Assessing the Efficiency of the Homogenizers – Fat globules and USPH index as an Indicator

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Abstract:
The efficiency of homogenizers were assessed using USPH index and the milk fat globules distributions as an indicator. A significant difference (p<0.01) in milk fat globules size and the USPH index was observed between raw and homogenized milk. However, there was no significant difference in the USPH index recorded for different homogenization time intervals. The mean fat globules size for the raw milk was 3.37 μm; and for milk homogenized at different times were 0.98 μm (1h) and 0.96 μm (3h). A significant difference (p<0.01) in a total surface area was also observed between the raw and homogenized milk. The mean total surface area estimated for the raw milk was 17965 cm² / g. Whereas, the mean total surface estimated for the homogenized milk at different homogenization times was 61483 cm² / g (1h) and 62767 cm² / g (3h), an increase in total surface area by more than three-folds in comparison to raw milk. There was significant differences (p<0.01) in the homogenization index between the homogenized and raw milk. However, significant differences was not observed between the different homogenization time intervals. The estimated average least square means of USPH index was 79.37 % for the raw milk; and 5.54 % (1 h) and 5.42 % (3 h) for the homogenized milk. In this study, about 70 % of fat globules in raw milk were observed less than 3 μm in diameters, and also a large fat globules of 12 μm in diameter was also encountered. Whereas, in homogenized milk about 75 % of fat globules were ≤ 1 μm in diameter, with maximum fat globules size of about 2.37 μm in diameter was recorded. In this study, almost above 90 % of the fat globules size observed was below 2 μm in diameter after homogenization of milk. Considering the USPH index, the fat globules size and fat globules distributions derived in this study as an indicator, the homogenizers used in this study were found equally efficient.

Keywords: Efficiency, fat globules distribution, total surface area, USPH index.

Introduction
Homogenization is a standard industrial process, universally practiced as a means of stabilizing the fat emulsion against gravity separation by reducing the fat globules to approximately 1 micron in diameter, accompanied by an increase in the fat/plasma interfacial surface area by a four to six-fold (Tetra Pak, 1995). Turbulence, shear and cavitation are the main physical causes that reduced the fat globule size (Tetra Pak, 1995; Thieband et al., 2003). Several methods such as consumer reaction, appearance, microscopic examination, fat rising during quiescent storage for 48 hours, centrifugal separation and refraction of light were used to determine the efficiency of homogenizer. However, today the microscope examination of fat globule size and USPH index (Tetra Pak, 1995) are the most common method adopted in measuring the efficiency of homogenizer. USPH index is defined as “the fat percentage of the milk in the top 100 ml (1/9 of volume) of milk in a quartz bottle, or of proportionate volumes in containers of other sizes, does not differ by more than 10 percent of itself from the fat percentage of the remaining milk (9/10 of volume) as determined after through mixing” (Trout, 1950). The microscopic examination and counting of fat globules would require a great skill and experience (Trout, 1950).

Homogenization efficiency would depend on several factors, (a) the velocity at which the liquid passes through the valve, (b) the force with which it strikes the wall of the chamber surrounding the valve, (c) the angle of impact, (d) the degree of mechanical perfection of operation of the machine and (e) temperature used in processing. Proper operation of the homogenizer could only be obtained by keeping the machine and homogenizing valve in good operating condition, free of grooves and worn place (Trout, 1950). The homogenizers used in the dairy plant were
old and their efficiency were not assessed, so far. Therefore, this study was carried out to assess its efficiency using USPH index and milk globules size as an indicator.

Materials and Methods

Study location
The study was conducted at the Dairy Centre, Kasetsart University, Bangkok. Two homogenizers, i.e. locally fabricated (made in Thailand) and imported homogenizer (Tetra pak) were used in the dairy plant to homogenized milk prior to processing into different products.

Sample collection and analysis
Milk preheated at 60-70 °C was homogenized using commercially imported (T) and locally fabricated (P) homogenizers at 2500 psi using standard homogenizing head. Milk samples about 500 ml were collected at 0 h (prior to homogenization), 1 h and 3 h after homogenization during the daily processing period. The samples collected were analyzed for the fat content using calibrated automatic ultrasonic milk analyzer (Eon Trading, 2001).

USPH Index
Milk samples (200 ml) raw and homogenized were transferred in plastic bottles of 200 ml and were kept in a refrigerator for 48 hrs at 5 °C (Harding, 1995). Then, the fat content of samples from the upper part, i.e. 1 / 10 (a) and samples from the bottom, i.e. 9 / 10 (b) of the plastic bottle were determined using automatic ultrasonic milk analyzer. The following equation (Ertugay et al., 2004) was adopted to calculate the homogenization index /USPH index of the sample:

\[
\text{USPH Index} = \frac{(a - b)}{a} \times 100
\]

Raw milk fat globules size determination
The samples were prepared and fat globule size for the raw milk and milk homogenized at different time intervals was determined. A sample was prepared for the determination of fat globules size distributions as follows. A dilution of 1 part of milk to 25 parts of cold distilled water was prepared and a drop of dilution was transferred to the center of the glass slide (75 x 24mm) using rubber dropper. A small drop of Rhodamine dye was transferred over the solution and the solution was thoroughly mixed by tilting the slides. Then a glass cover slide (22 x 22mm) was gently placed over the solution and the fat globule size was determined by light scattering microscope (Microscope model LM series 2000, MEIJI, Japan) equipped with a camera at 40 X magnification power. Five films per slide were observed and average diameter of fat globules was determined by the computer software.

Statistical analysis
The data collected was analyzed using simple model as hereunder:

\[
Y_{ijl} = \mu + H_i + T_j + \varepsilon_{ijl}
\]

Where, \(Y_{ijl}\) = the dependent variables;
\(\mu\) = overall mean;
\(H_i\) = blocked by homogenizer, where \(i=1\) and \(2\);
\(T_j\) = Time of homogenization \((j = 0, 1 \text{ and } 3 \text{ h})\);
\(\varepsilon_{ijl}\) = main error-NID \((0, \sigma_v^2)\)

Mean differences were compared by Duncan’s multiple range test (DMRT) according to Cody and Smith (1997).

Result and Discussions

USPH index as indicator to assess the efficiency of homogenizer
Table 1 and 2 illustrate the least square means of milk fat content (%) and USPH index of raw and homogenized milk. There was no significant difference \((p>0.05)\) in fat content between the raw and homogenized milk. However, a slight increase in fat content \((3.83 \text{ & } 3.84 \% \text{ fat for } 1 \text{ hr and } 3 \text{ hr, respectively})\) was observed after homogenization of milk at 2,500 pounds pressure. It was reported that the best fat content for the homogenized milk should be about 4 \% (Trout, 1950). In contrast, Trout (1950) reported that milk homogenized at 2,000 – 3,000 pounds pressure and tested for milk fat using Babcock test shows about 0.1 to 0.15 percent lower than the test of the milk before homogenization. The difference might have resulted due to one or more of several factors such as a) the specific
gravity of the acid used; b) the volume of acid; c) the maximum heats of reaction obtained; d) the length of time of shaking the acid-milk mixture and e) the completeness of digestion and the amount of foreign matter in or at the base of the fat column.

Table 1 Least square means and standard error of fat content (%) for raw and homogenized milk.

<table>
<thead>
<tr>
<th>Homogenizer</th>
<th>Homogenization Time (h)</th>
<th>N</th>
<th>0</th>
<th>N</th>
<th>1</th>
<th>N</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>3.87 ± 0.07</td>
<td>14</td>
<td>3.89 ± 0.07</td>
<td>13</td>
<td>3.92 ± 0.08</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>10</td>
<td>3.68 ± 0.08</td>
<td>10</td>
<td>3.75 ± 0.08</td>
<td>10</td>
<td>3.75 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>3.80 ± 0.05</td>
<td></td>
<td>3.83 ± 0.05</td>
<td></td>
<td>3.84 ± 0.06</td>
</tr>
</tbody>
</table>

There was a significant difference (P<0.01) in USPH index between the raw and homogenized milk. However, there was no significant difference (p>0.05) in the USPH index estimated at different time intervals of homogenization. The average least square means of USPH index estimated was 79.37 % for the raw milk; and 5.54 % (1 h) and 5.42 % (3 h) for the homogenized milk. The means USPH index decreased slightly with increasing time of operation for the homogenized milk. The slow decreasing trend in the USPH index with increasing time of operation was difficult to explain, however, a slight variation might have occurred during sampling or removing the cream layer from the top surface using pipette. Trout (1950) reported that in drawing off of the top portions it was impossible to get all the cream, a variable portion always remain because the tip of the pipette would be held at varying distances below the surface of the liquid.

Table 2 Least square means and standard error of USPH index for raw and homogenized milk.

<table>
<thead>
<tr>
<th>Homogenizer</th>
<th>Homogenization Time (h)</th>
<th>N</th>
<th>0</th>
<th>N</th>
<th>1</th>
<th>N</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>79.38 ± 0.58</td>
<td>14</td>
<td>5.35 ± 0.58</td>
<td>13</td>
<td>5.33 ± 0.58</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>10</td>
<td>79.36 ± 0.60</td>
<td>10</td>
<td>5.82 ± 0.60</td>
<td>10</td>
<td>5.53 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>79.37 ± 0.43</td>
<td></td>
<td>5.54 ± 0.43</td>
<td></td>
<td>5.42 ± 0.44</td>
</tr>
</tbody>
</table>

ab means within the same row are significantly different (p<0.01).

This differences in the USPH index between raw and homogenized milk could be attributed in part by an increase in the specific gravity of fat globules resulted by adsorption of casein particles in the newly formed fat globule surface. Trout (1950) reported that in non-homogenized milk about 2% of the casein was observed on the surface of fat globule; whereas in homogenized milk 25% of casein was found adsorbed on the surface of fat globules. Gravity creaming or skimming of homogenized milk was reported questionable (Trout, 1950). However, in this experiment gravity separation of fat globules was evident even in the homogenized milk. The findings could be substantiated by increasing fat content at the top surface (1/10 of the milk volume) of the container after 48 h of quiescent holding at 5 °C. Trout (1950) reported that the MFGs size in homogenized milk should be less than 2 μm to prevent creaming. In this study, substantial number of fat globules greater than 2 μm was observed after homogenization. The rising of these large fat globules individually could have triggered the gravity separation of fat globules in homogenized milk.

The means of USPH index for both homogenizers used in this study falls within the acceptable range value in between 1 to 10 (Tetra Pak, 1995). It was reported that a value of homogenization efficiency below 10 % indicates that homogenizer is very efficient (Ertugay et al., 2004). Therefore, the study concluded that both homogenizers were found equally efficient and it is functioning well.

Effects of homogenization on milk fat globules

Table 3 illustrates effects of homogenization on different parameters of milk fat globules, i.e. fat globule size, volume, surface area, total surface area and number of fat globules. A significant difference (p<0.01) was observed between the raw and homogenized milk in all the parameters studied with exception to fat content. However, there was no significant difference (p>0.05) between the milk homogenized at the different time intervals. This could be due to the same pressure (2500psi) used in the homogenization of milk. The greater the homogenization pressure used, the smaller would be the size of the fat globules (Tetra Pak, 1995).
A particles size of fat globules ranging from 0.1 – 10.10 μm with an average diameter of 3.37 μm was observed in this study for the bulk raw milk. Whereas, the mean fat globule size after homogenization was found to be 0.98 μm (1 h) and 0.96 μm (3 h) with particle size ranging in between 0.1 to 2.37 μm. In average, the raw milk fat globules size was reduced below 1μm, a decrease by about 3.44 (1h) and 3.51 (3h) times after homogenization. The result on the reduction of size after homogenization agrees with the previous report. Tetra Pak (1995) reported that the fat globules should be reduced to approximately 1 μm in diameter to stabilize the fat emulsion against gravity separation. Warner (1976) reported that the fat globules size in milk homogenized would a decrease by about 3.44 (1h) and 3.51 (3h) times after homogenization. Warner (1976) reported an increase in number of fat globules by about three folds after homogenization (Trout, 1950). An increase in total surface area after homogenization could be explained by increased in number of fat globules. Average total number estimated was about 1.96 x 10^6 fat globules per gram for the raw bulk milk. Whereas a total number of 8.27 x 10^10 (1h) and 8.83 x

Table 3 Least square means and standard error of different parameters for raw and homogenized milk fat globules.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time (h)</th>
<th>Homogenizers</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat content (%)</td>
<td>N</td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>0 6</td>
<td>3.66 ± 0.03</td>
<td>5</td>
<td>4.02 ± 0.11</td>
</tr>
<tr>
<td>1 6</td>
<td>3.74 ± 0.03</td>
<td>5</td>
<td>4.04 ± 0.11</td>
</tr>
<tr>
<td>3 6</td>
<td>3.76 ± 0.03</td>
<td>5</td>
<td>4.04 ± 0.11</td>
</tr>
<tr>
<td>Mean diameter (μm)</td>
<td>N</td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>0 6</td>
<td>3.39 ± 0.04</td>
<td>5</td>
<td>3.35 ± 0.08</td>
</tr>
<tr>
<td>1 6</td>
<td>0.99 ± 0.04</td>
<td>5</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>3 6</td>
<td>0.99 ± 0.04</td>
<td>5</td>
<td>0.93 ± 0.08</td>
</tr>
<tr>
<td>Surface Area (μm^2)</td>
<td>N</td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>0 6</td>
<td>36.22 ± 0.79</td>
<td>5</td>
<td>35.45 ± 1.70</td>
</tr>
<tr>
<td>1 6</td>
<td>3.12 ± 0.79</td>
<td>5</td>
<td>2.92 ± 1.70</td>
</tr>
<tr>
<td>3 6</td>
<td>3.07 ± 0.79</td>
<td>5</td>
<td>2.72 ± 1.70</td>
</tr>
<tr>
<td>Volume (μm^3)</td>
<td>N</td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>0 6</td>
<td>20.55 ± 0.66</td>
<td>5</td>
<td>20.05 ± 1.49</td>
</tr>
<tr>
<td>1 6</td>
<td>0.52 ± 0.66</td>
<td>5</td>
<td>0.47 ± 1.49</td>
</tr>
<tr>
<td>3 6</td>
<td>0.51 ± 0.66</td>
<td>5</td>
<td>0.43 ± 1.49</td>
</tr>
<tr>
<td>No. of fat globules/g</td>
<td>N</td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>0 6</td>
<td>1.86 x 10^8</td>
<td>5</td>
<td>2.09 x 10^8</td>
</tr>
<tr>
<td>1 6</td>
<td>7.49 x 10^10</td>
<td>5</td>
<td>8.96 x 10^10</td>
</tr>
<tr>
<td>3 6</td>
<td>7.68 x 10^10</td>
<td>5</td>
<td>9.79 x 10^10</td>
</tr>
<tr>
<td>Total Surface area/g (cm^2)</td>
<td>N</td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td>0 6</td>
<td>6721 ± 807.34</td>
<td>5</td>
<td>7405 ± 1749</td>
</tr>
<tr>
<td>1 6</td>
<td>23380 ± 807.34</td>
<td>5</td>
<td>26151 ± 1749</td>
</tr>
<tr>
<td>3 6</td>
<td>23582 ± 807.34</td>
<td>5</td>
<td>26626 ± 1749</td>
</tr>
</tbody>
</table>

Means within the same column against respective parameter are significantly different (p<0.01).

Note: Total no. of globules per g = fat content x 10^{12} \mu m^3 / density of fat x 100 x 1.032 \times volme of fat (\mu m^3)

The decrease in raw milk fat globules size after homogenization was accompanied by a significant increase (p<0.01) in the number of fat globules. Warner (1976) reported an increase in number of fat globules by about three times. There was a significant increase in the total surface area of fat globules after homogenization. However, a significant difference (p>0.05) was not observed in the total surface area among the milk homogenized at different time intervals. The mean total surface area of fat globules was about 7063 cm^2 per g of bulk raw milk. This finding was close to Attiae and Richter (2000), who estimated the total surface area of the bovine milk to be 17,117 cm^2/ml. It was low as compared to the findings of Warner (1976), who estimated a total surface area of bovine milk to be about 17,500 cm^2 per ml of milk. Whereas, total surface area for the milk homogenized at different times was estimated about 24,810 cm^2 (1 h) and 25,246 cm^2 (3 h) per g in this study, an average increased in the total surface area by about 4 times as compared to the raw milk (Table 30). This finding was close to Tetra Pak (1995); the fat/plasma interfacial surface area would increase by about four to six folds. The total surface area was reported to increase by 6 or 8 folds after homogenization (Trout, 1950). An increase in total surface area after homogenization could be explained by increased in number of fat globules. Average total number estimated was about 1.96 x 10^6 fat globules per gram for the raw bulk milk. Whereas a total number of 8.27 x 10^10 (1h) and 8.83 x
10^{10} (3h) fat globules per g was estimated after homogenization. The number of fat globules was reported to increase by many folds after homogenization. On the average, each fat globule in normal milk was reported to divide into approximately 1,200 smaller globules after homogenization (Trout, 1950). In this study, after homogenization the number of fat globules estimated increased by about 47 times, which means each fat globule was divided into approximately 43 smaller fat globules after homogenization. However, the extent of the increase in numbers resulting from the homogenization is seldom accurately determined mainly because it would depend on many factors, i.e. the size of the globules existing in the original milk and by the extent of their division. The fat globules size in homogenized milk would depend primarily upon the homogenizing pressure used and on other factors, such as condition of the valves and temperature of the milk (Trout, 1950). Since, it was derived by applying the mathematical formula; it would depend upon the respective radii of the fat globules. Thus, the result reported might greatly differ. It should also be noted that counting of fat globules required great skill and experiences in microscopic examination (Trout, 1950).

Particle distributions

Figure 1 illustrates particles size distribution of raw and homogenized milk fat globules. In this study, about 70 % of the fat globules were less than 3 μm in diameters, with fat globules as large as 12μm in diameter in raw milk was observed. Whereas, about 75 % of fat globules were ≤ 1 μm in diameter, with maximum fat globules size of about 2.37 μm in diameter was recorded in homogenized milk. In this study, almost above 90 % of the fat globules size observed was below 2 μm in diameter after homogenization of milk. Trout (1950) reported that the efficiency of homogenizer is considered good, when 90 % of the fat globules observed under a microscope were less than 2 μm in diameter. With this findings on fat particle distribution and using it as an indicator the homogenizers used in this study could be considered equally efficient. A significant decrease (p<0.01) in unit surface area and volume of fat globules was observed after homogenization.

![Fat globules size distributions curve of raw and homogenized milk.](image)

Conclusions

Homogenization greatly increases the number of fat globules and the total globule surface area; and reduces the average volume of fat per globule. It reduces the fat globules size by many-folds and renders even distribution of smaller fat globules drastically reducing the gravity separation. The gravity separation does occur even after the homogenization of milk but at a slower rate, and proper understanding of this gravity separation characteristic of modified fat globules can be used as a simple tool or method in any dairy plants in assessing efficiency of homogenizer and also in controlling the quality of homogenized milk. Considering the USPH index and the fat particle distribution determined in this study and using it as an indicator for the assessment, both homogenizers used in the dairy plant was found equally efficient.

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References


