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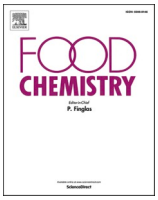

Does the age of milk affect its mid-infrared spectrum and predictions?

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ABSTRACT

Milk of dairy species commonly undergo standardized official analyses, these that may require chemical preservation and transportation to a certified laboratory. In this context, storage duration is an important factor that can potential affect both milk chemical analyses and its mid-infrared spectrum. We analysed milk samples at different time points/ages to assess repeatability and reproducibility of mid-infrared predicted traits (e.g., fat and protein). Using spectral data, we also evaluated the ability of spectroscopy coupled with chemometrics to discriminate samples according to their age. Although the main components of milk remained consistently reproducible across age (days), changes in the spectrum due to sample aging and deterioration of the matrix were detectable. Using a discriminant analysis, we achieved a classification accuracy of 77% in validation. Predicting milk age using mid-infrared spectra is feasible, allowing for sample monitoring within circuits where maximum reliability is needed, e.g., bulk or individual milk samples for legal/official use or payment systems.

1. Introduction

Milk information retrieved from official recording system is fundamental for management adh, milk pament and breeding purposes in dairy species. In Italy, the official recording system requires a representative milk sample to be collected longitudinally from each lactating animal, usually every four or five weeks in accordance with International Committee for Animal Recording (ICAR) guidelines. To avoid bacterial proliferation and impaired composition, milk samples have a preservative added at sampling (ICAR, 2022). As dairy farms can be located in marginal areas at non-negligible distance from the laboratory in charge of the analysis, it is not infrequent that milk samples are not processed within the conventional time (24–48 h). An optimal milk preservative should maintain the physical and chemical properties of the milk during the period between sampling and analysis under the locally applicable temperature and transport conditions (ICAR, 2020). The combination of cooling (4 °C) and short-term storage with a preservative such as Bronopol (2-bromo 2-nitro 1,3-propandiol) or Azidiol is recommended for conventional milk analysis worldwide, allowing for a good preservation of microbiologically uncontaminated milk (ICAR, 2020). According to ICAR (2012), Bronopol is the most popular preservative, at final milk concentrations from 0.02 to 0.1 %.

In addition to concentration and type of preservative, storage conditions can influence the results of milk analysis (Monardes et al., 1996; Wentz et al., 2018). During the aging of milk, bacterial, enzymatic, and chemical processes can occur naturally, altering the overall composition of milk. Sjaunja (1984) reported that preserved milk can undergo infrared analyses within five d from sampling without impairment of prediction accuracy. On the other hand, Damm et al. (2017) reported that the determination of somatic cell count (SCC) and differential somatic cell count (DSCC), the latter being the ratio of the sum of polymorphonuclear neutrophils and lymphocytes to the SCC, is less robust in milk samples added with preservative, stored at room temperature, and analysed after four d. This suggests that the output of benchtop instruments like CombiFoss^{TM} 7 (Foss Electric A/S, Hillerød, Denmark) equipped with both infrared scan and flow cytometer may be affected by sample age to a certain extent. A reduction in SCC and DSCC robustness can start to occur after three d in samples stored at 5 °C without preservative (Damm et al., 2017).

For these reasons, storage length is important also for bulk milk samples, whose composition is normally assessed for payment systems of milk buyers like dairy companies or cooperatives.

Mid-infrared spectroscopy, the gold standard for bovine milk gross composition (ISO, 2013; De Marchi et al., 2014), is routinely used worldwide to assess individual and bulk milk composition in different contexts and uses. Within the dairy industry, the use of Fourier

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transform infrared spectroscopy (FTIR), a technique to obtain an infrared spectrum of absorption, is fundamental to assess compounds such as content of fat, protein, and lactose as well as concentration of fatty acids and minerals. The FTIR spectral data coupled with chemometric and statistical analyses have also been used to predict complex traits at individual (e.g., cow health or metabolic status; Benedet et al., 2019) or bulk level (e.g., technological properties; Visentin et al., 2015). What is more, recently, studies have revealed how milk spectra can be used for prediction of cows' feed efficiency (Shetty et al., 2017) and methane emissions (Vanlierde et al., 2013). The use of FTIR spectra to predict difficult-to-measure traits is of high interest due to the possibility of simultaneously obtaining a wide variety of traits at population level and at low cost.

It, therefore, becomes obvious that good quality spectra are required to guarantee reliable predictions and that variation in milk storage conditions such as duration may be minimized to avoid biased spectra, imprecise phenotypes prediction, and fraud.

In the present study, we estimated repeatability (r) and reproducibility (R) of milk analyses obtained from FTIR on the same samples i) at different time points and ii) using two benchtop instruments. Finally, by combining FTIR data and chemometrics, we tested the ability of mid-infrared spectroscopy to discriminate the sample age of milk.

2. Materials and methods

2.1. Samples collection and analysis

An individual milk sample (800 mL) from each of 10 Simmental cows was collected on the same day in the experimental farm of the University of Padova (Legnaro, Italy). Cows were of different parity and, overall, represented all lactation stages (Table 1). Immediately after collection, samples were refrigerated and transported to the milk laboratory of the Breeders Association of Veneto Region (ARAV, Vicenza, Italy). After homogenization, each sample was partitioned into 16 aliquots of 50 mL each (Fig. 1): 15 aliquots had Bronopol preservative added and 1 was the blank control (no preservative) used for bacterial count via BactoScan™ FC+ (Foss Electric A/S, Hillerød, Denmark). The 150 aliquots with preservative were used for composition analysis and spectra acquisition through the CombiFoss™ 7 (Foss Electric A/S, Hillerød, Denmark) at different time points, in such a way that every day, from day 0 (sampling date) to four (sampling date + four d), three aliquots of each cow were analysed. Following the standard procedures (ICAR, 2020), before entering the benchtop instrument, aliquots were warmed at 37 °C and gently inverted. Each aliquot was processed in sequence using the two CombiFoss™ 7 devices available in the laboratory. The final dataset contained predicted milk composition traits and associated spectra of 300 analyses.

2.2. Variances estimation

The CombiFoss™ 7 machine, equipped with an infrared sensor and a flow cytometer, provided the following information for each sample: fat, protein, lactose, and casein content (%), milk urea (mg/dL), freezing point (°C), SCC (cells/mL), and DSCC (%). To achieve a normal distribution of data points and homogeneity of variance, SCC was transformed to SCS through the formula of Ali and Shook (1980): $SCS = 3 + \log_2(SCC/100{,}000)$.

Variance components subsequently used to assess r and R of milk traits, were estimated using the following mixed linear model in R software v 4.1.3, through the 'lme4' package (R Core Team, 2023):

$$y_{ijk} = \mu + S_i + I_j + Cow_k + (S \times I)_{ij} + (S \times Cow)_{ik} + (I \times Cow)_{jk} + e_{ijk}, \quad \text{(Eq. 1)}$$

where y_{ijk} is the milk trait predicted via FTIR; μ is the overall intercept of the model; S_i is the random effect of the ith sample age ($i = 0$ to four d); I_j is the random effect of the jth instrument ($j = 1, 2$); Cow_k is the random effect of the kth cow ($k = 1$ to 10); $(S \times I)_{ij}$ is the random interaction effect between sample age and instrument; $(S \times Cow)_{ik}$ is the random interaction effect between sample age and cow; $(I \times Cow)_{jk}$ is the random interaction effect between instrument and cow; and e_{ijk} is the random error term. All random effects were assumed to be independently and normally distributed, with mean zero and proper variance, i.e., σ^2_S, σ^2_I, σ^2_{Cow}, σ^2_{SxI}, σ^2_{SxCow}, σ^2_{IxCow}, and σ^2_e for sample age, instrument, cow, their interactions, and residual variance, respectively.

Formulas of r and R were retrieved from the International Organization for Standardization (ISO, 1994a; ISO, 1994b). In particular, r evaluates the agreement between repeated predictions of milk traits under the same conditions. Specifically, in the present study r was defined as the value below which the absolute difference between two measures of the trait on milk sample of the same cow in the same sample age and instrument is expected to lie within a probability of 95 % (ISO, 1994a; ISO, 1994b), and it was calculated using the σ^2_e from (Eq. 1), as:

$$r = 1.96\sqrt{2\sigma^2_e}$$

The R is conventionally used in ring tests to evaluate the consistency of measures provided by different laboratories. In this study, both the sample age R (R_S) and the instrument R (R_I) were calculated to evaluate the consistency of milk traits obtained in different sampling dates and from the two devices, respectively. They were defined as the value below which the absolute difference between two predictions of milk traits on the same milk sample under different conditions (i.e., different sample age or instrument) is expected to lie with a probability of 95 % (ISO, 1994a; ISO, 1994b). The R_S and R_I were computed using the appropriate variance components from (Eq. 1), as:

$$R_S = 1.96\sqrt{2(\sigma^2_S + \sigma^2_{SxCow} + \sigma^2_{SxI})}$$

$$R_I = 1.96\sqrt{2(\sigma^2_I + \sigma^2_{IxCow} + \sigma^2_{SxI})}$$

The coefficients of repeatability (r%) and reproducibility (R%) were also calculated. The first is an indicator of the degree of agreement between repeated predictions of milk traits obtained on the same milk sample, whereas the second is an indicator of the degree of agreement between predictions of milk traits for the same milk sample at different sample ages (R_S%) or obtained from different instruments (R_I%). They were computed as:

$$r\% = \frac{\sigma^2_{Cow} + \sigma^2_S + \sigma^2_I + \sigma^2_{SxCow} + \sigma^2_{IxCow} + \sigma^2_{SxI}}{\sigma^2_{Cow} + \sigma^2_S + \sigma^2_I + \sigma^2_{SxCow} + \sigma^2_{IxCow} + \sigma^2_{SxI} + \sigma^2_e} \times 100$$

$$R_S\% = \frac{\sigma^2_{Cow} + \sigma^2_I + +\sigma^2_{IxCow}}{\sigma^2_{Cow} + \sigma^2_S + \sigma^2_I + \sigma^2_{SxCow} + \sigma^2_{IxCow} + \sigma^2_{SxI} + \sigma^2_e} \times 100$$

$$R_I\% = \frac{\sigma^2_{Cow} + \sigma^2_S + +\sigma^2_{SxCow}}{\sigma^2_{Cow} + \sigma^2_S + \sigma^2_I + \sigma^2_{SxCow} + \sigma^2_{IxCow} + \sigma^2_{SxI} + \sigma^2_e} \times 100$$

2.3. Discriminant analysis

Milk spectra were paired with the reference value of sample age and

Table 1
Overview of the cows' characteristics on the sampling date.

Cow	Parity	Days in milk
1	8	57
2	6	131
3	3	57
4	2	177
5	2	49
6	1	281
7	1	169
8	1	217
9	1	56
10	1	80

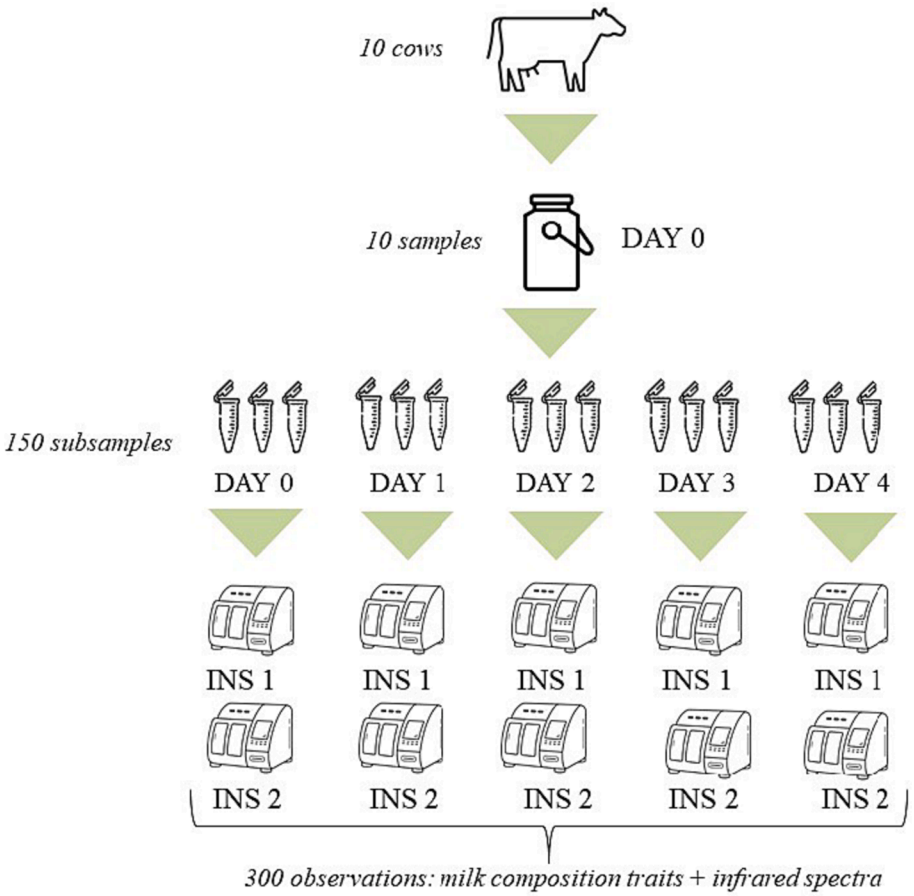

Fig. 1. Experimental design for the analysis of milk samples at different sample age[1] (DAY) and with two benchtop instruments (INS). [1]number of days between the sampling date and the date of analysis.

for each wavelength the value was transformed from transmittance (T) to absorbance (A) through the formula: $A = \log_{10}(1/T)$. Spectral regions known to be associated with noisy water absorption wavelengths were discarded (De Marchi et al., 2014), leading to 338 spectra wavelengths available in the following intervals: 945.5 to 1585.6 cm^{-1}, 1716.8 to 1929.0 cm^{-1}, 2507.7 to 2970.7 cm^{-1}. No spectral outliers were identified using the Mahalanobis distance (threshold = 3.0). Partial least squares discriminant analysis was carried out using the 'trainControl' function available in the R package 'caret' (Kuhn, 2008). The dataset was split into a calibration set of 210 observations (seven cows) and a validation set of 90 observations referred to as three "external" cows so that samples used for validation were independent from those used for calibration. Models were fine-tuned using 10-fold cross-validation repeated three times and the number of components was set automatically but capped at a maximum of 15 to avoid overfitting. Spectral data points were mean-centered and scaled, and discrimination was performed based on class probabilities. The model performance included sensitivity, specificity, positively predictive values, negatively predictive values, and balanced accuracy in both calibration and validation. The balanced accuracy is the mean of sensitivity and specificity for each sample age, and positively and negatively predictive values are the proportions of positive and negative results that are true positive and true negative results, respectively. The most important spectral regions for discrimination were identified using the importance score, which is based on weighted sums of the absolute regression coefficients. The 'varImp' function of the R package 'caret' was used. The weights are determined based on the reduction of the sums of squares across various components. In this case, the weights were computed separately for each outcome, and the contribution of the coefficients was weighted proportionally to the reduction in the sums of squares (Kuhn, 2008).

3. Results

3.1. Samples

Overall, 16 aliquots per cow were available: one was intended for BactoScan™ FC+ (Foss Electric A/S, Hillerød, Denmark) at day 0, whereas the others were processed as depicted in Fig. 1. In total, 150 aliquots were scanned with two CombiFoss™ 7 (Foss Electric A/S, Hillerød, Denmark) at different time points, ending up with 300 spectra. Bacterial count, determined on the subsample without preservative, averaged 38.20 ± 26.07 CFU/mL and had a median of 33.00 CFU/mL. Descriptive statistics of the investigated traits within each sample age are presented in Table 2.

3.2. r and R

The estimate of r was 0.03 % for protein, casein, and lactose contents, and 0.18 % for fat content, and the estimate of r% was > 99 % for fat, protein, casein, lactose, and SCS. The DSCC was the least repeatable trait, even if r% was > 90 % (Table 3). Fat, protein, casein, and lactose contents, and SCS were the most reproducible milk traits at different sample ages (R_S% >99 %) and across the two instruments (R_I% >99 %), whereas DSCC was the least reproducible trait across milk age (R_S% ~90 %) and freezing point the least reproducible trait across instruments (R_I% ~88 %). Overall, protein, casein, and lactose contents,

Table 2
Mean (SD) of milk composition traits across sample age.[1]

Trait[2]	Sample age, d				
	0	1	2	3	4
Fat, %	3.30 (0.83)	3.32 (0.83)	3.33 (0.84)	3.32 (0.83)	3.30 (0.83)
Protein, %	3.45 (0.38)	3.44 (0.38)	3.43 (0.38)	3.44 (0.38)	3.45 (0.38)
Casein, %	2.70 (0.33)	2.68 (0.33)	2.68 (0.33)	2.69 (0.33)	2.66 (0.32)
Lactose, %	4.67 (0.24)	4.67 (0.24)	4.67 (0.24)	4.67 (0.24)	4.66 (0.24)
Freezing point, °C	−0.520 (0.005)	−0.521 (0.005)	−0.520 (0.005)	−0.520 (0.005)	−0.522 (0.005)
Urea, mg/dL	33.62 (5.28)	32.81 (5.43)	32.73 (5.20)	32.70 (5.26)	32.67 (5.32)
SCS	2.14 (2.28)	2.19 (2.26)	2.13 (2.29)	2.16 (2.27)	2.17 (2.26)
DSCC, %	58.99 (20.07)	56.73 (19.45)	56.55 (20.35)	55.90 (18.81)	56.22 (20.47)

[1] number of days between the sampling date and the date of analysis.
[2] SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100,000), where SCC is somatic cell count (cells/mL); DSCC = differential somatic cell count.

freezing point, urea, and SCS were more reproducible across sample ages than between instruments, whereas fat content and DSCC were more reproducible between instruments (Table 3).

3.3. Prediction of sample age

The accuracy was the criterion to select the optimal model for sample age prediction from spectra. The greatest accuracy, given by the number of samples correctly classified through partial least squares discriminant analysis, was achieved with 14 latent variables. Overall, the greatest accuracy was 91 % in calibration and 77 % in validation with a confidence interval from 87 to 95 % and from 67 to 85 %, respectively. Each sample age differed in terms of sensitivity, specificity, positive and negative predictive values, and balanced accuracy (Table 4). Sensitivity, defined for each sample age as the percentage of samples which were correctly assigned to the right class (age), ranged from 74 (day one) to 100 % (day 0) in calibration and from 56 (day one and day three) to 100 % (day 0 and day four) in validation. Specificity, complementary to sensitivity, is the percentage of samples from other ages which are correctly attributed as inconsistent with the target age (Pomerantsev and Rodionova, 2018). Overall, specificity was ≥ 96 % in calibration and ≥ 89 % in validation; the lowest specificity was obtained for sample of day 0 and day two in calibration and validation, respectively. The balanced accuracy varied from 87 to 98 % in calibration and 74 to 99 % in validation. Positive predictive values ranged from 88 to 100 % in calibration and 62 to 91 % in validation, and negative predictive values ranged from 94 to 100 % in calibration and 89 to 100 % in validation.

The most important wavenumbers for sample age classification are depicted in Fig. 2. Even if each point within the milk spectrum contributed to the separation of the sample age, the most relevant predictors were in the regions between 2835.6 and 2827.9 cm^{-1}, at 1774.7 cm^{-1}, and between 979.9 and 964.5 cm^{-1}.

Table 3
Repeatability (r), coefficient of repeatability (r%), sample age[1] reproducibility (R_S), sample age coefficient of reproducibility (R_S%), instrument reproducibility (R_I), and instrument coefficient of reproducibility (R_I%) for milk composition traits.

Trait[2]	r	r%	R_S	R_S%	R_I	R_I%
Fat, %	0.18	99.49	0.08	99.38	0.03	99.47
Protein, %	0.03	99.94	0.02	99.90	0.04	99.78
Casein, %	0.03	99.93	0.05	99.64	0.07	99.30
Lactose, %	0.03	99.85	0.01	99.82	0.03	99.64
Freezing point, °C	3.15	95.28	3.18	90.43	3.43	87.8
Urea, mg/dL	2.13	98.19	2.03	96.56	3.63	91.8
SCS	0.40	99.65	0.06	99.64	0.12	99.61
DSCC, %	17.87	90.65	6.08	89.57	0.00	90.65

[1] number of days between the sampling date and the date of analysis.
[2] SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100,000), where SCC is somatic cell count (cells/mL); DSCC = differential somatic cell count.

4. Discussion

4.1. Influence of sample age on milk traits

Estimates of r and R across instruments and sample ages for the major milk components were in line with those of ISO (2013) that reported typical r and R for raw cow milk components of 0.04 and 0.11, respectively. The only exception was fat content, for which the t value was much greater than that of other major traits, probably due to inefficient homogenisation of milk samples which results in a non-uniform distribution of fat globule size (Iñón et al., 2004).

Some studies reported that sample age as well as storage method (e.g., preservative concentration and/or type) can affect milk composition predicted through FTIR (Monardes et al., 1996; Wentz et al., 2018). In samples with added preservative, milk deterioration due to aging is slowed down compared to samples without preservative (Wentz et al., 2018). In fact, endogenous enzymes drive most activity in the milk, while exogenous enzymes (produced by bacteria) have little or no activity (Fox et al., 2015; Wentz et al., 2018). In this case, proteolysis and lipolysis can be caused by endogenous enzymes. Milk quality decreases with increasing SCC and this phenomenon is associated with a consequent increase in lipases and proteases activity (Barbano et al., 1991; Barbano et al., 2006; Wickström et al., 2009).

Table 4
Performance[1] (%) of the discriminant analysis carried out on milk samples to detect sample age.[2]

Sample age, d	Calibration					Validation				
	Sensitivity	Specificity	TP	TN	Balanced Accuracy	Sensitivity	Specificity	TP	TN	Balanced Accuracy
0	100	96	88	100	98	100	97	90	100	99
1	74	100	100	94	87	56	99	91	90	77
2	98	98	91	99	98	72	89	62	93	81
3	88	97	88	97	93	56	93	67	89	74
4	98	98	93	99	98	100	93	78	100	97

[1] Sensitivity (%) = $\frac{TP}{TP+FN} \times 100$; Specificity (%) = $\frac{TN}{FP+TN} \times 100$; and Balanced accuracy (%) = $\frac{\text{Sensitivity} + \text{Specificity}}{2}$. Where: TP = true positives correctly classified, TN = true negatives correctly classified, FP = false positives correctly classified, FN = false negatives correctly classified.
[2] number of days between the sampling date and the date of analysis.

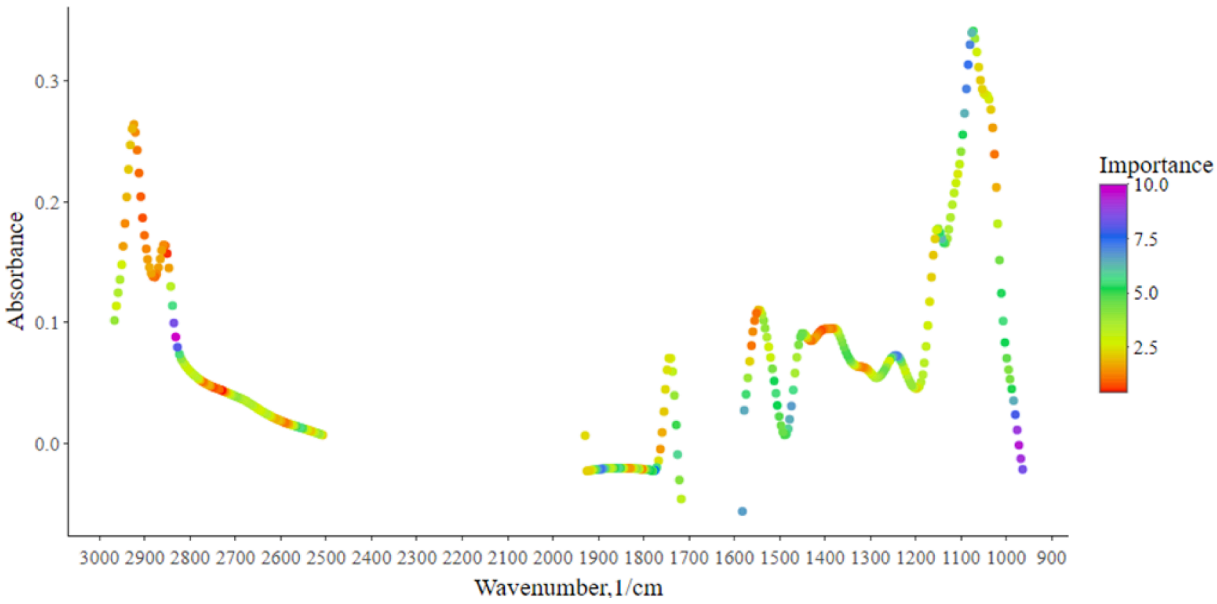

Fig. 2. Importance[1] of each spectral point for the discrimination of sample age[2]. [1]10 = highly important; greatest importance and 0 no importance. [2]number of days between the sampling date and the date of analysis.

Lipase, arguably the most significant endogenous enzyme in milk, is responsible for breakdown of triglycerides into free fatty acids (Fox et al., 2015). Literature details how storage time should not affect milk fat content (Wentz et al., 2018). However, Monardes et al. (1996), who investigated the best preservative and storage conditions for raw milk samples collected within an official recording system, reported lower fat content in seven d old samples compared to three d old samples. Chalermsan et al. (2004) reported that the lipolysis of fat in prolonged storage of preserved raw milk normally causes an increase in free fatty acids.

On the other hand, proteinase, in particular plasmin, hydrolyses peptide bonds, particularly those of β-casein (Fox et al., 2015). Vigolo et al. (2022) reported that milk protein composition is affected by storage time as well as by factors such as the type of preservative and temperature of analysis. In particular, Vigolo et al. (2022) observed that casein content determined through HPLC declined across storage time (from 0 to 30 d) regardless of the type of preservative used. The activity of the enzymes in milk during storage can decrease the percentage of protein and increase the fraction of non-protein-nitrogen, i.e., amino acids and peptides (Verdi et al., 1987). However, it is not possible to differentiate the true milk proteins (casein, albumin, lactalbumin, lactoglobulin, and immunoglobulins) from those formed by non-protein-nitrogen, when the physical–chemical constituents of milk are analysed by infrared methodologies (Wentz et al., 2018).

Chalermsan et al. (2004) reported that milk preserved with Bronopol had significant depression of lactose at the fourteenth day storage, in addition to fat and total solids. This reduction is probably due to deterioration, in which lactose is converted into acidic compounds, in addition to the deterioration caused by microorganisms that transform this constituent into lactic acid.

In our study the major milk composition was reproducible at different sample age, i.e., until four d after sampling. However, considering that it was possible to classify samples of different ages using spectra, it is reasonable to assume that a change in the milk occurs and can be detected by commercial FTIR instruments. Changes are likely due to the action and interactions of lipases and proteinases. Freezing point is expected to also be affected by the changes the milk undergoes during storage; in fact, it was the least reproducible trait at different sample ages. The freezing point of milk is an important indicator of quality and adulteration. As with other physical properties, it is influenced by the concentration and the type of preservative, rather than by solids content, especially lactose (Radeljevic et al., 2012). The low R_S of DSCC was probably due to SCC level of initial samples. The collected samples belonged to cows with highly variable SCC from 10,000 to 1,400,000 cells/mL and an average SCS of 2.16. This trait has been widely investigated by Damm et al. (2017) who reported good robustness of the values on samples stored for a maximum of four d and with added preservative. The same authors concluded that reliable DSCC can be obtained from samples whose SCC falls between 50,000 and 1,500,000 cells/mL.

4.2. *Sample age from FTIR spectra*

The bacterial, enzymatic, and chemical processes naturally occurring within the milk after harvesting act simultaneously and modify the overall composition progressively, making the spectra recorded at different days somehow different. Although milk composition presented good R_S (Table 3), the discriminant analysis succeeded to classify sample age from spectra. The most important spectral data points (Fig. 2) belonged to regions known to be attributed to fat, in particular the region "fat B" (3000 to 2800 cm^{-1}) and "fat A" (1800 to 1700 cm^{-1}; Iñón et al., 2004), which contain the major absorbance peaks for C—H bonds, C═O bonds, C—N bonds, and N—H bonds (Soyeurt et al., 2010). Another contributing band was in the region called the "fingerprint region" (1582 to 930 cm^{-1}) which brings important information regarding the chemical structure of the analysed matter (Karoui et al., 2011). This region contains peaks of absorbance relative to C—H bonds and C—O bonds (De Marchi et al., 2009). Again, although the main milk components remain reproducible within the four d period, changes in the structure of the analysed milk due to aging/deterioration make classification of milk according to age feasible and accurate. Performance of the FTIR model was better for days zero, two, and four, likely because the changes that take place within the milk in a single day is not sufficiently large nor as noticeable. On the other hand, over a two d period, we expect milk to undergo more pronounced changes, leading to better statistical fit for the FTIR model.

To our knowledge, this is the first study that has tested the ability of FTIR to classify milk age using individual samples. Grewal et al. (2017) performed a principal component analysis using FTIR spectra collected on the first day of delivery and then at 14 and 28 d of storage of ultra-

high temperature treated whole and skim milk samples. Those authors observed significant changes in FTIR spectra in samples stored above 40 °C within 14 d (Grewal et al., 2017).

Predicting sample age using FTIR can be useful for screening and allows monitoring of sample age within circuits where reliability of milk composition should be maximised like official recording schemes or dairy cooperatives with quality-based payment systems. In fact, adjusting for the sample age could be fair in situations where farmers are paid according to the quality of the delivered milk, particularly if the analysis is performed out of the conventional time window. These findings lay the ground for future studies on the effect of sample age on either treated milk (skimmed or UHT) or preservative-free milk. Raw milk stored without preservative is expected to be classified even more correctly compared to this study, as deterioration is accelerated by exogenous enzymes of the microorganisms present.

5. Conclusions

The major milk quality traits and SCS were reproducible at different sample ages. Sample age did not affect the investigated traits significantly and thus milk composition assessment from 0 to four d after sampling is expected to be reliable in commercial standardised laboratories. Nevertheless, findings reveal that sample age can be predicted with good accuracy from FTIR spectra as part of the wavelengths (i.e. the predictors) are enzymatic and subjected to changes, thus chemical bonds lead to spectral perturbations popping up in milk during the storage. Even if there are not significant differences in the final infrared-predicted content of fat and protein, taking into account milk age, and being able to determine it, can be useful for legal purpose in certain contexts. Being able to accurately classify milk according to its age can be pivotal for example to identify fraud. Future studies may attempt to predict the age of pooled samples such as bulk and/or multi-species milk.

CRediT authorship contribution statement

S. Magro: Data curation, Formal analysis, Software, Validation, Writing – original draft. **N.W. Sneddon:** . **A. Costa:** Conceptualization, Data curation, Investigation, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **E. Chiarin:** Data curation, Writing – review & editing. **M. Penasa:** Project administration, Supervision, Validation, Writing – review & editing. **M. De Marchi:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available upon request from the corres.

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