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Fourier transform infrared spectroscopy is used for analyzing goat milk samples

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Abstract

In this investigation, the FT-IR spectra of goat milk before and after feeding on mulberry leaves were recorded to obtain potential spectral characteristics for the detection and characterization of a discriminately distinct wavelength spectrum. From FT-IR spectra, it was possible to determine the visible intensities, infrared (IR) wave numbers (4000 cm1–400 cm1), and types of vibrations (stretching and bending) of notable peaks. The FT-IR data show that goat milk contains biological components like phenol, amine, alkene, alkane, alkyne, carboxyl, ester, ether, polysaccharide, nitrite, and sulfur compounds, as well as bound O-H, N-H, C-H, C-C, C-O, C=O, C-O-C, SO3, and O-N=O functional groups.

K**eywords:** FT-IR spectra, mulberry, milk, Infrared, components

Introduction

A goat subspecies that was domesticated from the wild goat of southwest Asia and eastern Europe is the domestic goat (*Capra aegagrushircus*). The goat and sheep are closely related and both belong to the Bovidae family, which includes the Caprinae goat-antelope subfamily. There are more than three hundred different goat breeds.The domestication of goats dates back many centuries. Throughout much of the world, goats are utilized for their milk, meat, skin, and hair. A healthy diet includes milk and dairy products, which include not only cow's milk but also sheep's milk and buffalo's milk (Hinrichs, 2004).

Milk is a complex mixture of lipids, proteins, carbs, minerals, vitamins, and other insignificant components that are spread or dissolved in water (Harding, 2004).Milk is an important part of the human diet and the nutritional significance of milk is apparent from the fact that daily consumption of a quart (1.14 liters) of milk furnishes approximately all the daily requirements from fat ,calcium ,phosphorus , riboflavin ,one half of the protein ,one third of vitamin A ,ascorbic acid ,thiamine and one fourth of calories needed daily by an average individual (Bilal and Ahmad ,2004).

Milk contains three different forms of globular proteins: caseins, lactoblumins and lactoglobulins .Globulins proteins fail to interest with themselves and form colloid suspensions more easily than fibrous proteins.

Casein is a mixture of several similar proteins, alpha, beta, kappa and gamma caseins.

Casein is a phosphoprotein and the difference between the forms of caseins is the number of phosphate groups contained in the protein. About 80% of the total protein in milk is casein; the differences is represented of the whey protein and in small amount of various enzymes (e.g. lipoprotein lipase ,alkaline phosphates, lactoperoxidase) .Casein ,the dominant protein in goats milk , can be estimated by titration methods.

The milk of small ruminants such as goats and sheep is of particular economic interest in certain areas of the world. In developing countries, the production of this type of milk has come to be a useful strategy to tackle the problem of under nutrition, especially among the infant population (Haenlein, 1996, 2001, 2004). An additional element of interest in the production of small ruminants is the fact that it is a sustainable resource with excellent possibilities of economic profitability and demographic stability, of special importance for arid, semi-arid and other problematic regions of the earth. These species, which are exploited in the latter type of region under an extensive or semi-extensive management regime prioritizing autochthonous breeds, are valuable in preserving genetic variability while production costs are held down by the appropriate use made of natural resources. The foods produced, namely milk and meat (from the young animals) are from a nutritional point of view of excellent quality (Boza, 1993).

According to Haenlein (1996, 2004), the consumption of milk from small ruminants may occur for a variety of reasons. In some instances, it may be done simply because this type of milk is the most readily available. In other instances, it may be the main ingredient in specially preferred foods (such as goat or sheep cheese).

Dairy goat and dairy sheep farming are a vital part of the national economy in many countries, especially in the Mediterranean and Middle East region (FAO, 2003), and are particularly well organized in France, Italy, Spain, and Greece (Park and Haenlein, 2006). However, large scale industrialization of the dairy goat and dairy sheep sectors in many countries is limited by low volume and seasonal cyclicity of individual milk production, around 50 kg annually (Juarez and Ramos, 1986; FAO,1997) .

Information on composition and physico-chemical characteristics of goat and sheep milk is essential for successful development of dairy goat and sheep industries as well as for the marketing the products. There are distinct differences in physico-chemical characteristics between goat, sheep and cow milks. The composition of market cow milk is expected to have minimal change throughout the year, because the milk entering bulk tank from the cow herds would vary little by seasons because of year-round breeding. On the other hand, this is quite different from sheep and goat milk, which is predominantly produced by seasonal breeding of ewes and does (Haenlein and Wendorff, 2006). Therefore, changes in goat and sheep milk compositions occur by seasons, because towards the end of the lactation, the fat, protein, solids and mineral contents increase, while the lactose content decreases (Brozos*et al*., 1998; Haenlein, 2001;2004).Goat milk differs from cow or human milk in having better digestibility, alkalinity, buffering capacity, and certain therapeutic values in medicine and human nutrition (Haenlein and Caccese, 1984; Park and Chukwu, 1989; Park, 1994). Sheep milk has higher specific gravity, viscosity, refractive index, titratable acidity, and lower freezing point than average cow milk (Haenleinand and Wendorff, 2006). Lipids in sheep and goat milk have higher physical characteristics than in cow milk, but there are variations between different reports (Anifantakis, 1986; Park, 2006a).

The differences between cow milk and goat and sheep milk in terms of their distinct physico-chemical properties, with a focus on the lipid and protein fractions, including bioactive peptides.Milk fat is an important component of the nutritional quality of goat dairy products. For example, some fatty acids (**FA**) found in milk triacylglycerol have been shown to exert positive effects on human health such as, for oleic and linolenic acid, a cardio protective effect through a direct vascular anti atherogenic action (Massaro*et al*., 1999). Furthermore, goat milk fat content and composition can be extensively modified by genetic and physiological factors as well as by nutritional factors(Chilliard*et al*., 2003a).

Among nutritional factors, fat supplementation of the diet is an efficient means to modify milk FA composition in lactating ruminants (Palmquist*et al*., 1993; Chilliard*et al*., 2000), which could be used to improve the nutritional quality of milk fat. Milk fatty acids have 2 main origins: they are synthesized de novo in the mammary gland or extracted from the arterial blood. These processes involve numerous mammary enzymes, including lipoprotein lipase, acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase. Particular attention has been focused on SCD in the last decade because of its implication in the synthesis of monounsaturated fatty acids [mainly 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) found in ruminant milk fat. Differences in mammary gland levels of SCD may explain, in part, the substantial variations of these fatty acids in milk fat.

Milk, which is the secretion of the mammary glands, is the only food of the young mammal during the first period of its life. The substances in milk provide both energy and the building materials necessary for growth. Milk also contains antibodies which protect the young mammal against infection (Bylund, 1995). Milk plays a tremendous role in building a healthy society and can be used as vehicle for rural development, employment and slowing down the migration of the rural population (Sarwar*et al.*, 2002). In the year 2008-2009, Pakistan produced 43,562 million tons of milk; of which 62.04% was contributed by buffaloes, 34.39% by cows, 1.65% by goats, 0.08% by sheep and 1.83% by camels (Anonymous, 2009). Buffalo is the most valuable animal and is being highly liked by the people of the sub-continent. Buffalo milk is preferred more than the cow's milk (Bilal *et al*., 2006).

Mulberry leaves (*Morusalba*) have long been the single feed for the silkworm (*Bombyxmori*). Mulberry trees are growing under varied climatic conditions, ranging from temperate to tropical, all over the world. The biomass yield of fresh leaves is often in the order of 25 to 30 tones/ha/year with a cutting interval of about 9 to 10 weeks, while leaves have a high protein content (18 to 25% in DM) and high (75 to 85%) in vivo DM digestibility (Ba et al. 2005). Therefore mulberry leaves have a high potential as a protein-rich forage supplement for animal production (Benavides 2000). The production of fresh mulberry leaves and total dry matter (DM) per hectare depends on climatic conditions, soil characteristics, variety, plant density, fertilizer application and harvesting techniques, but in terms of digestible nutrients, mulberry produces more than most traditional forages (Sanchez 2000).The chemical composition of the leaves varies according to variety, degree of maturity, leaf position Within the branch and fertilization level (Shayo 1997; Singh and Makkar 2002). Thus, leaves' contents of protein, soluble sugars and organic acids decrease with maturity, whereas fiber, fat and ash constituents increase. Moreover, the content of moisture, protein and carbohydrates of mulberry leaves is higher in temperate regions compared to the tropics (Singh and Makkar 2002).Mulberry is cultivated on a semi-extensive scale in various parts of Greece, particularly in the northern part, mainly for the leaves to support the sericulture industry. The total acreage of mulberry plantations cultivated in Greece has been reported at around 400 has .Mulberry grows rapidly in the early stages and reaches maturity at an early age; the growth rate falls off rapidly after approximately ten years. The mulberry leaves can also be used as the main feed for sheep (Prasad and Reddy 1991; Liu *et al*. 2001), goats (Omar *et al.* 1999; Sanchez 2000; Schmidek*et al*. 2000; Bakshi and Wadhwa 2007) and rabbits (Prasad *et al*. 2003; Martinez *et al.* 2005). Moreover, they have been used to replace concentrates in dairy cattle (Sanchez 2000; Mejia 2002) or goat diets (Anbarasu*et al*. 2004). They can also be used as an ingredient in the diets of monogastric livestock, such as pigs (Ly *et al*. 2001; Araque*et al*. 2005; Leterme*et al*. 2006) and laying hens (Narayana and Setty 1977). Recently, Srivastava*et al*. (2003) investigated the potential of powdered dried mulberry leaves, which in mixture with wheat flour can be utilized for human consumption in Indian diets. Due to its high digestibility and excellent level of crude protein, mulberry foliage can be a comparable source to commercial concentrates for ruminal feeding and production. The content of total phenols is very low (1.8% as tannic acid equivalent) and tannins by the protein precipitation capacity method were not detectable (Shayo and Uden 1999; Singh and Makkar2002).

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 to determine the effects of mulberry leaves, partially substituting lucerne hay and concentrates, to the digestibility of goat's 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, the mulberry cultivation was ruled by regulations which forbade to cut down this plant; but, after the coming in the market of the synthetic textile fibers, the silk production diminished and so the cultivation of mulberry was almost forgotten. At present, it can be found as 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 m and from humid tropics to semi-arid lands (FAO, 1990; Tipton, 1994). Beyond silkworm feeding, depending on regions, mulberry is also appreciated for its fruit (fresh, in juice, as preserve), for its medicinal properties (mulberry leaf tea), for landscaping, as a vegetable (leaves and young stems) and as a feed for ruminants and others animals (Zepeda, 1991). Due to high percentages of crude protein (15 – 25 %) and in vitro dry matter digestibility (75 – 85 %), together with perennial nature and adaptation to various soil types, mulberry leaves appear 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 also been several studies on the use of mulberry for cows and other domestic animals.

The main use of mulberry globally is as feed for the silk worm, 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 utilized traditionally as a feed in mixed forage diets for ruminants, like in certain areas of India, China and Afghanistan. In Italy there has been several studies on the use of mulberry for dairy cows and other domestic animals (Vezzani, 1938; 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). But 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 silk worm, which has high nutritional feedrequirements, received limited attention by livestock producers, technicians and researchers.

The main objective of goat milk analyzed the functional groups.

Materials and Methods

Collection of sample:

Two goats were selected from afarmerAjay, MadaiShikohabad. One goat was selected from a farmer,Satyam maurya and Kanthri of Shikohabad. One goat of Madai was black in colour and 3 years old, second goat was brown in colour and 4 year old and third goat was red in colour and 3year old.

The milk sample was collected before feeding of mulberry leaves, six samples were collected alternative days in 12 days feeding period. Two samples were collected of three goats after feeding of mulberry leaves (one day gape), sample were collected in morning time in sterile bottles and stored in freezer at -20 ^{o}C.

Materials required for functional groups estimation

Fresh goats milk samples, ZnCe plate, FTIR etc.

Procedure for functional groups analysis Fourier Transform Infrared Spectroscopy (FTIR),Model :TM 6700 and Make:Thermo Scientific. USA.

Freshgoat's milk samples run on ZnCe plate by FTIR and analyses the functional groups in samples.

Procedure for milk photo by Scanning Electron Microscope (SEM)

First of all made a film of milk sample onstub with the help of carbon tape, after then keep the sample in desiccators for 5-7 days for complete dry. After complete dry the sample coated by palladium afterthen take the photo by SEM.

Result of Fourier Transform Infrared Spectroscopy (FTIR) of three goats

For functional groups analysis by FTIR, I was first sample collected before feeding of mulberry leaves, 6 samples collected in 12 days feeding period (one day gape) and 2 samples collected after stop feeding of mulberry leaves. The FTIR spectra of goat milk were assigned to wave number 4000 cm^{-1}–400 cm^{-1} . The stacked FTIR spectra of before feeding and after feeding of mulberry leaves are shown in (Figures 1–2) respectively.

The absorption bands observed in after feeding of mulberry leaves at 3734 cm^{-1} and 3800.5 cm^{-1}, respectively due to bonded O–H stretching which show the presence of phenols and alcohols. The strong absorption bands observed between 3400.1 cm^{-1} and 3358.5 cm^{-1} due to the bonded N–H stretching show the presence of primary amine. The variable strong bands observed at 2928.6 cm^{-1}–2920.1 cm^{-1} due to asymmetric stretching of bonded C–H show the presence of alkene (CH_2) and alkane (CH_3) groups in the goat milk of after and before feeding. However, the multiple bands observed at 2967.8 cm^{-1}–2958.3 cm^{-1} due to bonded C–H asymmetric stretching attribute the presence of alkene and alkane mainly in feeding period(Table 3). In addition, the bands observed at 2855.0 cm^{-1}–2847.5 cm^{-1} in all due to C–H symmetric stretch show the presence of alkene as a common biochemical constituent.

The C–O bands at 2378.9 cm^{-1}–2325.1 cm^{-1} due to carboxyl group were predominantly observed. The multiple bands observed at 2140 cm^{-1}–2100 cm^{-1} and at 2287.1 cm^{-1}–2190.2 cm^{-1} due bonding of C–C stretching show the presence of monosubstituted and disubstituted alkyne, respectively in both. The spectral band observed at 1742.5 cm^{-1}–1723.5 cm^{-1} due to C=O stretch vibration corresponds to presence of saturated aliphatic esters.

The secondary structure of amine due to N–H bending observed between 1652.8 cm^{-1}–1549.3 cm^{-1}. The bands observed at 1452.1 cm^{-1}–1412.9 cm^{-1} may bedue to bonded C–O / O–H bending in alcohols and phenols. The band observed at 1372.6 due to C–H bending shows the presence of alkane in feeding period of mulberry leaves. The IR spectral range observed at 1319.5 cm^{-1}–1308.7 cm^{-1} due to S=O stretching shows the presence of sulphone.

The bonded C–O absorbed IR at 1255.7 cm^{-1}–1238.6 cm^{-1} show the presence ether functional groups. The strong bands observed at 1150.4 cm^{-1}–1054.2 cm^{-1} due to C–O–C symmetric stretching shows the presence of polysaccharides. The spectral band observed at 1077.6 cm^{-1}–

1068.5 cm^{-1} due to SO_3 symmetric stretching shows the presence of sulfur compounds. The medium bands observed at 795.5 cm^{-1}–770.9 cm^{-1} due to C–H out–of–plane bending show the presence of aromatic compound with substitution of hydrogen. The band observed at 685 cm^{-1}–615.3 cm^{-1} due to O–N=O bending shows the presence of nitrite. The weak absorption bands observed at 578.7 cm^{-1}–529.8 cm^{-1} may be due to S–S stretching show the presence of sulphide and disulphide compounds.

Conclusion

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.

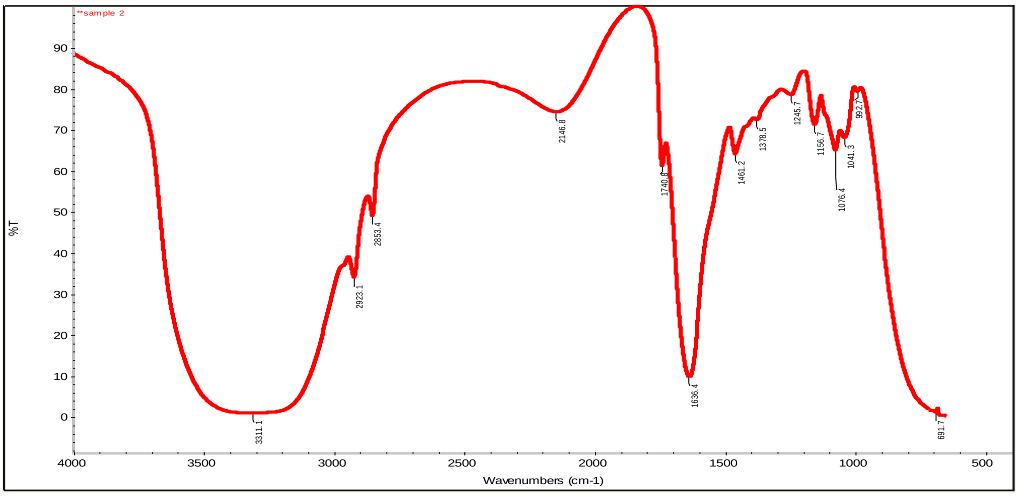

Figure 1.IR Spectra of goat milk (before feeding of mulberry leaves)

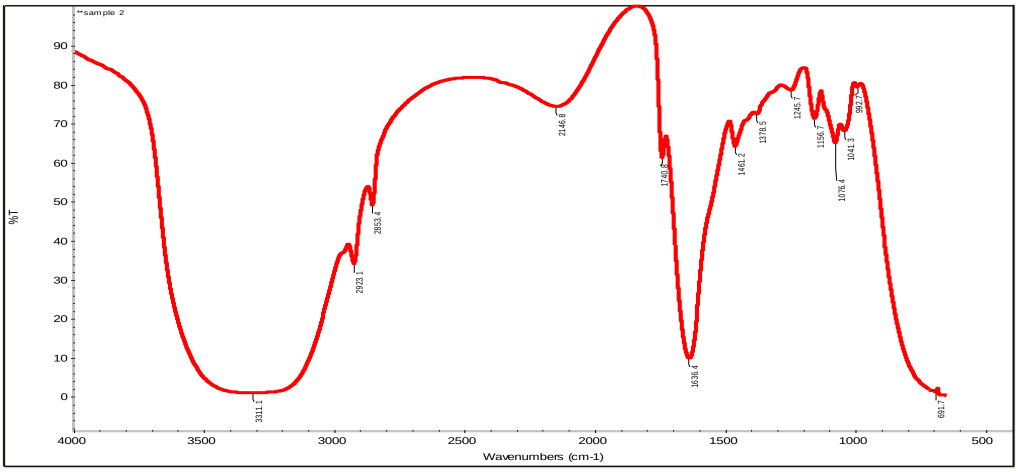

Figure2. IR Spectra of goat milk (feeding period of mulberry leaves)

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