Analysis of Commercial Curd Samples for the Nutrients and Adulterants

A thesis submitted in the partial fulfillment

Of the requirement for the degree of

MASTERS OF SCIENCE

IN

CHEMISTRY

Submitted by

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CERTIFICATE

This is to certify that the thesis entitled **Analysis of Commercial Curd Samples for the Nutrients and Adulterants** being submitted by **Maansi Sharma (Roll No. 302102007)** to the **School of Chemistry and Biochemistry**, Thapar Institute of Engineering and Technology, Patiala, in partial fulfillment of the requirements for the award of the degree of Master of Science in Chemistry, is an authentic record of the work carried out by the candidate under our guidance and supervision. She has fulfilled the requirements for submitting this dissertation, which has reached the requisite standard to our knowledge.

The results embodied in the dissertation have not been submitted in part or whole to any other University or Institute for the award of any other degree or diploma.

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DECLARATION

I, hereby declare that the dissertation entitled **Analysis of Commercial Curd Samples for the Nutrients and Adulterants** being submitted in the partial fulfillment of the requirements for the award of degree of Master of Science in Chemistry to the **School of Chemistry and Biochemistry**, Thapar Institute of Engineering and Technology, Patiala is a record of my work carried out under the supervision of **Dr. Manmohan Chhibber** Professor, **School of Chemistry and Biochemistry**, and **Mr. Mukesh Agarwal,** Research Scientist, **Sophisticated Analytical Instrumentation Lab,** Thapar Institute of Engineering and Technology, Patiala from Jan-July, 2023.

Further, the results embodied in the dissertation have not been submitted in part or whole to any other University or Institute for the award of any other degree or diploma.

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This is certified that the above statement made by the student is correct and true to the best of our knowledge and belief.

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ABSTRACT

Curd, an essential part of our diet, provides us with vital nutrients for the healthy growth of the body. These nutrients include protein, lactic acid, fat, pantothenic acid, mineral electrolytes – calcium, phosphorous, and vitamins. Milk, the precursor for the curd, also has similar nutrients that are either transformed or passed on as such in the curd.

Recently, there have been reports of adding adulterants in milk to match its physical and chemical properties after suppliers dilute it with water. These contaminants pass on from milk into curd, besides additional added for preservation and appearance.

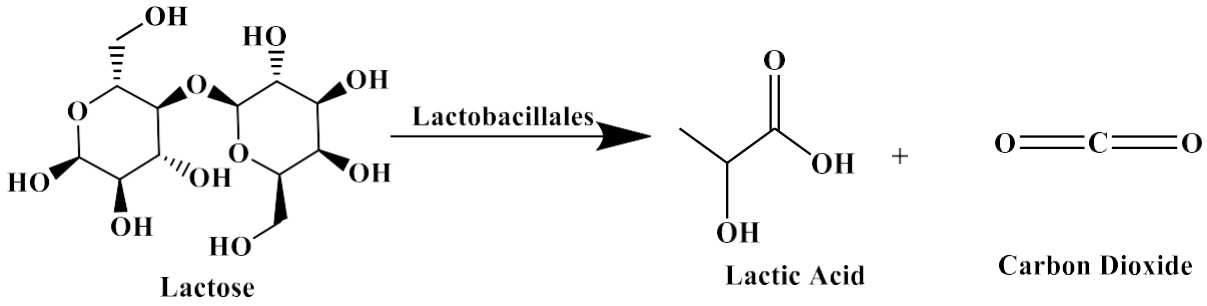
This work analyses commercial curd samples for their nutritional content, besides analyzing seven for the adulterants. The sample contained protein (4.14%), lactose (4.92%), lactic acid (<over 0.20%), fat (3.66%), calcium (0.12%), magnesium (0.014%), and sodium (0.06%). However, to our knowledge, these values are not comparable due to a lack of formal reference standard for the curd. Adulterants like starch, formalin, sodium bicarbonate, added sugar, vanaspati oil and bloating paper were investigated in seven commercial samples. Two samples were found laced with blotting paper, while six contained formalin. The formalin was also quantitively analyzed.

The work concludes by suggesting a need to develop convenient methods to detect contaminants accessible to consumers and the development of a reference standard for the curd.

CHAPTER – 1

INTRODUCTION

Curd is a fermented dairy product that has long been a staple of Indian cuisine and religious practices[1]. Metchnikoff popularized the idea of probiotic microbes, which led to a rise in the consumption of food preparations containing lactic acid bacteria (LAB). LAB includes strains of the thermophilic *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus bulgaricus* and mesophilic *Lactococcus lactis*, *Lactobacillus casei*, and *Leuconostoc lactis* that initiate the fermentation[2]. These organisms convert lactose, a milk sugar, into lactic acid **(Scheme-1)**.



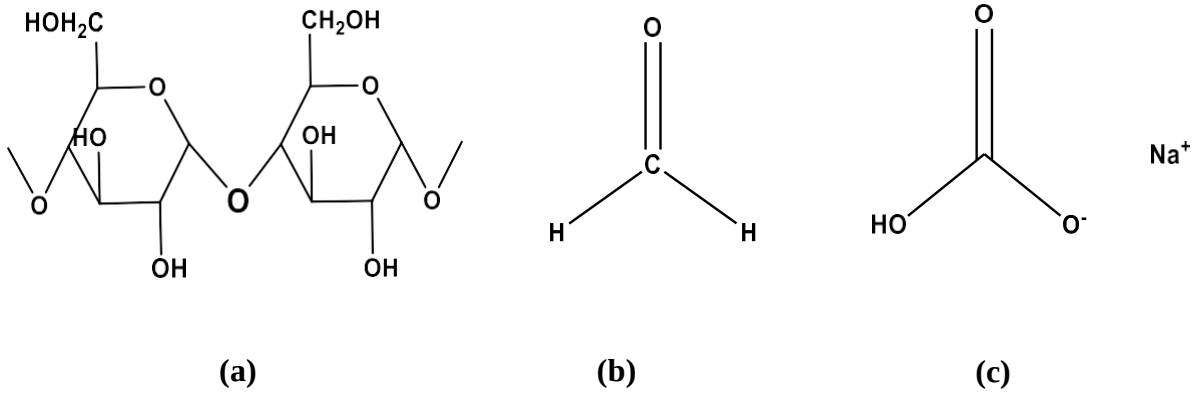
Scheme 1

These microorganisms-containing food products are known for their beneficial effects on health, like improving bowel habits, lactose digestion, boosting resistance to infectious disease, enhancing immunity, preventing colon cancer, lowering cholesterol and blood sugar levels and increasing mineral absorption.

Adulteration as Counterfeiting is the deliberate act of degrading a commercial product to imitate or replicate a pure or authentic commodity. This practice generally involves substituting an inferior item for a superior one, all to acquire financial gain illegally. Food adulteration is an age-old practice known for centuries. There is evidence that prohibited the addition of flavouring and colouring substances in wine in Rome and Athens. Hassall, a prominent British scientist in the nineteenth century, was credited with identifying methodologies for chicory in coffee[3].

There has been widespread dissemination of apprehension regarding the adulteration of milk and dairy products globally in recent years. Several factors, like a mismatch between supply and demand, the perishable nature of milk and its products, low customer purchasing power,

and a lack of appropriate detection techniques, are reasons for the adulteration. Milk and curd contain formalin, sodium bicarbonate, sugar, starch and water as significant adulterants. The structure of these compounds as shown in **Figure 1.1**[4].



(a) **(b)** **(c)**

Figure 1.1: Adulterants found in dairy products.

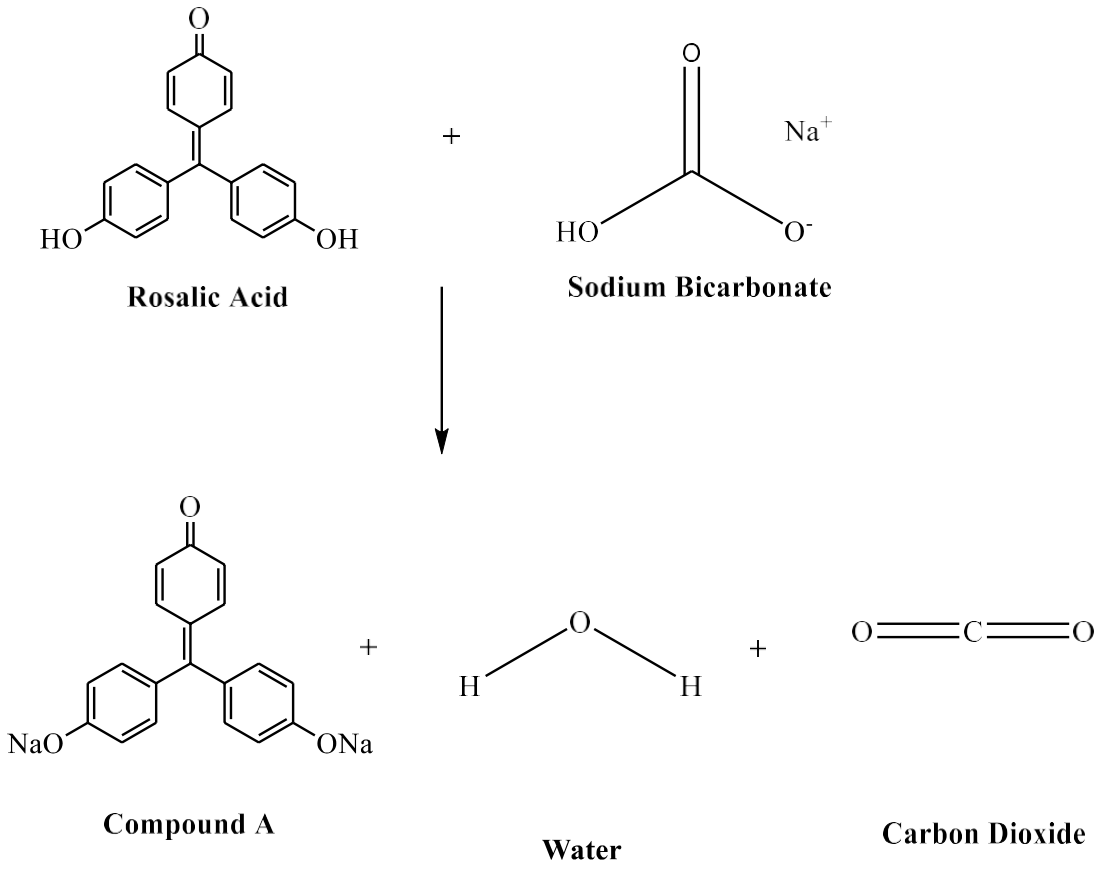
In light of the above reports, using milk from direct sources is generally recommended to avoid adulteration. The work presented here analyses both qualitatively and quantitively the commercial curd samples for their nutritional constituents and adulteration.

CHAPTER – 2

LITERATURE REVIEW

The Swiss Milk Scandal, discovered in 1850, resulted in the unfortunate loss of approximately 8000 infant lives in New York. Milk is commonly acknowledged as a highly nutritious dietary source due to its abundant supply of essential nutrients that benefit individuals of all ages, including infants and adults[5]. Melamine was added to infant formula and other milk products in China in 2008, which resulted in the deaths of both infants and other people. In 2007, wheat gluten and melamine samples were found in the human food supply and numerous U.S. pet food products, likely to increase protein amounts. There have been multiple reports of adulteration of milk worldwide, wherein ingredients like extra water, foreign proteins, whey proteins, melamine, urea, vegetable, or animal fats, have been added as potential adulterants in milk and milk products[6]. Milk is falsified to such an extent that it has very little nutritional value and may even be dangerous for public health due to the high consumer demand, which affects their profit margin. According to a 2011 and 2012 national survey on milk adulteration by the FSSAI (India), water is the most frequent adulterant in milk, followed by detergent. 68% of milk samples were contaminated, with 31% coming from rural areas. 16.7% were packaged or branded milk; the remaining samples came from dairies. In the cities, it was discovered that 68.9% of the milk was tainted with water, detergent, urea, and skim milk powder[7]. Food adulteration, intentional or unintentional, is an illegal fraud that alters food composition, nutritional value, and price[8]. Formalin, added to dairy products as an adulterant to extend its shelf life for long-distance transit, damages the kidneys and liver and is highly toxic[9]. It is the most popular and efficient preservative for keeping milk and some dairy products fresh. According to widely divergent scientific research findings, the effectiveness of formalin in maintaining the composition profile of milk and milk products is in dispute[10]. The renowned Hehner test was developed in 1895 and is still used today. A violet hue appears at the junction when H_2SO_4 is poured over a curd solution. The tryptophan in the proteins affects how they react with formaldehyde. The amino acid tryptophan is one of them. The test can be performed on proteins other than milk as long as they have a reasonably high tryptophan content. The test involves an aromatic amine aldehyde-oxidation process[11]. The Kjeldhal method is typically used to quantify formaldehyde.

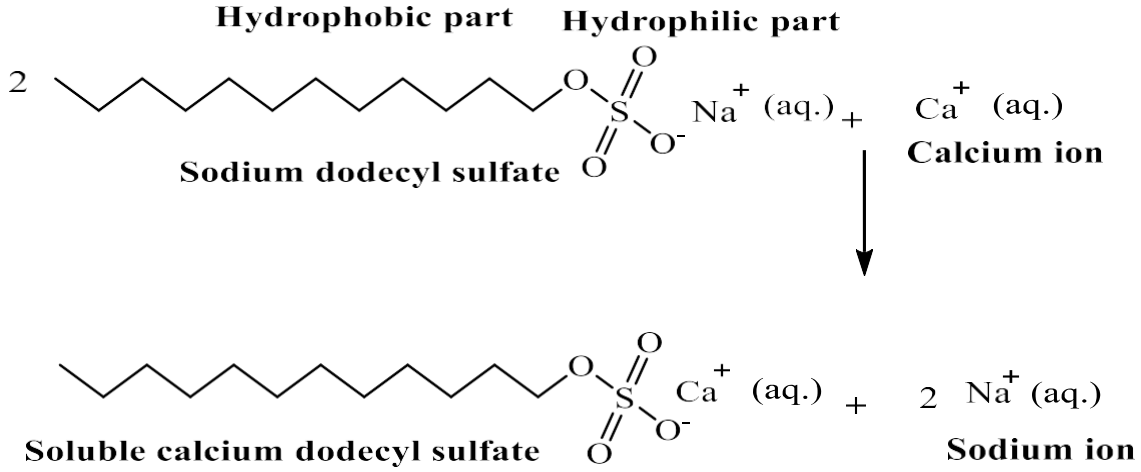
Before milk is pasteurized, neutralizers such as lime water or sodium bicarbonate are added to neutralize any generated acidity. These actions are not acceptable. Numerous techniques have been developed to find neutralizers in dairy products, including the pH test, the alkalinity of ash, and the rosalic acid test. **Scheme-2:** below shows the formation of (**Compound-A)** by the reaction of rosalic acid with sodium bicarbonate. Rosalic acid indicates that it exhibits a color change when added to alkaline milk. This test will be successful only when neutralizers are added in excess amounts and milk is naturally alkaline[12].



Scheme 2

The use of antimicrobial drugs to reduce the microbial population is one of the frequent issues in raw milk quality assurance. Detergents and other substances of this nature can be added to raw milk for this purpose. A rise in milk acidity that results in ageing and loss of milk quality could be caused by increased milk microbial load due to improper production, storage, and distribution circumstances. One of the microbial growth inhibitors added to raw

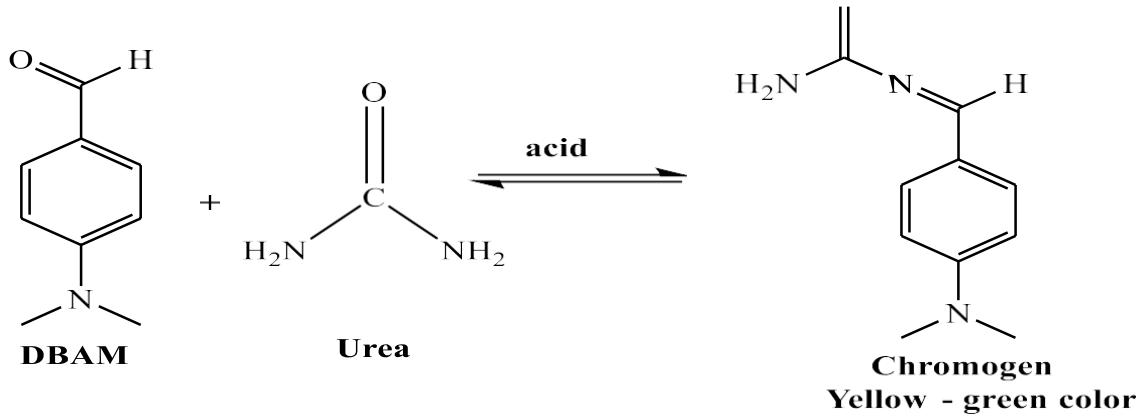
milk as a regulator or coating of milk acidity is detergent powder[13]. Detergents improve milk's cosmetic qualities, which reduce milk's frothy look and whitening. When water is added to milk, the frothy appearance reduces. Thus detergents are added to the milk to restore the foamy appearance artificially[14]. Water was added to the sample to determine the presence of detergent, which generated scum. **Scheme-3** below shows the combination of Ca^{+} present in the water with SDS to produce scum.



Scheme 3

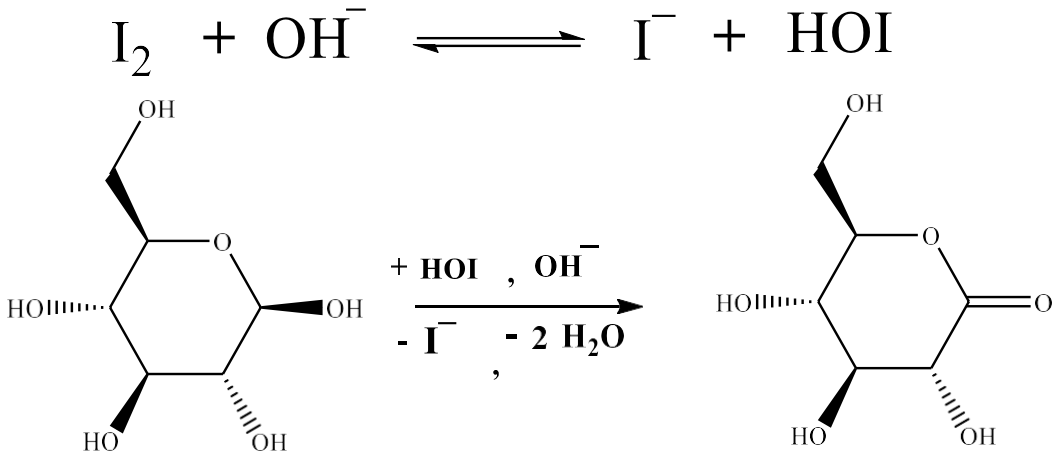
Milk naturally contains urea, which is a sizable portion of the non-protein nitrogen. The urea in raw milk ranges from 20 mg to 70 mg per 100 ml. However, a sample with a urea level above 70 mg/100 ml is considered to have "added urea."[15] It has been a very popular adulterant in the past. Adulteration raises urea levels, which are harmful to human health[16]. The most common method of tampering with milk is to mix water into it, then add urea to the resulting mixture to increase the amount of SNF, which gives the milk a concentrated and rich appearance. To make the specific gravity of the fabricated milk equal to that of natural milk, the urea content is changed depending on the volume of water added, preventing the lactometer from detecting any difference[17]. DMAB can be used to determine if urea has been added to milk. **Scheme-4** below represents the combination of H_2NCONH_2 and DMAB to generate a yellow-green complex at room temperature in a low acidic solution. The Food Safety and Standards Authority of India (FSSAI) conducted a national survey on milk adulteration in 2011 that found several pollutants across India. Starch, typically added to milk to raise the solid non-fat content, is one of these pollutants. To find starch, an iodine starch

test was conducted. Due to its non-polar nature, iodine is only weakly soluble in water. But when KI is present, a soluble linear tri-iodide ion complex is created. The helical coil structure of the starch absorbs this linear tri-iodide, giving it a dark bluish-brown hue[18].



Scheme 4

By adding a few drops of a diluted amylose ("starch") solution to a weakly alkaline solution, the presence of iodine can be detected due to the characteristic blue hue. If glucose is added to the solution, the colour is lost. The reaction's primary oxidizing agent is hypoiodous acid, or HOI, not iodine itself[19]. **Scheme-5** displays conversion of D-glucopyranose into D-gluconolactone by hypoiodous acid.



Scheme 5

Due to the contaminant's preservation properties, hydrogen peroxide is added to dairy products to adulterate it, extending its shelf life and making the product easier to market. Hydrogen peroxide is dishonestly used to stop microbial growth in samples close to expiring or unfit for drinking. Due to its harmful effects, hydrogen peroxide is prohibited in some

nations from being used as a preservative; nevertheless, in countries where it is allowed, such as the United States, its proportion cannot be more than 0.05% of the milk's weight[20].

The most common food preservative is benzoic acid. Even though benzoic acid is generally considered safe (GRAS), sensitive individuals have reported side effects such as asthma, urticaria, and convulsions at modest dosages. Benzoic acid and its salt are often prohibited as food additives in dairy products. However, cheese, sour cream, and yogurt all contain benzoic acid. Benzoic acid is produced by lactic acid bacteria in fermented milk[21]. Regression, a loss of acquired speech, and sensory problems are all brought on by ammonia. Typically, sugar is added to enhance the solids content rather than the fat content or to raise the lactometer reading of milk that has already been diluted with water. Additionally, other food colorings are added to enhance the appearance. Milk contains antibiotics in the form of antimicrobial residues. Tetracycline, aromatic amines, gentamicin leftover after treating mastitis, neomycin leftovers, sulfamethazine leftovers, chloramphenicol leftovers, aflatoxin M1 contamination, etc., are further severe concerns when it comes to milk adulterants. It disrupts bacterial fermentation, leading to significant losses in fermented products. Mastitis, which is described as the inflammatory reaction brought on by an infection of the udder tissue, has been documented in a wide range of species, particularly domestic dairy animals. Mastitis is one of the most expensive diseases for the dairy business because it causes decreased milk production, changes in the composition and quality of the milk, and other economic consequences[22]. Effective mastitis control programs have a greater emphasis on prevention than on treatment. Even today, antibiotic medication is a well-established component of mastitis preventive programs. Antibiotics are routinely used with other treatments, but their effectiveness is still subpar[23]. The hygienic level maintained during milking, cleanliness of the milking instruments, storage situation, mode of transportation, and cleanliness of the udder are all closely related to the microbiological quality of milk. Some manufacturers may also add potassium chromate or dichromate to stop spoilt milk from coagulating during heating. However, it is known that potassium dichromate can induce rhinitis, allergic contact dermatitis, and skin irritation. A cow with high milk output may be more susceptible to milk fever but not to most other frequent illnesses, such as dystocia with veterinary assistance, metritis, cystic ovary, ketosis, left displaced abomasum, and mastitis. But the majority of these illnesses have as a side effect, that is, reduced milk production[24]. When analyzing the financial effects of health issues on dairy farms, particularly their effects on feed consumption, it is common practice to assume a steady decline in concentrate intake per

kilogram of milk loss[25]. Lameness and ketosis both have a negative impact on milk production[26]. Substituting high-quality milk, like that from a buffalo, for cheap, widely accessible milk, like that from a cow, is the most frequent and straightforward purposeful adulteration. Although no adverse health effects are associated with this adulteration practice, it is done to maximize profits or close the supply-demand mismatch[27].

COMPONENTS OF CURD:

Due to the solubilization of CCP, acidification of milk causes the internal structural characteristics of casein micelles to be disrupted. When caseins **(Figure-2.1)** approach their isoelectric point (pH 4.6), the electrostatic interaction between charged groups, including the phosphoserine residues exposed when the CCP is solubilized, decreases. Electrostatic attraction and protein-protein attraction both rise thanks to enhanced hydrophobic interactions. As the pH from 6.6 to 6.0, the net negative charge on the casein micelles decreases, which lessens electrostatic repulsion. As the pH declines from pH 6.0 to pH 5.0, the net negative charge on casein micelles considerably diminishes, and the charged "hairs" of -casein may constrict. The electrostatic repulsion and steric stabilization, both of which are diminished, are responsible for the stability of the casein micelles in the original milk. When the pH approaches the isoelectric point of casein, the net negative charge on casein decreases, which reduces the elctrostatic attraction between casein molecules. On the other hand, casein-casein attractions, increase due to improved interactions between hydrophobic and plus-minus (electrostatic) charges[28]. Sugar is fermented by lactic acid bacteria to produce lactic acid **(Figure-2.2)**. To have any possibility of producing a positive effect in humans, at least f 1.0×10^{7} to 1×10^{9} live cells must be consumed daily. The homo fermentative organism Lb. delbrueckii subsp. bulgaricus mainly produces lactic acid from sugar. Grigoroff first separated it from fermented milk. They thrive in milk and can withstand greater acidities, like a pH range of 3 to 4. Growth is favored by a relatively high temperature, such as 32 to 38°C[29]. Oxalate, linked to age-related disorders such heart ailments, has been suggested to be the cause of lactic acid's structural similarities to oxalate. Lactic acid prevents oxalate synthesis, which could be the cause of for yogurt's heart-healthy properties[30]. Galactose and glucose combine to make lactose **(Figure-2.3)**, a disaccharide sugar present in milk. Lactose digestion is facilitated by yogurt, which also lessens lactose intolerance symptoms. These advantageous effects are brought about by microbial-galactosidases, which also increase gastrointestinal innate and adaptive immune responses while delaying gastrointestinal transit

and reducing sensitivity to symptoms. Dairy product fermentation also converts lactose intoV its monosaccharides[31].

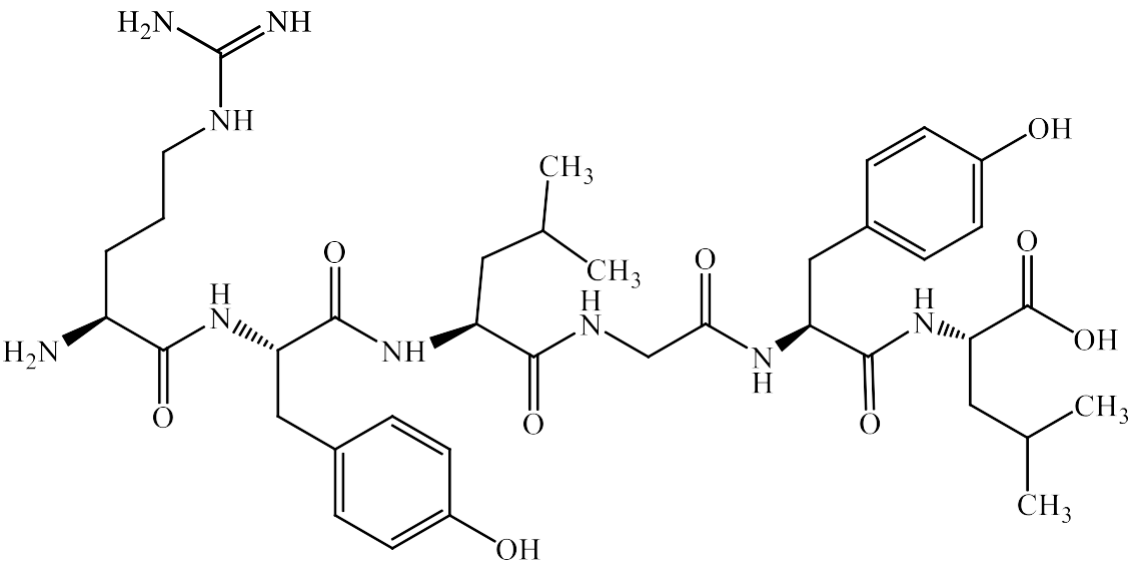


Figure-2.1 : Casein

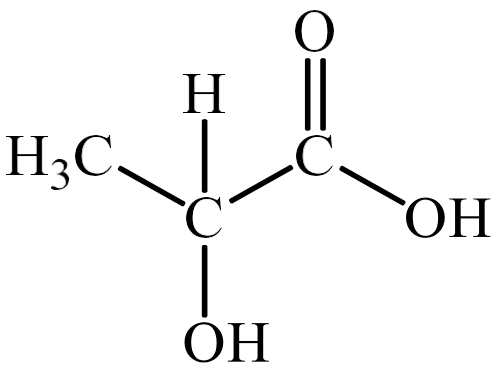


Figure 2.2: Lactic Acid

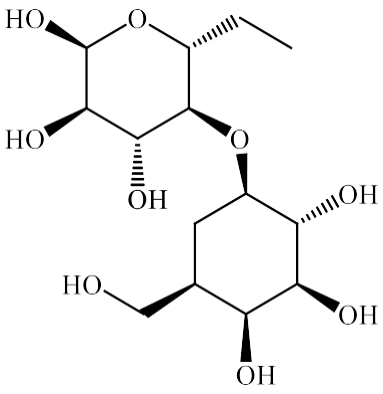


Figure 2.3: Lactose

Probiotic yogurt helped type 2 diabetic patients' fasting blood sugar levels and antioxidant status. Yogurt with probiotics has potential as a diabetic control tool[32]. Curds cannot be regarded as a vegetarian source of vitamin B12 because when cow's or buffalo's milk is curdled with a culture of domestic curds, roughly 30% of the vitamin B12 is lost. During the production and maturing of kefir, the vitamin B12 level of the milk dropped, with the loss being most noticeable during the 18-hour incubation at 22 °C with the kefir culture[33].The process of turning milk into curd impacted the ascorbic acid level of milk. It was possible that the warmth required to cause curdling would negatively impact the vitamin stability in milk. On the other hand, the vitamin may be stabilized by the lowering of pH caused by acid

production. The lactic acid bacillus might be able to synthesize the vitamin, in which case the ascorbic acid content would rise[34].A large amount of β-glucan, a linear, non-branched carbohydrate, can be found in oats and barley. By lowering serum cholesterol levels, β-glucan may also lower blood sugar and insulin levels. It may also strengthen the immune system, which could have an anti-cancer effect. It is feasible to produce low-fat curd structures that mimic those of full-fat products by replacing some fat in low-fat milk formulations with β-glucan. The ability to regulate the structure and, consequently, the texture and rheological characteristics of dairy products is made possible by the modulation of protein-fat-β-glucan interactions[35].

CHAPTER – 3

GAPS IN RESEARCH AND OBJECTIVES

GAPS IN RESEARCH

A bowl of curd has all the essential nutrients, including protein, carbohydrates, fat, minerals, electrolyte, and vitamins. However, despite being a significant component of our food, there aren't many research papers or Indian standards accessible to determine its nutritional content or whether it contains any adulterants. Milk has been the subject of several research, and numerous ways exist to assess its nutritional content and detect various forms of adulteration. To determine the components and their quantities of the curd in this investigation, curd samples were processed using the ISI's established procedures for milk. The reported methods for checking milk for adulterants also apply to curd.

OBJECTIVES

1. Complete analysis of different curd samples.
2. To determine adulteration, if any, qualitatively.
3. To quantify adulteration.

CHAPTER - 4

MATERIAL AND METHODS

CHEMICALS: All chemicals were bought from various sources with a 98% purity. Chemicals were purchased from Loba Chemie and Rankem. The washings were done with distilled water. The SAI Labs' distillation glass equipment was used to obtain it.

INSTRUMENTATION: Several instruments are used to quantify the sample. Atomic Absorption Spectrometer (GBC 932 AA), Hot Plate, Hot Air Oven, Microprocessor Flame Photometer (Esico model 1382), and Muffle Furnace were all used. All of the equipment was from SAI Labs, TIET, Patiala.

TEST OF COMPONENTS PRESENT IN CURD:

1. TOTAL SOLIDS DETERMINATION (GRAVIMETRIC METHOD)

Procedure: With the lid on, weigh empty dish. 5 ml of the prepared curd sample pipetted into the dish. The dish should be weighed with the cover on. Place the dish on boiling water, uncovered. Keep the dish's base horizontal to encourage even drying. Remove the dish after at least 30 minutes, wipe the bottom, and then place it in a well-ventilated oven set to 98 to 100°C with the cover next to it. The oven control thermometer's bulb must be positioned directly above the shelf holding the dish. Cover the dish and move it immediately to a desiccator after the three hours. After letting it cool for about 30 minutes, weigh it. Heat the dish for an additional hour in the oven with the lid off. Take out to the desiccator, and weigh as usual. If required, repeat until the weight loss between weighing does not exceed 0.5 mg. Observe the lowest weight.

Calculation:

$$\text{Total solids, percent by weight} \quad 100\,w$$

Where

w = weight of the residue after drying in g

W = weight of prepared sample taken for test in g

2. ASSESSMENT OF THE TOTAL NITROGEN (KJELDHAL METHOD)

Procedure: Weigh 10 g of the prepared curd sample to an 800-ml Kjeldahl flask. 25 ml conc. H_2SO_4 (98% by weight), was added. Pour this into the flask's neck to wash any curd into the flask's body, then add 0.2 g of copper sulfate. Gently rotate the flask to ensure all contents are thoroughly mixed. Placing the flask on a frame with the neck inclined at a 45º angle to the horizontal, and the bulb resting in an asbestos sheet hole will prevent the flame from touching the flask above the liquid's level. When frothing has stopped, bring to a mild boil and add 10g potassium or anhydrous sodium sulfate. Rapidly boil the flask's contents for one hour when they are clear and devoid of yellow color. Allow the liquid to cool before using a fine jet of distilled water to clean the sides. The flask's contents should be heated for an additional hour. Allow the liquid to cool before dilution with 200 ml of distilled water, and transfer to a 1000ml flask thoroughly rinsing. Add some granulated zinc pieces to the flask to prevent bumping. To create a layer beneath the acid liquor, carefully pour a suitable quantity of NaOH solution (50% by weight) about 75 to 80 ml down the flask's neck. Attach a splash head to the flask and a condenser to it. Pipette 50 ml of the standard H_2SO_4 (0.1N) into a 250 ml beaker. The apparatus should be put together. Ensure the condenser's tip extends below the H_2SO_4 surface in the beaker. Shake and distill the flask's contents until all the ammonia has been absorbed into the regular acid. Shut off the burner after removing the flask from the condenser. Fill the beaker with water, and thoroughly rinse the condenser. Carefully clean the dip tube to ensure that all condensate is delivered to the beaker. Use the indicator solution to titrate the excess acid in the beaker with the regular NaOH solution. Perform a blank determination by substituting 0.5 g of sucrose for the curd and using the exact amounts of the other reagents and test conditions.

Calculation:

$$\text{Total Nitrogen \%by weight} = \frac{1.4(A-B)N}{W}$$

Where

A = volume in ml of the standard NaOH necessary for the blank determination.

B = volume in ml of standard NaOH required for the test

N = normality of the standard NaOH

W =weight of the prepared sample taken for the examination in g

3. CASEIN DETERMINATION

Procedure: 10 g of the prepared sample should be put into two flasks. Only 0.4 ml of the potassium oxalate solution should be added after adding 1 ml of the phenolphthalein indicator solution. Wait for two minutes. Utilizing the other flask as a blank, neutralize the contents of one of the flasks with the standard NaOH (0.1N) solution. To achieve the same pink color, titrate with the NaOH solution once more after adding 2 ml of neutralized formaldehyde (40%).

Calculation: Although the result of the first titration is not necessary, it is important to record how many milliliters of the NaOH was used in the second titration. To determine the proportion of casein, multiply this value by 1.38.

4. ASSESSMENT OF LACTOSE (MUNSON AND WALKER GRAVIMETRIC METHOD)

Procedure: In a 500-ml graduated flask, dilute 25 g of the prepared sample, precisely weighed, with 400 ml of water. 10.8 ml of the standard NaOH solution (0.5N) and 8.8 ml of the Fehling solution (A) should be added. After mixing with the alkali solution, the combination must still undergo an acid reaction and contain copper. Water should be added until the mark is reached, mixed, then filtered through dry filter paper. A slight excess of copper should be seen in the filtrate as a faint blue color. Create a 6 mm thick asbestos film to use as a tared Gooch crucible. 10 ml of ethyl alcohol (95%) and 10 ml of ethyl ether were used to clean the crucible. Thirty minutes of drying at 98 to 100°C, they were then cooling in a desiccator and weighing. 50 ml of the filtrate should be added after 25 ml of each of the Fehling solutions (A) and (B) have been transferred to a 400 ml beaker made of alkali-resistant glass. If less sugar solution is used, add water to get the desired final volume of 100 ml. The beaker should be heated on asbestos gauze over a Bunsen burner. The flame should be controlled so that boiling starts in 4 minutes and continues for 2 minutes. (It is crucial that these instructions are followed precisely. Before making the accurate determination, conducting preliminary tests with 50 ml of the reagent and 50 ml of water is advisable to conduct initial tests with 50 ml of the reagent and 5O ml of water to adjust the burner.) During heating, keep the beaker covered with a watch glass. Using suction, simultaneously filter the heated solution via an asbestos mat in a porcelain Gooch crucible. With plenty of

water, carefully clean the cuprous oxide precipitate at around 60 °C and weigh the dried cuprous oxide directly. Use 50 ml of the reagent and 50 ml of water to do a blank determination, and if the weight of cuprous oxide obtained exceeds 0.5 mg, adjust the reducing sugar determination result appropriately. Upon standing, the alkaline tartrate solution degrades, producing more cuprous oxide in the blank. After carefully washing it with hot water, rinse the precipitate with 10 ml of each ethyl ether and ethyl alcohol if cuprous oxide is to be measured by weight. The precipitate should be dried in boiling water temperature for 30 minutes in oven before cooling and weighing.

Calculation - Using Munson and Walker's table for lactose, determine the proportion of lactose in the sample as follows:

$$\text{Lactose \%} = \frac{Factor}{Titrate\ X\ 100}$$

5. ESTIMATION OF SUCROSE (MUNSON AND WALKER GRAVIMETRIC METHOD)

Procedure: The filtrate from the lactose determination should be placed in a 250 ml flask together with 34 ml of hydrochloric acid (1:1 by volume). The flask should then be placed immediately in a briskly boiling water bath. (The contents must be at a temperature that, after the addition of acid, will display a temperature of 21±2°C). After keeping it in a water bath for 5 minutes, quickly bring to room temperature. Use NaOH solution (50% w/v) to neutralize. Ensuring that there isn't a local or overall excess of alkali in the warm solution to prevent the destruction of part of the sugar. With a 50 ml aliquot, determine the reducing sugar using the Munson and Walker gravimetric method, cool to room temperature, make up to the mark, and shake well. Filter using a tared Gooch filter, then wash four or five times in hot water and once in alcohol (95%) before drying for 30 minutes at 98 to 100 degrees Celsius. Weigh the dried cuprous oxide precipitate.

Calculation: Take the cuprous oxide precipitate's invert sugar equivalent from Munson and Walker's table and multiply the result by 0.95 to get its sucrose value in milligrams. The sample's sucrose content should be calculated as follows:

$$\text{Sucrose weight percent} = \frac{M}{5}$$

Where, M is the amount of sucrose needed to produce a cuprous oxide precipitate (mg).

6. ASSESSMENT OF CHLORIDE (TITRATION METHOD)

Procedure: Weigh 15 g of the prepared sample precisely and pour it into a 250 ml Erlenmeyer flask. 10 ml of silver nitrate solution should be added to it. 10 ml Nitric acid solution (0.05N) should be added, and the mixture should be digested until reddish-brown fumes are released. Saturated iron alum solution should be added in one milliliter. The excess silver nitrate is determined by titrating with the typical potassium thiocyanate solution (0.05N) until the first appearance of an orange color lasts for 15 seconds. Similarly, using the exact amounts of reagents and water, calculate the volume of the standard thiocyanate solution corresponding to 10 ml of silver nitrate.

Calculation:

Chlorine weight % = 0.01773 (B-A)

Chloride as Sodium chloride weight % = 0.02923 (B-A)

B = volume of the standard potassium thiocyanate solution required by the blank (ml)

A = volume of the standard potassium thiocyanate solution needed for the sample (ml)

7. ASSESSMENT OF ASH (GRAVIMETRIC METHOD)

Procedure: 10 g of the prepared sample should be precisely weighed in platinum, silica dish that has been ignited, cooled in a desiccator, filled with an effective desiccant, and weighed. Until the ash is free of carbon, evaporate to dryness and ignite in a muffle furnace at a temperature of 550°C. Desiccate and weigh after cooling.

Calculation:

$$\text{Ash \% by weight} = \frac{100\,w}{W}$$

w = weight of ash (in g)

W = weight of sample taken (in g)

8. OBSERVATION OF NITRATES (COLORIMETRIC METHOD)

Procedure: Mix 25 ml of curd and 25 ml of mercuric chloride solution (2.5% w/v in 1% HCl). After thoroughly blending, pass the mixture through an 11-cm filter. Add 4 ml of the diphenylamine sulphate reagent in a test tube after adding 1 ml of the filtrate. Mix it properly.

Observation: If nitrates are present, a blue color develops.

9. DETERMINATION OF ACIDITY (ALIZARIN- ALCOHOL TEST)

Procedure: Transfer a volume of 5 ml of curd into a test tube, adding an equivalent volume of the alizarin solution (prepared with 0.2% ethanol). Agitate the contents of the test tube through repeated inversions. Observe the color of the mixture and take note of the existence of any particulate matter in the form of flakes or clots. Additionally, it is essential to observe and document the size of any flakes, if present.

Observation: Observe the lactic acid percentage concerning the solution's color and the flakes' size.

10. DETERMINATION OF FAT (ROSE-GOTTLIEB METHOD)

Procedure: In the extraction tube, accurately weigh 10 to 11 g of the prepared sample, then add one milliliter of the concentrated ammonia solution and stir well. After thoroughly mixing again, add 10 ml of alcohol. Complete mixing at each stage is necessary for successful fat extraction. Before sealing the tube with the cork (or stopper), which has been moistened with water before insertion, add 25 ml of ether and shake fiercely for one minute. Remove the cork and wash the cork and tube neck with 25 ml of light petroleum, letting the washings flow into the tube. Once moistened with water, replace the cork and shake ferociously for 30 seconds. (The cork (or stopper) must be wetted with water before each insertion and solvent-washed after each withdrawal. Additionally, the tube should be somewhat cooled before each removal to reduce pressure to prevent solvent spurting. Stoppers made of rubber must not be utilized). It usually takes at least 30 minutes for the ethereal layer to become clear and totally separate from the aqueous layer. To transfer the ethereal layer to a suitable flask, remove the cork and insert the siphon (or wash bottle) fitting with the length adjusted so that the inlet is 2 to 3 mm above the interface between the ethereal and aqueous layers. Use 5 ml of the combined solvent to wash the inside of the extraction tube and the siphon or wash-bottle fitting, which has been elevated just enough to allow for this but has not been removed. Lower the fittings and pour the solvent into the flask without stirring. With an additional 5 ml of the combined solvent, repeat this procedure. Clean the tip of the siphon fitting into the flask with a mixture of solvents. Removing the siphon fitting will allow you to extract the curd residue again using 15 ml of ether and 1 ml of light petroleum. The other steps will be repeated as before. Wash the inner limb with ether when removing the siphon (or wash bottle) fitting from the tube. Reverse the extraction after 15 ml of each ether and petroleum were used.To safely remove all traces of volatile solvent, carefully distill the solvents from the flask and dry the remaining fat in an oven at 98 to

100°C for one hour. The flask should then be cooled to room temperature in a desiccator that has been charged with an effective desiccant. Repeat this process every half-hour until subsequent weighing does not reveal a weight decrease of more than one milligram. The fat can be extracted by repeatedly washing the flask with light petroleum, allowing any sediment to settle before each decantation, drying the flask in the oven, cooling it down, and weighing it beforehand. The weight of fat contained in the weight of curd collected is the difference between the weights before and after the petroleum extractions, subject, if necessary, to a correction for the blank. Make a blank determination using the water in place of the curd, and the amounts of reagents stated throughout, and then subtract the value discovered, if any, from the apparent weight of fat. The same heating and cooling processes will be applied to one flask, similar to the one used to hold the fat, and it will also be utilized as a counterbalance.

Calculation:

$$\text{Percentage of fat} = \frac{wX100}{W}$$

Where, w = weight of the sample after drying in g

W = weight of prepared sample taken for test in g

11. TO CHECK THE QUANTITY OF CALCIUM, COPPER, AND MAGNESIUM (AAS)

Procedure: To make the ash, 1 ml of curd samples were placed in silica crucibles and then heated on a hot plate until completely charred. The charred samples were burned at 550 °C for 3 to 4 hours in a muffle furnace to produce white ash. Then add Hydrochloric acid (1:4 v/v). Heat it on a boiling water bath. By using filter paper, filter it properly. Collect filtrate in a 100 ml volumetric flask. Make volume up to 100 ml by using distilled water. Then Atomic Absorption Spectroscopy was performed for calcium, copper, and magnesium. To calibrate the curve we took known conc. Standard of 1, 2, 4 ppm for calcium, 1, 3, 5 ppm for copper, and 0.1, 0.2, 0.4 ppm for magnesium[36].

Calculation:

$$\%\ \text{of Ca, Cu, Mg ions present} = \frac{w}{1000} X \frac{100\ X\ 100}{1000\ X\ W}$$

w = concentration of the sample

W = weight of the sample taken

12. DETECTION OF THE AMOUNT OF SODIUM PRESENT (FLAME PHOTOMETER)

Procedure: 1 ml of curd samples were put in silica crucibles and roasted on a hot plate until charred to generate the ash. The charred samples were burned at 550 °C for 3 to 4 hours to create white ash in a muffle furnace. Then mix in 1:4 volume of hydrochloric acid. On a boiling water bath, warm it. Filter it properly using filter paper. Fill a 100 ml volumetric flask with the filtrate. In the volumetric flask, use distilled water to increase the volume to 100 ml. Then perform the flame photometer method to detect the amount of sodium present in the curd sample.

Calculation:

$$\% \text{ of Na ion present in sample} = \frac{w}{1000} X \frac{100 \text{ X } 100}{1000 \text{ X W}}$$

w = concentration of the sample

W = weight of the sample taken

TEST OF ADULTERANTS PRESENT IN CURD SAMPLE:

1. ROSALIC ACID TEST FOR CARBONATE PRESENCE

Procedure: In a test tube, combine 5 ml of curd, 5 ml of ethyl alcohol (95%), and a few drops of rosalic acid solution (1% w/v). Mix it properly.

Observation: While pure curd merely exhibits a brownish tint, a rose-red color is visible if the carbonate is present.

2. FORMALDEHYDE DETECTION AND DETERMINATION

A) FOR DETECTION OF FORMALDEHYDE (HEHNER TEST)

Procedure: Add about half of the conc.H_2SO_4 to the 10 ml curd in a test tube with a wide opening. Carefully pour the acid down the tube's side so that it forms a layer at the bottom and does not mix with the curd.

Observation: Formaldehyde is present when the two liquids meet and exhibit a violet or blue tint.

B) QUANTITATIVE DETERMINATION OF FORMALDEHYDE (KJELDAHL METHOD)

Sample preparation: Combine 100 ml of water and 100 ml of curd in an 800-ml Kjeldahl flask using phosphoric acid to acidify. Add one extra milliliter. Slowly distill 50 ml by connecting to the condenser through a splash head with a trap.

Procedure: 50 ml of the regular NaOH solution (1N) and 50 ml of the hydrogen peroxide solution (3% H_2O_2) should be added to a 500 ml conical flask. Place a funnel in the neck of the flask, add 25 ml of the prepared distillate, and heat over a steam bath for 5 minutes while shaking the flask occasionally. Remove the flask from the steam bath, rinse it with water, let it cool to room temperature, and then titrate the excess NaOH solution with the H_2SO_4 (0.1N) using either a litmus test or bromothymol blue as an indicator. (The flask must be cooled before the procedure to get a clear endpoint with litmus during titration.)

Calculation:

Formaldehyde g/100 ml = 0.06006 X

V = volume in milliliters of the neutralizing NaOH solution used.

3. TO DETECT THE PRESENCE OF ADDED SUGAR (SELIWANOFF'S TEST)

Procedure: A test tube containing 5 mL of curd. Add 0.1 g of the resorcinol solution and 1 mL of concentrated Hydrochloric acid. For five minutes, submerge the test tube in the water bath. Then observe the color.

Observation: The presence of additional sugar is indicated by the color red.

4. DETECTION OF STARCH (STARCH-IODINE TEST)

Procedure: Put 3 ml of curd sample in a test tube. Cool it to room temperature after completely boiling it. Put one drop of 1% iodine solution in.

Observation: The presence of starch is indicated by the emergence of blue color[37].

5. DETECTION OF BLOTTING PAPER

Procedure: Put a teaspoon's amount of curd in a beaker. Add 3 ml of HCl and 3 ml of water to the beaker. Using a glass rod, mix it thoroughly.

Observation: The blotting paper appears on the top of the sample present in the beaker[38].

6. TO TEST THE PRESENCE OF VANASPATI OIL

Procedure: Place about one teaspoon of curd into a test tube. To initiate the reaction, incorporate ten droplets of Hydrochloric Acid into the test tube and a pinch of sugar, subsequently agitating the contents. After a duration of 5 minutes, proceed to analyze the composition of the mixture.

Observation: The presence of Vanaspati in the curd is indicated by the red coloration.

CHAPTER - 5

RESULT AND DISCUSSION

This work aimed to identify adulteration in the curd samples by chemical or instrument analysis. Therefore, as a prerequisite, it was essential to check curd for its nutritional content. Although commercially available curd has its nutritional contents on its packing, no literature other than IS 9617-1980 DAHI describes the dietary contents of the curd. The following table **(Table-1)** compares the nutritional contents mentioned in Indian Standard (IS 9617-1980 DAHI) with the study carried out in this work and two commercially available curd packs.

Table-1: Nutritional contents analysis carried out in this study compared to mentioned in Indian Standard (IS 9617-1980 DAHI) and on the packs of two commercial samples.

Nutrient content	IS 9617 1980	Commercial Sample-1	Commercial Sample-1	This Study	Procedure Used
Total Solid		-	-	✓	Gravimetric
Total Nitrogen		-	-	✓	Kjeldahl
Casein (Protein)		✓	✓	✓	Titration
Lactose		✓	✓	✓	Munson &Walker Gravimetric
Sucrose		✓	✓	✓	Munson &Walker Gravimetric
Chloride		-	-	✓	Titration
Ash		-	-	✓	Gravimetric
Nitrates		-	-	✓	Colorimetric
Acidity (Lactic acid)	✓	-	-	✓	Alizarin – Alcohol
Fat		✓	✓	✓	Rose-Gottlieb
Calcium		✓	✓	✓	AAS
Copper		-	-	✓	AAS
Magnesium		-	-	✓	AAS
Sodium		✓	✓	✓	Flame photometer

Without a detailed standard for the curd, the ISI Handbook of Food Analysis Part XI: Dairy Products for Analysis of Milk standard served as a reference with modifications as per requirement. This is justified considering that milk is the precursor for the curd formed by its fermentation by Lactic Acid Bacteria (LAB). As discussed in the literature, the fermentation of milk to transform into curd results in its varied composition from the precursor, besides additional components like free amino acids (Glycine and Proline) and Vitamin C. **Table-2** compares the composition of milk and curd with reference to ISI Handbook for dairy products.

Table-2: Comparison of the values for nutritional content of milk and curd

S.No.	Nutrient content	Values checked in the milk sample[a]	Values checked in the curd sample[b]
1.	Total Solid	6.57%	**10%**
2.	Total Nitrogen	0.43%	**0.65%**
3.	Casein (Protein)	3%	**4.14%**
4.	Lactose	4.09%	4.92%
5.	Sucrose	0.00%	0.00%
6.	Chloride	0.73%	0.80%
7.	Ash	0.61%	0.83%
8.	Nitrates	0.00%	0.00%
9.	Acidity (Lactic acid)	0.17-0.20 %	**Over 0.20%**
10.	Fat	2.38%	**3.66%**
11.	Calcium	0.109%	0.12%
12.	Copper	0.014%	0.012%
13.	Magnesium	0.016%	0.014%
14	Sodium	0.023%	**0.06%**

a: The precursor of the curd sample was not the same milk sample as mentioned above. The values have been shown as a reference for the comparison.

Notably, the table displays the values of most parameters for the milk and curd in the same range. However, total solids demonstrated an increased value for the curd due to its precipitate nature compared to milk. The acidity in curd also showed an enhanced value of lactic acid due to the conversion of lactose into the corresponding acid. Mathematically, a curd sample should show decreased lactose content compared to its precursor milk due to its conversion into corresponding lactic acid. However, this is not the case, as the precursor for the analyzed curd sample was not the same milk shown in **Table-2**. The difference in fat

content and casein values of milk and curd can also be attributed to the same. Calcium, magnesium and copper were determined as Ca2+, Mg2+ and Cu2+ using an atomic absorption spectrophotometer. Thus, a more systematic study is required to compare curd's nutritional contents with its precursor milk. However, it is challenging considering dairy products' perishability and composition variation due to their collection system.

Determination of the Adulteration in Curd

After analyzing the curd's nutrient parameters, the next objective was to determine and quantify the presence of adulterants' in the different commercial and end-use consumer curd samples. The suppliers add many contaminants to dairy products to increase its shelf life, appearance and visible quality.

Curd samples from seven suppliers were collected within Patiala City and analyzed for carbonate, formalin, added sugar, starch, blotting paper, and vanaspati oil as adulterants. The adulterants were shortlisted based on their presence in milk and possible addition while fermenting it into curd. The distribution of the suppliers was made uniform by considering samples from two national brands, two local brands and three local dairy shops. This work aims to conduct a survey and present an academic submission rather than a legal one. Therefore, all collected samples were coded from 1 to 7 to maintain anonymity. The procedure followed was as per ISI Handbook of Food Analysis Part XI: Dairy Products for Analysis of Milk. **Table-3** below displays an abstract of the results obtained for different samples.

Table-3: Qualitatively analysis of curd for adulterants from two national brands, two local brands and three local dairy shops carried out as per ISI Handbook of Food Analysis Part XI: Dairy Products for Analysis of Milk[a].

	Samples						
Adulterants	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Blotting paper	✗	✗	✓	✗	✗	✗	✓
Carbonate	✗	✗	✗	✗	✗	✗	✗
Formalin	✗	✓	✓	✓	✓	✓	✓
Added Sugar	✗	✗	✗	✗	✗	✗	✗
Starch	✗	✗	✗	✗	✗	✗	✗
Vanaspati Oil	✗	✗	✗	✗	✗	✗	✗

a: This work aims to conduct a survey and present an academic submission rather than a legal one. Therefore, all collected samples were coded from 1 to 7 to maintain anonymity.

Formalin, generally added in the test samples by legal analytical labs to preserve for a longer duration, was present in all the consumer samples except one **(Sample-1)**. **Figure-5.1a** shows an absence of violet color with the addition of H_2SO_4 in **sample-1** due to the absence of formalin. The other sample **(Sample-2, Figure-5.1b)** displayed violet colour due to reaction of formalin with proteins (tryptophan) present in curd on addition of concentrated H_2SO_4.

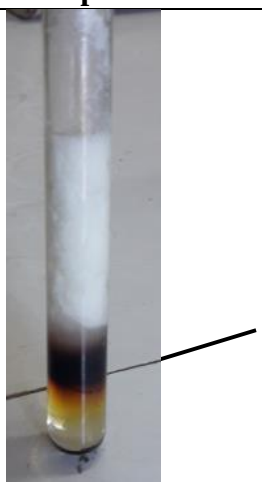
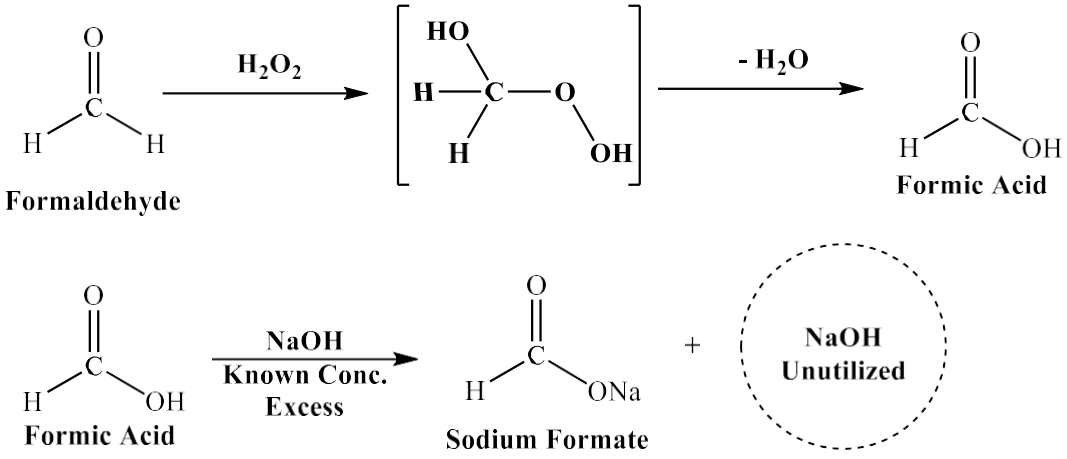
Sample-1	**Sample-2**
	
(a)	**(b)**

Figure-5.1: Formalin analyzed in curd samples by addition of sulphuric acid. **(a)** Negative Formalin test in **Sample-1** as violet colour is absent. **(b)** Sample-2 shows the presence of formalin as the violet colour appears with the addition of acid. A similar violet colour for samples 3-7 appeared on adding sulphuric acid in curd samples.



Scheme-6 : Hydrogen peroxide (H_2O_2) converts formalin to corresponding formic acid. Excess and known concentation of sodium hydroxide transforms it sodium formate. Titration of unutilized sodium hydroxide (circled) gives the amount of formalin present in the sample.

Scheme-6 describes the mechanism of formalin detection using a titration method. The formalin was also quantified by a known procedure described in the literature. Hydrogen peroxide (H_2O_2) was added to the sample that converted it to corresponding formic acid. The formic acid formed was neutralized by adding known concentration and volume of the excess NaOHthat transformed the former into sodium formate. Titration of unutilized NaOH and back-calculation gave the amount of formalin present in the sample.

Table-4: Results obtained after quantitative determination of formalin in the curd

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Formalin (mg/l)	Not determined	0.40 g/l	0.15g/l	0.49g/l	1.51g/l	0.76g/l	1.70g/l

Table-4 above describes the amount of formalin present in curd samples. Thus, except one, all commercial samples of contained formalin as an adulterant.

Blotting paper enhances the curd's visual appearance looking dense by absorbing excess water present. **Figure-5.2** shows the separation of blotting paper from the curd after adding concentrated hydrochloric acid, followed by stirring with equal water.

Sample-3	**Sample-7**
	
(a)	**(b)**

Figure-5.2: Adding concentrated hydrochloric acid in the curd sample followed by stirring with equal water separates blotting paper from the curd sample, as seen in **(a)** sample-3 and **(b)** sample-7.

The difference in the quantity of separated blotting paper was significant for **sample-7 (Figure 5.2a)** compared to **sample-3 (Figure 5.2b)**, suggesting no uniform parameter to add the adulterant.

Added carbonates, as adulterants, enhance the shelf life of curd by neutralizing its acidity and masking its taste during decay. None of the samples displayed alkalinity when evaluated using the Rosalic acid test **(Figure-5.3a)** due to the absence of the dianion **(Figure-5.3b)** that showed pink colour when tested using a positive control.

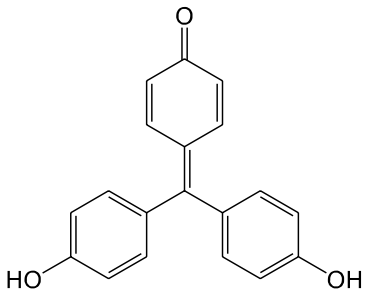
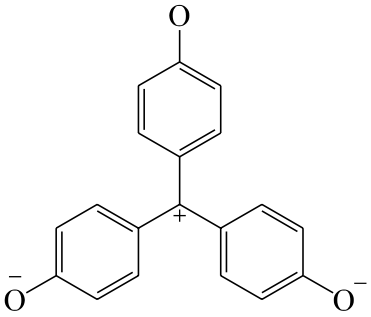
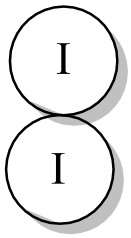
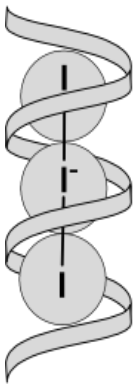
	Sample-6	**Positive Control**	
 **Rosalic Acid**			 **Rosalic Acid Dianion**
(a)	**(b)**	**(c)**	**(d)**

Figure-5.3: The presence of carbonates in the curd was analyzed by the Rosalic acid test. **(a)** Rosalic acid, in its neutral form **(b)** when added to **sample-6,** displayed yellow colour. **(c)** Red coloured in sample added with carbonate due to the formation of **(d)** Rosalic acid dianion due to reaction. None of the samples displayed a positive Rosalic acid test.

Starch is one of the harmful substances added to milk to raise the quantity of solid non-fat content**.** The starch detection is conventionally done using Starch-Iodine, where the addition of iodine gives blue color due to complexation. The test performed on all the collected commercial samples were found free from starch showing yellow color **(Figure-5.4a** and **b)** due to iodine molecule compared to the positive control that displayed a blue color4 **(Figure-5.4c)** due to the formation of triiodide ion after interacting with the starch polymer as shown in **Figure-5.4d**.

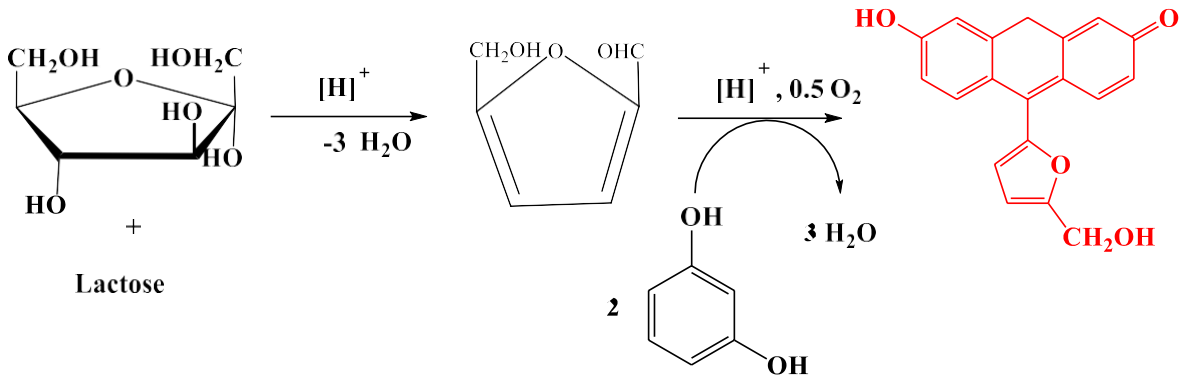
The addition of water to milk increases its quantity and thus profits. Sugars and vanaspati oils are added to milk to compensate for milk's diluted lactose and fat content. It harms persons with diabetic and cardiovascular medical conditions (12).

The curd fermented from such milk has passed on its sugar and fat content. None of the samples tested by adding hydrochloric acid displayed red or crimson blue, indicating the absence of sugar or fat in all the samples.

	Sample-4	Positive Control	
			
(a)	**(b)**	**(c)**	**(d)**

Scheme-5.4: The Starch-Iodine test is blue due to the complexation between starch and iodine. **(a)** The iodine molecule at low concentrations has a pale yellow color. **(b)** The test performed on **sample-4** showed yellow due to the iodine molecule compared to the **(c)** positive control, which displayed a blue color due to the **(d)** formation of triiodide ion after interacting with the starch polymer.

Seliwanoff's test can differentiate between lactose, naturally present in milk and curd, and added sucrose (sugar) with the help of resorcinol and hydrochloric acid. **Scheme-7** displays the mechanism for forming polyphenol **compound-a** that gives red color due to its reaction with sugar in the presence of lactose.



Scheme-7: Seliwanoff's test can differentiate between lactose, naturally present in milk and curd, and added sucrose (sugar) with the help of resorcinol and hydrochloric acid that forms red colour polyphenol.

In conclusion, seven commercial curd samples were analyzed for their nutrient content and adulteration. It was noticed that there is a vital requirement to update IS 9617-1980 DAHI and add additional chemical parameters to check adulteration, especially in light of the evolving commercial and processed food industry.

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