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ORIGINAL PAPER

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MILK QUALITY DETERMINED USING CHEMICAL ANALYSIS AND MICROWAVE PLASMA ATOMIC EMISSION SPECTROMETRY AS A FUNCTION OF SEASONALITY IN TWO CONVENTIONAL ITALIAN DAIRY FARMS

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ABSTRACT

Milk contains macrominerals and trace elements which are essential for animal and human nutrition, but it also contains elements whose biological role is not well known and which could even potentially be toxic. The investigation reported in this research paper aimed to evaluate the mineral content of milk from two different seasons (summer and winter) on two different conventional Italian farms within the Lombardy region (Italy). The mineral composition (macrominerals: calcium, potassium, magnesium; microelements: iron, copper, zinc, selenium, manganese; heavy metals: cadmium, chromium, barium) and proximate composition were analyzed on bulk tank milk collected weekly. The results of the proximate composition showed values comparable to other studies and the bovine milk market; however, the season influenced the protein content, which was higher ($p < 0.05$) in winter than summer. The mineral content of the analyzed milk varied between the seasons ($p < 0.005$) in all minerals, except for iron, chromium and zinc. No effects were found from the farm, except for in Fe and Mn. Cd was only detected on one farm as a trace element. The current study shows that milk composition and mineral concentration vary little between dairies, confirming the typical composition of Holstein Friesian milk cows.

Keywords: bulk tank milk, macroelements, microelements, heavy metals, chemical composition, season

INTRODUCTION

Milk is characterized by a particularly high nutritional value due to its balance of nutrients. Furthermore, it is an important source of water- and fat-soluble vitamins and minerals, including calcium, phosphorus and magnesium (Haug et al., 2007). The major milk minerals are essential not only for nutritional value, but also for the technological properties of milk. The mineral concentrations and distribution in milk are important contributors to optimal milk coagulation during cheese making (Manuelian et al., 2018). Numerous studies have investigated sources of variation in the mineral content of milk, showing that mineral content is influenced by the stage of lactation, nutritional status and climate (Gaucheron, 2005; Heck et al., 2009;

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Spiteri and Attard, 2017; Agius et al., 2019). A good knowledge of the influence of the season on the mineral content of milk could be helpful in improving dietary mineral content in the most efficient, economic and natural possible way. Moreover, trace elements in cow's milk are of interest because of their essential or toxic nature; for instance, Cr and Mn are essential but may become toxic at higher levels, whereas Pb and Cd are toxic (Gaucheron, 2013) and can accumulate over time. The latter two are amongst the elements that have raised concern in terms of adverse effects on human health because they are readily transferred through food chains (Liu, 2003).

Cadmium is classified as an element with a carcinogenic effect, and its embryotoxic and teratogenic effects have also been confirmed. The main source of cadmium contamination in soil is industry, phosphorus fertilisers and wastes (Sujka et al., 2019). The standard limit value of Cd in milk (0.0026 µg/g) is outdated (IDF, 1979), but it is still the only acceptable maximum limit of Cd level in milk, as recently reviewed by Boudebbouz et al. (2021).

Therefore, today milk producers and the dairy industry should provide the necessary information on the quality of milk to the consumer. In fact, the dairy industry and retailers include nutritional facts in their public communications linking the quality of dairy products with human health. The mineral content of macrominerals and microelements in particular is highly variable throughout the year. On the other hand, heavy metals, though investigated through research, are rarely taken into account in commercial dairy products. In a geographical area that is linked to a protected designation of origin (PDO), the characterization of milk to be used for traditional Italian cheese which takes into account the mineral content could be more relevant.

This study aimed to determine any seasonal and/or farm differences in a specific geographical area linked to Grana Padano production.

MATERIALS AND METHODS

Animals and management

A total of 127 multiparous Holstein Friesian cows on two farms (Farm 1 and Farm 2) located in northern Italy were used for the study. All housing and care conditions conformed with the standards recommended by the Guide for the Care and Use of Laboratory Animals (EU, 2010) and Directive 1998/58/EU (EU, 1998), concerning the protection of animals kept for farming purposes. Both farms belong to the geographical production area of Grana Padano (EU, 2011).

The herd of Farm 1 consisted of 73 lactating cows with an average milk production equal to 29.05 L/day ±1.31 with a lactation period of around 214 days. The herd on the second farm consisted of 54 cows with an average milk production equal to 37.9 L/day ±2.53.

Experimental organization

Unprocessed milk was sampled after agitation from dairy bulk tanks. Samples were collected once a week, in the morning, from two conventional Italian dairies (Farm 1 and Farm 2) of the Lombardy region, in April–July 2014, and in November–March 2015 period. Samples taken in April–July were classified as summer samples and those taken in November-March as winter samples. The milk samples were packed with ice and transported directly to the laboratory, divided into subsamples and kept at –20°C until analysis.

Milk pH was determined using a pH meter (Mettler Delta 340) liquid electrode, with direct immersion in tube containing the sample and taking three measurements. Milk fat content was quantified using the Gerber method based on the double action of sulphuric acid and isoamyl alcohol that separated fat percentage from other components. The total protein content and ash content were analysed using the method AOAC (2000) and AOAC (2005) respectively.

The concentration of minerals in the milk samples was determined using Microwave Plasma-Atomic Emission Spectrometry (MP-AES 4100, Agilent Technologies, Santa Clara, USA).

Sample preparation

Briefly, the milk samples (2 mL) were treated with 5% HNO_3 and heated on a hot plate at 80°C for approximately 75 minutes. The samples were ashed at a temperature of 500°C for 4 hours and made up to a 50 mL volume by dissolving the ashes in 5 ml of 5% nitric acid, before being topped with deionized water (Spiteri and Attard, 2017).

Each metal was calibrated against a known set of standardized concentrations at the optimal wavelengths (nm). The instrument and metal parameters are listed in Table 1 and Table 2. The MP-AES analyzed the samples in triplicates, and intensity values were converted into concentration values against the mineral calibration curves using Agilent MP-expert software.

Table 1. MP-AES configuration and operating conditions

Parameters	Settings
Pump speed (rpm)	15
Sample introduction	manual
Number of replicas	3
Stabilization time (s)	15
Uptake time (s)	15 (using fast pump)
Nebulizer	Agilent One-Neb (pneumatic concentric nebulizer)
Spray Chamber	Double-Pass Glass Cyclonic spray chambre for Agilent MP-AES

Table 2. Wavelengths of the minerals being studied

Elements	Wavelength, nm
Ba	455.403
Ca	616.217
Cr	425.433
Cu	324.754
Fe	259.940
K	766.491
Mg	518.360
Mn	403.076
Zn	213.857
Cd	228.802

Statistical analysis

The data are expressed as means ±SEM. Data concerning the chemical composition and the mineral content of milk were analyzed using multivariate analysis, inserting the farm and the season as a fixed effect. The effect of replication related to the triple reading of the instrument was tested separately and, considering that it was not significant for any of the minerals examined, it was subsequently excluded from the model. The effects were deemed to be significant at $p < 0.05$, and a trend was noted when at $p < 0.10$. Statistical analyses of the data were performed using SPSS software (SPSS Inc., Chicago, Illinois).

RESULTS AND DISCUSSION

Proximate analysis for bulk tank milk samples on Farm 1 and Farm 2 related to the summer and winter seasons are illustrated in Table 3; the pH value falls within an optimal range between 6.5 and 6.9 (Sinaga et al., 2017) without seasonal differences. Fat content was similar to the values found by Palladino et al. (2010) in Holstein Friesian milk; small but significant fluctuations of protein and ash were found between the farms and seasons. A low protein concentration in summer in comparison to that in winter agrees with other studies (Poulsen et al., 2015; Heck et al., 2009; Ivanova et al., 2017), confirming that protein is an indication of seasonal differences in feeding. In fact, it can be speculated that the effects of heat stress in ruminants as reported by Silanikove (2000) could lead to a decrease in milk protein composition. The results for ash content showed that summer milk had a significantly higher value, although the difference was rather small and the biological significance for the consumer is not relevant.

Table 4 shows the mineral content found in bulk milk samples and a comparison between them based on farms and seasonality. No difference between farms was found, except for in Fe, Cd and Mn content. Ca in milk is generally distributed between micellar and aqueous phases and has an average concentration of 1200 mg/L (Poulsen et al., 2015; Gaucheron, 2011). Rodríguez Rodríguez et al. (2001) and Tsioulpas et al. (2007) reported Ca values in the range of 1000-1758 mg/kg and 970 mg/L, respectively. The Ca concentration found here exhibited lower values than those listed before, but the average value of both farms was 600 mg/kg, which is in accordance with Gabryszczuk et al. (2010).

Table 3. Proximate analysis of bulk tank milk from Farm 1 and Farm 2 in two different seasons

	Farm		Season		P	
	farm 1	farm 2	summer	winter	farm	season
Fat, %	3.84 ±0.04	3.65 ±0.05	3.81 ±0.03	3.65 ±0.07	NS	NS
Protein,%	3.21 ±0.03	3.18 ±0.02	3.12 ±0.02	3.27 ±0.02	0.017	<0.05
Ash, %	0.71 ±0.04	0.66 ±0.08	0.70 ±0.00	0.65 ±0.00	0.007	NS
pH	6.83 ±0.00	6.84 ±0.00	6.85 ±0.00	6.84 ±0.01	NS	NS
DM, %	12.78 ±0.25	12.76 ±0.26	12.78 ±0.04	12.76 ±0.04	NS	NS

NS – not significant; $p < 0.05$; DM – dry matter.

Table 4. Main mineral values contained in bulk milk and their comparison based on farm and seasonality

	Farm		Season		P	
	farm 1	farm 2	summer	winter	farm	season
Ca, mg/kg	731.00 ±71.67	472.00 ±60.64	819.00 ±19.98	326.00 ±53.90	NS	<0.05
Fe, mg/kg	1.96 ±0.59	0.90 ±0.20	1.02 ±0.29	1.74 ±0.56	0.021	NS
K, mg/kg	618.00 ±111	493.00 ±72	823.00 ±88.05	243.00 ±49.59	NS	<0.05
Mg, mg/kg	92.95 ±8.9	75.58 ±7.5	104.00 ±5.91	59.00 ±8.33	NS	<0.05
Se, µg/kg	4.65 ±1.47	3.40 ±1.13	7.50 ±1.44	0.00 ±0.00	NS	<0.05
Ba, µg/kg	0.99 ±0.09	0.81 ±0.06	1.09 ±0.08	0.66 ±0.04	NS	<0.05
Cd, µg/kg	0.00 ±0.00	0.07 ±0.02	0.00 ±0.00	0.08 ±0.02	0.007	0.007
Cr, mg/kg	0.01 ±0.01	0.00 ±0.00	0.00 ±0.00	0.01 ±0.00	NS	NS
Cu, mg/kg	0.17 ±0.02	0.70 ±0.5	0.21 ±0.01	0.75 ±0.06	NS	NS
Mn, mg/kg	0.11 ±0.02	0.08 ±0.02	0.04 ±0.01	0.15 ±0.02	0.033	<0.05
Zn, mg/kg	3.79 ±0.20	3.19 ±0.15	3.65 ±0.20	3.23 ±0.14	NS	NS

NS – not significant; $p < 0.05$.

The K content in milk showed a mean value of 555.5 mg/kg. Our values are quite far from those reported in the literature. For instance, Park et al. (2007) reported an average K concentration of about 1520 mg/kg, Gaucheron (2005) a concentration equal to 1212 mg/kg and Bilandžić et al. (2015) values equal to 1900 mg/kg. However, our data agree with those found by Gabryszczuk et al. (2010) and Barłowska et al. (2006) in Polish Holstein Friesian cows (black-white variety).

The average content of Mg detected in the two different farms (84 mg/kg) agrees with that found by Sola-Larrañaga and Navarro-Blasco (2009) and Poulsen et al. (2015) (91.8 mg/L and 106–112 mg/kg respectively).

The Cu content found in the present paper (about 0.43 mg/kg) is comparable with results found by Moreno-Rojas et al. (1993) (0.1 mg/kg) and Barłowska et al. (2006) (0.49–0.56 mg/L). Furthermore, Bilandžić et al. (2010) stated that Cu concentrations between 0.1

and 0.9 mg/L are within the ordinary milk range. It is known that Cu deficiency is a common nutritional problem in ruminants farmed extensively (Gooneratne et al., 1985; Hill and Shannon, 2019) due to forages that have been reported to be marginal or deficient in Cu. More recently, rather than the copper content of the soil or herbage, the principal determinants of copper deficiency in grazing cattle are soil molybdenum, soil pH (high values encourage Mo uptake), sward maturity, rainfall, fertilizer use and soil ingestion. Low concentrations and poor availability of Cu is often due to interactions with other nutrients in the soil. Low concentrations were reported by other authors, for instance: Sola-Larrañaga and Navarro-Blasco (2009) and Bilandžić et al. (2015) reported an average Cu milk concentration of 0.055 mg/L and 0.01mg/kg respectively. Particularly low concentrations of 0.0377 mg/L and 0.0453 mg/L were found in Simmental and Friesian breed cows from organic farms respectively, indicating the lack of this element in animals traced back to the environment and ration (Pilarczyk et al., 2013).

Both the content of Fe and Zn in milk found in our study are comparable with the scientific literature (Park et al., 2007). There is no plausible explanation for the significant difference between Farm 1 and Farm 2 for Fe content. The content of selenium in both farms is in line with the range (3.42–25.08 µg/kg) reported by different authors and presented by Licata et al. (2004). Using the criteria proposed by Puls (1988) to diagnose the presence of selenium deficiency in bovine milk, our results fall in the lower range.

The Mn mean value of 0.09 mg/kg found herein is in line with the range of 0.04 mg/kg and 0.2 mg/kg reported by Barłowska et al. (2006), Bilandžić et al. (2015) and Park et al. (2007). Even if a statistical significance between farms was found, the numerical values are very similar and from a qualitative point of view negligible.

The presence of Cr was found only in Farm 1. Its extremely low value (0.01 mg/kg milk) agrees with the results of Zamberlin et al. (2012), who reported a mean value between 0.01 mg/kg and 0.04 mg/kg. Bilandžić et al. (2015) found a content equal to 34 µg/kg. However, the presence of chromium remains well below the limit set for human food by the Council of Europe, which is 60ppm for the trivalent Cr and 0.05 ppm for the hexavalent Cr.

Cadmium (Cd) is a heavy metal present in the environment both for natural phenomena and as a consequence of industrial and agricultural processes. For the general population, with the exception of smokers, the main source of cadmium exposure is food. This metal is responsible for a multiplicity of toxic effects on human health. The European Food Safety Authority (EFSA, 2009) further lowered the tolerable weekly intake for Cd from the initial 7 µg/kg to the current 2.5 µg/kg of body weight. In the present survey, we found traces of cadmium only on farm 2; the content found is absolutely negligible, and it was well below the values provided for by Regulation (EC) 1881/2006 (EU, 2006) and subsequent amendments in Regulation (EU) 488/2014 (EU, 2014). In other studies, high concentrations of Cd in milk were reported on dairy cattle farms situated close to sources of pollution (Swarup et al., 2005; Patra et al., 2008; Król et al., 2012). Farm 2 is located near a very busy road and the traces found can probably be attributed to the location of the farm. Barium was found in traces at levels comparable to a study by Norakmar et al. (2017).

In general, as shown in Table 4, for most of the minerals analysed (Ca, K, Mg, Se, Mn, Ba, Cd), significant differences emerged based on the season, where most of the minerals present a higher concentration in the summer. In fact, milk ash content, which is rich in minerals, is higher in the summer season than in winter. No effects of season were detected for Fe, Cr, Cu or Zn.

We observed a lower ($p < 0.05$) Ca average concentration in the winter period than in the summer, with a mean value between the two seasons of 572.5 mg/kg. In accordance with Ross et al. (2012), the higher Ca content is due to an increased amount of sunlight during the summer months leading to an increase in the production of vitamin D, and hence higher Ca levels in the milk.

In the present survey, the data collected related to the content of Fe and Cu were not affected by seasonal effects, which is in accordance with a study by Rodriguez et al. (2001). This study showed that the content was constant over twelve months of the year, amounting to 0.52 mg/kg and 0.08 mg/kg respectively.

It was observed that Se was found only in the summer season, with an average concentration of 7.5 µg/kg. In accordance with different authors, Se, Zn and Mg content is significantly higher in the summer season than in the winter (Rodriguez Rodriguez et al., 2001; Poulsen et al., 2015; Barłowska et al., 2006).

CONCLUSION

In conclusion, the present work provides information about the mineral composition of milk. The current study shows that milk composition and mineral concentration vary little between farms, confirming the typical composition of milk from the Holstein Friesian breed. The fact that the composition remained almost constant confirms its suitability in the manufacture of typical PDO cheese such as Grana Padano, which is produced in the production area.

Much of the data was consistent with earlier studies on seasonal variation. The variation in mineral concentration between spring-summer and autumn-winter confirms the influence of seasonality on the concentration of minerals in milk. In all the milk samples, toxic element concentrations were well below those set by the European Union Regulation. The location of the farm has a significant impact on the content of cadmium in the cow's milk. Further research is needed to establish the relationship between the composition and properties of milk and the production capacity and quality of products, taking into account more farms.

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