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Qualitative evaluation of buffalo cheese using FTIR spectroscopy

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Abstract. FTIR is a rapid technique based on infrared which has been used to analyze the following samples of cheese: traditional buffalo milk cheese, mouldy cheese traditionally produced (in Mesendorf), buffalo milk cheese (Napolact) and buffalo mozzarella (Italy). Here were highlighted main wavelengths at which the main components were observed in cheese, namely: fat, protein, lactose and water. These compounds have been outlined in terms of quality. The presence of water was observed in the region of 3600-3010 cm^{-1}, showing a high content in water for mozzarella, followed by buffalo milk cheese, traditional cheese and the lowest content being observed to mouldy cheese.
Key Words: FTIR, buffalo cheese, protein, fat, lactose.

Introduction. Infrared spectroscopy (IR) technique is the most common tests used to analyze organic and inorganic compounds. The objective of this analysis by spectroscopic techniques is to determine all major functional groups in the test sample.

Each functional group has a characteristic absorbance spectrum infrared radiation (Stuart 1996). Fourier Transform Infrared Spectroscopy (FTIR) has many advantages compared with conventional methods, is reproducible and accurate, with a capacity of handling precise spectral and could be coupled with chemometrics software for calibration in quantitative analysis purpose (Van de Voort et al 1994a). Due to these advantages, FTIR spectroscopy can provide information on the characteristics, composition and chemical changes during the technological process (Van de Voort et al 1994b). From a practical perspective, quantitative analysis using FTIR is a rapid (1-2 minutes) test, reducing the quantities of solvents and toxic reagents.

The FTIR analysis methods are used for different types of accessories, the most widely used being Horizontal Attenuated Total Reflectance (HATR). Its simplicity and convenience analysis (Sedman 1999) requires only small volumes (<50 ml) of reaction, just to cover crystal surface of Attenuated Total Reflectance (ATR). FTIR spectroscopy is a Scientific Association Dedicated to Excellence in Analytical Methods (AOAC) official method for determination of fat, protein and lactose in milk (AOAC 1995), and can be used in monitoring the hydrolysis of lactose in milk, showing that lactose can be quantified in the presence of its hydrolysates products, glucose and galactose. Hansen (1999) investigated the possibility of commercial use of infrared milk analyzer, but the calibration of the instrument has not been set to obtain quantitative results.

Meantime there were made quantitative analysis which assays to monitor the enzymatic processes that play a role in lactose reduced milk. Regarding Ceddar and Mozzarella cheese, there were obtained to IR analysis specific bands for fats and proteins. It was found that the intensity of absorbance of these bands increases proportionally with fat and protein content. IR technique became a fast analysis technique to determine fat and protein in milk (Chen & Joseph 1998; Lefier et al 1996; Koca et al 2007). Buffalo milk cheese is one of the cheese aged in brine, alongside sheep cheese, cow albumin added cheese and Fetta.

Achieving good quality cheese is conditioned by the quality of milk used as staple for cheese (including sensorial characteristics, physical-chemical, hygienic and microbiological).

Water content from buffalo milk cheese has an average of 47.62%, 3.28% for NaCl and fat on a dry basis is 46.17% (Pece 2008). Cheese production is a complex process consisting of protein concentration with a variable fraction of fat and minerals. The removal of a considerable quantity of water and lactose is required.

Material and Method. FTIR technique is a fast and simple method compared with other laborious and expensive techniques. In our case this technique has been used for the analysis of cheese. For the IR spectral analysis was used Nicolet 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. IR absorption spectrometry is therefore appropriate for liquid sample analyzing.

Cheese and other solids should be homogenized before performing the analysis (Biggs 1972).

Measurements were carried out on infrared scale of 650-4000 cm^{-1}, 100 scans per sample at 2 cm^{-1} resolution. After analysis of each sample the accessories were washed with acetone. Samples analyzed were: buffalo milk cheese, mouldy cheese traditionally produced (in Mesendorf), buffalo milk cheese (Napolact) and buffalo mozzarella (Italy). Cheese samples were well homogenized in order to perform proper analysis by infrared spectroscopy.

Results and Discussion. FTIR spectral recording was performed to identify the main types of structures present in the analyzed samples. There were selected spectral intervals between: 2900-2827 and 1782-1705 cm^{-1}, which correspond to fat content in cheese. The spectral region between 1701 and 1507 cm^{-1} corresponded to protein content and spectral between 1200 and 967 cm^{-1} corresponded to carbohydrates (Iñón et al 2003). Specific water absorption bands were located in accordance with the literature, in the regions between 3650-3000 cm^{-1} and 1680-1600 cm^{-1} (Hop et al 1993; Iñón et al 2003). The spectral circa values of 1800-700 cm^{-1} are considered to be "fingerprint" values (specific fingerprint of each compound). In literature this area has a coverage between 800-1200 and 1000-1500 cm^{-1} (Van de Voort et al 1994b) but in this study we extended it to 1800 cm^{-1}. The spectral values of circa 1700-1800 cm^{-1} are considered important because the absorption frequency around 1711 cm^{-1} corresponds to free fatty acids and this absorption is directly proportional to the percentage of free fatty acids contained in milk. In figure 1 are shown spectra of cheese samples analyzed.

Studies of milk and milk products by FTIR spectroscopic method have been made by many researchers: Belton et al (1987), Biggs (1972, 1979), McGann (1978) and McQueen et al (1995). IR spectroscopy is designed to identify the molecular vibrations of polar bonds in different molecules. IR spectra have the ability to identify signals in length frequencies between 400 and 4000 cm^{-1}.

Using FTIR spectrophotometric technique determined the secondary structure of proteins (Boye et al 1995). A similar study of that we had conducted was done by (Manxiang & Joseph 1996) on Cheddar and Mozzarella cheese.

Mendenhall & Brown (1991) described that proteins were observed to 3030, 2500, 1698, respectively 1656 cm^{-1} wavelength bands. According to researches conducted by (Belton et al 1988) wavelengths for the various chemical groups were as follows: 1477-1400 and 1195-1129 cm^{-1} for ester carbonyl C-H and C-O group; 1650 cm^{-1} for fat and 1540 cm^{-1} corresponds to the protein. Using FT-NIR and FT-IR spectroscopy (Cattaneo et al 2005) studied some parameters of Crescenza cheese. For our study the presence of water was observed in the region of 3600-3010 cm^{-1}.

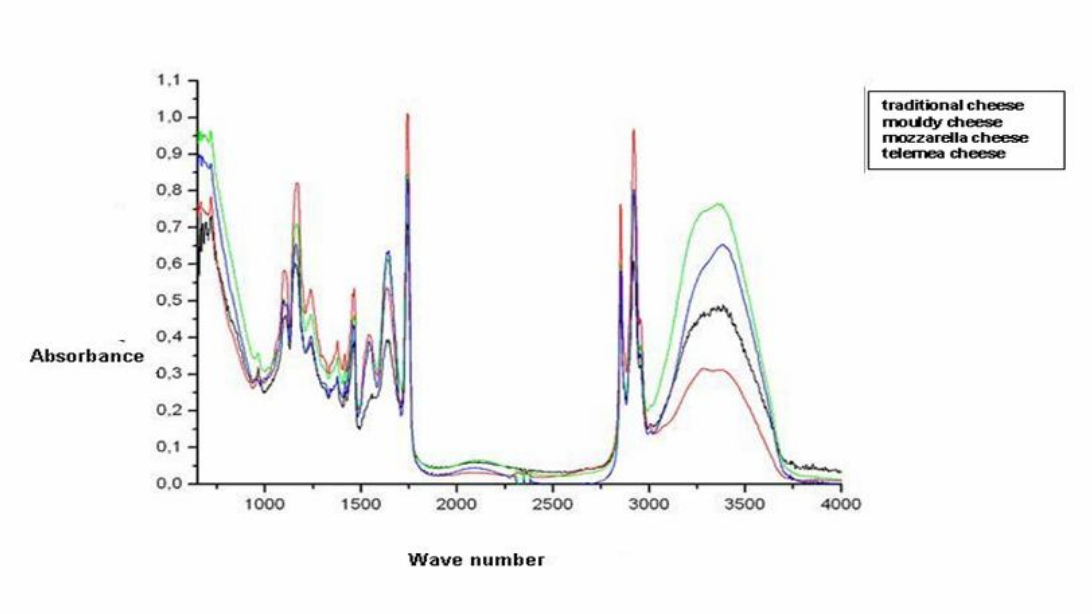

Figure 1. FTIR spectra of analyzed cheese samples.

Table 1

Representation of band (b) and shoulders (u) frequency of analyzed cheese using FTIR-ATR spectroscopy

Frequency no. (cm^{-1})	*Cheese sample*	*Band type*	*Vibration mode*
1.	3005	=C-H (cis-)	of spread
2.	2920	-C-H (CH_2)	of spread (asymmetrical)
3.	2850	-C-H (CH_2)	of spread (symmetrical)
4.	1741	-C=O (ester)	of spread
5.	1415	=C-H (cis-)	of deformation (rocking)
6.	1377	-C-H (CH_3)	of deformation (symmetrical)
7.	1240	-C-O, -CH_2-	of spread
8.	1161	-C-O, -CH_2-	of deformation of spread
9.	1112	-C-O	of deformation
10.	1097	-C-O	of spread, of spread
11.	964	-HC=CH- (trans-)	of deformation outside the plane
12.	719	-(CH_2)n-, -HC=CH-(cis-)	of deformation (rocking)

Mozzarella cheese presented the highest water content and the lowest content has been registered to mouldy cheese. The highest protein content (compared to the absorbance value) was identified in mouldy cheese. According to researches conducted by Luienge et al (1993) the corresponding region of proteins and amides I (C=O) was identified

between 1670-1630 cm^{-1} wavelength. In table 1 are presented the frequencies of bands and shoulders of cheese analyzed using FTIR-ATR spectroscopy.

Conclusions. Infrared spectroscopy can be used as a rapid method for cheese and other milk products legalization. IR absorption spectroscopy has practicability in liquid samples therefore cheese and other solids should be well homogenized before performing analysis. FTIR spectra revealed the main compounds from various types of cheese analyzed and the wavelengths at which these compounds can be observed in accordance with the literature, namely: fat has the appropriate interval range at 2900-2827 cm^{-1} and 1782-1705 cm^{-1}, proteins at 1701-1507 cm^{-1} and for water the appropriate range is 3650-3000 cm^{-1} and 1680-1600 cm^{-1}.

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