

Detection of common adulterants in bulk bovine milk using fourier transformed mid-infrared spectroscopy

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ABSTRACT

Fourier transformed mid-infrared spectroscopy is a widespread method for routine analysis in milk. This method can also be used for the detection of adulterants in bovine milk which represent a current risk for dairy industries and public health. This work focuses on the detection of seven adulterants present in three known concentrations in bulk milk sample. The adulterants were: sodium bicarbonate, sodium chloride, hydroxyproline, glucose, sodium citrate, water, and urea. Partial least squares – discriminant analysis was used to develop statistical models to predict the presence of adulterants in milk samples. The obtained models could provide an easy, efficient, and rapid tool for the dairy industry to detect specific adulterants in milk.

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Keywords: Adulteration; bulk milk; prediction; dairy industry; traceability; FT-MIR

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1. INTRODUCTION

Food adulteration refers to the practice of intentionally altering foodstuff for economic profit [1]. Adulteration can be realized in different ways, such as by adding unconventional/not permitted cheap ingredients or by adding non-food substances to increase product yield or weight [1]. The adulteration of certain foods is a growing problem that can impair the consumers' trust and health. It is therefore potentially harmful for the producers and the consumers. According to the database of food fraud developed by members of the US Pharmacopeia Convention's Food Ingredients Intentional Adulterants, milk is one of the most adulterated products [2]. Water is the most prevalent adulterant in dairy and is added to increase the volume supplied and thereby the profit. Adulteration with water reduces the nutritional value of milk and its derivatives; in addition, whether contaminated by chemicals or pathogens, the presence of water in milk could be harmful for human health [1]. Urea is a nitrogen-based compound, added to increase whiteness, the apparent solids non-fat content, and the apparent protein content of milk [3]. Adulteration with sodium chloride is used to

lower the freezing point of milk, which otherwise normally freezes below -0.520 °C [4]. The freezing point of milk is also highly dependent on the amount of water-soluble compounds. Henceforth, values approaching zero are indicative of exogenously introduced water [5]. Similarly, glucose is used to mask changes in milk density and freezing point [5]. Adding preservatives, such as sodium bicarbonate and sodium citrate can be a mean to disguise improper milk storage. These substances suppress proliferation and activity of micro-organisms responsible for milk spoilage [6]. Finally, the addition of hydroxyproline can increase the apparent milk total protein content [5]. Several methods of analysis have been studied for the detection of milk and dairy products adulterants. Certainly, quantitative detection techniques depend on the nature of the adulterant itself. For example, liquid chromatography and enzyme linked immunosorbent assay are the most common methods to detect external adulteration in the field [3]. Salomonsen et al. (2007) reported that, although costly, the proton nuclear magnetic resonance is a powerful technique for quality control of milk and adulterants detection (water and whey) [7]. Moreover, mid-infrared spectroscopy has been

Table 1. Amount of added adulterants (%).

Adulterant	Level 1 (L1) of adulterant	Level 2 (L2) of adulterant	Level 3 (L3) of adulterant
Sodium bicarbonate	0.005	0.010	0.020
Sodium chloride	0.075	0.150	0.300
Hydroxyproline	0.007	0.014	0.028
Glucose	0.031	0.062	0.124
Sodium citrate	0.019	0.038	0.076
Water	1.600	3.200	6.400
Urea	0.0125	0.025	0.050

successfully applied in combination with two-dimensional correlation spectroscopy to detect the fraudulent addition of urea and glucose [8]. Other authors reported that Fourier Transformed infrared spectroscopy interferometers are able to detect the differences between normal and abnormal milk [9]. Nowadays, it is important to establish a robust and cost-effective rapid method to be used in the dairy industry for the inspection of milk authenticity. Based on this scenario, the present study aimed to develop Fourier Transformed Mid-Infrared Spectroscopy (FT-MIR) prediction models to detect the presence of adulterants in bulk milk at different levels of contamination.

2. MATERIALS AND METHODS

One hundred samples of bulk milk were collected in one of the largest dairy companies in north-eastern Italy. Each sample was divided in 22 aliquots. One aliquot was kept as raw milk, while the others were contaminated with adulterants at different concentrations (7 adulterants, 3 concentrations each). The selected adulterants were water, urea, sodium bicarbonate, sodium chloride, hydroxyproline, glucose and sodium citrate. Concentrations of adulterants are presented in Table 1.

Mid-infrared spectra of each sample were obtained using two different MilkoScan FT3™ (Foss Electric A/S, Hillerød, Denmark), one located at the production site and the second located at the DAFNAE department (University of Padova, Legnaro, Italy). Spectra information of analysed samples was stored and used for further statistical analysis.

The effect of each adulterant on milk components, predicted using FT-MIR, was assessed using generalized linear model procedure in SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA). The fitted model was [1]:

$$y_{ijmn} = \bar{x} + L_i + C_j + S(H_n)_m + e_{ijmn} \quad (1)$$

where y_{ijmn} is the milk component of interest, \bar{x} is the overall intercept of the model, L_i is the fixed effect of the laboratory performing the analysis (i = dairy industry or DAFNAE), C_j is the fixed effect of the adulterant concentration (j = 0 to 3), $S(H_n)_m$ is the repeated effect of analysed sample nested within the effect of n herd source of the sample (m = 1 to 100), and e_{ijmn} is the random residual $\sim N(0, \sigma_e^2)$, where σ_e^2 is the residual variance.

A prediction model for adulterants presence was developed using PLS Regression module of python scikit-learn library [10]. As a first step, spectra were matched with the corresponding adulterant and its level. Subsequently, spectral regions associated to noisy water absorption wavelengths were excluded and the spectra were standardized using a standard normal variate transformation. Then, each discriminant model was developed using spectra from not adulterated milk and spectra from the

selected level of adulterant. Accordingly, the dataset for each model comprised 400 spectra, 200 from not adulterated milk (100 raw samples, 2 instruments each) and 200 from adulterated milk (100 adulterated samples, 2 instruments each). Prior to any further analyses, 4 randomly selected groups of samples, each from a single supplier and which in total represented about one fifth of the total number of samples, were assigned to the independent test set, while the remaining were used as the calibration set. The optimal number of latent variables for each model was selected using stratified 10-fold cross validation on the calibration set. For each model, the overall discriminant ability was evaluated on the independent test set using conventional performance indicators, namely true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), precision, recall, accuracy, and F1 score [11].

3. RESULTS

The estimated means of milk components assessed using FT-MIR on milk samples with different adulterants and concentrations are reported in Table 2. The tested adulterants had a significant impact on the predicted milk components. At the lowest concentration of added urea, all the IR predicted milk components increased except for freezing point, which showed an opposite trend. When water was added, a dilution effect was observed for all FT-MIR predicted components, even at a concentration of 1.6 %. Sodium citrate had a similar effect as urea addition, affecting milk components at the lowest tested concentration, except for FT-MIR predicted fat content, which significantly increased at 0.038 % sodium citrate. However, lactose content experienced a decreasing trend at increasing concentration of sodium citrate. Sodium chloride, sodium bicarbonate, and hydroxyproline addition had a minimal effect on FT-MIR predicted milk components, except for freezing point, which was extensively affected even at the lowest tested adulterant addition (0.075 %, 0.005 %, and 0.007 % for sodium chloride, sodium bicarbonate, and hydroxyproline, respectively). Increasing the added glucose concentration significantly increased FT-MIR predicted lactose content, total solids, and solids non-fat, while decreasing fat, urea, and freezing point.

Outcomes of the discriminant analyses for each adulterant (3 levels) on the independent test set are summarized in Table 3. Results demonstrated that a complete discrimination of adulterated samples using FT-MIR occurred for urea, water, sodium citrate, sodium chloride and sodium bicarbonate at concentrations of 0.025 %, 6.4 %, 0.038 %, 0.150 % and 0.010 %, respectively. Hydroxyproline and glucose were not fully identified at tested adulteration levels, but samples with 0.124 % of glucose were correctly identified and only 1 not adulterated sample out of 38 was incorrectly labelled as positive. The worst results were obtained for hydroxyproline, with an overall accuracy for the highest level of adulteration (0.028 %) of 0.67. Accuracy values had a similar trend of the F1 value score for all adulterants at every level of concentrations.

4. DISCUSSION

In this research, a dairy industry was selected to collect one hundred bulk milk samples. Although there have been some studies on the effect of adulterants on IR predicted milk components, they are limited. For instance, Cassoli et al. (2011) conducted a study on the impact of sodium bicarbonate and sodium citrate on FT-MIR predicted milk components. The findings revealed that adding up to 0.15 % and 0.075 % of

Table 2. Least squares means of milk component¹, assessed using FT-MIR, from samples with different adulterants and adulterants concentrations (%).

Adulterant	Concentration	Fat (%)	Protein (%)	Lactose (%)	Total solids (%)	Solids non-fat (%)	Casein (%)	Urea (g/dL)	Freezing point (°mC)
Urea	0	3.90 ^a	3.35 ^d	4.73 ^d	12.54 ^c	8.75 ^d	2.60 ^d	26.35 ^d	-521.66 ^d
	0.0125	3.87 ^b	3.36 ^c	4.75 ^c	12.55 ^c	8.78 ^c	2.62 ^c	34.13 ^c	-525.32 ^b
	0.025	3.88 ^b	3.37 ^b	4.76 ^b	12.57 ^b	8.81 ^b	2.63 ^b	41.79 ^b	-528.81 ^c
	0.050	3.87 ^b	3.38 ^a	4.78 ^a	12.61 ^a	8.86 ^a	2.65 ^a	55.97 ^a	-535.42 ^a
Water	0	3.89 ^a	3.35 ^a	4.73 ^a	12.54 ^a	8.74 ^a	2.60 ^a	26.25 ^a	-521.49 ^a
	1.600	3.84 ^b	3.29 ^b	4.66 ^b	12.34 ^b	8.60 ^b	2.56 ^b	25.73 ^b	-512.31 ^c
	3.200	3.77 ^c	3.24 ^c	4.58 ^c	12.13 ^c	8.45 ^c	2.51 ^c	25.24 ^b	-502.65 ^b
	6.400	3.64 ^d	3.13 ^d	4.45 ^d	11.73 ^d	8.18 ^d	2.43 ^d	24.60 ^c	-485.02 ^a
Sodium citrate	0	3.90 ^c	3.35 ^d	4.73 ^a	12.54 ^d	8.75 ^d	2.60 ^d	26.36 ^d	-521.63 ^d
	0.019	3.91 ^c	3.36 ^c	4.69 ^b	12.60 ^c	8.83 ^c	2.63 ^c	27.50 ^c	-518.52 ^b
	0.038	3.94 ^b	3.37 ^b	4.65 ^c	12.66 ^b	8.90 ^b	2.65 ^b	28.61 ^b	-514.89 ^c
	0.076	3.99 ^a	3.38 ^a	4.59 ^d	12.75 ^a	8.99 ^a	2.67 ^a	30.34 ^a	-507.79 ^a
Sodium chloride	0	3.89 ^a	3.35 ^d	4.73 ^b	12.54 ^a	8.75 ^d	2.60 ^d	26.25 ^d	-521.94 ^d
	0.075	3.86 ^b	3.35 ^c	4.73 ^{ab}	12.52 ^b	8.76 ^c	2.61 ^c	27.66 ^c	-564.97 ^c
	0.150	3.86 ^{bc}	3.36 ^b	4.74 ^{ab}	12.52 ^b	8.77 ^b	2.61 ^b	28.85 ^b	-607.29 ^b
	0.300	3.84 ^c	3.36 ^a	4.74 ^a	12.51 ^b	8.79 ^a	2.62 ^a	31.65 ^a	-691.86 ^a
Sodium bicarbonate	0	3.90 ^a	3.35 ^a	4.73 ^c	12.54 ^a	8.74 ^a	2.60 ^a	26.25 ^a	-521.79 ^d
	0.005	3.87 ^b	3.35 ^a	4.74 ^{ac}	12.53 ^b	8.74 ^b	2.60 ^b	25.78 ^{ab}	-544.18 ^c
	0.010	3.86 ^b	3.34 ^b	4.75 ^b	12.53 ^b	8.73 ^c	2.59 ^c	25.33 ^{ab}	-565.76 ^b
	0.020	3.85 ^b	3.33 ^c	4.76 ^a	12.53 ^b	8.70 ^d	2.57 ^d	24.59 ^c	-609.15 ^a
Hydroxyproline	0	3.90 ^a	3.35 ^d	4.73	12.54 ^b	8.74 ^d	2.60 ^d	26.22 ^b	-521.74 ^c
	0.007	3.87 ^b	3.36 ^c	4.73	12.53 ^b	8.76 ^c	2.61 ^c	26.44 ^b	-543.44 ^{bc}
	0.014	3.87 ^b	3.36 ^b	4.73	12.54 ^{ab}	8.76 ^b	2.62 ^b	26.66 ^b	-564.73 ^b
	0.028	3.87 ^b	3.37 ^a	4.73	12.55 ^a	8.78 ^a	2.63 ^a	27.25 ^a	-607.23 ^a
Glucose	0	3.90 ^a	3.35	4.73 ^d	12.54 ^c	8.74 ^d	2.60	26.22 ^a	-521.76 ^d
	0.031	3.87 ^b	3.35	4.76 ^c	12.55 ^c	8.77 ^c	2.60	25.65 ^b	-544.14 ^b
	0.062	3.87 ^b	3.35	4.79 ^b	12.59 ^b	8.78 ^b	2.60	25.05 ^c	-566.10 ^c
	0.124	3.88 ^b	3.35	4.85 ^a	12.66 ^a	8.81 ^a	2.60	23.83 ^d	-610.05 ^a

sodium bicarbonate and sodium citrate, respectively, did not significantly affect the content of fat, protein, casein, or total solids. However, a decrease in freezing point was observed at 0.05 % of both sodium bicarbonate and sodium citrate, and the FT-MIR predicted urea content reduced with increasing concentration of sodium bicarbonate. The discrepancy in results between the present study and Cassoli et al. (2011) may be due to the use of different statistical approaches to test the differences of FT-MIR predicted components between raw and adulterated milk [12]. The addition of urea is a popular practice for standardizing the levels of FT-MIR predicted solids non-fat in raw milk. According to Renny et al. (2014), an increase in the concentration of added urea results in a corresponding increase in the FT-MIR predicted solids non-fat content [13]. According to the present study, Yang et al. (2020) highlight a positive correlation between glucose adulteration and FT-MIR predicted freezing point depression, even if significance was not reported [14]. Nascimento et al. (2013), highlighted an impact on freezing point of water and sodium chloride similar to the one reported in the present paper [15].

The recall values for 0.005 % sodium bicarbonate were greater (0.79) than the ones reported by Gondim et al. (2017) (0.69) at similar contamination level (0.004 %) [16]. According to literature, compounds similar to the milk constituents, as in the case of water, are more difficult to be detected [17]. This explains the high concentration of water (6.4 %) needed to generate sufficient changes in the spectrum to achieve an acceptable

accuracy. A good accuracy value of prediction of sugar adulteration was obtained from PLS regression model using near infrared spectrometer [18]. In particular, it was observed that the coefficient of correlation for the percentage of glucose adulteration was greater than 0.90 [18]. These values are consistent with the results of the present study, in particular the good accuracy (0.97) obtained for 0.124 % glucose adulteration. Santos et al. (2013) observed that a concentration of 1.25 % of urea can be predicted using mid-infrared spectroscopy with a coefficient of determination of 0.98 [19]. Hansen et al. (2019), studied adulteration by hydroxyproline in milk at different concentrations, obtaining optimal accuracy at 0.1425 % of hydroxyproline, about five times higher than the highest concentration tested in the present study [17].

5. CONCLUSION

This study developed a straightforward model for detecting adulterants in bovine milk using FT-MIR; this model can offer a different approach for the dairy industry to identify potential adulterants in milk. The findings indicate that added urea, sodium citrate, sodium chloride, and glucose adulteration can be detected at concentrations that begin to affect the FT-MIR predicted milk composition. Moreover, the model displayed satisfactory results in differentiating between water and sodium bicarbonate addition, even at concentrations that had a more pronounced impact on IR predicted milk composition. However, the prediction performance was lower for hydroxyproline.

¹ a,b,c,d Different superscripts letters indicates significantly different ls-means ($P < 0.05$)

Table 3. Performance of the discriminant analysis carried out on bovine bulk milk for detection of 7 adulterants.

Adulterant	Concentration (%)	True positives	True negatives	False positives	False negatives	Precision	Recall	Accuracy	F1-score
Urea	0.0125	37	34	4	1	0.90	0.97	0.93	0.93
	0.0250	38	38	0	0	1.00	1.00	1.00	1.00
	0.0500	38	38	0	0	1.00	1.00	1.00	1.00
Water	1.600	23	28	10	14	0.70	0.62	0.68	0.66
	3.200	30	33	5	8	0.86	0.79	0.83	0.82
	6.400	38	38	0	0	1.00	1.00	1.00	1.00
Sodium Citrate	0.019	37	38	0	1	1.00	0.97	0.99	0.99
	0.038	38	38	0	0	1.00	1.00	1.00	1.00
	0.076	38	38	0	0	1.00	1.00	1.00	1.00
Sodium Chloride	0.075	37	38	0	0	1.00	1.00	1.00	1.00
	0.150	38	38	0	0	1.00	1.00	1.00	1.00
	0.300	38	38	0	0	1.00	1.00	1.00	1.00
Sodium Bicarbonate	0.005	30	24	14	8	0.68	0.79	0.71	0.73
	0.010	34	34	4	4	0.89	0.89	0.89	0.89
	0.020	38	38	0	0	1.00	1.00	1.00	1.00
Hydroxyproline	0.007	26	11	27	12	0.49	0.68	0.49	0.57
	0.014	26	11	27	12	0.49	0.68	0.49	0.57
	0.028	30	21	17	8	0.64	0.79	0.67	0.71
Glucose	0.031	35	30	8	3	0.81	0.92	0.86	0.86
	0.062	36	31	7	0	0.84	1.00	0.91	0.91
	0.124	38	37	1	0	0.97	1.00	0.99	0.99

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