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Comparative Study of Constituents of Fresh Raw Milk of Buffalo, Cow and *Equus asinus* (Donkey)

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The study investigates the qualitative differences in the spectrum of milk of three mammalian species buffalo, cow and *Equus asinus* (donkey) in the mid-infrared region corresponding to the amide I, amide II and amide III bands of casein micelles that form about 80 % of the protein in bovine milk. The absorptions of other principal constituents; fat content due to carbonyl stretching and lactose with characteristic C-OH band for all three species have been studied and compared with available quantification data. In addition, milk proteins also play an important role in dairy and food products.

Key Words: Raw milk, FTIR spectrum, Constituents, Analysis.

INTRODUCTION

The main constituent of milk is water that can range from a low content in marine mammals to a high content in human milk and others. Cow milk has 87 % water. Lactose which comprises of glucose and galactose range from 4.8-5.2 % of milk. The casein contents in milk constitutes 80 % of milk proteins (3-4 % of milk), 20 % are whey proteins and trace fractions of glycoproteins. The milk casein exist in structures called micelles having diameters 90 to 150 nm and the evidence from electron microscopy suggest that micelles have smaller units called submicelles having diameters 10-20 nm. The micelle structure are flexible and do not have well defined three dimensional structures. The principal casein fractions are α(S1) and α(S2) caseins, β-casein and κ-casein. The composition factor is that caseins are conjugated proteins most with phosphate groups. αS-caseins are the major casein proteins containing 8-10 seryl phosphate groups, while β-casein contains about 5 phosphoserine residues and it is more hydrophobic than αS casein while κ-caseins contain one phosphoserine residue. The phosphate group is important to the structure of the micelles and its stability in precipitation of Ca^{2+} ions. Besides, calcium and phosphate, the micelle structure contains citrate, minor ions, lipase, plasmin enzymes and entrapped milk

serum. The submicelle model is thought to have two different kinds of submicelle *i.e.*, with and without κ-casein. In the micelle structure proposed by Waugh there is a hydrophobic association of α and β casein that are roughly spherical and are coated with a monolayer of κ-casein while the model of Morr suggest that α and β caseins are coated by a layer of β-casein. The final size of the micelle will depend on the amount of β-casein forming the coating. κ-Casein will stabilize αS-casein from precipitation by calcium. A three dimensional molecular model describes the structure of αS-casein as containing 15 % α-helix, 22 %β structure, 45 % turns and 18 % that cannot be specified. The structure of κ-casein contains 16 % α-helix, 27 % β structure, 37 % turns and 20 % that cannot be specified. Caseins are among the most hydrophobic proteins and there is some evidence to suggest that they play a role in the stability of the micelle. They may have a preferred secondary and tertiary structure, they are often in other conformations. The other structures must expose hydrophobic groups to contact with water making the casein sensitive to structural alterations[1-4].

EXPERIMENTAL

The mid-FTIR spectrum of milk from three mammalian species buffalo(b), cow(c) and equus asinus(d) recorded on a Perkin Elmer spectrophotometer in the range 4000-500 cm^{-1} is presented in Figs. 1-3, respectively. The transmission measurements were effected with a cell path length of 0.03 to 0.05 mm for optimum quantitative performance. The fresh raw milk were procured from sources milked manually from the species, refrigerated and after *ca.* 4 h their spectrum was recorded.

RESULTS AND DISCUSSION

The analysis of protein components in milk is difficult for two main reasons. First, water, which is the continuous phase in milk, has intense absorbance bands centered *ca.* at 1640 cm^{-1} due to H_2O bending vibrations and 3300 cm^{-1} due to O-H stretching vibrations. The coincidence of O-H in-plane bending with amide I band a key band to casein structure poses experimental difficulty while the amide II band 1570 to 1550 cm^{-1} is much weaker than amide I band[5]. The formation of both intra- and intermolecular hydrogen bonds by proteins affects the position, magnitude and shape of the amide bands and are sensitive to a number of factors by which the amide bands are influenced by the protein environment. Hence adequate measurement configurations of optic path is required to keep water absorbance reduced and ensuring absorbance by fat globules that are at least one order of magnitude larger than the casein micelles[6]. The difficulty with this approach stems from the considerable effect that particle size, shape,

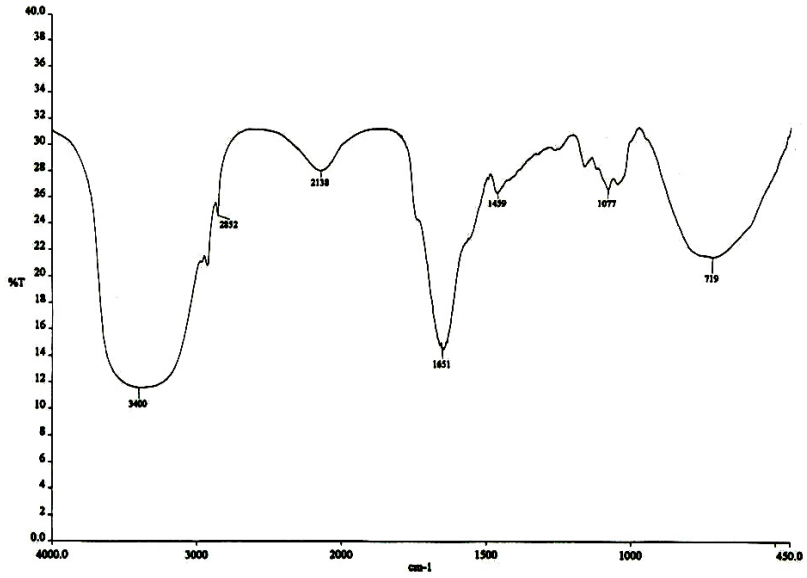

Fig. 1. FTIR Spectrum of buffalo milk (b)

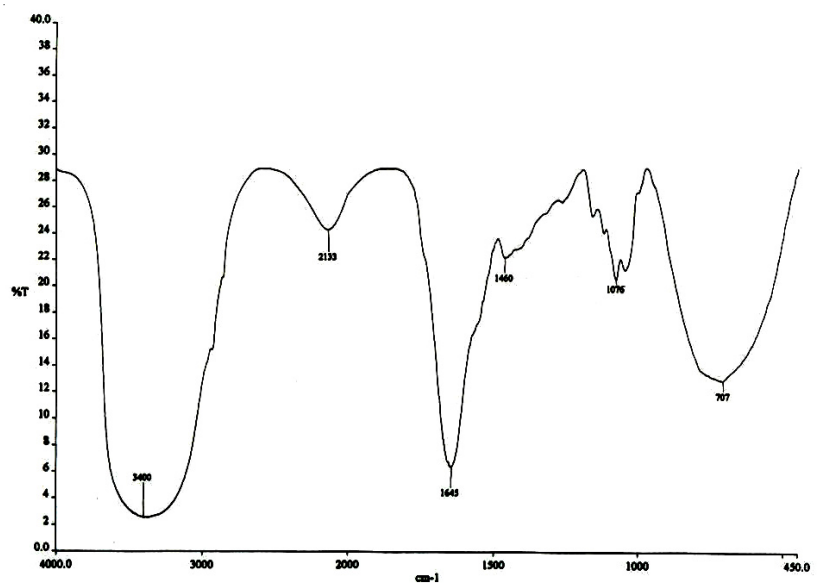

Fig. 2. FTIR Spectrum of cow milk (c)

density and distribution have on the spectrum. The study involved IR measurements with three mammalian species of raw milk without much affecting the actual environment of protein and other constituents of milk.

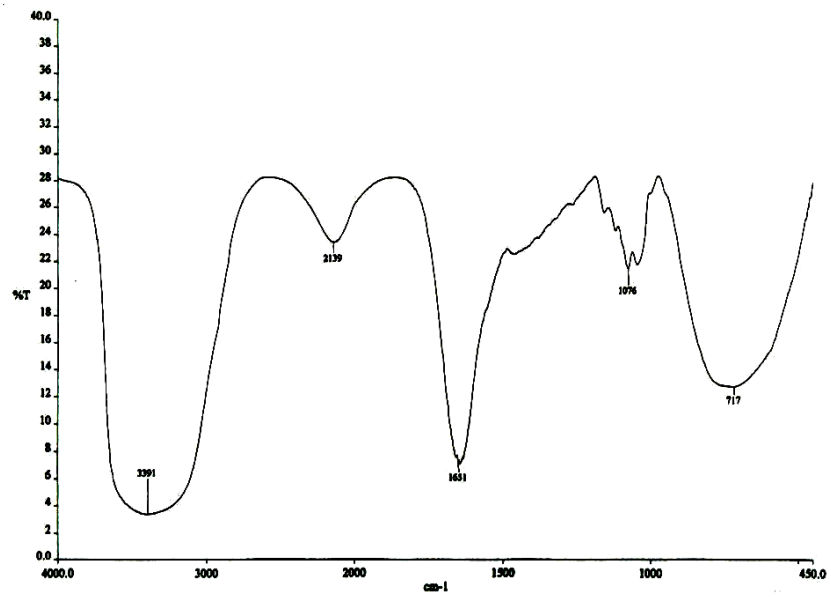

Fig. 3. FTIR Spectrum of Equus asinus (donkey) milk (d)

Casein exhibits characteristic amide I, amide II and amide III absorption bands. The amide I band is primarily a C=O stretching vibration. The amide II band is a combination of C-N stretching and N-H in-plane bending while the amide III is a mixed mode of C-N stretching and N-H in-plane bending with minor contributions from C-C stretching and C=O in-plane bending. The assignment of specific secondary structures to the amide I band is the α-helix and β sheet of the αS-casein at about 1663 and 1645 cm^{-1}, respectively for all the three species. The unordered structures of the side chain of caseins absorb considerably lower than the β sheet at about 1616 cm^{-1} in milk of buffalo and donkey while it is absent in milk of cow due to overlap with β sheet absorbance possibly a high α-helix and low β sheet content[7]. The investigations on proteins with well known structures have established a clear relation of the amide III band shape and the secondary structure and quantitatively their content. The amide III band corresponding to α-helix at 1290 cm^{-1} while the β sheet at 1250 cm^{-1} are indistinctive and weak absorptions for all three species[8]. The amide II is an out-of-phase combination of NH in-plane-bending and CN stretching with minor contributions from C=O in-plane bending, C-C stretching and N-C stretching. The amide II band is not seen for d but appears as a weak absorption at about 1547 cm^{-1} for milk of buffalo and cow. The 85-88 % water content of the milk causes very strong absorption of the intermolecular and bonded O-H.The broad and strong absorptions at 3400 cm^{-1} in milk of buffalo and cow and 3391 cm^{-1} in milk of donkey is the ν_1 mode of O-H. The out-of-plane bending of

O-H also appears as a strong broad absorption at about 719 cm^{-1} in milk of buffalo, 707 cm^{-1} in milk of cow and 717 cm^{-1} in milk of donkey. This band coincides with the absorption of lactose and the N-H out-of-plane bending corresponding to amide V band of casein structure. The phosphate ion (PO_4^{3-}) in the micelle structure of casein shows a ν_3 band due to P=O stretching at about 1076 cm^{-1} for all three species. The PO_4^{3-} ν_1 band can be seen to occur at 966 cm^{-1} in milk of buffalo and 977 cm^{-1} in milk of cow and donkey. The C-H stretching and CH_2 bending due to fatty acids at 2931, 2852 and 1459 cm^{-1} in milk of buffalo; 2932, 2862 and 1459 cm^{-1} in milk of cow and the only 1453 cm^{-1} band in milk of donkey assay the fat content in milk. The fat component of milk is composed of a complex mixture of lipids. Triglycerides are the major type of lipid in milk fat (98.3 %). Triglycerides are composed of three fatty acids covalently bound to a glycerol molecule by ester bonds. The carbonyl stretching due to fatty esters at 1733 cm^{-1} in milk of buffalo and 1721 cm^{-1} in milk of cow indicate the fat content of milk. The relative intensities of these bands in the spectra follow as milk of buffalo > milk of cow > milk of donkey coinciding with the available data of milk fat in the three species as buffalo(10.4 %), cow(4.1 %), donkey(1.2 %)[9]. A considerable difference in the absorbance for the three species at these frequencies support the low fat content of donkey milk. Further the C-C stretchings of fatty acids and esters show an absorption at 1163 cm^{-1} the band being more pronounced in milk of buffalo than in milk of cow and donkey. The lactose comprising of D-glucose and D-galactose joined by β-glycosidic linkage is the next major constituent of milk. Lactose is responsible for drawing water into the milk during formation and is the least variable component of milk approximately in buffalo (4.3 %), in cow (4.9 %) and in donkey (6.9 %)[9] for the species studied giving characteristic C-OH vibrations[10] at 1093 and 1041 cm^{-1} for all the three species. The lactose content in terms of band intensities appear as milk of buffalo less than milk of cow and donkey in the present study. A combination band consisting of the torsional oscillations and asymmetric bending of the NH_3 group of amino acids occurs at about 2135 cm^{-1} for all the three species of milk.

Conclusion

The results presented in this work show the potential usefulness of mid-FTIR spectroscopy for determining the major constituents of raw milk The water subtraction method and other spectral enhancement procedures have not been adopted so as to compare the spectra in a completely true hydrated environment of the fresh raw milk. Specifically the amide I region in the spectra of the three mammalian species show the bands of the secondary structure of casein being well ordered and more defined in milk of donkey than milk of buffalo and cow. The fat content and lactose content

of milk for the three species follow the same observations available from other other instrument methods like milk analyzers. All other absorption bands in the spectra of milk of buffalo, cow and donkey have been accounted and assigned to their respective mode of vibration.

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