–fat complexes on the newly formed surface during shearing, could be the main reason for the improvement in foamability and the foam stability of milk samples.[33,34] In addition, higher foam stability observed for ultra-turraxed, homogenised, and microfluidised milk samples are probably due to the increased viscosity of these milk samples (Fig. S2). High viscosity retards the destabilisation processes of foam, such as drainage of liquid from the foam, coalescence of air bubbles, and disproportionation of air bubbles, thereby improving foam stability.[9]

It is difficult to correlate the zeta potential with the foaming properties of the milk samples as the zeta potential of ultra-turraxed, homogenised and microfluidised milk samples exhibited a different trend to that of their foamability and foam stability (Figs 1 and 2). The zeta potential of milk is not only dependent on pH but is also determined by the interaction of proteins, fat, and fat globule membrane components on the newly formed surface of fat globules.[22]

Regarding surface tension, Kamath[14] reported that homogenised milk samples with high surface tension produced a higher foam volume and more stable foam than raw milk samples with low surface tension, which is highly compatible with the results of our study. However, Kamath[14] recommended that surface tension values of milk samples could not be used to predict foaming properties because foaming properties are greatly dependent on the presence of surface active components such as fat, proteins and phospholipids, and FFAs.

As indicated in Fig. 2, the foamability and foam stability of all milk samples in both foaming methods significantly declined with increasing FFA content. It is well known that FFA molecules are more surface active than proteins and dominate the foam interface at high concentrations but are unable to interact with their neighbour molecules, subsequently interfering with the formation of intermolecular interactions of proteins and destroying the integrity, cohesiveness, and viscoelastic properties of the interfacial films.[8] Similar negative effects of lipolysis products, especially FFAs, on the foamability and/or foam stability of milk were also reported.[2,4,7,9]

At the same FFA level, different shearing methods affected the foaming properties of milk differently. For mechanical mixing (Fig. 2(a)), at 1.5 μ-equiv. mL^{-1} FFAs, while ultra-turraxed and homogenised milk samples were almost unable to produce foam, microfluidised milk samples still exhibited very good foaming properties as their foamability and foam stability just slightly reduced compared with those of the control microfluidised milk samples. However, at 3.5 μ-equiv. mL^{-1} FFAs, the microfluidised milk samples could not produce a foam that could last for 10 min. A similar phenomenon was also observed for the steam injection foaming method (Fig. 2(b)). In response to an increase in FFA level, microfluidised and homogenised milk samples showed a steady decline in foamability and foam stability, with a higher rate being observed for the latter, while the foamability and foam stability of the ultra-turraxed milk samples sharply reduced, especially as the FFA level was at 3.5 μ-equiv. mL^{-1}. In a previous study, Deeth and Smith[5] reported increasing the FFA content from 0.8 to 1.2 μ-equiv. mL^{-1} decreased the foamability by 60%. At concentrations higher than 2.0 μ-equiv. mL^{-1} FFAs, the foamability of milk was negligible. Similarly, Kamath *et al.*[7] illustrated the negative effects

(a) Mechanical mixing
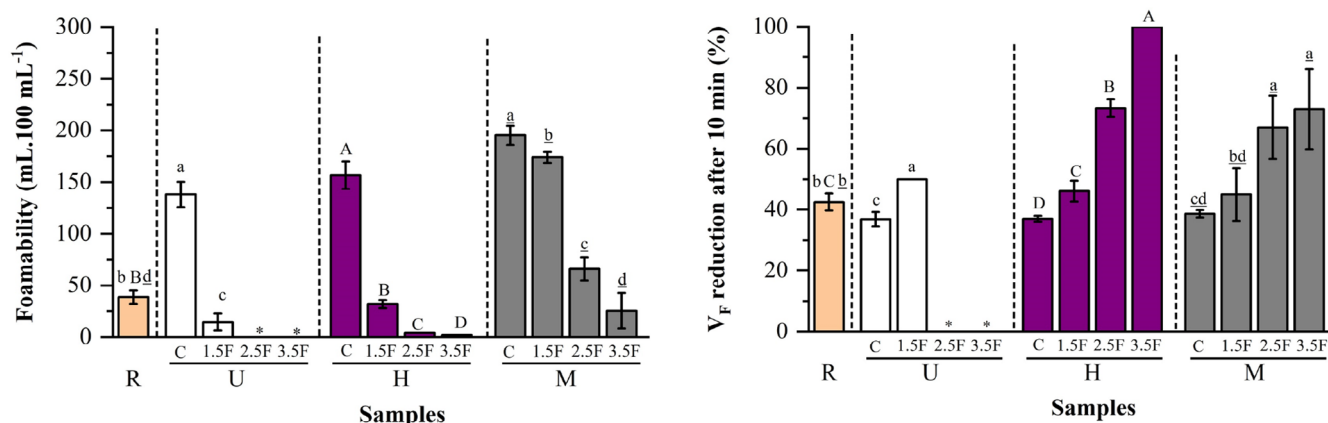
(b) Steam injection
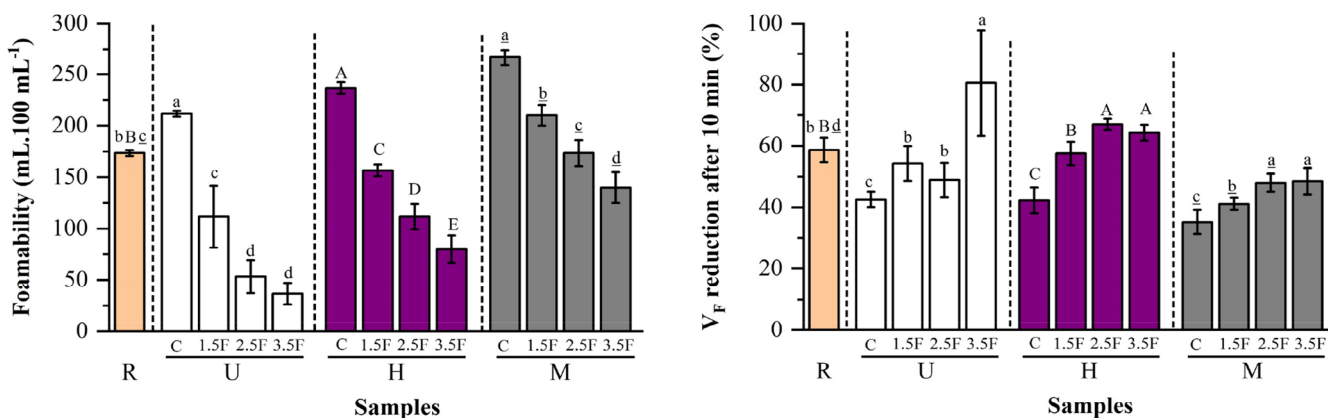
Figure 2. Foamability and foam stability using two foaming methods: mechanical mixing (a) and steam injection (b) of raw and control milk samples and those with different free fatty acids (FFAs) contents which were produced by lipolysis induced by ultra-turraxing (U), homogenisation (H) and microfluidisation (M). Foamability was calculated as millilitres of foam per 100 mL of milk samples. Foam stability was expressed as the percentage of foam volume (V_F) reduction after 10 min of destabilisation. In all figures, R, C, 1.5F, 2.5F and 3.5F represent raw and control milk samples, and those containing 1.5, 2.5 and 3.5 μ-equiv. mL^{-1} FFAs, respectively. An asterisk (*) in (a) indicated missing values as the foam was too unstable for the measurements. The data are presented as mean values ± standard deviation ($n = 3$). For each property and each shearing method (including raw milk samples), different letters indicate significant difference among samples ($P < 0.05$).

of FFAs on the foamability and foam stability of milk. However, the point at which the FFA level induced the negative effects on foamability was different from those reported by Deeth and Smith.[5] A high volume of stable foam was still obtained at FFA levels higher than 2.0 μ-equiv. mL^{-1}, although the same foaming method (steam injection at 65–70 °C) was employed. The differences in the results of these reports were similar to those in our study and are possibly explained by the dissimilarities in the way in which lipolysis was induced, which affected the particle size of fat globules, the composition and properties of the fat globule membrane, and structural changes associated with proteins. Under extremely high pressure, shear stress and high-speed collision of milk droplets occur in microfluidisation and casein micelles are likely to be disintegrated into casein monomers or caseins particles, which can interact with each other or whey proteins to form protein aggregates.[35] In foaming, these protein aggregates supposedly mask the negative effect of FFAs.

Foam structure

Images of the foam surface prepared from milk samples containing varied FFA levels induced by lipolysis with different shearing
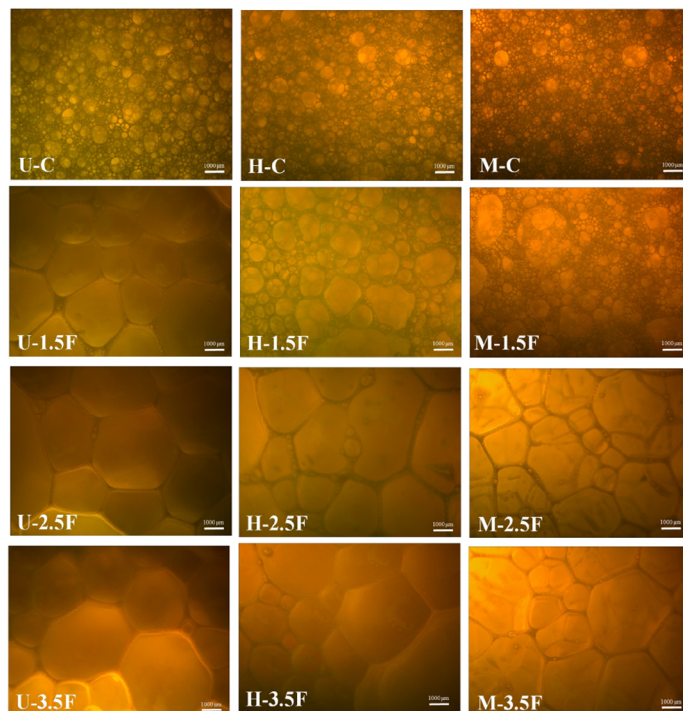
methods are shown in Fig. 3. It is noticed that these images were taken from foam produced by steam injection, and they were captured at a time of 0 and 10 min of the destabilisation process of foam at room temperature (25 °C). Foam appearance in all milk samples including raw milk samples (Fig. S3) was characterised by polyhedric air bubbles. It is well known that destabilisation of air bubbles due to the drainage of liquid film and coalescence of air bubbles, which occurs immediately after the foaming process is accomplished, results in the deformation of air bubbles from spherical to a polyhedric shape.[9]

A comparison between raw milk and control shearing-subjected milk samples [e.g., ultra-turraxed-control (U-C), homogenisation-control (H-C), and microfluidisation-control (M-C)] at both 0 and 10 min of the destabilisation process indicated that shearing actions significantly reduced air bubble size in foam, especially homogenisation and microfluidisation, which had very narrow size distribution curves (Fig. 4).
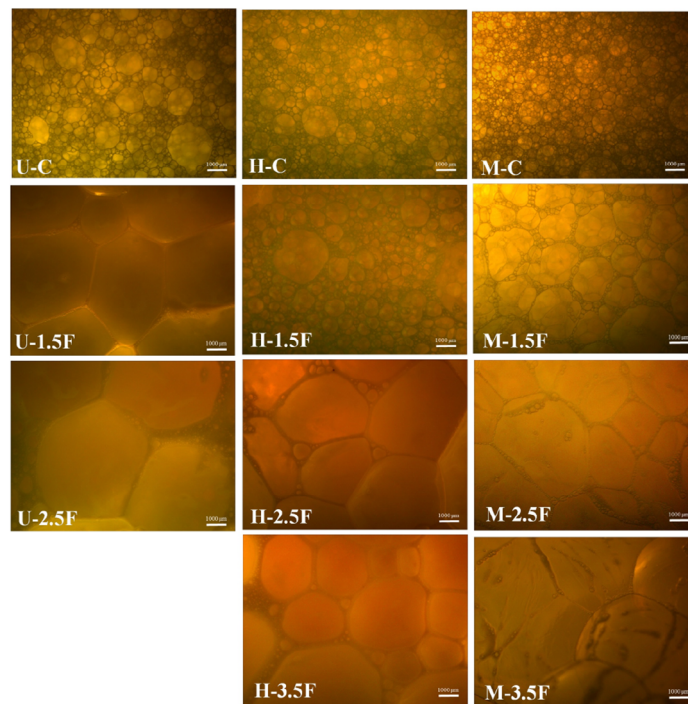
Visual observation during foaming, along with Fig. 3, indicated that FFAs greatly affected foam appearance and texture. Based on the foam appearance which was described by Kamath et al.[7] it can be concluded that foam with a smooth and creamy texture

J Sci Food Agric 2023   © 2023 The Authors.   wileyonlinelibrary.com/jsfa

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.
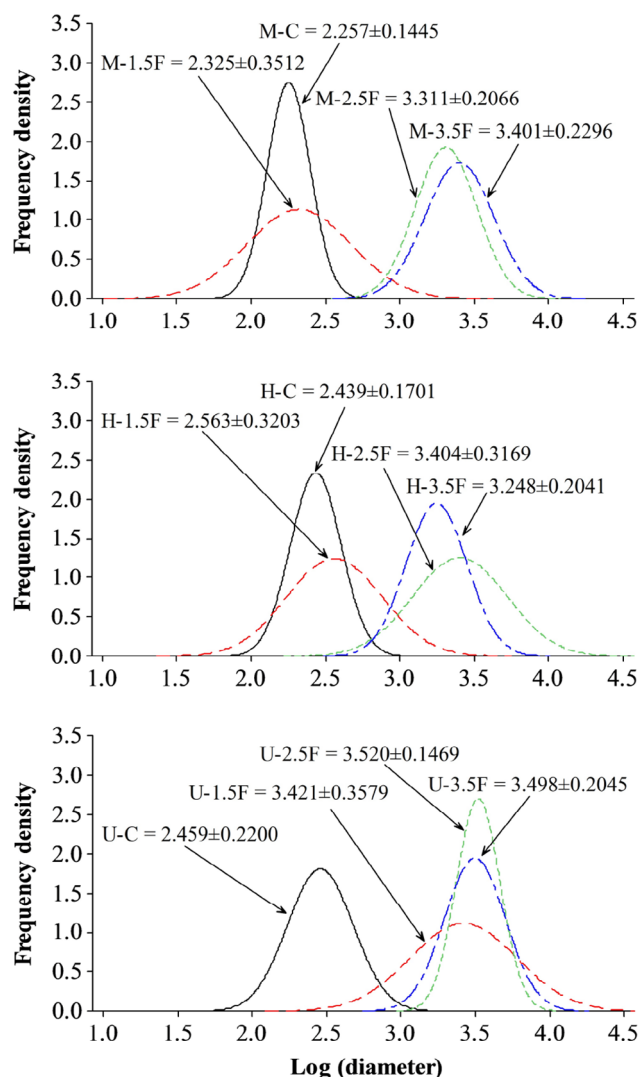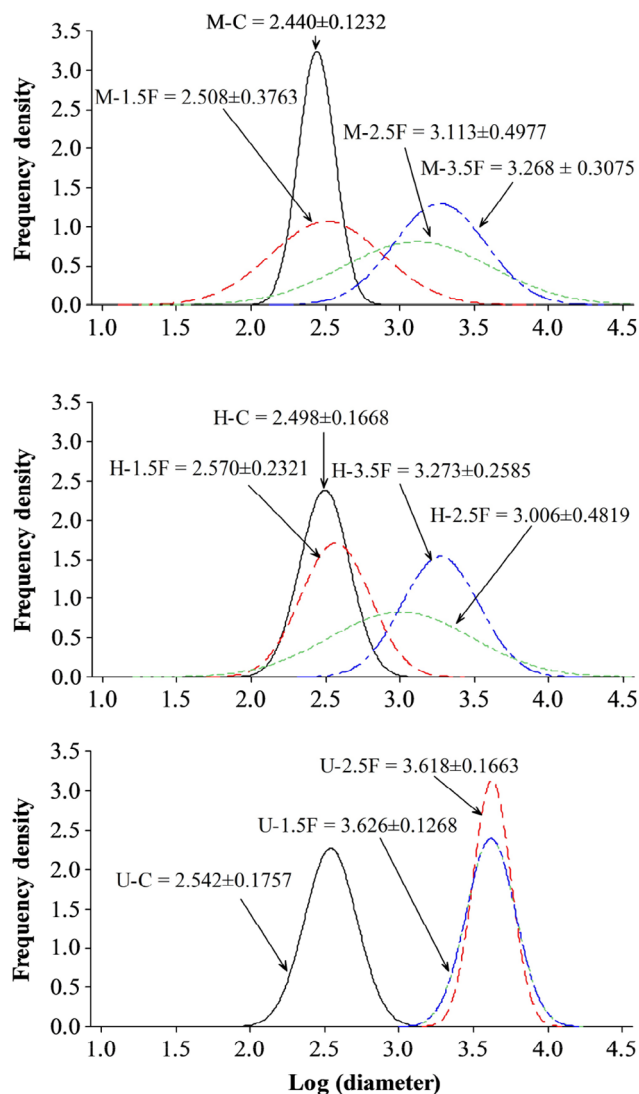
(a) t = 0 min

(b) t = 10 min

Figure 3. Images of foam surface captured at 0 and 10 min of destabilisation process and prepared from milk samples with varied free fatty acids (FFAs) contents and different shearing methods using the steam injection foaming method. U, H and M denote ultra-turraxing, homogenisation and microfluidisation, respectively. C, 1.5F, 2.5F and 3.5F denote control milk samples and those containing 1.5, 2.5 and 3.5 μ-equiv. mL^{-1} FFAs, respectively. Image of U-3.5F at t = 10 min could not be captured because the foam was too unstable for the measurements. Scale bar = 1000 μm.

was only obtained from control shearing-subjected milk samples, and foam became coarser and air bubble size in foam larger with increased FFA level. At an FFA level of 1.5 μ-equiv. mL^{-1}, only microfluidised and homogenised milk samples could produce a homogenous foam with a large proportion of small air bubbles. The other milk samples produced two-layered foam with a coarse foam layer on the top and a fine foam layer at the bottom. Therefore, compared with the air bubble size distribution of control milk

Figure 4. Size distribution curves of air bubbles in foam captured at 0 and 10 min of the destabilisation process and prepared from milk samples with varied free fatty acid (FFA) content and different shearing methods using the steam injection foaming method. U, H and M denote ultra-turraxing, homogenisation and microfluidisation, respectively. C, 1.5F, 2.5F and 3.5F denote control milk samples and those containing 1.5, 2.5 and 3.5 μ-equiv. mL^{-1} FFAs, respectively.

samples, the air bubble size distribution of milk samples containing an FFAs level higher than 1.5 μ-equiv. mL^{-1} was much larger (Fig. 4). These results were compatible with the findings reported by Kamath.[14] It is noticed that foam produced from ultra-turraxed milk samples at an FFA level of 3.5 μ-equiv. mL^{-1} collapsed too rapidly to capture the surface images at 10 min of the destabilisation process. During the destabilisation process, the air bubble size of foam produced from milk samples containing an FFA level of 1.5 μ-equiv. mL^{-1} became larger, while the air bubble size of foam produced from the other milk samples (e.g., $\geq$ 2.5 μ-equiv. mL^{-1} FFAs) was smaller. This is because of a quick collapse of the coarse foam layer on the top. However, the analysis of the Pearson correlation coefficient (*r*) between air bubble size at *t* = 0 (Fig. 4) and percentage V_F reduction (Fig. 2) revealed a high degree of correlation between the size of air bubbles and foam stability (*r* = 0.6).

CONCLUSION

Results in this study indicated that the foaming properties of milk were highly dependent on not only FFA content but also lipolysis-inducing methods which induced the changes in the physical properties of milk samples. Microfluidised milk samples exhibited the highest foamability and foam stability and the smallest air bubbles, followed by the homogenised milk samples. The changes in particle size, surface tension, zeta potential, and viscosity of milk under high-speed shearing forces could be responsible for the improvement in the foaming properties of milk. With the same shearing method, increasing the FFA content led to significantly low foamability, poor foam stability, and a coarse foam structure. However, the effects of FFAs on the foaming properties were different among the shearing methods. At the same FFA level, the foaming properties of the microfluidised milk samples were less affected by an increase in FFA level than those of

J Sci Food Agric 2023   © 2023 The Authors.   wileyonlinelibrary.com/jsfa

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

homogenised and ultra-turraxed milk samples. Regarding foam structure, the foam produced from all milk samples containing an FFA level higher than 2.5 μ-equiv. mL^{-1} had an unacceptable appearance.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTION

Thao M. Ho: conceptualisation, methodology, investigation, formal analysis, visualisation, data curation, writing – original draft, writing – review and editing. Bhesh R. Bhandari: conceptualisation, methodology, data curation, writing – review and editing. Nidhi Bansal: conceptualisation, methodology, supervision, investigation, data curation, visualisation, project administration, funding acquisition, writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

Research data are not shared.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Kontkanen H, Rokka S, Kemppinen A, Miettinen H, Hellström J, Kruus K et al., Enzymatic and physical modification of milk fat: a review. Int Dairy J 21:3–13 (2011).
2 Deeth HC, Lipoprotein lipase and lipolysis in milk. Int Dairy J 16:555–562 (2006).
3 Santos MV, Ma Y, Caplan Z and Barbano DM, Sensory threshold of off-flavors caused by proteolysis and lipolysis in milk. J Dairy Sci 86:1601–1607 (2003).
4 Buchanan R, Lipolysis and the frothing of milk. Aust J Dairy Technol 20:62–66 (1965).
5 Deeth H and Smith R, Lipolysis and other factors affecting the steam frothing capacity of milk. Aust J Dairy Technol 38:14 (1983).
6 Kitchen B and Cranston K, Lipase activation in farm milk supplies. Aust J Dairy Technol 24:107–112 (1969).
7 Kamath S, Wulandewi A and Deeth H, Relationship between surface tension, free fatty acid concentration and foaming properties of milk. Food Res Int 41:623–629 (2008).
8 Nylander T, Arnebrant T, Bos M and Wilde P, Protein/emulsifier interactions, in Food Emulsifiers and Their Applications, ed. by Gerard LH and Richard WH. Springer, New York, USA, pp. 89–171 (2008).
9 Huppertz T, Foaming properties of milk: a review of the influence of composition and processing. Int J Dairy Technol 63:477–488 (2010).
10 Ho TM, Bhandari BR and Bansal N, Functionality of bovine milk proteins and other factors in foaming properties of milk: a review. Crit Rev Food Sci Nutr 62:4800–4820 (2021).
11 Goh J, Kravchuk O and Deeth H, Comparison of mechanical agitation, steam injection and air bubbling for foaming milk of different types. Milchwissenschaft 64:121–124 (2009).
12 Ho TM, Le THA, Yan A, Bhandari BR and Bansal N, Foaming properties and foam structure of milk during storage. Food Res Int 116:379–386 (2019).
13 Breville, Breville—The Milk café™ Instruction Booklet. Breville Pty. Ltd., New South Wales (2010).
14 Kamath S, Foaming of Milk. PhD Thesis. School of Argiculrue and Food Sciences, University of Queensland, Brisbane, Australia (2007).
15 Ho TM, Dhungana P, Bhandari B and Bansal N, Effect of the native fat globule size on foaming properties and foam structure of milk. J Food Eng 291:110227 (2021).
16 Kamath S, Huppertz T, Houlihan AV and Deeth HC, The influence of temperature on the foaming of milk. Int Dairy J 18:994–1002 (2008).
17 Thiebaud M, Dumay E, Picart L, Guiraud JP and Cheftel JC, High-pressure homogenisation of raw bovine milk. Effects on fat globule size distribution and microbial inactivation. Int Dairy J 13:427–439 (2003).
18 Ruettimann K and Ladisch M, Casein micelles: structure, properties and enzymatic coagulation. Enzyme Microb Technol 9:578–589 (1987).
19 Hayes MG and Kelly AL, High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. J Dairy Res 70:297–305 (2003).
20 Antonelli M, Curini R, Scricciolo D and Vinci G, Determination of free fatty acids and lipase activity in milk: quality and storage markers. Talanta 58:561–568 (2002).
21 Borcherding K, Lorenzen PCHR and Hoffmann W, Effect of protein content, casein–whey protein ratio and pH value on the foaming properties of skimmed milk. Int J Dairy Technol 62:161–169 (2009).
22 Tunick MH, Ren DXX, Van Hekken DL, Bonnaillie L, Paul M, Kwoczak R et al., Effect of heat and homogenization on in vitro digestion of milk. J Dairy Sci 99:4124–4139 (2016).
23 Gallier S, Ye A and Singh H, Structural changes of bovine milk fat globules during in vitro digestion. J Dairy Sci 95:3579–3592 (2012).
24 Yang T, Li H, Wang F, Liu X and Li Q, Effect of cattle breeds on milk composition and technological characteristics in China. Asian-Australas J Anim Sci 26:896–904 (2013).
25 Fox PF ed, Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts and Vitamins, 2nd edn. Springer Science+Business Media, Dordrecht (2013).
26 Michalski M-C and Briard-Bion V, Fat-related surface tension and wetting properties of milk. Milchwissenschaft 58:6–29 (2003).
27 Pereda J, Ferragut V, Quevedo J, Guamis B and Trujillo A, Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. J Dairy Sci 90:1081–1093 (2007).
28 Amador-Espejo G, Suàrez-Berencia A, Juan B, Bárcenas M and Trujillo A, Effect of moderate inlet temperatures in ultra-high-pressure homogenization treatments on physicochemical and sensory characteristics of milk. J Dairy Sci 97:659–671 (2014).
29 Khezri M, Shahriari S and Shahsavani L, The effect of xanthan gum and temperature on foam stability of milk-based espresso coffees. J Food Biosci Technol 7:15–22 (2017).
30 Walstra P, Principles of foam formation and stability, in Physics, Chemistry and Structure Foams, ed. by Wilson A. Springer, London, pp. 1–15 (1989).
31 Silva S, Espiga A, Niranjan K, Livings S, Gumy JC and Sher A, Formation and stability of milk foams, in Bubbles in Food 2: Novelty, Health and Luxury, ed. by Campbell GM, Scanlon MG and Pyle DL. AACC International, Inc, Minnesota, pp. 153–162 (2008).
32 Augustin MA and Clarke PT, Skim milk powders with enhanced foaming and steam-frothing properties. Dairy Sci Technol 88:149–161 (2008).
33 Harte FM, Martinez MC and Mohan MS, Foaming and emulsifying properties of high pressure jet processing pasteurized milk. Patent: US20160374359A1 (2016).
34 Tran M, Roberts R, Felix T and Harte F, Effect of high-pressure-jet processing on the viscosity and foaming properties of pasteurized whole milk. J Dairy Sci 101:3887–3899 (2018).
35 Hettiarachchi CA, Corzo-Martinez M, Mohan MS and Harte FM, Enhanced foaming and emulsifying properties of high-pressure-jet-processed skim milk. Int Dairy J 87:60–66 (2018).