Estimation of milk fat globule distribution in milk using scattering in the near infrared region

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Introduction

Leewenhoek in 1674 discovered milk fat globules by using a microscope to observe the diffraction of light caused by refractive index differences between fat and serum. The first systematic studies of the dimensions of milk fat globules and the technological significance of this size distribution were conducted by Walstra.[1] Milk fat globule distribution depends on cattle breed,[2] milking period and on the fat production of the individual cow.[3] When milk fat concentration increases an increase of the large globule fraction and stearic, palmitic, linoleic and oleic acid content in the backbone of triglycerides may be observed. At the same time, the enzymatic activity associated with the fat globule membrane decreases. Milk with bigger fat globules has faster creaming and better skimming properties. The cream obtained from this milk is better for whipping, but gives rise to butter with lower water content, bigger fat crystals and is less spreadable.[4] Fat globule dimension distribution affects physico-chemical, functional and sensory characteristics of cheeses. Using microfiltration techniques it is possible to standardise milk fat globule size. Recent studies of Camembert and Emmental manufacturing using milk either enriched in big (average diameter 6 µm) or small (average diameter 3 µm) fat globules highlighted effects on syneresis, proteolysis and rheology of cheeses.[5,6] In milk pasteurised products and longlife milk the dimension of homogenised globules plays a pivotal role in emulsion stability and palatability of the final product.[7]

Despite the importance of knowing fat globule size distribution in several technological dairy processes, laser diffractometers and other instrumentation for particle size analysis are often not available in dairy laboratories and therefore this information is not easily attainable. Near infrared (NIR) instrumentation is largely available in dairy laboratories. The interaction between electromagnetic radiation in the near infrared region and suspensions of small particles in continuous media, such as fat globules in milk, gives rise to scattering phenomena. The NIR spectrum of whole milk arises both from absorbance due to molecular vibrations and elastic scattering. The number of photons that deviate from straight trajectories is dependent on the wavelength and on the size of the scattering particles. Therefore, it is possible get information on particle size distribution from a NIR spectrum. The extraction of this information requires the development of a suitable physical model and is presented in this study..

Materials and Methods

Milk fat globule analyses were performed on raw milk of 50 individual cows (43 Friesian and 7 Jersey) from 6 different farms with different feeding types. The Friesian cows were descended from 6 paternal lines and the Jersey cows from 3. Milk samples were taken monthly. Sodium azide was added directly to the sampling tubes, samples were stored at 4°C and analysed within 3 days. The reference particle size analyses of fat globules were performed using a Mastersizer 2000 (Malvern instruments Ltd., Malvern Worcestershire, UK) granulometer equipped with a single laser source at 633 nm. The instrument software was set to calculate particle size distribution according to Mie theory using the “general purpose model” for spherical particles. The working parameters were chosen in accordance with;[8] water was used as a dilution medium (~1:600). To avoid multiple scattering phenomena, refractive indices were set to 1.330 for water and 1.458 for milk fat; the absorption coefficient was measured for liquid fat and was set to 0.5×10^{-5}. In order to avoid fat crystallization, all the measurements were done at 40 ± 1°C. The effect of casein micelles was mitigated by the high dilution factor.

Spectral data were recorded using a Büchi NIRFlex N-500 (Büchi Italia, Assago, MI, Italy) in transmission mode and a quartz flux cuvette with a pathlength of 200 µm. During measurements, milk samples, either diluted or undiluted, were placed in 40 ml tubes, heated at 40 ± 1°C and fluxed through the cell using a peristaltic pump. Before processing, the spectrum of the flow cuvette filled with pure water was subtracted from each milk spectrum in order to obtain the signal due to scattering and milk constituents. The physical model was developed using both Matlab (The Mathworks, Natick, MA, USA) and Microsoft Visual Basic for Excel.

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Results and discussion

The NIR milk spectrum measured in transmission mode arises from both true absorptions due to constituents and from the varying intensity at different wavelengths of photon deviation due to scattering particles represented mainly by fat globules. Vibrational absorptions in the spectral region from 900 to 2500 nm are very small for all the milk constituents, except for the strong water absorptions. In the spectral regions void of water absorptions the extinction of radiation recorded by spectrometers is mainly due to scattering phenomena, which prevent photons from reaching the detector. To a first approximation, given the geometry of a transmittance measurement in a FT-NIR spectrometer it can be assumed that only the photons diffracted at a very small angle θ (θ→0) reach the detector. The photons that are deflected by a bigger angle from the straight trajectory do not reach the detector and give rise to an increase in optical density of the samples which is not dependent on true absorptions. Figure 1 depicts spectra of different milk samples showing different scattering behaviour due to either cow breed or fat concentration.

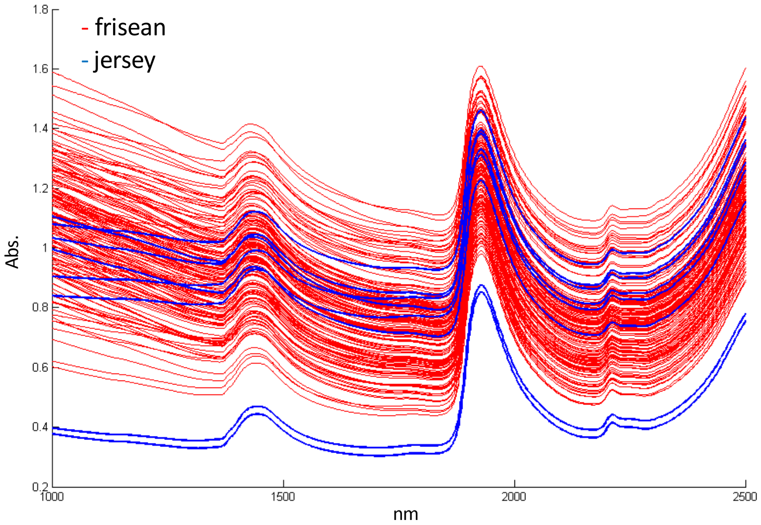

Figure 1. Transmission spectra of raw milk samples.

The specific extinction arising from scattering is distinguishable together with water and quartz cuvette bands. On the basis of these observations a model was developed which calculated, given the fat concentration, the optical density produced by milk fat globules. In the model it was assumed that n(d), the probability density function in number, follows a Weibull distribution:[9]

$$n(d) = \frac{\beta}{\eta}\left(\frac{d-d_0}{\eta}\right)^{\beta-1} e^{\left[-\left(\frac{d-d_0}{\eta}\right)^{\beta}\right]}$$

where β, the shape parameter, and η, the scale parameter, are the two coefficients which describe and define the particle distribution.

In a specific milk sample having an amount of fat equal to *G*, the volume of fat contributed by the globules with diameter between *d* and $d+\delta d$ per unit volume of milk is:

$$\frac{G\rho_{milk}}{100\rho_f}\, p(d)\delta d \quad [\mathrm{cm^3_{fat}/cm^3_{milk}}],$$

where:

d is the fat globule diameter [μm]

G is the weight % of fat in the milk [$\mathrm{g_{fat}.100\ g^{-1}_{milk}}$]

ρ_{milk} is the serum density [$\mathrm{g.cm^{-3}}$]

ρ_f is the fat density [$\mathrm{g.cm^{-3}}$].

Assuming as a first approximation that the fat globules are spherical and introducing the expression for the spherical volume, the following equation can be derived:

$$\frac{G\rho_{milk}}{100\rho_f \frac{\pi}{6}\left(\frac{d}{10^4}\right)^3}\, p(d)\delta d \quad [\text{number of particles}/\mathrm{cm^3_{milk}}]$$

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which describes the total number of fat particles having a diameter between d and $d+\delta d$ per unit volume of milk. It is also possible to introduce a new probability density function $n(d)$, such that $n(d)\cdot\delta d$ yields the fraction of the number of fat particles having a diameter between d and $d+\delta d$; the expression will be as follows:

$$\int_0^\infty \frac{N_{tot} 100\rho_f \frac{\pi}{6}\left(\frac{d}{10^4}\right)^3}{G\rho_{milk}} n(d)\delta d = \int_0^\infty p(d)\delta d = 1$$

This equation allows calculation of N_{tot}, the total number of particles, which otherwise cannot be measured experimentally:

$$N_{tot} = \frac{G\rho_{milk}}{100\rho_f \frac{\pi}{6}\left(\frac{1}{10^4}\right)^3 \int_0^\infty n(d)d^3\delta d} \quad \text{[number of particles/ cm}^3{}_{\text{milk}}\text{]}$$

Once N_{tot} is known, it is possible to introduce a new quantity $N(d)$, such that: $N(d)\delta d = N_{tot} n(d)\delta d$

This equation describes the amount of globules with diameter between d and $d+\delta d$ in number instead of in volume and yields the total number of fat particles with diameters between d and $d + \delta d$ per unit volume of milk. Making use of this equation, the extinction coefficient can be written as:

$$\tau(\lambda) = \int_0^\infty \mu_{scatt}(d,\lambda)\cdot N(d)\delta d \quad \text{[dimensionless]}$$

where $\mu_{scatt}(d,\lambda)$ is the scattering section. For the calculation of $\mu_{scatt}(d,\lambda)$ the Evans Fournier approximation of the Mie theory as reported by Kokhalnovsky and Zege[10] was adopted. The refractive index of water at each wavelength was calculated using the formula proposed by the International Association for the Properties of Water and Stems (IAPWS, 1997), and the fat refractive index was adopted from the equation proposed previously[9].

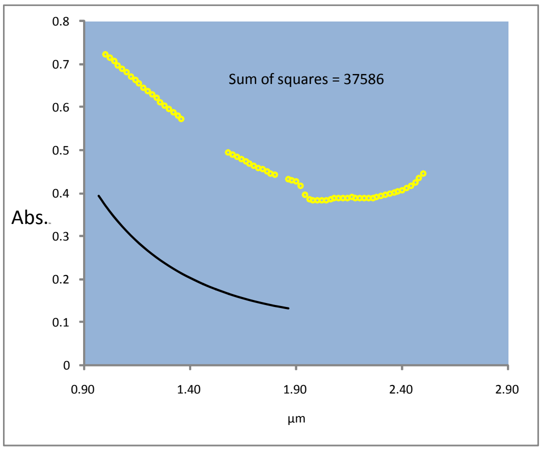

Figure 2. Yellow rings: measured milk absorbance after water subtraction; black line: model first estimate of scattering extinction. Optimisation regions 1000–1360 nm and1580–1800nm.

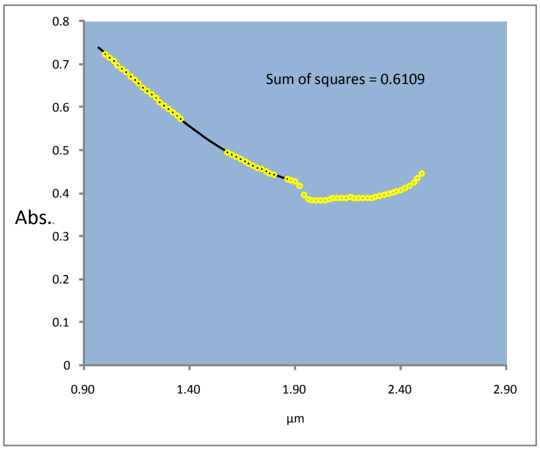

Figure 3. After optimisation of distribution parameters the differences between the measured and calculated spectra were reduced.

The problem of analytical inversion of the model to obtain particle size distribution from extinction measurements was solved using the generalised reduced gradient (GRG) method, a non-linear optimisation system. The model inversion was performed by minimising the sum of squared differences between the measured spectrum of each sample and the calculated one. Using the estimated values of β and η from the calculated spectrum it is possible to calculate other distribution parameters, such as Sauter mean diameter d(3,2) and span. The behaviour of the model optimisation is illustrated in Figures 2 and 3.

The optimal sample dilution rate to avoid multiple scattering events was identified by comparing the estimations obtained using the NIR model and the reference data obtained using the Mastersizer granulometer. A good correlation between laser diffraction measurements and NIR estimates was obtained using a dilution factor of 4. Determination coefficients of 0.914 and 0.942 were obtained for the median

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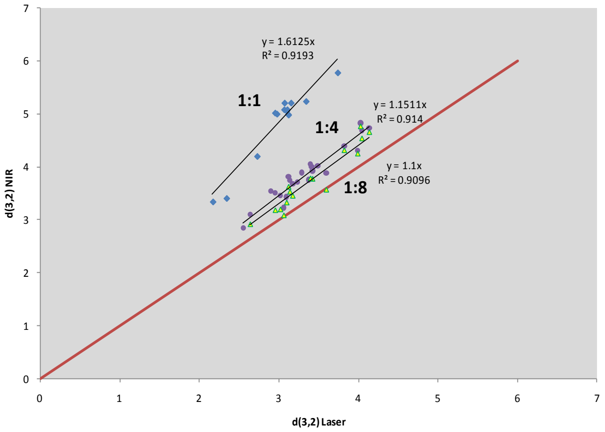
value of particle size distribution (on volume basis; Figure 4), and for distribution span measured as difference between 20th and 80th percentile (Figure 5), respectively. No further improvements were achieved by increasing the dilution factor.



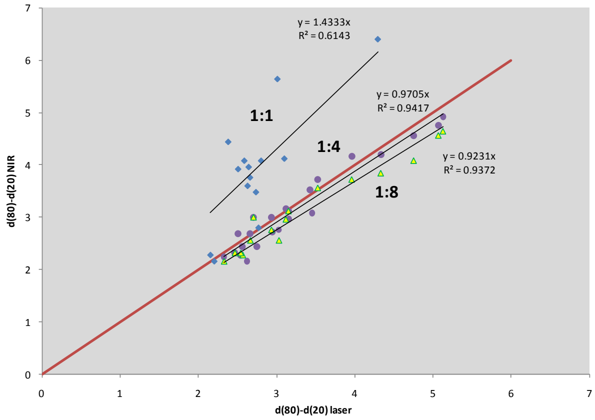


Figure 4. Correlation between Sauter mean diameter obtained using laser diffractometer and NIR scattering model at varying dilution rates (1:1 blue, 1:4 lilac, 1:8 green).

Figure 5. Correlation between fat globule size distribution span obtained using laser diffractometer and NIR scattering model at varying dilution rates (1:1 blue, 1:4 lilac, 1:8 green).

Using 1:4 dilution a set of 180 samples was analysed by FT-NIR and the reference diffractometry technique. The estimation of Sauter mean diameter d(3,2) had an R^2 of 0.94 and a SEP (standard error of prediction) of 0.120 μm (over a range from 2.22 to 5.34 μm; Figure 6); the R^2 for the distribution span was 0.88 (over a range from 0.79 to 1.70 μm) and the SEP was 0.185 μm.

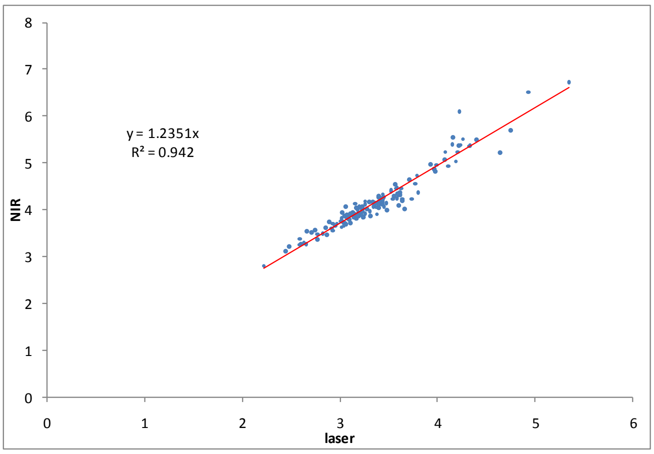


Figure 6. Correlation between d(3,2) estimates.

Conclusions

Although it is known that dairy composition can be monitored by NIR, the use of this technique for the study of fat globule distribution on the basis of scattering principles is new. The model developed here could be useful for the rapid evaluation of milk fat globule distribution in place of a dedicated instrument. Rapid measurement of milk fat globule size in dairy plant laboratories would be useful for better evaluation of the technological fate of milk. Small fat globules are richer in phospholipids and well-suited for new functional food development; big fat globules are better for whipping purposes. The method can be useful for selecting breeding bulls for milk fat globule size trait and for monitoring creaming processes.

Acknowledgements

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