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COMPARATIVE ANALYSIS OF COW MILK SAMPLES USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

Umesh Chandra & Harendra Kumar Department of Zoology, J.S.University, Shikohabad, Firozabad- 283135 (U.P.), India :: k.harendra82@gmail.com

Abstract

In this study, Fourier Transform Infrared Spectroscopy (FT-IR) was used to record the spectra of cow milk both before and after feeding mulberry leaves. The goal was to identify spectral changes and characterize different wavelengths. The FT-IR spectra were analyzed to determine the types of vibrations (stretching and bending), visible intensities and infrared (IR) wave numbers ranging from 4000 cm^{-1} to 400 cm^{-1} for prominent peaks.

The FT-IR data revealed the presence of various functional groups, including N–H, O–H, C–H, C–O, C–C, C=O, SO_3, O–N=O and C–O–C as well as biochemical constituents such as phenols, amines, alkenes, alkanes, alkynes, carboxyl groups, esters, ethers, polysaccharides, nitrites and sulfur compounds in the cow milk. The O–H stretching band at 3384.2 cm^{-1} increased after the goats were fed mulberry leaves. Additionally, bands corresponding to C–H asymmetric stretching were observed at 2924.2 cm^{-1} and 2854.6 cm^{-1}.

These findings suggest that mulberry leaves significantly influence the biochemical composition of cow milk, indicating their potential as an exceptional feed for cow's and other small ruminants.

Keywords–Characterization, functional groups, mulberry, milk, FT–IR spectra.

Introduction

The domestic cow is a subspecies of wild cattle that were domesticated in Southwest Asia and Eastern Europe. Cows belong to the family Bovidae and are closely related to sheep, as both are part of the subfamily Caprinae, which includes cows and antelopes. There are over three hundred distinct breeds of cows.

Cows are among the oldest domesticated species and have been utilized for their milk, meat, hair, and skins throughout much of the world. Milk and dairy products, including cow's milk, sheep's milk, and buffalo's milk, are integral to a healthy diet (Hinrichs, 2004). Milk is a complex mixture of fats, proteins, carbohydrates, minerals, vitamins, and other minor constituents dispersed or dissolved in water (Harding, 1999). Its nutritional significance is evident from the fact that consuming a quart (1.14 liters) of milk daily provides approximately all the daily requirements for fat, calcium, phosphorus, riboflavin, half of the protein, one-third of vitamin A, ascorbic acid, thiamine, and one-fourth of the daily calorie needs for an average individual (Bilal and Ahmad, 2004).

Milk contains three types of globular proteins: caseins, lactalbumins, and lactoglobulins. Globular proteins interact less with themselves and form colloidal suspensions more easily than fibrous proteins. Casein is a mixture of several similar proteins, including alpha, beta, kappa, and gamma caseins. It is a phosphoprotein, and the differences among casein forms are due to the number of phosphate groups in the protein. About 80% of the total protein in milk is casein, with the remainder consisting of whey proteins and small amounts of various enzymes (e.g., lipoprotein lipase, alkaline phosphatase, lactoperoxidase). Casein, the dominant protein in goat milk, can be estimated using titration methods.

The milk of small ruminants, such as goats and sheep, is of particular economic interest in various regions of the world. In developing countries, this type of milk has become a valuable strategy to address undernutrition, especially among infants (Haenlein, 1996, 2001, 2004). Additionally, small ruminants offer a sustainable resource with potential economic benefits and demographic stability, particularly in arid, semi-arid, and other challenging regions. These species often managed extensively or semi-extensively, contribute to preserving genetic diversity while keeping production costs low by utilizing natural resources effectively. The milk and meat produced from young animals are of high nutritional quality (Boza, 1993).

According to Haenlein (1996, 2004), the consumption of milk from small ruminants may be due to its availability, its use in popular foods like goat or sheep cheese, or its nutritional composition, which may meet specific needs for certain populations. Dairy goat and sheep farming play a vital role in the national economies of many countries, especially in the Mediterranean and Middle Eastern regions (FAO, 2003), with well-organized industries in France, Italy, Spain, and Greece (Park and Haenlein, 2006). However, large-scale industrialization of dairy goat and sheep sectors is limited by the relatively low volume and seasonal cyclicity of milk production, averaging around 50 kg annually (Juarez and Ramos, 1986; FAO, 1997).

Understanding the composition and physicochemical characteristics of goat and sheep milk is crucial for the successful development of dairy industries and the marketing of products. There are notable differences in physicochemical characteristics among goat, sheep, and cow milk. Cow milk typically experiences minimal changes in composition throughout the year due to year-round breeding. In contrast, sheep and goat milk production is predominantly seasonal, with variations in composition occurring due to seasonal breeding. Towards the end of lactation, fat, protein, solids, and mineral contents in goat and sheep milk increase, while lactose content decreases (Brozos et al., 1998; Haenlein, 2001; 2004).

Goat milk differs from cow or human milk in its digestibility, alkalinity, buffering capacity, and certain therapeutic values in medicine and nutrition (Haenlein and Caccese, 1984; Park and Chukwu, 1989; Park, 1994). Sheep milk generally has higher specific gravity, viscosity, refractive index, titratable acidity, and a lower freezing point compared to cow milk (Haenlein and Wendorff, 2006). The lipids in sheep and goat milk also exhibit higher physical characteristics than those in cow milk, though variations between different reports exist (Anifantakis, 1986; Park, 2006a).

The specific characteristics of the physico-chemical properties of goat and sheep milk, in comparison with cow milk, with an emphasis on lipid and protein fractions, including bioactive peptides, are of significant interest. Milk fat is a crucial component of the nutritional quality of goat dairy products. For instance, some fatty acids (FAs) found in milk triacylglycerol, such as oleic and linolenic acids, have been shown to exert positive effects on human health, including a cardioprotective effect through direct vascular anti-atherogenic action (Massaro *et al*., 1999). Moreover, the fat content and composition of goat milk can be extensively modified by genetic, physiological, and nutritional factors (Chilliard et al., 2003a).

Among nutritional factors, dietary fat supplementation is an effective method to modify milk FA composition in lactating ruminants (Palmquist et al., 1993; Chilliard et al., 2000). This approach can enhance the nutritional quality of milk fat. Milk fatty acids originate from two main sources: they are either synthesized de novo in the mammary gland or extracted from the arterial blood. These processes involve various mammary enzymes, including lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD). Recent focus has been on SCD due to its role in the synthesis of monounsaturated fatty acids, such as oleic (cis-9 C18:1) and palmitoleic (cis-9 C16:1) acids, and the major conjugated linoleic acid isomer (cis-9, trans-11 C18:2; Corl et al., 2001). Variations in mammary gland levels of SCD may partially explain the substantial differences in these fatty acids in milk fat.

Milk, the secretion of the mammary glands, is the sole food source for young mammals during the early stages of life. It provides energy and essential building materials for growth and contains antibodies that protect against infections (Bylund, 1995). Milk plays a critical role in building a healthy society and can be used as a vehicle for rural development, employment, and reducing rural-to-urban migration (Sarware et al., 2002). In the year 2008-2009, Pakistan produced 43,562 million tons of milk; of which 62.04% was from buffaloes, 34.39% from cows, 1.65% from goats, 0.08% from sheep, and 1.83% from camels (Anonymous, 2009). Buffalo milk is preferred over cow's milk in the sub-continent (Bilal et al., 2006).

Mulberry leaves (*Morus alba*) have long been the primary feed for silkworms (*Bombyx mori*). Mulberry trees thrive in various climatic conditions, from temperate to tropical regions, and yield fresh leaves at a rate of 25 to 30 tons per hectare per year with a cutting interval of 9 to 10 weeks. These leaves are high in protein (18 to 25% in dry matter) and have high in vivo dry matter digestibility (75 to 85%) (Ba *et al*., 2005). Thus, mulberry leaves are a high-potential protein-rich forage supplement for animal production (Benavides, 2000). The production of fresh mulberry leaves and total dry matter per hectare depends on climatic conditions, soil characteristics, variety, plant density, fertilizer application, and harvesting techniques. In terms of digestible nutrients, mulberry surpasses many traditional forages (Sanchez, 2000). The chemical composition of the leaves varies according to variety, maturity, leaf position within the branch, and fertilization level (Shayo, 1997; Singh and Makkar, 2002). As leaves mature, their contents of protein, soluble sugars, and organic acids decrease, while fiber, fat, and ash content increase. Additionally, the moisture, protein, and carbohydrate content of mulberry leaves is higher in temperate regions compared to tropical ones (Singh and Makkar, 2002).

One of the main features of mulberry leaves is their high palatability. Small ruminants avidly consume the fresh leaves and the young stems first, even if they have never been exposed to them before. Then, if the branches are offered unchopped, they may tear them off and eat the bark. Cattle consume the whole biomass if it is finely chopped. For ruminants, the preferred method of feeding has been branch cutting by hand, although mechanical harvesting could be employed in the future for direct feeding of fresh material on a large scale, for processing or drying. Since mulberry leaves are rich in nitrogen, sulfur, and minerals (Singh and Makkar 2002), their use for ration supplementation could increase the efficiency of utilization of crop residues in ruminant feeding systems. Thus, mulberry leaves have the potential to be used as a supplementary feed for improving livestock productivity and play a valuable role in world agriculture. However, little information is available on their nutritive value and benefits as a high-quality supplement to low-quality roughages or as a replacement for grain-based concentrates in ruminant feeding. Therefore, studies to determine intake and optimal levels of supplementation with mulberry leaves for growing ruminants and milk yield should be carried out.

The objective of the present study was to evaluate the nutritional value of mulberry leaves in terms of chemical composition, nitrogen solubility, non-protein nitrogen, protein fractionation, and in vitro true digestibility of dry matter (DM), and to determine the effects of mulberry leaves, partially substituting lucerne hay and concentrates, on the digestibility of goats' diets. There is evidence that domestication, selection, and improvement of mulberry started about 5,000 years ago in China together with sericulture. In Italy, until the past century, mulberry cultivation was regulated to prevent cutting down the plant. However, after the advent of synthetic textile fibers, silk production diminished, and mulberry cultivation was almost forgotten. At present, it can be found as an ornamental or wild plant in marginal areas. Wild or cultivated mulberry varieties are spread in countries all over the world from temperate to tropical areas, from sea level to altitudes of 4,000 meters, and from humid tropics to semi-arid lands (FAO, 1990; Tipton, 1994). Beyond silkworm feeding, mulberry is also appreciated for its fruit (fresh, in juice, as preserves), for its medicinal properties (mulberry leaf tea), for landscaping, as a vegetable (leaves and young stems), and as a feed for ruminants and other animals (Zepeda, 1991). Due to high percentages of crude protein (15 – 25%) and in vitro dry matter digestibility (75 – 85%), together with their perennial nature and adaptation to various soil types, mulberry leaves appear to be excellent forage for feeding and supplementing ruminants. In fact, there are several places where mulberry leaves are used traditionally as a feed in mixed forage diets for ruminants, and there have been several studies on the use of mulberry for cows and other domestic animals.

A group of nutritional scientists in India has suggested that the powdered leaves of white mulberry (*Morus alba* L.) might make a good, nutritious, non-toxic, and low-cost food ingredient for paratha, the traditional food item for breakfast and dinner in the Indian diet. Moreover, in Korea and Japan, mulberry fruit and leaves are used as functional foods. In Korea, an ice cream with an agreeable taste, containing powder of mulberry leaves as an ingredient, has shown to reduce blood glucose levels instead of rising, as could be expected for a food with high sugar content. The blood serum glucose reducing effect is only one of the healthy properties attributed to mulberry in traditional Chinese herbal medicine, ancient Latin texts, and folk medicine. Other examples include lowering men's blood cholesterol and lipid levels, fighting arterial plaques, and antiphlogistic, diuretic, and expectorant effects. Many of these properties have been proven by clinical studies, and various compounds present in mulberry (flavonoids, alkaloids, steroids), responsible for these therapeutic benefits, have also been recognized. Nevertheless, these substances can limit the use of mulberry leaves as a food or food ingredient, especially for people affected by pathologies that mulberry extracts can help control, due to interactions with other medicines or physiological intolerance. Therefore, in order to use mulberry leaves as animal feed or a safe human food source, it is necessary to remove these substances. While this increases processing costs, it could be advantageous by recovering such substances for industrial (e.g., natural dyeing) or medical uses under controlled conditions. This paper describes experiments carried out for the extraction of flavonoids from mulberry leaves, believed to be the major component responsible for the therapeutic benefits attributed to mulberry in folk medicine. The aim of this study was to find a process, easily practicable without the use of toxic solvents, for the recovery of both the flavonoids and the extracted leaf material, to be used as a potential food source. In order to evaluate the suitability of the process, the aqueous extracts from leaves were also tested for dyeing power on textile fibers.
The main use of mulberry globally is as feed for silkworms, but depending on the location, it is also appreciated for its fruit (consumed fresh, in juice, or as preserves), as a delicious vegetable (young leaves and stems), for its medicinal properties in infusions (mulberry leaf tea), for landscaping, and as animal feed. In Peru, the multiple uses of mulberry have been recognized (Zepeda, 1991). There are several places where mulberry is traditionally utilized as a feed in mixed forage diets for ruminants, like in certain areas of India, China, and Afghanistan. In Italy, there have been several studies on the use of mulberry for dairy cows and other domestic animals (Maymone *et al*., 1959; Bonciarelli and Santilocchi, 1980; Talamucci and Pardini, 1993), and in France, there was a research project to introduce mulberry in livestock production (Armand, 1995). However, it was only in the eighties that specific interest in the intensive cultivation and use of mulberry as animal feed started in Latin America. It is surprising that a plant which has been improved for leaf quality and yield to feed an animal, the silkworm, which has high nutritional feed requirements, received limited attention from livestock producers, technicians, and researchers.
Like several significant breakthroughs in science and technology, the discovery of the value of mulberry as high-quality feed in Latin America happened serendipitously. A Costa Rican farmer of Chinese origin, whose silk project failed, fed mulberry leaves to his goats and was impressed by their palatability and the performance of his animals. He communicated his observations to scientists at the Tropical Agriculture Research and Training Center (CATIE), who were receptive to the farmer's news and smart enough to include mulberry in their tree fodder evaluations and later in agronomic and animal performance trials (J. Benavides, personal communication). In Africa, the International Centre for Research in Agroforestry (ICRAF) in Kenya and the Livestock Production Research Institute in Tanzania have conducted successful agronomic and animal trials independently, apparently without being aware of the interest elsewhere.
Mulberry belongs to the Moraceae family (subtype Angiosperms; class Dicotyledons; subclass Urticales), and there are several species: *Morus alba*, *M. nigra*, *M. indica*, *M. laevigata*, M. bombycis, etc., which have been used directly, or through crossings and induced mutations, for the development of varieties to support silkworm production. The diploid M. alba (2n=2x=28) is the

species most widely spread, but polyploid varieties, which originated in various research stations in Asia, show greater leaf yields and quality. In general, polyploid varieties have thicker and larger leaves with darker green color and produce more leaves. There is a large variation in leaf production and quality (e.g., protein content) among mulberry species and varieties grown at different locations under a wide range of soil and environmental conditions, indicating the huge potential for identifying suitable germplasm for most sites. Many references on mulberry in the literature do not specify which species or varieties were used. Often, names are given based on leaf features. In many cases, locally grown varieties (native or "criolla") seem to perform adequately since they are probably well adapted to local conditions.

To determine the functional groups of cow milk.

Materials and Methods
Collection of Samples
Four cows were selected for the study from two farmers in Shikohabad. Two cows were from Ajay's farm in Madai Shikohabad, and one cow was from Satyam Maurya's farm in Kanthri of Shikohabad. The first cow from Madai was black and 3 years old, the second cow was brown and 4 years old, and the third goat was red and 3 years old.
Milk samples were collected before feeding the cow’s mulberry leaves. Six samples were collected on alternate days during a 12-day feeding period. After feeding the cows mulberry leaves, two samples from each goat were collected (with a one-day gap) in the morning in sterile bottles and stored in a freezer at -20°C.

Materials Required for Functional Group Estimation
Fresh cow milk samples
ZnCe plate
FTIR (Fourier Transform Infrared Spectroscopy) equipment
Procedure for Functional Group Analysis
Fourier Transform Infrared Spectroscopy (FTIR) was used for the analysis. The model TM 6700, manufactured by Thermo Scientific, USA, was employed. Fresh goat milk samples were run on a ZnCe plate using FTIR to analyze the functional groups present in the samples.

Results and Discussion
Results of Fourier Transform Infrared Spectroscopy (FTIR) of three cow’s
For functional group analysis by FTIR, the first sample was collected before feeding the cow’s mulberry leaves. Six samples were collected over a 12-day feeding period (with one-day gaps), and two samples were collected after stopping the feeding of mulberry leaves.
Conclusion
In the present work, the effect of mulberry leaves on milk composition of goats should a high crude protein, high apparent digestibility, casein %, carbohydrate % and lipid % .The functional groups and elements of three goats milk increases due to feeding of mulberry leaves, however further study is needed to investigate the response to increasing inclusion levels of mulberry leaves in diets of goats.

Result of cow’s milk (before feeding)
Cow milk (before feeding of mulberry leaves)

S.N.	**Frequency (cm^{-1})**	**Functional group**	**Type of vibration**	**Intensity**
1	3329.2	Alcohol (O-H)	Stretch-bonded	Strong, broad
2	2387.3	Amino acid (N-H)	Stretch	Medium

3	2146.2	Alkyne(-C=_C)	Stretch	Variable ,asymmetrical
4	1636.9	Amide(N-H)	Bending	Strong,sharp
5	1459.3	Aromatic alkene(C=C)	Stretch	Medium,weak,multiple band
6	1156	Amine(C-N)	Stretch	Medium ,weak
7	1075.7	Alcohol (C-o)	Stretch	Strong
8	1041.3	Ester (C-O)	Stretch	Two band or more
9	992.8	Alkane (C-C)	Stretch	Weak
10	665.1	Alkyl halide(C-Cl)	Stretch	Strong

Result of cow milk (before feeding of mulberry leaves)

Cow milk (before feeding of mulberry leaves)

S.N.	Frequency (cm^{-1})	Functional group	Type of vibration	Intensity
1	3311.1	Alcohol (O-H)	Stretch,H-bonded	Strong,broad
2	2923.1	Alkane(C-H)	Stretch	Strong
3	2853.4	Alkane(C-H)	Stretch	Strong
4	2146.8	Alkyne(-C=_C)	Stretch	Variable ,asymmetrical
5	1740.8	Ester (C=O)	Stretch	Strong
6	1636.4	Amide(N-H)	Bending	Strong,sharp
7	1461.2	Aromatic alkene(C=C)	Stretch	medium,weak,multiple band
8	1378.5	Alkane(C-H)	Bending	variable
9	1245.7	Amine(C-N)	Stretch	Medium ,weak
10	1156.7	Alkyl halide(C-F)	Stretch	Strong
11	1076.4	Alcohol (C-o)	Stretch	Strong
12	1041.3	Ester (C-O)	Stretch	Two band or more
13	992.7	Alkane (C-C)	Stretch	Weak
14	691.7	Alkyl halide(C-Cl)	Stretch	Strong

Changes of functional groups in due to feeding of mulberry leaves

Result of cow milk (Mulberry leaves feeding period)

1	2853.4	Alkane(C-H)	Stretch	Strong
2	2923.1	Alkane(C-H)	Stretch	Strong
3	1740.8	Ester (C=O)	Stretch	Strong
4	1378.5	Alkane(C-H)	Bending	variable
5	1245.7	Amine(C-N)	Stretch	Medium ,weak
6	1075.9	Ester (C-O)	Stretch	Two band or more
7	1041.1	Ester (C-O)	Stretch	Two band or more
8	991.8	Alkane (C-C)	Stretch	Weak

Fourier Transform Infrared Spectroscopy (FT-IR) analysis of cow’s milk

Red cow (before feed)

S.N.	Characteristic’s absorptions (cm^{-1})	Functional Group	Types of vibration	Intensity
1	3338.4	Alcohol(O-H)	Stretch,H-bonded	Strong,broad

2	2924.5	Alkane (C-H)	Stretch	Strong
3	2854.4	Alkane (C-H)	Stretch	Strong
4	2381.3	Amino acid (N-H)	Stretch	Medium
5	2041.4	Amino acid (N-H)	Stretch	Medium
6	1741.3	Ester (C=O)	Stretch	Strong
7	1635.6	Amide N-H)	Bending	Strong ,sharp
8	1463.3	Aromatic alkene(C=O)	Stretch	Medium weak
9	1164.5	Amine (C-N)	Stretch	Medium ,weak
10	1118.3	Amine (C-N)	Stretch	Medium ,weak
11	687.8	Alkyl halide(C-Cl)	Stretch	Strong
12	654.7	Alkyl halide(C-Cl)	Stretch	Strong

Red cow (first sample of feeding period)

S.N.	Characteristic's absorptions (cm^{-1})	Functional Group	Types of vibration	Intensity
1	3321.4	Alcohol(O-H)	Stretch,H-bonded	Strong,broad
2	2383.2	Amine (C-N)	Stretch	Medium
3	2142.3	Nitrile (CN)	Stretch	Medium
4	1636.1	Amide N-H)	Bending	Strong ,sharp
5	1462.1	Aromatic alkene(C=O)	Stretch	Medium weak
6	1157.5	Amine (C-N)	Stretch	Medium ,weak
7	1076.4	Ether (C-O)	Stretch	Strong
8	1041.7	Ester (C=O)	Stretch	Two band or more
9	685.1	Alkene(=C-H)	Bending	Strong
10	656.5	Alkyl halide(C-Cl)	Stretch	Strong

Red cow (second sample of feeding period)

S.N.	Characteristic's absorptions (cm^{-1})	Functional Group	Types of vibration	Intensity
1	3261.4	Alcohol(O-H)	Stretch,H-bonded	Strong,broad
2	2145.9	Alkyne(-C=_C-)	Stretch	Variable ,asymm.
3	1635.9	Amide N-H)	Bending	Strong ,sharp
4	1459.9	Aromatic alkene(C=O)	Stretch	Medium weak'
5	1156.3	Amine (C-N)	Stretch	Medium ,weak
6	1075.9	Ether (C-O)	Stretch	Strong
7	1041.6	Ester (C=O)	Stretch	Two band or more
8	991.2	Alkane (C-C)	Stretch	Weak
9	674.3	Alkyl halide(C-Cl)	Stretch	Strong

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