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Measurement of homogenisation efficiency of milk by laser diffraction and centrifugation

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ABSTRACT

For ultra high temperature (UHT) milk, the development of a cream layer is a shelf life limiting factor that can be controlled by homogenising the milk correctly. This article suggests a method to measure homogenisation efficiency using laser diffraction. Milk was diluted with a protein cluster dissolving solution before measurement with a Malvern Mastersizer 3000. To avoid multiple scattering obscuration needed to be 1.5–3.5%. The D[5;3] (μm) value extracted from the particle size distribution could, with the equation developed, be directly correlated to a homogenisation efficiency (NIZO value) obtained by centrifugation of the milk sample. The equation presented is only valid for monomodal distributions and analysis should be performed on freshly produced milk.

1. Introduction

For milk, and ultra high temperature (UHT) milk in particular, creaming is often a shelf life limiting factor. The formation of a cream layer can be described according to Stokes law, where fat globule size, the density difference between fat globules and milk serum and the milk serum viscosity are input parameters (Mulder & Walstra, 1974). From a shelf life perspective, fat globule size, storage time, storage temperature, package size and shape as well as raw milk quality will influence the quantitative amount of creaming but also the visual appearance of the cream layer. Homogenisation is used to decrease the fat globule sizes and thereby prolonging the time it takes before a cream layer is formed. In a homogeniser, milk is forced by high pressure through a narrow gap (Tetra Pak, 2015). The fat globules get elongated and when leaving the gap will be broken up into smaller droplets (Håkansson et al., 2011). The geometry of the gap and the pressure applied will influence the droplet sizes. Higher pressure will give smaller fat globules. It is, however, not only the homogenisation pressure that influences the fat globule size. Other factors such as homogenisation temperature (Goulden & Phipps, 1964; Kurzhals, 1977), fat content (Kurzhals, 1977), and type of homogenisation device (Kurzhals, 1977), are also important. The placement of homogeniser (upstream or downstream of final heat treatment) and type of heat treatment (indirect or direct steam injection) seems to be of minor importance regarding the fat globule size (Hillbrick, McMahon, & McManus, 1999).

Homogenisation efficiency can be measured in several ways and a summary of different methods has been done by Ridgway (1957). In the industry centrifugal methods are still common but the use of laser diffraction for fat globule size determination is increasing.

In centrifugation methods the natural creaming is accelerated using a centrifugal force. Centrifugation methods are cheap and easy to perform but they are labour intense (about 1 h is needed to get a result), and inaccuracies easily arise due to manual work. Many different types of centrifuges and centrifugation tubes exist and to be able to compare results the same equipment and settings must be used. A centrifugation method used in the industry is the "NIZO Centrifugation" method. Milk is filled into special homogenisation pipettes and centrifuged. Homogenisation efficiency is calculated according to equation (1), the higher the value (max 100%) the slighter the creaming (Tetra Pak, 2015). Several variations of this method exist (Kurzhals, 1977; VDLUFA, 2000).

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$$Homogenisation\ \ efficiency\ (\%) = \frac{fat\ content\ in\ bottom\ 20\ mL\ of\ centrifuged\ sample}{fat\ content\ in\ non\ centrifuged\ sample} * 100 \quad (1)$$

Laser diffraction particle size analysers can be used for analysis of milk fat globule size distributions. All particles scatter light, larger particles in narrow angles and small in wide. Together with sensitive detectors and advanced computer models utilising the Mie theory about particle scattering a particle size distribution (PSD) can be calculated (ISO, 2009). It is a fast method and a result is obtained within 10 min.

When performing a PSD measurement there are several instrumental settings to consider. Some parameters, like measurement temperature, obscuration and reactant solution cannot be changed after measurement and must be set and verified prior to analysis, whereas software parameters (refractive index and absorption coefficient) can be also changed after measurement. The measurement temperature will affect the crystallinity of the fat in the milk fat globules, which affects the absorption coefficient; however, the refractive properties remain unchanged (Michalski, Michel, & Briard, 2001). Obscuration, or amount of added sample to the dispersion unit, should be low enough to avoid multiple scattering (light reflected from one particle bounces on other particles and is detected a second time or more by other detectors and results in a shift towards smaller particles), but high enough for noise not to interfere (Malvern, 2013).

Milk contains both casein micelles, fat globules, and fat/protein aggregates that will scatter light. When measuring homogenisation efficiency, it is the size of single fat globules that are of interest. Prior to analysis the milk sample should be mixed with a reactant solution, the purpose of which is to dissociate casein micelles and potential protein aggregates as well as disintegrate potential fat globule aggregates. In literature there is reported use of several different reactant solutions (McCrae & Lepoetre, 1996; Nowak, Kiełczewska, Murach, & Dabrowska, 2017; Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003). When choosing one it is important that it contains substances not only to dissociate casein micelles but also an emulsifier that prevents fat globules from clumping. As casein dissociating substances EDTA or trisodium citrate have been reported; as emulsifier SDS or tween have been reported (Haugaard & Pettinati, 1959; Huppertz, Fox, & Kelly, 2003; Michalski et al., 2001). The refractive index and absorption coefficient of milk fat has been thoroughly studied and the use in PSD measurements is well described by Michalski et al. (2001), who found that the refractive index did not change upon homogenisation of the milk nor by washing the fat globules to remove attached casein micelles.

From a particle size distribution several values can be extracted. There is no standard set in the industry of what to use, but D[4;3] (volume moment mean), D[3;2] (surface area moment mean), Dv10, Dv50 and Dv90 (volume percentiles whereof 10, 50 and 90% of the volume is smaller than) are frequently used. Walstra and Oortwijn (1975) showed that for calculations of cream layer formation in milk the volume proportion of fat that has reached the cream layer after a certain time is proportional to the D[5;3] diameter derived from a particle size distribution. The equation applies as long as the D[5;3] value remains unaltered, i.e., until the largest globules have reached the cream layer. D[5;3] is shown in equation (2), N_i being the number of globules and D_i the diameter:

$$D[5;3](\mu m) = \left(\frac{\sum_1^n N_i D_i^5}{\sum_1^n N_i D_i^3}\right)^{0.5} \quad (2)$$

The aim of this study is to define a method for determining homogenisation efficiency from fat globule size distributions measured by laser diffraction that are correlated with current, commonly used centrifugal methods. The advantage with particle size distribution determination compared with centrifugal methods will be a more defined method as well as a faster and more repetitive method.

2. Material and methods

2.1. Milk processing

Milk was processed at Tetra Pak, Lund, Sweden in a Tetra Therm® Aseptic Pilot (300 L h^{-1}) connected to a Tetra Alex® 150 homogeniser. Milk was obtained from the local dairy. For UHT milk pre-pasteurised and homogenised milk standardised to either 1.5 or 3% fat was used (i.e., final UHT milk had been homogenised twice). For pasteurised and ESL milk samples pre-pasteurised skim milk and unhomogenised cream (40% fat) was mixed to either 1.5% or 3% fat. The milk was not protein standardised and protein content was consistent around 3.5%. Different heat treatment with upstream or downstream homogenisation was investigated, homogenisation

Table 1
Heat treatment, homogenisation and number of samples analysed by centrifugation and particle size distribution.

Heat treatment	Homogeniser placement	Fat content (%)	Number of samples
Indirect ESL 125 °C/2s	Upstream	3	1
Direct steam injection ESL 127 °C/2s	Downstream	1.5	3
		3	3
Direct steam injection ESL 138 °C/4s	Downstream	3	4
Indirect UHT 137 °C/4s	Upstream	1.5	2
		3	17
Indirect UHT 137 °C/4s	Downstream	3	4
Direct steam injection UHT 140 °C/4s	Downstream	1.5	2
		3	5
Commercial samples – exact heat treatment unknown	Unknown		
Pasteurised		3	1
ESL		3	1
UHT		3.5	5

pressures ranging between 9 and 40 MPa. Milk was filled in 250 mL aseptic Tetra Brik packages. Additionally, some commercial samples of milk were analysed, in total 48 samples (Table 1).

2.2. Homogenisation efficiency - centrifugation

For centrifugation a Funke Gerber Nova Safety centrifuge was used. Twenty-five millilitres of milk were filled in special homogenisation pipettes (Funke Gerber 3639). They were heated to 40 °C in a water bath and transferred to the centrifuge. The centrifuge was run at a fixed speed of 350×*g*. It could not heat a sample for more than 12 min; therefore, centrifugation took place three consecutive times. The first two times for 10 min and the third time for 10.5 min. The extra 0.5 min was added to compensate for the time it took for the centrifuge to reach full speed. A centrifugation temperature of 40 °C was chosen as a higher temperature equals more fat separation due to lower viscosity that will yield in a better resolution for highly homogenised samples. Furthermore, most centrifuges generate some heat during centrifugation, therefore centrifugation at room temperature will cause unstable temperature conditions. The bottom 20 mL of the centrifuged pipettes were emptied out in a container and the fat content of the milk was measured in a MilkoScan™ FT120 (Fourier-transform infrared spectroscopy; FOSS, Hillerød, Denmark) on the milk channel. The homogenisation efficiency was calculated according to eq. (1).

2.3. Particle size distribution

Particle size distribution measurements was conducted with a Malvern Master Sizer 3000. Spherical particles were assumed and the refractive index for milk fat was set to 1.46 for the red laser (632.8 nm) and 1.47 for the blue laser (470 nm). The refractive index of water for the red laser was set to 1.33. Dispersant (water) temperature was 20 °C ± 2 °C. The temperature of 20 °C was chosen because it is a standard indoor temperature in many countries thus any temperature gradients in the media and any degassing causing bubbles will be avoided. Michalski et al. (2001) suggested to use absorption index of melted fat [0.5×10^{-5} (red laser) and 1.7×10^{-5} (blue laser)] and heating the sample to 65 °C followed by promptly measurement at 20 °C. This has been evaluated together with an absorption index of 0.001 without any pre-heating. The Malvern Mastersizer 3000 "general purpose" analysis model based on volume distributions was used to evaluate data. Laser obscurations in the range of 1%–8% was investigated.

Before analysis 2 mL milk sample was mixed with 2 mL water and 2 mL of a casein micelle dissociating agent (solA) and let to stand for 5 min. SolA was 6.25 g Tween 20 and 18.75 g EDTA added to 400 mL deionised water, heated while stirring to 40 °C, pH adjusted to pH 10 with 0.1 M NaOH. The procedure for solA preparation was adapted from Haugaard and Pettinati (1959) with modifications in concentration. Samples were also analysed without solA added.

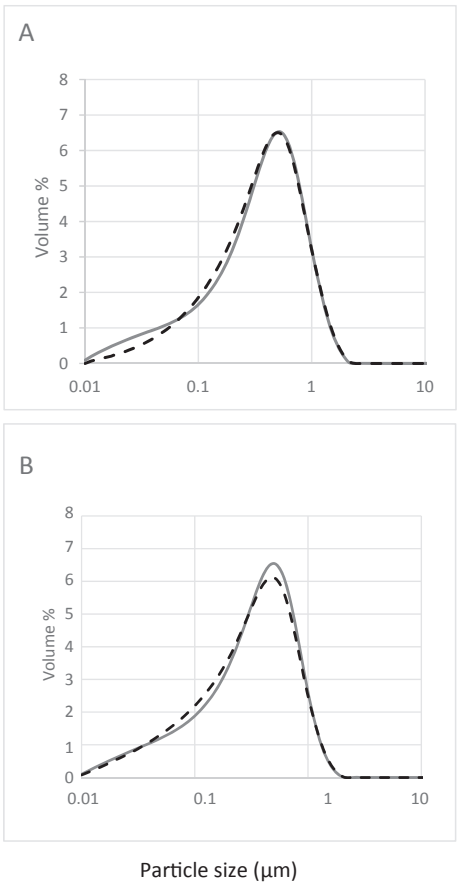

Fig. 1. Effect of absorption index and pre-heating to 65 °C on particle size distribution for (A) a commercial pasteurised milk and (B) a commercial UHT milk (——, AI = 0.001, T = 20 °C; – – – -, AI = 0.5×10^{-5} and 1.7×10^{-5}, T = 65 °C).

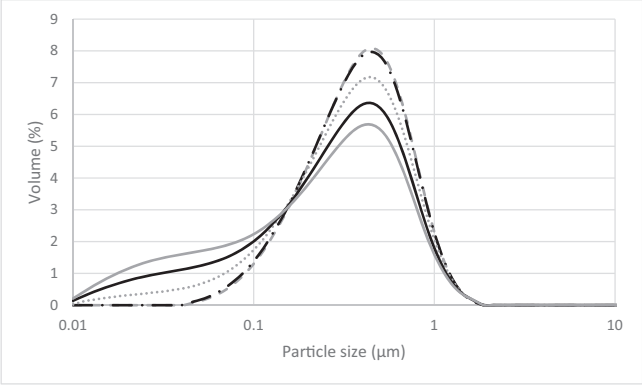

Fig. 2. Effect of varying sample obscuration on the shape of the particle size distribution: – – – -, 1%; — · -, 2%; ·····, 4%; ——, 6%; ——, 8%.

3. Results and discussion

3.1. Optimising laser diffraction measurement

Pre-heating of the milk to 65 °C and measurement with AI of melted fat showed minor differences compared with no pre-heating and AI of 0.001, Fig. 1 shows the example of one commercial pasteurised milk and one commercial UHT milk, both 1.5% fat. To keep the method as fast and simple as possible it was therefore decided to use AI 0.001 and exclude the pre-heating.

When measuring a particle size distribution with laser diffraction it is of high importance to adjust the obscuration to avoid

multiple scattering as this will result in false small particles. From Fig. 2 one can conclude that an obscuration of 4% or higher shifts the PSD towards the left indicating multiple scattering. For low obscuration noise may start to interfere. Based on this data it was concluded that the optimal obscuration was between 1.5 and 3.5%.

In addition, as mentioned in section 1 it is of importance to only measure the fat globules and not the proteins. This can be assured by addition of solA. In Fig. 3A particle size distributions with and without solA added to UHT milk heat treated with direct steam injection are compared. In direct UHT milk large casein protein aggregates exist (Malmgren, 2007), and they will be present in the particle size distribution if solA is not added. Fig. 3B compares particle size distributions for indirect extended shelf life (ESL) milk with and without solA added for two different milk fat contents, 1.5 and 3%. In milk with different fat content the volume ratio between protein and fat differs (3.4:3 and 3.4:1.5). This becomes visible in the PSD if solA is not added as a difference in heights of the two peaks in the bimodal distribution, the first peak showing the casein micelles and the second the fat globules; however, the two distributions intersect due to some overlapping sizes. By addition of solA this difference disappears.

Based on its mathematical correlation to creaming the D[5;3] value was chosen to be the output parameter from the PSD. From

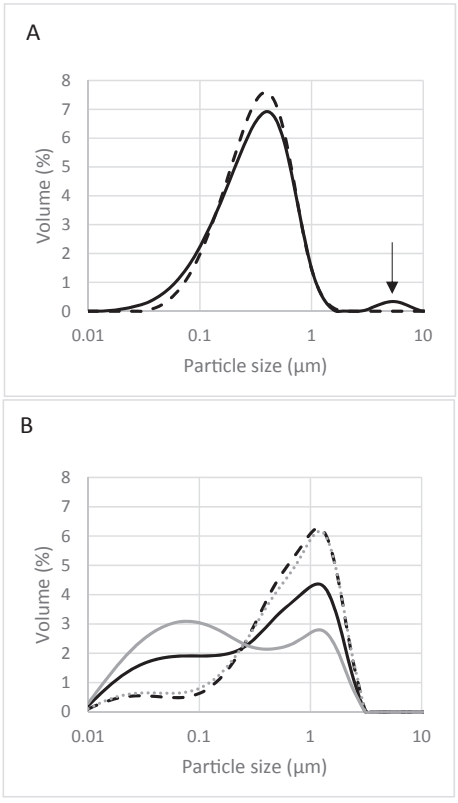

Fig. 3. Influence of addition of protein dissolving solution (solA): (A) direct UHT milk (——, 3% fat; – – – -, 3% fat + solA) with downstream homogeniser 20 MPa, the arrow shows the protein aggregates; (B) indirect ESL milk (——, 1.5% fat; ·····, 1.5% fat + solA; ——, 3% fat; – – – -, 3% fat + solA) with upstream homogeniser 11 MPa.

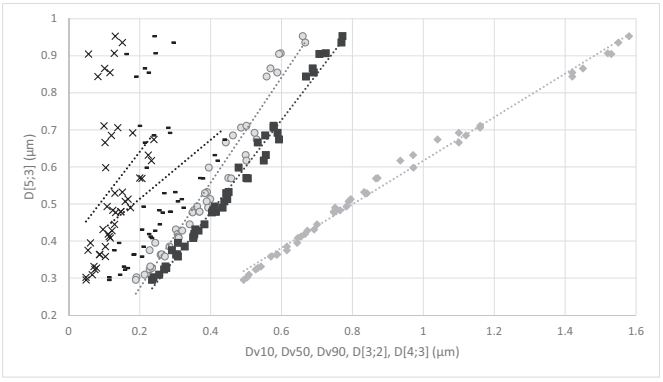

Fig. 4. Correlations between different particle size distribution parameters (×, Dv10; ●, Dv50; ◆, Dv90; –, D[3;2]; ■, D[4;3]) and D[5;3]; dotted lines show linear regressions.

Table 2
Fit between D[5;3] and other particle size distribution data.[a]

Parameter	Linear regression	R^2
Dv90	D[5;3] = 0.5881Dv90 + 0.0288	0.9928
Dv50	D[5;3] = 1.4213Dv50−0.0107	0.9534
Dv10	–	0.0987
D[3;2]	–	0.1084
D[4;3]	D[5;3] = 1.2464D[4;3]−0.0208	0.9866

[a] For Dv10 and D[3;2] linear regression equations are omitted out due to the bad R^2 value.

Fig. 4 it can however be shown that the use of D[4;3], Dv90 or Dv50 would also be possible, since a linear correlation with good coefficients of determination (R^2 values) exists; however, Dv10 and D[3;2] had bad correlations (Table 2).

3.2. Correlation between particle size distribution and homogenisation efficiency obtained by centrifugation

With obscuration 1.5–3.5% and solA added to the milk prior to the measurement, the D[5;3] values from PSD measurements could be plotted against the homogenisation efficiency obtained from centrifugation, Fig. 5. An equation correlating D[5;3] to homogenisation efficiency was developed (Equation (3)) and is referred to as homogenisation efficiency D[5;3] (HEff. D[5;3]). The equation correlates well to the measured data, root mean square (RMS) of the deviation was 1.24 (n = 48).

$$HEff.(D[5;3]) = 100 * exp^{-\frac{\left|D[5;3]-0.0802\right|^{2.0669}}{1.6309}} \quad (3)$$

The equation is valid for correlating the above specified methods. If any measurement conditions change, so will the constants in the equation. The equation is only valid for plain milk, any addition of a stabiliser which will change the viscosity of the milk will result in different results. The equation is only valid for monomodal distributions, if for some reason the PSD with addition of solA shows several peaks or has a non-Gaussian shape it will not apply. Furthermore, the analyses should be performed on newly produced milk, if any creaming process has started this can result in non-representative sampling.

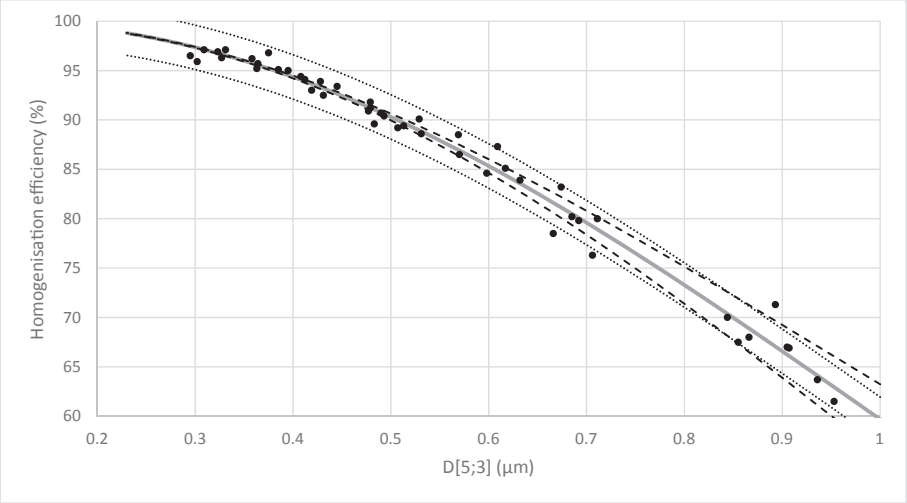

Fig. 5. Correlation between the D[5;3] value obtained from the particle size distribution and homogenisation efficiency measured by centrifugation (●). The solid line (——) shows homogenisation efficiency (D[5;3]) calculated using of Eq. (3); the dotted lines (·····) are 3 stdev for NIZO centrifugation and the dashed lines (– – –) are 3 stdev for D[5;3] measurement.

4. Conclusions

The proposed equation to calculate a homogenisation efficiency D[5;3] value based on the results from a particle size distribution measurement from Malvern Mastersizer 3000 correlates well with the in the industry used homogenisation efficiency (NIZO centrifugation) value. When measuring the particle size distribution, a protein dissolving solution must be added, and care should be taken to add the correct amount of sample. It should be noted that changing any parameter in the measurement methods will give different results.

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