Towards in-flow monitoring of fat content and fluid composition of dairy milk using microfluidic confocal Raman spectroscopy

P. Canyelles Pericàs*[a], A. Sundararajan [a], R. Wiegerink [a], J.C. Lötters [a,b]
[a] Department of Integrated Devices and Systems, MESA+ Institute for Nanotechnology, University of Twente, Enschede, 7522 NB, The Netherlands
[b] Bronkhorst High-Tech BV, Ruurlo, 7261 AK, The Netherlands

ABSTRACT

Dairy milk composition analysis is critical for quality control and pricing purposes. Particularly, fat content in dairy milk determines its market value, and free fatty acids can be used as a proxy for milk quality. Milk with high fat contents (4% and above) is progressively skimmed to obtain a chain of products (butter, cheese, milk, powder milk, etc.), increasing the profitability in a traditionally low-margin industry. Besides total fat percentage, the composition of fatty acids is also relevant, as C14, C16 and C18 free fatty acids can be used to monitor cattle food intake. Established chemical separation techniques to measure fat content, such as the Gerber method, are off-line, manual, labor intensive, cannot distinguish fats and have a high error margin. We present a monitoring system concept, free of labelling and sample processing, for fat milk content using Raman spectroscopy in flowing microfluidic channels. The technique has been tested using several microfluidic flow rates (20 μl/min to 1000 μl/min) with Raman measurements of 5 seconds of exposure time and with a single accumulation. We show that Raman spectra remain the same even after continuously refreshing the fluid during measurements at increasing flow rates. Proof of concept Raman measurements at the region of interest for undiluted whole (3.5% of fat), semi-skimmed (1.5%) and buttermilk (0.5%) are presented under different microfluidic flows, showing the potential of the technique. The technique can also be used to identify different fats, proteins, and minerals.

Keywords: Raman spectroscopy, microfluidic sensing, in-flow measurements, milk, fluid composition.

1. INTRODUCTION

Milk consists of 87% water and 13% of total solids, of which 4% are milk fat globules (MFG) and 9% solids-non-fat (SNF) such as proteins, lactose, minerals, and vitamins[1]. Milk composition delivers dairy's unique taste and texture. It also allows the making of dairy products that require solubility, gelation, water-binding, foaming, heat stability and emulsification. Fat content is the main industrial proxy for market value and quality. The average content of fat in raw milk is in the order of 4.4%, with over 400 various fatty acids present. Fatty acids are derived from the feed and the microbial activity in the rumen of the cow, which is linked to cattle health. If these factors change, the fat content changes too, highlighting the need for in-line, continuous monitoring. The golden standard technique for fat content determination in milk is the Gerber method[2]. This consists in butyrometers specifically designed for fat content measurements in which a skilled operator mixes a milk sample with sulfuric acid. The chemical reaction of the sulfuric acid to the fatty acids inside the butyrometer separates fats from proteins. The method is off-line, time and labor consuming, and prone to errors. Above all, it has a 0.8% error margin. The process takes multiple sample preparation steps, including sulfuric acid manipulation and cooling the sample at 4°C for 12 hours. Dairy production is becoming more industrialized with a process flow that results in many products. New instruments for at-line and in-line dairy production analysis are being called by the regulatory bodies to transition to industry 4.0[1].

2. CONFOCAL RAMAN MEASUREMENTS FOR FAT MILK CONTENT DETERMINATION IN STATIONARY LIQUID

Raman spectroscopy is a molecular identification technique that detects and analyzes the absorption and emission of light of molecules in various regions of the electromagnetic spectrum. It is based on the principles of Raman scattering, where a tiny portion of light impacting a sample is absorbed and then re-emitted at a different frequency. The frequency shift is characteristic of the material's structure and can be employed for chemical fingerprint and sensing.

* j.canyellespericas@utwente.nl, phone +31534899076 , website https://people.utwente.nl/j.canyellespericas

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Raman spectra of stationary dairy milk reveal its fat content[3]. Fatty acids display the brightest Raman peaks in the 2700-3000 cm^{-1} Raman shift region, with their intensity having a relation with the fat content. The potential of the technique is then assessed by the following measurements of Raman spectra in commercial dairy milk samples of whole milk (3.5% fat content), semi-skimmed (1.5%) and buttermilk (0.5%). The equipment used to take the measurements is a commercial confocal Raman microscope (WiTEC Alpha 300), with excitation light at 633nm, 20mW of power, 100x/0.9NA objective, an exposure time of 5 seconds and just one accumulation.

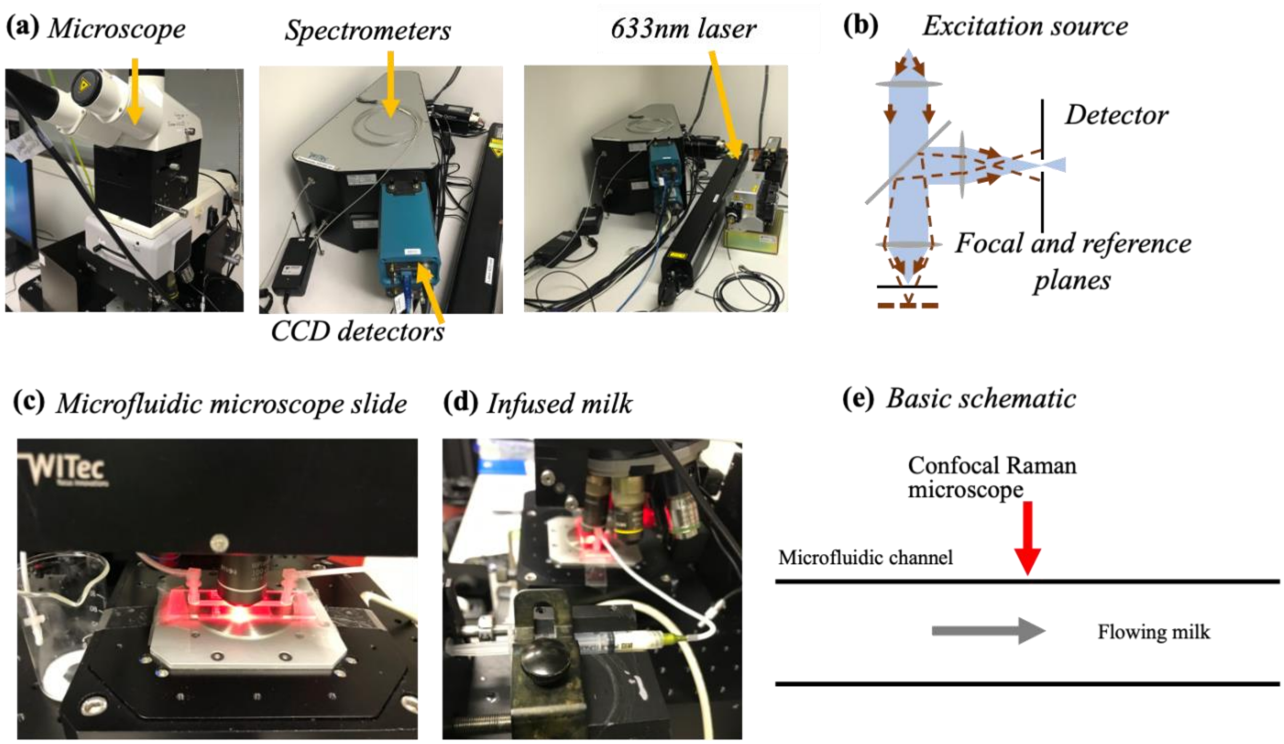


Figure 1. a) Raman confocal microscope, b) Raman schematic, c) Microfluidic slide, d) Harvard pump, e) In-flow schematic

The measurements were made using undiluted milk samples inside a microfluidic slide (Ibidi μ-Slide I Luer uncoated, polymer coverslip, 200μm height channel, 5mm wide, 50mm long, reservoir volume of 60μl). All measurements were taken at room temperature.

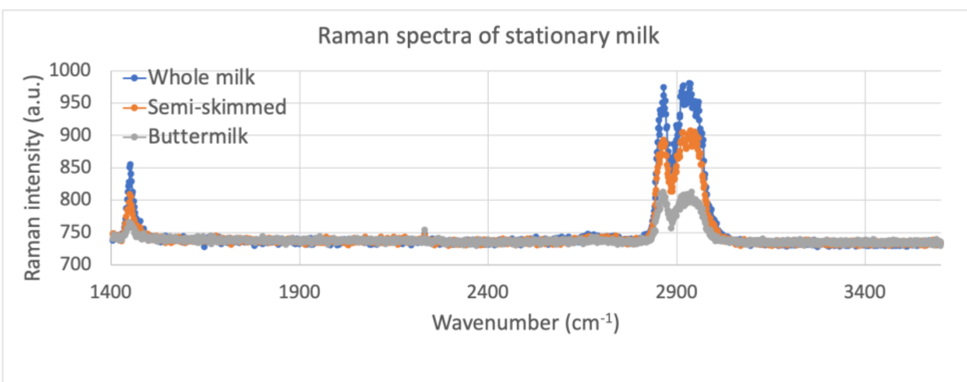


Figure 2. Raman spectra of stationary dairy milk

In literature we can find reports on employing Raman for fat content determination for stationary measurements of liquid and dried milk for dairy milk[3], and for human lactation fluid composition analysis[4]. However, to our knowledge, in-flow Raman measurements of undiluted milk have not been demonstrated yet.

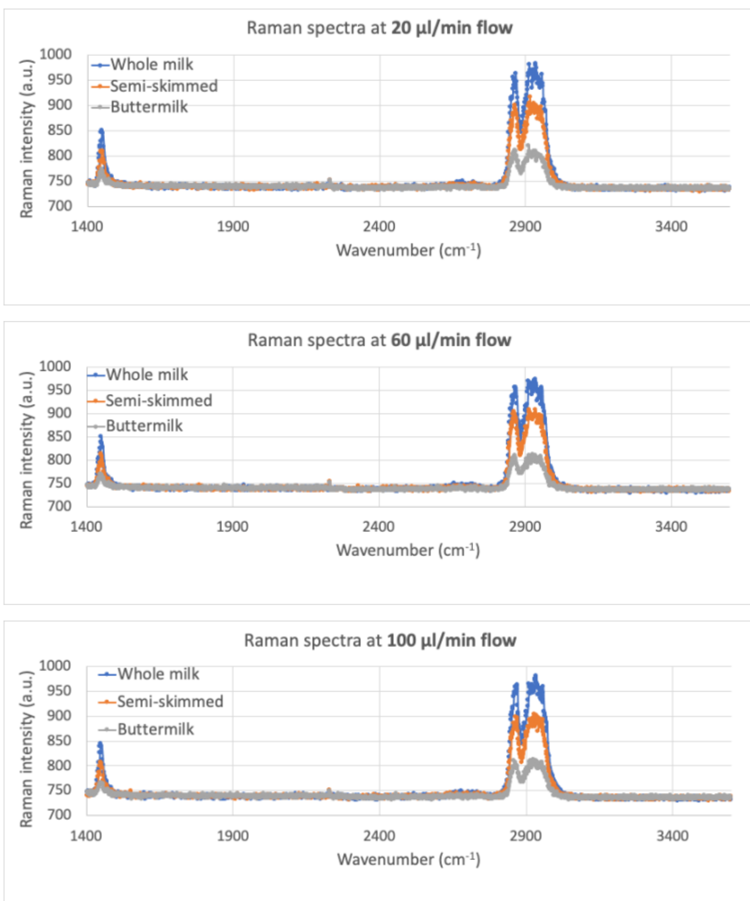
3. CONFOCAL RAMAN MEASUREMENTS FOR FAT MILK CONTENT DETERMINATION IN FLOWING LIQUID

We propose performing microfluidic Raman measurements of flowing milk for in-line and continuous sensing purposes. Recently, a similar concept has been demonstrated for Raman measurements of flowing blood plasma[5]. In that case, the motivation was to avoid photodegradation on a single Raman spot measurement due to high-power laser use, resulting in reproducible measurements with high signal to noise ratio and low acquisition time. With dairy milk, it is expected that the Raman spectra will be the average of the fluid flow over the exposure time. If the fluid is relatively homogenous (as with blood plasma), Raman measurements are hypothesized to be representative of the fluid composition. In table 1, we calculate the distance travelled by flowing milk in each measurement (5 second exposure), considering that the area of the microfluidic channel is $1mm^2$.

Table 1. Flow distance covered for each measurement at different flow speeds.

Flow speed (µl/min)	**Flow speed (mm^3/sec)**	**Distance covered per measurement (mm)**
20	0.33	1.67
60	1	5
100	1.67	8.33
500	8.33	41.67
1000	16.67	83.33

We have then tested the concept with several flow rates (20, 60, 100, 500 and 1000 µl/min) using infused milk with a Harvard pump (PHD 2000 series). The measurements obtained show that in-flow microfluidic Raman measurements are consistent at increasing different speeds, regardless of the distance that the fluid has travelled during the exposure time. Figure 3 suggests that the fluid is sufficiently homogenous, with the spectra being the fluid flow average which is independent of the flow rate:



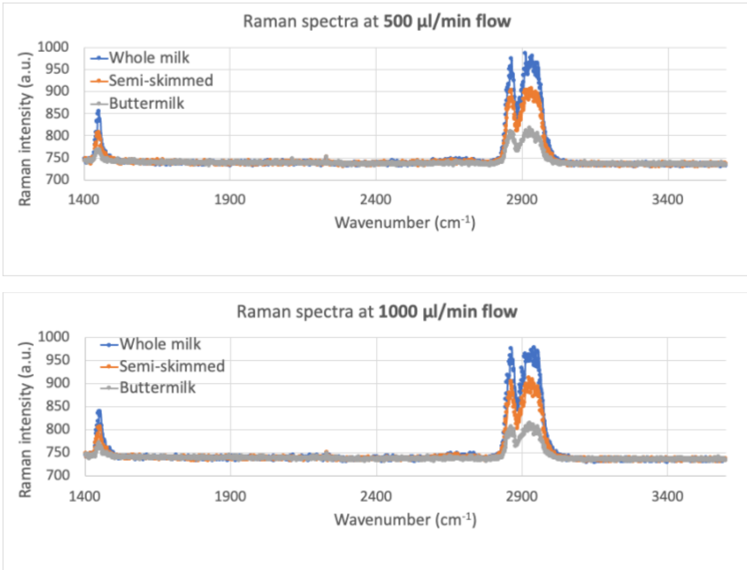


Figure 3. Raman spectra of flowing dairy milk at different flow rates

Raw dairy milk presents a separation effect, where MFG tend to sit on top of the channel. However, in commercial dairy, fat separation can be neglected due to industrial processing. Hence, a focus of 100x/0.9NA (0.14µm^3 measured) is representative enough. For raw dairy milk measurements, the focus needs to be adjusted to the channel or capillary tube.

4. CONCLUSIONS

In this work we study flow composition of milk for dairy industries, exploring the suitability of in-flow Raman measurements for in-line sensing purposes. Proof of principle results show that Raman spectra remain the same at different flow rates independent of the amount of fluid measured. We have measured specifically fat content in commercial dairy, but the principle might also be potentially suitable for composition analysis (minerals, proteins, lactose). Further work includes the optimization of confocal Raman parameters to increase the measurement speed and scanned volume as well as the miniaturization and integration of the setup with MEMS fluid composition technology platforms.

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