# PHYSICO-CHEMICAL ANALYSIS OF MILK SAMPLES

**DISSERTATION SUBMITTED TO THE UNIVERSITY OF KERALA**
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# CERTIFICATE

This is to certify that the dissertation bound here with is an authentic record of the project work entitled "**PHYSICO-CHEMICAL ANALYSIS OF MILK SAMPLES**" carried out by **MANNA SARA THOMAS, NEETHU REGHUNATH, SHERIN GEEVARGHESE** under my supervision in partial fulfillment of the requirement for the award of the Degree of Bachelor of Science of University of Kerala and further that no part thereof has been presented before for any other degree.

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# DECLARATION

We hereby declare that the dissertation entitled **“PHYSICO-CHEMICAL ANALYSIS OF MILK SAMPLES”**is the original project work carried out by me under the supervision of **Dr.Tressia Alias PrincyPaulose**, Assistant Professor, Department of Chemistry, Bishop Moore College, Mavelikara, and it has not previously formed the basis of award of another degree, diploma, or other title.

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# ABSTRACT

Physico-chemical analysis were conducted on four commercially available milk samples sold in retail shops and milk collected from farmers. Physical parameters such as moisture, pH and fat content in the milk samples were investigated using standard procedures. Fat content shows different values for commercial milk samples and farmers. Moisture content and pH were found to be close to each other for all samples. Commercial milk fat content closely matches the levels declared by the industries. Two farmer-provided milk samples that were tested for fat content yielded average values of 3.58% and 3.64%.

# INDEX

# CHAPTER 1
# INTRODUCTION

## INTRODUCTION

Humans and other mammals, especially those with mammary glands, produce milk. Infants prefer breast milk over other foods because it is well-tolerated while their digestive systems are growing and maturing. If tolerated well, dairy milk may be offered at a later age. Although any mammal can produce dairy milk, cows, goats, buffalo, and sheep are the most common breeds. About 87% of whole cow's milk is made up of water. Protein, fat, carbohydrates, vitamins, and minerals make up the remaining 13%(1).

| Component | Average Content percentage (w/w) | Range Percentage (w/w) | Average % of Dry matter |
|---|---|---|---|
| Water | 87.3 | 85.5 -88.7 | |
| Solids not fat | 8.8 | 7.9-10.0 | 69 |
| Lactose | 4.6 | 3.8-5.3 | 36 |
| Fat | 3.9 | 2.4-5.5 | 31 |
| Protein | 3.25 | 2.3-4.4 | 26 |
| Casein | 2.6 | 1.7-3.5 | 20 |
| Mineral substances | 0.65 | 0.53-0.80 | 5.1 |
| Organic acids | 0.18 | -- | 1.4 |
| Miscellaneous | 0.14 | -- | 1.1 |

**Table 1 -Composition of Cow Milk**

### 1.1 Different Types of Milk & Its Composition

The species of dairy animal, its breed, age and diet, along with the stage of lactation, parity (number of parturitions), farming system, physical environment and season influence the colour, flavour and composition of milk and allow the production of a variety of milk products(2).

- Cow milk: Fat constitutes approximately 3 to 4 percent of the solid content of cow milk, protein about 3.5 percent and lactose 5 percent, but the gross chemical composition of cow milk varies depending on the breed. For example, the fat content is usually higher in Bos indicus than B. taurus cattle. The fat content of milk from B. indicus cattle can be as much as 5.5 percent.
- Buffalo milk: It has a very high fat content, which is on average twice as high as that of cow milk. The fat-to-protein ratio in buffalo milk is about 2:1. Compared with cattle milk, buffalo milk also has a higher casein-to-protein ratio. The high calcium content of casein facilitates cheese making.
- Camel milk: It has a similar composition to cow milk but is slightly saltier. Camel milk can be three times as rich in vitamin C as cow milk and represents a vital source of this vitamin for people living in arid and semi-arid areas, who often cannot obtain vitamin C from fruits and vegetables. Camel milk is also rich in unsaturated fatty acids and B vitamins. Milk from Bactrian camels has a higher percentage of fat than milk from dromedaries, but levels of proteins and lactose are similar. Generally, camel milk is consumed raw or fermented.
- Sheep milk: It has higher fat and protein contents than goat and cow milk; only buffalo and yak milk contain more fat. Sheep milk also generally has a higher lactose content than milk from cows, buffaloes and goats. The high protein and overall solid contents of sheep milk make it particularly appropriate for cheese and yoghurt making. Milk from sheep is important in the Mediterranean region, where most of it is processed into cheeses such as pecorino, caciocavallo and feta.
- Goat milk: It has a similar composition to cow milk. In Mediterranean countries and in Latin America, goat milk is generally transformed into cheese; in Africa and South Asia, it is usually consumed raw or acidified.
- Yak milk: It tastes sweet and has a fragrant, sweetish smell. Yak milk has between 15 and 18 percent solid content, 5.5 to 9 percent fat and 4 to 5.9 percent protein. It therefore has higher solid, fat and protein contents than cow and goat milk, and resembles buffalo milk. Raw milk is used mainly by herders and their families in milky tea. Yak milk can be processed into a variety of milk products including butter, cheese and fermented milk products.

- Equine milk: Horse and donkey milk have very similar compositions. Equine milk, like human milk, is relatively low in proteins (particularly caseins) and ashes and rich in lactose. Compared with that of other dairy species, equine milk contains low levels of fat and protein. Most equine milk is consumed fermented and it is not suitable for cheese making.

## 1.2 Milk Fat Chemistry

In the human diet, milk and milk products are well-balanced, nutrient-rich foods. The high concentration of n-3 fatty acids, conjugated linoleic acid (CLA), and fat-soluble vitamins in milk fat are all factors that contribute to the superior nutritional quality of dairy products. Additionally, milk fat acts as a taste and scent transporter and affects how raw materials are processed. Cow's milk typically contains 3.3% to 4.4% fat (3). 3.25%–4.2% and 7.1% of fat, respectively, are found in goat's and ewe's milk, respectively. Breed, nutrition, personality qualities, and the length of lactation are only a few examples of the variables that affect milk's fat content.

Milk contains approximately 3.4% total fat. Milk fat has the most complex fatty acid composition of the edible fats. Over 400 individual fatty acids have been identified in milk fat. However, approximately 15 to 20 fatty acids make up 90% of the milk fat. The major fatty acids in milk fat are straight chain fatty acids that are saturated and have 4 to 18 carbons (4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0), monounsaturated fatty acids (16:1, 18:1), and polyunsaturated fatty acids (18:2, 18:3). Some of the fatty acids are found in very small amounts but contribute to the unique and desirable flavour of milk fat and butter. For example, the C14:0 and C16:0 ß-hydroxy fatty acids spontaneously form lactones upon heating which enhance the flavour of butter.

The fatty acid composition of milk fat is not constant throughout the cow's lactation cycle. The fatty acids that are 4 to 14 carbons in length are made in the mammary gland of the animal. Some of the 16 carbon fatty acids are made by the animal and some come from the animal's diet. All of the 18 carbon fatty acids come from the animal's diet. There are systematic changes in milk fat composition that are due to the stage of lactation and the energy needs of the animal. In early lactation, the animal's energy comes largely from body stores and there are limited fatty acids available for fat synthesis, so the fatty acids used for milk fat production are obtained from the diet and tend to be the longer chain 16:0, 18:0, 16:1 and 18:2 fatty acids. Later in lactation more of the fatty acids in milk are formed in the mammary gland so that the concentration of the short chain fatty acids such as 4:0 and 6:0 are higher than they are in early lactation (4). These changes in fatty acid composition do not have a great impact on milk's nutritional properties, but may have some effect on processing characteristics for products such as butter.

Milk fat contains approximately 65% saturated, 30% monounsaturated, and 5% polyunsaturated fatty acids. From a nutritional perspective, not all fatty acids are created equal. Saturated fatty acids are associated with high blood cholesterol and heart disease. However, short chain fatty acids (4 to 8 carbons) are metabolized differently than long chain fatty acids (16 to 18 carbons) and are not considered to be a factor in heart disease. Conjugated linoleic acid is a trans fatty acid in milkfat that is beneficial to humans in many ways(5). These issues are discussed in the Milk and Human Health section. The fatty acids are arranged on the triglyceride molecule in a specific manner. Most of the short chain fatty acids are at the bottom carbon position of the triglyceride molecule, and the longer fatty acids tend to be in the middle and top positions. The distribution of the fatty acids on the triglyceride backbone affects the flavor, physical, and nutritional properties of milk fat.

In the present study, we investigated some physical and chemical components of commercially available milk samples and samples collected from farmers to assess their nutritional quality. The main goal of this present study therefore was to evaluate various physicochemical properties of some commercially available milk samples for nutritional quality assessment.

# CHAPTER – 2

# REVIEW OF LITERATURE

# Review of Literature

Quality requirements in the food industry stimulate the development of analytical techniques capable of precise component quantification at a reasonable price of analysis. Therefore, several analytical methods are reported for milk fat determination and quantification. In general, the efficiency of reference method results are usually more reliable, and also more expensive, than routine method results. For this reason, more routine methods for milk fat determination have been developed during the last hundred years(6).

The Butyrometric method is an operating method which can be used as a reference method for routine analytical procedures. The method was developed by the Swiss chemist and dairy-owner Niklaus Gerber . In principle, proteins (mainly phospholipid envelopes of milk fat globules) are dissolved by sulfuric acid. Then, the addition of amyl-alcohol results in a sharp interface. Subsequently, the fat is quantitatively released and separated by centrifugation. Fat volume is read on the butyrometer scale which is calibrated to indicate the fat content in percentage by weight (ISO-International Standards Office, 1976). Under the conditions in the Czech Republic, this method is regulated by the COSMT-Czech Office for Standards, Metrology and Testing (2001) – CSN ISO 2446:2001. This method can be applied to raw milk, drinking milk (whole, semi-skimmed and skimmed milk), milk powder,cream and dairy products (cheese, yoghurt). In the USA and Canada the Babcock method is used with the same analytical principle as a modification of the Butyrometric method .The Folch method (1957) is another method for fat content determination. In principle, lipid extraction is carried out with chloroform and methanol. Then, the homogenate is filtered. The procedure enables extraction of lipid classes for subsequent fatty acid analysis(7).After extraction, transesterification (formation of fatty acid methyl esters) is carried out. This method is commonly used for marine products(8). However, the Folch method was used for milk fat determination of Weddell seal (Leptonychotes weddellii) and bovine milk .Similarly, the Bligh and Dyer method (1959) is used for extraction of fat. In principle, this method is also based on extraction using chloroform and methanol, but it was developed for lipid extraction of lean fish tissue (9).

After extraction, the homogenate is filtered and filtrate is transferred to a graduated cylinder. After allowing a few minutes for complete separation and clarification, the volume of the chloroform layer is recorded and the alcoholic layer removed by suction. Nevertheless, this method was used by Nascimento et al. (2017) for determination of fat content in commercial dried dairy products. Maxwell et al. (1986) used a “dry-column method” for determination of fat. Firstly, the sample is dried by anhydrous sodium sulphate and diatomaceous earth. Subsequently, extraction is accomplished using a mixture of dichloromethane and methanol. The lipids may be isolated and simultaneously separated into neutral and polar fractions by a sequential elution procedure. Neutral lipids (without polar lipids) are eluted first with dichloromethane, followed by elution of polar lipids with the dichloromethane/methanol (9:1) mixture. This method can be applied to raw milk, drinking milk (whole,semi-skimmed and skimmed milk) and buttermilk(10).

Supercritical fluid extraction (SFE) was investigated by Eller and King (1996) as an alternative to solvent-based extraction methods(11). The sample is weighed to the extraction thimble, previously loaded with 1 g of diatomaceous earth. a temperature of 100 °C and sc-CO2 (supercritical carbon dioxide) fluid density of 0.60 mg·ml-1 are used for extraction. Every fluid is characterized by a critical point, which is defined in terms of the critical temperature and critical pressure, thus CO2 is supercritical above 31.1 ºC and 7.38 MPa. Sc-CO2 is removed exclusively nonpolar lipid material. Wolf et al. (2003) used SFE for analysis of milk infant formula powder whereas Astaire et al. (2003) applied method on buttermilk powders (with using microfiltration).The Weibull-Berntrop gravimetric method involves Soxhlet extraction and it is used for special dairy products–infant formula (ISO–Part 1, 2005), ice creams (ISO–Part 2, 2005) or special cases (ISO–Part 3, 2005). In principle, the method include hydrolysis (by hydrochloric acid) and extraction (by n-hexane).Next, the solvent was released in a rotary evaporator,followed by cooling in a dessicator and weighing(12).

# CHAPTER - 3

# AIM AND OBJECTIVES

## **AIM AND OBJECTIVES**

- Collection of different cow milk samples
- Evaluation of pH of the samples
- Determine the Moisture content in milk samples
- Determine the fat content of the samples by Rose Gottlieb method

# CHAPTER -4

# MATERIALS AND METHODS

## 4.1 MATERIALS

Five different cow milk samples were collected. Three samples were commercial milk from shop and two were collected from farmers

| Sample | Name of milk samples |
|---|---|
| A | Milma (Milk Fat -3%) |
| B | Malanad(Milk Fat -3%) |
| C | Muralya(Milk Fat -3%) |
| D | Milk from farmer |
| E | Milk from farmer |

**Table 2- Milk Samples Collected**

## 4.2 METHODS OF ANALYSIS

### 4.2.1 pH

**Apparatus Required**

1. pH meter
2. Beaker

**Procedure**

- All the samples are taken in beaker one by one.
- pH value is recorded for all the sample using pH meter

### 4.2.2 Moisture content

Moisture content was determined using moisture analyser (Wensar ( PGB-1MB))

### 4.2.4 Fat content

**<u>Rose Gottlieb Method</u>**(13)

**<u>Apparatus and Chemicals</u>**

Separating Funnel, Petri dish, conical flask, Ammonium hydroxide solution with specific gravity 0.88 at $20^0$C, Ethanol 95%, Diethyl ether, Petroleum ether (40-$80^0$C)

**<u>Procedure</u>**

- Weigh 5g of milk sample in a 50 ml beaker
- Add 1ml of $NH_3$ (Specific Gravity 0.88 at $20^0$C) and mix well
- Add 10 ml of ethanol and mix well
- Transfer the sample into a 100ml separating funnel with help of 10-15ml distilled water
- Add 25ml diethyl ether, stop the funnel and shake vigorously for one minute
- Wait for few minutes and add 25 ml petroleum ether and shake vigorously for 30 seconds
- Allow to stand for 30 minutes
- Two layers were get separated then decant the ether layer into conical flask.
- Repeat the extraction twice using diethyl ether(25ml) and petroleum ether(12.5ml)
- After completion of separation organic layer dried over anhydrous $MgSO_4$
- Weight of the beaker with organic layer was taken
- Place the beaker on a water bath to evaporate organic solvent and weight of the beaker taken again.

$$\textbf{Total Fat ( \% )= (W1 − W2/ W)} \times \textbf{100}$$

Where:

W = Weight of the sample

W1 = Weight of flask and organic layer

W2 = Weight of flask after evaporating organic layer

# CHAPTER 5

# RESULT AND DISCUSSION

# RESULT AND DISCUSSION

## 5.1 pH

The pH of milk accounts for the amount of lactic acid produced by microbial activity. The more lactic acid present, the higher the acidity. This would result in a change in taste and smell, making it unsuitable for human consumption.

| Sample | pH |
|---|---|
| A | 6.80 |
| B | 6.75 |
| C | 6.82 |
| D | 6.78 |
| E | 6.83 |

**Table 3-pH values of milk samples**

## 5.2 Moisture Content

Water, the most important diluent in foodstuffs, has an important influence on the physical, chemical and microbiological changes. The water content is the principal component, by weight, in most dairy products, including milk, cream, ice cream etc. The moisture content in milk ranges from 85.5 to 89.5%(14).

| Sample | Moisture Content(%) |
|---|---|
| A | 85.98 |
| B | 86.02 |
| C | 85.65 |
| D | 86.98 |
| E | 86.57 |

**Table 4-Moisture content of milk samples**

## 5.4 Fat Content

Fat content in different milk samples were determined by Rose Gottlieb method

| Sample | Fat content in 100ml (%) |
|---|---|
| A | 2.78 |
| B | 2.94 |
| C | 2.77 |
| D | 3.58 |
| E | 3.64 |

**Table 5-Fat content of milk samples**

**Figure 1: Different stages of Rose Gottlieb method**

## CONCLUSION

Physical characteristics such as moisture, and pH are important parameters in studying the physicochemical compositions and nutritional aspects of milk. Tables 3-5 shows the various physical/chemical parameters of the different milk samples analysed in this work. The physical properties of milk collected from farmers and commercial milk found to be close values. The fat content in the commercial milk shows very close values to the fat content claimed by the industries. Fat content of two milk samples collected from farmers shows an average value 3.58% and 3.64%.

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