

Microstructure of α -Crystalline Emulsifiers and Their Influence on Air Incorporation in Cake Batter

G. Richardson,¹ M. Langton,^{1,2} P. Fäldt,³ and A.-M. Hermansson¹

ABSTRACT

Cereal Chem. 79(4):546–552

The microstructure of α -gel and β -crystalline emulsifiers and their effects on cake batter foam have been studied with polarized light microscopy, confocal laser scanning microscopy (CLSM) and image analysis, freeze-etching, and transmission electron microscopy (TEM). The emulsifiers Colco and Aroplus, which are commercial α -gels, and the monoglyceride Dimodan P in its α -gel and β -crystalline forms were added to the batter in concentrations of 0.8, 2.0, 3.1, and 4.2%. Dimodan P α -gel was also prepared with three NaCl concentrations (0.05, 0.67, and 1.35%). The distribution of air in the foam was evaluated with density

measurements and with image analysis of bubbles in optically sectioned batter. In the cake batter, all the α -gel emulsifiers decreased the density, thereby increasing the incorporation of air, more than the β -crystalline emulsifier, which did not have any effect on the density. There were noticeable differences in microstructure between the different α -crystalline emulsifiers. Large, regular α -structures seemed to increase the batter volume and interfacial area more than smaller aggregates. Adding salt in the emulsifier gel changed the structure, probably into α -lamellar liposomes, which impaired the aerating effect at lower concentrations.

Emulsifiers are widely used products in the food industry. The most apparent field of application is the dispersion of air into a foam or an emulsion, since the surfaces are stabilized by either proteins or emulsifiers. In the baking industry, the emulsifiers are also used for other effects. A sponge cake batter with emulsifier can be whipped in a one-stage process, simplifying the production. The incorporation and stability of air bubbles are improved, and a finer dispersion gives a better cake quality. The emulsifiers may also improve the shelf life by interactions with starch, slowing down the recrystallization process.

One way of studying the foam stability and air incorporation is to measure the air bubbles with some kind of microscopy. Cauvain and Cyster (1996) showed a bubble size diagram in which the air bubbles in the batter decrease from 25 to 10 μm as the emulsifier level rises. Unfortunately, they did not reveal how this measurement was performed. Shepherd and Yoell (1976) squashed the batter under a cover glass to make the measurements under a light microscope. A more gentle variant of transmission microscopy was used by Brooker (1993). This did not include any squashing, but the analyzed layer was 1–2 mm thick and several bubbles were on top of each other. It would be more practical to use a method that makes optical sections of the foam, such as confocal laser scanning microscopy (CLSM). In combination with image analysis, the method should give information about the bubble size distribution and the stability. The theories about image analysis and the interpretation of two-dimensional images are well described by Russ (1990).

The performance of an emulsifier in a foam will depend on factors such as the chemical content, the hydrophilicity, the content of the foam, etc. One very important factor is the physical state of the emulsifier. Experience has shown that the emulsifiers used for cake baking should be in the α -gel state. When the emulsifier, originally in the β -crystalline conformation, is heated in water, a lamellar phase is formed. This consists of nonrigid lipid bilayers separated by water that is dissolved between the polar head groups. Upon cooling, the lipid chains rearrange into a rigid conformation and the lamellar phase turns into an α -gel, which still has a water layer between the polar groups (Krog 1997). The α -gel has a smooth, ointment-like consistency. It is believed that the water layer in the α -gel improves the ability to move the lipid bilayers relative to each other. Thus, these flexible layers can easily cover the bubble surfaces and create a stabilizing film (Howard 1972; Silva 2000). Wootton et al (1967)

have seen such a solid α -crystalline film between cottonseed oil and water. The emulsion films will not necessarily consist of several lipid layers, although they are produced with α -gels, but if they do, this may be revealed by freeze-etching. The coalescence rate will depend on whether the drops or bubbles are covered by several layers or just a monolayer (Friberg and Jansson 1976).

The viscoelastic properties of the film that is created by α -crystalline emulsifiers on the bubble surfaces are interesting for the consideration of stability. Wootton et al (1967) made DuNuoy tensiometer tests to show that viscoelastic properties are directly related to the stability of cake batter. Krog (1975) considers that elasticity and viscosity both affect the stability of foam. The stronger the film, the more air it incorporates. Although many emulsifiers may form α -gels themselves, the interfacial viscoelastic properties are often improved by blending two emulsifiers in the right proportions. For example, α -gels with propylene glycol monostearate and monoglycerides have been more effective than the single components (Lee and Hosney 1982). It is believed that a higher packing density contributes to this increase in stability (Silva 2000).

The electrolyte concentration in the emulsifier gel affects the interaction between the gel layers, and a high concentration may cause the structure to collapse. Salt shields the repulsive forces between the layers and will thus decrease the swelling of the gel (Larsson and Krog 1973). Monolayers of monostearin on a sub-phase with electrolytes, examined with a Langmuir balance, exhibit an earlier collapse when the salt concentration is increased (Rodríguez Patino and Ruíz Domínguez 1996). The addition of 0.04% NaCl to a neutralized monoglyceride gel with 70% water decreased the water layer from 136 to 9 Å, as determined with X-ray studies by Krog and Borup (1973). Effects of NaCl on a saccharose ester gel were recorded by Carlotti et al (2000).

As the water layer in the α -gel decreases due to the salt addition, two things can happen. Because the electrolyte reduces the swelling of the water layers, the amount of water that can be dissolved in the gel is reduced. Either the gel structure collapses completely into β -crystals and water, or closed vesicles are formed in excess water. It has been suggested that the lamellae are enclosed because it is unfavorable to have associated water mixed with free water. Larsson and Krog (1973) reported that addition of 0.1% sodium chloride to a monoglyceride neat phase with 70% water is enough to transform the gel into liposomes. They observed that the X-ray spacing of the liposomes was similar to that of the noncurved α -structure and the optical properties resembled those of dispersed particles. For transformation into β -crystals and water, more than 2% NaCl was required at 25°C.

The objective of this work was to investigate whether the aeration performance of mixed emulsifiers and of α -gels in which the stacking of the lipid bilayers differed could be related to the micro-

¹ SIK—The Swedish Institute for Food and Biotechnology, PO Box 5401, S-402 29 Göteborg, Sweden.

² Corresponding author. Phone: +46-31-3355600. Fax: +46-31-833782. E-mail: ml@sik.se.

³ Aromatic AB, PO Box 440 40, S-100 73 Stockholm, Sweden.

structure. The structure was examined with several kinds of microscopy to obtain information about anisotropic structures, the influence of shear, and the packing of lipid bilayers and water. The performance in a foam was evaluated by density measurements on the cake batter and by image analysis of the foam structure as seen under CLSM. Measured parameters were bubble number, size, and volume fraction. The total interfacial area was calculated to ensure that the emulsifier was able to cover the bubble surfaces.

MATERIALS AND METHODS

Materials

Colco α -gel with a polyglycerol ester and monoglyceride (fat $31 \pm 2\%$, iodine value <1), and Aroplus α -gel with a propylene glycol ester and monoglyceride (fat $28 \pm 2\%$, iodine value <2), were commercial ready-to-use α -gels obtained from Aromatic AB, Sweden. Dimodan P was obtained from Danisco Cultor, Denmark. Dimodan P is a distilled monoglyceride from fully hydrogenated lard (monoester content $>90\%$, iodine value <2 , free glycerol $<1\%$, acid value <3). Sodium palmitate, purum $\approx 97\%$, was from Sigma-Aldrich Chemie, Germany.

Emulsifier Preparation

The Dimodan emulsifier was prepared to achieve the desired physical states of α -gel or β -crystalline. Dimodan P in α -gel form was prepared by mixing 5.0, 65.0, or 130.0 mg of NaCl (0.05, 0.67, and 1.35%, w/w), 1.5 g of sodium palmitate, and 75 g of deionized water. The mixture was heated to 70°C . When the sodium palmitate was melted, 20 g of Dimodan P was added with gentle stirring and the mixture was held at 70°C for 2 hr. The weight was checked and water was added if needed, after which the sample was stirred and cooled in a refrigerator. The gel was used within 24 hr to avoid recrystallization.

Dimodan P was originally in β -crystalline form, but to examine the β phase with a composition similar to that in the α -gel (with medium salt concentration), salt, sodium palmitate, and water were mixed, melted at 70°C , and cooled, after which 20 g of Dimodan P was added and the mixture stirred.

Cake Batter Preparation

The ingredients used for the experimental sponge cake batter were 350 g of sugar, the standard quality (K5) from Danisco Sugar, Sweden; 10–55 g of emulsifier (0.8, 2.0, 3.1, and 4.2% of total batter weight); 300 g of fresh egg; 200 g of water; 350 g of wheat flour (protein 12.5% and ash 0.68% of dry substance, 14.5% water) from Kvarn AB Juvel, Sweden; 50 g of wheat starch C \star Gel 20006 (starch $>85.7\%$, protein 0.3%, moisture 12%) from Cerestar, The Netherlands.

All the ingredients were premixed to ensure even distribution, after which the batter was whipped for 3 min with a Hobart AE 200 mixer at highest speed. The batter was immediately weighed in a cup with a specific volume for density calculations and stained for microscopic examination. Three replicates were evaluated for each emulsifier concentration except the β -crystalline form, which was only tested twice for each concentration. The eggs were used within 28 days, which was the expiration date. The age was noted but not found to have any effect on the density of the batter. Baking powder was not added because this affected the air bubble volume during the measurements.

Three levels of emulsifier were used in the batters. The medium concentration of Colco and Aroplus (2.0%) was the normal amount used in the original formula; the low and high concentrations (0.8 and 3.1%) were chosen so that the batter would still have acceptable properties for the measurements. Because the Dimodan P emulsifier had a poorer aerating effect, it was used in higher amounts (2.0, 3.1, and 4.2%).

Microscopy

For examination of emulsifier structure with polarized light, the gel was smeared onto an object glass and covered with a glass. The sample was examined with a Nikon Microphot FXA microscope using a $4\times$ objective and crossed polarizers. Images were taken with a Hamamatsu 3CCD camera C6157.

Small cups with emulsifier were rapidly frozen in liquid propane and transferred to a Balzer BAF 400 freeze-etching system. The samples were fractured under vacuum at -100°C , etched for 1 min,

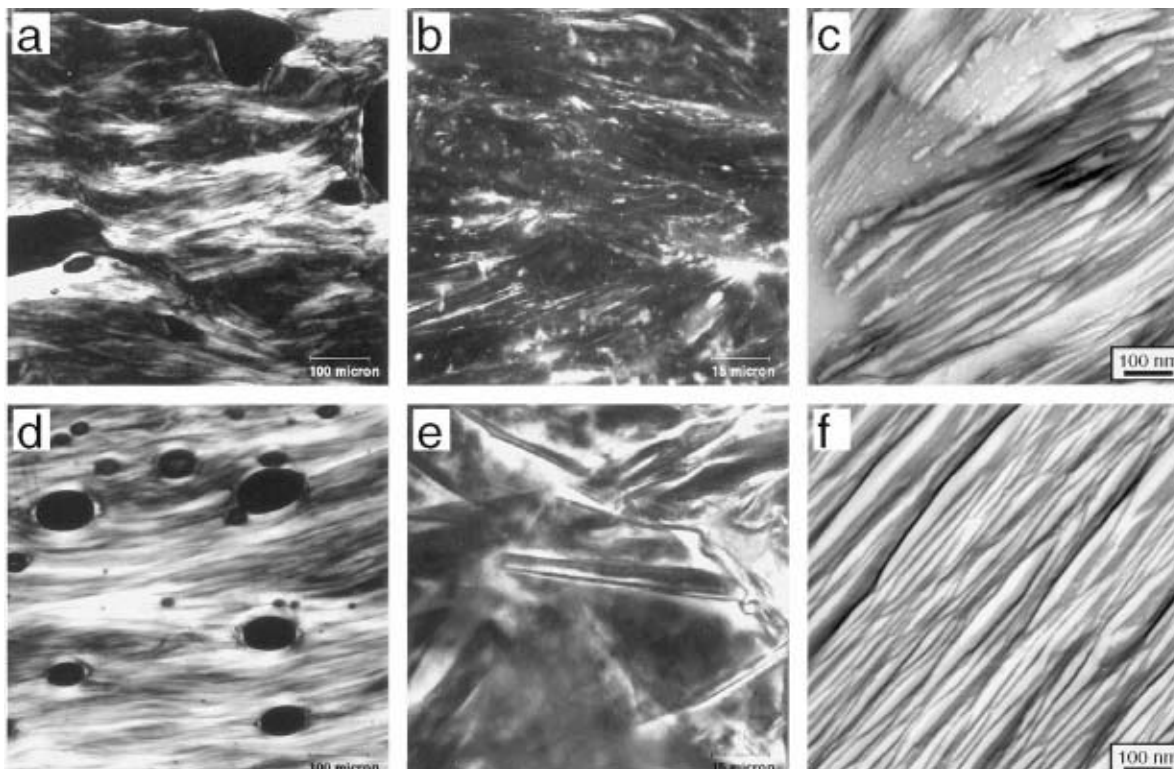


Fig. 1. Emulsifiers Colco (a–c) and Aroplus (d–f) viewed with polarized light microscopy (a and d); confocal laser scanning microscopy (CLSM) (b and e); and transmission electron microscopy (TEM) (c and f).

and rotary shadowed with Pt/C at an angle of 45° and C at an angle of 80°. The thickness was controlled with a quartz crystal monitor. The replicas were cleaned in methanol, chloroform, and de-ionized water and mounted on copper 400 mesh grids. The samples were examined with a LEO 906 E transmission electron microscope (TEM) at an accelerating voltage of 100 kV.

For examination under CLSM, small cylinders of emulsifier were cut out, placed on an object glass, and covered with a glass stained with Nile red, which stains the fat phase. The samples were examined under the Leica TCS 4D CLSM (Heidelberg, Germany) with a 100× oil immersion objective (N.A.=1.4). The excitation wavelength from the Ar/Kr laser was 488 nm, and emission wavelengths >515 nm were detected. Images of 512 × 512 pixels were obtained from a depth of 8 μm in the sample.

Whipped cake batter was also studied under the CLSM. Batter was placed in a concave object glass and a stained cover glass was placed on top. Nile red and acridin orange were mixed to stain fat, protein, and starch, so that only the air bubbles that were to be measured would remain unstained. The same setup was used as for the emulsifiers. Images were obtained from 8 μm under the

surface, which was the optimal depth considering loss of intensity and resolution. Thus, very large air bubbles could not be well represented in the measurements. The objective was chosen so that the focus would be on the distribution of smaller bubbles and also to achieve as high a resolution as possible. Images were taken over a period of 1 hr, which resulted in 20–60 images for each sample. Three replicates for each sample condition were examined.

Image Analysis

Images were converted into binary images with the Contextvision microGOP 2000/S (Linköping, Sweden) program. The conversion consisted of a smoothing median step, followed by two gray scale thresholds. The first threshold was set low, so that only the desired objects were marked. The second threshold was used to fill all the objects thoroughly. After some noise-removing steps, the first threshold image was used as a mask over the second one, so that only the objects existing in the first one appeared in the final image. The mask was needed because some starch granules in the flour,

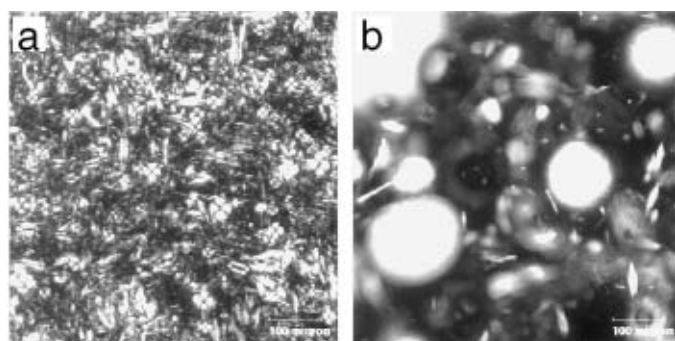


Fig. 2. Polarized light microscopy of emulsifier Dimodan P in α-gel form (a) and β-crystalline form (b).

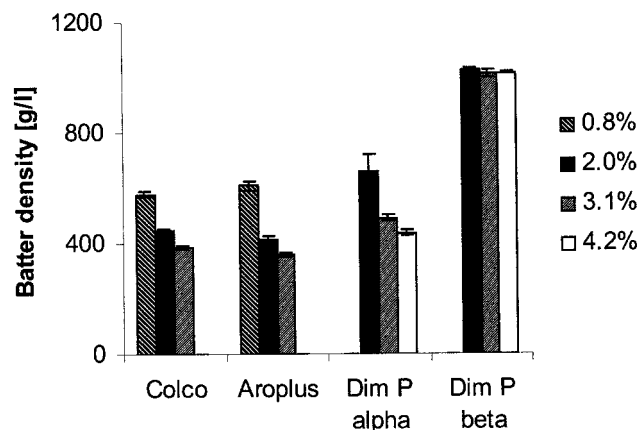


Fig. 3. Density of cake batter with four emulsifiers.

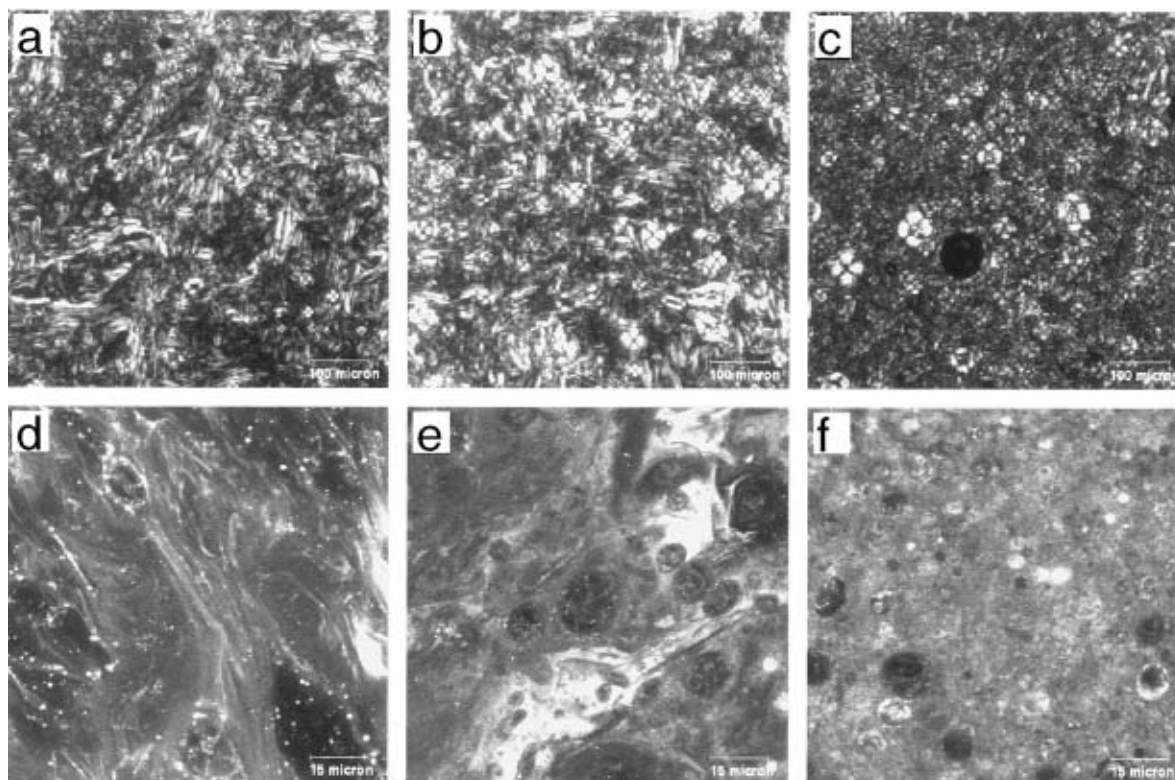


Fig. 4. Polarized light microscopy (a–c) and confocal laser scanning microscopy (CLSM) (d–f) of Dimodan P α-gels with NaCl added at 0.05 (a and d), 0.67 (b and e), and 1.35% (c and f). Scale bars indicate magnification.

similar in size to the air bubbles, were poorly stained and might have disturbed the measurements. Objects $< 2 \mu\text{m}^2$ (50 pixels) were considered as noise and were removed because they might have been influenced by lack of resolution.

The binary images were measured using 2D as well as 3D (stereological) techniques. To avoid border effects, a measure frame that included objects touching on two sides and excluded them on the other two sides was used in the image. The 2D measurements were used to measure the areas of the air bubbles in the optical section. The bubble diameters, calculated from the areas, were presented in a histogram. The area fraction of air in the images was also obtained by dividing the total bubble area by the total area of the image. This measurement was done without a measure frame.

The stereological measurements resulted in one parameter, the surface area per unit volume (S_v). This is the statistically estimated total interface between air bubbles and batter, divided by the total volume. A number of test lines are drawn across the image and the transitions between the two phases are counted. The surface is given by the equation $S_v = 2 P_L$, where P_L is the number of object-background transitions divided by the total line length (Russ 1990). S_v was recalculated as total surface area by multiplying by the total batter weight and dividing by the density.

Statistics

A surface response model was used to evaluate the effects of the emulsifier concentration in the batter and of the salt concentration in the emulsifier on the batter density. A design with three concentration levels of salt and of emulsifier and three replicates of each sample condition was evaluated in SYSTAT v. 10. The statistical evaluation resulted in the equation:

$$Y = \beta_0 + \beta_1 x_{em} + \beta_2 x_{salt} + \beta_3 x_{em}^2 + \beta_4 x_{salt}^2 + \beta_5 x_{em} \times x_{salt}$$

where Y is the density, β are the estimated coefficients, x_{em} is the concentration of emulsifier in the batter (normalized to -1, 0, and 1),

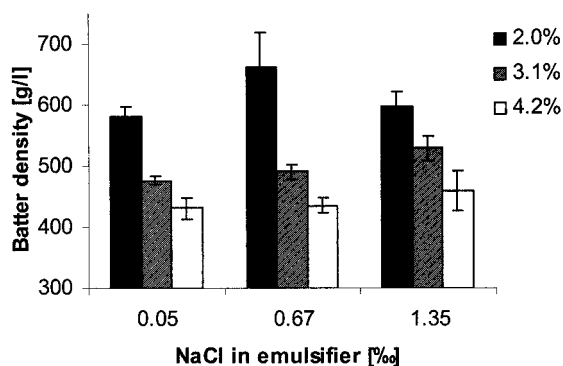


Fig. 5. Density of cake batter with Dimodan P α -gels of varying salt content.

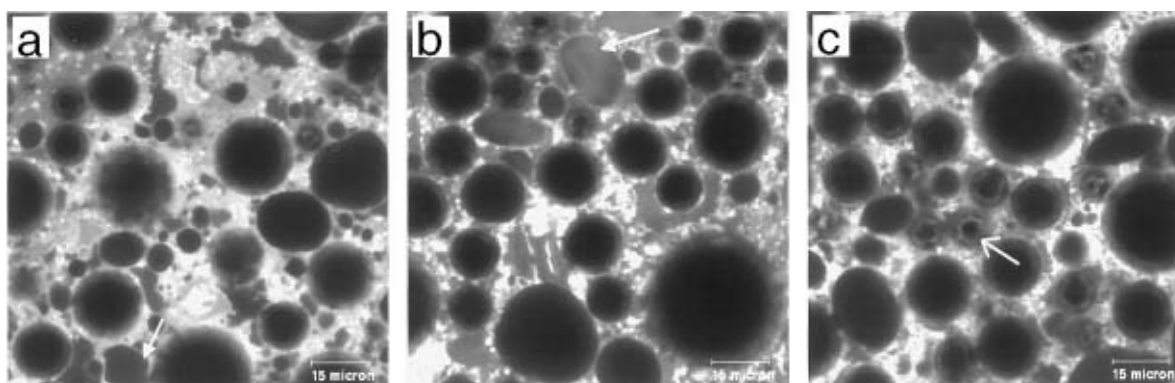


Fig. 6. Confocal laser scanning microscopy (CLSM) of cake batter with emulsifier Colco added at 0.8 (a), 2.0 (b), and 3.1% (c). Air bubbles are black. Arrows indicate badly stained starch granules in (a) and (b) and Becke lines inducing bright reflections inside the air bubbles in (c).

and x_{salt} is the concentration of salt in the emulsifier. The square and interaction terms were also included.

RESULTS AND DISCUSSION

Structure of Emulsifiers

The structure of the emulsifier gels was examined with three different microscopic methods: polarized light microscopy, CLSM, and TEM. Four emulsifier mixtures are shown in Figs. 1 and 2. Two of them, Colco (Fig. 1a-c) and Aroplus (Fig. 1d-f), are considered very efficient mixed α -gels used for cake batters. With polarized light that shows anisotropic structures, the streaky α structure could be seen clearly, especially in Aroplus (Fig. 1d), which was clearly arranged in the smearing direction. The black objects are air bubbles. Colco (Fig. 1a) was also smeared but did not show the same linearity, rather a more needle-like structure. As these samples were smeared out, they became orientated. However, non-deformed bulk samples were examined with CLSM and the tendency for long streaks was similar (Fig. 1b and e). The magnification was now higher but Colco (Fig. 1b) still showed a needle-like appearance.

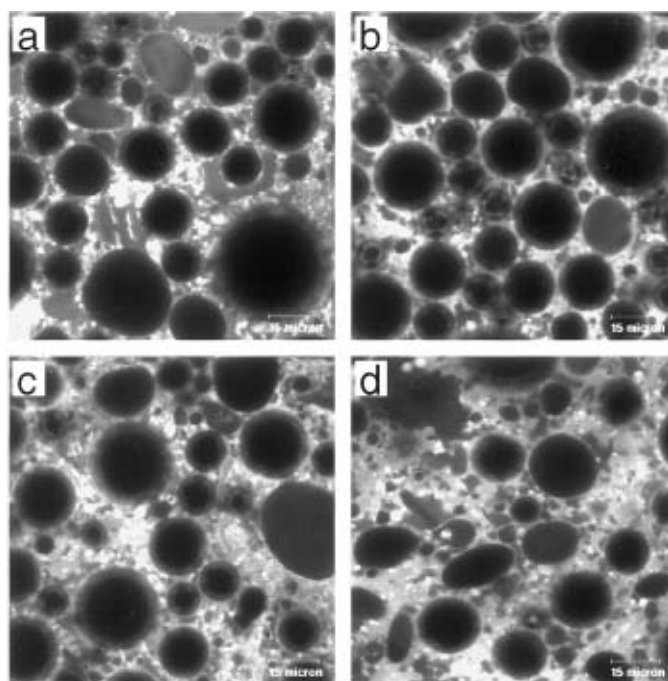


Fig. 7. Confocal laser scanning microscopy (CLSM) of cake batter with 2.0% added emulsifiers Colco (a), Aroplus (b), or Dimodan P with low-salt (c) and medium-salt (d) concentrations (0.05 and 0.67% NaCl, respectively). Oval or irregular dark objects are badly stained starch granules. Air bubbles are round and somewhat diffuse at the edges.

Aroplus seemed to have longer and thicker aggregates than Colco. The freeze-fractured samples (Fig. 1c and f) were etched and rotary shadowed, so if the α -gel was fractured perpendicular to the layers, the lipid layers should be seen as black and the water layer, which was etched, as bright under TEM. Some areas of this kind were found where the thickness of the black streaks was in the magnitude of 5 nm and the total width of the lipid and water layer was ≈ 20 nm. The appearance corresponds to the α -gel image presented by Krog (1997) and to X-ray studies on α -gels (Krog and Borup 1973). Colco seemed to have quite irregular α -gel structures, while it was easier to find large structured areas in the Aroplus sample. When the α -gels were handled, it was obvious that Colco was creamy but not very stretchable. It did not seem to be very much affected by the shearing; the results of the different microscopy methods were similar. Aroplus was more flexible and easily extended into long strands and it seemed to be more easily linearized in the shearing direction. Considering all this, it seems reasonable that the α -gel aggregate size and flexibility differed in the two products.

The Dimodan P gel in its α -gel form with a medium concentration of salt (Fig. 2a) also showed some streaky structure in polarized light, although not as perfect as the previous ones, but mixed with a phase showing Maltese crosses. Dimodan P in β -crystalline form consisted of large lumps of emulsifier crystals (≤ 100 μm in diameter) and small needles of sodium palmitate in water that could be seen in polarized light (Fig. 2b). The consistency of the β -crystalline form was thick but fluid.

Batter Density with Different Emulsifiers

The aerating effect of these emulsifiers in a cake batter was measured as the batter density after whipping (Fig. 3) with three

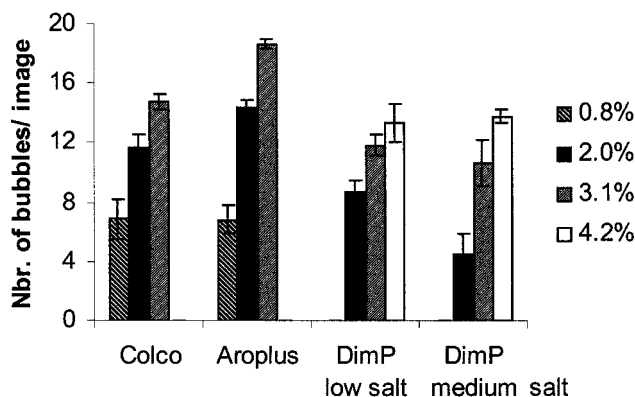


Fig. 8. Mean number of bubbles calculated from image analysis (of cake batter images such as in Fig. 6 or 7).

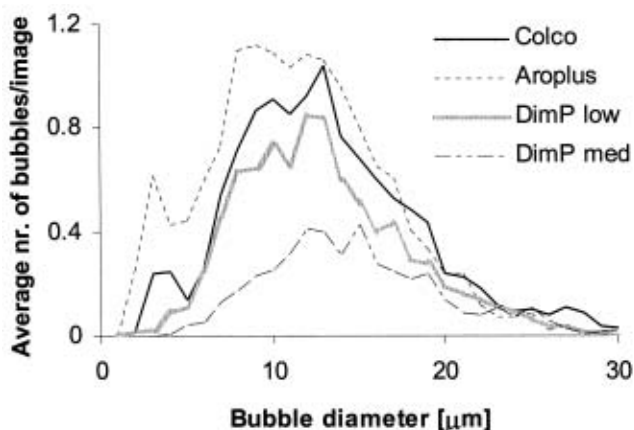


Fig. 9. Bubble diameters in cake batter with emulsifiers added at 2.0% grouped in 30 classes, each 1 μm wide. Dimodan P emulsifiers with 0.05 and 0.67% NaCl are low-salt and medium-salt, respectively.

levels of emulsifier. When α -gels were used, such as Colco, Aroplus, and Dimodan P in α -gel form, a higher amount of emulsifier improved the air incorporation into the batter (i.e., it lowered the density at each emulsifier concentration). The commercial emulsifiers Colco and Aroplus had the best aerating properties and were quite similar, as expected. Colco was slightly more effective at low concentration (580 g/L compared with 610 g/L for Aroplus), but increasing the concentration of the Aroplus α -gel gave a lower batter density. At the highest emulsifier concentration, Colco had a density of 390 g/L and Aroplus 360 g/L. It is possible that the emulsifiers are unable to stabilize more than a critical amount of air, which is lower for Colco than Aroplus. The difference between the two emulsifiers might also be a matter of distribution of the emulsifier in the batter. Kim and Walker (1992) have commented on air bubbles increasing viscosity due to sterical hindrance. They also stated that an increase in viscosity aids the incorporation of air. If this higher apparent viscosity could improve the distribution of the emulsifier, it is probable that Aroplus would be more susceptible to concentration changes, due to the increased flexibility seen in Fig. 1.

Dimodan P, used in two variants, showed that the α -gel form was better than the β -crystalline form at lowering the density, which supports the experience of the α -gel form being effective for aeration in cake batters. The Dimodan P α -gel showed the same behavior as the other α -gels, but a higher amount was required. The β -crystalline form did not improve the batter aeration at all; the density was constant at $\approx 1,000$ g/L. For comparison, the batter was also whipped twice in the same manner (a one-step process) without any emulsifier addition at all. The same density was obtained ($\approx 1,000$ g/L). A traditional process with 30 min of whipping of egg and sugar before addition of the other ingredients, without emulsifier addition, resulted in a density of ≈ 960 g/L, which was slightly better because the egg components could stabilize the foam surfaces. A centrifuged batter had a density of $\approx 1,250$ g/L (results not shown).

Effect of Salt on Emulsifier Properties

To further examine the importance of the emulsifier microstructure, the Dimodan P α -gel was modified by changing the salt concentration. The experiments described above used medium-salt concentration (0.67%). This was now supplemented with measurements made at lower and higher salt concentrations.

Three Dimodan P α -gels had slightly varying optical properties. The one with a low salt concentration was quite transparent, the other two were more opaque. They were examined under the microscope to determine the effect of salt concentration in the α -gel (Fig. 4). Appearance was affected by sample thickness and smearing under polarized light, but there was a small tendency toward a gradual change from streaky to an aggregated Maltese cross structure with increasing

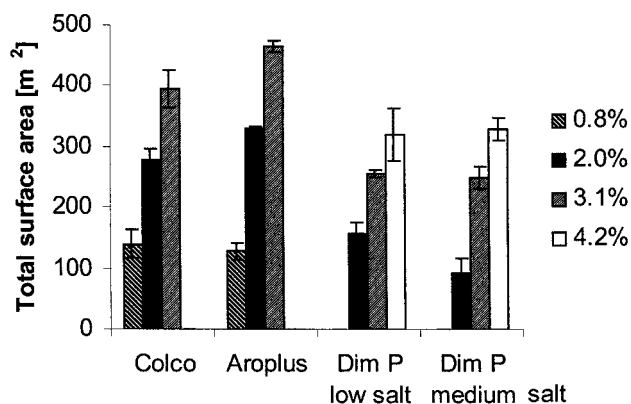


Fig. 10. Total surface area calculated from stereological measurements of cake batter with added emulsifier.

concentrations of salt (Fig. 4a–c). When the nondeformed samples were examined at higher magnification under CLSM (Fig. 4d–f), it was evident that the emulsifier with a low salt concentration showed a more α -gel-like streaky structure than the emulsifier with a high salt concentration, which seemed to consist of smaller, poorly stained aggregates $\leq 10 \mu\text{m}$ in diameter. The medium concentration was something between these two, with long streaks and aggregates mixed. The exact composition of the aggregates could not be decided solely with these methods.

Batter Density with Emulsifiers of Differing Salt Content

The Dimodan P α -gels with three salt concentrations were used in cake batter with three emulsifier concentrations (2.0, 3.1, and 4.2%). Batter density range was 430–660 g/L. The α -gels differed slightly in their performance (Fig. 5). When 2.0% emulsifier was added, the low salt concentration resulted in much better air incorporation than the medium salt concentration; the high salt concentration could not be distinguished from the other two. When 3.1% emulsifier was added, the low and medium salt samples performed almost similarly (the low salt sample was still somewhat better), but the high salt concentration had a worse air incorporation. When 4.2% emulsifier was added, there were no longer any differences in density between the three samples. The equation for the surface response model including all the replicates and concentrations was:

$$\text{Density} = 500 - 90x_{\text{em}} + 20x_{\text{salt}} + 30x_{\text{em}}^2$$

all with a significance of $P < 0.05$. The interaction term and square term of salt were not significant. If only the samples with a high emulsifier concentration in the batter were evaluated, the salt addition had no significant effect at all, which indicates that the salt concentration induced a difference in microstructure, but it only had an effect on the batter aeration at lower emulsifier concentrations.

The most well-known effect of salt addition to an α -gel is that the water layer gets thinner until it collapses, when it turns into a β -crystal. But if β -crystals were formed in the emulsifier with a high salt concentration, they would not have any positive effect on the air incorporation, whatever the amount added. This was shown with the β -crystalline emulsifier in Fig. 3. However, when a higher amount of emulsifier was added, the same batter density was obtained in all three samples. Thus, the transformation was probably not from α -gel to β -crystalline. Neither could it be a situation of simply reducing the water layer thickness because this would not give the appearance under the microscope shown in Fig. 4. It is more probable that the water layer reduction induced a transformation into closed α -gel layer vesicles. This structure is easily formed when the water concentration exceeds the solubility limit between the lipid bilayers (Larsson 1997), which could very well be the case with 70% water. Krog (1997) and Heertje et al (1998) have shown these aggregates appearing as Maltese crosses in polarized light. These liposomes are normally spherical but can be flattened out into cylinders if they are forced to flow, which leads to a gel-like structure (Krog and Larsson 1968). Thus, under certain conditions, it could be possible to transform α -gels with high salt concentration into structures similar to α -gels with low salt concentration. This might be the explanation for the similar performance at higher emulsifier concentrations in the batter.

Structure of Cake Batter

To estimate the effect of the α -gel emulsifiers in a foam, the microstructure of the batters was examined with CLSM and the images were analyzed. The emulsifiers used were Colco, Aroplus, and two Dimodan P α -gels with low and medium salt concentrations. All emulsifiers were used at three levels.

The change in the appearance of the batter with increasing emulsifier concentration can be seen in Fig. 6, which shows cake batter with three levels of Colco (densities of 580, 450, and 390 g/L). The bubbles are black and the surrounding phase is visible. The number of bubbles increases successively, especially small bubbles.

The effect on batter of changing the emulsifier but using the same amount (2.0%) can be seen in Fig. 7. The Colco batter (Fig. 7a) has a density of 450 g/L. The Aroplus batter (Fig. 7b) has a density of 420 g/L and shows foam with more small bubbles. The Dimodan P α -gel batter (Fig. 7c and d) with low and medium salt concentrations (580 and 660 g/L) showed many fewer bubbles than the commercial emulsifier batters. There is a large difference in the number of bubbles in the two images, which is related to the batter density.

To see whether the binary images obtained could be used for measurements, the area fraction of air in the images was calculated as the total object area divided by the total image area. The area fraction in the image is equal to the volume fraction in the sample, as explained by Russ (1990). Thus, it should be related to the batter density. When the area fraction was compared with the density of the same replicate, a linear relationship was obtained ($R^2 = 0.98$): Area fraction = $0.5345 - 0.0006 \cdot (\text{density [g/L]})$. In the perfect case, with an area fraction of 1 when the density approaches 0 and a density of 1,250 g/L without air (a centrifuged batter), the equation would have been: Area fraction = $1 - 0.0008 \cdot (\text{density [g/L]})$

The slope is quite similar but the constant deviates; thus, the area fraction was constantly underestimated. This was an effect of several factors such as the difference in the refractive index of air and bulk sample, inducing bright Becke lines in the bottom part of the air bubbles, which made them appear smaller. However, because the R^2 was so high, it was considered that all the images had been treated in the same way and could be used for comparative measurements.

The average number of air bubbles per image, calculated as the total number of bubbles in all images of one replicate divided by the number of images, is shown in Fig. 8. The number of bubbles increased as more emulsifier was added, which is quite natural. As an example, the Colco batter had 7 counted bubbles at a concentration of 0.8%, and 15 bubbles at a concentration of 3.1%. At higher concentrations, Aroplus incorporated more bubbles than Colco. If the two Dimodan P samples were compared, the low-salt sample already had quite a number of bubbles at the low concentration, while the medium-salt sample had an inferior effect at the low concentration but reached the same number of bubbles at a higher concentration. This reinforces the observations from the density measurements that the two α -gels have a different microstructure but that the effect is not observed at higher concentrations of emulsifier.

The size distribution of the bubbles in the batters with 2.0% emulsifier is shown in Fig. 9. Because the distribution is measured in a section, it does not represent the true bubble distribution in the batter (Russ 1990), but it can be used for comparisons. The diameter peak is between ≈ 10 and $15 \mu\text{m}$, depending on the sample. It seems that in a more stable batter with more bubbles, such as Aroplus, the distribution shifts toward smaller diameters. These two emulsifiers also give rise to an extra peak for objects with a diameter of 2–4 μm . The extra peak only appeared at higher emulsifier amounts ($>2.0\%$ Colco or Aroplus or $>4.2\%$ Dimodan P), so it should be an indication of the higher stability. The Becke lines, arising from diffraction between air and the bulk sample, might contribute to this extra peak; tight packaging of the bubbles could make them appear smaller. Aroplus had more small bubbles than Colco at all concentrations, but fewer large ones at low emulsifier concentrations. In Fig. 9, there is a large difference in the batters with Dimodan P, depending on the salt concentration. The shift in distribution toward smaller bubbles in a more stable batter is very clear, as the low-salt sample contains many more small bubbles than the medium-salt sample. At an addition of 3.1% emulsifier, the low-salt sample has only a slight dislocation to the left in the histogram; at the highest amount (4.2%), there is no difference any more.

The total interfacial area between air and surrounding substance in the batter (total surface area) is shown in Fig. 10. Depending on the sample, these measurements show that the whole batter has a bubble interface of 100–500 m^2 . The question is now, whether the added emulsifier is sufficient to cover this area. In all the emulsifier

gels, the fat concentration is $\approx 30\%$. Assuming a monolayer 2.5 nm thick, 10 g (0.8%) of emulsifier would give a surface of 1,000 m². Thus, at this stage, there must be a great surplus of emulsifier in all the batters, even though the area fraction in the image is not as large as it should have been according to the batter density. It is probable that the egg also contributed somewhat to the surfaces, which would cause an even larger excess of emulsifier. The surplus could be situated on the same surfaces, in the form of stacked α -gel layers, or on other surfaces, such as the starch granules. The total surface area shows the same behavior as the number of bubbles, with more surface in the batter with a high concentration of Aroplus rather than Colco; the low-salt Dimodan P was more effective than the medium-salt variant at low concentrations only.

CONCLUSIONS

The results of this study show that the microstructure of the emulsifier has an important effect on its aerating properties. A β -crystalline structure did not have any effect on a cake batter made in a one-stage process, while an α -gel had a very positive influence on the air incorporation. When Colco and Aroplus, two commercial α -gels with similar performance, were compared, Colco had an evenly increasing effect on air incorporation with concentration. It did not seem to be much affected by shear forces as seen in the emulsifier images. Aroplus, on the other hand, had larger and more regular α -gel structures and was more easily smeared out. The structure probably facilitated its distribution. The density differences and all the measurements on the air bubbles in the batter indicated that, at higher concentrations, Aroplus could incorporate more air in smaller bubbles. These commercial mixed emulsifiers had a pure α -lamellar appearance and were much more effective for air incorporation than the monoglyceride.

Changing the salt concentration in the monoglyceride Dimodan P α -gel showed that the salt concentration may influence the microstructure of the gel. There seemed to be a gradual change from the normal α -gel structure to some smaller aggregates when the salt concentration increased. However, all the measurements on the batter showed that this change only had an effect on the foaming when the amount of emulsifier in the batter was low. At high emulsifier concentrations, there was no difference in batter density or bubble sizes. If the transformation had been into β -crystals, they would not have contributed to the air incorporation, as was shown with the β -crystalline emulsifier. The most probable explanation is that the α -gel formed closed α -lamellar liposomes that could become stretched out into α -gels during whipping.

It was also shown that the amount of emulsifier added, which is a normal amount in cake batter, was more than enough to cover the bubble surfaces after whipping, even without considering the proteins that are present. Whether the surplus is located on the interfaces in multiple layers or on other surfaces such as starch granules could not be determined at the magnification used.

ACKNOWLEDGMENTS

The financial support from KK-stiftelsen and Aromatic AB is gratefully acknowledged, as well as the ingredients received from Kvarn AB Juvel, Danisco Cultor and Aromatic AB. Special thanks to Björn Bergenstahl at Lund University for all the discussions about emulsifiers and to Elvy Jordansson and Paula Olofsson for laboratory help.

LITERATURE CITED

- Brooker, B. E. 1993. The stabilisation of air in cake batters—The role of fat. *Food Structure* 12:285-296.
- Carlotti, M. E., Gallarate, M., Trotta, M., and Canova, V. 2000. On the use of saccharose esters in the preparation of o/w emulsions with liquid crystals. *J. Dispersion Sci. Technol.* 21:31-48.
- Cauvain, S., and Cyster, J. 1996. Sponge cake technology. *Campden and Chorleywood Food Res. Assoc. Rev.* 2:1-43.
- Friberg, S., and Jansson, P. O. 1976. Surfactant association structure and emulsion stability. *J. Colloid Interface Sci.* 55:614-623.
- Heertje, I., Roijers, E. C., and Hendrickx, H. A. C. M. 1998. Liquid crystalline phases in the structuring of food products. *Lebens. Wiss. Technol.* 31:387-396.
- Howard, N. B. 1972. The role of some essential ingredients in the formation of layer cake structures. *Baker's Dig.* 46(5):28-37.
- Kim, C. S., and Walker, C. E. 1992. Interactions between starches, sugars, and emulsifiers in high-ratio cake model systems. *Cereal Chem.* 69:206-212.
- Krog, N. 1975. Structures of emulsifier-water mesophases related to emulsion stability. *Fette, Seifen, Anstrichmittel* 77:267-271.
- Krog, N., and Borup, A. P. 1973. Swelling behaviour of lamellar phases of saturated monoglycerides in aqueous systems. *J. Sci. Food Agric.* 24:691-701.
- Krog, N., and Larsson, K. 1968. Phase behaviour and rheological properties of aqueous systems of industrial distilled monoglycerides. *Chem. Phys. Lipids* 2:129-143.
- Krog, N. J. 1997. Food emulsifiers and their chemical and physical properties. Pages 141-188 in: *Food Emulsions*, 3rd Ed. S. E. Friberg and K. Larsson, eds. Marcel Dekker: New York.
- Larsson, K. 1997. Molecular organization in lipids. Pages 111-139 in: *Food Emulsions*, 3rd Ed. S. E. Friberg and K. Larsson, eds. Marcel Dekker: New York.
- Larsson, K., and Krog, N. J. 1973. Structural properties of the lipid-water gel phase. *Chem. Phys. Lipids* 10:177-180.
- Lee, C. C., and Hosney, R. C. 1982. Optimization of the fat-emulsifier system and the gum-egg white-water system for a laboratory-scale single-stage cake mix. *Cereal Chem.* 59:392-395.
- Rodríguez Patino, J. M., and Ruíz Domínguez, M. 1996. Study of mono-stearin films in the presence of electrolytes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 114:287-296.
- Russ, J. C. 1990. *Computer assisted microscopy: The measurement and analysis of images.* Plenum Press: New York.
- Shepherd, I. S., and Yoell, R. W. 1976. *Cake emulsions.* Pages 215-275 in: *Food Emulsions*, 1st Ed. S. E. Friberg, ed. Marcel Dekker: New York.
- Silva, R. F. 2000. Uses of alpha-crystalline emulsifiers in the sweet goods industry. *Cereal Foods World* 45:405-411.
- Wootton, J. C., Howard, N. B., Martin, J. B., McOsker, D. E., and Holme, J. 1967. The role of emulsifiers in the incorporation of air into layer cake batter systems. *Cereal Chem.* 44:333-343.

[Received September 25, 2001. Accepted March 18, 2002.]