Whipped Cream Structure Measured by Quantitative Stereology

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ABSTRACT

Whipping cream, with 35% milk fat, was high temperature, short time pasteurized and ultra-high temperature sterilized, with and without the addition of stabilizer, to study the effect of these processing conditions on the stability of foam structure. Processed creams were whipped to maximum overrun using a double beater system and were immediately prepared for low temperature scanning electron microscopy. Duplicate foams were refrigerated for 24 h before processing for low temperature scanning electron microscopy. Air bubble sizes, lamella lengths, and volume fraction of air in the foams were measured using quantitative stereology. A significant increase was noted for bubble size and lamella length in aged foams. Comparison between aged foams showed a significant difference caused by heat treatment. Foams prepared from unstabilized or stabilized pasteurized creams had significantly larger bubbles than those prepared from comparable sterilized creams. Therefore, differences between foams whipped from stabilized creams were primarily due to effect of heat treatment.
(**Key words**: whipping cream, heat treatment, stabilizer, stereology)

Abbreviation key: **LT** = low temperature, **SEM** = scanning electron microscopy.

INTRODUCTION

Whipping cream has traditionally been HTST pasteurized (75°C, 16 s) without homogenization. The trend today is to use UHT sterilization (140°C, 4 s) to provide a product with a longer shelf-life. However, it has been suggested that higher temperature during UHT treatment affects the properties of the cream and results in lower stability of the foamed product. Bruhn and Bruhn (4) reported that whipping of cream following UHT sterilization required a longer whipping time and resulted in a foam with lower overrun than following HTST pasteurization. Therefore, UHT-treated creams did not whip into foams that fit the definition by Graf and Muller (7) of an ideal foam as one that has a rigid structure, a high overrun (100 to 120%), and is stable against deformation. The stability of the foam against collapse is dependent on structure formed during the whipping process with partially coalesced fat globules supporting the air bubbles, a membrane of proteins at the fat-water interface, and proteins and fat in the aqueous phase (2). The addition of stabilizers and emulsifiers affects the viscosity of the aqueous phase and the viscoelastic properties of the fat-protein interface. The response of these components to heat treatment is manifest in the physical properties of the foam. Foam collapse has been attributed to three main forces: drainage or loss of water from the foam, coalescence as air bubbles fuse, and disproportionation as air migrates from small to large air bubbles (11). Measurement of foam drainage has been used to evaluate foam stability (7, 16, 17). However, because foam drainage has proved to be an unreliable measure of loss of structure, in this study image analysis was used to evaluate the differences between cream treatments by measuring air bubbles in fresh and aged foam samples. This approach was facilitated by low temperature (**LT**) scanning electron microscopy (**SEM**) and computer-assisted quantitative stereology.

Low temperature SEM is an effective tool to examine the development of whipped cream structure (1, 3, 9, 10, 14). To freeze the foam preserves its original structure and provides a basis to assess the changes in foam morphology brought about by heat treatment and the addition of stabilizer. Storage of whipped cream samples allows time for destabilization processes to alter the structure. Quantitation of these changes can be accomplished through the acquisition of digital images directly from the microscope and measurement of structural features. Quantitative stereology is an efficient and unbiased approach to derive three-dimensional image measurements from two-dimensional images (6, 12, 13). This estimation technique facilitates the sampling of a large number of images and provides global measurements of image

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features as opposed to size specific measurements that have been used to characterize foam samples in previous studies (9, 14). By overlaying a grid on foam images and marking bubble-line intersects, global measurements can be made of the percentage of volume of sample area occupied by bubbles, mean bubble diameter, and mean lamella length, which is the mean distance between bubbles. This technique is only valid if the structure is random, uniform, and isotropic (13). Foam images are random with respect to a grid, are uniform to support comparable measurements from any location in the image, and are isotropic such that the structure is the same in all directions. The LT SEM sampling technique allowed these criteria to be met. Measured parameters could be statistically validated by ANOVA, which allowed comparison of the physical structure of foam samples.

MATERIALS AND METHODS

Cream Preparation

Raw cream was obtained on three different dates from GayLea Foods (Guelph, ON, Canada) after hot separation (50°C) and at a variety of fat contents (41.01 to 45.18%). The cream was refrigerated overnight at 5°C. On the following day the fat content was standardized to 35% by addition of commercial skim milk, and half of the cream was stabilized with 0.25% Aertex® cream stabilizer (Food Specialties, Mississauga, ON, Canada). The Aertex® cream stabilizer is a proprietary mixture of emulsifiers and hydrocolloids including mono- and diglycerides, disodium phosphate, guar gum, carrageenan, locust bean gum, and soya lecithin, listed in order of predominance. The stabilizer mix was added directly to the cream just prior to heat treatment and was thoroughly mixed. Heat treatment was conducted with the No-Bac Unitherm IV® processing system (Cherry-Burrell Corp., Cedar Rapids, IA) under the following conditions: HTST pasteurization at 75°C for 16 s and UHT sterilization at 140°C for 4 s. After being processed, the cream was refrigerated (5°C) overnight to provide sufficient solid fat content to support formation of foam structure during whipping (5). Samples of 200 ml were whipped using an automatic mixer (Sunbeam®, Toronto, ON, Canada) with a double beater system at a rotational velocity of 925 rpm (power level 10) and a refrigerated bowl and beaters. After 30 s of whipping, the overrun was calculated (16) by weighing a volume of unwhipped cream with the same volume of foam as follows:

$$\text{overrun percentage} = (\text{weight of unwhipped cream} - \text{weight of whipped cream} \times 100)/\text{weight of whipped cream.}$$

Measurements were then conducted at 10-s intervals, and whipping was stopped when the foam reached maximum overrun. Fresh foam samples from each treatment were processed immediately for LT SEM. A second 200 ml sample of each treatment was whipped to the same overrun, stored in the refrigerator (5°C) in closed containers for 24 h, and then processed for LT SEM. Therefore, a total of eight foam samples was analyzed as shown in Table 1.

LT SEM and Stereology

Whipped cream samples were prepared for LT SEM by mounting two 2- to 3-mm^3 samples from the top of the foam onto each of two copper holders for the cryopreparation unit (Emscope SP2000A; Emscope Ashford, Kent, United Kingdom) and then by freezing the samples and holders by conduction in liquid nitrogen (−196°C). Frozen samples were transferred under vacuum into the cryopreparation unit (−166°C), where the samples were fractured and coated with 300 Å of gold. Fully hydrated foam samples were transferred under vacuum onto the cold stage (−130°C) and were scanned in the SEM (S-570; Hitachi, Tokyo, Japan) at 10kV. A total of ten images was captured from three or more samples of each foam preparation (Table 1) with Voyager® Active Scan Interface (Noran Instruments, Middleton, WI) at 512 × 512 pixels of resolution.

The dimensions of acquired images were calibrated with a three-point calibration procedure at each magnification used. Magnification was 200× for all fresh samples. Some aged foam images were collected at

TABLE 1. Whipped cream treatments prepared for analysis and number of bubbles counted.

Treatment	Code[1]	Bubbles counted
		(no.)
1 HTST pasteurized, unstabilized, fresh	HTST	2094
2 HTST pasteurized, stabilized, fresh	HTST-S	1610
3 UHT sterilized, unstabilized, fresh	UHT	2131
4 UHT sterilized, stabilized, fresh	UHT-S	1851
5 HTST pasteurized, unstabilized, aged 24 h	HTST-A	924
6 HTST pasteurized, stabilized, aged 24 h	HTST-SA	684
7 UHT sterilized, unstabilized, aged 24 h	UHT-A	1216
8 UHT sterilized, stabilized, aged 24 h	UHT-SA	1083

[1]S = stabilized, A = aged, and SA = stabilized and aged.

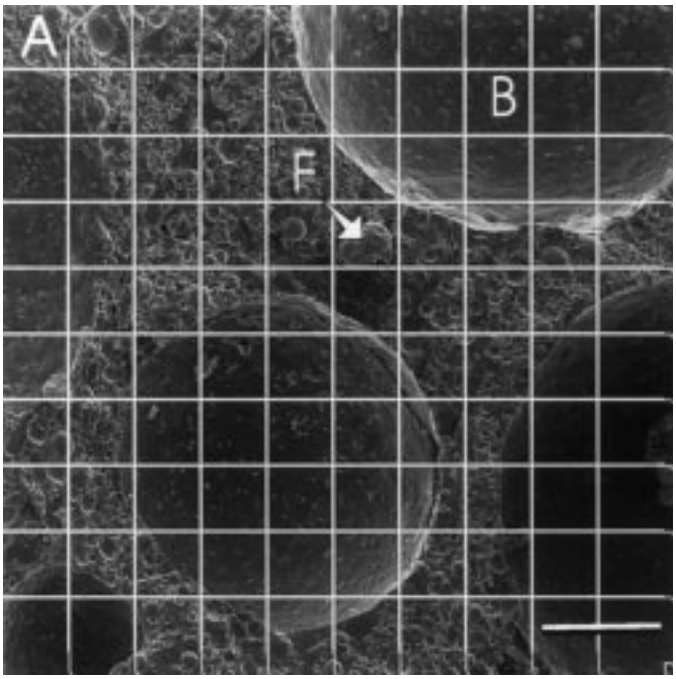

Figure 1. Low temperature scanning electron image of whipped cream overlayed with a grid to provide a basis for stereological measurement. Line spacing is 50 pixels. B = Bubble; F = fat. Bar = 100 μm

100× magnification to ensure that enough air bubbles were available for measurement. A total of 500 features (bubbles or lamellae) must be measured to provide the basis for the statistical evaluation of image measurements (8). A grid image program (Labtronics Inc., Guelph, ON, Canada) was used to generate a grid with a lattice spacing of 50 pixels. Grid size was selected to meet the sampling criteria of 0.6 to 1 hit point per bubble interface. This grid was overlayed on foam images, as shown in Figure 1, through Optimas® image analysis software (Optimas Corp., Bothwell, WA). Intersection points between the vertical lines of the grid and the air-serum interfaces of the bubbles were marked using the mouse, and their position was recorded on a spreadsheet in Optimas®. The bubble lengths and the lamella length (distance between bubbles) were calculated, and the number of bubbles was counted. The number of bubbles counted in each treatment are included in Table 1. Mean bubble size and mean lamella length were estimated from the total air bubble area divided by the number of bubbles or lamella counted in each image. These measurements were global not size specific; global measurements are useful for evaluating differences between treatments. The difference between treatments was statistically evaluated with ANOVA and protected least significant difference test for planned comparisons using general linear model (SAS®, The SAS® System, Release 6.12.1994. SAS Inst. Inc. Cary, NC).

RESULTS AND DISCUSSION

Effect of Heat Treatment and Stabilizer on Whipping Time and Overrun Percentage

Maximum overrun of the whipped cream was selected as the point of comparison for all cream treatments. The values for whipping time to reach maximum overrun (Table 2) indicated that UHT sterilized creams required a significantly longer whipping time than did HTST pasteurized creams, as previously reported by Bruhn and Bruhn (4). Although the addition of stabilizer tended to increase the whipping times for both heat treatments, it did not have a significant ($P \leq 0.05$) effect in the present experiment. The addition of the stabilizer did cause a significant ($P \leq 0.05$) decrease in the overrun within heat treatments (Table 2) as reported by Bruhn and Bruhn (4). However, heat treatment did not have an effect on the maximum foaming ability of the cream. The overrun tended to be slightly greater for both UHT treatments, stabilized or unstabilized, than for the comparable HTST treatments, although not significantly ($P \leq 0.05$).

Microstructure of Foams

The LT SEM provided excellent preservation of whipped cream structure at the resolution used to study foam morphology. Samples were frozen by conduction, with liquid nitrogen, on copper holders to avoid bubble distortion, which can occur in larger bubbles during conventional plunge freezing. The fracture of frozen foams under vacuum provided a fresh surface that was undistorted by the sampling procedure. Furthermore, because the sample did not undergo sublimation, the structure was fully hydrated. Low magnification (200×) facilitated the imaging of many bubbles and a wide range of bubble sizes with the resolution and depth of field offered by SEM.

The top of the foam was sampled to exaggerate the effect of destabilization mechanisms including drainage, disproportionation, and coalescence. Visual characteristics must be quantified to determine whether heat treatment, addition of stabilizer, or both affected stability of the foam. Changes in microstructure that characterize these phenomena are bubble

TABLE 2. Whipping times and overrun percentages for cream treatments[1] whipped to maximum overrun.

Treatment[2]	Whipping time(s)		Overrun %	
	$\bar{X}$	SD	$\bar{X}$	SD
HTST	53.3[b]	4.7	130.3[a]	10.1
HTST-S	66.7[b]	12.5	89.3[b]	9.0
UHT	73.3[a]	4.7	132.7[a]	5.6
UHT-S	83.3[a]	9.4	106.0[b]	10.5

[a,b]Whipping times and overrun percentages with different superscripts are significantly different ($P \leq 0.05$).

[1]Comparisons made for single variable differences.

[2]S = Stabilized.

size, reduction of the number of bubbles, thinning of lamellae between bubbles, and ultimately reduction in the volume fraction of air in the foam. Figure 2 shows representative images of four pasteurized whipped creams. The fresh unstabilized and stabilized foams exhibited uniform bubbles of more intermediate size than did their respective aged foams, which appeared to have a few large bubbles and many smaller bubbles in comparison. This change is a manifestation of coalescence and disproportionation such that large bubbles grow at the expense of small bubbles. Channels that form in the foam are caused by breaches in the air bubble interface or by coalescence of visible bubbles with others that are deeper in the sample. Channels were identified visually as black areas inside the bubbles when the bottom of the bubble was beyond the depth of field of SEM. A lateral view of the coalescence process can be seen in Figures 2D and 3D. As foams aged, gravity caused the bubbles to deform. Deformation was most visible in those foams prepared from cream with added stabilizer, which increased the water-holding capacity of the aqueous phase and, thus, increased the weight on the system. The effect of gravity was particularly pronounced when foams were prepared from HTST treated cream.

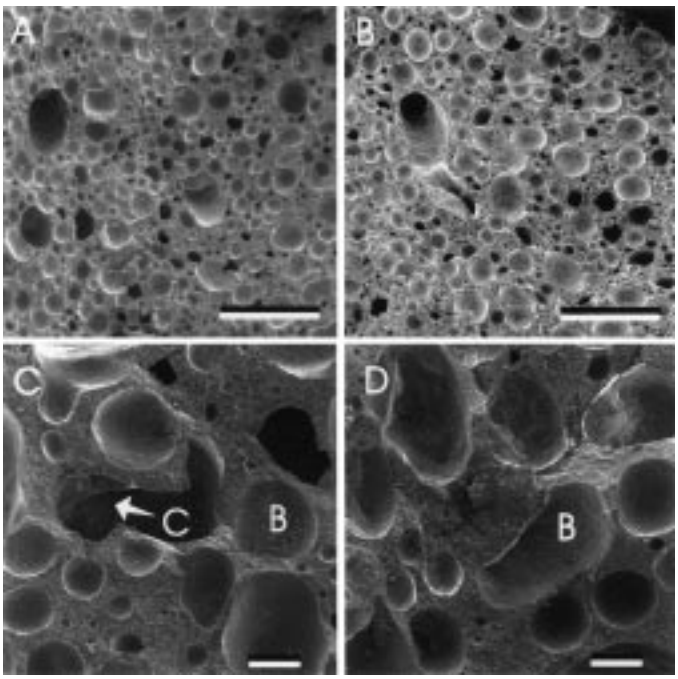

Figure 2. Low temperature scanning electron micrographs of foams prepared from HTST treated cream. Freshly whipped, unstabilized cream (A), freshly whipped, stabilized cream (B), aged, unstabilized cream (C), and aged, stabilized cream (D). B = Bubble; C = channel. Bar = 150 μm.

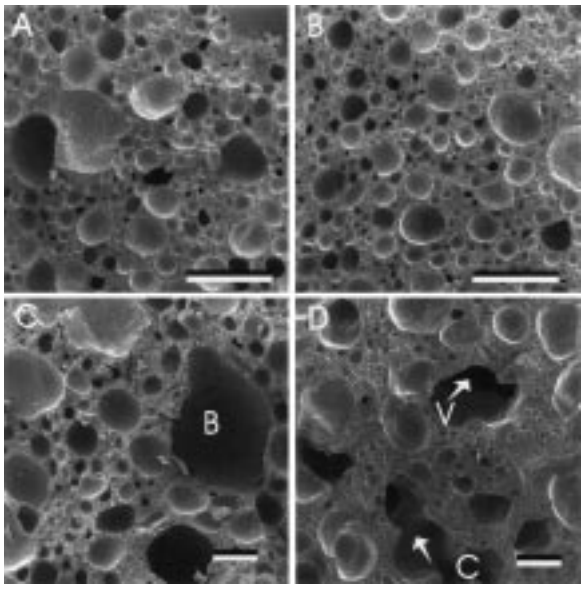

Figure 3. Low temperature scanning electron micrographs of foams prepared from UHT treated cream. Freshly whipped, unstabilized cream (A), freshly whipped, stabilized cream (B), aged, unstabilized cream (C), and aged, stabilized cream (D). B = Bubble, C = channel, and V = void. Bar = 150 μm.

Figure 3 shows the fresh and aged foams prepared from UHT sterilized creams with and without added stabilizer. Changes in bubble size distribution were apparent in aged and stabilized aged treatments but were less extreme than in pasteurized treatments with fewer large bubbles and less visible deformation. However, the channeling effect appeared to be greater and suggested that the bubble interface in UHT stabilized foams may be less elastic and therefore, less likely to deform and more likely to rupture over time. Paquin and Dickinson (10) attribute foam stability to the mechanical resistance of the air-serum interface to collapse and propose that long term stability of

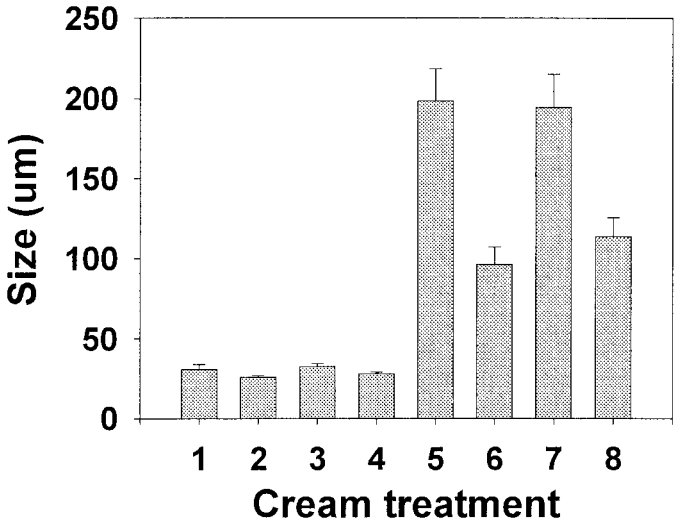

Figure 4. Bubble sizes in fresh (bars 1 to 4) and aged (bars 5 to 8) foams prepared from four cream treatments. Results are the average of thirty images per treatment; error bars represent standard error. 1 = HTST pasteurized, unstabilized, fresh foam; 2 = HTST pasteurized, stabilized, fresh foam; 3 = UHT sterilized, unstabilized, fresh foam; 4 = UHT sterilized, stabilized, fresh foam; 5 = HTST pasteurized, unstabilized, foam aged 24 h; 6 = HTST pasteurized, stabilized foam aged 24 h; 7 = UHT sterilized, unstabilized foam aged 24 h; and 8 = UHT sterilized, stabilized foam aged 24 h.

foam is related to partially coalesced fat globules, polysaccharides, and the composition of protein films at the air-serum interface. Differences appear to be due to heat treatment and not to the addition of stabilizer. Thus, protein composition at the air-serum interface may account for the increased elasticity observed at the air-serum interface in foams whipped from HTST stabilized cream and may result in more stable foams.

Quantitative Stereology

Quantitation of foam characteristics by measuring changes in microstructure lends objectivity to visual assessment of images. Quantitative stereology facilitates the measurement of many fields including many bubbles, as shown in Figures 4, 5, and 6, to provide an efficient estimate of the characteristics of different whipped cream treatments. Lines were placed at 50-pixel intervals. Grid size was selected to meet the sampling criteria of 0.6 to 1 hit point per bubble interface. A total of 500 hits is recommended to support the statistical evaluation of image measurements (8). Because air bubbles were the feature of interest, this number was doubled so that a minimum of 1000 hits or 500 bubbles were evaluated per treatment.

Figures 4, 5, and 6 represent mean bubble size, mean lamella length, and percentage of foam volume occupied by air. A great deal of variation occurred between replicate cream processes such that a difference ($P \leq 0.05$) existed between some trials. However, the statistical model for each parameter proved to be significant ($P \leq 0.05$). Comparison of treatments was restricted to single variable changes in an attempt to establish the independent effects of heat treatment, stabilizer, and storage time on foam structure.

The mean bubble size estimated from measurements of fresh foams did not demonstrate significant differences caused by heat treatment or the addition of stabilizer. However, Figures 2 and 3 and mean bubble size represented in Figure 4 show a general increase in bubble size caused by storage for both treatments compared with the same fresh samples. Statistical results for mean bubble size differences, reported in Table 3, confirmed that the increase in bubble size with age, whether or not the sample was stabilized, were significant ($P \leq 0.05$). The increase in bubble size that occurred during aging of the foam was different ($P \leq 0.05$) when treatments of whipped cream that were HTST unstabilized and aged or stabilized and aged were compared with the same UHT treatments. Therefore, measurement of mean

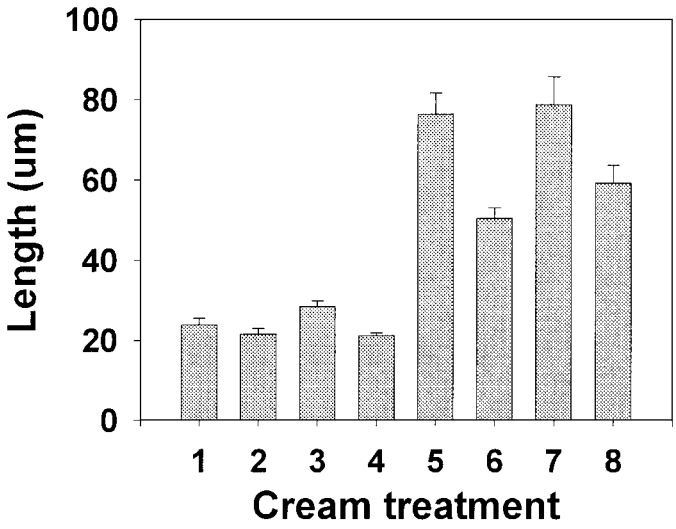

Figure 5. Lamella lengths in fresh (bars 1 to 4) and aged (bars 5 to 8) foams prepared from four cream treatments. Results are the average of thirty images per treatment; error bars represent standard error. 1 = HTST pasteurized, unstabilized, fresh foam; 2 = HTST pasteurized, stabilized, fresh foam; 3 = UHT sterilized, unstabilized, fresh foam; 4 = UHT sterilized, stabilized, fresh foam; 5 = HTST pasteurized, unstabilized, foam aged 24 h; 6 = HTST pasteurized, stabilized foam aged 24 h; 7 = UHT sterilized, unstabilized foam aged 24 h; and 8 = UHT sterilized, stabilized foam aged 24 h.

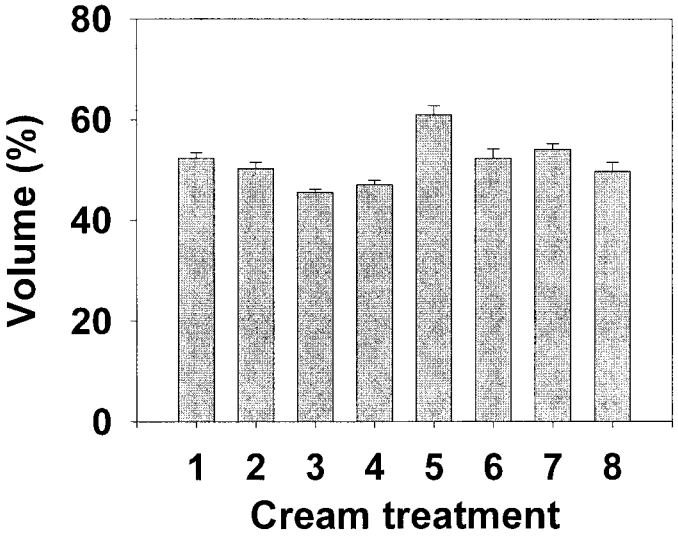

Figure 6. Volume fraction of air in the fresh (bars 1 to 4) and aged foams (bars 5 to 8) prepared from the cream treatments. Results are the average of thirty images per treatment, error bars represent standard error. 1 = HTST pasteurized, unstabilized, fresh foam; 2 = HTST pasteurized, stabilized, fresh foam; 3 = UHT sterilized, unstabilized, fresh foam; 4 = UHT sterilized, stabilized, fresh foam; 5 = HTST pasteurized, unstabilized, foam aged 24 h; 6 = HTST pasteurized, stabilized foam aged 24 h; 7 = UHT sterilized, unstabilized foam aged 24 h; and 8 = UHT sterilized, stabilized foam aged 24 h.

bubble size demonstrated changes in foam stability caused by the combined effect of heat treatment and aging but not because of the addition of stabilizer alone. It could be argued that the difference in the size distribution of bubbles, rather than differences in mean bubble size, may be a better measure of changes in overall structure. The standard deviation in bubble sizes increased in aged foam samples and could be assessed as a measure of size distribution.

Lamella length, the distance between the bubbles, did not change ($P \leq 0.05$) because of the effect of heat treatment or the addition of stabilizer in fresh foams. Lamellae between bubbles in unstabilized, aged foams prepared from cream that was processed by either heat treatment were longer than in unstabilized, fresh foams, as seen in Figure 5. The differences are significant ($P \leq 0.05$) as reported in Table 3. With age, as air bubbles coalesced, larger areas of the aqueous phase were created, which was reflected in longer lines being measured, as shown in Figure 5. These lengths were longer ($P \leq 0.05$) as a result of aging foams regardless of heat treatment or addition of stabilizer. No significant ($P \leq 0.05$) difference existed in lamella length because of the addition of stabilizer within heat treatments when aged foams were compared. A difference ($P \leq 0.05$) existed in stabilized aged foams between heat treatments. Therefore, heat treatment had a primary effect of increase in lamella length for UHT unstabilized and stabilized foams, which exhibited longer lamellae than did comparable HTST foams. Stanley et al. (16) suggested that thicker lamellae in stabilized foams may lead to a less rigid and more viscous structure, which would seem to be more conducive to foam stability. In this study, longer lamellae would appear to be indicative of greater air bubble coalescence and would reflect a loss of foam structure. This condition is more pronounced in foams whipped from UHT-treated creams (Figure 5). Addition of stabilizer produced foams with shorter lamellae regardless of heat treatment (Figure 5), but the effect was not significant (Table 3).

The volume fraction of air in the foam was estimated by the proportion of sampling line length taken up by bubbles. Heat treatment did not result in a

TABLE 3. Significance of whipped cream treatment contrasts defined by the mean air bubble size and mean lamella length.[1]

Cream treatment[2]	HTST	HTST-S	HTST-A	HTST-SA	UHT	UHT-S	UHT-A	UHT-SA
HTST								
HTST-S	NS[3]							
HTST-A	*							
HTST-SA		*	NS					
UHT	NS							
UHT-S		NS			NS			
UHT-A			*		*			
UHT-SA				*		*	NS	

[1]Statistical differences between treatments were the same for bubble size and lamella length. Comparisons were limited to single variable differences between treatments.

[2]S = Stabilized, A = aged, SA = stabilized and aged.

[3]No significant difference.

*$P \leq 0.05$.

TABLE 4. Significance of treatment contrasts defined by the air bubbles as a percent of the volume. Comparison were limited to single variable differences between treatments.[1]

Cream treatment	HTST	HTST-S	HTST-A	HTST-SA	UHT	UHT-S	UHT-A	UHT-SA
HTST								
HTST-S	*							
HTST-A	*							
HTST-SA		*	*					
UHT	NS[2]							
UHT-S		NS			NS			
UHT-A			*		NS			
UHT-SA				*		NS	NS	

[1]S = stabilized, A = aged, SA = stabilized and aged.

[2]No significant difference.

*$P \leq 0.05$.

significant change in volume fraction (Table 4) or overrun percentage (Table 2), but addition of stabilizer caused a significant difference ($P \leq 0.05$) (Tables 2 and 4) in foams whipped from HTST-treated cream. Addition of stabilizer to UHT cream resulted in a lower overrun when measured by the whipping data (Table 2) but not when measured by stereology. Aged unstabilized and stabilized foams prepared from cream, which was UHT sterilized, had a lower percentage of their volume taken up by air than did the comparable HTST sample as shown in Figure 6. These differences are significant ($P \leq 0.05$) as shown in Table 4. The addition of stabilizer to the cream caused a reduction ($P \leq 0.05$) in the amount of air in the foam prepared after HTST pasteurization but not after UHT sterilization. The amount of air increased in foams prepared from the UHT-treated cream and the whipped cream when the sample was taken from the top of the foam. When foams were aged percentage of total volume occupied by air increased significantly ($P \leq 0.05$) for HTST but not UHT-treated samples. Differences because of heat treatment were expressed for unstabilized as well as stabilized samples and suggested that heat treatment had a more significant effect than stabilizer when the two variables were in combination. Stabilizer effect was expressed as a significantly ($P \leq 0.05$) lower volume of air in the foam prepared from HTST processed creams. The effect was not significant within UHT treatments. It has been reported (1, 15) that heat treatment affects the proteins in the cream, specifically, increasing the denaturation of β-LG. This effect leads to a change in protein interaction with the stabilizer at the milk fat globule membrane and in the aqueous phase of the foam. An increased interaction between stabilizer and proteins may explain the visual differences illustrated in HTST stabilized, aged whipped cream (Figure 2D) and UHT stabilized, aged whipped cream (Figure 3D) and account for the increased volume of air in the former, expressed in Figure 6.

The addition of stabilizer resulted in a lower overrun as seen in our whipping data, Table 2, and as previously reported (4, 16). This effect is supported by an expected decrease in bubble size and an increase in lamella length at the top of the foam for stabilized whipped creams (Figures 4 and 5). The reduction in air volume in the foam caused by the addition of stabilizer was also borne out by the reduction in bubble volume fraction in HTST pasteurized and whipped creams. This reduction did not occur for the same UHT treatments (Figure 6) in which the addition of stabilizer resulted in an increase in the volume fraction of air in the fresh foam. Destabilization mechanisms of coalescence and disproportionation resulted in larger bubble sizes and increased lamella length and volume fraction of air in the foam of stabilized and stabilized, aged treatments. All measures demonstrated a difference between HTST and UHT stabilized, aged treatments. Further study could be conducted to determine whether the difference in these treatments was due to heat treatment alone or to differences in heat treatment affecting the interaction between proteins and the hydrocolloids in the stabilizer mix. Components of the stabilizer mix could be evaluated for their individual effect on stability of foam.

CONCLUSIONS

Quantitative stereology provided a fast, efficient, and unbiased approach to computer-assisted measurement of foam microstructure and supported visual assessment of the effect of heat treatment and added

stabilizers on whipped cream structure. Micrographs from LT SEM showed differences in whipped cream structure caused by the aging of the foam and by differences in heat treatment. Bubbles were uniform and intermediate in size in fresh foams, but because of Ostwald ripening and disproportionation, aged foams exhibited many small bubbles and a few large bubbles. Bubbles in foams prepared from aged HTST pasteurized creams appeared to have interfaces with greater viscoelasticity. This result was suggested by their resistance to rupture that was visible in foams whipped from UHT sterilized creams. Stereology results identified heat treatment as the primary cause of differences between fresh and aged foams. The HTST unstabilized or stabilized whipped creams held significantly more air after 24 h of refrigerated storage than did comparable UHT treatments and were, therefore, more stable foams.

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