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2024-08
Wiley Blackwell
http://hdl.handle.net/10138/593491
Ho, M T, Lu, Y-J, Xiong, X, Bhandari, B R & Bansal, N 2024, 'Ability to re-foam frothed milk at different solid concentrations and their foam structure', International Journal of Dairy Technology, vol. 77, no. 3, pp. 874-883. https://doi.org/10.1111/1471-0307.13074

International Journal of Dairy Technology

SDT Society of Dairy Technology

doi: 10.1111/1471-0307.13074

RESEARCH ARTICLE

Ability to re-foam frothed milk at different solid concentrations and their foam structure

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Left-over frothed milk in most coffeehouses is typically discarded, leading to wastage of a lot of milk due to a common belief that frothed milk is unable to re-foam. We investigated the foaming and physical properties of frothed reconstituted skim milk (1.5–15%, w/w) foamed up to four times. The results showed that at all investigated solid concentrations and foaming times, milk samples retained their high foamability and foam stability, and uniform micro-foam structure. Similarly, properties of milk samples including viscosity (1.9–3.3 Pa.s), absolute zeta-potential (25.5–27.2 mV) and surface tension (54.8–59.7 mN/m) remained unchanged even after re-foaming multiple times.

Keywords Foaming times, Foamability, Foam stability, Foam structure, Steam injection, Solid concentration.

INTRODUCTION

A layer of foam provides a critical contribution to the quality of many cappuccino-style drinks regarding their appearance, volume and texture (Walstra 1989; Khezri *et al*. 2017). Milk proteins can produce highly stable foam at a very low concentration due to their distinctive structure and properties. In foaming, the diffusion rate of milk proteins is controlled by their size, surface hydrophobicity and structural flexibility, determining their foamability. Meanwhile their foam stability is decided by the mechanical and rheological properties of the interfacial adsorbed layers. Therefore, the rapid adsorption and unfolding on the air–liquid interface give milk proteins a high foamability, while the high strength and viscoelasticity of the adsorbed protein layers enable a good foam stability. Milk proteins with a high foam ability are not necessary to produce a highly stable foam and *vice versa* (Wilde and Clark 1996). Due to lack of tertiary structure, caseins have a great ability to diffuse to the interfacial region, and highly susceptible to the conformation alteration to form reversibly hydrophobic and ionic intermolecular interactions while whey proteins with disulphide bridges and tertiary structure have an ability to form a highly rigid interfacial film via intermolecular disulfide bonds (Martin *et al*. 2002; Ipsen and Otte 2004; Marinova *et al*. 2009; Ho *et al*. 2022). However, there has been an inconsistency in the reported results about foamability and foam stability of individual proteins. Many studies reported that milk samples with the higher proportion of caseins produced higher foam volume while those with the higher proportion of whey proteins produced more stable foam (Lee *et al*. 1992; Borcherding *et al*. 2009; Martinez-Padilla *et al*. 2014; Ewert *et al*. 2016). Meanwhile, Dombrowski *et al*. (2016) reported that β-lactoglobulin exhibited a significantly higher foamability and foam stability than casein micelles. Similarly, Xiong *et al*. (2020) found that increasing the proportion of whey proteins in milk protein dispersions increased foamability, but decreased foam stability and induced the formation of large foam air bubbles. The differences in the results reported in these studies indicate that foaming properties of milk proteins are very complicated as they are not only determined by protein properties (type, concentration, molecular weight and structure) but also by protein solution conditions (pH, temperature and

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electrolyte concentrations) and foaming methods (air injection, steam injection and mechanical mixing).

In most coffeehouses, steam injection is the most common foaming approach in which milk foam is generated by the steam injected directly into the milk via a very small opening nozzle placed just under the milk's surface. During foaming process, milk is simultaneously heated to 65–70°C, which is the desirable temperature for the consumption of many hot beverages. However, in this foaming method, controlling the final temperature is very difficult, which requires practical experience of a highly trained steaming operator. Thus, there is a very high risk of overheating of the foaming milk, leading to the extreme denaturation of proteins and scalding of milk, followed by a loss of milk flavour and bitter undertones to the foam surface. The rearrangement of hydrophilic and hydrophobic groups of milk proteins on the interfacial layers, and the intermolecular interactions to form an interfacial film during foaming possibly cause the partial denaturation of proteins, possibly leading to the loss of their integrity and inherent functionality (Zayas 1997). Additionally, the shearing action of mixing during steam injection could be detrimental to the surface properties of proteins (Maa and Hsu 1997). All these could be reasons for the common belief that frothed milk is unable to be re-foamed. Therefore, a large amount of left-over frothed milk is always discarded, leading to much milk waste. Another possible reason for disposing of frothed milk is dilution of milk. In steam injection, steam is unavoidably condensed into water and incorporated into milk, which accounts for 10–12% by mass (Deeth and Smith 1983), leading to a reduction of the protein concentration in the milk and its viscosity. These changes have been reported to have great impact on foamability and foam stability as they determine the amount of proteins available for formation of high viscoelastic interfacial layer (Kelly and Burgess 1978; Britten and Lavoie 1992; Kamath 2007; Borcherding *et al*. 2009; Marinova *et al*. 2009; Tamm *et al*. 2012; Martinez-Padilla *et al*. 2014). However, there is still a lack of scientific evidence on poor foamability and foam properties of frothed milk.

A study by Deeth and Smith (1983) is likely an unique report on re-frothing ability of milk, but it was not comprehensive as it focused solely on the milk foamability. The authors found that the foamability of raw and pasteurised milk after second frothing was much higher than that in the first. No further analyses were performed on the properties of frothed milk and other foam properties like foam stability and structure. Therefore, further comprehensive investigation on the multi-foaming ability of milk is warranted. In this study, re-foaming ability up to four times of reconstituted skim milk solutions at different solid contents (1.5, 5.0, 8.5, 10 and 15%, w/w) was investigated employing two common foaming methods namely mechanical mixing and steam injection. These total solid contents (i.e. 1.5–15%) of milk samples were chosen based on the previous study by Kamath (2007) to cover a wide range of protein contents in the milk samples which were about 0.5–5.0% (w/w) and resulted in a significant difference in their foamability and foam stability. In most coffeehouses, not only the left-over frothed milk is discarded but also the milk jugs must be carefully washed to remove the frothed milk residue which can undergo multiple foaming times if the same unwashed jugs are used. To evaluate if frothed milk is able to maintain their foaming ability after foaming multiple times and if the frothed milk residues have a negative impact on foaming properties, we chose to re-foam milk samples up to four times. The physical properties of milk samples obtained from collapsed foam at each foaming times, foamability, foam stability and foam structure were evaluated.

MATERIALS AND METHODS

Materials

Low-heat-treated skim milk powders (SMP) were purchased from Tatura Milk Industries Ltd. (Tatura, NSW, Australia), and its main composition, according to the manufacturer, includes 32.5% protein, 0.8% fat and 55.0% lactose. All chemicals (which were of analytical reagent grade) were bought from Sigma-Aldrich (Castle Hill, NSW, Australia).

Preparation of reconstituted skim milk at different solid concentrations

Reconstituted SMP with different solid concentrations (1.5, 5.0, 8.5, 10 and 15%, w/w) were prepared as reported by Kamath (2007) with some modification. For each concentration, the pre-weighed SMP amount was mixed to the predetermined amount of MilliQ water under the stirring at a speed of 100 rpm (IKA® overhead stirrer, LabGear, Milton, QLD, Australia). The solution was further mixed at 100 rpm for 1 h. All milk samples were equilibrated for 12 h in the cold room at 4°C before being used for further experiments.

Foaming of milk samples

Two common foaming methods, namely mechanical mixing (Breville cafe milk frother, BMF600; Breville Group Ltd, Alexandria, NSW, Australia) and steam injection (Café Series EM6910; Sunbeam, Corrimal, NSW, Australia) were investigated in this study. The step-by-step details of these methods were reported in our previous studies (Ho *et al*. 2019, 2021; Xiong *et al*. 2020). Briefly, in both foaming methods, milk samples were firstly cooled to 5°C using an ice bath, and the foaming process was stopped as the foaming milk's temperature reached to 65°C. In mechanical mixing, the mechanical mixer is integrated with a heating unit allowing to heat the milk during foaming process to the preset value (65°C), and due to technical requirement, 250 mL of milk was used to foam. For steam injection, 100 mL of

milk was used, and during foaming process, the milk container was moved gradually down to maintain the steam wand position which was 2 mm under the milk surface to facilitate the air incorporation into the milk bulk. As the milk temperature reached 45°C, the milk container was moved up to lower the steam wand tip into the milk to texturize and heat the milk up to 65°C.

After the first foaming, the whole container of foamed milk was kept at 4°C overnight to allow the foam to completely collapse. This milk sample was then foamed again using the same technique. This foaming procedure was repeated up to four times. For each foaming, the same amount of milk samples was used by combining collapsed foam from at least two foam containers. It was noticed that the incorporation of condensed steam into milk samples happened only for steam injection. The amount of water addition at each of foaming times was estimated from the volume difference between the initial volume of milk used for foaming (i.e. 100 mL) and the volume of frothed milk measured as the foam was completely collapsed at 4°C. To simplify the presentation, foam produced at the initial foaming time, and at the first, second and third re-foaming times were denoted as initial, RF1, RF2 and RF3 respectively.

Determination of foaming properties

Foamability and foam stability

Foamability was evaluated as a ratio (foam ratio) of foam volume (which was immediately measured as the interfacial layer between liquid and foam was observed) and volume of initial milk sample used to generate foam (which was 100 mL in steam injection and 250 mL in mechanical mixing). Foam volume was measured either directly from the graduated foaming jug (steam injection), or gently pouring and scrapping (using a plastic spatula) the foam into a 500 mL plastic cylinder (mechanical mixing).

Foam stability was expressed as per Eq. 1, which was the percentage reduction in foam volume after 10 min of the destabilisation process at room temperature (%). This time was chosen for evaluating of foam stability because most cappuccino-style drinks are typically consumed to half their volume within 10 min after being processed/severed (Xiong *et al*. 2020). In the Eq. 1, V_{F0} and V_{F10} are the foam volume at $t = 0$ and $t = 10$ min respectively.

$$V_{\text{Foam}} \text{ reduction after 10 min } (\%) = \frac{V_{F0} - V_{F10}}{V_{F0}} * 100 \quad (1)$$

Foam structure

The light microscope (Prism Optical, Eagle Farm, QLD, Australia) equipped with a 5.0 MP camera system (Tucsen Image Technology Co., Ltd., Fuzhou, China) was employed to image the surface of foam immediately after it was generated ($t = 0$) and after 10 min of destabilisation at 25°C ($t = 10$ min). During imaging, a fibre optic light source (Olympus LG-PS2 lamp, Eagle Farm, QLD, Australia) was used to illuminate the foam. Image-Pro Plus 6.0 software (Media Cybernetics Inc, Bethesda, MD, USA) was used to determine the diameter the longest length of air bubbles in foam.

Determination of properties of milk samples

Physical properties of milk samples including viscosity, surface tension and zeta-potential were characterised by following the methods reported in our previous article (Ho *et al*. 2021), and here we briefly described their principle. The shear-dependent viscosity curves at 25°C in the shear rate range of 0–100 s^{-1} of milk samples was determined with an AR 1500 Rheometer (TA Instruments, Cheshire, UK), using a cone and plate geometry (40 mm in diameter and 0.2 mm in gap). Viscosity at 50 s^{-1} was extracted from the curves and reported. The surface tension of milk samples was measured using a tensiometer (ST9000, Nima technology, Coventry, UK), which was the maximum force acting on the plate as it was raised out of the samples. Before the measurement, a platinum Wilhelmy plate was flamed with a strong flame source to remove any residue, and the tensiometer was checked by measuring the surface tension of ultrapure Millipore water (72 mN/m). Between measurements, the plate is rinsed with ultrapure Millipore water and absolute ethanol solution, and flamed if required. Zeta-potential of milk samples diluted with MilliQ water at a ratio of 1:100 was measured using a Zetasizer Nano (Malvern Panalytical Ltd., Great Malvern, UK) using disposable polycarbonate cuvette (DTS1061; ATA Scientific, Caringbah, NSW, Australia). Measurements were performed with a minimum of three runs and a minimum of 10 times per run.

Determination of protein components in the fractions of foam and liquid, and collapsed frothed milk

Individual protein components, including kappa-casein (κ-CN), alpha-casein (α-CN), beta-casein (β-CN), alpha-lactalbumin (α-La) and beta-lactoglobulin (β-Lg) of foam and liquid fractions and collapsed frothed milk prepared from milk samples at solid concentrations of 1.5 and 10% (w/w) were determined. A duration of 1–2 min after foaming, when an interfacial layer between foam and liquid fractions was clearly observed, some portions of foam fraction were carefully collected from the top of the foaming container using a plastic spoon without touching the interfacial layer, and then some portions of liquid fraction from the bottom of the foaming container were carefully withdrawn using a plastic pipette. Collapsed frothed milk was obtained by keeping the whole container of frothed milk at 4°C for 12 h to collapse all foam. Foam in the foam fractions was also collapsed in a similar way before analysis.

Individual protein components were determined by reversed-phase high-performance liquid chromatography (RP-HPLC), following the approach reported by Bonfatti

et al. (2008) with minor modification. A mixture solution (pH 6.8) of 0.1 M Bis-Tris buffer, 6 M guanidine hydrochloride (GdnHCl), 5.37 mM sodium citrate and 19.5 mM dl-dithiothreitol (DTT) was mixed into milk samples at a volume ratio of 1:1. After shaken for 10 s, the mixed samples were incubated at 25°C for 1 h and followed by centrifugation at 16 000 *g* for 5 min. After carefully removing the fat layer, the remaining liquid was diluted 1:3 (v/v) with a solution containing 4.5 M GdnHCl in a solvent consisting of acetonitrile, water, trifluoroacetic acid in a volume ratio 100:900:1. Individual protein components were separated using an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an analytical column Zobax 300SB-C18 (4.6 × 150 mm, 5 µm, Agilent Technologies, Waldbronn, Germany). The chromatographic conditions were of injection volume: 50 µL, column temperature: 25°C, flow rate: 1.20 mL/min and detection wavelength: 226 nm.

Experimental design and statistical analysis

The experiments were executed with three replications from three different SMP bags and at least two measurements were done for each replicate. The results are reported as mean values (± SD). The experimental data were subjected to one-way ANOVA and then post hoc Tukey's test to differentiate the mean values at a significance level of $P = 0.05$ using the Minitab 16.0 statistical programme (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Foaming properties of milk samples at different solid concentrations and different foaming times

Foamability and foam stability

Table 1 exhibits foamability and foam stability of milk samples at various solid concentrations and different foaming times, determined by mechanical mixing and steam injection. Based on Eq. 1, the higher percentage of foam volume reduction after 10 min indicates less foam stability. Regarding effects of solid concentrations, at the same foaming times, there was not significant difference in foamability and foam stability among milk samples with different solid concentrations ($P > 0.05$) despite their differences in the content of protein, lipid and lactose and viscosity (Table S1). This phenomenon was observed in both foaming methods. The results indicate the good foaming properties of milk proteins even at a very low concentration. Milk proteins, for example, caseins at a concentration of as low as 0.02 ppm can absorb at the air–liquid interface and reduce the surface tension, facilitating formation of stable foam (Kinsella and Morr 1984; Ho *et al*. 2022). Studies reported that milk proteins at a low concentration were able to produce a high volume of stable foam, for example, reconstituted SMP solutions at 0.5% protein (Kamath 2007), sodium caseinate and whey protein isolate at 0.25% protein (Britten and Lavoie 1992), sodium caseinate at 0.3% and whey protein concentrate at 1.0% protein (Marinova *et al*. 2009), skimmed milk at 1.5% protein (Borcherding *et al*. 2009) and reconstituted milk protein concentration dispersions at of 1.5–4% protein (Xiong *et al*. 2020). It is noticed that there is an inconsistency in the reported results on foaming properties of milk samples at different solid concentrations. Kamath (2007) reported that increasing solid concentration of reconstituted SMP solutions from 1.5 to 15% markedly increased foamability, but significantly reduced foam stability. Similar results were also reported for foaming properties of either whey protein isolate or whey protein hydrolysate solutions with an increase of protein content from 1 to 3% (Tamm *et al*. 2012). However, Martinez-Padilla *et al*. (2014) reported that despite an four-fold increase of the viscosity of reconstituted SMP solutions as their protein content increased from 3.6 to 9.0% (w/w), both their foamability and foam stability significantly increased. Meanwhile, Borcherding *et al*. (2009) found that the foam stability of skim milk was almost identical with an increased protein content from 1.0 to 6.0% (w/w). It is noticed that in these studies, foam was prepared via different foaming methods and from milk sources, which could explain the inconsistent reported results.

Regarding the effects of foaming times, in steam injection foaming method, at the same solid concentration, there was no significant difference in foamability of milk samples at different foaming times ($P > 0.05$). Meanwhile, under mechanical mixing foaming method, foamability slightly increased with the foaming times, especially that of milk samples at 5–10% which was significantly improved at the fourth foaming times ($P < 0.05$). However, in both foaming methods, regardless of solid concentration of milk samples, foam stability was not affected by the foaming times ($P > 0.05$). The results are similar to those reported by Deeth and Smith (1983), who reported that foamability of raw and pasteurised milk in the second frothing times was much higher than in the first times. The improvement of foamability of frothed milk samples is possibly caused by the unfolding of proteins in the previous foaming times, which facilitates their re-unfolding during subsequent foaming times. The results contradict the common belief that unfolding/denaturation of milk proteins induced by the heating during foaming process makes milk unable to foam. It is reported by Brown (1988) that unfolding of milk proteins is reversible if the heating process is stopped before aggregation begins. Thus, the structure of milk proteins is easily renatured during foam collapsing at cold temperature (4°C). Moreover, Deeth and Smith (1983) reported that there was no change in the foaming properties of milk samples initially heated at conditions similar to those in steam injection foaming method (e.g. heating to 75°C), followed by cooling

Table 1 Foam ratio and foam stability of milk samples at different solid concentrations and foaming times under mechanical mixing and steam injection foaming methods.

		Foaming times				
	Solid concentration, (%)	Initial	RF1	RF2	RF3	*P*-values
Steam injection						
Foam ratio (−)	1.5	2.73 ± 0.05	2.74 ± 0.05	2.78 ± 0.04	2.91 ± 0.15	>0.05
	5.0	2.69 ± 0.22	2.80 ± 0.16	2.77 ± 0.13	2.86 ± 0.14	>0.05
	8.5	2.55 ± 0.17	2.72 ± 0.20	2.73 ± 0.28	2.89 ± 0.16	>0.05
	10	2.51 ± 0.18	2.58 ± 0.16	2.66 ± 0.17	2.90 ± 0.16	>0.05
	15	2.43 ± 0.15	2.56 ± 0.14	2.75 ± 0.20	2.82 ± 0.16	>0.05
	P-values	>0.05	>0.05	>0.05	>0.05	
V_{Foam} reduction after 10 min (%)	1.5	56.46 ± 2.99	54.97 ± 2.31	55.96 ± 1.92	57.03 ± 5.42	>0.05
	5.0	54.81 ± 5.94	53.95 ± 3.56	52.74 ± 3.41	57.65 ± 1.74	>0.05
	8.5	49.59 ± 2.64	49.43 ± 1.94	51.36 ± 2.71	53.93 ± 3.64	>0.05
	10	47.97 ± 5.57	50.30 ± 3.22	47.82 ± 4.70	50.85 ± 1.86	>0.05
	15	45.50 ± 4.16	48.26 ± 3.13	48.52 ± 2.01	50.93 ± 4.40	>0.05
	P-values	>0.05	>0.05	>0.05	>0.05	
Mechanical mixing						
Foam ratio (−)	1.5	1.85 ± 0.05	1.86 ± 0.05	1.93 ± 0.04	1.98 ± 0.06	>0.05
	5.0	1.82 ± 0.04^{a}	1.83 ± 0.01^{a}	1.85 ± 0.03^{ab}	1.93 ± 0.06^{b}	<0.05
	8.5	1.73 ± 0.09^{a}	1.77 ± 0.02^{ab}	1.83 ± 0.05^{ab}	1.89 ± 0.02^{b}	<0.05
	10	1.72 ± 0.05^{a}	1.75 ± 0.04^{a}	1.81 ± 0.05^{ab}	1.89 ± 0.06^{b}	<0.05
	15	1.74 ± 0.05	1.79 ± 0.07	1.84 ± 0.08	1.81 ± 0.11	>0.05
	P-values	>0.05	>0.05	>0.05	>0.05	
V_{Foam} reduction after 10 min (%)	1.5	37.03 ± 2.20	36.34 ± 0.36^{A}	36.56 ± 1.87	37.43 ± 2.81	>0.05
	5.0	37.25 ± 1.13	34.40 ± 0.33^{AB}	36.56 ± 1.87	37.86 ± 2.88	>0.05
	8.5	33.89 ± 3.11	33.47 ± 1.30 ^{B}	35.90 ± 1.90	36.90 ± 2.63	>0.05
	10	33.79 ± 3.23	33.13 ± 1.33^{B}	34.65 ± 1.01	35.83 ± 2.06	>0.05
	15	34.27 ± 0.48	34.37 ± 0.73^{B}	35.39 ± 0.66	35.67 ± 0.73	>0.05
	P-values	>0.05	<0.05	>0.05	>0.05	

Initial, RF1, RF2 and RF3 denote the initial foaming time, and the first, second and third re-foaming times respectively. With the same solid concentration or the same row (e.g. 1.5, 5, 8.5, 10 and 15), different letters (a, b, c, …) indicated significant difference in the means values among foaming times ($P < 0.05$). With the same foaming times or the same column (e.g. 1.5, 5.0, 8.5, 10.0 and 15), different capitalised letters (A, B, C, …) indicated significant difference the means values among solid concentrations ($P < 0.05$).

to room temperature. Kamath (2007) also reported that denaturation of whey proteins happened only when the milk was heated at 75−85°C for 1 min. The steam injection foaming method added a certain amount of condensed steam into milk samples after each foaming, which was about 4–6% (v/v), while this phenomenon does not occur with the mechanical mixing foaming method. This could be the reason for the differences in the foamability of milk samples at different foaming times in two foaming methods. However, the addition of condensed steam during the foaming process did not affect foam stability of frothed milk.

Foam structure

Foam images and size of the air bubbles in foam produced generated from the milk samples at solid concentration of 1.5 and 10% (w/w) via steam injection at four foaming times were analysed. Figures 1 and 2 indicated that at both solid concentrations, foaming times did not markedly affect foam appearance, foam structure or size of air bubbles. Foam obtained from all the investigated milk samples was of good structure and appearance having a significant portion of very small air bubbles. At both $t = 0$ and $t = 10$ min, and the same foaming times, milk samples at 1.5% solid concentration produced larger air bubbles than those at 10% solid concentration. These results are similar to those founded by Borcherding *et al*. (2009) where it was found that average air bubble diameter decreased with increasing of milk protein from 1.0 to 4.0%. In addition, it can be seen from Figure 2 that the foaming times did not affect average air bubble size in foam.

For all milk samples regardless of the solid concentrations and foaming times, after 10 min of foam preparation, the air bubble size became larger and the size distribution pattern shifted to the side with a larger air bubble size, which can

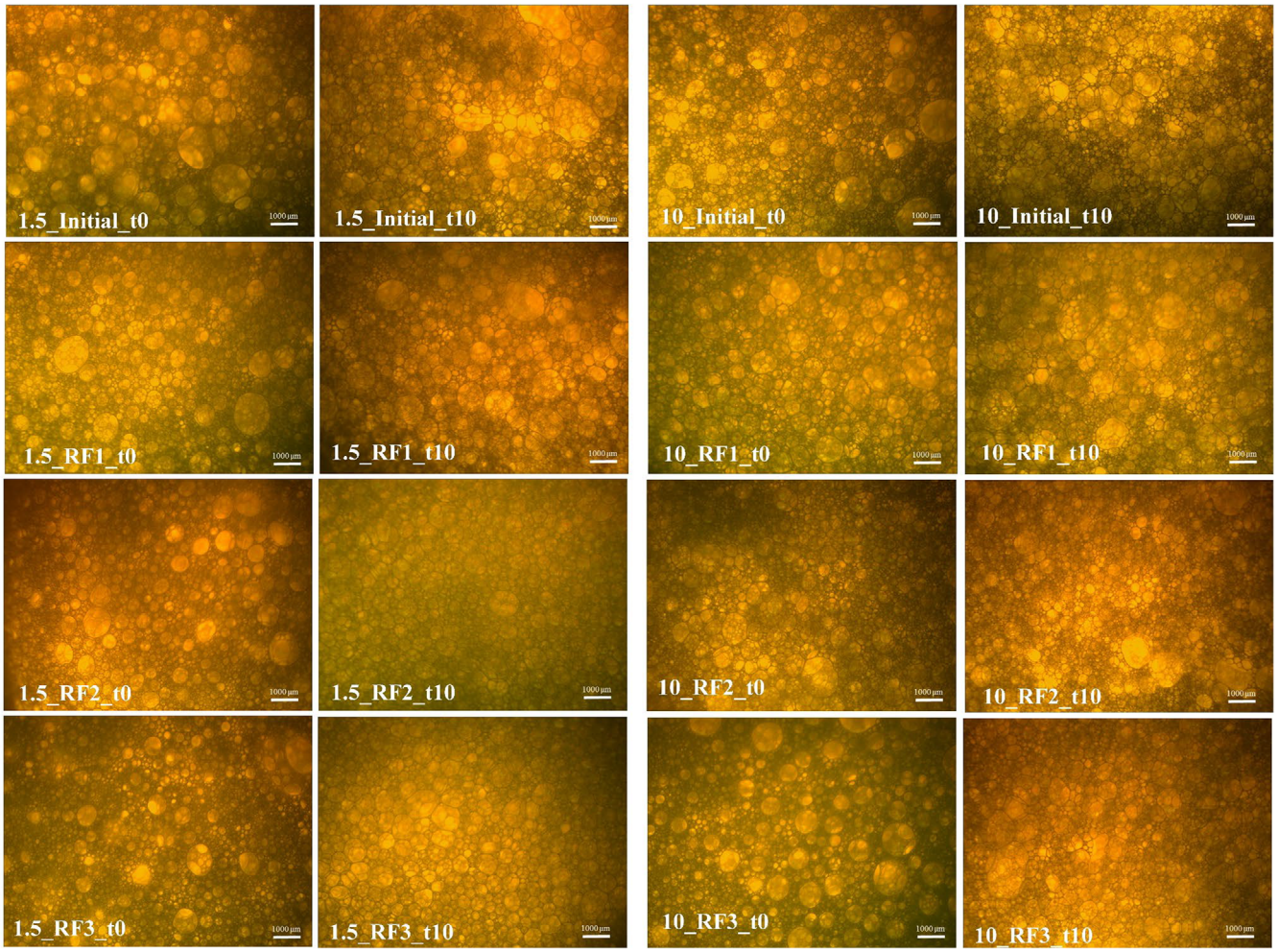

Figure 1 Images of foam surface prepared from milk samples at solid concentrations of 1.5 and 10% (w/w) at four foaming times via steam injection. Initial, RF1, RF2 and RF3 denote initial foaming time, and at the first, second and third re-foaming times, respectively. These images were taken at time 0 (t_0) and 10 min (t_{10}) of destabilization process at 25°C. Scale bar = 1000 µm.

be a result of drainage of liquid film and coalescence of air bubbles (Walstra 1989; Huppertz 2010).

Properties of collapsed foamed milk

Table 2 and Figure 3 exhibit viscosity, and surface tension and zeta-potential of milk samples at 1.5 and 10% (w/w) solid concentrations, and those of milk samples obtained from collapsed frothed milk at different re-foaming times (RF1, RF2 and RF3) under two foaming methods (mechanical mixing and steam injection). For viscosity, milk samples at 1.5% was 1.93 mPa.s, which was significantly lower than that of milk samples at 10% (i.e. 3.21 Pa.s). Similarly, Kamath (2007) reported that viscosity of reconstituted SMP increased with increase in the total solids content of the milk. Regardless of foaming methods and solid concentrations, re-foaming did not induce any alteration of viscosity ($P > 0.05$). As mentioned, there was water addition due to steam condensation in steam injection method, which resulted in dilution of milk samples after each foaming (i.e. 4–6%, v/v). However, the amount of water addition could still not be enough to induce the reduction of viscosity. It was reported that the denaturation and/or aggregation of whey proteins led to an increase of viscosity (Tari *et al.* 2021). Thus, the unchanged viscosity of milk samples at different foaming times in our study could indicate that an extensive denaturation of whey proteins did not happen during repeated foaming. However, this assumption must be further investigated.

As shown in Figure 3, milk samples at 1.5 and 10% solid concentrations had a similar values of surface tension (56.9–58.6 mN/m) and absolute zeta-potential (26.2–27.2 mV), and these values did not change among different foaming times in both foaming methods ($P > 0.05$). Surface tension and zeta-potential of milk are highly dependent on the

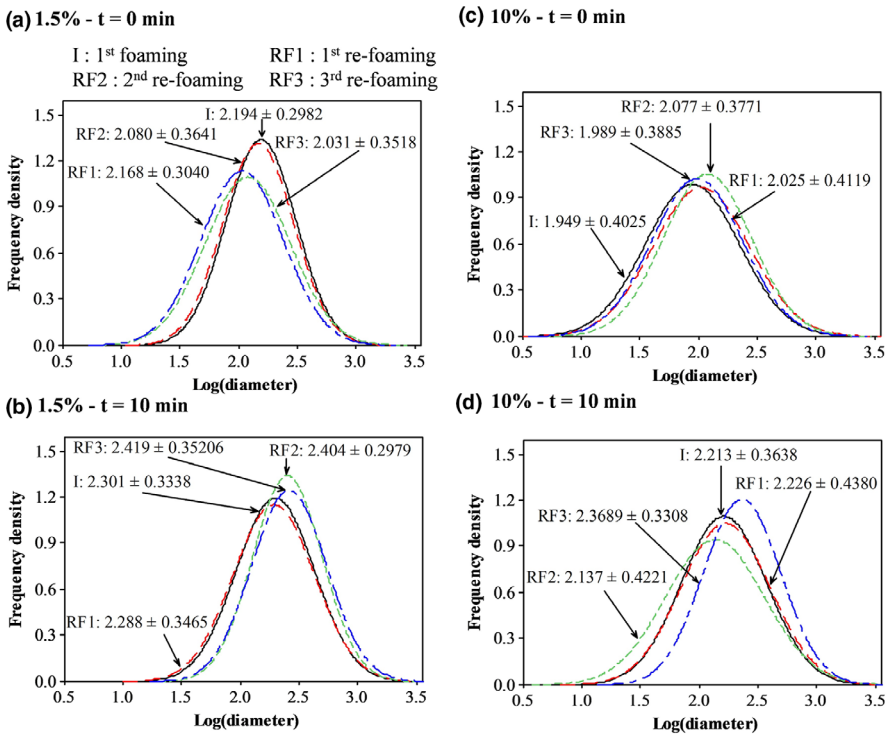

Figure 2 Size distribution of air bubbles in foam prepared from milk samples at solid concentrations of 1.5 and 10% via steam injection, at time 0 and 10 min of the destabilization process. Initial, RF1, RF2 and RF3 denote initial foaming time, and at the first, second and third re-foaming times, respectively.

Table 2 Viscosity (mPa.s, at shear rate of 50 s^{-1}) of milk samples at solid concentrations of 1.5 and 10% (w/w) and different foaming times in mechanical mixing and steam injection foaming methods.

Samples	Initial	RF1	RF2	RF3
Mechanical mixing				
M-1.5	1.93 ± 0.18^{a}	2.37 ± 0.15^{a}	2.38 ± 0.35^{a}	2.32 ± 0.33^{a}
M-10	3.21 ± 0.14^{a}	3.51 ± 0.22^{a}	3.42 ± 0.37^{a}	3.60 ± 0.60^{a}
Steam injection				
S-1.5	1.93 ± 0.18^{a}	1.78 ± 0.09^{a}	1.90 ± 0.09^{a}	1.99 ± 0.20^{a}
S-10	3.21 ± 0.14^{a}	3.10 ± 0.18^{a}	3.58 ± 0.10^{a}	3.54 ± 0.31^{a}

1.5 and 10 indicated the sample concentrations of 1.5 and 10% respectively; M and S were the initials of mechanical mixing and steam injection foaming methods respectively. Initially, RF1, RF2 and RF3 denote the initial foaming time, and the first, second and third re-foaming times respectively. In each row, different letters indicate a significant difference among foaming times ($P < 0.05$). For each of foaming methods and each of foaming times, viscosity values of milk samples at 1.5 and 10% of solid concentrations were significantly different ($P < 0.05$).

presence of surface active components such as fat, proteins and phospholipids and their interaction (Kamath 2007; Tunick *et al*. 2016). Similarities in these properties of milk samples among foaming times indicated ability of milk proteins to renature during foam collapsing at 4°C, and explained for the similarities in foamability and foam stability of milk samples at different foaming times.

Individual protein components in foam faction, liquid fraction and collapsed frothed milk

Concentration of individual protein components in foam faction, liquid fraction and collapsed frothed milk at different foaming times is shown in Table 3. Unlike mechanical mixing, steam injection is the most widely used foaming method in most coffeehouses and results in water addition due to steam condensation which possibly leads to the distribution of individual proteins in liquid and foam factions. Therefore, this analysis was carried out only for foam produced by steam injection. There was a reduction in the concentration of these individual proteins in foam faction, liquid fraction and collapsed frothed milk after each foaming time. The results were observed for milk samples at both solid concentrations of 1.5 and 10% (w/w). After each foaming time, there was water incorporation into milk due to the condensed steam, which diluted protein content in milk samples. Despite the decline in concentration of individual milk proteins in milk samples after each foaming time, both foamability and foam stability were unchanged. As reported by Deeth and Smith (1983), the frothing properties of milk was unchanged with an addition of water into

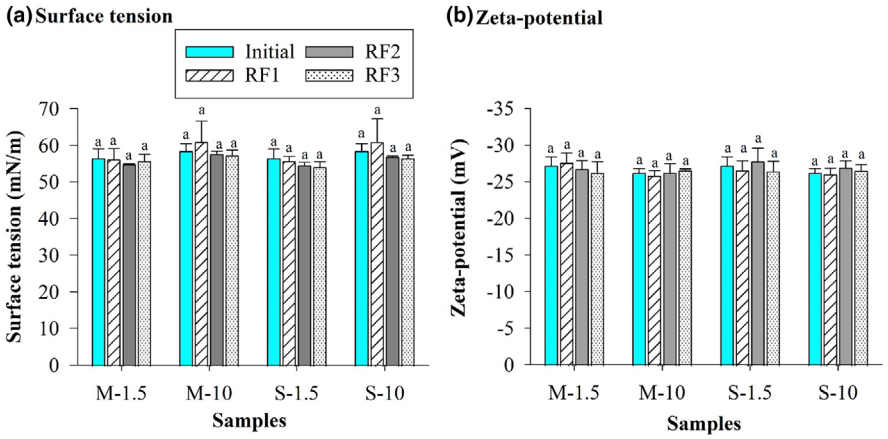

Figure 3 Surface tension (a) and zeta-potential (b) of milk samples solid concentrations of 1.5 and 10% (w/w) in mechanical mixing (M) and steam injection (S). Initial, RF1, RF2 and RF3 denote initial foaming time, and at the first, second and third re-foaming times, respectively. For each property of milk sample (e.g., M-1.5, M-10, S-1.5 and S-10), different letters indicated significant difference among the foaming times ($P < 0.05$).

Table 3 Concentration of individual protein components (mg/mL) in foam faction, liquid fraction and collapsed foamed milk.

Samples			κ-CN	α_CN	β-CN	α-La	β-Lg
1.5	Milk sample		0.39 ± 0.01^{abf}	1.78 ± 0.04ab	1.61 ± 0.08^{abc}	0.09 ± 0.00^{a}	0.39 ± 0.00^{ab}
	Foam fraction	F1	0.43 ± 0.04^{a}	1.93 ± 0.15^{a}	1.91 ± 0.36^{a}	0.08 ± 0.00^{a}	0.41 ± 0.00^{a}
		F2	0.37 ± 0.04^{abcf}	1.72 ± 0.18^{abc}	1.77 ± 0.34^{ab}	0.07 ± 0.00^{abd}	0.36 ± 0.02^{abe}
		F3	0.33 ± 0.03^{bcdf}	1.55 ± 0.11^{bcd}	1.53 ± 0.34^{abc}	0.07 ± 0.00^{abc}	0.32 ± 0.01^{abc}
		F4	0.29 ± 0.01^{cdef}	1.37 ± 0.09^{cdeg}	1.39 ± 0.24^{abcd}	0.06 ± 0.00^{bc}	0.32 ± 0.03^{abc}
	Liquid faction	L1	0.33 ± 0.22^{bcdf}	1.49 ± 0.06^{bcde}	1.25 ± 0.12^{abcd}	0.08 ± 0.00^{abd}	0.33 ± 0.03^{abc}
		L2	0.28 ± 0.02^{cdef}	1.28 ± 0.00^{defg}	1.05 ± 0.12^{bcd}	0.07 ± 0.00^{abdf}	0.29 ± 0.02^{bcd}
		L3	0.24 ± 0.02^{def}	1.14 ± 0.10^{efg}	0.92 ± 0.09^{cd}	0.06 ± 0.00^{bc}	0.23 ± 0.07^{cd}
		L4	0.21 ± 0.02^{eg}	1.00 ± 0.08^{fg}	0.76 ± 0.04^{d}	0.05 ± 0.01^{c}	0.20 ± 0.05^{de}
	Collapsed frothed milk	M1	0.35 ± 0.00^{abcf}	1.62 ± 0.02^{abd}	1.49 ± 0.03^{acd}	0.08 ± 0.00^{ab}	0.35 ± 0.00^{abe}
		M2	0.32 ± 0.01f^{g}	1.44 ± 0.03^{bcdeg}	1.22 ± 0.07^{acd}	0.06 ± 0.01^{cd}	0.29 ± 0.01^{bcd}
		M3	0.28 ± 0.01^{cde}	1.32 ± 0.02^{defg}	1.09 ± 0.05^{bcd}	0.06 ± 0.01^{cd}	0.26 ± 0.03^{ce}
		M4	0.25 ± 0.03^{deg}	1.14 ± 0.04^{g}	0.97 ± 0.03^{cd}	0.05 ± 0.01^{cf}	0.24 ± 0.02^{cd}
	P-values		<0.05	<0.05	<0.05	<0.05	<0.05
10	Milk sample		2.81 ± 0.19^{A}	11.82 ± 0.77^{A}	11.05 ± 0.18^{A}	0.56 ± 0.03^{A}	2.74 ± 0.27^{A}
	Foam fraction	F1	2.61 ± 0.12^{AF}	11.07 ± 0.51^{AB}	10.32 ± 1.15^{AB}	0.54 ± 0.02^{AB}	2.62 ± 0.10^{AG}
		F2	2.51 ± 0.06^{AB}	10.63 ± 0.29^{BC}	9.87 ± 0.87^{ABC}	0.52 ± 0.02^{ABD}	2.40 ± 0.04^{ABH}
		F3	2.24 ± 0.06^{BCG}	9.52 ± 0.29^{CD}	8.83 ± 0.80^{BCD}	0.46 ± 0.03^{BCDG}	2.12 ± 0.04^{BCE}
		F4	1.95 ± 0.08^{CEG}	8.54 ± 0.11^{DE}	7.85 ± 0.55^{CD}	0.41 ± 0.02^{CDG}	1.91 ± 0.03^{CDE}
	Liquid faction	L1	2.52 ± 0.00^{AB}	10.72 ± 0.01^{AB}	9.93 ± 0.60^{ABC}	0.53 ± 0.03^{AB}	2.46 ± 0.13^{AB}
		L2	2.28 ± 0.01^{BDG}	9.37 ± 0.20^{DG}	9.03 ± 0.26^{ABCD}	0.46 ± 0.02^{BCD}	2.23 ± 0.12^{BCD}
		L3	2.01 ± 0.01^{CDEG}	8.75 ± 0.13^{DE}	7.91 ± 0.30^{CD}	0.43 ± 0.02^{DEG}	1.93 ± 0.00^{CDE}
		L4	1.84 ± 0.06^{E}	7.99 ± 0.16^{E}	7.26 ± 0.55^{D}	0.38 ± 0.01^{CG}	1.75 ± 0.05^{E}
	Collapsed frothed milk	M1	2.59 ± 0.02^{AF}	10.89 ± 0.10^{AB}	10.53 ± 0.11^{AB}	0.51 ± 0.01^{ABE}	2.46 ± 0.00^{AB}
		M2	2.36 ± 0.01^{BFG}	9.99 ± 0.09^{BCG}	9.53 ± 0.08^{ABC}	0.47 ± 0.01^{ABCD}	2.24 ± 0.01^{BDG}
		M3	2.16 ± 0.02^{G}	9.32 ± 0.08^{DG}	$8.69 \pm 0.0.15^{BCD}$	0.42 ± 0.03^{CEG}	2.03 ± 0.04^{CDEH}
		M4	1.82 ± 0.09^{E}	8.39 ± 0.03^{DE}	7.92 ± 0.06^{CD}	0.37 ± 0.04^{G}	1.86 ± 0.05^{CDE}
	P-values		<0.05	<0.05	<0.05	<0.05	<0.05

1.5 and 10 indicated the sample concentrations of 1.5 and 10% respectively; letters F, L and M denote the foam faction, liquid fraction and collapsed foamed milk respectively; and numbers 1, 2, 3 and 4 denote the foaming times. In each column and for each solid concentration, different letters (a, b, c, … or A, B, C, …) indicate a significant difference in each of individual protein components on the foam faction, liquid fraction and collapsed foamed milk ($P < 0.05$).

milk up to 15%. In addition, at each foaming time, protein compositions of collapsed frothed milk, and foam and liquid factions were marginally different. Similar findings were also reported for protein compositions analysed in skim milk and its foam (Borcherding *et al*. 2008, 2009), and in foam and bulk phases (Kamath *et al*. 2011). These results suggested that milk foams are not notably enriched in proteins. Also, it can be seen from Table 3 that regardless foaming times, foam fractions were more enriched with caseins (i.e. α-CN, β-CN and κ-CN) than whey proteins (i.e. α-La and β-Lg), indicating rapid adsorption of caseins on the interfacial region during foaming. Similarly, Zhang *et al*. (2004) reported that during foaming of solutions prepared from mixtures of skim milk powder and whey protein isolate, caseins were more enriched in foam than whey proteins; and among casein subunits, β-casein was the most enriched into the foam phase. Lorient *et al*. (1989) also reported that β-casein with amphipathic structure showed the greatest ability to decrease the surface tension of the air–water interface and to produce foam, followed by α-casein and then κ-casein. However, the competitive adsorption of individual proteins to the foam interfacial region is determined not only by protein types, but also by many other extrinsic factors such as protein concentration, ionic strength and pH.

CONCLUSION

The results indicated that foaming properties and foam quality of milk samples at various solid concentrations (1.5–15%, w/w) did not change as the milk samples were re-foamed up to four times. Therefore, the left-over of frothed milk can be cooled and re-foamed without changing the properties and quality of foam. The results of this study could be the basis for the milk processing company and coffee shops to consider to re-use the frothed milk for the next foaming times. However, the maximum foaming temperature investigated in this study was 65°C, which is close to temperature achieved in most domestic and coffeehouse milk frothers. Foaming at higher than this temperature might affect protein properties, consequently foaming properties and re-foaming ability of milk. Further studies on re-foaming ability of other types of milk such as whole milk and skim milk as well as changes in the properties of milk components, especially denaturation of proteins after each foaming are needed to provide a comprehensive understanding about re-foaming of milk.

ACKNOWLEDGEMENT

The authors acknowledged the Australian Research Council's Industrial Transformation Research Hub (ARC-ITRH) for the research funding, and the facilities, and the scientific and technical assistance, of the School of Agriculture and Food Sustainability at The University of Queensland. Open access publishing facilitated by The University of Queensland, as part of the Wiley - The University of Queensland agreement via the Council of Australian University Librarians.

AUTHOR CONTRIBUTIONS

Thao M Ho: Conceptualization; methodology; investigation; formal analysis; supervision; visualization; data curation; writing – original draft; writing – review and editing. **Yu-Jen Lu:** Conceptualization; methodology; investigation; formal analysis; writing – review and editing. **Xiaoying Xiong:** Writing – review and editing; conceptualization; investigation; methodology; formal analysis. **Bhesh R Bhandari:** Conceptualization; methodology; investigation; formal analysis; writing – review and editing. **Nidhi Bansal:** Conceptualization; methodology; investigation; supervision; funding acquisition; data curation; project administration; visualization; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

FUNDING INFORMATION

This study was funded by Australian Research Council's Industrial Transformation Research Hub (ARC-ITRH).

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

The following supporting information is available for this article:

Table S1 Calculated amounts (w/w) of protein, lipid and lactose, and physical properties of milk samples at various solid concentrations.

