

Measuring the lactose content in milk

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Abstract. Raman spectroscopy has become a powerful and popular tool for food systems analyses lately. Based on characteristic vibrations of the studied material, the information on its content and structure can be answered. In the paper, Raman spectroscopy is studied for a purpose of lactose content in milk assessment. Lactose, the milk disaccharide, in a human organism decomposes during digestion by the act of enzyme lactase to more easily digestible monosaccharides – glucose and galactose. The lack of enzyme lactase causes symptoms of lactose intolerance what limits lactose-intolerant individuals in the intake of milk and dairy products. Lactose-free products in the diet can be a solution. Raman spectroscopy offers rapid measurement independent of the number of chemicals and other in the paper listed benefits. Raman spectra of lactose, glucose and galactose exhibit enough differences to distinguish the content of lactose in milk. C-O-H bending mode at 1087 cm^{-1} is used for lactose quantification. The method accuracy for measuring content of lactose was tested on dried milk droplets. Evaluation of the spectroscopic data was related to two different substances - phenylalanine contained generally in the milk and crystal violet used as an internal standard.

1 Introduction

Milk and dairy products are commonly considered as an important part of human diet as a good source of proteins, calcium, phosphorus, magnesium, and other crucial macro- and micronutrients.

The simple disaccharide lactose represents about 5% of the milk content. In a human organism is decomposed by an enzyme lactase to monosaccharides. Different extent of lactase deficiency is described in most of the world's population. Nowadays, approximately 70% of adult population worldwide suffer from lactose intolerance. In most cases the intolerance is gained during the lifetime as genetically programmed decrease of the amount of lactase; however, in a small content can be inborn. Many people should control lactose intake in their diet. A number of nutritional specialists warn of complete exclusion of dairy products from the diet because of the increased risk of inadequate intake of calcium and other minerals, resulting in risk of osteoporosis, etc.

To avoid elimination milk and dairy product from diet, producers have to manufacture lactose-free products. For meeting this requirement it is necessary to handle appropriate equipment for fast, simple and real-time determination of lactose content in milk or dairy products.

Raman spectroscopy is one of rapidly developing modern spectroscopic methods expanding into many industry sectors including dairy industry. Raman spectroscopy brings many advantages over conventional techniques.

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2 Focus of the study

Lactose assessment is necessary in terms of food technology and analyses of food product in connection with nutritional value and also lactose intolerance. The development and use of modern methods offers fast experimental procedures independent of a number of chemical reagents. Using Raman spectroscopy for quantification requires finding signal of any constant part of sample, and a calibration set of samples. In this paper two procedures are presented - the constant share of protein phenylalanine as a natural part of milk; and addition of crystal violet to the samples as an internal standard.

3 Theory

3.1 Milk and lactose

Milk and dairy products are important part of human nutrition. Mammalian milk is first food for infants, and milk consumption lasts to adulthood in many parts of population on the world. In European and American population cow milk is the most frequently consumed, but sheep and goat milk is consumed also. The chemical composition of milk is influenced by animal species, environmental conditions, nutrition of animals, their lactation state and others. Main characteristics of milk are

Table 1. Average composition of goat, sheep, bovine and human milk [1].

	Milk			
	Bovine	Sheep	Goat	Human
Fat [%]	3,6	7,9	3,8	4,0
Lactose [%]	4,7	4,9	4,1	6,9
Protein [%]	3,2	6,2	3,4	1,2
Calcium [mg/100 g]	122	193	134	33
Phosphorus [mg/100 g]	119	158	121	43
Vitamin A [IU]	126	146	185	190
Vitamin D [IU]	2,0	0,18	2,3	1,4
Energy [kcal/100 g]	69	105	70	68

described in Table 1. Bovine milk is composed of 87% water, 4-5% lactose, 3-4% fat, 3% proteins, 0,8% minerals and 0,1% vitamins, on average.

As was mentioned above, lactose content can be individually important for many people. Lactose is the main carbohydrate present in milk. It is a disaccharide molecule composed by glucose and galactose. In human digestion there is enzyme called lactase (connected to small intestine membrane) that hydrolyses lactose to glucose and galactose. Chemical structure is shown in Figure 1. This lactase activity decreases significantly after weaning, but it is individual and it does not happen at the same grade [1].

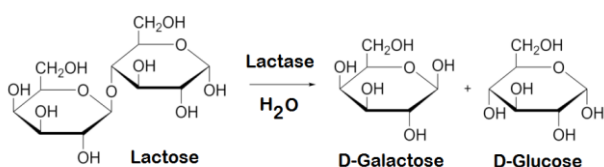


Figure 1. Chemical structure of lactose, glucose and galactose.

It is estimated approximately 70% adult population worldwide suffer from lactose intolerance. This is strongly influenced by human origin. In adult population in Europe and USA it is approximately 7-20%, in some Asian countries it is close to 100% [2]. Similar situation is in African countries. Lactose intolerance causes problems in gastrointestinal tract due to non-hydrolysed lactose, which is fermented by microorganisms in the colon. The main symptoms are flatulence, abdominal cramps and bloating, diarrhoea, nausea and others [1].

Some years ago, it was necessary to avoid all products with lactose content. Today, milk producers offer many kinds of milk and dairy products with low lactose content. Cheese, curd cheese and fermented dairy products content low amount of lactose due to their technological origin. On the other hand, products with high lactose content are milk, dried milk and cream. It is possible to found some lactose-free milk on the market.

3.2 Determination of lactose

Common methods for lactose determination in milk include gravimetric analysis, gas chromatography and most often high-performance liquid chromatography (HPLC). These methods are time consuming, need reagents and they are difficult for on-line analysis [3]. This is the reason for novel techniques development. For this purpose modern spectroscopy methods are often used. Near-infrared spectroscopy is one of those popular methods in dairy industry. However, it is necessary to adjust the determination due to water absorption interferences. Other new methods were developed using enzyme biosensors, which could be utilized for real-time lactose quantification in milk and dairy products [4].

3.3 Raman spectroscopy

Raman spectroscopy as a vibrational spectroscopic method reflects chemical composition and structure of materials. This feature makes the method proper for material identification. Moreover, considering the advantages of the method, Raman spectroscopy becomes popular and valuable part of laboratories around the world in recent years.

Raman spectroscopy provides very specific chemical „fingerprint“ of every single chemical substance in the form of the Raman spectrum. The method is based on so called Raman scattering. Raman scattering is an inelastic scattering resulting from an interaction of a photon and a molecule. In inelastic scattering photons have slightly changed wavelengths that are characteristic for specific bonds in surveyed material. Since most photons are on molecules scattered elastically (Rayleigh scattering without changing the wavelength), it is necessary to filter out of the spectrum of the strongly present wavelength of laser.

Although the fundamental phenomenon is known since thirties of the 20th century, its effective use in Raman spectroscopy occurs in about last decade. The rebirth of this method goes hand in hand with advances in a laser, detectors and computer technology. Raman spectroscopy brings many advantages as the method is relatively rapid, non-destructive, contactless, usable for measuring through transparent glass or polymeric covering layers or containers, applicable to all states of matter and different forms, without special requirements for sample preparation, usable as *in situ* analysis

The greatest drawback of the method is the fact that Raman scattering is a weak effect. Luminescence as much stronger quantum effect with bigger intensity can overlap Raman spectra and mask spectral information. Another disadvantage is eventual degradation of a sensitive sample when using intense laser beam.

Raman spectroscopy finds many applications in recent years in a number of scientific areas such as chemistry, biochemistry, material science, mineralogy, arts, medicine, also is used for pharmaceutical or forensic and security purposes.

4 Experimental part

4.1. Samples and chemicals

Samples were prepared from commonly sold milk (containing lactose) and lactose-free milk. Lactose, glucose and galactose from Sigma Aldrich were used as standards for HPLC calibration and to obtain Raman spectra of pure saccharides.

A set of samples were prepared from a common milk with additions of lactose to final amounts from 5,5 g/ 100 ml to 9,5 g/ 100 ml. Another set of samples contained also crystal violet (CV) as an internal standard [3].

After experiences with previous measurements of milk for milk fat content analyses [5] and the appearing luminescence at liquid milk samples, the form of dried milk droplets on aluminium plates was considered for the measurements and evaluation.

4.1.1 Sugar content in milk by HPLC

The accurate contents of individual monosaccharides and disaccharide were determined by HPLC. The data for common milk (LM) and lactose-free milk (LFM) are listed in Table 2.

Table 2. Content of lactose (LAC), glucose (GLU) and galactose (GAL) in milk. NS = not specified.

Sample	LAC [g/100 ml]	GLU [g/100 ml]	GAL [g/100 ml]
LM	5,46 ± 0,12	NS	NS
LFM	NS	2,34 ± 0,06	2,41 ± 0,05

4.1.2 Phenylalanine content in milk by IEC

Phenylalanine (Phe) is one of essential amino acids. It occurs in all organisms, especially as a part of protein and must be supplied from a diet of animal of vegetable origin. Naturally it is found in a breast milk of mammlas.

Amount of phenylalanine and 14 other amino acids in milk samples obtained after acid hydrolysis was assessed using ion-exchange liquid chromatography (IEC) as described in [6]. Phenylalanine was determined in common milk, lactose-free milk and also in all tested samples. The content was considered as constant (0,93 ± 0,04) g/kg. Under this condition, it is possible to use peak corresponding to phenylalanine for spectra normalization as is shown below in the result section.

4.2 Instrumentation

4.2.1 Raman microscope

InVia Basis Raman microscope (Renishaw) was used to measure Raman spectra of all samples. The Raman microscope uses two lasers as light sources: argon ion laser with the maximum power 20 mW and 785 nm NIR

diode laser with maximum output power 300mW. Both were tested, however, more accurate and by luminescence less affected results were those, obtained using NIR laser. A Leica DM 2500 confocal microscope with the resolution 2µm was coupled to the Raman spectrometer.

The acquisitions were collected with 5 s exposure time and 20 accumulations for milk and 1 s exposure time and 10 accumulations for carbohydrates. The samples were firstly scanned in common range 100 to 3200 cm⁻¹ with 2 cm⁻¹ spectral resolution. After determining the principle vibrational response the spectral range was then reduced to area from ca 300 to 1700 cm⁻¹.

4.2.2 HPLC-RI

Determination of lactose content in milk samples was carried on HPLC chromatograph Shimadzu LC-20AD Prominence with diferencial refractometric detector RID-20A, autosampler SIL 20AC (all Shimadzu Scientific Instruments) and Agilent Zorbax NH₂ column. Solution acetonitril: water in ratio 80:20 was used as mobile phase (acetonitil for HPLC, Sigma Aldrich).

4.2.3 IEC

Amount of phenylalanine in milk samples was determined by ion-exchange liquid chromatography (IEC). Amino Acid Analyzer AAA400 (Ingos, Prague, Czech Republic) was used for this analysis [6].

5 Results

All spectroscopic measurements were performed on dried milk droplets. Firstly Raman spectra of lactose, glucose and galactose were acquired. Spectrum of lactose, as can be seen in Figure 2, exhibits more peaks most likely due to the composition of two monosaccharides.

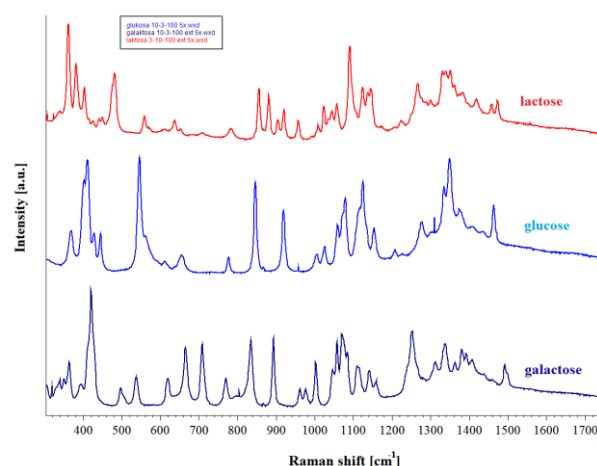


Figure 2. Raman spectra of lactose, galactose and glucose.

Secondly Raman spectra of milk and lactose-free milk were measured (see Figure 3). The Raman peaks of milk are listed in [5]. The main differences can be observed in region 400 cm⁻¹ – 600 cm⁻¹ area for endocyclic and exocyclic deformation bands [7], band 918 cm⁻¹

corresponding to glucose and mainly area around 1070 cm^{-1} – 1090 cm^{-1} . Here is clearly visible intense band 1087 cm^{-1} corresponding to lactose vibrations C-O-H bending mode. Band 1087 cm^{-1} is used for lactose content evaluation in sample sets.

Raman spectra of samples with addition of lactose were acquired. Baseline correction was applied on the spectral data in a uniform manner. Two methods were used for assessment of the lactose content. First method based on constant content of phenylalanine as was proved by HPLC. Second one uses CV as an internal standard.

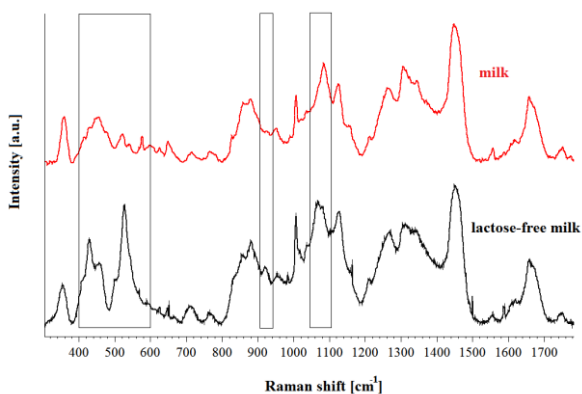


Figure 3. Raman spectra of milk and lactose-free milk.

5.1 Phenylalanine normalization

Raman spectra were normalized according to 1003 cm^{-1} band, which is characteristic for phenylalanine ring breathing band [8]. Phenylalanine is indicative of protein content in the sample. The dependence of the ratio of Raman intensities from bands 1087 cm^{-1} and 1003 cm^{-1} and the content of the lactose exhibits a steady increase, with quite well correlation as can be seen from Figure 4.

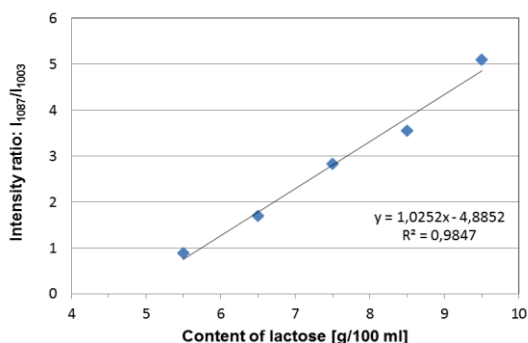


Figure 4. The increase of Raman intensity ratio I_{1087}/I_{1003} corresponding to content of lactose in milk using Phe as the standard.

5.2 Crystal violet normalization

As an internal standard CV was used and band 1173 cm^{-1} was taken for normalization as the most intense band in spectrum of CV, with a solitary position. Results acquired from Raman spectral data by linear regression shows (shown in Figure 5.) not so precise linear behavior ($R^2 = 0,9122$) in comparison with the phenylalanine

normalization ($R^2 = 0,9847$). However, the linearly rising trend is observed also when using CV.

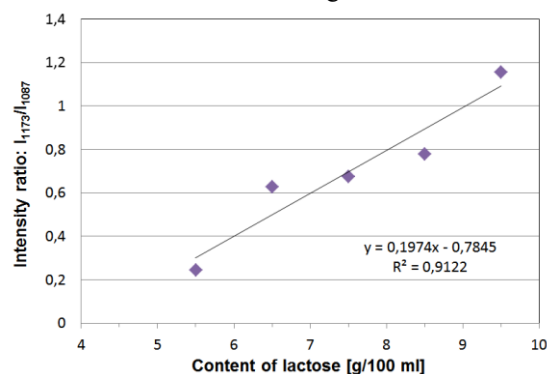


Figure 5. The increase of Raman intensity ratio I_{1173}/I_{1087} corresponding to content of lactose in milk using CV as an internal standard.

6 Conclusion

Raman spectroscopy was used as an innovative method for measuring the lactose content in milk. Measurements were performed on dried milk droplets in order to obtain more precise spectral response. Acquired spectral data show the possibility to distinguish different lactose concentrations on the basis of characteristic bands for lactose, phenylalanine or crystal violet. Normalization using phenylalanine exhibits better accuracy and also easier procedure without adding other substances. Raman spectroscopic evaluation brings advantages mainly in terms of simplicity, rapidity, no use of chemical reagents with the only demand to prepare the milk droplets.

Acknowledgement

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