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Annual and seasonal trends in cow's milk quality determined by FT-MIR spectroscopy in Hungary between 2011 and 2020

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RESEARCH ARTICLE



ABSTRACT

We analysed and monitored the major chemical composition of cow's bulk milk by Fourier transform mid-infrared (FT-MIR) spectroscopy over a 10-year period in the whole territory of Hungary. In addition, the two most important key parameters for milk quality assessment, total bacterial count (TBC) and somatic cell count (SCC) were also followed. Production parameters showed significant seasonal and yearly changes. The overall mean fat, protein, lactose and solids-non-fat (SNF) contents of cow's milk were 3.81%, 3.32%, 4.74% and 8.76%, respectively. A circannual variation was observed in the chemical composition and yield of milk components of samples examined between 2011 and 2020. Concerning milk fat, milk protein and SNF, the values were the lowest in summer and the highest in winter. In the case of lactose, the minimum values were measured in autumn and the maximum values in spring. An obvious trend of long-term elevation of lactose and SNF was found in the raw cow milk samples over the observed period. The overall mean TBC and SCC of cow's milk were 52×10^3 CFU ml⁻¹ and 270×10^3 cells/ml, respectively. Although there were differences in the monthly average values, no seasonal cyclicity was observed.

KEYWORDS

cow's milk, FT-MIR spectroscopy, chemical composition, annual changes

INTRODUCTION

During milk production the concentrations of milk components vary according to the season across the year (Auldist et al., 1998; Heck et al., 2009; Salfer et al., 2019). Changes in the composition of raw milk have a great impact directly on the dairy industry and the quality of milk products (Li et al., 2019; Yener et al., 2021; Jensen, 2022). In addition to the routinely tested and mentioned milk contents, there are many special components in milk that must be taken into consideration in the case of a change in milk quality. As an example, 514 proteins have been identified in cow milk and colostrum (Hejel et al., 2021). Those components are less interesting for food production considerations but much more because of the positive health effects of milk. There is evidence that the raw milk of domestic animals (bovine milk and ewe's milk) contains beneficial growth factors like pituitary adenylate cyclase-activating polypeptide (PACAP). This peptide plays a key role in the regulation of physiological processes (e. g. reproduction, feeding, thermoregulation and motor activity) (Czeplédi et al., 2011). The importance of several bioactive factors has already been demonstrated in human milk, too (Vass et al., 2019). These results help to improve the composition of infant formulas (Almeida et al., 2021).

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There is an increasing demand for monitoring annual changes in raw milk worldwide (Heck et al., 2009). It has been determined that the quality and composition of raw milk are not constant within a given country. Considerable changes have been observed in the composition of raw milk due to seasonality (Auldist et al., 1998; Lindmark-Mansson et al., 2003; Heck et al., 2009; O'Connell et al., 2015). Only small-scale studies performed with a limited number of cows from a few herds have studied the seasonal effects on raw milk samples (Auldist et al., 1998; Lindmark-Mansson et al., 2003; Heck et al., 2009; More, 2009; Poulsen et al., 2017). In a larger-scale study in the United States in dairy herds, annual rhythms of milk and milk components were quantified and cow-specific factors affecting these changes have been evaluated (Salfer et al., 2019). Rearte et al. (2022) carried out a 14-year-long large-scale study in Argentina on associations of SCC with daily milk yield and reproductive performance. However, a systematic, large-scale and decade-long study focusing on annual and seasonal trends in overall milk quality from the entire territory of a country has not been published so far.

In Hungary, a quality-based raw milk control and compensation system was introduced in 1984. Ever since, dairy farmers within the scope of the raw milk testing regulation have been required to send samples for comprehensive quality tests on a regular basis (Unger, 2001; Császár et al., 2011). According to the relevant EU legislation (Regulation No. 853/2004/EC), dairy farmers must send at least two raw milk samples per month to the raw milk testing laboratory for further qualification. However, prior to Hungary's entry into the European Union in 2004, domestic legislation was more stringent and required dairy farmers to test at least three qualifying raw milk samples (ten-day-period samples) per month.

There was a significant milk quality improvement between 1984 and 1995 in Hungary (Unger, 1996). The main reason was the implementation of a price-dependent raw milk purchase system introduced by the authorities. The quality improvement was primarily manifested in a significant decrease in the total bacterial count (TBC) and in the somatic cell count (SCC), as well as in a decrease in the foreign water content of milk which is present in the case of milk adulteration as water is added to milk (Montgomery et al., 2020).

The ten-day-period raw milk testing system has become so closely linked to the milk production and the contractual accounting system that the majority of producers/purchasers have agreed to send regularly three samples per month to the Raw Milk Testing Laboratory of the Hungarian Dairy Research Institute Ltd. (HDRI Ltd.) for certification purposes, maintaining the former customary right and the greater certainty resulting from the higher number of tests on which compensation is based (Unger, 2001; Császár et al., 2011).

The objective of our study was to analyse and document trends and cyclicity in bulk raw milk composition focusing on fat, protein, lactose and SNF content, TBC and SCC of Hungarian raw milk samples tested by the Hungarian Raw

Milk Testing Laboratory between 2011 and 2020 over a 10-year period, covering the whole territory of the country.

MATERIALS AND METHODS

Sampling

Raw milk sampling from dairy farms was carried out according to the requirements and procedure laid down in the *Codex Alimentarius Hungaricus* (2013) and the relevant national legislation. The raw milk sample was taken by the sampler in the presence of the dairy farmer at the time specified in the sampling schedule. The sample was safely sealed by the milk producer in order to ensure its integrity. The raw milk sample was 100 ± 10 ml. Sampling jars contained 0.9% (vol./vol.) sodium azide–Bronopol–Furacine preservatives. Raw milk samples were stored and transported at 2–8 °C until analysis. Raw milk samples had to be tested within 48 h (about two days) of sampling. The raw milk samples were collected in Hungary from January 2011 through December 2020.

Physicochemical analyses

Raw milk produced in Hungary for human consumption and processing is examined and qualified by the Raw Milk Testing Laboratory of HDRI Ltd. according to the requirements of Decree 16/2008 (15 February) FVM SZMM. The determination of fat, protein, lactose and SNF content of raw milk samples was carried out by Fourier transform mid-infrared spectroscopy (FT-MIR) method and the determination of TBC and SCC by the flow cytometry method according to the ISO 9622:2013 (IDF 141:2013) standard and according to the requirements of Annex III to Directive 3-2-1/2004 of the *Codex Alimentarius Hungaricus* in the 'Examination' chapter (2013).

The chemical composition (i.e., fat, protein, lactose, SNF) was determined with an automatic milk analyzer (Milko-Scan; Foss A/S, Hillerød, Denmark) which is a Fourier transform infrared spectroscope based on the infrared absorption principle, operating in the mid-infrared range (FT-MIR spectroscope). The results of chemical composition analysis are given in g/100 g. The TBC was determined with Bactoscan and the SCC was measured with Fossomatic (both instruments were made by Foss A/S, Hillerød, Denmark). The unit of measure is 10^3 CFU ml⁻¹ and 10^3 cells/ml for TBC and SCC, respectively. The equipment has been validated against reference methods by the manufacturer and has also been calibrated for raw cow milk.

Statistical analysis

Statistical analyses were carried out by R software (R Foundation, General Public License). The seasonality of monthly trends was tested using Hewitt's test for seasonality (Rogerson, 1996). Correlation coefficients and R^2 values were calculated using Microsoft Excel (Microsoft, Redmond, WA, USA).



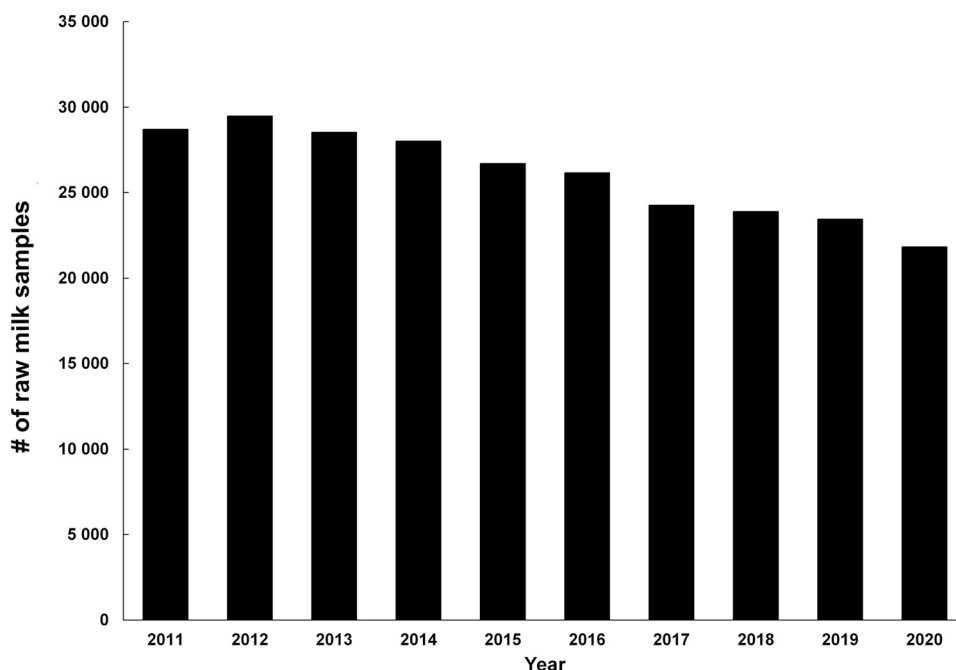


Fig. 1. Number of processed raw milk samples per year

RESULTS

During the period 2011–2020, a total of 261,151 samples of raw milk from producers were analysed in the Raw Milk Testing Laboratory of HDRI Ltd. The annual number of raw milk samples produced by Hungarian dairy farmers have shown a steadily decreasing trend from 2012 onwards, which is a 26% reduction over the analysed 10-year period (Fig. 1).

Our analysis has revealed that the content of fat, protein, lactose and SNF examined in raw milk samples changes seasonally within a year, and this cyclicity was observed every year between 2011 and 2020 in Hungary (Figs 2–5). Concerning milk fat, milk protein and SNF, the values were the lowest in the summer months and the highest during the winter months ($P < 0.001$).

Milk fat content of the analysed raw milk samples showed obvious cyclicity, and the minimum values were typically measured in summer (June, July and August) and the maximum values in the winter months (November, December and January) (Fig. 2). Annual milk fat content within the monthly samples ranged between 3.76 and 3.86 g/100 g (mean 3.81 ± 0.14 SD, SE = 0.013) with the lowest values detected in 2019 and the highest in 2012. Milk fat content was stable, as there was no obvious change in the trend of mean milk fat content in the observed 10-year period ($R^2 = 0.007$).

Milk protein content in the analysed milk samples also showed obvious cyclicity, and the minimum values were typically also measured in the summer (June and July) and the maximum values in the winter months (October,

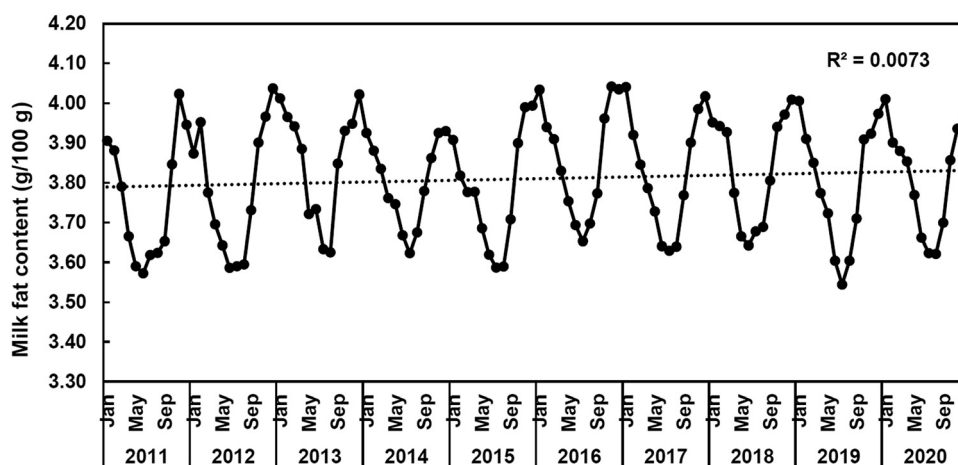


Fig. 2. Average milk fat content (g/100 g) between 2011 and 2020



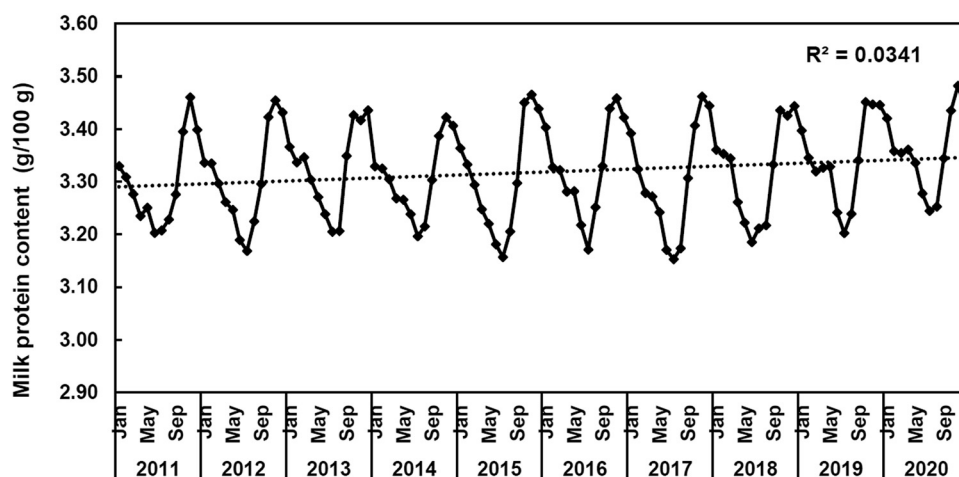


Fig. 3. Average milk protein content (g/100 g) between 2011 and 2020

November and December) (Fig. 3), like the milk fat values. Annual mean milk protein content within the monthly samples ranged between 3.30 and 3.36 g/100 g (mean 3.32 ± 0.09 SD, SE = 0.008) with the lowest values detected in 2017 and the highest in 2020. There was no obvious trend of mean milk protein content changes in the observed 10-year period ($R^2 = 0.012$).

In the case of SNF, the minimum values were typically measured in the summer (July and August) and the maximum values in the winter months (November, December and January) (Fig. 4). Mean SNF content within the monthly samples ranged between 8.68 and 8.84 g/100 g (mean 8.76 ± 0.10 SD, SE = 0.009) with the lowest values detected in 2011 and the highest in 2019. There was an increasing trend of mean SNF content in the observed 10-year period ($R^2 = 0.2$), and this obvious upward trend was steady.

In the case of lactose, the minimum values were typically measured in autumn (September, October and November) and the maximum values in spring (March, April and May) (Fig. 5). Annual lactose content within the samples ranged on average between 4.70 and 4.81 g/100 g (mean 4.74 ± 0.05 SD,

SE = 0.005) with the lowest values detected in 2012 and the highest in 2020. There was an obvious increasing trend of mean lactose content ($R^2 = 0.487$) during the analysed 10-year period.

For all four milk composition parameters, a one-factor analysis of variance was performed to determine whether this seasonal variation is significant. The values of the three lowest average months of milk fat, milk protein and lactose content in 10 years and the three highest average months of milk fat, milk protein, and lactose content do not differ significantly. The analysis of each of the components over 10 years shows that the annual average values of lactose content have a steadily increasing trend between 2011 and 2020. For the other parameters, there is no such change present.

Interestingly, in case of the monthly average values of TBC cyclicity cannot be found (Fig. 6), and values within the samples ranged on average between 35 and 93×10^3 CFU ml⁻¹ (mean $52 \pm 10 \times 10^3$ SD, SE = 873) with the lowest values detected in 2014 and the highest in 2019. There was no obvious trend of mean TBC content changes in the observed 10-year period ($R^2 = 0.007$).

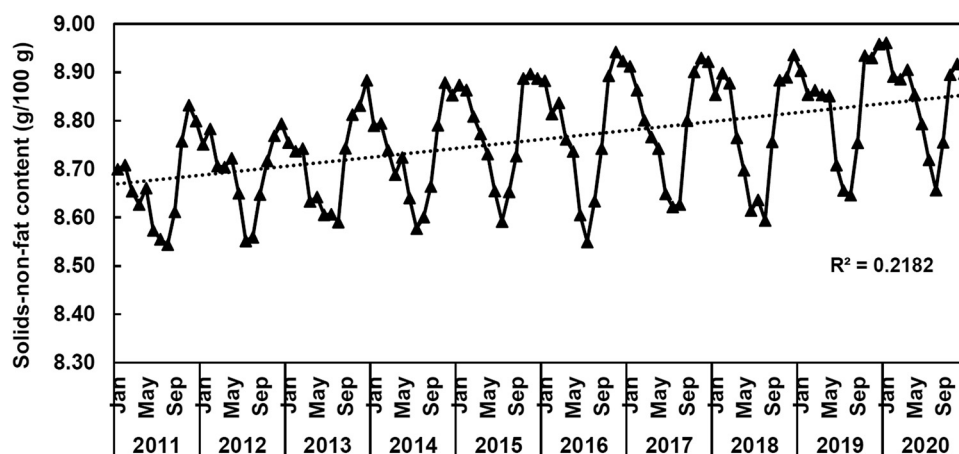


Fig. 4. Average solids-non-fat (SNF) content (g/100 g) between 2011 and 2020

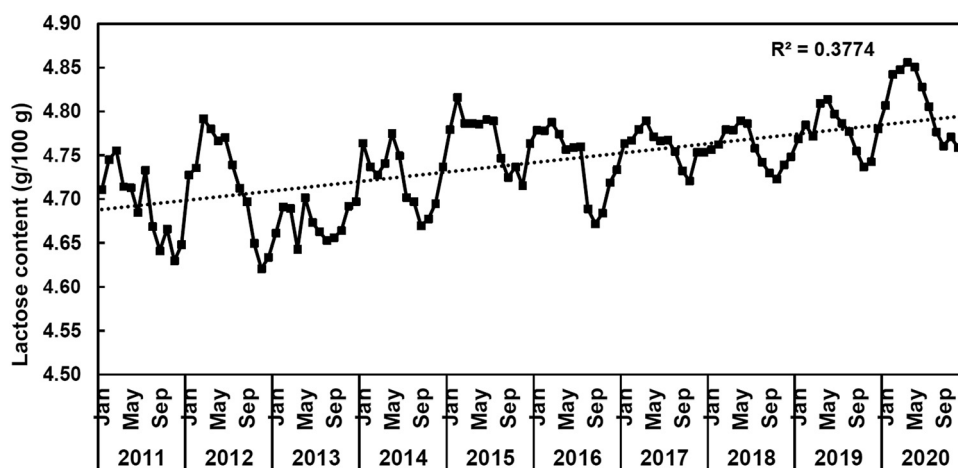


Fig. 5. Average lactose content (g/100 g) between 2011 and 2020

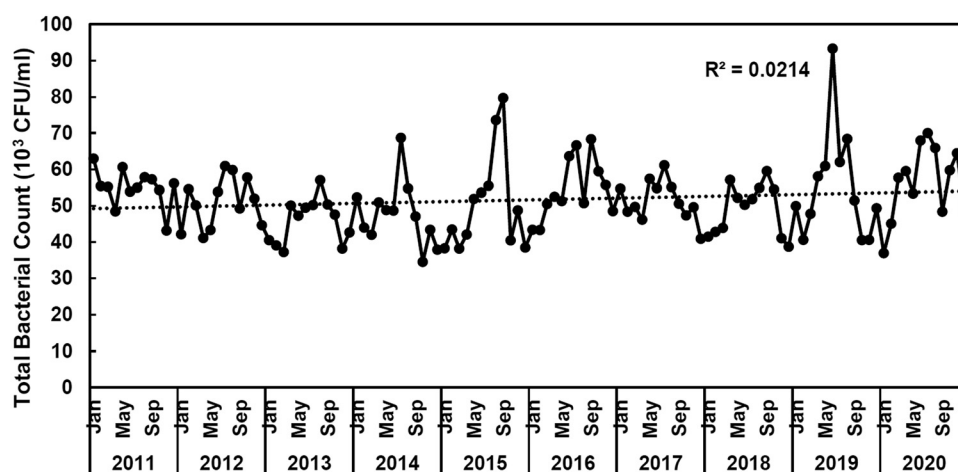


Fig. 6. Average total bacterial count (TBC) (10^3 CFU ml^{-1}) between 2011 and 2020

In the case of SCC the minimum values were measured either in spring (March and April) or in late autumn (November and December), but the maximum values were concentrated in summer (July and August) during the

analysed 10-year period (Fig. 7). Monthly SCC values within the samples ranged on average between 238 and 328×10^3 cells/ml (mean $270 \pm 21 \times 10^3$ SD; SE = 2×10^3) with the lowest values detected in 2014 and the highest in 2018.

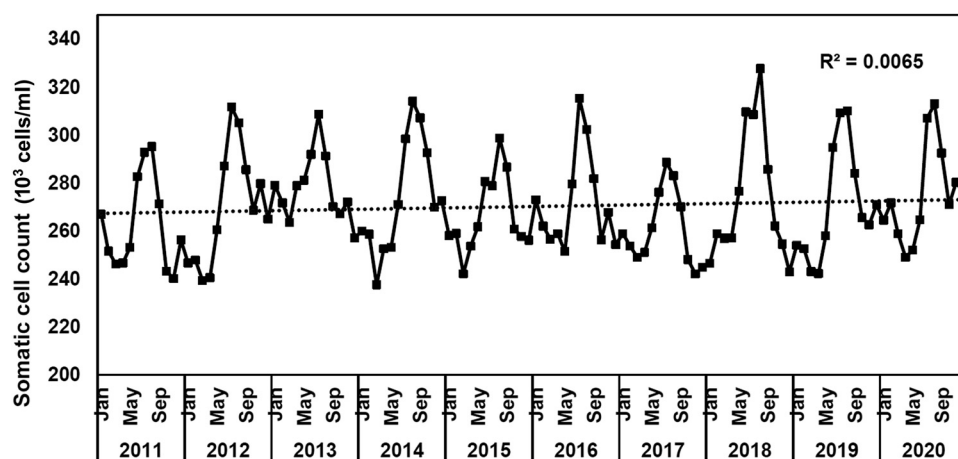


Fig. 7. Average somatic cell count (SCC) (10^3 cells/ml) between 2011 and 2020



There was a slight but not remarkable increasing trend of mean SCC content in the observed 10-year period ($R^2 = 0.06$).

DISCUSSION

Here we present results from a large-scale raw milk study spanning a decade up to the year 2020. Such an extended period of scientific study on the various chemical parameters of raw milk on a country-wide scale is unique. Similar studies have been carried out in several countries but focusing over a much shorter period (Auldist et al., 1998; Jensen, 2002; Lindmark-Mansson et al., 2003; Heck et al., 2009; More, 2009; O'Connell et al., 2015). For example, it has been reported that the lactose concentration of Dutch raw milk was constant during the season from February 2005 until February 2006 (Heck et al., 2009). Milk protein content changed according to the season. The lowest protein content was detected in June (3.21 g/100 g) while the highest content in December (3.38 g/100 g). Increasing concentration of milk fat was observed during the milking season. An increasing tendency of milk fat content from a minimum of 4.10 g/100 g in June to a maximum of 4.57 g/100 g in January was detected by Heck et al. (2009). General changes in the Dutch dairy diet composition could induce seasonal variation of protein and fat concentration in the raw milk (Heck et al., 2009).

Salfer et al. (2019) analysed the annual rhythms of milk yield, milk fat and milk protein concentration using the data of the national milk market and cow-level data in the United States. Milk fat concentration reached its maximum value in January, while the milk protein peak was observed in December. Annual changes in the components of milk (fat and protein) and milk yield were observed at both regional and cow levels in the USA. These changes were independent of environmental factors (e. g. heat stress).

From November 1995 until November 1996 a complex investigation of changes in the composition of raw milk was carried out in Sweden (Lindmark-Mansson et al., 2003). Every other month samples were collected from nine dairies in the country. The average fat, protein and lactose content was 4.34%, 3.37% and 4.62%, respectively. From the examined 140 parameters most components (~90) showed seasonal variation. The values of 27 parameters showed the effect of geographical location. A significant increase in the milk fat content (from 4.03% to 4.34%) was detected in comparison with a previous monitoring of raw milk quality in the 1970s (Lindmark-Mansson et al., 2003). In our ten-year-long, nationwide study in Hungary a decrease in yearly sample numbers was observed between 2011 and 2020. The reason for this is related to a decrease in the number of dairy farms in Hungary. The number of dairy farms included in this study was 984 in 2011 and 797 in 2020. As the number of dairy cows is constantly growing in Hungary, the decrease in the number of dairy farms is caused by the concentration of milk production on fewer but bigger farms. Another reason for the decrease in the number

of samples is that several farmers switched from sending three samples per month (as required by the previous, so-called ten-day test system) to sending two samples per month allowed by the current legislation in force.

The evaluation of the average fat, protein, SNF and lactose content of domestic raw milk shows a seasonal cyclicity of those parameters, with a similar trend every year from 2011 to 2020. The highest fat, protein and SNF contents are found in the winter months, while the lowest contents in the summer months.

The cyclicity of milk content parameters within a year may result from the effect of a number of factors affecting milk production and their interaction with one another.

Such influencing factors may be the breed, the genetic stock, the feeding system, animal husbandry conditions, lactation phase, milking systems, climatic effects, etc. The Holstein-Friesian breed of cattle is widespread on dairy farms in Hungary, with only a small proportion of the Jersey breed (Béri, 2013). Therefore, the domestic dairy stock can be considered constant for the evaluation of the average data. Feeding dairy cows significantly affects the quality characteristics and content composition of the produced milk. In Hungary, three types of feeding systems are typically used in dairy farms: seasonal, monodietic and combined feeding (Gál et al., 2016). Grazing dairy cattle are not typical in Hungary, so milk production is continuous and can be planned throughout the year. The effect of Hungarian feeding systems on the cyclicity of milk content parameters within a year was not examined in our present work. It would be interesting to examine whether the animal husbandry systems and milking systems typically used in Hungary have an effect on milk content parameters. Seasonal changes in milk content parameters are most likely related to climatic effects. Nagy et al. (2019) experimentally proved the hypothesis that environmental factors (e.g. the photoperiod) can significantly influence the composition of bulk dromedary camel milk.

Hungary has a temperate continental climate. In the largest part of Hungary (the majority of the Great Plain and the Little Plain), where dairy stocks are concentrated, the moderately warm, dry climate range is typical. The relationship between climatic characteristics and the seasonal variation of milk content parameters requires further investigation. Varga (2021) studied the agro-climatological conditions of Mosonmagyaróvár for the period 1991–2020. An increasing number of summer days (57.2), heat days (33.2) and hot days (4.1) was registered in this area in the period between 2011 and 2020. It can be stated that the frequency of summer days above 25 °C increased by 15% compared to 1961–1990; moreover, in the last decade of the last 30 years, these occurred 7% more frequently than in 1991–2000. However, the frequency of above 30 °C daily maximum temperatures increased to a greater extent compared to the mean of the earlier 30 years' average values. Based on the current 30-year average, twice as many hot days, while in the 10-year period between 2011 and 2020 three times as many such hot days were experienced (Varga, 2021). It would be worthwhile to carry out comparative



studies to find the correlations between the change in individual local agrometeorological characteristics and the change in the values of regional milk quality parameters. Interestingly, lactose content shows a different annual periodicity compared to fat, protein and SNF. Although some literature references have found a winter maximum and a summer minimum in lactose values, for example in Croatia (Dobranic et al., 2008) and in Pakistan (Yasmin et al., 2012), our large-sample study clearly showed an autumn minimum and a spring maximum pattern. There could be several reasons for the upward trend in lactose content, which needs further investigation. Possible reasons at the national level could include the increase in the proportion of first-calving animals due to herd expansion or due to a decrease in the average total number of cows in the herds (resulting in an increase of the proportion of animals in the first lactation). Another reason could be a genetic shift (breeding with a sire herd that inherits this trait) having an impact on the lactose level.

It is worth mentioning that in terms of the TBC and SCC of domestic raw milk, the values of the qualifying samples were in line with the legal requirements, thus it can be stated that the quality of domestic raw milk was excellent during the period under study. Although there are differences in the monthly average values of TBC at different periods of the year, no clear seasonal cyclicity can be detected. In the case of the SCC, it can be stated that the minimum values are characteristic of a much wider period, from November to April. This observation is similar to the results obtained in Croatia (Dobranic et al., 2008) and contradicts that previously described in the United States (Coleman and Moss, 1989).

The following conclusions can be drawn from our large-scale, decade-long study conducted in Hungary, in which we focused on the development of the annual sample number, the most relevant milk content parameters (fat, protein, SNF and lactose) and the two most important key parameters for milk quality assessment (TBC and SCC).

Analysis of the monthly mean values of Hungarian bulk milk samples for fat, protein and SNF revealed a similar pattern, showing recurring cyclicity with elevated values in winter and decreased values in summer. These significant differences are most likely due to the annual changes in climatic factors in Hungary.

The monthly mean values of lactose content show different annual periodicity compared to the fat, protein and SNF, and compared to results of other studies carried out in other countries. In Hungary, the minimum lactose values were measured in autumn and the maximum values in spring. We suggest that in Hungarian milk production an influencing factor stronger than climate may contribute to the development of milk lactose content. Possible reasons could be an increase in the proportion of first-calving animals or a genetic shift, which needs further investigation.

Concerning the average monthly values of TBC, no clear seasonal cyclicity was observed in Hungary, possibly meaning that there is no such general factor that significantly influences the nationwide TBC average values. In the case of SCC the minimum values are characteristic of a

much wider period, from November to April. The maximum values are concentrated in a two-month period in summer. Since SCC values are highly dependent on the health status of herds, the maximum values observed in the summer months are related to the adverse health effects of higher monthly mean temperatures. In order to monitor the tendencies of raw milk quality and to analyse whether the detected changes can negatively influence the properties of milk, this large-scale study should be continued in the future.

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