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Review Article

Reliability results in the assessment of milk composition by an ultrasonic analyzer

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Abstract

The objectives of the study were to evaluate the accuracy and the repeatability of ultrasonic milk analyzers compared to reference methods. At the same time, milk composition was assessed using an ultrasonic analyzer, Fourier transform mid-infrared milk analyzer, and standard methods. The results obtained by the different analytical methods for fat, protein, and lactose contents were compared using different statistical parameters. The repeatability and reliability of the results obtained with the calibrated ultrasonic analyzer were comparable with those obtained using mid-infrared, Gerber method, and Kjeldahl method. The ultrasonic method underestimated the fat concentration by about 1 g/L and overestimated protein content by about 1.2 g/L, while the mid-infrared method showed results very close to the direct methods for all milk components. Well calibrated ultrasonic analyzers might be an alternative to conventional methods of milk analysis in small dairy processing units and milk collection centers in Tunisia because Fourier transform mid-infrared instruments are very expensive and need highly trained technicians.

Keywords: comparison of methods, fat content, milk, protein content, quality

1. Introduction

In Tunisia, milk composition is usually evaluated using a Fourier transform mid-infrared (MIR) milk analyzer by the dairy industry and official laboratories. In small dairy processing units and milk collection centers, small and inexpensive analyzers based on ultrasonic waves are used to evaluate the quality of raw milk; however, inaccuracy is a main concern for milk producers. Since there is no national certified organization to ensure the accuracy of different types of equipment, analysis results obtained by the ultrasonic method are always contested by the dairy industry laboratories. In Europe, MIR analyzers are routinely used for milk payment purposes. However, in Tunisia MIR technology is limited to large dairy plants and some state laboratories. Analysis of milk by MIR is an indirect method based on the principle that different functional groups absorb MIR energy at different wavelengths (Keylegian, Houghton, Lynch, Fleming & Barbano, 2006). MIR instruments are based on infra-red spectroscopy of the whole spectrum by Michelson's interferometer and use of Fourier's transformations with advantages such as a very high throughput, ease of use, and availability (Soyeurt *et al.*, 2006). On the other hand, MIR analyzers are very expensive in Tunisia and need accurate maintenance. Therefore, most small dairy processing units and milk collection centers use ultrasonic analyzers to evaluate milk quality of their providers. Ultrasonic spectroscopy presents a practical alternative to infrared spectroscopy for milk analysis. Because of its affordable cost and facility of

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use, the ultrasonic technique is used more and more by small dairies and farmers in Tunisia to rapidly evaluate milk composition. However, the results carried out by ultrasonic analyzers are always considered to be imprecise by big dairy buyers who purchase the milk mainly for the big dairy plants. For many years, ultrasound at the higher frequencies and low power has good sensitivity and has been successfully utilized to study the physicochemical and structural properties of liquid foods (McClements, 1997). Many studies have demonstrated the capability of ultrasound for analytical applications by identifying different properties of dairy products (Narsaiah & Jha, 2012). Ordolff (2005) tested an ultrasonic device to measure fat, protein, and lactose and concluded that the method adequately fulfils the requirements for herd management. In general, any kind of instrument whatever its operating principle or its cost, needs suitable calibration to be accurate. Lynch, Barbano, Fleming and Nicholson (2004) reported that the analytical accuracy of milk analyzer results can have a large effect on both buyers and sellers of dairy products. Barbano and Lynch (2006) described the improvement in the accuracy of MIR measurement of all milk components. According to Svennersten-Sjaunja, Sjögren, Andersson and Sjaunja (2005), MIR spectroscopy has high accuracy and repeatability, both when the milk is analyzed in the laboratory and when it is analyzed with a portable on-farm device. The accuracy of milk analysis equipment can be affected by instrumental factors such as homogenization efficiency, signal to noise ratio, repeatability, linearity or bad calibration (Smith, Barbano, Lynch, & Fleming, 1993b). Keylegian *et al.* (2006) mentioned that deterioration of preserved, refrigerated calibration samples during storage due to lipolysis and proteolysis may cause uncorrected readings to change, resulting in incorrect calibration adjustments. Any instrumental method of milk component determination requires the use of calibration standards that are produced according to official methods that have known chemical composition. Instrument calibration is generally maintained by a trained lab technician or lab manager because it needs a minimum level of experience. O'Sullivan, O'Connor, Kelly and McGrath (1999) stated that reference methods usually expose laboratory personnel to toxic substances which, subsequently, lead to potential health hazards. Harmful gases from current analytical methods are no longer tolerated by international administrative agencies.

The objective of this study was to determine whether ultrasonic milk analyzers are sufficiently accurate to evaluate raw milk composition in small dairies and at the farm scale and compare the results to infrared and reference methods and carry out mutual comparison of the results.

2. Materials and Methods

2.1. Milk collection

Sixty raw bulk milk samples were collected during six days from a milk collection center during the morning milk reception in September 2017. Each 300-mL sample was thoroughly mixed in a sealed container by manually inverting 10 times and then partitioned into three subsamples of 100 mL. All samples were immediately refrigerated at 4 °C until analyzed.

2.2. Milk analysis

All milk samples were analyzed in duplicate and at the same time with the three testing methods. The direct reference analyses carried out on all milk samples were fat content (according to Gerber method) (IDF Standard 105: 2008) and crude protein content (according to Kjeldahl method) (IDF Standard 20-1: 2014). Fat content and protein content in all milk samples were determined also by two indirect methods. The first indirect method was carried out using the MIR analyzer (Milkoscan FT1 Foss-Electric A/C, Hillerod, Denmark) (IDF Standard 141B: 1996) and the second indirect method was carried out using an ultrasonic analyzer (Lactoscan MCC, Milkotronic Ltd Bulgaria). The MIR and ultrasonic instruments were previously calibrated using high quality standards provided by CECALAIT France.

2.3. Statistical analysis

The results of milk composition obtained with the different methods for fat and protein content were compared using different measures of statistical fitness. The coefficient of determination (r^2), the concordance correlation coefficient (CCC), the bias correction factor (BCF), the mean prediction error (MPE) and the intraclass correlation coefficient (ICC) were calculated. When $r^2=1$, this indicates 100% of precision between the methods. The concordance correlation coefficient (CCC), used by Lin (1989), was calculated to determine overall agreement between the methods. The following criteria were used as described by Lin (1992): If the CCC values are 0.21-0.40, agreement is fair. If the CCC values are 0.41-0.60, agreement is moderate. If the CCC values are 0.61-0.80, agreement is substantial, and if the CCC values are 0.81-1.00, agreement is almost perfect. In order to determine if the line of the best fit is close to equal, the BCF was calculated. To describe the predictive performance of each method, the MPE was used. When MPE is above 10%, predictability is very poor as indicated by Sheiner and Beal (1981). The accuracy of the methods was evaluated using the ICC. When ICC is equal to 1, this indicates that the two different analytical methods will estimate the same concentration of a milk component when measured from the same sample. The SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA) was used to create a multiple linear regression model. The ANOVA model included the type of instrument as a factor to account for any variation of milk composition.

2.4. Unitary cost calculation

In order to compare both analyzers from an economic perspective, we made simple calculations of the unitary costs of analysis of one milk sample under both technologies of ultrasonic and mid-infrared. These unitary costs were calculated assuming a lifetime of 8 years for the mid-infrared (Milkoscan FT1) analyzer and 6 years for the ultrasonic analyzer. We also assumed a maximum of 100 analyses per hour for the mid-infrared technology and 60 per hour for the ultrasonic technology. We also considered that each machine is used only 8 hours per day during the whole year. All of these assumptions were made based on current observed practices and data. We calculated the unitary costs of a given sample using equation 1.

$$UC_t = \frac{CM_t}{N_t \times L_t} + \frac{AMC_t}{N_t} + \frac{cc_t}{100} \quad (1)$$

UC is the unitary cost per sample analysis for technology t, CM is the investment cost of the technology t, and N is the total number of analyses conducted during a year for a given type of machine. For the analysis of 100 milk samples, AMC and CC are the annual maintenance costs and consumable costs, respectively, which are different among machines. N is multiplied by L, which is the lifetime of each machine expressed in years, in order to obtain the average unitary costs that result from using the machine for its entire lifetime.

3. Results and Discussion

All of the correlation coefficients obtained for the MIR and ultrasonic technologies and reference methods (Gerber & Kjeldahel) for determination of the milk components were statistically significant (P≤0.05) (Tables 1 and 2). Statistical measures of repeatability, reliability, and precision of the results, obtained by analyzing milk with the ultrasonic analyzer were acceptable for the fat and protein content. Moreover, different statistical tests indicated that the performance of the ultrasonic method was satisfactory compared with the MIR method but there were some differences in results. The results of the milk analysis showed that the ultrasonic method underestimated the fat concentration by approximately 1 g/L and overestimated protein content by approximately 1.2 g/L, while the MIR method showed results very close to the direct methods for all milk components (Table 1).

3.1. Milk fat content

There was a strong and significant correlation (r=0.99, P<0.05) between the results of the MIR and the standard Gerber method for milk fat determination (Table 2). The agreement between the Gerber and MIR methods according to the CCC was 0.99 which indicated almost perfect agreement. The BCF value of 1.0 indicated that the line of best fit was close to equal to the perfect agreement line, whereas r^2 (0.98, P<0.05) indicated a very high precision of the MIR instrument compared to the reference Gerber method (Table 2 and Figure 1). The MPE value (3.86%) indicated very good predictability of the MIR, and the ICC value of 0.99 (P<0.05) indicated high accuracy (Table 2). Barbano and Lynch (2006) reported that MIR milk analysis is a rapid and very accurate testing method. When the MIR analyzers are suitably calibrated, they can be used as a method for payment to the farmer based on the milk fat and protein content (Karen & Barbano, 2016). No other instrument has been approved or used to any significant degree other than the MIR method (Lynch *et al.*, 2006).

A significant correlation (r=0.91, P<0.05) was found between the results of the ultrasonic and MIR instruments for milk fat determination. The agreement between the MIR and ultrasonic methods according to the CCC was 0.78 which indicated almost a good agreement. The BCF value of 0.80 indicated that the line of best fit was close to the perfect agreement line, whereas the r^2 value of 0.83 (P<0.05) indicated acceptable precision (Figure 2). The MPE value of

Table 1. Description of samples analyzed using different methods.

Constituent	Nbr Samples	Method	Mean	Sd
Fat (g/L)	220	Gerber	32.22[a]	3.7
		MIR	32.20[a]	4.6
		Ultrasonic	31.21[b]	4.1
Protein (g/L)	220	Kjeldahl	29.90[a]	4.8
		MIR	29.77[a]	4.2
		Ultrasonic	31.01[b]	4.4

[a, b] Values in the same column with different superscript letters were significantly different (P<0.05)

Table 2. Measures of statistical fitness to assess agreement between standard methods (Gerber and Kjeldahel) and mid-infrared and ultrasonic methods.

Measures of statistical fitness	CCC	r	BCF	MD (±95% CI)	MPE (%)	ICC
Milk fat method						
Gerber vs. MIR	0.99	0.99	1.00	0.02±0.58	3.86	0.99
MIR vs. US	0.78	0.91	0.80	0.98±2.08	17.45	0.69
Milk protein method						
Kjeldahl vs. MIR	0.97	0.96	0.99	0.12±0.41	5.14	0.99
MIR vs. US	0.75	0.90	0.79	1.26±2.02	21.01	0.63

CCC=concordance correlation coefficient; r=correlation coefficient; BCF=bias correction factor; MD=mean difference; MPE=mean prediction error; ICC=intraclass correlation coefficient; MIR=mid-infrared; US=ultrasound.

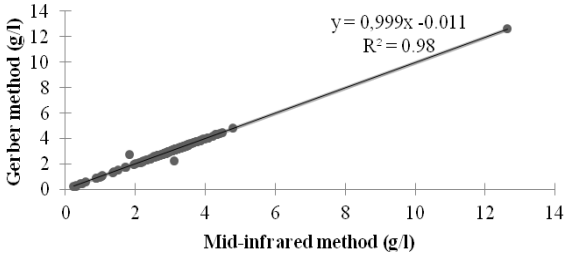

Figure 1. Correlation between fat analysis of Gerber and mid-infrared methods.

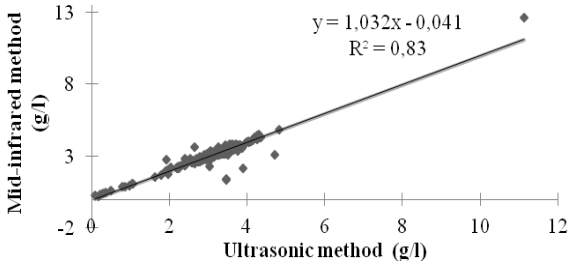

Figure 2. Correlation between analysis of mid-infrared and ultrasonic methods.

17.45% was above 10% which indicated poor predictability of the ultrasonic method, and the ICC value of 0.69 (P<0.05) indicated moderate accuracy (Table 2). Compared to the MIR instrument, the ultrasonic analyzer showed acceptable performance for the determination of the milk fat concentration. In general, the ultrasonic method is repeatable as indicated by the values of r^2, CCC, r, and BCF (Table 2). The ultrasonic analyzer underestimated the mean fat concentration by approximately 0.99 g/L. The reason for the discrepancy between the MIR and the ultrasonic results and the low predictability of the latter method is unclear but can be attributed to some factors. Indeed, the ultrasonic analyzer isn't equipped with an automatic stirrer, so the lab handler simply inverts the sample bottle to homogenize the fat before analysis. However, the MIR instrument (MilkoScan FT1) is equipped with a high pressure homogenizer, which makes an adequately homogeneous mixture of the milk fat and the fat globules have a uniform size. Therefore, fat globule size could be one of the causes of the difference in the fat results between the two methods. According to McClement (2005) the ultrasound technique is based on the interaction of sound waves with the emulsion components, which alters both the velocity and attenuation of the sound waves via absorption or scattering mechanisms or both. Awad, Moharram, Shaltout, Asker and Youssef (2012) reported that sound wave velocity is very sensitive to molecular organization and is affected by the mean droplet diameter and droplet size distribution in emulsions.

3.2. Milk protein content

A strong and significant correlation (r=0.96, P<0.05) was found between the results of the Kjeldahl and MIR methods for milk protein determination with high agreement between the two methods (CCC=0.97, P<0.05) (Table 2). The line of best fit was very close to the perfect agreement line (BCF=0.99, P<0.05), whereas precision (r^2=0.92, P<0.05) was classified as very high (Table 2 and Figure 3). The estimates for MPE and accuracy (ICC, P<0.05) were within the predetermined limits of acceptability (Table 2). Brenda and Barbano (2017) reported that the precision of the MIR instrument compared to the official methods makes it an easy and effective way for cheese producers to evaluate milk protein content and to determine protein recovery in making cheese. Because of its accuracy, MIR spectroscopy has been implemented in the measurement of milk protein content as an alternative to official methods (Etzion, Linker, Cogan, & Shmulevich, 2004).

There was a significant correlation between the MIR and ultrasonic methods for protein content (r=0.90, P<0.05). The results represented almost substantial agreement (CCC=0.75, P<0.05). The accuracy of the ultrasonic method to determine milk protein concentration was acceptable according to the ICC (ICC=0.63, P<0.05), whereas precision (r^2=0.81, P<0.05) was classified as acceptable (Table 2 and Figure 4). However, the MPE value of 21.01% was above 10%, which indicated poor predictability of the ultrasonic method for the determination of protein. The fit to the perfect line of agreement was moderate (BCF=0.79, P<0.05) (Table 2). The results from the duplicated analysis of the protein concentration in milk, with the ultrasonic analyzer showed a difference of 1.24 g/L compared to the MIR instrument. The

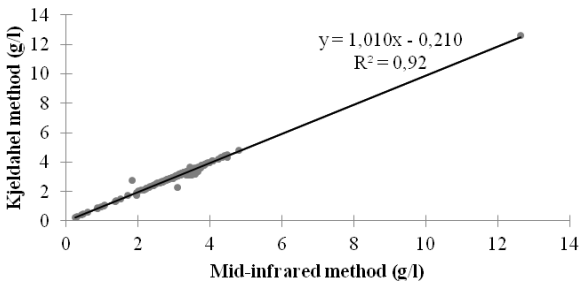

Figure 3. Correlation between protein analysis of Kjeldahel and mid-infrared methods.

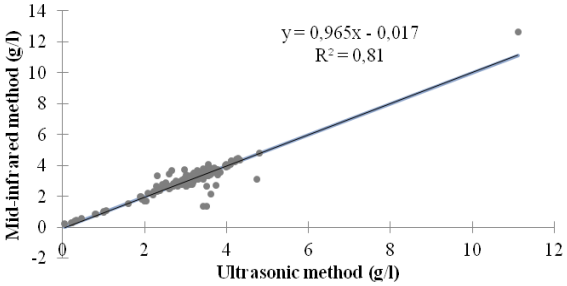

Figure 4. Correlation between protein analysis of mid-infrared and ultrasonic methods.

reason for the different results is not apparent, but the discrepancies could be attributed to the difference between the two measurement principles of the two methods. In general the MIR instruments are calibrated to determine true protein and not non-protein nitrogen, which represents less than 1.5% of the total nitrogen content of the milk. Barbano and Lynch (2006) described the improvement in accuracy of MIR measurement of all milk components that could be expected when true protein rather than crude protein is used as a reference, because MIR only detects true protein at the classical protein measurement wavelength. The ultrasound method is very sensitive to the viscosity and to the chemical characteristics of the analyzed solution. The ultrasound velocity is very sensitive to molecular organization and intermolecular interactions, which may cause differences in measurements (Buckin, O'Driscoll, & Smith, 2003). Pavlovskaya, McClements, and Povey (1992) studied the properties of aqueous solutions of a globular protein using the ultrasound method and found that the measurements of compressibility, density, and attenuation of the solutions were linearly dependent on protein concentration. Importantly, the measurements were sensitive to the isoelectric point and to casein content. Buckin & Kudryashov (2001) stated that fluid viscosity, thermal conduction, and molecular relaxation are the reasons for generating differences in emulsions and suspensions using ultrasonic methods.

3.3. Costs calculations

Given the high difference in the prices of the MIR and ultrasound technologies (Table 3), it is clear that an ultrasonic analyzer could be a better option from an economic perspective. This argument was further supported by cost calculations which showed that the unitary cost per analysis of

Table 3. Economic characteristics of both milk analyzers.

Analyzer type	Mid-infrared	Ultra-sonic
Investment costs (analyzer cost) (USD)	46.200	1155
Maximum life time (in years)	8	6
Annual maintenance costs (USD)	1925	77
Consumable expenses for 100 analysis (USD)	3.85	0.77
Maximum number of analyses per hour	100	60

USD=United States Dollar

a single milk sample was about 0.07 USD and 0.009 USD for the mid-infrared and ultrasonic technologies, respectively. However, these calculations represent the results of a unitary cost analysis of a single milk sample when both machines are used at their full capacities. In reality at a collection center, they would like to analyze the milk samples of all suppliers from an estimated 250 farms in production. This would be about 175,000 analyses per year which allows for full control of the milk quality. If we take this assumption into consideration, the unitary costs to analyze a single milk sample would be better represented by changing **N** in equation 1 with $\bar{\mathbf{N}}$ which is a scalar parameter representing the total number of analyses effectively needed to be conducted by the collection center. Taking this into consideration, the results based on 175,000 analyses per year showed that the unitary costs of milk analysis using MIR and ultrasonic technologies would be 0.082 USD and 0.009 USD, respectively.

Based on the technical results obtained in this paper and the economic calculations regarding costs of both machines and their respective capacities, we can conclude that it would be interesting to promote the ultrasonic technology not only for collection centers, but possibly for medium and large milk farms. This could help farmers adjust their feeding strategies to obtain higher milk quality and prices.

4. Conclusions

The ultrasonic method could be an appropriate alternative method to determine the fat and protein concentrations on a small scale when high precision is not needed. The most significant advantages of the ultrasonic method are the low cost and the ease of handling that make it a good instrument for rapid evaluation of milk in small dairies and milk collection centers. The ultrasonic method can also be used in an advisory service to provide farmers with daily information on variations of major milk constituents which would offer considerable support for farm management and as an additional benefit compared with monthly records.

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