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ISOLATION AND IDENTIFICATION OF CASEIN FROM VARIOUS SOURCES OF MILK

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ABSTRACT

Milk is an important part of human life and supposed to be a nutritious food which contain about 80% proteins. Milk protein contain 80% casein (soluble protein), 2-8% lactose (milk fat) and remaining is whey (by product of cheese and casein manufacture). Casein is more important and contain almost all essential amino acids. The purpose of the present work was to Estimate the amount of casein in different milk samples including natural milk (cow, buffalo, goat, sheep). Milk products have long-term health, cultural, and financial implications. Current analytical methods for the detection of milk adulteration are slow, laborious, and impractical for use in routine milk screening by the dairy industry. Fourier transform infrared (FT-IR) spectroscopy is a rapid biochemical fingerprinting technique that could be used to reduce this sample analysis period significantly. To test hypothesis we investigated 4 types of milk: cow, goat, sheep, sheep.

KEYWORDS: Casein, Milk, Amino acids, Precipitation, Fourier transform infrared spectroscopy.

INTRODUCTION[1]

Amounting to nearly 80% of the total nitrogen in milk, casein is the most significant protein component in terms of both quantity and nutritional value. It was used in industries producing paper, textiles, paint, leather, fibre, and other types of materials. Dairy by products such as edible casein and caseinates have a long history and are used in a variety of dishes. Except for cysteine, casein is a fairly abundant supply of all the essential amino acids. Mammary glands

produce the phosphorylated and glycosylated complex. Three distinct polypeptide chains—s1, s2, and ß—are what make up the substance, and they are held together by noncovalent interactions. The casein portions are arranged in 20–300 nm diameter micellar aggregates that also include bivalent cations (calcium and lower quantities of magnesium). With the help of this structure, hydrophobic fractions can be dispersed depending on the animal breed and lactation stage, there are different amounts of casein in whole milk.

It typically weighs between 24 and 29g. 0.7– 0.9% of the phosphorus in casein is covalently linked to the protein by a serine ester bond. As a result, casein is referred to as a phosphoprotein. Aside from cysteine, casein contains significant concentrations of all the necessary amino acids for humans. So, casein is a very nutrient-dense protein. It is present in milk as intricate molecule clusters known as micelles.

The micelles typically have a molecular weight of several hundred million Daltons and are made up of casein molecules, calcium, inorganic phosphate, and citrate ions. In terms of physical chemistry, milk has relatively stable colloidal dispersion of casein micelles. Depending on the pH of the milk system, each of the hundreds of individual amino acids that make up casein's protein structure may have a positive or negative charge.

The isoelectric point (IEP), which is 4.6 for casein, is the pH value at which all of the positive charges and all of the negative charges on the protein stay in balance (i.e., there is no net charge on the protein).

The protein is least soluble at the IEP, which is the pH. The casein micelles in milk have a net negative charge and are relatively stable at a pH of about 6.6.Casein is made up of numerous distinct casein components (s1-, s2-, s-, and s-casein), each of which has a few varying characteristics.

Skim milk can either be processed with rennet to make rennet casein or it can be acidified to produce acid casein. Separated from the whey, the precipitated casein curd is cleaned, dried, and washed.

Caseinates are the water-soluble by products of the interaction between acid caseins and alkalis. A well-known dairy by product called edible casein is utilised as an ingredient in a variety of foods, including dairy products.

The production and demand for casein have expanded as a result of the general advancement of food technologies and their applications. Its manufacture differs from that of non edible casein (also called industrial casein) in that casein for food is produced under sanitary conditions. Further, during its manufacture, food-grade chemicals are used and sufficiently heat-treated to make the case in safe for human consumption.

The intensive investigation into manufacturing technologies over the years and the introduction of efficient plant designs has immensely improved the technology for edible casein production**.**

USES AND APPLICATION OF CASEIN[2]

Edible Casein can be used in Food, Beverage, Pharmaceutical, Health & Personal care products, Agriculture/Animal Feed/Poultry. Edible Casein is used in cheese making and protein supplement such as in cream-based soups, sherbet, pudding and custard. Casein is the principal protein of cow's milk. It is the curd that forms when milk is left to sour. It is the most commonly used milk protein in the food industry and contains 21 amino acids.

Edible acid casein is highly nutritional, low in fat and cholesterol, and flavorful, making it ideal for medical and nutritional applications. Edible acid is used in coffee whiteners, infant formulas, processed cheese, and for use in pharmaceutical products.

Hydrolyzed casein is casein that has been broken down partially or completely to its constituent amino acids.

IN FOOD

Casein can be used as nutritional supplements, thickener, emulsifier and texture stabilizer in food such as in cream-based soups, sherbet, pudding and custard.

IN BEVERAGE

Casein can be used as nutritional supplements, thickener, emulsifier and texture stabilizer in beverage.

IN PHARMACEUTICAL

Casein has been used for both microencapsulation and nanoencapsulation of biologically active agents, including pharmaceuticals, probiotic cells, nutraceuticals and nutrients.

IN HEALTH AND PERSONAL CARE

Casein used as Hair Conditioning Agent; Skin-Conditioning Agent – Miscellaneous; Antistatic; Skin Conditioning in Cosmetics and personal care products.

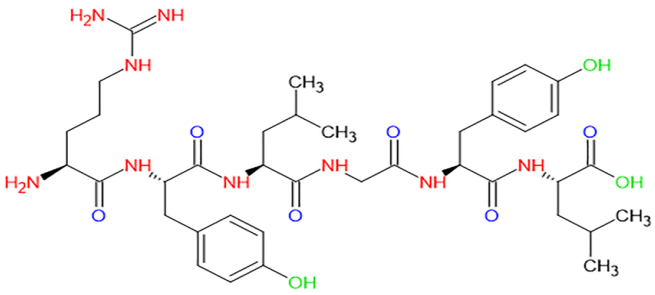
IN AGRICULTURE/ANIMAL FEED/POULTRY

Casein can be used in Agriculture/Animal Feed/Poultry feed.

IN OTHER INDUSTRIES

Casein can be used in ointments, paper, paints, glues, textiles, varnishes and early plastics

STRCTURE[3]



COW MILK[4]

Cow's milk is a nutrient-rich food that contains different levels of protein, fat, and carbohydrates. Human milk has 7% lactose, compared to cow's milk's 4.8% (12-12.5 g lactose per cup). Whey proteins are more labile than caseins, which are more heat-stable. Alpha, Beta, Kappa, and Gamma casein are the four main fractions of casein.

Dairy products and cow's milk are significant sources of dietary iodine. A variety of products are made from Milk, including Cream, Butter, Yoghurt, Kefir, Ice cream, and Cheese.

HEALTH BENEFITS[5]

- Low blood pressure.
- Good for all ages' development of healthy bones.
- Contains protein of true quality.
- Aids in weight loss prevention.
- Keeps the heart healthy.
- Reduces inflammatory problems
- Increases immune.

GOAT MILK[6]

Goat milk is high in essential nutrients and is a good source of vitamins. it is also rich in medium – chain fatty acids, which are heart healthy fats associated with good health.

Since goat milk comes from a mammal, it is theoretically regarded as a sort of dairy. however, it's fantastic substitute for cow milk because it's simpler to digest, less inflammatory and contains fewer allergens than cow milk generally dose**.**

HEALTH BENEFITS[7]

- Good for Skin.
- It indicates good weight gain
- It's easier to Digest Goat milk.
- Boosts the platelet count.
- Kids' milk allergies can be prevented with goat milk.
- Arteriosclerosis is prevented by goat milk.
- Protects against insulin resistance.
- Assists in lowering Cholesterol Levels.

BUFFALO MILK[8]

Buffalo milk has a thick, creamy texture that is ideal for making butter, cream, and yoghurt because of its high protein and fat content. A creamy dairy product made primarily from water buffaloes, The largest producers of buffalo milk are India and Pakistan.

HEALTH BENEFITS[9]

High levels of calcium, a mineral essential for bone formation, are present in buffalo milk.

It is also a source of peptides generated from casein, which may support bone health and lower your risk of osteoporosis, a condition marked by bone thinning and a higher risk of fractures**.**

Sheep milk[10]

Sheep milk when it comes to non –human milk, there are only a few minerals on which the human population relies, such as cows, goats, sheep.

Sheep s milk is the milk of domestic sheep. It is commonly used to make cultured dairy products such as cheese. some of the most popular sheep cheeses include feta(Greece), ricotta(Italy), and Roquefort(France).

In comparison to other milks, sheep milk has a high level of solids and a very high concentration of fat and conjugated linoleic acid (CLA). Because of this, it is excellent for creating cheese. Particularly, compared to cow's milk, sheep's milk yields far more cheese.

HEALTH BENEFITS[11]

- An ability to lower cholesterol levels.
- Strengthens the bones.
- Boost the immune system.
- Stimulate growth and development.
- Prevent birth defects.
- Reduce inflammation.
- Fight cancer and lower blood pressure.

COMPARISON OF NUTRITION FACTS OF CASEIN IN DIFFERENT MILK SAMPLE[12]

S.NO	TYPES OF MILK	NUTRITION VALUE				
		Fat (%)	Water (%)	Protein (%)	Lactose (%)	Ash (%)
1	Cow milk	4.4	87	3.8	4.9	0.8
2	Goat milk	4.5	88	3.7	4.2	0.9
3	Buffalow milk	11.5	84	3.6	5.0	0.9
4	Sheep milk	8.6	82	6.7	4.8	0.1

METHODS[13]

1. A Clean dry beaker has been taken, and transfers 20ml of warmCow milk, Goat milk, Sheep milk, Buffalo milk and add 20ml of Acetic acid solution slowly and with stirring.
2. Fat along with Casein was precipitated out.
3. The solutions is filter and transfer the precipitates in another beaker. Add about 30ml of water to the precipitate. Only Casein dissolves in water forming milky solution leaving fat undissolved.
4. The milky solution was heated to about40°C.
5. Collect the precipitate, washed with water and the precipitate was allowed to dry.
6. Weigh the dry solid mass in a previously weighed watch glass.

MATERIALS[14]

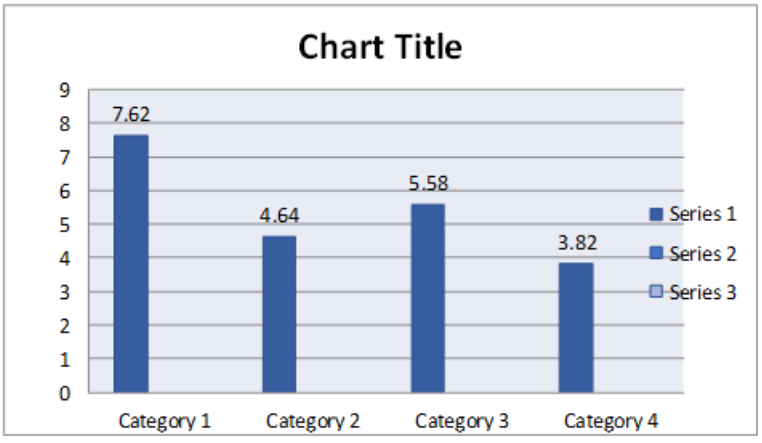
1) Conical flask
2) Beakers
3) Funnel
4) Measuring cylinder (100 mL)
5) Watch glass
6) Filter paper
7) 1% acetic acid
8) Different milk
9) Glass rod and Spatula
10) Tissue paper
11) Cylinder

RESULT

S.NO	MILK SAMPLE (100 ml)	AMOUNT OF CASEIN (g)
1.	Cow milk	7.62
2.	Goat milk	4.64
3.	Buffalo milk	5.58
4.	Sheep milk	3.82

100 ml of milk sample taken from four different milk samples namely cow milk, goat milk, sheep milk, buffalo milk. We identified and isolated the casein, the amount of casein is described in the above table. The yield of casein in cow milk is high in compare to other milk.

Sheep milk < goat milk < buffalo milk < cow milk



Category – 1: Cow milk

Category – 2: Goat milk

Category – 3: Buffalow milk

Category – 4: Sheep milk

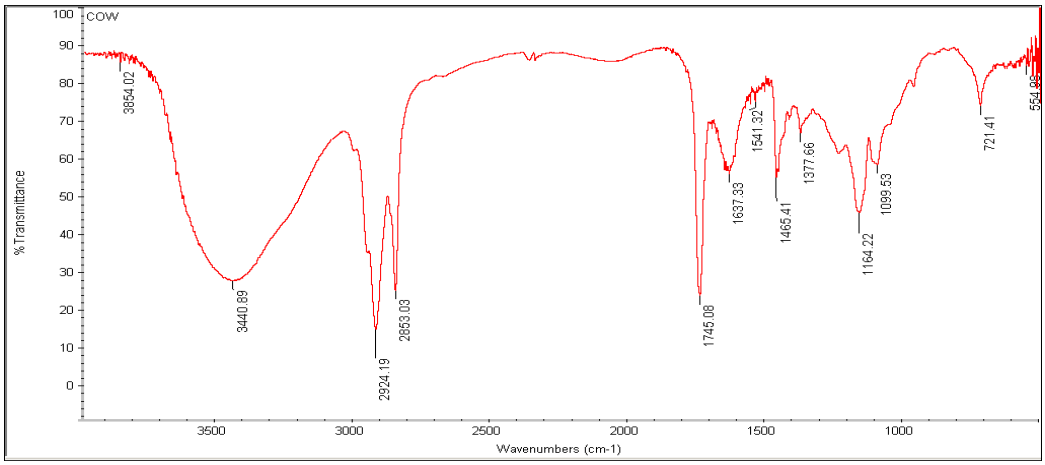
IR SPECTRUM OF COW MILK

IR spectrum was taken using KBR on **IR Affinity 1S** model FTIR spectrometer, the spectrum attached. The IR peak and the groups assigned are shown in Table No.

COW MILK IR SPECTRAL DATA

S.No	Absorption	Group	Appearance	Compound class
1.	3440	O-H stretching	Strong,broad	Alcohol
2.	2924	C-H stretching	Medium	Alkane
3.	1745	C=O stretching	Strong	Ester
4.	1637	C=C stretching	Medium	Alkene
5.	1541	N-Ostretching	Strong	Nitro compound
6.	1164	C-O stretching	Strong	Ester
7.	1099	C-O stretching	Strong	Alcohol
8.	721	C=C bending	Strong	Alkene

COW MILK



IR SPECTRUM OF BUFFALOW MILK

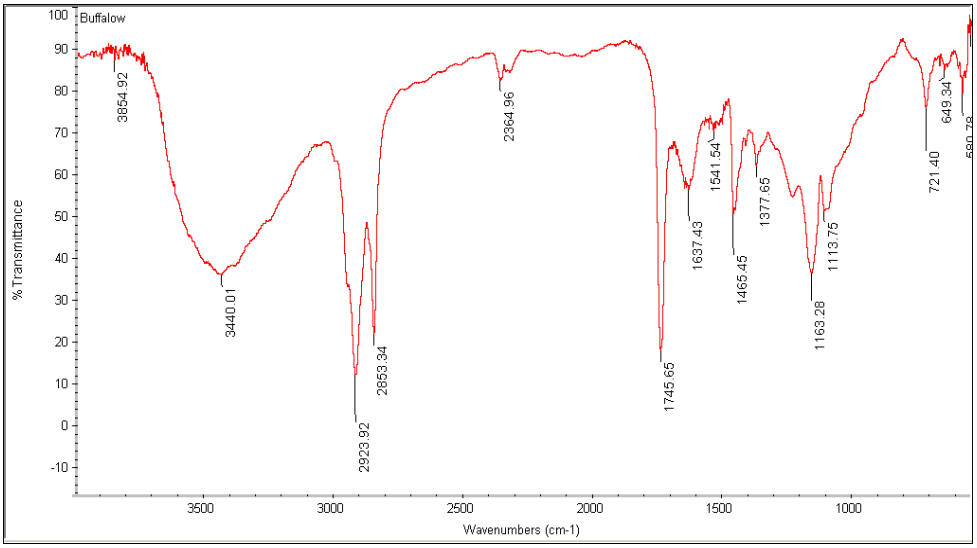
IR spectrum was taken using KBR on IR Affinity 1S model FTIR spectrometer, the spectrum attached.

BUFFALOW MILK IR SPECTRAL DATA

S.No	Absorption (cm^{-1})	Group	Appearance	Compound class
1.	1630	C=C Stretching	Strong	Alkene
2.	3854	O-H Stretching	Medium,sharp	Alcohol
3.	3440	O-H Stretching	Strong,broad	Alcohol
4.	2923	C-H Stretching	Medium	Aldehyde

5.	1745	C=O Stretching	Strong	Ester
6.	1377	O-H Bending	Medium	Phenol
7.	1465	C-H Bending	Medium	Alkane
8.	1541	N-OStretching	Strong	Nitro compound
9.	1163	C-O Stretching	Strong	Ester

BUFFALOW MILK



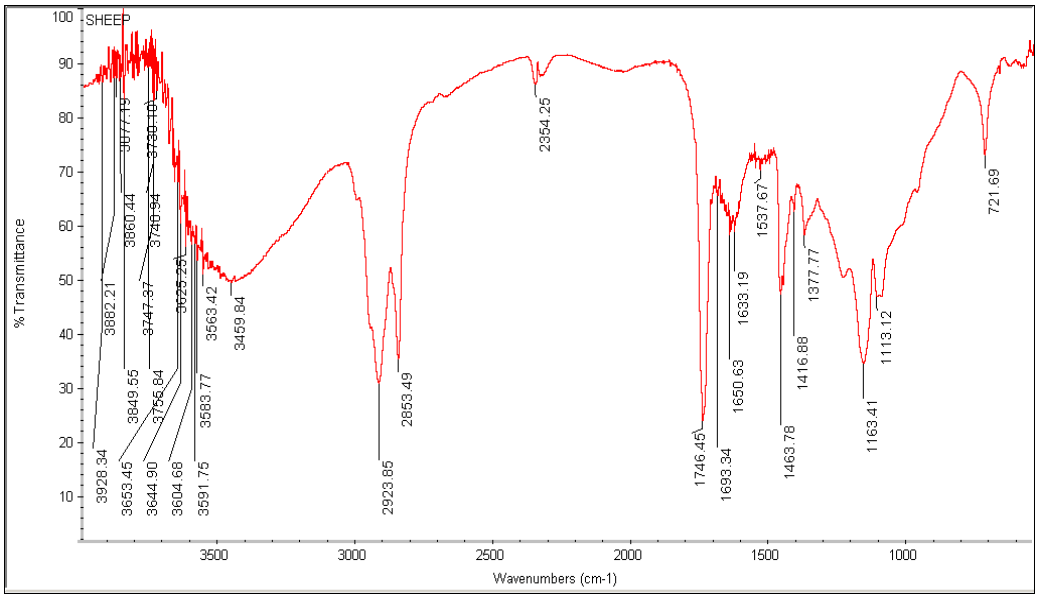
IR SPECTRUM OF SHEEP MILK

IR spectrum was taken using KBR on **IR Affinity 1S** model FTIR spectrometer, the spectrum attached.

SHEEP MILK IR SPECTRAL DATA

S.No	Absorption	Group	Appearance	Compound class	Comments
1.	3928	O-H stretching	Medium,sharp	Alcohol	Free
2.	3653	O-H Stretching	Medium,sharp	Alcohol	Free
3.	3077	C-H Stretching	Medium	Alkene	
4.	3591	O-H Stretching	Medium,sharp	Alcohol	Free
5.	3459	N-H Stretching	Strong,broad	Alcohol	Intermolecular bonded
6.	2853	C-H Stretching	Medium	Alkane	
7.	746	C-H Bending	Strong	monosubstituited	
8.	1693	C=O Stretching	Strong	Conjucated aldehyde	
9.	1633	C=C Stretching	Medium	Conjucated alkene	

SHEEP MILK



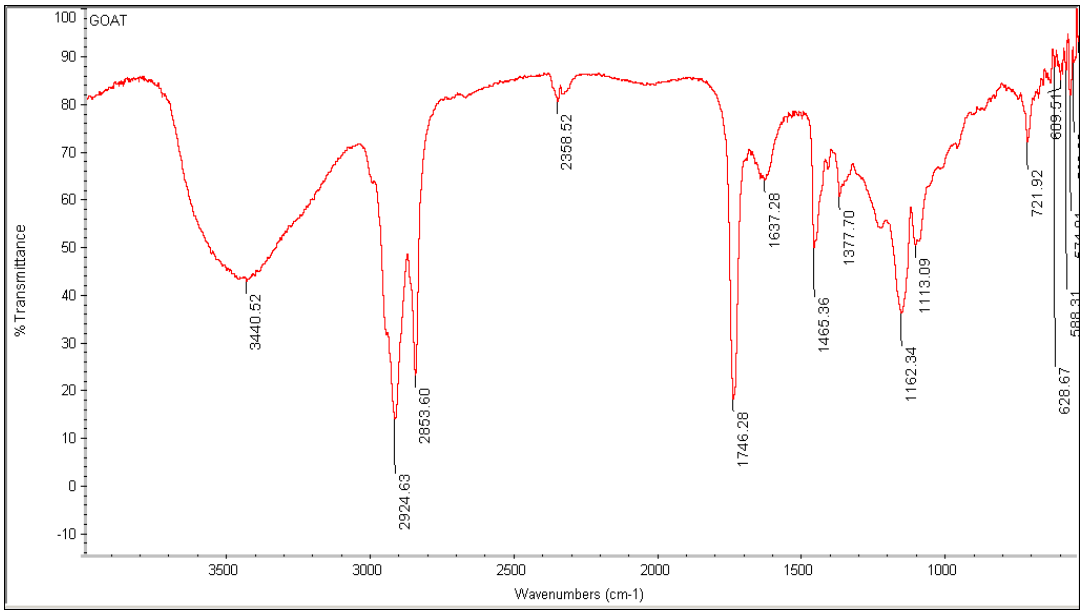
IR SPECTRUM OF GOAT MILK

IR spectrum was taken using KBR on **IRAffinity1S** model FTIR spectrometer, the spectrum attached.

GOAT MILK IR SPECTRAL DATA

S.No	Absorption	Group	Appearance	Compound class	Comments
1.	3440	O-H Stretching	Strong,broad	Alcohol	Intermolecular bonded
2.	2924	C-H Stretching	Medium	Alkane	
3.	2853	C-H Stretching	Medium	Alkane	
4.	1746	C=O Stretching	Strong	Ester	6 membered lactone
5.	1637	C=C Stretching	Strong	Alkene	Mono substituited
6.	1465	C-H Bending	medium	Alkane	Methylene group
7.	1113	O-H Bending	Strong	Secondary Alcohol	
8.	721	C=C Bending	Strong	Alkene	Disubstituitd (cis)

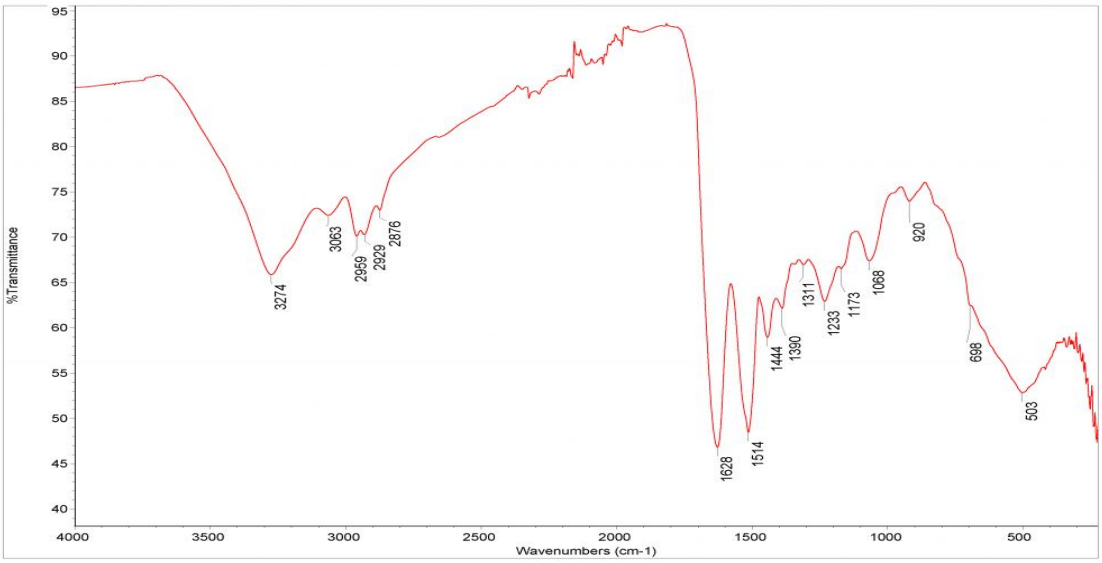
GOAT MILK



PURE MILK CASEIN IR SPECTRAL DATA

S.No	Absorption	Group	Appearance	Compound class
1.	3274	O-H stretching	Strong,broad	Alcohol
2.	3063	C-H stretching	Medium	Alkene
3.	2959	N-H stretching	Strong ,broad	Amine salt
4.	2876	C-H stretching	Medium	Alkane
5.	1628	C=Cstretching	Medium	Conjugated alkene
6.	1444	O-H Bending	Medium	Alcohol
7.	1311	O-H Bending	Medium	Phenol
8.	1173	C-N Stretching	Medium	Amine

PURE MILK CASEIN IR SPECTRAM



CONCLUSION

Casein is the most important protein component in milk, both quantitatively and nutritionally. It is a very rich source of essential amino acids also.

The amount of casein isolated from different milk samples are collected and calculated. This study clearly shows that the amount of casein obtained from Cow milk is greater than other sources of milk.

IR spectral data of all isolated casein is compared with IR spectral of pure casein, the cow milk casein IR spectral data is very close to pure casein, so the cow milk is suitable for the casein isolation.

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