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Review on the advances in dairy milk chemistry

Pregled novih spoznaja u kemiji mlijeka

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ABSTRACT

Advances in milk chemistry analytics are in a continuous development for more than one century. Recently, more sophisticated analytical methods became available for a routine performance in the laboratory. On the other hand, some of the methods routinely performed in the laboratory became available for the analyses performed at farm level. Overall, the amount and the resolution of data acquired on daily bases today, enables timely monitoring in the dairy production process. Determining the individual components of milk and dairy products in the laboratory can be accomplished using reference methods, or by using routine methods, which are calibrated using the results of the reference methods. Advanced laboratory analysis of milk and dairy products include sophisticated analytical techniques and equipment such as centrifugation, electrophoresis, chromatography, electron microscopy, dynamic light scattering, neutron and X-ray scattering, rheology, mass spectrometry and proteomic approach, all summarized in this review. Additionally, a synopsis of the methods available in laboratory analytical approach of milk components (protein, fat, lactose, minerals and vitamins) and recent on-farm analytical methods using sensor technology is provided.

Keywords: milk composition, milk chemistry, analytical techniques, sensors

SAŽETAK

Tijekom dvadeset i prvog stoljeća nove spoznaje u istraživanju kemijskog sastava I kvalitete mlijeka su kontinuirane. U zadnjih nekoliko godina istraživanja koja su se obavljala u laboratoriju postaju puno detaljnija i sofisticirana, dok dio rutinskih laboratorijskih analiza postaje moguć na farmama. Laboratorijske analitičke metode za određivanje komponenti mlijeka i mliječnih proizvoda mogu biti referentne ili rutinske koje su kalibrirane uz pomoć referentnih metoda. Nove analitičke metode u laboratorijskoj analitici uključuju opremu za elektroforezu, kromatografiju, određivanje aminokiselinskih sekvenci, elektronsku mikroskopiju, DLS-metoda (metoda dinamičkog rasipanja svjetlosti), neutronsko i radioaktivno zračenje, reologija, masenu spektrometriju i proteomske analize koje su detaljno objašnjene u ovom preglednom radu. Pregledni rad opisuje analitičke metode analize sastava mlijeka u laboratoriju (bjelančevine, mliječnu mast, laktozu, minerale i vitamine), te analitičke metode na farmi koje koriste senzorsku tehnologiju.

Ključne riječi: sastav mlijeka, kemija mlijeka, analitičke tehnike, senzori

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INTRODUCTION

Throughout history, milk is one of the most important nutrients in the world. Several animal species are producing milk used for human consumption, for example, cow, goat, sheep, mare, buffalo, camel, and yak. Milk composition varies between the species considering the main milk nutrients (milk fat, protein, lactose, minerals, vitamins, solids-non-fat etc.). Their physical and chemical properties determine which kind of processing and treatment of dairy product will be applied. The knowledge of milk chemistry and components has changed a lot during the last century. Recent review articles (Zhou et al., 2014) showed the difference between the classic, so-called, off-line methods, and rapid detection methods (chromatography, spectroscopy, dielectric properties, and sensors). New analytical techniques applied in the laboratory enabled fractionating and charactering milk components in detail (Lucey et al., 2017). Furthermore, sensor techniques applied on farm in the recent period of no more than a decade (Brandt et al., 2010) improved further in their specificity and sensitivity. With the advantages of conventional and robotic milking, these new techniques moved further towards farm usage. Thus, in the current situation very detailed description of milk components is still provided by laboratories. However, everyday description of the milk constituents is moving towards the farm level ensuring better quality of the herds' management, timely abnormality of the milk detection and timely udder health monitoring and management. Moreover, a combination of day-to-day milk composition measurements together with the use of high throughput techniques can be applied in the genetic evaluation of the animals. Additionally, milk components differ between robotic milking, depending mainly on milking interval, day of lactation and season (Quist et al., 2008; Pavel and Gavan, 2011). Therefore, importance of the sensor usage on farm to determine the main milk constituents in conventional and robotic milking will be addressed within this review.

The review article will provide a detailed overview of the analytical techniques used in dairy milk chemistry, the analytical approach in milk proteins, milk fat, lactose, and other milk compounds analysis, and on-farm dairy milk analytics that became recently available.

ANALYTICAL APPROACH

Accurate, precise, and sensitive analysis is crucial for determining the nutritional value of milk. It is also important for the control of the technological processes and the milk standardization with the aim of producing high quality milk and dairy products. Analytical methods for the analysis of the individual milk components can be reference or routine. Reference methods are most often internationally standardized (ISO, FIL-IDF or AOAC methods) requiring more time to perform and higher costs, but their analytical results are more reliable than the routine methods. However, because routine analytical methods in dairy are often automated and robotic, they can achieve better values for example in the parameter of measurement repeatability as compared to reference methods. Routine methods are usually calibrated by using the results of reference methods, generally faster to perform and can also be standardized. Laboratories often create in-house routine analytical methods which, like all not standardized methods, have to be validated. Standardized methods have to be verified by testing different applicable validation parameters separately in each laboratory which apply them.

Over the last 100 years, accurate and fast analytical techniques and equipment have been developed that include centrifugation, electrophoresis, chromatography, amino acid (AA) sequencing, electron microscopy, dynamic light scattering, neutron and X-ray scattering, rheology, mass spectrometry (MS), genomic approach, etc. (Lucey et al., 2017). In the last few decades, these techniques have been improved and advanced and have made possible to understand the structure of casein micelles, milk fat globules, chemical processes that occur during protein binding, chemical changes that occur during milk heating and the action of various enzymes. Modern analytical methods are computerized, which enables automatic output, monitoring and better-quality control of analysis results.

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Due to the high efficiency of separation and detection, it is necessary to single out modern chromatographic methods. Thus, high performance liquid chromatography (HPLC) and gas chromatography (GC) are very effective for complex analysis of milk and dairy products. HPLC is a non-destructive technique for breaking down complex mixtures into individual fractions or components for detection. There are various technical solutions for fractionation such as Normal Phase Chromatography, Reverse Phase Chromatography, Flash Column Chromatography, Ion Exchange Chromatography, Affinity Chromatography, Chiral Chromatography, Size Exclusion Chromatography etc. Reversed-Phase HPLC (RP-HPLC) is most commonly used. A large number of HPLC detectors have been designed that work on different principles, such as MS, ultraviolet-visible spectrophotometry (UV-VIS), evaporative light scattering (ELSD), photo diode array (PDA), pulse amperometric detection (PAD), as well as on fluorescence, electrical conductivity and refractive index (RID) principles. Gas chromatography is mostly used for the resolution and detection of low molecular weight compounds in the gaseous phase and/or for the analysis of the composition of thermally stable compounds. The most used detectors for GC analysis are flame-ionization detector (FID), MS and thermal conductivity detector (TCD).

The development of spectroscopic methods such as Raman, MIR (2500 - 25000 nm), NIR (780 - 2500 nm) and VIS-NIR (400 - 2500 nm) enabled rapid routine analysis of milk macromolecules and are used today in all major farms and dairies and in dairy laboratories for the purpose of paying producers on the basis of the quality of the delivered milk. Raman spectral analysis is based on vibrational, rotational, and other low-frequency modes of operation and is suitable for a wide range of liquid products (Jha et al., 2016). Spectroscopy (MIR, NIR and VIS-NIR) uses the concept that molecules absorb certain frequencies of light that are characteristic to the corresponding molecule structure. The amounts of energy absorbed depend on the shape of the molecular surfaces, the vibration of the covalent bonds, and the atomic mass. The resulting spectrum contains quantitative information on the amount of the absorbed infra-red (IR) radiation from which the proportion of different compounds in milk and liquid milk compounds is derived (Zhu and Guo, 2021). The reliability and accuracy of MIR and NIR instruments significantly depends on the success of their calibration performed using certified reference material (CRM). Early MIR instruments were entirely filter-based, using pairs (sample and reference) of optical filters to select a band of wavelengths for the measurement of milk fat, protein, and lactose. Today, more recent instruments utilize an interferometer to acquire the complete spectrum information within the MIR region using Fourier Transform Infrared Spectroscopy (FTIR). In this way, it is possible to obtain an extensive computing and data manipulation capabilities (Gambelli, 2017).

To analyze the composition of milk it is possible to use its inherent dielectric properties. Namely, the dielectric properties of the material do not depend only on the temperature and frequency of the electric field but also on physicochemical properties of the analyzed material (Zhu and Guo, 2021). Dielectric properties are defined by the interaction between a material and an electric field. The dielectric constant represents the ability of a sample to store electricity, and the loss factor represents the ability of a sample to transform electricity into heat (Skierucha et al., 2012).

In the recent years, several types of analytical instruments have been produced in which the analysis of milk and dairy products is rapidly performed using different types of sensors including microwave sensors, resonant cavity sensors, biosensors, and photoelectric sensors (Zhu and Guo, 2021). Easy-to-use, portable devices for fast material analysis have been produced which operate within smartphones and perform various complicated analytical protocols without the need to invest in expensive equipment that requires highly educated and professional staff (Nelis et al., 2020).

The components of milk that are most often determined using the above listed analytical methods are protein, milk fat and lactose. These macromolecules, together with the mineral composition, mostly make up

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the dry matter of milk. Vitamins are also a very important ingredient in milk from a nutritional standpoint.

ANALYSIS OF MAJOR MILK CONSTITUENTS

Protein

Milk proteins, casein and whey proteins, are inactive in milk and their activation occurs after their partial hydrolysis. The formation of bioactive peptides is particularly significant in fermented milk where they are formed due to the action of the lactic acid bacteria enzymes from the composition of microbial culture (Martinez-Villaluenga et al., 2016). Active milk protein peptides in the human body play a significant role in the body's natural defense against infection by pathogenic bacteria (Divyang and Subrota, 2018). In addition to antimicrobial function, the antioxidant potential has been determined for bioactive peptides (Tonolo et al., 2020).

Proteins are difficult to define biochemically because of their different primary structure. The secondary and tertiary structure may differ depending on the environment in which the protein is located and the purification methods used. Posttranslational modification (e.g., glycosylation) can further alter protein characteristics. Several definitions have been established depending on the scientific field in which the proteins are being investigated and the reason for their identification and quantification (Mariotti et al., 2008; Moore et al., 2010). In the food science, analyses are performed to establish the quality and value of its ingredients, as well as for nutritional labels. Therefore, the total protein is defined according to the method of its determination, rather than biochemically. For milk and dairy products, total protein is defined empirically as Kjeldahl nitrogen multiplied by a conversion factor of 6.38. The Kjeldahl method is often used as a reference for the calibration of other routine methods. Method includes transformation of all nitrogen in a weighed sample into ammonium sulfate by digestion with sulfuric acid, alkalizing the solution, and determining the resulting ammonia by distilling it into a measured volume of standard acid, the excess of which is determined by titration. This principle determines the appropriate proportion of nitrogen in the dairy analytical medium, depending on whether milk, a protein precipitate, a casein precipitate or a whey protein solution is used as a sample, i.e. total nitrogen, protein N, casein N and whey protein N. According to the convention, after conversion, this corresponds to determining the proportion of crude proteins, true proteins, casein or whey proteins and also by calculation the proportion of non-protein nitrogenous substances. Routine methods are the most often (for milk trade and milk recording) calibrated according to the results of the reference method for crude protein content. In addition to the Kjeldahl method, the Dumas method is also sometimes used as a reference for determining the total nitrogen. The method is based on incineration, i.e. mineralization of a milk of known mass using the temperature in the range of 800 to 900°C in the presence of oxygen, which leads to the release of carbon dioxide, water and nitrogen oxides.

The formol titration method can be applied to directly determine the total protein in milk and dairy products (milk powder and ice cream). The principle of the method is based on the fact that free amino acids, protein-bound amino acids and peptides react with formaldehyde and produce methylene amino acid derivatives and alter the pKa of their amino groups which is actually a measure of groups' tendency to donate a proton (Hu et al., 2018; Kala et al., 2019).

Many colorimetric methods can be used to directly determine total protein, using the chemical dyes to determine the protein content. The proportion of the colored compound is then determined by a spectrophotometer, i.e. by measuring the absorbance at specific wavelengths.

The proportion of total protein is routinely determined during the commercial milk production and in the final product by Raman, MIR or NIR spectroscopic methods. By measuring the proportion of the total protein in commercial milk by the Raman spectroscopic method (30.1 g L^{-1}), a good comparability was found with the results obtained by Lowry colorimetric method (29.8 g L^{-1}) and Kjeldahl method (30.7 g L^{-1}) (Mazurek et al.,

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2015). However, the presented accuracy of the results is acceptable only in the case of in-line measurements on farms or in smaller dairies. Measurements of total protein for scientific purposes, nutritional labels or as part of milk payment schemes require higher accuracy compared to the result of the reference method. Authors who performed protein analysis in milk by MIR and NIR methods found different accuracy and suitability of these methods (Zhu and Guo, 2021). However, the accuracy of these instrumental methods also depends on the quality of their calibration, which analysts perform more or less successfully.

Scientific research on milk proteins is based on proteomics, which includes the separation, identification and quantification of various proteins as well as their modifications that occur naturally or are the result of technological processing and storage of milk. Milk protein separation techniques can be based on different ways of applying one-dimensional (SDS / polyacrylamide gel electrophoresis (PAGE) or isoelectric focusing (IEF)) or two-dimensional gel electrophoresis (2-DE) which involves the separation of proteins based on their specific molecular weight and isoelectric point which therefore overcomes the problem of separating proteins with a similar molecular weight or isoelectric point. Furthermore, in addition to gel techniques, gel free methods can be used for protein separation. These include liquid chromatography (LC) and capillary electrophoresis. LC methods are more sensitive, possess better dynamic range, and are more robust for peptide separation. In combination with mass spectrometry (MS), LC/MS, it is possible to overcome the challenges in identifying hydrophobic proteins as well as low molecular weight proteins and basic proteins that cannot be determined using 2-DE gel techniques (Le et al., 2017). MS techniques are often used in proteomics. The two most commonly used mass spectrometers that differ in ion source are electrospray ionization (ESI) and matrix-assisted laser-desorption ionization (MALDI). MALDI is often associated with time-of-flight (TOF) mass analyzers, while ESI ionization is used with TOF, quadrupole, ion trap or hybrid mass analyzers. Different combinations of mass spectrometers can determine the molecular weight, amino acid sequence, the site of attached groups, and the types of post-translational modifications. These applications have enabled significant advances in milk protein analysis (Le et al, 2017). Chromatographic methods, most often RP-HPLC, using different detectors can determine the proportions of individual proteins and their genetic variants in different types of milk (Ruprichova et al, 2014; Vincent et al., 2016; Ma et al., 2017). Furthermore, Zhu et al. (2015) measured the dielectric properties of milk at different protein contents and found that they can also be used to accurately determine the protein content.

Milk fat

Approximately 98% of milk fat is made up of many different triacylglycerols (TAG) that largely determine the properties of milk fat, such as the solubility ratio, chemical reactivity, and nutritional value. The composition of TAGs is mostly saturated fatty acids (approximately 65%) which have 4 to 18 carbon atoms in their chain. The monounsaturated fatty acids C16:1 and C18:1 in the TAGs composition are represented by approximately 30%, and the polyunsaturated fatty acids C18:2 and C18:3 are represented by approximately 5% of the total lipids. Of the other lipids, milk fat contains diacylglycerols, monoacylglycerols, phospholipids, free fatty acids, cholesterol and its esters. Thus, more than the half of the total fatty acids in milk are saturated fatty acids. The health effects of individual saturated fatty acids are very well known (Haug et al., 2007). The most important polyunsaturated fatty acids (PUFA) in milk are linoleic (C18:2 omega-6) and alpha-linolenic (C18:3 omega-3) acids which are essential. The results of scientific studies show that PUFA and lower ratios of omega-6 and omega-3 can affect metabolism and physiological mechanisms by preventing the onset of some diseases (Bell et al., 2012; Guida et al., 2014).

The official reference method for determination of the total milk fat content in milk is the gravimetric method (ISO 1211, 2010) or the Röse-Gottlieb method in which milk fat extraction is performed with diethyl ether and petroleum ether. The solvent mixture is evaporated, and the residue is dried in an oven and weighed to constant

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weight. The Mojonnier method, which is essentially the same as the method according to Röse- Gottlieb (Choi et al., 2015), is mostly used in the United States and Canada.

The most used routine method for determining the milk fat content in milk is the standardized butyrometric method (ISO 19662, 2018), developed in the 19th century by the Swiss chemist Niklaus Gerber. Protein and lactose are dissolved by the addition of sulfuric acid, and the milk fat is quantitatively separated by centrifugation into a graduated butyrometer column. Very similar to Gerber's method is the Babcock method mainly used in the United States. Between 1950 and 1980, a turbidimetric routine method based on a calculated correlation between the milk fat content and a dispersed light by sample at a given wavelength was most used in milk payment schemes in Europe. Today, this method is considered obsolete, among other things, because the result of the analysis depends on the size of milk fat particles in the sample (Kucheryavskiy et al., 2014; Kala et al., 2018). MIR analyzers are most used today for routine rapid determination of milk fat content in dairies and laboratories. For their accurate and precise operation calibration using CRM is required. Raman spectroscopy (El-Abassy et al., 2011), MIR (Hanuš et al., 2014; Samková et al., 2020), NIR (Kawasaki et al., 2008) and VIS-NIR spectroscopy (Bogomolov et al., 2017) of different spectral ranges can be used for routine determination of milk fat content.

The method commonly used for the separation and analysis of individual fatty acids (FA) in milk (total or free) is GC with FID detector as well as GC-MS. After lipid extraction, total fatty acids (TFA) are converted to their methyl esters (FAME) before chromatographic analysis. In contrast, free FA (FFA) can be analyzed after conversion to FAME or directly as FFA after extraction from the product. One of the most important steps in the analysis of FAME from TFA is the selection of a suitable column for their separation, which depends on the purpose of the analysis (Amores and Virto, 2019). More recently, NIR, Raman spectroscopy and nuclear magnetic resonance (NMR) have been shown to be suitable methods for the analysis of FA in milk and dairy products (Amores and Virto, 2019). Samková et al. (2020) studied the correlation between the FAs determined by the routine MIR spectroscopy (in connection with FTIR) and the reference GC method thus concluded that the MIR method can be used for routine determination of mainly saturated and unsaturated FAs. The HPLC method with MS detector is widely used for the analysis of TAGs. Beccaria et al. (2014) used ultra-high-performance chromatography and MS to characterize TAGs in milk. Ultra-performance convergence chromatography and MS are used to determine TAGs and diacylglycerol (Zhou et al., 2014).

The milk fat content of milk can be determined on the basis of dielectric properties under certain conditions. The relationship between the dielectric constant and/or both the dielectric loss factor and the milk fat content is determined by different models for determining milk fat using the full dielectric spectrum and the extracted characteristic dielectric variables (Zhu and Guo, 2021).

Analysis of milk fat content is possible using different sensor designs, such as VIS-NIR sensor, moisture sensor, optical fiber sensor and annular photoelectric sensor (Zhu and Guo, 2021), however, in those cases the relative errors of milk fat content results are too high for scientific research but can be used for milk analysis on farms.

Lactose

Lactose or milk sugar is the most abundant carbohydrate in milk. It is a disaccharide consisting of glucose and galactose and is synthesized in the mammary gland with the participation of α-lactalbumin. In the form of individual sugars, glucose and galactose are present in milk in negligible proportions. For humans and animals, lactose is an easily available source of energy, and helps the intestinal absorption of calcium, magnesium, and phosphorus, as well as the utilization of vitamin D. Other carbohydrates in milk are oligosaccharides, glycopeptides, glycoproteins, and nucleotide sugars, present in low concentrations. Intolerance, maldigestion and malabsorption of carbohydrates are common disorders in clinical practice, with lactose-intolerance being the

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most frequently diagnosed, afflicting 10% of the world's population. Four clinical subtypes of lactose intolerance may be distinguished, namely lactase deficiency in premature infants, congenital lactase deficiency, adult-type hypolactasia and secondary lactase intolerance (Seoane et al., 2020).

The standard reference method for determining the lactose content in milk and dairy products is the HPLC method (ISO 22662, 2007). Namely, HPLC methods combined with different types of detectors can effectively differentiate carbohydrates. Schuster-Wolff-Buhring et al. (2011) used the HPLC-ELSD method to determine lactose and established a detection limit (LOD) and a quantification limit (LOQ) of 3.8 and 17.3 mg L^{-1}. Fusch et al. (2011) quantified the lactose content of milk by HPLC with tandem mass spectrometry (MS/MS) and the LOD <0.005 mg L^{-1}. Several other methods and detectors (LC-ELSD, LC MS/MS, RP-HPLC, and UV-HPLC) have been used successfully (Zhou et al., 2014). In contrast, the HPLC-RID method was found to be unsuitable for determining the lactose content of lactose-free milk (Trani et al., 2017). Van Scheppingen et al. (2017) and Garballo-Rubio et al. (2018) described LC methods for determining the proportion of lactose in which it is present in low concentrations (<0.01%) or as residual lactose in lactose-free milk.

The GC-FID method can also be used to determine the proportion of the most present monosaccharides and disaccharides in milk. The excellent results of LOD qualify this method as one of the most suitable for routine analysis and quality control in the dairy industry (Zhou et al., 2014; Idda et al., 2016).

Routinely used for determining the lactose content of milk is the standard enzyme method measuring the pH difference. The analysis can be performed by the CL10 instrument, and is based on changes in pH value depending on enzymatic reactions, i.e. measuring the pH change that occurs due to the reaction of glucose and adenosine triphosphate (ATP) in the presence of hexokinase, before and after treatment of the sample with β-galactosidase enzyme (ISO 26462, 2010).

In addition to the above, there are several enzymatic methods for the determination of lactose. They are characterized by the reaction of enzymatic hydrolysis of lactose into lactose and galactose and the determination of one of the released monosaccharides. The content of lactose in the sample is determined by the difference between the content of monosaccharides before and after hydrolysis. Probably the most widely used enzymatic UV method for the determination of galactose is based on its oxidation by β-galactose dehydrogenase to galacturonic acid in the presence of nicotinamide-adenine dinucleotide (NAD) which is reduced in NADH. The absorbance of NADH at 340 nm is calculated as the difference between the readings before and after the addition of the enzyme galactose dehydrogenase (Lynch et al., 2007).

As in the case of other macromolecules in milk, for routine rapid determination of lactose content in milk in dairies and laboratories, MIR analyzers (ISO 9622, 2013) are most often used today, whose accurate and precise operation requires calibration using CRM samples.

Polarimetric methods are based on measuring the specific rotation of polarized light using chiral molecules such as lactose (AOAC 896.01, 2005), while the gravimetric method for determining lactose is based on the change of copper sulfate to cuprous oxide precipitated by the addition of potassium hydroxide in the presence of aldoses and ketoses. The lactose content is calculated after weighing the cuprous oxide formed, by using empirical tables that allow the conversion of the cuprous oxide formed in terms of lactose (AOAC 930.28, 2005; Gambelli, 2017).

Due to the growing demand for fast online measurements imposed by milk and dairy producers in the last years, several amperometric, conductometric and chemiluminescence flow-through biosensors based on different enzymes have been developed (Yang et al., 2010; Soldatkin et al., 2013; Portaccio and Lepore, 2017) for the determination of carbohydrates (lactose, maltose, sucrose, and glucose).

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Other milk components

In addition to macromolecules, the most prominent components of milk are minerals and vitamins that play an indispensable role in the functioning of the human body, and their determination by analytical methods is necessary to define the nutritional value.

Minerals

Minerals in milk are present in colloidal and soluble form, and only slightly in the pure ionic form. For the most part, they form inorganic salts in milk or are an integral part of organic molecules such as proteins, fats, carbohydrates, and nucleic acids. Due to the presence of minerals in the complex equilibrium between the colloidal and soluble phases, minerals are responsible for the stability of the physicochemical balance in milk. Essential minerals, according to their presence in the human body, are very often classified into two groups, macroelements or macrominerals and microelements (microminerals) or trace elements. The chemical form of individual minerals in milk determines its availability to the human and animal organism. The body then uses them for several physiological functions such as bone building, transport of various nutrients, maintaining ionic balance in body fluids, and for a number of vital functions for example catalysis, activation or regulation of cellular processes (Zamberlin et al., 2012).

Most analytical techniques used to determine the mineral content of milk and dairy products involve the preliminary incineration of samples at a temperature of 550°C until the appearance of white ash and the complete disappearance of organic matter. The ash is then dissolved in concentrated strong acid and diluted with ultrapure water. Each of these sample preparation steps can lead to an error in the analysis result. Therefore, direct methods for determining minerals are more acceptable. Total concentration of elements in complete samples are mainly determined by atomic spectrometry and mass methods, including inductively coupled plasma mass spectrometry (ICPMS), inductively coupled plasma optical emission spectroscopy (ICPOES), graphite furnace atomic absorption spectroscopy (GFAAS) and flame atomic absorption spectroscopy (FAAS) (Mir-Marqués et al., 2016). One of the complexities in food analysis is that the essential elements like K, Mg, Ca, Na, Fe and Zn are present at relatively high concentration levels, while toxic elements like Pb, Cd, Hg and As are present at trace or ultra-trace levels. So, for toxic elements and trace minerals determination in food ICPMS and GFAAS are often used due to the ability to work at ppb level with very low limit of detection values (LOD) (Mir-Marqués et al., 2016).

Determination of the content of individual minerals in milk can be successfully performed by ion specific and HPLC methods, but the HPLC method has been found to be ideal due to high accuracy, precision, selectivity and LOD (Zhu and Guo, 2021). Asada et al. (2017) recommended a procedure for the simultaneous determination of K, Mg, and Ca in milk by ion chromatography (IC) after deproteinization of the sample with perchloric acid. Determination of the mineral content in milk by the IC method was performed by Wei et al. (2017).

Vitamins

Milk contains all vitamins which are known to date but regarding the amounts necessary for the needs of the human body, it can be stated that milk is rich in vitamins B2 and B12, moderately rich in vitamins A and B1, while poor in vitamins D, E and C. Retaining certain vitamins from milk in dairy products depends on the type of product. If the product is rich in milk fat, it will also contain fat-soluble vitamins (A, D, E, K), while water-soluble vitamins (B-complex and C) follow the transition of plasma components. Vitamins are essential nutrients for human metabolism that play an important role as coenzymes in many processes that are crucial for the normal functioning of the human body. Thus, for example, they are known to be important for the prevention and treatment of various types of cancer (Mamede et al., 2011).

Determination of the vitamins in milk is most often based on chromatographic methods that can determine individual vitamins or several different vitamins at the

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same time.

Before performing an LC analysis, it is advisable to adopt some preventive measures to restrain losses due to instability problems. The most important factors that cause inactivation are light, air, temperature, pH, trace metals, and ionic strength (Gentili and Caretti, 2017). Because most vitamins are photosensitive, the use of low actinic amber glassware and subdued light is recommended for the entire duration of the analysis. Another precaution that cannot be disregarded is the addition of a proper antioxidant to the solvents employed for the preparation of standard solutions and for the extraction. To date, the most used approach consists in analyzing each vitamin individually, by hydrolyzing all bound forms (acidic, alkaline, or enzymatic digestion) and/or by converting the several vitamins into the most stable form (Gentili and Caretti, 2017).

Barba et al. (2011) identified and determined vitamins E and D by LC using a UV detector. Trenerry et al. (2011) described a robust method with LC-MS and LC-MS/MS to determine the proportion of vitamin D3 in fresh milk. Schmidt et al. (2017) determined all possible natural B6 vitamers in milk by combining ultra-high-performance chromatography with simplified sample preparation. They suggested that this method could be used for large sets of samples due to the duration of analysis and the possibility of simultaneously determining several different vitamins (Zhu and Guo, 2021). Short duration of the analysis was observed by Plozza et al. (2012) who simultaneously determined vitamins A, E and ß-carotene in milk using HPLC - ion trap mass spectrometry (HPLC-MSn).

ON-FARM DAIRY MILK ANALYTICS

Recently, on-farm dairy milk composition analytics is improving and changing rapidly and that it is adapted by producers of the equipment. Moreover, there is no unique standard for this kind of equipment and therefore it is hard to compare results obtained with the equipment of different producers. Even though there are some common initiatives to make the obtained data comparable, overall, the protocols for calibration, maintenance, and validation are missing. Use of such obtained data is for management purposes, animal health, animal welfare and for genetic evaluation. These devices can be used in conventional and robotic milking systems. Recently, AfiLab system (Afimilk, Kibbutz Afikim, Israel) which measure milk fat, protein, lactose content and presence of blood in milk was compared with regular DHIA (Dairy Herd Improvement Association) analysis which is done in the laboratory each month (Kaniyamattam and De Vries, 2014). The results of comparison showed that protein content had the best estimate with correlation coefficient 0.67, milk fat 0.59 and lactose 0.46. The authors explained that the part of the variation may be due to a bit different milk sample collection method. Another study which was conducted for two years in 47 dairy herds in Israel (Weller and Ezra, 2016) showed that the milk yield was correctly measured by AfiLab, but milk fat was underestimated for 125 days of lactation and almost equal afterwards. On the contrary milk protein content was overestimated for 150 days of lactation and thereafter underestimated. It was suggested that optimal interval between the two calibrations of AfiLab measuring device should be determined in the future. Robotic milking systems are increasing rapidly all over the World and they are equipped with sensors for milk components estimate and somatic cell count (SCC). It is known that the milking system (conventional vs robotic) does not influence milk composition (milk fat, protein, and lactose) (Jacobs and Siegford, 2012). However, milking interval and amount of milk per milking have an influence on the milk composition especially milk fat and SCC (Friggens and Rasmussen, 2001). It is important to know that in the conventional milking parlor milking frequency can be mainly 2, while in robotic milking systems it was on average 2.91 (Tremblay et al., 2016). Moreover, day to day variation of the milking interval can cause further differences in the milk composition. However, recent study performed on two milking robot systems, one equipped with both SCC and milk composition sensor (Lely, MQC) and one equipped only with SCC sensor (DeLaval, OCC) showed that the sensor-based estimates tended to overestimate milk component levels below average and underestimate high milk components

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concentrations (Fadul-Pacheco et al., 2018). The authors found differences in SCC measurements between the robots, and they claim that the main problem for these differences would be the consistency of calibration, especially when the farm has two milking robots and one milk tank and calibrates both robots from the same milk tank. The newest studies which observed patterns of SCC with OCC sensors concluded that changes in herd prevalence of subclinical intramammary infections (IMI) can be predicted using newly developed dynamic transmission models (Dalen et al., 2019).

CONCLUSION

Advances in dairy milk chemistry should be divided into laboratory and on-farm ones. Laboratory advances go towards the very specific determination of milk compounds, while on-farm dairy milk chemistry requires further standardization and development to provide comparable results. Analytical methods for the determination of individual components of milk and dairy products can be either reference or routine methods. Reference methods are most often internationally standardized (e.g., ISO, IDF or AOAC methods). Routine methods can also be standardized and are, if possible, calibrated by using the results of reference methods.

Although there is no official reference method for determining protein content in milk and dairy products, the Kjeldahl method is often used as a reference for the calibration of other routine methods. The official reference method for determination of the total milk fat content in milk is the gravimetric or Röse-Gottlieb method (ISO 1211, 2010). Furthermore, the standard reference method for determining lactose content in milk and dairy products is the HPLC method (ISO 22662, 2007). There is no official reference method for determination of minerals and vitamins. Total concentration of macro and micro elements is mainly routinely determined by atomic spectrometry, including inductively coupled plasma mass spectrometry (ICPMS), inductively coupled plasma optical emission spectroscopy (ICPOES), graphite furnace atomic absorption spectroscopy (GFAAS) and flame atomic absorption spectroscopy (FAAS). Vitamins in milk are most often analyzed by chromatographic methods that can determine individual vitamins or several different vitamins at the same time.

On-farm analysis of the milk composition is quite precise in determination of SCC, while in dairy milk compound analysis best results are obtained in milk protein than in lactose and milk fat content. Main problem is the calibration of these on-farm sensors and therefore further research is needed. Recognizing the potential of standardization of on-farm routine methods could enable joint endeavors in milk quality improvement and dairy farm management, provided the data sharing mechanisms and pipelines are created.

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