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Light scattering and transmission measurement using digital imaging for online analysis of constituents in milk

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ABSTRACT

Milk is an emulsion of fat globules and casein micelles dispersed in an aqueous medium with dissolved lactose, whey proteins and minerals. Quantification of constituents in milk is important in various stages of the dairy supply chain for proper process control and quality assurance. In field-level applications, spectrophotometric analysis is an economical option due to the low-cost of silicon photodetectors, sensitive to UV/Vis radiation with wavelengths between 300 - 1100 nm. Both absorption and scattering are witnessed as incident UV/Vis radiation interacts with dissolved and dispersed constituents in milk. These effects can in turn be used to characterize the chemical and physical composition of a milk sample. However, in order to simplify analysis, most existing instrument require dilution of samples to avoid effects of multiple scattering. The sample preparation steps are usually expensive, prone to human errors and unsuitable for field-level and online analysis. This paper introduces a novel digital imaging based method of online spectrophotometric measurements on raw milk without any sample preparation. Multiple LEDs of different emission spectra are used as discrete light sources and a digital CMOS camera is used as an image sensor. The extinction characteristic of samples is derived from captured images. The dependence of multiple scattering on power of incident radiation is exploited to quantify scattering. The method has been validated with experiments for response with varying fat concentrations and fat globule sizes. Despite of the presence of multiple scattering, the method is able to unequivocally quantify extinction of incident radiation and relate it to the fat concentrations and globule sizes of samples.

Keywords: spectrophotometry, turbidimetry, scattering, digital imaging, milk

1. INTRODUCTION

Milk is an emulsion of fat globules and casein micelles dispersed in an aqueous medium with dissolved lactose, whey proteins and minerals. Quantitative analysis of these constituents is important in various stages of the dairy supply chain for proper process control and quality assurance. Analysis of raw milk is especially important in the early stages of production for elimination of abnormal milk prior to pooling, and for proper management of cattle feed and health. Online and non-destructive instruments for raw milk analysis are particularly useful in automatic milking systems in dairy farms for continuous measurements during production. However, existing such instruments usually provide poor sensitivity, accuracy and precision[1] compared to their traditional counterparts. The presence of multiple analytes, in different physical forms, and varying concentrations significantly complicates analysis. Fat globules in raw milk have an average spherical diameter ranging from 3 - 10 μm, which in processed milk is reduced to below 2 μm by homogenization. Casein micelles have an average spherical diameter ranging from 50 - 500 nm. Current laboratory instruments like FTIR spectrometers hence require homogenization and dilution of samples before analysis. They are sensitive to the sample preparation process, and dependent significantly on operator skill or expensive sample preparation systems. Present analysis techniques therefore cannot be directly adapted to online analysis to fit within the functional and economic requirements for field-level instrumentation.

Recent works[2-7] on developing new methods for analysis of milk have shown an increasing interest in optical measurements using UV/Vis spectrophotometry for estimating fat, protein and lactose concentrations. UV/Vis spectrum is especially attractive for field-level instrumentation because of the low cost of silicon photodetectors sensitive to wavelengths between 300 - 1100 nm. In spectrophotometry, interaction of UV/Vis radiation with analytes present in a sample is measured and related to the physiochemical characteristics of the analytes, in order to quantify their respective

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concentrations and physical forms. Radiation in the UV/Vis spectrum incident on a sample of milk attenuates as it interacts with the dispersed phase, the aqueous medium and the solutes dissolved in the aqueous medium. This attenuation or extinction is a combined effect of the absorption and scattering characteristics of the multiple interacting substances. With traditional spectrophotometric instruments, only pure absorption of incident radiation is measured, while scattering is intentionally suppressed or purged from observations. Samples are usually diluted and homogenized to be able to ignore multiple scattering events. Further, scatter correction methods like standard normal variate transformation are employed to process data before chemometric regression methods are used for calibration and analysis. Such instruments usually require sample pretreatment that is not practical in an online instrument, especially when continuous monitoring is desired, or sample wastage is unacceptable. Further, results from absorption based UV/Vis spectrophotometry have been found to be less precise compared to those from NIR spectroscopy[4]. However, the results have been shown to improve when scatter correction methods are not employed in analysis[5]. Reflectance measurements, indicative largely of the scattering characteristic, have also been shown to give more precise results[4] than pure absorption measurements.

Particle size variations in raw milk samples, and the polydisperse nature of milk as an emulsion reinforce the importance of scattering measurements for unequivocal quantification of constituents, especially fat and casein protein. Scattering measurements are traditionally done using turbidimeters and nephelometers to estimate the fat content in milk. Turbidimetry, in other applications, is used as a method for particle size analysis in colloids and suspensions[8]. Typically, samples of milk are diluted and homogenized, and mixed with a chelating agent like Na_4EDTA to isolate fat globules in the dispersed phase[9]. Radiant power along or at an angle to the incident beam of light is measured as it exits the sample. Depending on the average globule size relative to the wavelength, Rayleigh or Mie light scattering theories are used to relate observations with the globule size distribution and volume fraction, assuming spherical isotropic globules and absent multiple scattering events. For an online instrument, it is essential to develop methods to measure scattering without modification or pretreatment of samples. Scattering measurements may be combined with absorption measurements to characterize samples of raw milk on both physical and chemical parameters.

This paper introduces a novel method for online spectrophotometric measurements on raw milk and for extraction of both scattering and absorption characteristics of the samples from captured data. The method employs multiple LEDs as discrete wavelength sources of radiation, and a CMOS image sensor. The use of LEDs and conventional digital image sensors is of particular interest due to their low cost, high availability, and ease of repair and replacement. The method has been systematically analyzed with experiments on raw and processed milk for response with different fat concentrations and fat globule sizes. Results have been presented here with a discussion on inferences, developments and applications.

2. COMPOSITION OF MILK

Milk is a complex biological fluid with several constituents dispersed or dissolved in an aqueous medium. Table 1 gives the typical concentrations of major constituents and the total solids in milk obtained from common milch animals. The composition further varies between milk from animals of the same species, and between milk from the same animal depending on the season, feed and lactation stage. Milk may also include other minor constitutes like somatic cells, urea (non-protein nitrogen), microorganisms and residual compounds, including antibiotics, pesticides and heavy metals.

Table 1. Typical composition of milk from common milch animals[10]

(wt /wt %)	Cow	Buffalo	Goat	Sheep
Total Solids	12.8	17.8	13.2	17.1
Fat	3.8	8.0	3.9	6.3
Protein	3.3	4.0	3.7	5.3
Lactose	4.8	5.1	4.8	4.7
Minerals	0.7	0.8	0.8	0.9

Fat in milk is present in the form of dispersed spherical globules[11]. Depending on several intrinsic and extrinsic factors, it may or may not be in a crystalline form. A layer of Milk Fat Globule Membrane (MFGM) emulsifies the globules. It is a complex biological layer mainly composed of proteins and phospholipids. The diameter of the globules in raw milk varies with species and seasons. Its diameter is usually between 3 - 5 μm and up to 10 μm, and follows a lognormal distribution. In processed milk, the size is decreased to 0.7 - 2 μm by homogenization to increase globule surface area and promote emulsion stability. The difference in globule sizes in raw and processed milk is visible under an optical microscope, as shown in Figure 1.

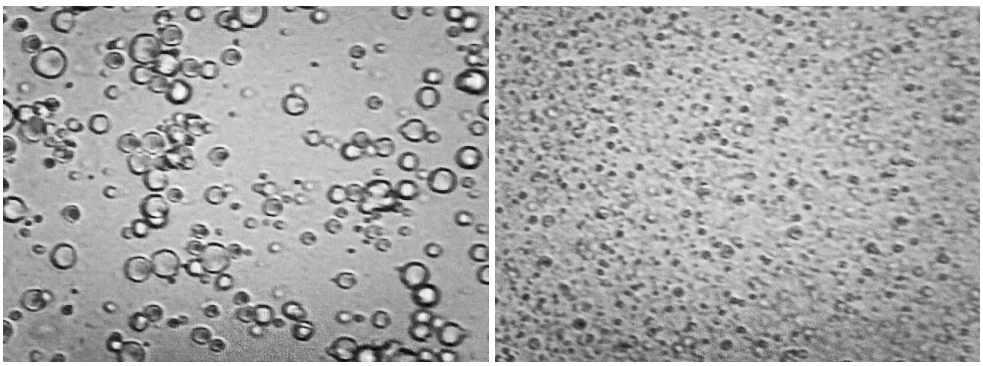

Figure 1. Raw milk (a - left) and processed milk (b - right) viewed under an optical microscope with 40X magnification (The two images are at the same scale.)

Protein in milk is present as dispersed casein micelles and dissolved whey or serum[11]. The former constitutes approximately 80 % of the total milk protein. Casein micelles are stable spherical particles with mean diameter between 100 - 150 nm, and range between 50 - 500 nm. Milk is hence a polydisperse emulsion with dispersed particles (fat globules and casein micelles) having widely different particle size distributions. In homogenized milk, both casein and whey proteins are adsorbed to smaller fat globules as emulsifiers and form a layer usually thicker than the original MFGM layer.

Lactose is the dominant dissolved carbohydrate in milk[11]. Its concentration varies with lactation stage of the animal. In processed milk, it typically remains unchanged after homogenization. Other dissolved substances in the aqueous phase include vitamins and minerals. Phosphates, chlorides and citrates of potassium, sodium, magnesium and calcium are present as dissolved ions or in complexes with caseins[11].

3. SPECTROPHOTOMETRY OF MILK

Absorption and Scattering in Milk

The presence of multiple chemical constituents, in different physical forms, and varying concentrations significantly complicates interaction of electromagnetic radiation with milk. Most fundamental signatures due to atomic or molecular absorption from lipids, proteins and lactose lie in the mid-infrared region (2500 - 25000 nm) of the electromagnetic spectrum. Near-infrared region (780 - 2500 nm) also exhibits absorption signatures from milk constituents, however with overtones and band overlapping. Both mid-infrared and near-infrared spectrums are hence used in laboratory instruments for constituent analysis of milk. Electromagnetic radiation is also scattered by milk due to the presence of particles of different sizes. In laboratory measurements, samples are hence diluted and homogenized to prevent the effects of scattering on observations.

In the UV/Vis region (300 - 1100 nm), instead of specific bands and peaks of absorbance, a continuum is observed. Interaction is dominated by the scattering of light from fat globules and casein micelles that is strongly dependent on the ratio of particle diameter and incident wavelength. Turbidimeters used for estimation of fat concentration measure the extinction of incident light due to scattering by fat globules. To isolate the scattering effect of fat globules, casein protein is dissolved by addition of chelating reagents. Milk is also diluted to prevent multiple scattering that is not accounted for in analysis.

Effect of Multiple Scattering

The radiative transfer equation explains the absorption, scattering and extinction of electromagnetic radiation passing through an interacting medium. If a sample of milk is present between two parallel transparent flats with a discrete light source at one end, the system can be modeled as a plane-parallel problem, as illustrated in Figure 2.

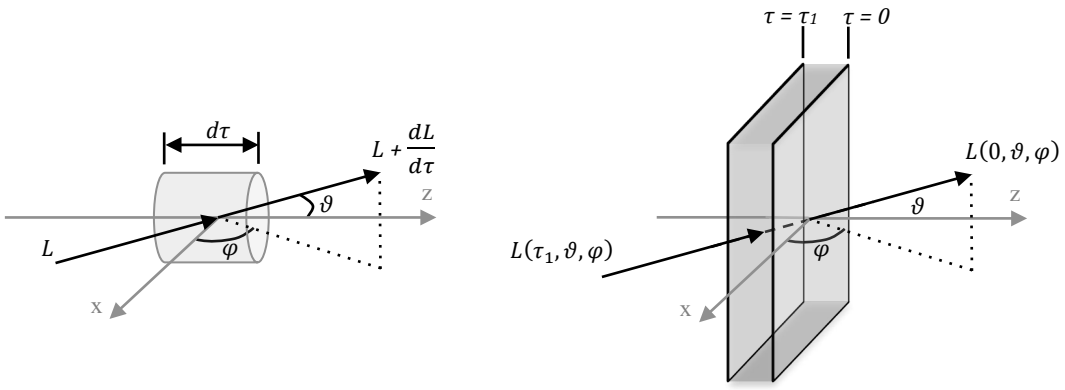

Figure 2. Extinction of a beam passing through an infinitesimal mass of optical thickness $d\tau$ (a - left); Extinction of a beam passing through a finite plane-parallel system of optical thickness τ_1 (b - right)

The equation of transfer for the problem, adapted from Chandrasekhar[12], is then given by:

$$\mu \frac{dL(\tau,\vartheta,\varphi)}{d\tau} = L(\tau,\vartheta,\varphi) - S(\tau,\vartheta,\varphi) \tag{1}$$

L is the radiance and S is the source function along a vector at any given point. τ is the optical thickness of the sample, and dependent on the extinction coefficient and density of the material, and the total path length. $\vartheta = cos^{-1}\mu$ and φ are polar and azimuth angles respectively according to the chosen coordinate system. The formal solution to the problem described in Figure 2 is as follows:

$$L(0,\vartheta,\varphi) = L(\tau_1,\vartheta,\varphi)e^{-\tau_1/\mu} + \int_0^{\tau_1} S(t,\vartheta,\varphi)e^{-t/\mu}\frac{dt}{\mu} \tag{2}$$

The scope function S depends on the radiance L and a phase function p. The phase function p is the angular distribution of scattered radiation about a point scattering source, and is defined by the scattering regime applicable (Rayleigh or Mie). The Mie scattering regime is applicable for particles with diameters comparable with or greater than incident wavelength. This is true for milk fat globules and casein micelles subjected to UV/Vis radiation. Forward scatter is dominant in the Mie scattering phase function.

From the equations, it is evident that absorption follows the Beer-Lambert Law stating an exponential relation between extinction, and analyte concentration and path length. Further, it is apparent that scattering or spread depends also on the power of incident radiation because of the radiance-dependent source function S.

4. METHOD

A method for online spectrophotometric measurements on raw milk and for extraction of both scattering and absorption characteristics of the samples from captured data is introduced here. Samples of milk are channeled into a flow-through sampling cell of a known uniform path length. UV/Vis radiation is incident on one transparent window of the sampling cell from an optical fiber. One end of the fiber is held against a light source with multiple LEDs and the other end is held with its face flat on a transparent window of the sampling cell. LEDs of different emission spectra are used as discrete light sources to individually illuminate the samples. Resultant images on the opposite window are captured using a coaxially placed digital camera with a CMOS sensor.

Multiple images are captured for every combination of milk sample and peak wavelength, at different values of LED brightness. LEDs of different peak wavelengths are powered separately and in increments of output power. The captured

images are processed to derive the radial distribution of transmitted radiation, which is further used to derive the extinction and scattering response of the samples.

Experimental Setup

An experimental setup was prepared to correspond with the outlined method. Figure 3 illustrates the major components of the setup. A plastic optical fiber with a 1 mm core and polished terminations with SMA connectors was used. Standard red, green and blue LEDs were used as discrete light sources. The spectral response of the LEDs, observed using a calibrated spectrophotometer, is presented in Figure 4. The milk samples were confined between two quartz windows such that the path length through the sample was 1.5 mm. The quartz windows were 1.59 mm (1/8") in thickness and 25.40 mm (1") in diameter. A 5 MP off-the-shelf CMOS camera with manually adjustable focus was used for capturing images. The setup was placed inside an enclosure to isolate it from external radiations in the UV/Vis spectrum. All surfaces and inside walls of the enclosure were painted black to prevent stray reflections.

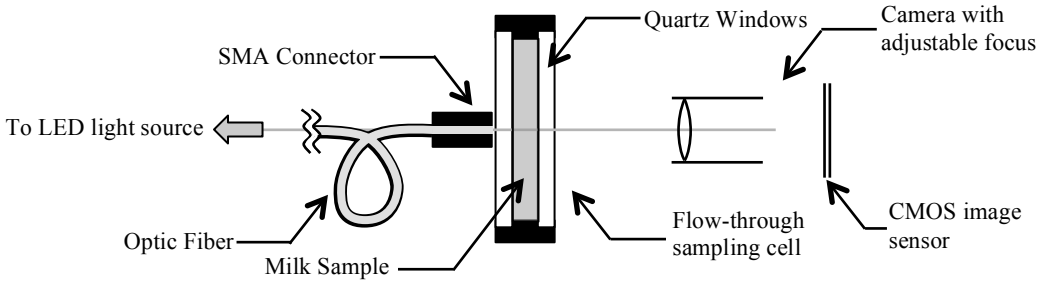

Figure 3. Major components of the experimental setup (Illustration not to scale with actual dimensions)

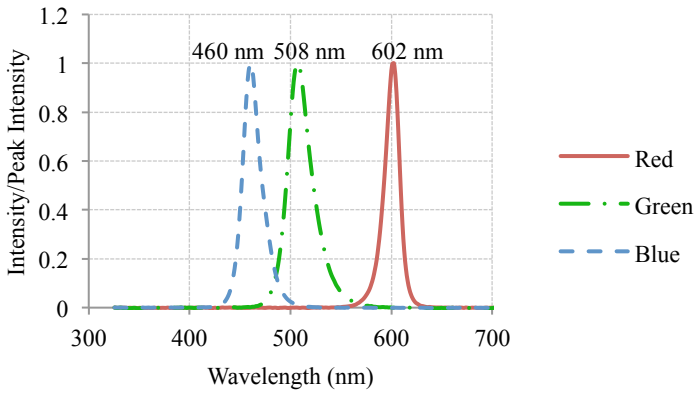

Figure 4. Emission spectra of the three LEDs used (Intensities are shown relative to measured peak intensities)

Samples

Multiple samples (A - M) of raw milk, processed milk, and combinations of raw and processed milk were examined on the described setup. Six raw milk samples (A - F) were derived from one sample of raw cow's milk (C) by separating layers in standing milk. Five processed milk samples (G - K) were prepared by mixing off-the-shelf skim milk (G) and whole milk (K) in different proportions. Two combined samples (L, M) were prepared by mixing raw milk (C) and whole milk (K) in different proportions. The major constituents in original samples were quantified using a calibrated FTIR instrument. Figure 5 shows the different samples on a plot of measured fat concentration vs. average particle size. The average particle size was assumed as 10 μm for all raw milk samples and 1 μm for all processed milk samples. The particle sizes are assigned strictly for convenience and have no bearing on current measurements, analysis and results.

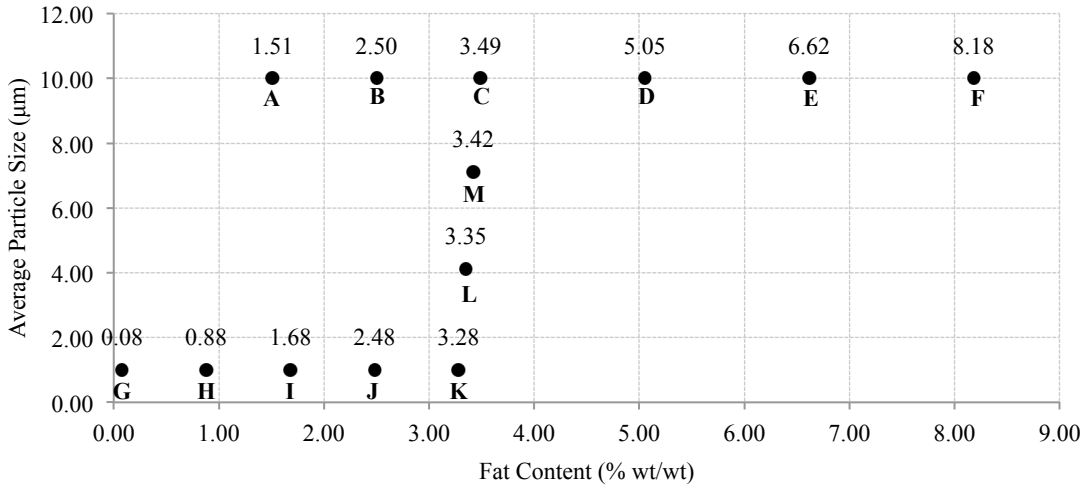

Figure 5. Samples of milk on a plot of measured fat concentration versus expected particle size

Image Capture and Processing

A sample-wavelength combination is a pair of one sample from among A - M and one peak wavelength from among 460, 508 and 602 nm. It is represented as (K, 602) for sample K subjected to 602 nm radiation. Images were captured for each combination at different values of LED brightness varying from 5% to 95% of the peak brightness by supplying a PWM of corresponding duty cycle. The peak brightness values were different for different LED wavelengths.

The CMOS image sensor and the onboard signal processor were programmed to have a constant shutter speed and gains in order to get the same exposure in all images. Images were captured in full resolution, denoised and demosaiced, and stored with a lossless compression. Any errors due to the image signal processing steps were ignored. The eight-bit readings (0 - 255) of the RGB channels for each pixel were assumed proportional to the power of incident EM radiation in the corresponding wavelength bands. Readings only from the most sensitive channel were used in the analysis. While all channels were sensitive to all incident wavelengths, the R, G and B channels were individually most sensitive to the 602, 508 and 460 nm radiations, as expected. Images with saturated pixel readings were rejected due to blooming.

For every combination and LED brightness, one image was obtained by averaging three captured images to reduce temporal noise. The images visually appeared like blurred discs with bright centers. The centers were at the same location in all images. A radial distribution of the chosen channel's pixel readings about the center was hence obtained for every combination and LED brightness. Image spread was quantified as standard deviation in normalized radial distributions. Figure 6 (a), (b) and (c) show the original image, the pixel readings, and the radial distribution of readings respectively for (K, 602) combination at 50% LED brightness.

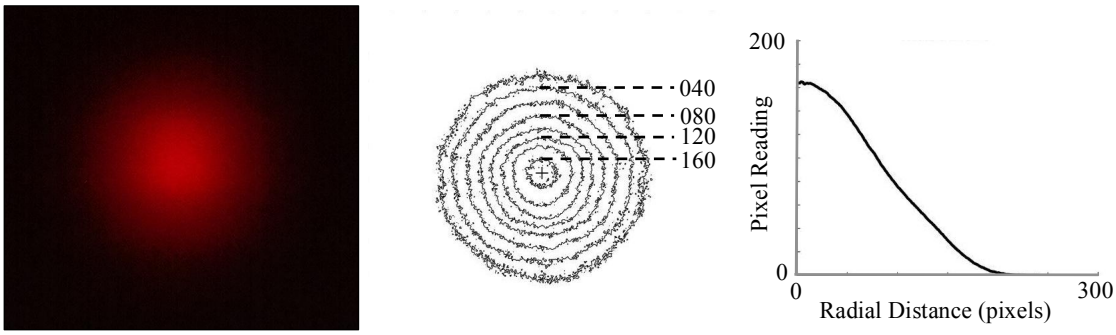

Figure 6. For (K, 602) at 50% brightness: Captured image (a - left); Readings from R channel (b - center); Radial distribution of readings (c - right)

5. RESULTS AND DISCUSSION

For any chosen combination, the pixel readings were observed to scale non-linearly with LED brightness. The spread of the radial distribution was found to vary with LED brightness. The non-linear scaling and the varying spread qualitatively indicate that the total scattering of transmitted radiation is dependent on the power of incident radiation (LED brightness). This effect is attributed to multiple scattering, as was inferred from eq. (2). Figure 7 (a) compares the distributions obtained with 5% - 95% LED brightness for the (K, 602) combination.

In order to estimate the power of transmitted radiation I_t, the sum of all pixel readings in the dominant channel was calculated for each image. I_t may be estimated in absolute units from the sum if the setup is calibrated with a reference spectrophotometry instrument. Without calibration, the sums allow quantitative comparison of transmitted power among different images belonging to the same combination. Figure 7 gives the transmitted power I_t for different levels of LED brightness for the (K, 602) combination. Since the estimates are not in absolute units, the values have been omitted from the discussion here for clarity. I_t is observed to scale linearly with incident power I_i (equivalent to LED brightness). Linear fit on the plot had a coefficient of determination (R^2) equal to 0.996, confirming linearity. The R^2 values for all other combinations were between 0.994 and 0.999.

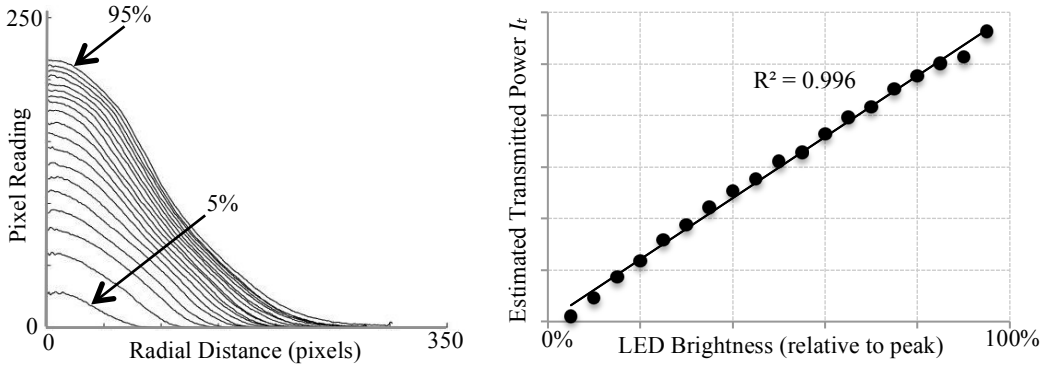

Figure 7. For (K, 602): Radial distributions of pixel readings at LED brightness from 5% to 95% of peak brightness (a - Left); Estimated power of transmitted radiation I_t at different values of LED brightness (b - Right)

In traditional turbidimeters, I_t and I_i are used to estimate the sample's optical thickness τ and eventually the analyte concentration c. Given that I_t can be estimated as the sum of pixel readings, I_t / I_i is proportional to the slope of the sum versus LED brightness plot. The proportionality holds for all combinations with the same LED wavelength. According to Beer-Lambert law,

$$\frac{\text{Sum of Pixel Readings}}{\text{LED Brightness}} \propto \frac{I_t}{I_i} \propto e^{-\tau} \tag{3}$$

The same inference can be drawn from the formal solution of the radiative transfer equation, described in eq. (2).

For (K, 602), the ratio was estimated from the slope of the plot in Figure 7 (b). The ratios were similarly estimated for all combinations. The natural log of the ratio gives an estimate of the optical thickness τ, which in turn is proportional to analyte concentration c for a given path length. Figure 8 gives the estimated optical thickness, in relative units, in comparison with reference fat concentrations for all samples at different incident wavelengths. Results were not obtained for sample G due to saturation of pixel readings. The linear relation between estimated optical thickness and reference fat concentrations is confirmed by $R^2 > 0.99$ for samples with the same average particle size.

From the results obtained, it is validated that the optical thickness of the samples can be determined without dilution. The optical thickness is linearly related to the reference fat concentration with extinction coefficient as the slope. The extinction coefficient of milk samples is observed to decrease with increasing particle size. It is also dependent on the wavelength of incident radiation, especially for processed milk due to comparable particle sizes. The fat concentrations could be estimated independent of the total solids as the samples were intentionally chosen to have similar non-fat solids. However, preliminary qualitative examination reveals that both the particle size and non-fat solids can be estimated by using multiple wavelengths and by quantifying the spread of transmitted radiation with the same method.

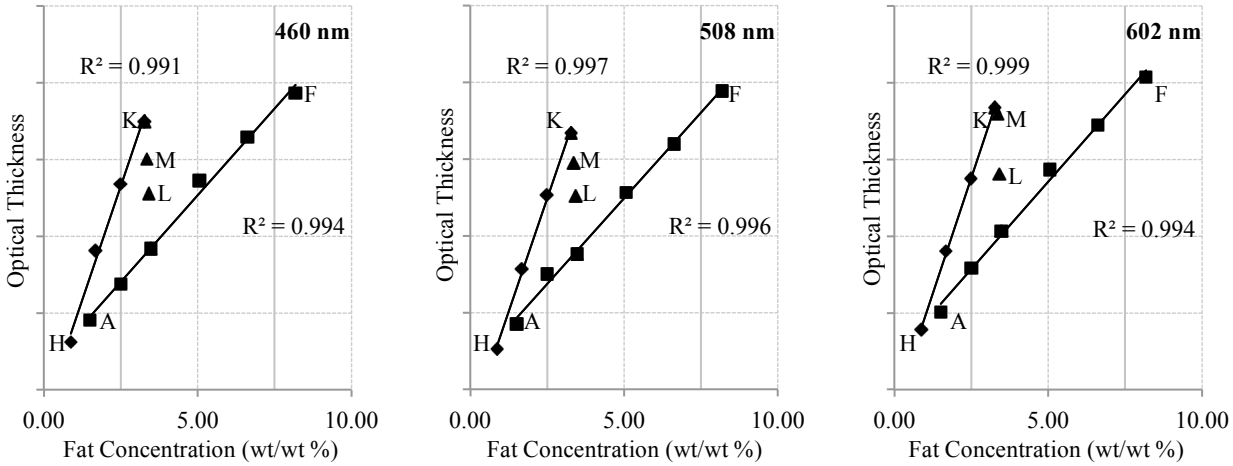

Figure 8. Estimated optical thickness τ, in relative units, compared to the reference fat concentrations for all samples at LED wavelengths: 460 nm (a - left); 508 nm (b - center); 602 nm (c - right)

6. CONCLUSION

Quantitative analysis of constituents in raw milk is important in early stages of the dairy supply chain for elimination of abnormal milk prior to pooling, and for proper management of cattle feed and health. Existing field-level instruments, however, require sample preparation and are prone to human errors. They are especially unsuitable for online analysis, required for automatic milking systems.

A novel method for online spectrophotometric analysis of raw milk is introduced. The method employs multiple LEDs as discrete wavelength sources of radiation, and a CMOS camera as an image sensor. Radiation at discrete peak wavelengths is transmitted across a given sample with known path length and the transmitted radiation is captured as an image. Multiple images are captured for each sample with LEDs of different peak wavelengths powered separately, in increments of output power or brightness. The optical thickness and hence the extinction characteristic of the sample are derived from the captured images. The dependence of multiple scattering on the power of incident radiation is exploited to distinguish the scattering and absorption components of the sample's extinction characteristic for each peak wavelength. These are used to quantify the physical and chemical composition of the samples based on system calibration.

Results from experiments on raw and processed milk validate the method's ability to derive optical thickness and extinction characteristic from images without any sample preparation or dilution. The results revealed that the extinction coefficient of milk samples decreases with increasing particle size. It is also dependent on the wavelength of incident radiation, especially for processed milk due to comparable particle sizes. For in-field applications, it is essential to distinguish samples with different particle sizes for accurate quantification of constituents. It is also important to distinguish the effects of fat and non-fat solids on the extinction characteristic. Preliminary qualitative examination reveals that both the particle size and non-fat solids can be independently estimated by using multiple wavelengths and by quantifying the spread of transmitted radiation.

The method has significant advantages over existing field-level instruments for analysis of milk. It allows rapid online analysis without any sample preparation or wastage. It is especially useful for automatic milking systems, where a large number of small milk volumes may be tested during production. It is also useful in clusters of small-scale dairy farmers where a wide variety of milk compositions may be handled simultaneously. Although the method has been described and validated solely for analysis of milk, it is equally applicable for other existing or new uses of absorption or scattering based spectrophotometry, including food and beverage, environmental or medical applications.

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