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The Effects of Polysorbate 80 on the Fat Emulsion in Ice Cream Mix: Evidence from Transmission Electron Microscopy Studies

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THE EFFECTS OF POLYSORBATE 80 ON THE FAT EMULSION IN ICE CREAM MIX: EVIDENCE FROM TRANSMISSION ELECTRON MICROSCOPY STUDIES


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Abstract

Emulsifiers are used in ice cream to produce a dry, smooth textured product with desirable melting properties. They function by promoting a partial destabilization of the fat emulsion. Polyoxyethylene sorbitan monooleate is used very commonly in the ice cream industry for this purpose. The objective of this research was to examine by transmission electron microscopy the differences in the fat globules in typical ice cream mix emulsions prepared with and without 0.08% polyoxyethylene sorbitan monooleate.

Ice cream mix was combined 3:1 with a 2% solution of ultralow gelling temperature agarose at 20°C, fixed with 4% glutaraldehyde, postfixed with 1% OsO4 in imidazole/phosphate buffer, embedded in Spurr resin and thin-sectioned for viewing. The emulsifier reduced the number of casein micelles adsorbed to the fat globules as determined by both TEM and quantification of membrane protein with Kjeldahl analyses. The fat/ serum interfacial tension was also significantly reduced by the presence of emulsifier in the mix. The data suggest that emulsifiers promote fat destabilization through reduction of membrane protein, based on their ability to reduce the fat serum interfacial tension. In the presence of crystallized fat, the emulsion then becomes less stable to shear forces during the whipping and freezing of ice cream.

Introduction

Emulsifiers, such as mono- and di-glycerides or polyoxyethylene sorbitan esters, are used in ice cream to improve the whipping quality of the mix, to produce a drier ice cream with smoother body and texture, and to achieve good drawing qualities at the freezer (Arbuckle, 1976). These effects result from a destabilization of the fat emulsion (Keeney, 1958; Berger, 1976) as occurs during the whipping of heavy cream (Schmidt and van Hooydonk, 1980; Brooker et al., 1986) in which chains and clusters of partially ruptured fat globules form a network of fat that envelops and stabilizes the air cells.

During ice cream manufacture, the whipping and concomitant freezing process imposes large shear forces on the mix. The combination of ice crystallization and shear is responsible for destabilization of the milkfat globules (Lin and Leeder, 1974; Goff and Jordan, 1986). The magnitude of destabilization facilitated by an emulsifier is related to its hydrophilic/lipophilic balance (HLB) (Govin and Leeder, 1971), and to the interfacial tension between the serum and lipid phases in the presence of the emulsifier (Walstra, 1983). In a protein stabilized emulsion such as a dairy emulsion, the addition of surfactant prior to homogenization reduces the amount of protein adsorbed per surface area of fat (Oortwijn et al., 1977; Oortwijn and Walstra, 1979, 1982; Barford and Krog, 1987).

Alsafar and Wood (1966) used transmission electron microscopy to examine the fat dispersion in ice cream. They reported that the surface layer of fat globules appeared darker and thicker without an emulsifier than with emulsifier. Berger and White (1971), using freeze etching techniques, reported the presence of a granular coating, considered to be casein subunits, on the outside of the fat globules. They stated that the strength of the fat globule membrane is the overriding factor in predicting fat destabilization. It was suggested that the membrane is only weakly bound and is easily stripped off to expose a crystalline lipid shell composed largely of the high-melting glycerides (HMG). Liquid fat escaping from these ruptured HMG shells then becomes the cementing agent in fat destabilization during freezing (Berger and White, 1976). However, this liquid core/solid shell model has been challenged (Walstra and van Beresteyn, 1975; Walstra, 1983).

Polyoxyethylene sorbitan monooleate (Polysorbate 80 or Tween 80) is the agent most commonly used by the ice cream industry for destabilization of the fat emulsion (Thomas, 1981; Keeney, 1982). This research project was initiated to elucidate the mechanism of emulsifier action in promoting fat destabilization during the manufacture of ice cream. The objective of this study was to examine the microstructural differences in ice cream mix emulsions prepared both in the presence and absence of polyoxyethylene sorbitan monooleate.

Key Words: agarose, casein micelles, emulsifiers, emulsion, fat destabilization, ice cream, milk fat globules, Polysorbate 80, transmission electron microscope, Tween 80

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Materials and Methods

Ice cream mix preparation
Four replicates of ice cream mixes with the following compositions were prepared: 10% milkfat, 11% milk solids-not-fat (msnf), 10% sucrose, and 5% corn syrup solids; and 10% milkfat, 11% msnf, 10% sucrose, 5% corn syrup solids, and 0.08% polyoxyethylene sorbitan monolaurate (Tween 80, ICI Americas Inc., Wilmington, DE). Fresh cream, skim milk, and nonfat dry milk were used as the sources of milk solids. The 8 kg mixes were blended, pasteurized at 74°C for 30 min, homogenized at 17.2 MPa (2500 psig), 3.4 MPa (500 psig) second stage (Manton Gaulin 75E Homogenizer), cooled to 5°C, and aged 24 h, as in conventional ice cream manufacturing procedures.

Fat destabilization analysis
One two-litre aliquot of each frozen mix was thawed in a Taylor batch freezer to -5°C and held in the freezer for a total of 15 min of agitation. Samples (40mL) were removed from the barrel every 2.5 min and analyzed for the degree of fat destabilization by turbidimetry. Samples were diluted 1:500 with distilled water and absorbance was measured at 540nm. Percent fat destabilized was calculated as [(Amix- Aice cream)/Amix] x 100% (Keeney, 1958).

Interfacial tension
The interfacial tension was measured with a duNuoy ring apparatus (Fisher Surface Tensiometer) by placing the ring in water or in aqueous solutions of 11% milk solids-not-fat in a jacketed beaker at 70°C, with and without 0.08% polyoxyethylene sorbitan monolaurate, layering the solution with anhydrous milkfat at 70°C, aging 10 min while maintaining constant temperature, and then drawing the ring from the one phase into the other.

Transmission electron microscopy
The two ice cream mix emulsions, after aging, were warmed to 15°C and combined with a 2% solution of ultralow gelling temperature agarose (SeaPrep agarose, FMC Marine Colloids Div.) at a rate of 3 parts sample to one part agarose. The agarose solution had been previously heated and cooled to 20°C. The ice cream mix and agarose combination was allowed to solidify at 4°C overnight. Pieces of the mix were then cut and fixed in 4% glutaraldehyde in 0.1M phosphate buffer (pH 7.0) overnight. After several rinses in phosphate buffer, the samples were postfixed in 1% osmium tetroxide in 0.1M phosphate/imidazole buffer (1:1 v/v; pH 7.0) overnight (Aungersmuller and Fahimi, 1982; Kalab, 1985). Following several buffer rinses, the samples were dehydrated in successive ethanol concentrations, embedded in Spurr resin molds, and held at 70°C overnight. Samples were then thin-sectioned (Sorvall MT-2 Ultra-microtome), placed on carbon-coated Formvar copper grids, and viewed using a Philips EM-300 Transmission Electron Microscope.

Fat globule measurement
Cross section diameters of all of the fat globules in at least 40 different random fields from each treatment were measured from the micrographs. A total of 911 globule sections were determined and grouped in 0.5 µm categories. The number of discrete protein particles adhering to each of the globules was also counted. The average diameter of the globule cross sections was determined as was the average number of adsorbed protein particles per globule as a function of globule diameter.

Quantification of membrane protein
Total protein in the fresh ice cream mix was determined by Kjeldahl nitrogen. Mix samples were then centrifuged at 26,000g, 20°C, 30 min, the centrifuge tubes were then frozen, the fat layer was removed, and the serum was thawed, mixed, and reanalyzed for Kjeldahl nitrogen. Adsorbed protein was calculated as the difference between total protein and serum protein (Oortwijn and Walstra, 1979).

Results and Discussion
The differences observed in the degree of fat destabilization when the two ice cream mixes were concomitantly whipped and frozen into the ice cream state are illustrated in Figure 1. The addition of Tween 80 to the mix prior to homogenization clearly produced an emulsion which was less stable to the applied shear forces than an emulsion stabilized solely by the milk proteins. Fat destabilization also manifested itself in dryness, smoothness, and meltdown of the two ice cream mixes when the ice cream mixes were frozen.

Both mixes, however, were equally stable following homogenization when not subjected to the freezing process (Table 1).

The interfacial tension between the aqueous phase and anhydrous butteroil was taken as representative of the interfacial tension at the surface of the globule in an emulsion. While the absolute numbers may be different due to the greatly different surface to volume ratio, the relative trends can be observed. The interfacial tension at the aqueous phase/oil surface was reduced by the milk proteins and was also reduced by the emulsifier (Table 2). This decrease in interfacial tension correlated with the increase in fat destabilization in the presence of the emulsifier. A good correlation has been shown between the destabilizing power of several emulsifiers and the serum/lipid interfacial tension in the presence of the emulsifier and has been reported elsewhere (Goff et al., 1987).

In examining electron micrographs of the two emulsions, differences in the number of casein micelles adhering to the fat globules were observed. In the absence of the emulsifier, more casein micelles adsorbed to the fat globules (Figure 2, Figure 4).
Effects of polysorbate 80 in ice cream mix

Figure 2. Transmission electron micrograph of the ice cream mix emulsion in the absence of emulsifier. F=fat globule, C=casein micelle, A=agarose matrix, arrow points to fat crystal within the globule. Bar=1 μm.

Figure 3. Transmission electron micrograph of the ice cream mix emulsion in the presence of 0.08% Tween 80. F=fat globule, arrow points to fat crystal within the globule. Bar=1 μm.

Figure 4. Side-by-side comparison of fat globules from the two ice cream mixes. 4a has no emulsifier. 4b contains 0.08% Tween 80. F=fat globule, C=casein micelle. Bar=1 μm.

than when the emulsifier was included in the mix (Figure 3, Figure 4). This difference in micellar adsorption was quantified by counting number of micelles adsorbed per globule in random micrographs from each emulsion. The statistical analyses performed demonstrated a significant difference (p<0.01) between emulsions; nearly twice as many micelles were adsorbed to the fat globules in the mix without emulsifier than in the mix containing Tween 80 (Table 3).

Adsorbed protein was also determined by Kjeldahl analysis as described above. A small portion of the fat was found to remain in the serum after centrifugation as was found by Oortwijn and Walstra (1979). The results indicated that 15.9% of the total mix protein was adsorbed to the fat globule in the absence of emulsifier whereas only 7.8% was adsorbed to the fat globule in the

Table 2. Interfacial tension between anhydrous butteroil and an aqueous phase with or without 10% skim milk powder and with or without Tween 80 as determined by a duNuoy ring at 70°C.

<table>
<thead>
<tr>
<th>INTERFACIAL TENSION</th>
<th>INTERFACIAL TENSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhydrous butteroil vs. water (dynes/cm)</td>
<td>Anhydrous butteroil vs. 10% skim prd. (dynes/cm)</td>
</tr>
<tr>
<td>No Emulsifier</td>
<td>0.26</td>
</tr>
<tr>
<td>Polyoxyethylene sorbitan monoooleate (Tween 80)</td>
<td>2.53</td>
</tr>
</tbody>
</table>

n = 4
a not significant at p < 0.05

Table 3. Summary of the number of micelles adhering to fat globules in the presence and absence of Tween 80 as a function of the globule section diameter. Mean section diameters of the two emulsions are also shown.

<table>
<thead>
<tr>
<th>Globule Section Diameter (μm)</th>
<th>No Emulsifier</th>
<th>0.08% Tween 80</th>
</tr>
</thead>
<tbody>
<tr>
<td># Globules</td>
<td>Av. # Adsorbed micelles per globule</td>
<td># Globules</td>
</tr>
<tr>
<td>0.00 - 0.50</td>
<td>256</td>
<td>3.35</td>
</tr>
<tr>
<td>0.55 - 1.00</td>
<td>108</td>
<td>4.27</td>
</tr>
<tr>
<td>1.05 - 1.50</td>
<td>47</td>
<td>4.47</td>
</tr>
<tr>
<td>1.55 - 2.00</td>
<td>14</td>
<td>6.43</td>
</tr>
<tr>
<td>&gt;2.00</td>
<td>6</td>
<td>4.67</td>
</tr>
<tr>
<td>Total</td>
<td>431</td>
<td>3.82 ± 0.25</td>
</tr>
</tbody>
</table>

Table 4. Differences in the amount of protein adsorbed to the fat globules in each of the mixes as determined by centrifugal separation and Kjeldahl analyses.

<table>
<thead>
<tr>
<th>Mix</th>
<th>Protein (%)</th>
<th>No Emulsifier</th>
<th>Tween 80 (0.08%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein in serum</td>
<td>3.43 ± 0.07</td>
<td>3.76 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Adsorbed Protein (%) of total protein</td>
<td>15.9</td>
<td>7.84</td>
<td></td>
</tr>
</tbody>
</table>

n = 4
presence of Tween 80 (Table 4). This difference was statistically
significant and the 95% confidence intervals are shown in Table 4.

The information gained from measurement of the fat
globule diameters on the micrographs is relevant only to the cross­
sectional diameters and not to the true diameters of the globule
(Table 3). The mean section diameters and size distribution of the
sections between the two emulsions, however, were not
significantly different. Turbidity or absorbance of the two emulsions
was also determined at both 400 nm and 540 nm using two
different procedures (Table 5). There was no difference in light
scattering between the two emulsions. Thus it appears that the
addition of the emulsifier had no significant effect on fat globule
size distribution of the two populations. The high homogenizing
pressures involved were the overriding factor in size distribution.

Emulsifier action, therefore, may be summarized as follows.
The added emulsifiers reduce the interfacial tension between the
serum and lipid (fat globule) phases of the mix. It is thus more
favourable for the emulsifiers rather than caseins to adsorb to the
fat surface at the time of homogenization, as this leads to a lowering
of the net free energy of the system. This reduction in the amount
of casein, however, produces an emulsion which is less stable to
the shear forces applied during ice cream freezing. As a result, the
fat emulsion is destabilized to a greater extent in the presence of the
emulsifiers. Destabilization leads to the production of a
smoother ice cream with good melt resistance due to the
enhanced structure of the foam caused by the fat network.

Examination of the electron micrographs revealed
crystalline fat within the cross section of the fat globule. The
needle-like crystals were distributed evenly and randomly
throughout the globule (Figures 2-6) and no evidence of a solid
shell around the fat globule was observed; however, a crystalline
periphery was slightly evident in a few globules (Figure 5). This
structure conforms to that proposed by Walstra (1983); however,
concentric crystalline layers of fat at the globule boundary have
been shown by freeze etch techniques (Berger, 1976; Buchheim
and Precht, 1979; Precht and Buchheim, 1979).

Many of the globules also showed evidence of fat crystals
which influenced the shape of the globule (Figure 6). van Boeke!
and Walstra (1981) have suggested a model for crystallization
within a fat globule whereby tangentially oriented crystals protrude
from the surface of the globule and are able to pierce the film
between two existing droplets upon close approach, thus
promoting rupture of the globule membrane and subsequent
coalescence. This may partly explain the necessity of a partially
crystalline fat to enhance fat destabilization or partial churning.

Table 5. Differences in fat globule size distribution of the two ice
cream mixes as determined by light scattering techniques (Walstra,
1968).

<table>
<thead>
<tr>
<th>ICE CREAM MIX</th>
<th>ABSORBANCE</th>
<th>ABSORBANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(400nm)</td>
<td>(540nm)</td>
</tr>
<tr>
<td></td>
<td>w/ Soln. A</td>
<td>w/ Soln. A</td>
</tr>
<tr>
<td>(1:500 dilution)</td>
<td>(2%)</td>
<td>(2%)</td>
</tr>
</tbody>
</table>

| NO EMULSIFIER | 0.83 | 0.92 | 0.63 | 0.70 |
| 0.08% TWEEN 80 | 0.83 | 0.92 | 0.63 | 0.70 |

n = 4

Soln. A = 0.375% Disodium EDTA
0.125% Tween 20
adj. to pH 10 with NaOH

Figure 5. Milkfat globule from an ice cream mix showing the intricate
structure of needle-like crystals present in the cross section of the
globule. F=fat globule, arrow points to fat crystal. Bar=1μm.

Figure 6. Milkfat globule from an ice cream mix. Arrow points to
surface distortion of the globule caused by the presence of the
internal crystalline structure. F=fat globule. Bar=1μm.

Figure 7. Transmission electron micrograph of an ice cream mix
illustrating two fat globules joined together by the adsorption of a
casein micelle onto each globule. F=fat globule, arrow points to
casein micelle attachment. Bar=1μm.
under the influence of applied shear forces. The pendicular and
tangential crystal orientations observed in many of the fat globules
from the two ice cream mixes support this theory. The presence of
adsorbed casein micelles, however, would tend to physically block
the influence of these crystal protrusions on fat coalescence and
destabilization through steric repulsions.

Another observation made from the micrographs was the
presence of homogenization clusters formed by the sharing of an
adsorbed casein micelle between two fat globules, thus holding
them together (Figure 7). Ogden et al. (1976) have proposed this
type of structure as one of the possible mechanisms involved in
clustering of fat globules post homogenization. The two stage
homogenization process involved in the preparation of the mixes
for this experiment is intended to reduce the amount of fat globule
clustering. Thus, only a few such clusters were seen in all of the
micrographs examined.

Conclusions

In conclusion, a significant difference was observed in the
number of casein micelles adhering to the fat globule when the
ice cream mix was homogenized in the presence of poloxymethylene
sorbitan monooleate, more protein being adsorbed in the absence
of the emulsifier than in its presence. The total surface area of fat in
the emulsion was not affected by the presence or absence of the
emulsifier. Crystalline fat within the fat globules exhibited a random
distribution throughout the cross section of the globule. Several fat
globules exhibited crystals which appeared to influence the
peripheral shape of the globule.

Acknowledgments

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Board for their support of this research. The authors also wish
to thank Dr. M. Kalab for helpful suggestions.

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Discussion with Reviewers

B.E. Brooker: Are any problems encountered when using
agarose as a gelling agent? Would you like to say more about the
use of agarose gel?

Authors: We followed the protocol recommended by Strausbach et.
cell suspensions in ultra-low gelling temperature agarose: improved
specimen preparation for transmission electron microscopy. J.
Electron Microsc. Technique. 2: 361-362). They found that 2% agarose
solutions were easy to prepare and resulted in a firm gel. Agarose
concentrations below 1% did not allow to boil during preparation. This
could cause difficulties when sectioning. Therefore the agarose
preparation was removed from the heat source as soon as the agarose
went into solution and before boiling commenced. Some emulsions
did not gel firmly when prepared in a 3 part emulsion: 1 part agarose
solution but still gave satisfactory TEM results. However, the proportion of agarose
to sample could be raised in order to produce a firmer gel if
needed.

B.E. Brooker: Is it possible, by adding sufficient emulsifier, to
displace all protein from the fat-water interface of the fat globules? If
so, what can you say about the stability of the resulting globules?

Authors: It is known that the excessive use of emulsifier in ice
cream manufacture will cause churning to occur very quickly
(Keeney, 1958). Excessive surfactant is also used in the isolation
of membrane material from washed cream (Mulder H, Walstra P,
...
The Milk Fat Globule. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., England., p. 117. Desorption of membrane protein is facilitated by this technique. The stability of the resulting emulsion is greatly reduced.

P. Walstra: Have you compared the results of Table 3, no emulsifier, with those of Walstra and Oortwijn, Neth. Milk Dairy J. 36(1986)103-113, Fig. 1? You find a similar decrease in number of micelles attached per μm circumference of the fat globule with increasing globular diameter.

Authors: Our findings do agree with the prediction by Walstra and Oortwijn that smaller globules acquire more large particles per unit surface area, although the globule diameters in Table 3 refer only to the cross sections and, since this was not systematically studied by us, there are insufficient observations in the larger size ranges to independently conclude this relation.

I. Heerlie: Ice cream is made by cooling to about -5°C while beating in air, and under these conditions coalescence of fat takes place. On the other hand, ice cream emulsions were analyzed for their microstructure by a preparation scheme involving a temperature regime with a lowest temperature of 4°C. This preparation scheme and these conditions are apparently very well suited to show the interaction between casein and the fat globules. Can these conditions be considered to be representative for the situation at -5°C, in particular for aspects such as the amount and orientation of fat crystals and the deformation of fat globules? If not, would it be advisable and possible to perform these types of microstructural observations at this temperature?

Authors: During ice cream manufacture, a mix is prepared by processes similar to the one employed by us. This mix is then aged at 4°C prior to being concomitantly whipped and frozen into the ice cream state. We were interested in determining what microstructure existed prior to freezing such that fat coalescence during freezing was enhanced in the presence of the surfactant. We did not examine samples of frozen ice cream using this technique, although more information regarding the time and temperature dependent fat crystallization process may be gained from such a study.

I. Heerlie: Differences in fat destabilization were found only when the mixes were subjected to the freezing process. Does this not imply that, apart from the fat crystals, at least ice crystals also may induce coalescence of fat globules, which also, in that case, may be prevented by steric repulsion of the adsorbed casein micelles?

Authors: Indeed that is the case. It was reported by us (Goff and Jordan, 1986) that the combination of ice crystallization, air incorporation, and shear forces were all necessary for fat destabilization to occur in ice cream. When the fat globules lack the physical protection of the adsorbed protein layer and are subjected to the conditions of the freezer, coalescence is enhanced. Fat crystallization is also necessary for this destabilization to occur as the fluidity or mobility of the globule is greatly reduced and the potential for interglobule contact through protruding fat crystals is hypothesized by van Boekel and Walstra is enhanced.

I. Heerlie: Large differences are found in the amount of fat crystals in the different emulsion droplets (compare fig. 5 with 6 and 7). This is in accordance with earlier observations of Walstra (Neth. Milk Dairy J. 1967, 21, 166) by light microscopy and Buchheim and Precht (Milchwissenschaft 1979, 34, 657,720) by freeze fracture electron microscopy. There is some debate (Walstra, 1983) whether fat globules with birefringent outer layers as viewed by polarized light microscopy consist of small tangentially oriented crystals or of large concentric layers of fat crystals, forming a more or less solid shell. In fig. 5, a concentric arrangement of large crystalline layers is observed. Should this morphology, obtained by a chemical fixation procedure, be considered as independent proof for the existence of this type of arrangement?

Authors: Figure 5 demonstrates the presence of a crystalline peripheral orientation to some of the more extensively crystallized globules; however, the crystals are also distributed throughout the cross section of the globule in figure 5 and in figs. 2 to 6. There seems to be no evidence to support the presence of a solid shell of crystallized fat and a liquid core; however, this arrangement is still open to debate. This research does not prove or disprove the stability of these crystal arrangements. Precht and Buchheim (1979) found globules with a high melting glyceride shell of 0.1-0.5 μm thickness with crystalline aggregates and liquid fat in the interior to possess the stability necessary to withstand shear forces during buttermaking. However, Walstra (1983) suggested that globules with the birefringent outer layer were the unstable ones. This remains open to debate as well.

J. M. deMan: When Kjeldahl nitrogen was determined in the serum, was a correction applied for the volume of fat removed?

Authors: Yes. In the calculation of protein adsorbed to the fat globules, non-adsorbed protein was determined as Kjeldahl nitrogen in the serum multiplied by the Kjeldahl factor (6.38) multiplied by the fraction of serum in the mix (0.90). Adsorbed protein was calculated as total protein in the mix subtract non-adsorbed protein.