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# Mathematical separation of the main components of milk from kinetic data obtained by attenuated total reflection infrared spectroscopy †

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**Abstract:** The possibilities of using mid-infrared (IR) spectroscopy in combination with the method of attenuated total reflection (ATR) to analyze the quality of dairy products are shown. Application of chemometrics, in particular, the curve resolution method (MCR) to spectral data of the milk drop drying process allows us to obtain spectra of its individual components and make quantitative estimates of the sample composition.

**Keywords:** milk analysis; fat; protein; infrared spectroscopy; attenuated total reflection; curve resolution method

## 1. Introduction

Milk is one of the most important foods in the human diet. Therefore, quality control of dairy products is an important task. Fat, protein, and lactose are the main components of milk, which determine its nutritional value and, consequently, the product cost. Optical spectroscopic methods of analysis are widely used in modern dairy. Mid-infrared (IR) spectroscopy (4000–400 cm-1) is one of the most common methods adopted as the industry standard for quality assessment of dairy products [1]. Vibrational IR spectra carry rich structural information about the sample constituents, and the observed absorption signal depends linearly on the component concentrations. Due to this fact, the method suits well for both qualitative and quantitative analysis [2].

In the present work, the analytical capabilities of IR spectroscopy in combination with the attenuated total reflectance (ATR) measurement have been investigated. ATR analysis makes use of a weak interaction of light, reflected from the inner surface of the crystal, with the outer sample. This method is rarely used in the analysis of milk components due to its low (3-6 μm) penetration depth into the sample, which makes it difficult to analyze colloidal particles, such as fat globules and protein micelles [3]. Here, an original approach to the ATR-analysis of dairy products has been proposed. It consists in IR spectral observation of the drying process of a milk drop on the surface of ATR-crystal followed by a chemometric analysis of the obtained three-dimensional data array. It opens new opportunities for in-depth investigation of milk and its derivatives. This approach can be used for both qualitative and quantitative analysis of milk and can be put into the basis of new methods of quality control in the dairy industry.

## 2. Materials and Methods

### *2.1. Milk samples*

Commercial cow's milk from Pestravka with fat content of 3.2% was used as samples. A 20 μl drop of milk was placed on a diamond pad, dried at 40°C and spectra were taken.

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The sample was dried for at least 40 minutes until complete water evaporation. The drying was additionally monitored using a built-in camera in the ATR attachment.

### *2.2. Spectral studies*

The experiment was carried out on FT-805 FT-IR spectrometer with ATR attachment with a diamond element and temperature controller (SIMEX, Novosibirsk). The spectra were taken every 15 sec. Right before each measurement, the background measurement (air) was recorded.

### *2.3. Data Analysis*

The spectra were represented in absorption units and the multivariate curve resolved method (MCR) was used to analyze the milk constituents [4, 5] The MCR is a resolution method that is used in many different fields such as hyperspectral image analysis. In this context, the algorithm provides distribution maps and pure spectra of image components from the only information in the unprocessed image measurement. Additional information such as constituent identification can be easily derived based on the resulting distribution maps and spectra [6].

Data analysis and visualization were performed using PLS_Toolbox v 7.8 (Eigenvector Research Inc., USA) for Matlab R2022b (The Matworks, USA).

The main experimental difficulty of the IR spectroscopy method is the high extinction coefficients in this region, which requires small optical paths to obtain absorption spectra (about 500 μr).

The attenuated total reflection (ATR) method is a promising method of analysis due to its ability to use fibre optic probes. The ATR method is based on the reflection coefficient whereby IR radiation is directed through a crystal in contact with the sample. The beam is reflected several times before reaching the detector. For MIR, the penetration depth is less than 10 μm, which is similar to thin transmission cells [7].

NIR spectroscopy is rarely used in milk analysis because of a sample penetration depth of 3-6 μm the colloidal components of milk, primarily fat globules, remains invisible to measurement.

In this work 50 milk samples were analyzed on a FT-805 FT-IR spectrometer using a ATR attachment with a diamond crystal cell.

As water evaporates, the spectrum shows signals of all major components, the spectra of which were mathematically extracted by curve resolution [6] along with their corresponding kinetic curves.

## 3. Results and Discussion

Figure 1 shows the spectra of a drop of milk dried on an ATR attachment at this temperature and time. The changes in the spectra are due to the drying of the milk sample. The spectra show the charactered bands of water, milk fat and lactose. [8, 9]. During the drying of the droplet, the signals in the 3200-3500 region decrease. At the same time a group of signals in the region 2800-3000 grows, which refer to valence CH vibrations of hydrocarbon residues of fat, lactose and protein molecules, first of all CH2-molecule of fat. The lactose molecule does not have its own distinct signals that do not overlap with those of other milk components.

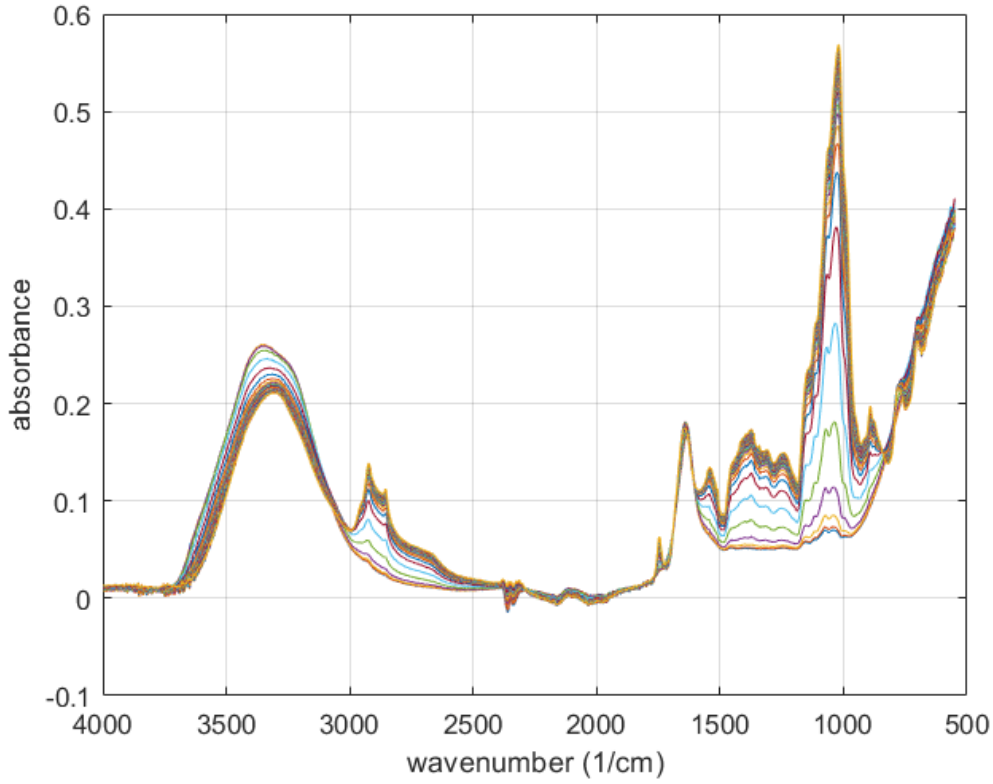
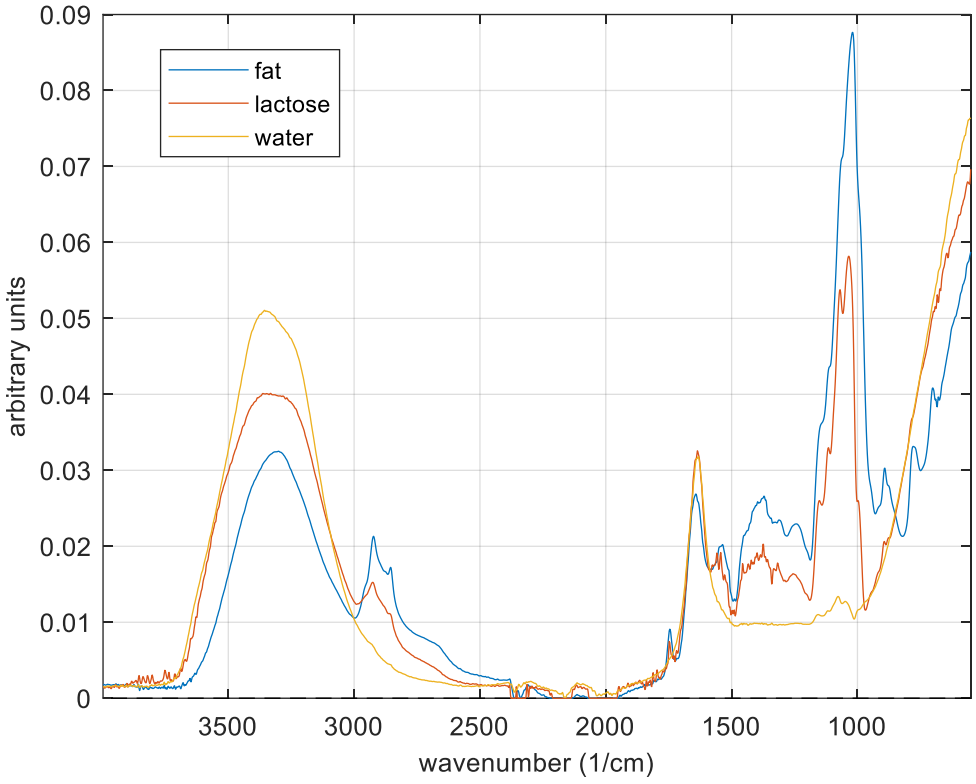


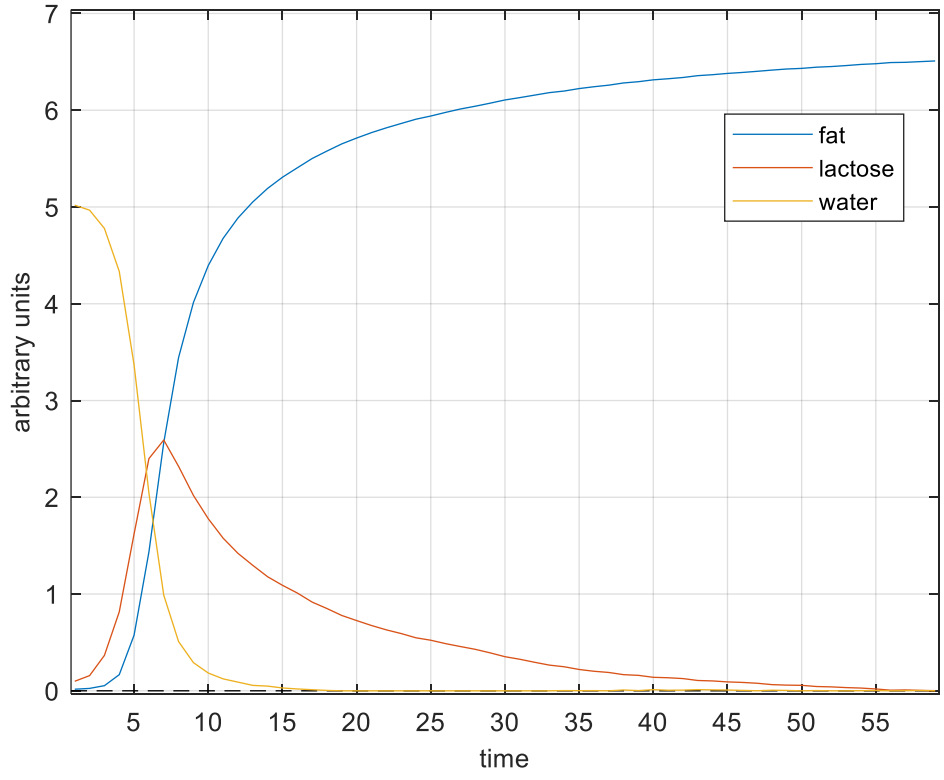

***Figure 1.*** IR-spectra of a milk drop.

The temporal changes of the signals of the different components in the milk spectra during drop drying are of different nature. This brings additional information that can be used in data analysis. Application of the MCR method on the data in Fig 1 gives three pairs of complementary curves: fat, lactose and water spectra (Fig 2(a)) and their corresponding concentration profiles.

As can be seen, the obtained resolved spectra are close to the known spectra of the components [10]. The main difficulty in resolving the spectra of milk components is that the spectra of lactose contain signals that largely coincide with those of fat and water. The obtained kinetic patterns confirm the correctness of the obtained resolved spectra. The water concentration falls expectedly during drying, slowing down towards the end of drying. The growth curve of the spectral contribution of fat is antisymmetrical to the decreasing water. Interestingly, at the beginning of the process, colloidal particles-globules of fat are practically invisible for the ATR method, being at a distance from the surface of the ATR-crystal, while at the end of drying the fat signals become the main ones in the spectrum.


fat
lactose
water
arbitrary units
wavenumber (1/cm)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
3500
3000
2500
2000
1500
1000

(a)


fat
lactose
water
arbitrary units
time
7
6
5
4
3
2
1
0
5
10
15
20
25
30
35
40
45
50
55

(b)

**Figure 2.** Resolved (a)-spectra and (b)-concentration profiles of milk components.

The observed ATR signal of lactose passes through the maximum. We explain this by the fact that in the process of drop drying, lactose is first concentrated and then its crystallization begins, with lactose crystals being essentially displaced by the fat film formed on the crystal surface at the end of the process.

In this experiment it was not possible to isolate weak protein signals. It may be possible to do this through a more detailed study and with the use of advanced data analysis methods, including fuse data analysis of milk samples of different compositions

## 4. Conclusions

The suggested method equipped with the chemometric analysis can be put the basis of a new technique for qualitative and quantitative analysis of milk and milk products.

The reported preliminary study results indicate the potential utility of Fourier transform ATR infrared spectroscopy for the rapid, possibly online, determination of the components of normalized and raw milk.

Resolution of weak spectrum of the milk protein is the goal of our subsequent research.

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