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ASSESSMENT OF BUFFALO MILK NUTRITIONAL COMPOSITION USING FT-IR SPECTROSCOPY

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Abstract

In the last decade, FT-IR spectroscopy has been introduced in methodological portfolio specific for analyzing food products, because it is a very efficient, and non-destructive analytical tool. Vibrational spectral techniques, as FT-IR, offer several advantages in the context of current research and using this techniques we can identify molecular components in the studied samples. In this study FT-IR spectroscopy technique is applied to detect fat, protein, and lactose content of buffalo milk, and compositional differences between samples corresponding to different lactations. The results emphasizes specific evolutions corresponding to the increase of protein, lactose, and fat buffalo milk contents, from the first lactation up to the fifths/fourths lactations.

Key words*: buffalo milk, stage of lactation, FT-IR.*

INTRODUCTION

Buffalo milk is characterized by specific chemical composition. Fat, lactose and protein content, which vary function of different factors such as area (Tăpăloagă et al., 2012) breeding, or stage of lactation, confer the special traits of the milk. In order to be used in different aims (consumption, raw material for cheese production, etc.) the buffalo milk must be collected properly and it also has to be free of colostrum.

Fourier - transform infrared spectroscopy (FT-IR) is one the most widely used method for detection of the compositional differences between samples, and it relies on the basic vibration of various chemical groups at specific wave lengths within the interval 400-4000 cm^{-1}.

This technique may be considered, by the food industry, as potential tool for testing the quality of food products, based on the fingerprint region (1800-200 cm^{-1}) because it can provide large information about functional groups characterizing the chemical composition of samples (Babushkin et al., 2016; Ketty et al., 2017; Kučević et al. 2017).

FT-IR technique, which is a non-invasive method, and involves minimum effort for sample preparation, may be considered a simple and fast alternative to other laborious and expensive analyze techniques.

For this reason it is used in many fields such as food science, chemical industry, pharmaceuticals study, Chinese medicine, food control, medicine (Liu at al., 2006; Xu et al., 2006; Andronie et al., 2011; Geghardt et al., 2011).

FT-IR technique was applied with great success for classification of raw of milk, collected from cooperatives located in three areas of Morocco (Elbnassbasi et al., 2010).

Lei Yu et al. (2010) performed a study where IR spectroscopy was used in combination with two dimensional (2D) correlation infrared spectroscopy for analysis of crystalized lactose, protein and fat in milk powder.

Grewal et al. (2017) used the advantages of this method in order to detect physico-chemical changes that lead to sedimentation or gelation under accelerated storage temperature in UHT (ultra-high temperature) milk and was used by for their study.

The aim of this study was to investigate the buffalo milk quality, in terms of fat, protein, and lactose content, depending on the stages of lactation, using FT-IR spectroscopy.

MATERIALS AND METHODS

The trial was carried out on a private farm located in Zalău, Sălaj County, Romania (47°11'28''N, 23°3'26''E). 50 buffalo females (*Bubalus bubalis* L.), Romanian buffalo breed, in different stage of lactation, were used. They were milked twice daily, in the morning and in the afternoon. Function of lactation, the buffalo females were divided in five groups.

The milk was centrifuged at 13,000 x g for 5 minutes, previously to further analysis. Lactose, protein, and fat, from the fresh buffalo milk samples were analyzed function of lactation, using FT-IR spectroscopy.

FT-IR spectroscopy was performed with Nicole FT-IR spectrophotometer equipped with Horizontal Attenuated Total Reflectance (HATR) with ZnSe accessory. IR frequencies are expressed by a light number that is directed to the sample. When radiant energy is equal to the vibrational frequency of the molecule, it realizes the suction and vibrating. Absorption intensity for each frequency of vibration is monitored by a detector. Specific footprint is a specific combination between molecular vibration and rotational vibration and has a great significance to identify specific molecules. Measurements were carried out on infrared scale of 650-4000 cm^{-1}, 100 scans per sample at 2 cm^{-1} resolution (Fig. 1).

These spectra were analyzed by comparing the obtained vibrational bands with those of similar functional groups from the literature (Ley el.al., 2010; Murphy et a., 2014; Jaiswal et. Al. 2015; Mendelsohn et.al., 2010).

The IBM SPSS v.19.0 for windows, was used for statistical analysis. Basic statistics, was implemented in order to emphasize the mean (X), standard deviation (SD) and coefficient of variation (CV%), of protein, fat, and lactose, by lactations. The mean concentration of milk components were compared across the various lactations using one-way analysis of variance (one-way ANOVA). Differences of the means were considered to be significant when p-value < 0.05 (Kittivachra et al., 2007). The Box-Plot diagrams were used for showing the distributions of the lactose, protein, and fat, by lactations. Minimum, maximum concentration values, and quartiles (first, median, third, and forth) are emphasized.

RESULTS AND DISCUSSIONS

The FT-IR spectra of milk in different stage of lactation present the same band positions and relative intensities with some differences.

The obtained FT-IR spectra emphasize the main types of structures present in the analyzed milk samples.

In order to determine the fat content of milk is very important to check the intensity of the peak 1743 cm^{-1} and 1161 cm^{-1}. In the region between 3000-2800 cm^{-1} are reported substantial changes in intensity, ad IR spectra show two peaks at 2920, 2852 cm^{-1} rending to fat content in the buffalo milk. The content of fat decreases in concentration depending on lactation. Both, peak characteristic for C=O bond in fat, corresponding to 1743 cm^{-1}, and peak representing C-O vibration in fat, corresponding to 1161 cm^{-1} are noticeably decreasing in intensity from lactation 1 compared to lactation 5 (Fig. 1).

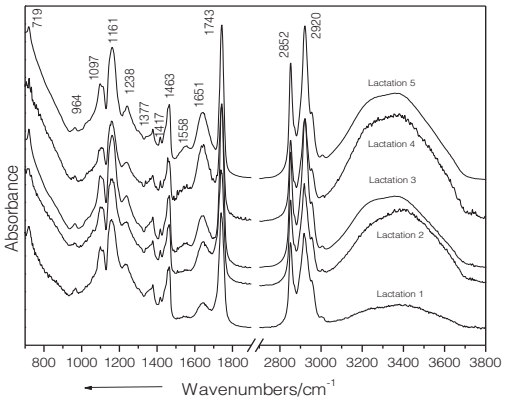

Figure 1. FT-IR spectra of milk in different stages of lactation

The region 1700-1500 cm^{-1} has two main parts, namely amide I (1700-1600 cm^{-1}) corresponding to C=O stretching mode of the peptide bonds, and amide II (1600-1500 cm^{-1}) attributed to C-N stretching vibration (Fig. 1). Another peak that increases in intensity at lactation 5 was found at 1651 cm^{-1} for the vibration of amide I. The peak reported at 1558 cm^{-1} was more emphasized in the spectrum that corresponds to lactation 5 (Fig. 1).

Region 1500-1200 cm^{-1}, amide III spectral region, respectively, also corresponds to secondary structure of proteins. In this region

was reported a peak at 1463 cm^{-1} were the increase in intensity is not very emphasized (Fig. 1).

For lactose, specific spectrum ranges from 1150 to 1030 cm^{-1} and the peak corresponding to C-O groups of the lactose is reported at 1097 cm^{-1}. The peak reported at 964 cm^{-1}, characterized by very low intensity was assigned to C-O vibration for carbohydrates (Iñón et. al. 2004). The mean content of protein in buffalo milk gradually increases from the lactation 1, when 4.98% content is reported, to the lactation 5, when 6.44% content is emphasized, by 1.48%, respectively.

The differences between mean protein buffalo milk content, function of lactations were not statistically assured at significance threshold 1%, between lactations 1, 2, and 3, on one hand, and 4, and 5, respectively, on other hand, but they were assured at significance threshold of 1% between lactation 1 (4.98%), and lactation 4 (6.07%), and at significance threshold of 0.1%, between lactation 1 and lactation 5 (6.44%), respectively. The standard deviation emphasizes a normal distribution of the individual protein concentration, and the values of coefficients of variations within the interval CV=1.51% (Lactation 3), and CV=4.57% (Lactation 1), confirm the representativeness of the means (Table 1).

Concerning the mean lactose content in buffalo milk, our study also emphasizes a gradual increase from lactation 1 to lactation 5, by 0.62%. The differences between mean lactose buffalo milk content, function of lactations were statistically assured at significance threshold 1%, only between lactation 1 (4,69%), and lactation 5 (5.62%).

The standard deviation, in this case, too, emphasizes a normal distribution of the individual lactose concentrations.

The coefficients of variations with values within the interval CV = 2.55% (Lactation 1), and CV = 3.47% (Lactation 6), emphasizes the representativeness of the means (Table 1).

The buffalo milk analyzed our study had a fat content, which increase from the lactation 1, when a mean of 8.33% is reported, to lactation 4, when it is reported a mean of 9.02%, by 0.69%, respectively. In lactation 5, the mean lactose content slowly decrease to 8.96%, by 0.62% higher, compared to lactation 1.

Table 1. The basic statistics and significance of differences, for protein, lactose, and fat content quantified in buffalo milk, function of lactation (%)

Issue		Protein (%)	Lactose (%)	Fat (%)
Lactation 1	n	10	10	10
	X	**4.98^{abc}**	**4.69^{ab}**	**8.33^{ab}**
	SD	0.22	0.12	0.7
	CV%	4.57	2.55	8.34
Lactation 2	n	10	10	10
	X	**5.36^{a}**	**4.98^{a}**	**8.64^{a}**
	SD	0.14	0.16	0.21
	CV%	2.69	3.12	2.50
Lactation 3	n	10	10	10
	X	**5.64^{a}**	**5.14^{a}**	**8.90^{a}**
	SD	0.09	0.15	0.44
	CV%	1.51	2.90	4.90
Lactation 4	n	10	10	10
	X	**6.07^{ba}**	**5.31^{ab}**	**9.02^{ab}**
	SD	0.17	0.16	0.32
	CV%	2.87	2.92	3.57
Lactation 5	n	10	10	10
	X	**6.44^{ca}**	**5.62^{ab}**	**8.96^{ab}**
	SD	0.15	0.20	0.42
	CV%	2.3	3.47	4.63

X – mean; SD – standard deviation; CV – coefficient of variation;
a – $p > 0.05\%$, b – $p < 0.05\%$; c – $p < 0.01\%$.

The differences between mean fat buffalo milk content, function of lactations were statistically assured at significance threshold of 1% between lactation 1 (8.33 %), and lactation 4 (9.02%), and 5 (8.96%), respectively.

The standard deviation emphasizes a normal distribution of the individual protein concentration, and the values of coefficients of variations within the interval CV = 2.50% (Lactation 2), and CV = 8.34% (Lactation 1), confirm the representativeness of the means (Table 1).

According to Box-Plot diagram (Fig. 2), different individual values are recorded for mean protein content identified in buffalo milk, function of lactations, but equilibrate distributions are emphasized only for lactations 2, 3, and 5, emphasizing similar individual values, meaning high samples homogeneity.

In lactations 1 and 4, the protein contents show asymmetric distributions, which suggest high variability of individual values.

In lactation 1, where the quartiles 1, and 2 are considerably bigger, predominate smaller individual values, compared to the mean, while

in lactation 4, where third quartile is bigger, slight predominance of bigger values compared to the mean, is suggested.

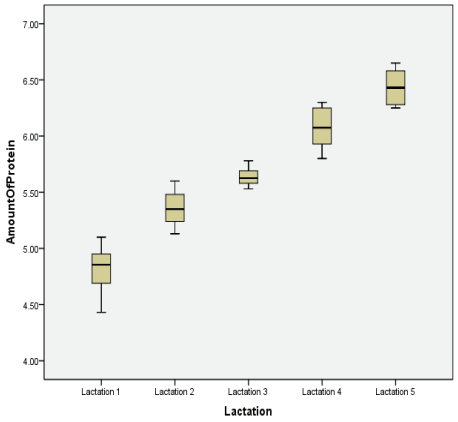

Figure 2. Comparison of protein content in different lactation stages

The distributions of the individual values of the lactose content in buffalo milk, by lactations, are predominant asymmetric. One exception is recorded, and it corresponds to lactation 2, where Box-Plot diagram emphasize equilibrate quartiles.

In lactations 1, and 3, quartiles 1 and 4 are bigger, and this signifies that smaller individual values are predominant, compared to the mean.

Corresponding to lactations 3, and 5, quartile 4, and 3 and 4, respectively, are more extended, meaning that, in this case, bigger individual values are predominant, compared to the mean (Fig. 3).

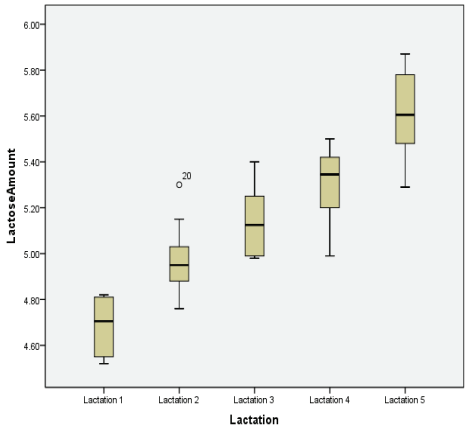

Figure 3. Comparison of lactose content in different lactation stages

The mean fat content shows different individual distributions, by lactation, as emphasized by Box-Plot diagrams (Fig. 4).

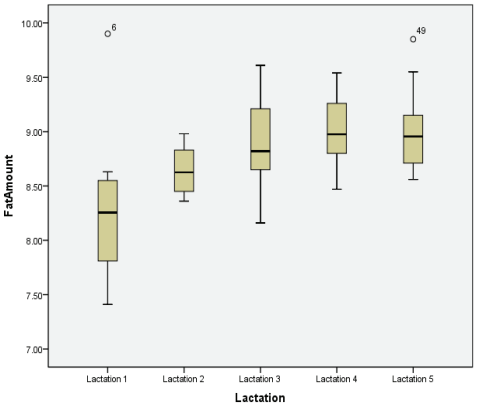

Figure 4. Comparison of fat content in different lactation stages

In lactation 2 a symmetric distribution is recorded.

Lactation 1 is characterized by asymmetric distribution, where quartiles 1, and 2 are bigger, meaning predominant lower values compared to the mean.

Lactations 3, and 4, also with asymmetric distributions, have the biggest extent correspondent to quartile 3. Lactation 5, also characterized by asymmetry, is characterized by the predominance of mean fat content values corresponding to quartiles 1, and 4.

CONCLUSIONS

The protein is the nutritional trait of buffalo milk, which is most affected by the lactation stage, exhibiting an increase of 1.48%, from lactation 1 to lactation 5. Less affected by lactation is fat content, where an increase of 0.69% is recorded between lactation 1, and lactation 4, and lactose content, where an increase of 0.62% is reported from lactation 1 to lactation 5. Continuous increase of protein and lactose buffalo milk content is reported function of lactations, while concerning lactose, discontinuous evolution is observed, with an increase from lactation 1 to lactation 4, followed by a slight decrease in lactation 5. Based on the results of our study, it can be concluded that the FT-IR spectroscopy is a reliable instrumental technique for the

determination of lactose, protein and fat in milk. This method offer highly specific, precise, and accurate and linear data across the analytical range. The study has show that the FT-IR method is indeed a acceptable instrumental technique for analyzing the chemical parameters characterizing the buffalo milk in different stages of lactation.

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