COMPREHENSIVE ASSESSMENT OF MILK AND MILK PRODUCTS: QUALITY PARAMETERS AND PESTICIDE RESIDUE ANALYSIS VIA GAS CHROMATOGRAPHY

Dissertation submitted to

School of Food Science and Technology

Mahatma Gandhi University, Kottayam

In partial fulfilment for the award of the degree of

MASTER OF SCIENCE
In
M.Sc. Food Science and Technology
By

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Reg. No: 242208

Under the guidance of

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SCHOOL OF FOOD SCIENCE AND TECHNOLOGY

MAHATMA GANDHI UNIVERSITY

KOTTAYAM, KERALA, INDIA

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CERTIFICATE

This is to certify that the project work entitled **"COMPREHENSIVE ASSESSMENT OF MILK AND MILK PRODUCTS: QUALITY PARAMETERS AND PESTICIDE RESIDUE ANALYSIS VIA GAS CHROMATOGRAPHY"** is a result of experiments carried out by Ms. HARITHA ANAND A, a bonafide student of Mahatma Gandhi University, Kottayam, Kerala in partial fulfilment of master of science in food science and technology, Mahatma Gandhi University, Kottayam, Kerala, during the period from February 2024 to May 2024 in the Department of Food Safety and Analytical Quality Control Laboratory, CSIR-Central Food Technological Research Institute, Mysuru, under my guidance.

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ANNEXURE I

UNDERTAKING BY THE SELECTED STUDENT FOR PROJECT WORK AT CSIR-CFTRI

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The college certificate in the project report prescribed by college / University shall categorically reflect that the place of project work is CSIR-CFTRI and acknowledge the name of the staff member who has guided. (Sl. No. 18 of Annexure I)

The successful execution of the project work by the students is solely under the guidance of scientific/technical guide of CFTRI. **No interference from the College/University is entertained.**

We have read the prescribed rules and regulations (Sl. Nos. 1 to 18 of Annexure I) and we agree to abide by them during our project work at CFTRI, Mysore and to the Conduct and Discipline mandate of the Institute.

HARITHA AANAND A
(Name of student in capital letters)

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DECLARATION

I, Haritha Aanand A hereby declare that the dissertation entitled – **Comprehensive assessment of milk and milk products: Quality parameters and pesticide residue analysis via gas chromatography "**submitted in the partial fulfilment for the award of degree master of science in Food Science and Technology, Mahatma Gandhi university Kottayam, Kerala; Human Resource Development, CSIR-Central Food Technological Research Institute, Mysuru; is the record of work carried out by me under the guidance of Dr. Sindhu R Nambiar, Senior Scientist, Department of Food Safety and Analytical Quality Control Laboratory, CSIR-Central Food Technological Research Institute, Mysuru.

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COMPREHENSIVE ASSESMENT OF MILK AND MILK PRODUCTS: QUALITY PARAMETERS AND PESTICIDE RESIDUE ANALYSIS VIA GAS CHROMATOGRAPHY

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ABSTRACT

This project endeavours to comprehensively evaluate the quality of milk and milk products through a combined quantitative and qualitative analysis approach. Milk and its derivatives are essential components of the human diet, providing vital nutrients and serving as versatile ingredients in various food products. However, ensuring their quality and safety is paramount to safeguarding public health. The study will employ both quantitative and qualitative analytical methods to assess different aspects of milk and milk products. Quantitative analysis will involve the measurement of key parameters such as fat content, protein content, total solids, titratable acidity, moisture content, total ash and presence of contaminants like pesticides. These analyses will be conducted using chemical methods and techniques such as chromatography.

Milk and milk products are fundamental components of the human diet, providing essential nutrients. Ensuring their quality and safety is paramount for consumer health and industry standards. This work involves assessment of quality of milk and milk products through various analytical methods. The study encompasses physiochemical analysis as well as advanced instrumental techniques. Physicochemical analyses include parameters such as total solid, fat content, protein and titratable acidity. Advanced instrumental techniques such as chromatography, UV-visible spectroscopy, and Gas chromatography-ECD are highlighted for their precision in detecting adulterants, pesticides, and compositional variations.

This study investigates the quality and composition of 70 milk and milk product samples using a comprehensive analytical approach. The specific methods employed will depend on the type of milk product being analysed. The results be analysed to identify trends and potential variation from prescribed quality standard within the sample. The comprehensive approach outlined in this paper underscores the importance of utilizing a multi-faceted methodology to achieve robust quality control in the dairy industry. By implementing these diverse analytical techniques, producers can ensure the safety, nutritional value, and overall quality of milk and milk products, thereby maintaining consumer trust and meeting regulatory standards.

Data interpretation will be done to identify correlations between different parameters, and assess the overall quality of milk and milk products. The findings of this study will provide valuable insights into the factors influencing the quality of dairy products and facilitate the implementation of quality control measures to ensure consumer safety and satisfaction. Furthermore, the results can inform regulatory agencies and stakeholders in the dairy industry

to implement measures aimed at improving milk quality and ensuring consumer safety. Overall, this project seeks to enhance the quality control processes in the dairy industry and promote the production of safe and nutritious milk products. Ultimately, this project aims to contribute to the enhancement of quality assurance practices in the dairy industry, promote the production of high-quality milk and milk products, and support consumer confidence in these essential food items

Chapter 1

INTRODUCTION

Milk and milk products hold significant importance in our diets and culinary traditions worldwide. As a rich source of essential nutrients like calcium, protein, vitamins, and minerals, milk plays a crucial role in supporting overall health, particularly bone strength and growth, muscle development, and immune function. Its versatility makes it a staple ingredient in countless dishes, from breakfast cereals to creamy sauces and desserts. Beyond milk itself, products like cheese, yogurt, butter, and ice cream offer diverse flavors, textures, and nutritional profiles, enriching our meals and culinary experiences. Moreover, milk products contribute to economic livelihoods, as dairy farming sustains many rural communities and provides income for farmers. Culturally, milk and its derivatives hold symbolic significance in various societies, often associated with nurturing, hospitality, and celebration. Overall, milk and milk products are not only nutritious but also integral to our diets, cultures, and economies. Milk and milk products, also known as dairy products, have been a staple food for human for long time. Often called “nature’s perfect food” because they are a rich source of |essential nutrients include calcium, protein, vitamin D, vitamin B12, Potassium and other vitamins and minerals. Considering healthy image of milk products in the society and rising prosperity consumption of milk and milk products is growing steadily in India. Today’s dairy industry, ensuring milk quality is paramount to meet consumer expectations and regulatory standards. These is the biggest challenge facing nowadays numerous factors, including animal health, feeding procedures, cleanliness during milking, transportation, processing, and storage conditions, all affect the product quality. Customers anticipate their milk to be pure, wholesome, and safe. In order to maintain these requirements, dairy farmers and processors must put strict quality control systems in place at every stage of the manufacturing process. Measures that determine whether raw milk is fit for human consumption, how the milk is processed into dairy products, and the health of the individual milk-producing animals can all be used to determine the quality of milk. Several measures including regulatory control, increasing awareness and extensive testing/analysis of milk by dairy industry personnel are in place to control adulteration. However, still it is going on and hence qualitative and quantitative tests for analysis of milk, which are precise and effective are most desired. Milk and milk

products quality can be evaluated by assessing some parameters like moisture, fat, total solids and qualitative tests. The parameters can be varying according to the milk products.

Figure 1: Milk and milk products

1.1 Milk Composition

Milk is a remarkably complex liquid, boasting a diverse composition that renders it a cornerstone of human nutrition. Comprising primarily water, it also contains an array of essential nutrients crucial for human health. Proteins, such as casein and whey, form a substantial portion, offering a complete source of amino acids vital for growth and repair. Lactose, the primary carbohydrate, not only provides energy but also fosters a healthy gut microbiome. Meanwhile, milk fats, including various fatty acids and fat-soluble vitamins, contribute to its rich flavour and provide essential nutrients like vitamin A and D. Furthermore, milk is a notable source of vitamins such as riboflavin, vitamin B12, and minerals like calcium and phosphorus, essential for bone health and metabolic functions. Beyond its macronutrient and micronutrient profile, milk contains bioactive compounds like enzymes and immune factors, enhancing its nutritional value and potential health benefits. In sum, milk's intricate composition underscores its significance as a fundamental dietary staple, offering a comprehensive array of nutrients crucial for human growth, development, and overall well-being.

MAJOR CONSTITUENTS	MINOR CONSTITUENTS
• Water	• Vitamins
• Fat	• Enzymes
• Protein	• Pigment
• Mineral matters	• Non protein nitrogenous

Table 1: Constituents in milk

1.1.1 Major Constituents

a) Water: Milk is primarily composed of water, making up about 87% to 89% of its total weight. This water content is essential for maintaining the fluidity and overall consistency of milk. Water serves as a carrier for various dissolved nutrients, including proteins carbohydrates, vitamins and minerals this make them easily accessible for absorption by the body. Any variation in the constituents can reflected upon the water percentage.

b) Fat: Milk fat, or butterfat, contributes to the creaminess and flavour of milk. The fat content varies depending on the type of milk, with whole milk containing around 3.5% fat, while skim milk has minimal fat content. Milk fat is a source of fat-soluble vitamins like A, D, E, and K. Milk contains various types of fats, including saturated fats, unsaturated fats, and cholesterol. The proportion of these fats varies depending on factors like the animal's diet and breed. Saturated fats are the primary type found in milk, but there are also significant amounts of unsaturated fats, including monounsaturated and polyunsaturated fats, which are considered healthier options. Milk fat exists in milk in the form of minute globules in a true emulsion of the oil-in-water type, the fat globules being in the dispersed phase. The fat globules are invisible to the naked eye, but are seen readily under the low power of a microscope. Size is about 3 microns. If cool raw milk kept for some time without mixing there is a tendency for the fat globules to cluster and rise at surface forming cream layer. Milk fat is not a single chemical compound but a variable mixture of several different glycerides. Each glyceride is the result of the union of glycerol and one or more organic acid. Milk lipids are present in three phases in milk namely the fat globule, the surrounding membrane called fat globule membrane and milk serum.

c) Proteins: Milk contains several types of proteins, with casein and whey proteins being the most abundant. Casein forms about 80% of the total protein content, while whey proteins make up the remaining 20%. These proteins are rich in essential amino acids, making milk a complete protein source. They contain carbon, hydrogen, oxygen, nitrogen, Sulphur, and sometimes phosphorus. Milk is a rich source of protein. There are two main types of protein found in milk: casein and whey protein. Casein is the slow-digesting protein. It forms micelles, which are tiny spheres that trap calcium and other nutrients. Casein is a good source of all nine essential amino acids, which are the building blocks of protein that your body cannot produce on its own. Whey is the fast-digesting protein that makes up about 20% of the protein in milk. Whey protein is absorbed quickly by the body and is a good source of branched-chain amino acids (BCAAs), which are important for muscle growth and repair. Both casein and whey protein have been

shown to have a number of health benefits, including muscle building and repair, weight loss, improved blood sugar control, lower blood pressure, reduced risk of heart disease.

d) Lactose: Lactose is the primary carbohydrate found in milk, comprising about 4.5% to 5% of its composition. It provides a source of energy and serves as a prebiotic, promoting the growth of beneficial bacteria in the gut. Milk sugar "lactose" is found only in milk. It is reducing disaccharide which upon hydrolysis yield 1 molecule of galactose and 1 molecule of glucose. It making up about 2-8% of its weight. Lactose is broken down in the small intestine by an enzyme called lactase. Lactase is produced by cells in the lining of the small intestine. Some people are lactose intolerant, which means they do not produce enough lactase to digest lactose. This can cause digestive symptoms such as bloating, gas, diarrhoea, and abdominal cramps. Lactose intolerance is more common in adults than in children. It is also, more common in some ethnic groups than in others. There are a number of lactose-free milk products available for people who are lactose intolerant. These products have had the lactose broken down into glucose and galactose by lactase.

e) Minerals: Milk is rich in minerals, particularly calcium, phosphorus, potassium, and magnesium. These minerals are essential for bone health, muscle function, and overall metabolism.

1.1.2 Minor Constituents

a) Vitamins: Milk is a good source of several vitamins, particularly vitamin D, which is essential for calcium absorption and bone health. It also contains significant amounts of vitamin A, B vitamins (such as riboflavin, vitamin B12, and pantothenic acid), and vitamin K. However, the amount of some vitamins, particularly vitamin D, can vary depending on the type of milk. Whole milk contains the most natural vitamins because the fat is t removed during processing. Reduced-fat milk (2% fat) and low-fat milk (1% fat) lose some vitamins along with the fat. These milks are usually fortified with vitamin A to make up for the loss. Skim milk usually has vitamin A and D added back in because nearly all the fat-soluble vitamins are removed during processing. Fortification is the process of adding additional nutrients to food. In the case of milk, vitamin A and D are commonly added to make sure people who drink milk get enough of these important vitamins.

b) Enzyme in Milk: Milk contains a variety of enzymes, each with its own specific function. Lipase enzymes break down fats into smaller molecules. This can be undesirable during processing, as it can lead to off-flavours in milk. Pasteurization, the process of heating milk to kill harmful bacteria, also inactivates lipase enzymes. Protease enzymes break down proteins.

Some proteases are naturally present in milk, while others are introduced by bacteria during storage. Protease activity can be undesirable, as it can lead to bitter flavours or a slimy texture in milk. Lactoperoxidase this enzyme is one of the most heat-stable enzymes found in milk. It has antibacterial properties when combined with hydrogen peroxide and thiocyanate. However, since fresh milk does not contain hydrogen peroxide or thiocyanate, lactoperoxidase alone does not have a significant antibacterial effect in raw milk. Lysozyme this enzyme has some antibacterial activity, but the amount present in milk is very low.

c) Pigment in Milk: Milk white colour is actually a bit of a trick of the light, not due to a pigment. Casein Micelles and Fat Globules: These are the major players. Casein micelles are tiny protein particles suspended in milk, and fat globules are droplets of milk fat. When light hits these particles, they scatter it in all directions, making the milk appear white. Carotenoids not the main reason for colour, milk does contain pigments called carotenoids, specifically beta-carotene and lutein. These fat-soluble pigments originate from the cow's diet and contribute a slight yellow hue to milk. This is more noticeable in whole milk than skimmed milk, where much of the fat (and carotenoids) are removed.

d) Non-protein Nitrogenous substances: The non-protein nitrogenous substance in milk like urea, nitrogen, amino nitrogen, creatinine, uric acid, adenine and guanine. These substances are measured in parts per million, ranging from 1.5 to 10 in milk. The level of non-protein nitrogen in milk can be influenced by various factors such as the cow's diet, health, and stage of lactation. Milk urea nitrogen commonly measured indicator of a cow's protein nutrition and overall health. commonly measured indicator of a cow's protein nutrition and overall health.

1.2 Milk and Milk Products

1.2.1 Cow milk:

Cow milk, produced by the mammary glands of cows, is a staple in many diets globally, esteemed for its nutritional richness and versatility. Renowned for its creamy texture and distinct flavour, cow milk stands as a primary source of essential nutrients. Rich in high-quality proteins, including casein and whey, it provides amino acids vital for growth and repair. Moreover, cow milk is a significant reservoir of calcium, vitamin D, vitamin B12, riboflavin, and phosphorus, all pivotal for bone health, metabolism, and overall well-being. Available in various forms, from whole milk to skimmed varieties, it caters to diverse dietary preferences and needs. Processed through pasteurization to ensure safety and homogenization for uniformity, cow milk retains its nutritional integrity while being widely utilized in cooking, baking, and as a standalone beverage. However, for those with lactose intolerance, alternative

options exist. Despite this, cow milk endures as a cherished cornerstone of nutrition and culinary traditions worldwide.

Nutrients	Cow milk
Water %	88.0
Energy kcal	61.0
Protein %	3.2
Fat %	3.4
Lactose %	4.7
Mineral %	0.72

Table 2: Nutritional Value for Cow Milk

Prescribed Standards for Cow Milk

Quality Characteristics	Requirement
Milk fat %	Not less than 4.5
Milk solid not fat %	Not less than 8.5
Test for starch	Shall be negative
Test for cane sugar	Shall be negative
Test for urea	Not more than 700 ppm
Test for Neutralizer	Shall be negative
Test for Detergent	Shall be negative

Table3: FSSAI Standards for Cow Milk

1.2.2 Buffalo Milk

Buffalo milk, sourced from the mammary glands of buffaloes, represents a rich and nourishing alternative to cow milk, prized in many cultures for its distinct properties. With a higher fat and protein content compared to cow milk, buffalo milk offers a creamy texture and a slightly sweeter taste, making it a favourite for many dairy enthusiasts. This milk is notably rich in nutrients, including calcium, phosphorus, vitamin A, and vitamin D, essential for bone health, immune function, and overall well-being. Its versatility in

culinary applications are extensive, commonly used to produce cheese, yogurt, butter, and traditional dairy delicacies in various parts of the world. Buffalo milk's composition, along with

its unique flavour profile, renders it a valued ingredient in both traditional and modern cuisine. Moreover, for individuals who may experience lactose intolerance, buffalo milk presents a potential alternative due to its lower lactose content compared to cow milk. In essence, buffalo milk stands as a testament to the diversity and richness of dairy products, offering a delightful and nutritious option for consumers worldwide. Buffalo milk contains more calcium a better calcium to phosphorous ratio and less sodium and potassium which makes it a better nutritional supplement for infants. Buffalo milk contains less cholesterol compared to cow milk

NUTRIENTS	BUFFALO MILK
Water %	84.0
Energy %	97.0
Protein %	3.7
Fat %	6.9
Lactose %	5.2
Mineral %	0.79

Table 4: Nutritional Value for Buffalo Milk

Quality Characteristics	Requirement
Milk fat %	Not less than 5
Milk solid not fat %	Not less than 9
Test for starch	Shall be negative
Test for cane sugar	Shall be negative
Test for urea	Not more than 700 ppm
Test for Neutralizer	Shall be negative
Test for Detergent	Shall be negative

Table 5: FSSAI Standards for Buffalo Milk

1.2.3 Mixed Milk

Mixed milk refers to a blend of milk from different animal sources, typically cow, goat, and sheep. This combination offers a unique flavour profile and nutritional composition, showcasing the best attributes of each type of milk. Blending milk from multiple species creates a harmonious balance, resulting in a rich and complex taste that appeals to a wide range of palates. Moreover, mixed milk often boasts a diverse array of nutrients, including proteins, vitamins, and minerals, contributing to its reputation as a nutritious dietary option. Commonly

used in the production of artisanal cheeses, mixed milk provides cheesemakers with an opportunity to experiment with flavour profiles and textures, yielding products that are both distinctive and delicious. Additionally, mixed milk cheeses often exhibit enhanced complexity and depth, making them prized delicacies in the culinary world. Beyond cheese production, mixed milk may also be used in other dairy products, such as yogurt or ice cream, further highlighting its versatility and appeal. Overall, mixed milk exemplifies the artistry and innovation within the dairy industry, offering consumers a delightful and flavourful alternative to single-source milks.

Quality characteristics	**Requirement**
Milk fat %	Not less than 4.5
Milk solid not fat %	Not less than 8.5
Test for starch	Shall be negative
Test for cane sugar	Shall be negative
Test for urea	Not more than 700 ppm
Test for Neutralizer	Shall be negative
Test for Detergent	Shall be negative

Table 6: FSSAI Standards for Mixed Milk

1.2.4 Toned milk

Toned milk is a type of milk that has been standardized to reduce the fat content while retaining the essential nutrients found in whole milk. It is commonly produced by combining whole milk with skim milk or milk powder, adjusting the fat content to a predetermined level, typically around 3% to 3.5%. The resulting product is often referred to as "toned" or "reduced-fat" milk.

Quality characteristics	**Requirement**
Milk fat %	Not less than 3
Milk solid not fat %	Not less than 8.5
Test for starch	Shall be negative
Test for cane sugar	Shall be negative
Test for urea	Not more than 700 ppm
Test for Neutralizer	Shall be negative
Test for Detergent	Shall be negative

Table 7: FSSAI standards for toned

1.2.5 Paneer

Paneer, a fresh cheese widely consumed in South Asian cuisine, holds a cherished place in culinary traditions for its versatility, taste, and nutritional benefits. Made by curdling milk with an acidic agent like lemon juice or vinegar and then straining the whey, paneer boasts a firm yet crumbly texture that is ideal for various cooking methods. Rich in high-quality proteins and calcium, paneer serves as a valuable source of nutrition, particularly for vegetarians and those seeking alternatives to meat. Its mild flavour allows it to absorb the aromas and spices of accompanying dishes, making it a popular addition to curries, stir-fries, and grilled dishes. Paneer is also celebrated in sweets and desserts, where its creamy texture and subtle taste complement ingredients like sugar, nuts, and spices. Beyond its culinary appeal, paneer's simplicity of preparation and long shelf life contribute to its widespread popularity and ubiquity in South Asian cuisine. Whether enjoyed in savoury or sweet dishes, paneer embodies the richness and diversity of flavours that characterize the region's culinary heritage. It is obtained by heat and acid coagulation of milk, entrapping almost all the fat casein complexed with denatured whey protein and a portion of salts and lactose. Paneer have marble white in appearance, firm texture and spongy body, sweetish-acidic nutty flavour. Paneer doesn't melt when heated, making it suitable for various dishes.

Quality characteristics	Requirement
Moisture % by wt.	Not less than 60
Milk fat (in dry wt. basis)	Not less than 50.0
Test for starch	Shall be negative
Test for cane sugar	Shall be negative
Test for urea	Shall be negative
Test for Neutralizer	Shall be negative
Test for Detergent	Shall be negative

Table 8: FSSAI standards for paneer

1.2.6 Mawa or Khoya

Mawa or khoya is a traditional dairy product that holds a special place in Indian cuisine, renowned for its rich and creamy texture and its ability to enhance the flavours of various sweet and savoury dishes. Made by evaporating milk over low heat until most of the moisture evaporates, khoya is essentially concentrated milk solids. This process results in a dense,

granular, and slightly caramelized product that is prized for its unique taste and versatility in cooking. Khoya is an essential ingredient in numerous Indian desserts, including gulab jamun, peda, and barfi, where it lends a creamy richness and depth of flavour. It is also used in savoury dishes like curries and gravies to thicken sauces and add a luxurious creaminess. Beyond its culinary uses, khoya holds cultural significance, often being prepared at home during festive occasions and religious celebrations. While commercially produced khoya is readily available, many prefer homemade versions for their superior taste and authenticity. In essence, mawa or khoya embodies the essence of Indian culinary craftsmanship, adding a touch of indulgence and nostalgia to a wide array of dishes.

Quality characteristics	Requirement
Milk fat (in dry wt. basis)	Not less than 30
Total solids	Not less than 55.0
Total ash	Not more than 6.0
Titratable acidity (as % lactic acid)	Not more than 0.9
Test for starch	Shall be negative
Test for cane sugar	Shall be negative
RM value	Not less than 24
B.R.R of extracted fat at 40°C	40-44

Table 9: FSSAI standards for khoya

1.2.7 Butter

Butter, a beloved dairy product derived from cream, holds a prominent place in culinary traditions worldwide, valued for its rich flavour, creamy texture, and versatility in cooking and baking. Produced by churning cream until the fat globules coalesce and separate from the buttermilk, butter is cherished for its distinct taste and aroma, often varying depending on factors such as the animal source, diet, and processing methods. It serves as a fundamental ingredient in a myriad of dishes, from savoury sauces and spreads to decadent pastries and desserts. Butter's unique ability to enhance the flavours of other ingredients while imparting its characteristic richness makes it a staple in both home kitchens and professional culinary settings. Beyond its culinary applications, butter holds cultural significance in many societies, symbolizing indulgence, hospitality, and comfort. While modern dietary trends have prompted the development of various butter alternatives, traditional butter remains a timeless favourite, cherished for its unrivalled taste and irreplaceable role in culinary traditions around the globe.

Quality characteristics	Requirement
Moisture % by wt.	Not more than 16
Fat % by wt.	Not less than 80
Milk solids not fat % by wt.	Not more than 2.0
Salt content % by wt.	Not more than 3.0
RM value	Not less than 24
B.R.R of extracted fat at 40°C	40-44

Table 10: FSSAI standards for Butter

1.2.8 Curd

Curd, also known as yogurt, is a versatile dairy product enjoyed worldwide for its creamy texture, tangy flavour, and numerous health benefits. Made by fermenting milk with beneficial bacteria cultures, curd is rich in probiotics, which promote digestive health and strengthen the immune system. Its smooth consistency and refreshing taste make it a popular breakfast staple, often enjoyed on its own or paired with fruits, granola, or honey. Curd is also a key ingredient in various savoury dishes, such as raita, tzatziki, and lassi, where it adds a cooling contrast and creamy texture. Additionally, curd serves as a natural tenderizer in marinades for meats and poultry, lending succulence and flavour to grilled or roasted dishes. Beyond its culinary uses, curd has cultural significance in many societies, often associated with purity, fertility, and auspiciousness. Whether enjoyed as a simple snack or incorporated into elaborate recipes, curd's delightful taste and health-promoting properties make it a beloved addition to diets worldwide.

Quality characteristics	Requirement
Milk fat % by wt.	Not less than 4.5
Milk protein % (Nx 6.38)	Not less than 2.9
Milk solids not fat % by wt.	Not less than 8.5
Titratable acidity % as lactic acid	Not less than 0.45

Table 11: FSSAI standards for curd

1.2.9 Skimmed Milk powder

Skimmed milk powder, also known as non-fat dry milk powder, is a dairy product derived from evaporating milk to remove the water content, resulting in a fine powder with minimal fat content. Skimmed milk powder is produced by extracting water from skim milk, typically through a process called spray drying, where the milk is atomized into a hot air stream to quickly evaporate the moisture. This process preserves the nutritional components of milk

while extending its shelf life. Skimmed milk powder is valued for its versatility and convenience. It serves as a convenient alternative to fresh milk, particularly in situations where refrigeration is limited or when a longer shelf life is required. Skimmed milk powder is widely used as an ingredient in various food products, including baked goods, confectionery, beverages, soups, and sauces. It can also be reconstituted with water to produce skim milk for drinking or cooking purposes. Nutritionally, skimmed milk powder retains most of the protein, vitamins, and minerals found in fresh milk, but with significantly reduced fat content. It is low in calories and cholesterol, making it a popular choice for individuals seeking to reduce their fat intake while still obtaining the nutritional benefits of dairy products.

Quality characteristics	**Requirement**
Moisture % by wt.	Not more than 5.0
Milk fat % by wt.	Not more than 1.5
Milk protein in MSNF % by wt.	Not less than 34
Titratable Acidity	Not more than 34
Total Ash	Not more than 9.3
Insolubility Index	Not more than 2.0

Table 12: FSSAI standards for Skimmed milk powder

1.2.10 Ice Cream

Ice cream is a frozen dessert made from cream, sugar, and often flavouring\s or additives. It's typically churned to incorporate air, creating a smooth and creamy texture. Ice cream is a dairy based product which typically contains 6-12% fat, 7.5-11.5% non-fat milk solids and 13-18% sugars. Stabilizers, emulsifiers colours and flavours are also added. Contains two to three times as much fat and slightly more protein than does milk. In addition-other food products such as fruits, nuts, eggs, and sugar which enhance its food value. While vanilla and chocolate are classic flavours, there's a vast array of options available, including fruity flavours, nut-infused varieties, and even savoury options in some regions. Ice cream can be served in cones, cups, or used as a topping or ingredient in various desserts. It's a beloved treat enjoyed by people of all ages around the world.

Quality characteristics	Requirement
Milk fat % by wt.	Not less than 10.0
Total solid % by wt.	Not less than 36.0
Milk protein %	Not less than 3.5

Weight g/t	Not less than 525
Added synthetic colour mg/kg	Max 100

Table 13: FSSAI standards for ice cream

1.2.11 Cheese

Cheese is a delicious and versatile food made by curdling milk. It comes from the milk of cows, sheep, goats, or even buffalo! The curdling process separates the milk solids (protein and fat) from the liquid whey. Cheesemakers can control the flavour, texture, and appearance of cheese in many ways. They use different cultures of bacteria and sometimes moulds, and they vary the length of aging. This results in a vast array of cheeses, from mild and creamy to sharp and crumbly. Cheese is a good source of protein, calcium, and fat. It's also a great way to preserve milk, lasting much longer than fresh milk.

Quality characteristics	**Requirement**
Moisture % by wt.	Not more than 54
Milk fat % on dry wt. basis	Not less than 35
Test for cane sugar	Shall be negative

Table 14: FSSAI standards for cheese

1.3 Adulteration in milk and milk products

Adulteration in milk, unfortunately, is a widespread concern in many parts of the world. It involves the addition of various substances to milk to increase its volume or alter its composition, often at the expense of its quality and nutritional value. Common adulterants include water, starch, urea, detergent, and even harmful chemicals like formalin. These additives can pose serious health risks to consumers, ranging from digestive issues to long-term health complications. Detection of milk adulteration requires vigilant testing and analysis. Various methods, including chemical tests, spectroscopy, and chromatography, are employed to identify adulterants and assess milk quality accurately. Additionally, regulatory bodies often set standards and guidelines to monitor milk production, processing, and distribution, aiming to curb adulteration practices and ensure consumer safety. Efforts to combat milk adulteration involve a combination of regulatory measures, public awareness campaigns, and technological advancements. Strict enforcement of regulations, regular monitoring of dairy farms and processing facilities, and fostering transparency in the supply chain are essential steps toward mitigating this problem. Consumers can also play a crucial role by being vigilant, purchasing milk from trusted sources, and reporting any suspicious activities to relevant authorities. In

essence, addressing milk adulteration requires a concerted effort from various stakeholders, including government agencies, dairy industry players, and consumers, to safeguard public health and maintain the integrity of this essential food staple.

1.3.1 Types of Adulterants in milk and milk products

Adulterants added to milk can vary widely, ranging from harmless substances used to dilute milk to more dangerous chemicals that pose serious health risks. Here are some common types of adulterants added to milk:

a) Water: One of the most common adulterants, water is added to increase the volume of milk, thereby diluting its nutrient content and quality.

b) Starch: Starch, often derived from sources like flour or tapioca, is added to thicken milk, giving it a semblance of creaminess and richness.

c) Urea: This nitrogen-rich compound is sometimes added to milk to artificially increase its protein content during testing, thereby misleading quality assessments.

d) Detergents: Detergents are added to milk to enhance its frothiness and viscosity, giving the illusion of freshness. However, they can be harmful when ingested, causing digestive issues and other health problems.

e) Formalin: A highly toxic substance, formalin is occasionally added to milk as a preservative to prolong its shelf life. However, consumption of formalin can lead to severe health complications, including organ damage and cancer.

f) Vegetable Oil: Cheaper vegetable oils are sometimes added to milk to mimic the creamy texture of natural milk fat. However, this adulteration reduces the nutritional value of milk and may lead to digestive discomfort.

g) Melamine: In some cases, melamine, a chemical compound used in plastics and fertilizers, has been added to milk to artificially inflate its protein content. However, melamine ingestion can cause kidney damage and other serious health issues, particularly in infants and young children.

h) Colouring Agents: Artificial colouring agents may be added to mask variations in milk colour or to give it a more appealing appearance. However, these additives offer no nutritional value and may have adverse health effects.

i) Detection and prevention of milk adulteration require stringent regulatory measures, including regular testing and monitoring of milk samples, as well as public awareness campaigns to educate consumers about the risks associated with adulterated milk.

Figure 2: Adulterants in milk

1.4 Importance of Qualitative analysis of Milk and Milk Products

Qualitative analysis of milk quality and safety holds immense significance in ensuring consumer health and confidence in dairy products. By focusing on the characteristics and properties of milk without necessarily quantifying each component, qualitative analysis provides valuable insights into its overall purity, freshness, and nutritional value. Qualitative analysis helps in early detection of contaminants such as pesticides, antibiotics, heavy metals, and microbial pathogens. By identifying abnormalities in taste, smell, texture, or appearance, analysts can flag potential issues before they escalate, preventing foodborne illnesses and protecting public health. Qualitative analysis allows for the assessment of milk's freshness by evaluating its sensory attributes such as odour, taste, and appearance. Any deviations from the expected qualities may indicate spoilage or improper handling, prompting further investigation to maintain product quality and safety. Adulterants added to milk, such as water, starch, or detergents, can compromise its quality and nutritional integrity. Qualitative analysis enables the detection of such adulterants through visual inspection, sensory evaluation, and simple chemical tests, helping to preserve the authenticity and purity of milk products. Qualitative analysis contributes to consumer satisfaction by ensuring that milk meets sensory expectations in terms of taste, aroma, and texture. By delivering a pleasant sensory experience, milk products build consumer trust and loyalty, driving repeat purchases and positive brand perception. Qualitative analysis is essential for ensuring compliance with regulatory standards and industry guidelines governing milk quality and safety. By assessing sensory attributes and

visual characteristics, analysts can verify adherence to established quality benchmarks, supporting regulatory compliance and consumer protection efforts. Qualitative analysis enables dairy producers to differentiate their products based on sensory attributes and quality characteristics. By emphasizing factors such as creaminess, richness, or freshness, producers can create distinct product offerings that cater to specific consumer preferences and market segments. In summary, qualitative analysis of milk quality and safety serves as a vital tool for detecting contaminants, ensuring freshness, detecting adulteration, enhancing consumer satisfaction, ensuring regulatory compliance, and fostering product differentiation. By combining sensory evaluation with scientific expertise, qualitative analysis contributes to the overall assurance of milk quality and safety, thereby safeguarding public health and consumer confidence in dairy products.

1.5 Quantitative analysis of pesticides in milk samples by GC-ECD

Pesticide residues are organic contaminants which are able to damage to endocrine, nervous and immune system as well as causing to cancer, by accumulating in the fatty tissue in human body. They can easily reach the food chain and concentrate in human and animal tissues. For food quality assurance, maximum residue limits (MRLs) of pesticides have been established by European Community for food and animal products [9]. Reason behind for causing pesticides contamination in milk through animal feed, various pesticide sprays that are used on the same farm. Thus, ruminants are at danger from various types and sources of anthropogenic contaminants, and it is possible that these components are transferred to the milk [19], there is some concern about the potential health effects of even low levels of pesticide exposure. Main sources of pesticide contamination can enter to the milk supply through contaminated feed or water consumed by cows. There are varies types of pesticides found in milk Organochlorines, organophosphates, synthetic pyrethroids, and triazines etc. Different types of organophosphorus and organochlorine have been identified in milk sample [18].

Gas chromatography with electron capture detection (GC-ECD) is a widely employed method for analysing pesticides in milk. The process typically begins with sample preparation, where the milk sample undergoes extraction to isolate the pesticides from the matrix using techniques such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), or QuEChERS. Following extraction, a clean-up step may be employed to remove interfering substances that could compromise the analysis. The cleaned-up sample is then injected into a gas chromatograph where it is vaporized and carried through a column by an inert gas. Within the column, compounds separate based on their affinity for the stationary phase and the mobile phase. Upon

elution, the compounds pass through the electron capture detector (ECD), where they are bombarded with high-energy electrons, resulting in the formation of negatively charged species. Pesticides, particularly those containing halogen atoms, exhibit a high affinity for capturing electrons, generating a signal response. This signal is recorded and analysed using appropriate software, with calibration against known standards to quantify the pesticides present in the milk sample. Quality control measures, including blank runs and spiked samples, ensure the accuracy and precision of the analysis. Ultimately, the results are reported in terms of pesticide concentrations, allowing for assessment against regulatory limits to determine the safety of the milk for consumption.

Aim and Objectives

This project endeavours to comprehensively evaluate the quality of milk and milk products through a combined quantitative and qualitative analysis approach. Milk and its derivatives are essential components of the human diet, providing vital nutrients and serving as versatile ingredients in various food products. However, ensuring their quality and safety is paramount to safeguarding public health. The study will employ both quantitative and qualitative analytical methods to assess different aspects of milk and milk products. Quantitative analysis will involve the measurement of key parameters such as fat content, protein content, total solids, titratable acidity, moisture content, total ash and presence of contaminants like pesticides. These analyses will be conducted using chemical methods and techniques such as chromatography. Data interpretation will be done to identify correlations between different parameters, and assess the overall quality of milk and milk products. The findings of this study will provide valuable insights into the factors influencing the quality of dairy products and facilitate the implementation of quality control measures to ensure consumer safety and satisfaction. Furthermore, the results can inform regulatory agencies and stakeholders in the dairy industry to implement measures aimed at improving milk quality and ensuring consumer safety. Overall, this project seeks to enhance the quality control processes in the dairy industry and promote the production of safe and nutritious milk products. Ultimately, this project aims to contribute to the enhancement of quality assurance practices in the dairy industry, promote the production of high-quality milk and milk products, and support consumer confidence in these essential food items.

Chapter 2
REVIEW OF LITERATURE

A study conducted in the central highlands of Ethiopia assessed the safety and quality of raw whole cow milk produced and marketed by smallholders. The study found that the average composition of protein, total solids, and ash were below the Ethiopian Standard Agency's standards. Factors contributing to these issues include unhygienic milking practices, use of local unsensitized containers, dirty milking areas, poor personnel hygiene, and lack of milk cooling systems. The study recommends strict monitoring and quality control measures at all levels from production to consumption, as well as sustainable awareness on good manufacturing practices (6).

In this research focused on assessing the chemical adulteration and hygienic quality of cow milk in the northwest of Iran. Customer need to aware of frequent adulterations as well as the quality standards for milk and dairy products. Total of 100 samples of raw cow milk were randomly collected. According to the finding, 8 and 10 % respectively of raw milk samples contained formalin and sodium bicarbonate. The methylene blue reduction test shows that only 44% of raw milk samples had proper hygienic quality (10). This study conducted in Gujarat, India, aimed to assess the quality and detection of adulteration in buffalo milk collected from different areas of Gandhinagar. The study involved 30 samples from different areas and tested for adulteration extent using standard milk adulteration methods. The results showed that the extent of adulteration varied significantly, with the least percentage for glucose (30%), sodium chloride (46%), sucrose (50%), and the highest for ammonium sulphates (96%) and urea (100%). All percentage values indicated the presence of these adulterants. From the study it was found that the quality of most of samples was bad and hardly few samples were found to be none adulterated [8]

This study was to assess the important quality parameter of goat-cow and buffalo-cow milk combinations by utilising chemometric technique to analyse Fourier-transform infrared (FTIR) spectroscopy data. FTIR spectra of pure and mixed samples were obtained at 4000–650 cm−1, when raw goat and buffalo milks were combined with cow milk at concentrations of 1–50% (v/v). As a result, this method could be used for rapid analysis of milk sample for quality parameters but also adulteration at level higher than 5% could be determined with a single run [18]. The study focused on qualitative analysis of market paneer of Odisha. And the sample were analysed for physical appearance, chemical analysis and bacteriological analysis. The

microbiological examination included standard plate count, coli form count, yeast, and mould count this result compared with BIS standards. This finding suggests the need for using standardized milk and adoption of sanitary practices. This research conducted in Nagpur; they collected samples from different zones of city. They evaluate the bacteriological quality of paneer. The samples were found to be contaminated with *Staphylococcus spp., Salmonella, E. coli* in 97%, 34% and 72% of the samples respectively. It indicates poor hygienic condition and faults in manufacturing/handling during the process of preparation [2]

This study aimed to compare the prevalence of foodborne pathogens in Malaai Pedha made from khoya. Samples were collected from a HACCP certified shop versus non-certified shops. 19 non-certified sweet shops were selected from seven localities in Pune City, and one HACCP certified shop was selected. Samples were collected from each shop, and microbial analysis was performed. Microbial results of samples from the HACCP certified shop less compared to non-certified shop it indicates the effectiveness of HACCP plan[20] . This study focused on to compare the practices of conventional and progressive dairy farms and their effects on quality of milk. Results show that conventional farms have poorer composition of milk compared to progressive farms. Good quality milk samples vary based on factors like fat, acidity, moisture, ash, protein, and pH. Additionally, conventional farms have a higher ratio of good quality samples for pesticide residues [11]. This study conducted to assessing the quality of curd (Dahi) in Bangladeshi markets found that curd samples from five popular breeds (Bg, Bo, A, M, and G) showed standard plate coliform (SPC), total colony fungus (TCC), and total fungus fungus (TFC) levels. The study also measured titratable acidity, pH, protein, fat, ash, and total solid of the collected curd samples. The results showed that curd Bg was considered excellent, while B, M, and G were considered very good. The findings are expected to contribute to the production of good quality curd in the future [13].

The study evaluating the quality, environmental safety, and biological value of a functional curd product. The researchers used a tasting scale to estimate organoleptic parameters, and a profilogram was built to determine the maximum quantity for taste, smell, appearance, and consistency. The experimental sample showed increased nutritional value due to the introduction of whey proteins and sesame seeds. The number of lactic acid microorganisms in the sample exceeded normal and control values. which prove its probiotic properties and indicate a high physiological value [5]. This research conducted for evaluating the quality of butter. There are sixty-six samples examined. Thirteen were gathered in Argentina, eighteen in France, and thirty-six in Brazil. Free fatty acids, peroxide value, total

lipid, cholesterol, and fatty acid composition were used to assess the samples. The studied brands of butter met the requirements of the Technical Regulation of Identity and Butter Quality of the Ministry of the Agriculture in Brazil, with exception of one brand only, which presented higher peroxide value than the maximum limit established by the present legislation [17]. This study focused on determination adulterant in butter sample. For finding the adulteration fatty acid composition by GC-MS, sterol concentration by GC-MS. TAG profile and tocopherol concentration through RP -HPLC/DAD/FLD. The results of the finding indicate that sterol and tocopherol analysis with an additional TAG profile examination can confirm if butter is adulterated, this analytical method can be successfully applied not only to confirm milk fat adulteration but also to quantitative analysis of the adulteration [4].

In this research 25 sample of skim milk powder were collected from various places of Maharashtra, their physio chemical properties were tested. Significant differences ($P<0.05$) were found in the moisture, fat, protein, lactose, and ash content of SMP samples collected from different areas. Additionally, it was discovered that samples' burnt particle content, solubility index, dispersibility, and wettability characteristics differed considerably between locations and brands [12]. This study investigated whether headspace analysis of volatile components can be used for monitoring the quality of raw cows' milk. The detection of different quality defects caused by cows' feed, microbiological and chemical contamination, as well as enzymatic deterioration was studied. It has been demonstrated that the seven volatile components of fresh raw milk are always present in unexplained quantities. The quantity of volatile chemicals in raw milk increased by up to 10 times when it was heated and homogenised, among other severe changes to this fundamental pattern. This headspace analysis is a supplementary method for raw milk quality control [14].

The aim of this study to determine the level of organochlorine pesticide residue in dairy products. A study in Ghana found that six organochlorine pesticides, including lindane, aldrin, dieldrin, endosulfan, dichloro diphenyltrichloroethane(DDT), and dichlorodiphenyldichloroethylene (DDE), were present in three dairy products from six communities. The concentrations of DDT and DDE were well below WHO's recommended levels, with the highest concentration found in Aboabo is the one site were sample is collected. The study aims to provide information on pesticide residue levels in dairy products to assess the impact of pesticides on public health, agriculture, and the environment in Ghana [3].

The study investigated the presence of organochlorine pesticide residues in raw buffalo milk and milk products from Sharkia Governorate, Egypt. Results showed that the only detected

residues were p,p'-DDE. Yogurt and cottage cheese samples had no residues. Butter samples had lindane, heptachlor epoxide, and p,p'-DDE residues. Heat treatment on raw buffalo milk, pasteurization, boiling, and sterilization reduced residues. Heat treatment of butter produced ghee with a significant decrease in organochlorine residue content [1].

A study in Jordan has found that 233 dairy product samples, including milk, butter, cheese, labaneh, and yoghurt, are contaminated with organochlorine pesticides (OCPs). The study found that 9%, 8.5%, 6%, and 2.1% of the samples were contaminated with b-HCH, pp0-DDE, a-HCH, and c-HCH, respectively. Heptachlor and a-endosulfan were present in less than 2% of the samples. The results will help assess the implications of pesticide residues on human risks in Jordan [16].

This study aimed to determine the presence and concentration of organochlorine pesticide residues in milk and dairy products from the Bacau district area. 54 samples were analyzed for residual content of hexaclorocyclohexane isomers (HCHs) and dichlorodiphenyl trichloroethane (DDT) and its analogues. Results showed that all samples were contaminated with α-HCH, β-HCH, and γ-HCH, respectively. DDT and its analogues were non-detectable. The study represents a step towards understanding the human health risks associated with OCP exposure through milk and dairy product consumption in Romania [7].

Chapter 3
MATERIALS AND METHODS

The Food Safety and Standards Authority of India (FSSAI) employs a range of robust methods for the analysis of milk, ensuring its safety and quality for consumers. These methods cover various parameters including fat content, solid not fat (SNF) content, protein content, and detection of adulterants. For fat content analysis, techniques such as the Gerber method, involving sulfuric acid and amyl alcohol separation, and the Rose-Gottlieb method, utilizing chloroform and methanol extraction, are commonly utilized. SNF content analysis may involve lactometer-based methods or gravimetric techniques, providing indirect measures of milk composition. Protein content is determined through methods like the Kjeldahl method, relying on nitrogen determination, or the Dumas method, employing high-temperature combustion. Additionally, FSSAI utilizes ultrasonic methods for simultaneous SNF and protein content analysis. To safeguard against adulteration, chemical tests are conducted to detect common adulterants such as water, urea, starch, detergent, and hydrogen peroxide. Chromatographic techniques like HPLC and GC further aid in detecting contaminants like antibiotics and pesticides. These comprehensive analytical methods ensure the adherence of milk products to stringent safety and quality standards mandated by FSSAI.

3.1 Assessment of the Quality Characteristics of Milk (Cow Milk, Buffalo Milk, Mixed Milk, Skimmed Milk}

3.1.1 Determination of total solids

The total solids in milk generally include proteins, fats, lactose (milk sugar), vitamins, and minerals. The exact composition can vary depending on factors such as the breed of cow, its diet, and processing methods. On average, cow's milk contains about 12-13% total solids, with fat making up around 3-4% of that, proteins about 3-4%, and lactose about 4-5%. However, these values can vary slightly. For instance, whole milk tends to have a higher fat content, while skim milk has a lower fat content but slightly higher protein and lactose concentrations. Determination of total solids in milk involves evaporating the water from a known volume of milk and weighing the remaining solids.

Apparatus Required:

- Analytical Balance
- Desiccator

- Hot air oven
- Aluminium dish

Method of Analysis:

- Take 5g of milk sample in aluminium dish [sample should be in room temperature (25±3°C)]
- Keep the dish in hot air oven for 3hour, oven temperature maintained at 102±2°C
- Allow to cool to room temperature (at least 30 min) and weigh the dish.

Calculation:

$$\text{Moisture} = W_1 - W_2 / W_1 \times 100$$

$$\text{Total Solid} = 100 - \text{Moisture}$$

W_1 - Weight of the dish in g with test portion

W_2 - Weight of the dish and dried test portion

3.1.2 Determination of Fat

The fat content of milk can vary depending on factors such as the animal's breed, diet, and processing methods. In cow's milk, the fat content typically ranges from around 3.5% to 4% by weight. However, this can vary, with whole milk containing a higher fat percentage, while skim milk has most of the fat removed, resulting in a much lower fat content, typically around 0.1% to 0.5%. It's important to note that different types of milk, such as goat's milk or sheep's milk, may have different fat contents compared to cow's milk. Additionally, milk products like cream can have much higher fat percentages, sometimes upwards of 30% or more.

Fat content in milk can be estimated by Gerber method. It is an empirical method and reproducible results can be obtained. The milk is mixed with sulphuric acid and isoamyl alcohol in special Gerber tube, permitting dissolution of the protein and release of fat. The tube is centrifuged and fat rising into calibrated part of the tube is measured as a percentage of the fa content of the milk sample

Apparatus Required

- Analytical Balance
- Butyrometer
- Water bath
- Gerber Centrifuge
- Milk pipette

Reagents

- Sulphuric acid 98%
- Iso amyl alcohol

Method of Analysis

- Transfer 10 ml of sulphuric acid into Gerber tube
- Add 10.75ml of milk sample
- Add 1ml of isoamyl alcohol and close with lock stopper, shake until it gets homogeneous.
- Kept the tube in hot water bath for 10 min at 60°C
- Cool it and centrifuge for 10 min, so as to conform to radial symmetry, and as evenly spaced as possible.
- Allow the centrifuge to come to rest and read the percentage of fat after adjusting the height in the tube as necessary by movement of the lock stopper with the key.
- Note the scale reading corresponding to the lowest pint of the fat meniscus and the surface of separation of the fat and acid.

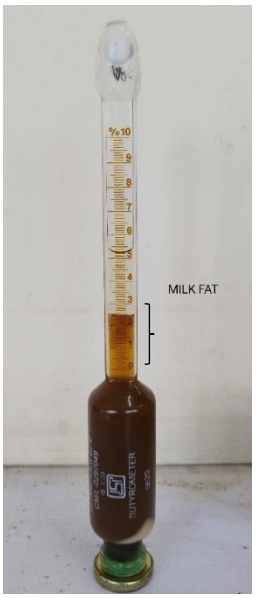

Figure 3: Fat separation of milk

Figure 4: Gerber Centrifuge

3.2 Qualitative Analysis of adulterants in Milk

3.2.1 Detection of sugar in milk

Milk naturally contains a sugar called lactose. Lactose is a disaccharide sugar composed of two simple sugars, glucose and galactose. It is the primary carbohydrate found in

mammalian milk, including cow's milk, goat's milk, and human breast milk. Sugar is added to milk as adulterant to increase the density of milk and also increase the carbohydrate content.

AIM: To detect the presence of sugar in the milk

PRINCIPLE: Seliwanoff's test is a chemical test which distinguish between aldose and ketose group. Sugar contains a keto group it is a ketose, if a sugar contains an aldehyde group is an aldose. when ketose is heated, they get rapidly dehydrated than aldose and gives a red colour colour. When added to solution containing aldose forming light pink is observed. The reagent consists of resorcinol and Conc. HCl the dehydrated ketose than reacts with two equivalents of resorcinol in a series of condensation reaction to produce a molecule with a deep cherry red colour.

Apparatus required

- Test tube
- Plastic filler
- 200 ml Beaker

Reagents

- Conc. Hydrochloric acid
- Seliwanoff's reagent

Method of analysis

- Take milk sample in a test tube
- Add few drops of Conc. HCl to the test tube for curdle the milk
- Vortex the mix and keep it for 5 min
- Filter the mix with a filter paper
- To that filtrate add equal volume of Seliwanoff's Reagent
- Keep boiling for 3 to 5 minutes in a water bath
- Observe the colour change

Inference

Development of brick red colour indicates the presence of sugar in the milk, or pink coloured complex formation also indicates the presence of sugar in low concentration.

Figure 5: Test for sugar (negative)

3.2.2 Detection of starch in milk

Starch is cheaply available in various forms such as wheat flour, corn flour and commercially manufactured starch. It is added to milk as adulterant to raise the SNF and also for thickening the milk.

Aim

To detect the presence of starch in the milk

Principle

Starch is composed of amylose and amylopectin. Iodine solution gives intense blue colour with starch due to the formation of starch- iodo - compound (blue colour unstable complex). Amylose in starch is responsible for the formation of deep blue colour. Iodine is not soluble in water, therefore the iodine reagent is made by dissolving iodine in water in the presence of KI this makes a linear triiodide ion complex with is soluble that slip into the coil of starch and form blue colour. Then the starch amylose is not present then the colour stays orange or yellowish.

Apparatus required

- Test tube
- Plastic filler

Reagents

- 1% Iodine solution

Method of analysis

- Take 1-2 ml of sample in a test tube
- Add few drops of 1% iodine solution
- Mix the contents and observe the colour

Inference

Blue or dark blue colour formation indicates adulteration of milk with starch, whereas pure milk remains yellow due to colour of iodine.

3.2.3 Detection of detergent in milk

Detergent may be added to milk as an adulterant to increase the frothiness or whiteness of the milk, making it appear fresher or richer than it actually is. This practice allows sellers to dilute the milk or mask its impurities. Adding detergent to milk is illegal and poses serious health risks.

Aim

To detect the presence of detergent in milk

Principle

Bromo cresol purple is a pH indicator that changes colour depending on the acidity or alkalinity of a solution. In the context of detecting detergent in milk, the principle behind using bromo cresol purple is based on the fact that detergents are typically alkaline or basic in nature. When added to milk, if detergent is present, it will increase the alkalinity of the milk. This change in pH will cause bromo cresol purple to undergo a colour change, indicating the presence of detergent. Typically, bromo cresol purple will shift from its initial colour to a different colour in the presence of alkaline substances like detergents.

Apparatus required

- Test tub
- Plastic filler

Reagents

- 1% Bromo cresol purple indicator

Method of analysis

- Take milk sample in a test tube
- Add drops of 1% of bromo cresol purple indicator
- Mix the contents and observe the colour change

Inference

Presence of violet colour indicate that detergent is present.

3.2.4 Detection of neutralizer in milk

Neutralizers, such as baking soda, washing soda, caustic soda other alkaline substances, may be added to milk as adulterants to mask acidity or sourness. By increasing the pH of the milk, neutralizers can hide the signs of spoilage or contamination, making the milk appear fresher than it actually is.

Aim

To detect the presence of neutralizer in milk

Principle

Rosalic acid is an indicator which shows a change in colour on addition to alkaline milk, Rosalic acid gives rose-red colour with carbonates and bicarbonates, whereas with pure milk it gives brownish coloration.

Apparatus required

- Test tube
- Plastic filler

Reagent

0.1% Rosalic acid

Method of analysis

- Take milk sample in a test tube
- Add 3-4 drops of 0.1% Rosalic acid
- Mix the content and observe the colour change

Inference

Development of pinkish orange colour indicates neutralizer adulterated in milk.

3.2.5 Detection of urea in milk

Urea is sometimes added to milk as an adulterant because it increases the nitrogen content, which can falsely elevate measures of protein content during testing. This can make the milk appear to have higher protein levels than it actually does level of urea to such an extent that on consumption of this adulterated milk cause toxicological hazards.

Aim

To detect the presence of urea in milk

Principle

The DMAB (dimethylaminobenzaldehyde) method is commonly used for detecting urea in milk. The principle behind this method is based on the reaction between urea and DMAB under low acidic conditions, which forms a yellow-coloured complex. The intensity of the colour is proportional to the concentration of urea present in the milk sample.

Apparatus required

- Test tube
- Plastic filler

Reagent

- 1.7% Dimethylaminobenzaldehyde

Method of analysis

- Take 2-3 ml of milk sample
- Add equal amount of DMAB solution
- Mix the content and observe the colour change

Inference

Formation of distinct yellow colour indicates the presence of added urea in milk sample, whereas pure milk shows light yellow colour due to natural urea.

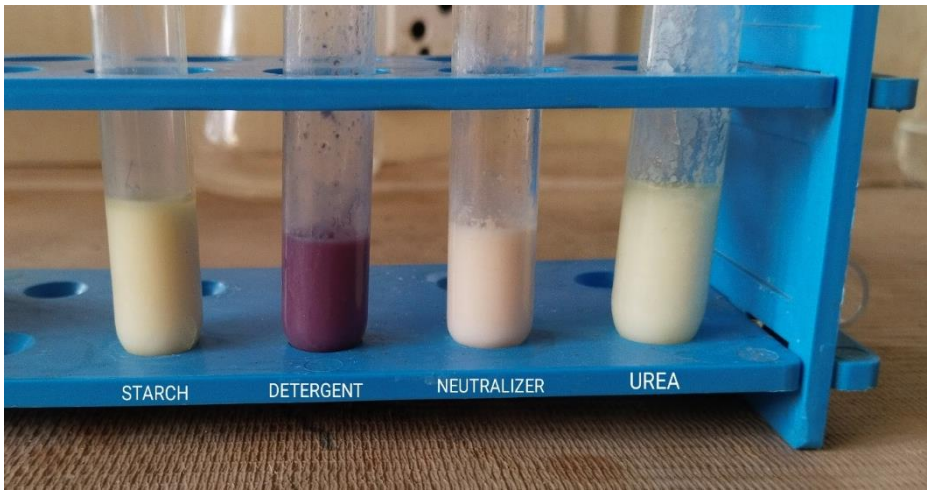

Figure 6: Test for qualitative analysis of adulterant

3.3 Assessment of quality characteristics of paneer

3.3.1 Determination of moisture

Principle

The moisture content of paneer is the loss of mass, expressed as a percentage be mass when the product is heated in hot air oven at 102±2◦C of constant mass.

Apparatus required

- Aluminium dish
- Hot air oven

Procedure

- Take 5g of milk sample in aluminium dish [sample should be in room temperature (25±3°C)]
- Keep the dish in hot air oven for 3hour, oven temperature maintained at 102±2°C
- Allow to cool to room temperature (at least 30 min) and weigh the dish.

Calculation:

$$\text{Moisture content} = \frac{M_1 - M}{M_2 - M} \times 100$$

Where,

M is mass in g, of the empty dish

M1 is initial mass in g of the dish with lid and test portion;

M2 is the mass in g of the dish with lid and dried test portion

3.3.2 **Determination of fat** (on dry matter basis)

Principle

In this method fat is extracted from the sample by semi continuously with organic solvent. The solvent builds up in the extraction chamber for 5-10 min and completely surrounds the sample, then siphons back to the boiling flask. Fat content is measured by weight of the fat removed.

Apparatus required

- Soxhlet apparatus
- Weighing balance
- Round bottom flask
- Tissue paper
- Cotton balls
- Thimble

Reagents

- Petroleum ether

Procedure

- Weigh about 5 g of predried sample into a predried extraction thimble
- Weigh the predried round bottom flask
- Add petroleum ether and assemble boiling flask, Soxhlet flask, condenser
- On the tap water for condensing the vapour
- As the petroleum ether flask is heated, the liquid will boil and rise up to a condenser tube, Here the cooling water surrounding the tube will turn the vapourized ether back into liquid.
- The liquid will drop on the top of the thimble with our sample and soak throughout the bottom taking along non polar lipids with it.
- The process allowed to run for 13 hours
- After 13 hr collect thimble and recover the petroleum ether.

- Dry the RB flask with extracted fat in a hot air oven at 100°C for 30 min and cool in desiccator and weigh the flask

Calculation

Percentage of fat = $\frac{\text{Initial weight of flask- final weight of flask}}{\text{Weight of sample}} \times 100$

Figure 7: Soxhlet Apparatus

3.4 Assessment of quality characteristics of khoya

Khoya, also known as mawa or khoa, is a popular dairy product in South Asian cuisine, particularly in Indian sweets and desserts. It is made by slowly evaporating milk to remove moisture, resulting in a thick, creamy, and granular solid**.**

3.4.1 Determine total ash

Principle

Total ash of khoa relies in incineration, in this process all the organic matter present in the sample like protein, fats, carbohydrates are combusts, only inorganic mineral present in the original sample remain. This residual mineral content is the total ash.

Apparatus required

- Crucible
- Muffle furnace
- Desiccator
- Safety tongs
- Analytical balance
- Gloves

Method of analysis

- Weigh accurately about 3 g of the khoa sample in the crucible
- Heat the crucible gently on a burner or hot plate at first to become completely black colour
- Kept in muffle furnace at 550 ± 20 ℃ for 6 hrs till grey ash is obtained
- Cool the crucible in a desiccator and weigh it

Calculation

$$\text{Total Ash \% by mass} = \frac{M_2 - M}{M_1 - M} \times 100$$

M - is mass in g, of the empty crucible

M_1 - is initial mass in g of the crucible with the material taken for the test

M_2 - is the mass in g of the crucible with ash

3.4.2 Determination of Titratable Acidity

Principle

A known quantity of sample is neutralized with standardized sodium hydroxide solution with phenolphthalein indicator. The amount of sodium hydroxide required is a function of the amount of natural buffering substances present in the product, and of developed or added acid or alkaline substances.

Apparatus required

- Analytical balance
- Conical flask
- Burette

Reagents

- Sodium hydroxide
- Phenolphthalein indicator

Method of analysis

- Weigh accurately about 2 g of the material in a suitable dish or basin,
- Add 3 mL of hot water and render it to paste; add further 17 mL of hot water washing off any adherents.
- Add 1 mL of phenolphthalein indicator, shake well and titrate against standard Sodium hydroxide solution; complete the titration in 20 sec
- Keep a blank by taking 2 g of material diluted with 20 mL of water in another dish for comparison of colour.

Calculation

$$\text{Titratable acidity (as lactic acid)} = \frac{9\times \text{Normality of NaOH} \times \text{Titratable value}}{\text{Sample weight}}$$

3.4.3 Estimation of Reichert Meissl (RM value)

Principle

The Reichert-Meissl (RM) value determines the volatile short-chain fatty acids present in a fat or oil sample, specifically those with a carbon chain length of 4 to 10. In the context of khoa, a dairy product, the RM value indicates the content of butyric acid, a key short-chain fatty acid.

Hydrolysis: The fat/oil sample, in this case khoa, is treated with an acid or enzyme to break down the triglycerides (fats) into their constituent fatty acids.

Distillation: The liberated fatty acids are then steam distilled. This process separates the volatile short-chain fatty acids (including butyric acid) from the longer-chain fatty acids that remain in the non-volatile fraction.

Titration: The collected distillate containing the short-chain fatty acids is then neutralized with a standardized sodium hydroxide (NaOH) solution. The amount of NaOH used indicates the quantity of short-chain fatty acids present.

Apparatus required

- Round bottom flask
- Heating coil
- Analytical balance
- Glass beads
- Measuring cylinder
- Distillation unit
- Burette
- Filter paper
- Pipette

Reagents

- Glycerol
- 50% Sodium hydroxide
- 1 N Sulphuric acid
- Phenolphthalein

Method of analysis

- Take 2.5-5g sample in 500ml RB flask
- Add 20 ml Glycerol
- Add 2ml 50% NaOH solution along with glass beads
- Boil until clear solution
- Stir in regular intervals while boiling
- Add 90 ml boiling water slowly along the sides
- Add 50ml 1N H_2SO_4
- White precipitate appears then connect to distillation
- Boil till volatile fatty acids is collected in volumetric flask
- Filter through qualitative paper to a conical flask
- Pipette or measure 100ml to fresh conical flask
- Titrate against 0.1 N NaOH with phenolphthalein as indicator
- End point is appearance of pink colour

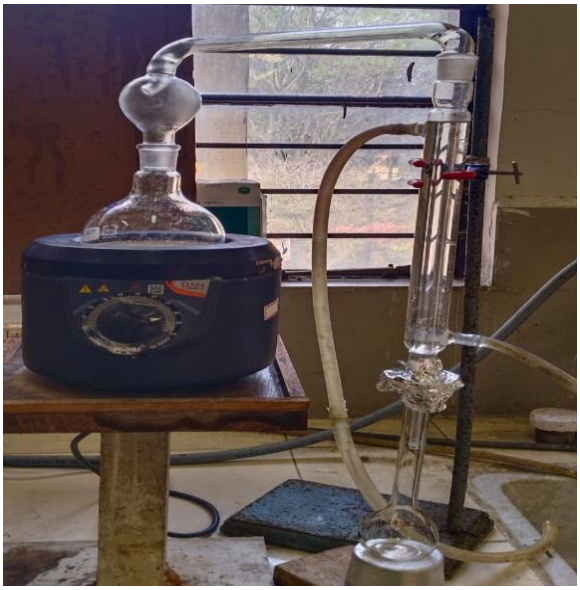

Figure 8: RM apparatus

3.4.4 Determination of Butyro Refractometer Reading Value

Principle

The butyro refractometer is a handy tool used to assess the q It works on the principle of measuring the refractive index of the substance. Light travels at different speeds through various materials. The refractive index (RI) is a physical property that indicates how much a material bends light. Khoa, with its specific fat and moisture content, bends light in a particular

way. The butyro refractometer measures this bending of light and translates it into a numerical value on a scale.

Apparatus required

- Butyro Refractometer

Procedure

- Clean the glass area of refractometer with tissue paper
- Calibrated with distilled water and wipe it
- Add the sample and take the reading

Figure 9: Butyro Refractometer instrument

3.5 Assessment of the quality characteristics of curd

3.5.1 Determination of fat (Rose - Gottlieb Method)

Principle

The Mojonnier method is a gravimetric technique used to determine the fat content in dairy product.

Disruption and Dissolution: The process starts with adding ethanol and ammonia to the sample. This disrupts and dissolves casein micelles, which are protein structures that encapsulate some of the milk fat. Then, a mixture of ethyl ether and petroleum ether is added. These organic solvents dissolve and extract the fat globules from the sample. After complete evaporation of the solvents, the remaining residue in the container is the extracted fat. The container is

weighed again, and the difference in weight before and after evaporation corresponds to the fat content in the original milk sample.

Apparatus required

- Mojonnier flask
- Measuring cylinder
- Analytical balance
- Conical flask
- Water bath

Reagents

- Ethanol
- Ammonia
- Petroleum ether
- Diethyl ether

Procedure

- Take 3g of sample in Mojonnier flask
- Add 10 ml warm water
- Add 10 ml ethanol
- Add 2 ml ammonia
- Keep in water bath for 15 min and cool it
- Add 25 ml Diethyl ether and shake well leave it for 45 min
- Decant the upper layer to weighed conical flask
- Add 25 ml petroleum ether and shake well
- Decant the layer to conical flask
- Add 15ml Diethyl ether and 15 ml petroleum ether shake well
- Decant the clear layer
- Evaporate the solvent and cool it
- Weigh the conical flask along with extracted fat

Calculation

$$\% \text{ of Fat} = \frac{\text{Initial weight of flask- Final weight of flask}}{\text{Sample weight}}$$

3.5.2 Determination of total nitrogen in curd (Kjeldahl method)

Principle

The method involves three major steps. In the first the protein is digested using concentrated sulphuric acid in presence of a catalyst (potassium sulphate/ copper sulphate). In this step all the organic material is oxidized except nitrogen, when is converted to ammonium sulphate. In the second step the digest is neutralized with alkali to liberate ammonia. The ammonia distilled is collected in boric acid. In the third step the collected ammonia in boric acid is titrated with standard hydrochloric acid in the presence of a methyl red bromocresol green indicator until the green distillate changes from colourless to pink (methyl red methylene blue indicator can also be used.

Reaction

$$\text{Protein} \xrightarrow[\text{Heat}]{K_2SO_4,\ CuSO_4,\ H_2SO_4} (NH_4)_2SO_4$$

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

$$NH_3 + H_3BO_3 \longrightarrow NH_4^+ . H_2BO_4^-$$

$$NH_4^+ H_2BO_4^- + HCl \longrightarrow NH_4Cl + H_3BO_3$$

The quality of acid required for titration is equivalent to the concentration of ammonia in the distillate and to the nitrogen content of the original protein containing sample

Apparatus required

- Kjeldahl flask
- Digestion heater block
- Fume hood
- Kjeldahl distillation apparatus
- Condenser
- Steam generator
- Distillation flask
- Receiving flask

Reagents

- Potassium sulfate
- Copper(II) sulfate solution
- Concentrated sulphuric acid
- Boric acid
- Sodium Hydroxide solution
- Standard hydrochloric acid

Method of analysis

- Add the 1.5g of sample and 1 spatula of digestion mixture
- Add 20ml conc. sulphuric acid
- Keep it for digestion
- After digestion make up to 100 ml volumetric flask
- From that again pipette out 10ml in the digestion tube
- Keep the digestion tube for distillation
- After distillation titrate with 01 N HCl
- Note the reading

Calculation

$$\text{Protein} = \frac{(\text{Titratable value-blank value}) \times \text{Nitrogen factor Protein factor} \times \text{Makeup vol} \times \text{N of HCl}}{\text{Pipetted volume} \times \text{Sample weight}}$$

Figure 10:Kjeldahl apparatus

3.6 Assessment of the quality characteristics of butter

3.6.1 Determine the salt content

Principle

In this method, salt present in the butter sample is extracted with hot water from the dried fat free residue obtained in moisture determination. Chloride ions, major component of salt (sodium chloride), react with silver nitrate ($AgNO_3$) to form insoluble silver chloride (AgCl). This reaction allows to determine the amount of chloride ions present, which can then be converted to the amount of salt in the buffer.

$$AgNO_3 + NaCl \longrightarrow AgCl + NaNO_3$$

$$2AgNO_3 + K_2CrO_4 \longrightarrow Ag_2CrO_4 + 2KNO_3$$

(Brick red ppt)

Apparatus required

- Conical flask
- Burette
- Pipette
- Conical flask

Reagents

- Silver Nitrate
- Potassium chromate

Method of analysis

- Take 5 g of sample in a conical flask
- Pour some hot water and dissolve the sample
- Then make this sample to 25 ml volumetric flask
- Take 5 ml sample using pipette
- Add 2 drop of potassium chromate titrate against silver nitrate till it turns to brick colour.

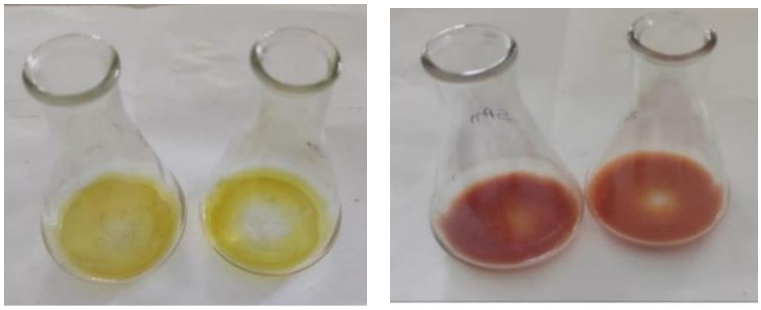

Figure 11: Test for common salt content in sample

3.6.2 Determination the curd + salt

Principle

In this method, the fat portion is removed using petroleum ether and residue is dried for determination of curd content. In case of table butter, it is curd and salt content, and thus salt content has to be determined separately.

Apparatus

- Funnel
- Filter paper
- Hot air oven

Reagents

- Petroleum Ether

Method of analysis

- Remove moisture from butter
- Take the weight of empty filter paper
- To the dish add petroleum ether and filter thoroughly with filter paper
- Keep in hot air oven for 30 min
- Weigh the filter paper after cooling

Calculation

$$\text{Curd +Salt \%} = \frac{\text{Final weight -Initial weight}}{\text{Sample weight}} \times 100$$

3.6.3 Determination of fat

Principle

There are two type of method direct and indirect method, indirect method is widely used method for determining the fat in butter. This approach focus on measuring the non-fat components of butter (moisture, protein, lactose, and minerals)

Calculation

$$\text{Fat} = 100 - (\text{Moisture} + (\text{Curd} + \text{Salt}))$$

3.7 Assessment of the quality characteristic of ice cream

3.7.1 Determination of weight per unit volume

Principle

Over-run is usually defined as the volume of ice-cream obtained in excess of the volume of the mix. It is usually expressed as a percentage. This increased volume is composed mainly of the air incorporated during the freezing process. The amount of air which is incorporated depends upon the composition of mix and the way it is processed. In this test, the volume of water and alcohol used corresponds with the volume of air originally contained in the ice-cream and the

difference between the sum of these two and capacity of the flask is equivalent to the volume occupied by the sample.

Apparatus required

- Measuring cylinder (25 ml)
- Analytical balance

Procedure

- Take measuring cylinder pour the sample up to 10ml marking
- Note the weight

Calculation

$$\text{Weight} = \frac{10 \times 1000}{\text{Weight of the sample}} = \text{ml /1000g}$$

3.7.2 Estimation of added synthetic colour

Principle

Qualitative and quantitative of added synthetic colour is measured. For qualitative test chromatography is used by isolating coloured band and comparing them to known standards. Estimating the amount of synthetic colour added to ice cream. Spectrophotometry is a scientific technique that measures the amount of light absorbed by a substance at different wavelengths. it is possible to estimate the concentration of the colour in the ice cream

Apparatus required

- Beaker
- Glass rod
- Wool
- Chromatography paper
- Micropipette
- Photo spectrophotometer
- Cuvette
- Volumetric flask (25ml)
- Heating coil

Reagents

- Ammonia
- Acetic acid
- Hexane

- Distilled water
- 0.1N Hydrochloric acid
- Trisodium citrate

Method of analysis

Extraction and qualitative analysis of water-soluble colours for paper chromatography

- Take 5 g of sample and add hexane to remove fat from the sample
- Extract the hexane layer
- Add the sample to a beaker add 20 ml of distilled water and mix well
- Add wool to it, add few drops of glacial acetic acid and boiled till the colour present in the sample is absorbed by the wool
- Wash the wool with water and transfer the wool add distilled water and 2 ml ammonia solution is added.
- The solution is boiled till the colour in the wool leaches out to the solution
- When the colour leaches out completely the wool is removed and boil the solution till it concentrated.
- This concentrated solution was used for spotting on chromatographic paper
- Take capillary tube and spotted the concentrated solution along with reference standard colour in a chromatography paper
- Place the chromatography paper in mobile phase to run for 30 min
- Take the paper out and keep it for dry
- And analysis which colour is present in the sample

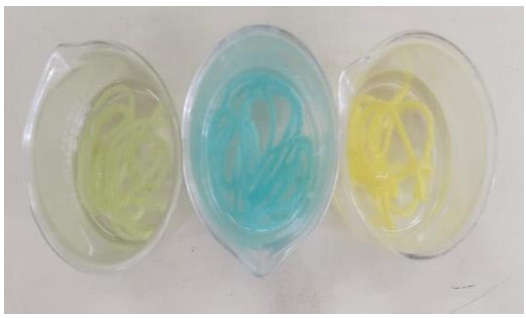

Figure 12: Extraction of colours

Quantitative analysis of synthetic colour

Procedure

- Concentrated solution again add little distilled water mix well

- Steak 2 ml of sample to a chromatographic paper
- Keep in mobile phase to run for 30 min
- Taken out and keep it for drying
- After drying isolated band are cut into pieces and dipped in in 0.1 N HCl till all colour leaches out.
- Filter the solution and make up into 25 ml
- Makeup solution is used for spectrophotometer reading
- Take the reading note it down

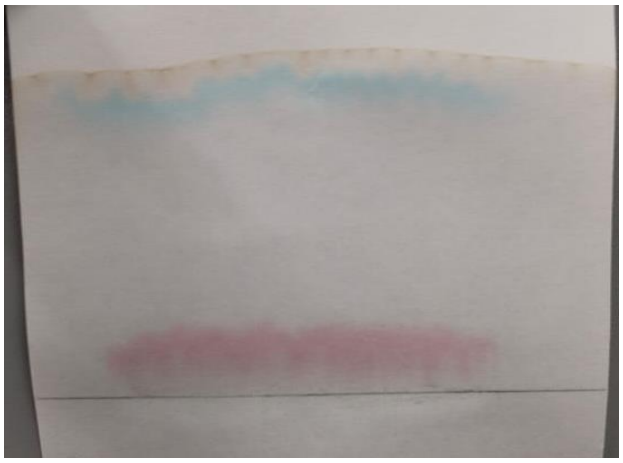

Figure 13: Isolated band Carmosine and Brillant blue

Figure 14: UV -Visible spectrophotometer

Calculation

$$\text{Concentration} = \frac{\text{Absorbance of UV} \times \text{Conc.Volume} \times \text{makeup Volume} \times 10000}{\text{Epselon value} \times \text{Sample weight} \times \text{Streak volume}}$$

$$= \_\_\_\_\_ \text{ ppm}$$

3.8 Assessment of quality characteristics of skimmed milk power

3.8.1 Determination of insolubility index

Principle

Distilled water is added to a test portion, which is reconstituted using a special mixer. After a specified standing period, a specified volume of the reconstituted milk or milk product is centrifuged in a graduated tube. The supernatant liquid is removed and the sediment is re-dispersed after the addition of water at the same temperature as used for the reconstitution. The mixture is centrifuged and the volume of sediment (insoluble residue) obtained is recorded.

Apparatus required

- Graduated tube
- Centrifuge
- Vortex mixer

Procedure

- Take 1.4g sample and 10 ml of distilled water in a graduated tube and vortex properly
- Allow the sample to settle for 10 min and remove if any foam formed on the top of sediment layer
- Again, after removal of foam add fresh water and and keep for centrifuge
- After centrifuging remove the upper layer and add water making up to 10ml
- Don’t disturb the sediment layer
- Once again centrifuge and note the sediment reading in the 15ml centrifuge tube

3.9 Analysis of pesticide by Gas chromatography- Mass spectrometer

Pesticides are chemicals used in agriculture to kill pests like insects, rodents, and weeds. Unfortunately, these chemicals can sometimes end up in our food, including milk. Pesticide residues in milk are a concern because they can be harmful to human health. The effects of exposure to pesticides can vary depending on the type of pesticide, the amount of exposure, and the individual's age and health. Gas chromatography- Electron Capture Detector is widely used analytical technique for separation, identification and quantification of various compounds including pesticide. Mass spectral data provides confirmation of the identity of detected pesticide residue.

Principle

The sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (usually helium). The sample flows through the column and the compounds comprising the mixture of interest are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile

phase). The latter part of the column passes through a heated transfer line and ends at the entrance to ion source where compounds eluting from the column are converted to ions and detected according to their mass to charge m/z ratio. Mass information can be used to identity, quantify, and determine the structural and chemical properties of molecules.

Apparatus Required

- Homogenizer
- Centrifuge
- Vortex mixer
- Turbovap concentrator
- Micropipette
- Gas chromatograph- Electron Capture Detector

Reagents

- 1% Acetic acid in Acetonitrile
- Magnesium sulphate
- C18
- Sodium acetate
- Primary Secondary Amine (PSA)
- Hexane

Standard Preparation

Alpha -BHC, Hexachlorobenzene, Beta-BHC, Gamma-BHC, Heptachlor, Aldrin, Heptachlor epoxide, Gamma-Chlordane, Alpha-endosulfan, Alpha-Chlordane, DDE, dieldrin, Endrin,4,4'-DDD, Beta-endosulfam, Endrin aldehyde, 4-4'DDT, Endosulfan sulfate, Endrin ketone, methoxychlor, Delta -BHC of various concentration of mg/kg were used as standards

Preparation Of Stock Solution

10 mg of pesticide standards were weighed, dissolved in ACN and made up to 10ml with Hexane for in volumetric flask and the resultant is stock solution.

Preparation Of Working Standard

The Standard of 10 mg/kg was prepared by pipetting 100ul from 1000 mg/kg stock and made up to 1ml with suitable solvent. (Here-ACN and Hexane)

The working standards are 10ppb, 20ppb, 50ppb, 100ppb, 150ppb and 200ppb prepared by serial dilution with n-hexane.

Procedure For Pesticide Extraction

- 4 ml of milk sample
- Add 10 ml of 1% acetic acid prepared with ACN
- Vortex and add 3g magnesium sulfate, 0.5 g of sodium acetate
- Vortex well and centrifuge at 5000rpm for 10 minutes
- Transfer 80ml supernatant into a 15ml centrifuge tube containing 1gm of $MgSO_4$,200mg PSA and 300 mg C18
- Vortex well centrifuge at 8000 rpm for 10 minutes
- Transfer 5 ml supernatant into a clean test tube and evaporate by using turbovap evaporator
- Reconstitute with 1ml hexane and filtered into GC-MS vials

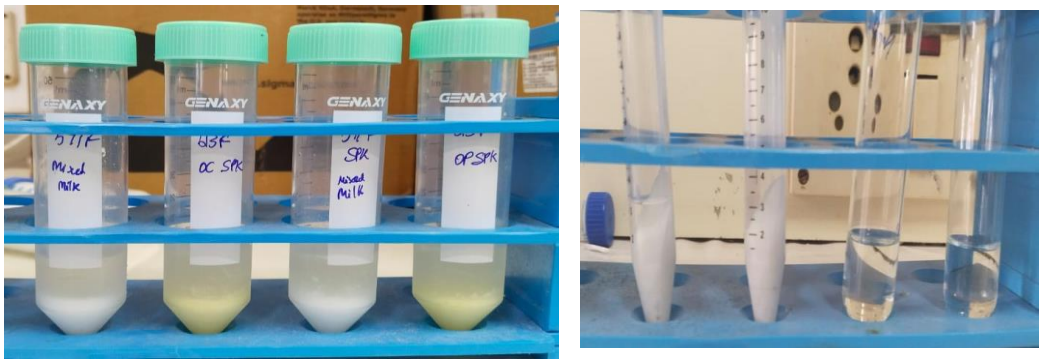

Figure 15: Pesticide extraction using solvent and salts

Figure 16: Mass chromatograph- Electron Capture Detector

Chapter 4

RESULTS AND DISCUSSIONS

Ensuring the quality of milk is paramount to safeguarding public health and meeting consumer expectations. Key quality parameters, including fat content, protein content, and microbiological safety, are rigorously monitored throughout the production and distribution chain. Maintaining standardized levels of fat and protein ensures consistency in nutritional composition, while microbiological testing detects and prevents the presence of harmful pathogens. Sensory evaluation further assesses the appearance, flavour, and texture of milk, enhancing consumer satisfaction. Implementing robust traceability systems and quality assurance programs further enhances transparency and accountability in the dairy industry. By prioritizing these quality parameters and implementing stringent quality control measures, stakeholders in the dairy sector can uphold the safety, nutritional value, and sensory appeal of milk for consumers worldwide. Milk samples of around 37 were collected from different states of India like Madhya Pradesh, Rajasthan, Maharashtra, Kerala. Collected milk samples includes different types of milk like cow milk, buffalo milk, mixed milk, toned milk etc. Quality parameters which include moisture, fat, total solids, milk solids not fat as well as qualitative test for starch, sugar, detergent, neutralizer were analysed. All the test was analysed in compliance with FSSAI, as per the regulation no 2.1.2.

4.1 Analysis of the quality parameters in milk samples

4.1.1 Cow milk (FSSAI 2.1.1)

Cow milk					
SL. No.	Moisture	Total Solids	Fat	Milk Solid Not Fat	Qualitative Test
Sample 1	84.5	15.5	7.75	7.78	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 2	65.0	35.0	26.5	8.51	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative

Sample 3	87.0	13.0	2.75	10.2	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 4	90.1	9.95	2.65	7.31	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 5	89.7	10.3	3.25	7.06	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 6	89.8	10.1	3.75	6.5	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 7	85.3	14.7	7.17	7.52	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative

Table 15: Results of cow milk analysis

Cow milk samples were collected from different states and quality parameters analysed. Among the tested samples Milk Solids Not Fat (MSNF) was found to be in the range 6.5 to 8.5%. As per FSSAI regulations MSNF should not be less than 8.5% for cow milk. MSNF for five milk samples was found to be less than 8.5% which means that the samples contain lower concentration of non-fatty components than its acceptable standards. When fat % were analysed, two samples contain fat which is less than 3.2% as per FSSAI regulations. One sample have extremely high fat content 26.5 % which shows that fat like vanaspati is added so as to increase the fat content. In that case have taken butyro refractometer value which was found to be 60.23, indicating that the sample is adulterated with added fat. Qualitative tests for starch, sugar, detergent, neutralizer and urea are negative for all the cow milk samples. Based on the quality parameters none of the cow milk samples conforms to FSSAI regulations.

4.1.2 Buffalo milk (FSSAI 2.1.2)

Buffalo milk					
Sl. No:	Moisture	Total Solids	Fat	Milk Solid Not Fat	Qualitative Test
Sample 8	86.3	13.7	5.75	7.95	Sugar-Negative

					Starch-Negative Detergent-Negative Neutralizer-Negative Urea - Negative
Sample 9	81.2	18.8	2.4	16.4	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 10	84.2	15.8	5.6	10.3	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 11	44.8	55.1	43.1	11.7	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 12	82.5	17.5	7.45	10.0	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 13	85.4	14.6	3.05	11.5	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 14	86.6	13.4	2.65	10.8	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative

Table 16: Results of Buffalo milk analysis

Buffalo milk samples were collected from different states and quality parameters analysed. Among the seven samples tested, fat content present in three samples was found to be less than 5%. As per FSSAI regulations fat content in buffalo milk should not be less than 5%. As explained in the previous case, one sample contained fat around 43.1% and BRR value of 61.5 which means that milk is adulterated with any fat alternatives like vegetable or even industrial fats. Milk solid not fat should not be less than 9 according to FSSAI standards. Among 7 samples one sample has a value less than 9. All the samples showed negative for qualitative test for adulterants. Among 7 buffalo milk samples tested two of the samples conforms to FSSR.

4.1.3 Mixed milk (FSSAI 2.1.2)

Mixed milk					
Sl. No:	Moisture	Total Solids	Fat	Milk Solid Not Fat	Qualitative Test
Sample 15	86.6	13.4	2.65	10.7	Sugar-**Positive** Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 16	86.4	13.6	3.9	9.62	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 17	85.6	14.4	4.8	10.0	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 18	85.5	14.5	3.1	11.4	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 19	85.7	14.3	3.75	9.95	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 20	85.5	14.5	3.7	10.8	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 21	87.1	12.9	2.7	10.3	Sugar-**Positive** Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 22	90.5	9.50	0.6	8.90	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative

Sample 23	87.1	12.95	4.1	8.89	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 24	88.8	11.2	1.2	9.99	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 25	82.6	17.4	7.05	10.4	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 26	85.6	14.4	4.9	9.5	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 27	87.5	12.5	4.75	7.74	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 28	85.5	14.5	5.35	9.13	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 29	82.8	17.2	7.05	10.1	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 30	84.0	16.0	2.55	13.4	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 31	85.9	14.2	5.15	9.01	Sugar-**Positive** Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 32	89.3	10.7	1.82	8.91	Sugar-**Positive** Starch-Negative Detergent-Negative

					Neutralizer-Negative Urea – Negative
Sample 33	86.5	13.5	5.15	8.31	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 34	86.5	13.5	2.5	11.0	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative

Table 17: Results of mixed milk analysis

Mixed milk samples were collected from different states and quality parameters analysed. As per FSSR, fat content in milk samples should not be less than 4.5%. Among the twenty samples analysed 12 samples contain fat % less than 4.5. For mixed milk samples MSNF should not be less than 8.5% as per FSSR. Only one sample showed a value less than 8.5%. Four samples have given positive for sugar test. Sugar is added to milk so as to increase the density of milk, masking the dilution or increase the sugar content in milk. Hence it was observed that among the 20 samples only 6 samples conform to FSSR standards.

Figure17: Positive for Sugar (Low Concentration)

4.1.4 Toned milk (FSSAI 2.1.2)

Toned Milk					
Sl No	Moisture	Total Solids	Fat	Milk Solid Not Fat	Qualitative Test
Sample 35	90.22	9.78	3.05	6.72	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 36	88.31	11.7	4.1	7.59	Sugar-Negative Starch-Negative

					Detergent-Negative Neutralizer-Negative Urea – Negative
Sample 37	87.04	13.0	3	9.96	Sugar-Negative Starch-Negative Detergent-Negative Neutralizer-Negative Urea – Negative

Table 18: Results of toned milk analysis

Among the three toned milk samples fat content was found to be more than 3% (FSSR limit). All the samples contain prescribed amount of fat. As per FSSR, MSNF for toned milk should not be less than 8.5%. Among the three samples analysed 2 samples have shown SNF below 8.5%. All the samples showed negative test for qualitative test for adulterants. Among three samples one sample conforms to FSSR.

4.2 Analysis of the quality parameters in paneer samples

Paneer samples were collected from different states and analysed. In paneer sample quality parameters like moisture, milk fat on dry wt. basis and qualitative test for starch, sugar, detergent, urea, neutralizer was analysed as prescribed standards as per FSSAI regulation no 2.1.16.

Paneer			
Sl. No	Moisture%	Fat % (On Dry Wt. Basis)	Qualitative Test
Sample 38	45.0	57.9	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea – Negative
Sample 39	59.3	43.3	Sugar - **Positive** Starch - Negative Detergent - Negative Neutralizer - Negative Urea – Negative\
Sample 40	50.9	40.7	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea - Negative
Sample 41	41.6	43.1	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea - Negative

Sample 42	48.1	59.2	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea - Negative
Sample 43	43.4	59.4	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer- Negative Urea - Negative
Sample 44	48.3	48.4	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea - Negative
Sample 45	57.5	50.2	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer- Negative Urea – Negative
Sample 46	59.6	38.8	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer -Negative Urea - Negative
Sample 47	58.4	44.7	Sugar - Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea - Negative
Sample 48	54.6	52.7	Sugar – Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea – Negative
Sample 49	58.05	54.89	Sugar – Negative Starch - Negative Detergent - Negative Neutralizer - Negative Urea – Negative

Table 19: Results of paneer analysis

Twelve samples of paneer were analysed for quality parameters. As per FSSAI regulation, moisture of paneer should not more than 60%. All the tested samples have moisture within the limit. Based on FSSR standards regulations fat content of paneer should not be less than 50%. Among tested samples 6 samples have shown fat % less than 50, not satisfying FSSAI

standards. One of the samples shows positive result for sugar in qualitative test. Among 12 samples, 6 samples conform to FSSR.

4.4 Analysis of the quality parameters in Khoya samples

Total six samples of khoya were collected from different states of India and analysed. In khoya sample, moisture, total ash, titratable acidity, fat, RM value, BRR value and qualitative test for adulterants like starch and sugar were analysed according to FSSAI regulation 2.1.6.

Khoya								
Sl No	Moisture	Total Ash	Total Solid	Titratable Acidity	Fat	RM Value	BRR Value	Qualitative Test
Sample 50	38.1	3.49	61.9	0.81	23.7	12.7	48.1	Starch-negative Sugar-negative
Sample 51	44.8	3.35	55.5	0.27	20.2	8.54	40.6	Starch- -negative Sugar-negative
Sample 52	40.0	3.12	60.1	1.36	37.8	34.9	42.2	Starch-negative Sugar-negative
Sample 53	46.4	3.15	53.6	1.34	27.4	13.9	48.4	Starch-negative Sugar-negative
Sample 54	41.7	3.11	58.3	0.25	38.3	37.9	41.2	Starch-negative Sugar-negative
Sample 55	33.8	3.87	66.2	0.6	27.5	28.2	41.6	Starch-negative Sugar-negative

Table 20: Results of Khoya analysis

For khoya, as per FSSAI, milk fat should not be less than 30%. Among the six samples tested 4 samples have fat content less than 30%. As per FSSAI total solids should not be less than 55, and for all the 6 samples total solids was found to be more than 55%. Ash content ensure a consistent mineral content across different khoya, potentially impacting taste or texture. High ash indicates the presence of fillers or additives not naturally found in khoya. Total ash for khoya should not be more than 6 as per regulations. Among the tested samples all the samples have ash content less than 6. Titratable acidity of khoya should not be more than 0.9 according to FSSAI. Two samples showed titratable acidity more than 0.9 indicating that the samples might have the sign of spoilage due to bacterial action. RM value for khoya should not be less than 24 as prescribed standards as per FSSAI. Three of the samples have less RM value than its limit which indicate a lower milk fat content and potentially the presence of additive or substitutes for milk fat. BRR value for khoya should be in the range 40-44, but two samples have greater value than its limits which indicates the presence of added fat. Qualitative test for sugar and starch for all samples are negative. Among the six samples only one sample conforms to FSSR standards.

4.4 Analysis of the quality parameters in Butter samples

Three samples of butter were collected from different places. For butter sample, moisture, milk fat, milk solids not fat, salt content, RM value and BRR value were analysed as per FSSAI regulation 2.1.9.

Butter							
Sl No	Moisture %	Total Solids	Fat%	Common Salt	Milk Solid Not Fat	RM value	BRR value
Sample 56	27.3	72.7	68.7	1.18	3.9	15.8	45.1
Sample 57	15.1	84.9	83.9	1.20	1.00	30.0	42.1
Sample 58	15.0	85.0	83.9	1.40	1.04	28.0	41.9

Table 21: Results of Butter analysis

For butter samples, moisture should not be more than 16% as per FSSAI standards. One of the sample have moisture content more than 16 which indicates it has shorter shelf life and also texture of butter will be softer and easier to spread but more difficult to baking application. Fat content should not be less than 80, one of the sample showed less fat %. Milk solids not fat should be not more than 2, but one sample have more than its limit which may affect the texture, shelf life. This may happen because of wrong churning process. Salt content of butter should not be more than 3 and all the samples conforms with the FSSR limit. RM value should not be less than 24, one of the samples have lower RM value which means it contain any kind of adulterants. In order to check the purity BRR value was analysed and was found to be higher, not in the range 40-44, which indicates the presence of added fats. Among the samples analysed, two samples conform to FSSR standards.

4.5 Analysis of the quality parameters in Ice cream samples

Total four ice cream sample were collected from groceries. For each sample, milk fat, total solid, milk protein, weight, added synthetic colour were tested as per FSSAI regulation 2.1.14 and FCS 1.7.

Ice Cream						
Sl No	Ice cream flavour	Total Solids	Weight	Fat%	Protein	Added Synthetic Colour
Sample 59	Strawberry ice cream	39.9	937/1000g	11.56	3.97	Erythrosine - 0.77ppm
Sample 60	Pista ice cream	38.2	915/1000g	12.32	4.00	Tartrazine - 5.36 ppm

Sample 61	Black current	40.9	937/1000g	12.89	4.50	Brilliant blue - 4.26 ppm Carmoisine - 10.39 ppm
Sample 62	Butterscotch	41.7	950/1000g	13.0	4.35	Tartrazine - 32.39ppm

Table 22: Results of Ice cream analysis

As per FSSAI regulations milk fat should not be less than 10, and all the samples contain prescribed amount of fat. Total solids of ice cream should not be less than 36, all the samples met its total solids requirement. The protein should not be less than 3.5, and protein content of all samples was found to be within the limit as per FSSAI standards. Weight of the ice cream should not be less than 525/1000g. In weight parameter all the sample met the requirement. Added synthetic colour should not be more than 100ppm. In strawberry icecream added colour is Erythrosine and the concentration of added colour was 0.77 ppm. In pista ice cream Tartrazine was present and concentration of tartrazine was found to be 5.36 ppm. In black current icecream two added colours were present, brilliant blue and carmoisine and its concentration was found to be 4.26ppm and 10.39ppm respectively. In butterscotch, tartrazine was present and its concentration is 32.39 ppm. All colours are within the limit. All four samples conform to FSSR standards.

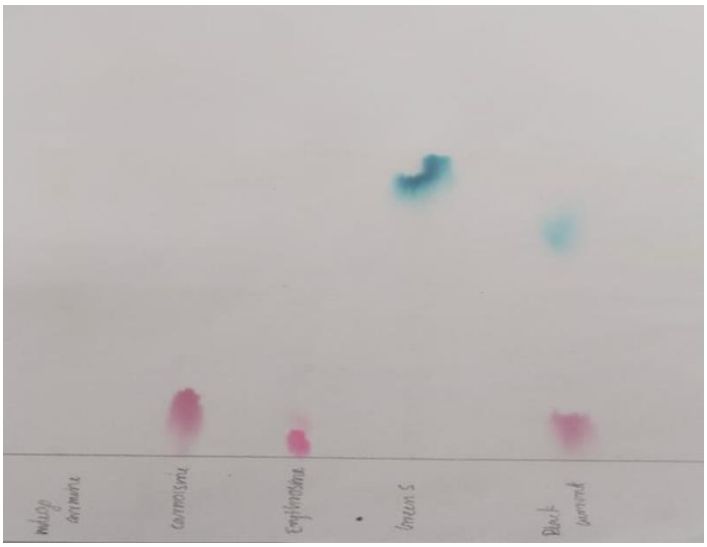

Figure 18: Interpretation of thin layer chromatography

4.6 Analysis of the quality parameters in skimmed milk powder

Two samples of skimmed milk powder were collected from different places. Moisture, milk fat, milk protein, titratable acidity, total ash, insolubility index was tested for each samples as per FSSAI regulation 2.1.10

Skimmed Milk Powder						
Sl No	Moisture%	Total Ash %	Fat%	Titratable Acidity	Milk Protein In MSNF	Insolubility Index
Sample 63	3.07	8.30	1.10	16.3	35.2	0.1
Sample 64	4.01	7.86	0.89	16.7	34.2	0.2
Sample 65	2.18	8.30	1.35	14.1	38.6	0.7

Table 23: Results of Skimmed milk powder analysis

As per FSSR, moisture should not be more than 5%. Among the tested samples, all samples have moisture content within the limit. Milk fat should not be more than 1.5, all of the samples met FSSAI requirement for fat content. Titratable acidity should not be more than 18, all the samples have less value for Titratable acidity. Total ash (% on moisture and fat free basis) should not be more than 9.3, and all samples have less than 9.3, which shows that all samples conform to the FSSR limit. Insolubility index should not be more than 2, all samples have values within limit. Milk protein should not be less than 34. Protein content for all samples is within the limit. Lower protein content may result of spoilage or contamination. All samples do not conform to FSSR standards.

4.7 Analysis of the quality parameters in cheese samples

Two sample of cheese were brought from groceries. Moisture, milk fat and qualitative test for cane sugar are analysed as per FSSAI regulation 2.1.17.

Cheese			
SL No	Moisture	Milk Fat	Qualitative Test
Sample 66	46.0	83.8	Sugar – negative
Sample 67	46.5	86.5	Sugar – negative

Table 24: Results of cheese analysis

Two cheese samples were analysed. Moisture content should not be more than 54, both the samples have moisture within the limit. Moisture test has been carried out in vacuum oven. Milk fat should not be less than 35, both samples satisfying FSSAI standards for milk fat. Qualitative test for cane sugar was negative for both samples. So, both samples conform to FSSAI regulations.

4.8 Analysis of the quality parameters in curd samples

Two samples of curd were collected from different places. Milk fat, milk solid not fat, milk protein, titratable acidity was analysed as per FSSAI regulation 2.1.13.

Curd				
Sl No	Fat%	Titratable Acidity	Milk Solid Not Fat	Protein
Sample 68	2.89	1.04	10.5	4.44
Sample 69	4.22	0.76	11.8	4.55
Sample 70	6.12	1.18	11.6	4.65

Table 25: Results of Curd analysis

As per FSSR, Milk fat should not be less than 4.5, among the tested samples two samples have lower fat content than its prescribed value. Milk solid not fat should not be less than 8.5 all three samples have higher value. Protein should not be less than 2.9, and all three samples have protein content within limit. Titratable acidity should not be less than 0.45, and all samples met the requirement for titratable acidity. Among the three tested samples one sample conform to FSSR.

4.9 Calibration of Pesticicides standards by GC-ECD

Calibration is a method used to determine the concentration of the sample by comparing a standard with a known concentration to the sample with unknown concentration. Calibration curves were drawn by plotting the peak area against the corresponding concentrations of the pesticides. Most commonly found pesticides in milk are organochlorine pesticides. Standard OC mix used for calibration includes Alpha -BHC, Beta-BHC, Gamma-BHC, Delta-BHC, Heptachlor, Aldrin, Heptachlor epoxide, Gamma-Chlordane, Alpha-endosulfan, Alpha-Chlordane, DDE, dieldrin, Endrin,4,4'-DDD, Beta-endosulfan, Endrin aldehyde, 4-4'DDT, Endosulfan sulfate, Endrin ketone and methoxychlor. Standard solutions of OC pesticides was preparted in the 10ppb, 20ppb, 50ppb, 100ppb and 150ppb. The linearity curve for the chosen pesticides is shown in the Figure 20,Figure 21, Figure 22, Figure 23, Figure 24 Figure Figure 25, Figure 26, Figure 27, Figure 28, Figure 29, Figure 30,Figure 31.Figure 32, Figure 33, Figure 34, Figure 35, Figure 36, Figure 37, Figure 38, Figure 39, Figure 40 .Five samples of milk were analysed for pesticides. Samples includes cow milk, mixed milk, buffalo milk, toned milk and pineapple flavoured milk. Pesticide concentration in the sample is calculated by the equation given below.

$$\text{Pesticide Con.} = \frac{\text{Sample area} \times \text{Con. of std} \times \text{Dilution factor} \times \text{Final volume}}{\text{Std. Area} \times \text{Weight of Sample} \times \text{Vol for Evaporation}}$$

Retention time means the amount of time a particular compound spends in a chromatographic column before being detected. RT is specific to each compound under a given set of condition, allowing for the identification of substance when comparing to known standards.

Table 26 shows the OC pesticides and corresponding retention time of the individual pesticides.

OC PESTICIDES	RETENTION TIME
Alpha BHC	8.01
Beta-BHC	8.76
Gamma – BHC	8.96
Delta – BHC	9.68
Heptachlor	11.20
Aldrin	12.33
Heptachlor epoxide	13.66
Gamma - Chlordane	14.46
Alpha endosulfan	14.87
Alpha chlordane	14.97
Dieldrin	15.76
DDE	15.71
Endrin	16.50
4,4’ -DDD	16.82
Beta endosulfan	17.13
Endrin aldehyde	17.47
4-4’ – DDT	18.27
Endosulfan sulfate	18.40
Endrin ketone	19.85
Methoxychlor	20.41

Table 26 : OC pesticide and their retention time

Figure 20 shows the chromatogram for OC pesticide mix standard with concentration 100ppb which includes Alpha –BHC (Retention Time RT=8.01), Beta-BHC (RT=8.76), Gamma-BHC (RT=8.96), Delta-BHC (RT=9.68), Heptachlor (RT=11.20), Aldrin (RT=12.33), Heptachlor epoxide (RT=13.66), Gamma-Chlordane (RT=14.46), Alpha-endosulfan (RT=14.87), Alpha-Chlordane (RT=14.97), DDE (RT=15.71), dieldrin (RT=15.76), Endrin (16.50),4,4’-DDD (RT=16.82), Beta-endosulfan (17.13), Endrin aldehyde (RT=17.47), 4-4’DDT (RT=18.27), Endosulfan sulfate (RT=18.40), Endrin ketone (RT=19.85) and methoxychlor (RT=20.41).

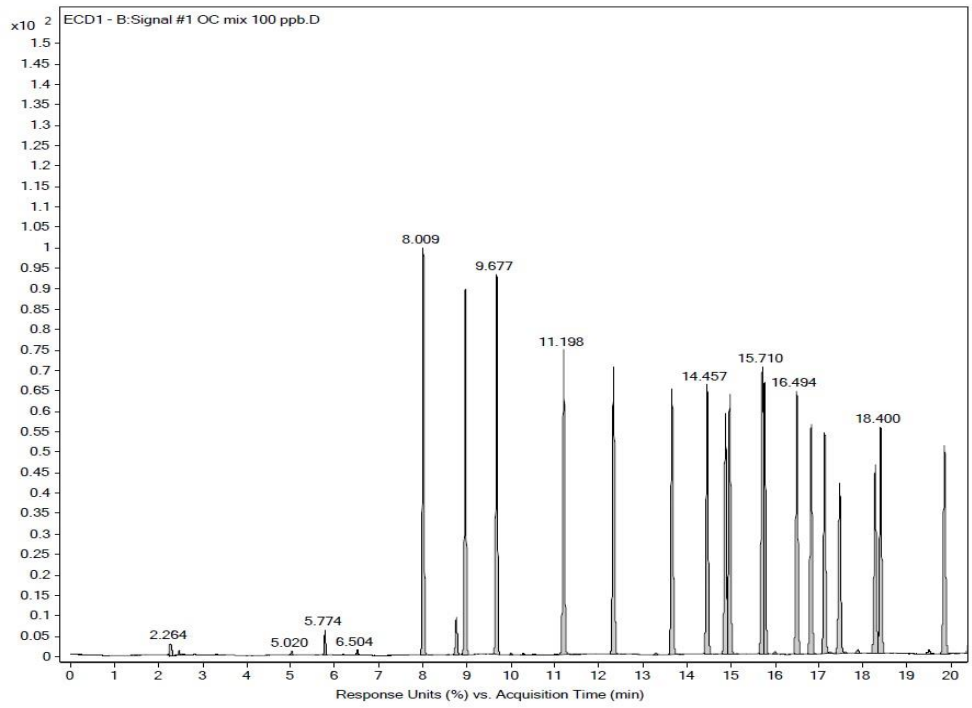

Figure 19: Chromatogram of Standard OC mix with Conc.100 ppb

The calibration plot for the OC pesticide standards with concentrations 10, 20, 50, 100 and 150ppb are shown in the figure. The area of the standards will be used for the calculation of the concentrations of pesticides of the samples under study.

a) Calibration plot for Alpha BHC (10, 20,50,100,150ppb)

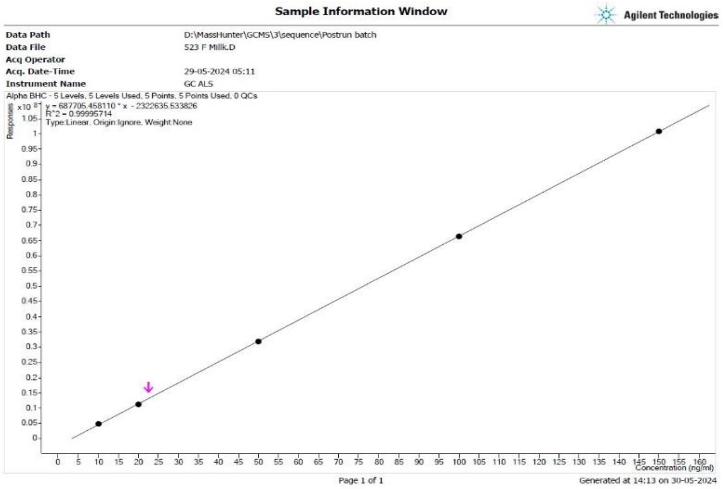

Figure 20: Standard calibration graph of Alpha BHC

b) Calibration plot for Beta BHC (10, 20,50,100,150ppb)

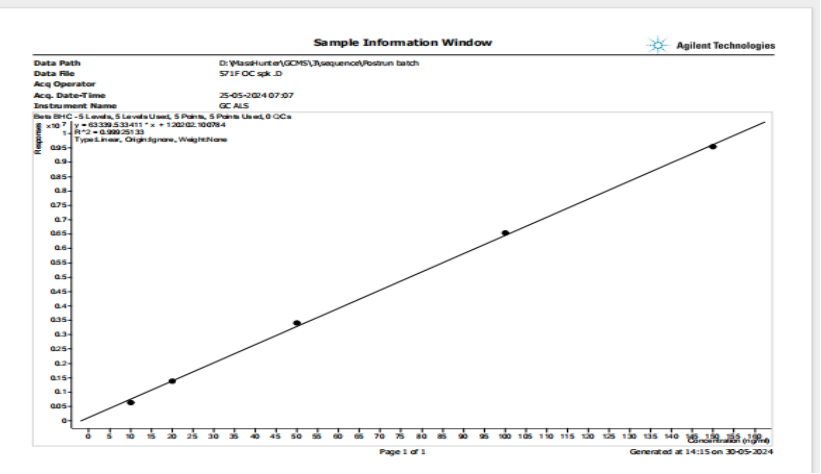

Figure 21: Standard calibration graph of Beta BHC

c) Calibration plot for Gamma BHC (10, 20,50,100,150ppb)

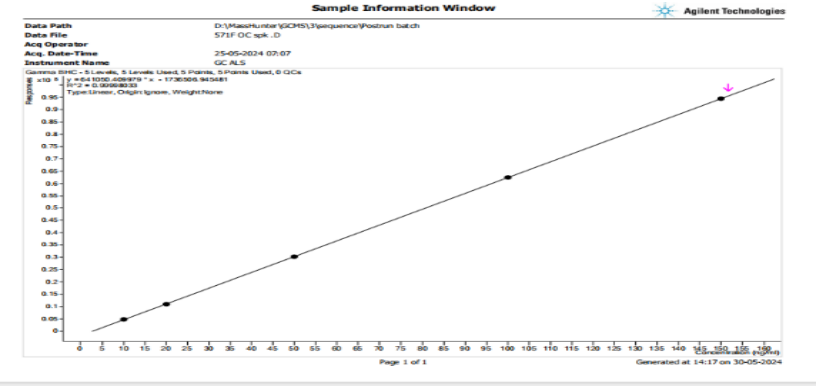

Figure 22: Standard calibration graph of Gamma BHC

d) Calibration plot for Delta BHC (10, 20,50,100,150ppb)

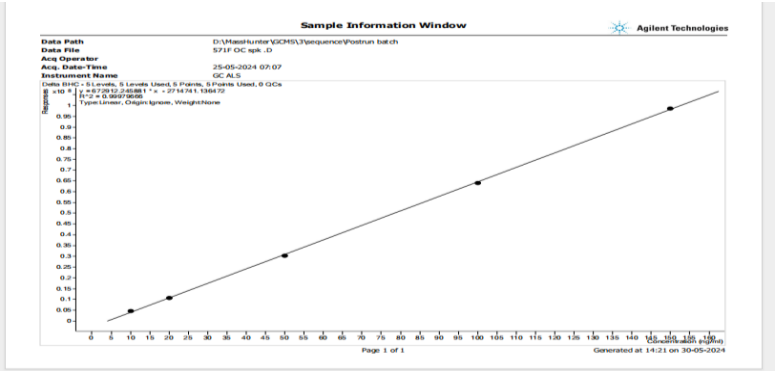

Figure 23: Standard calibration graph of Delta BHC

e) Calibration plot for Heptachlor (10, 20,50,100,150ppb)

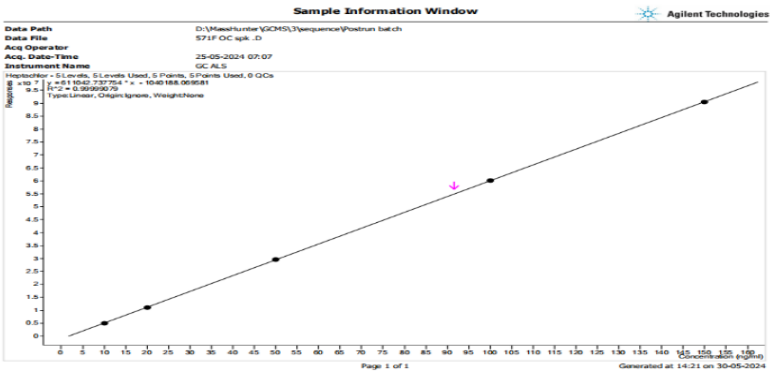

Figure 24: Standard calibration graph of Heptachlor

f**)** Calibration plot for Aldrin (10, 20,50,100,150ppb)

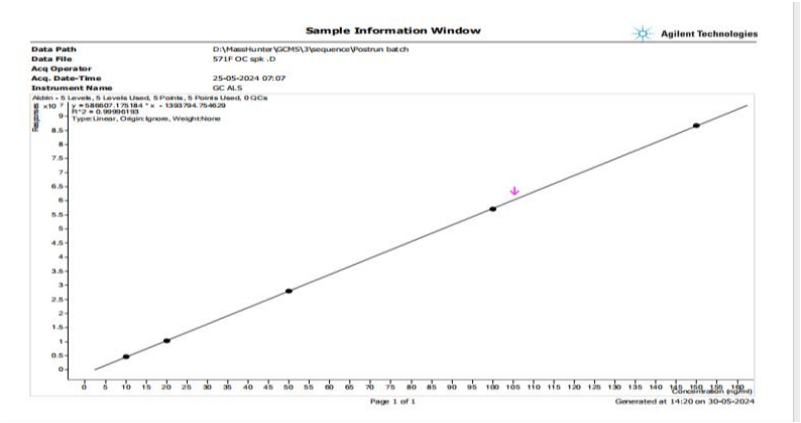

Figure 25: Standard calibration graph of Aldrin

g) Calibration plot for Heptachlor epoxide (10, 20,50,100,150ppb)

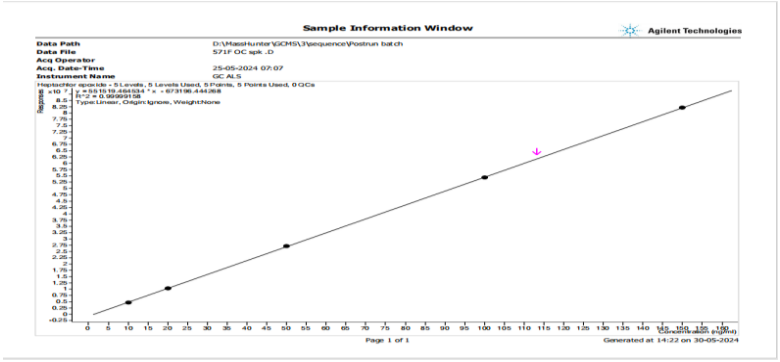

Figure 26: Standard calibration graph of Heptachlor epoxide

h) Calibration plot for Gamma Chlordane (10, 20,50,100,150ppb**)**

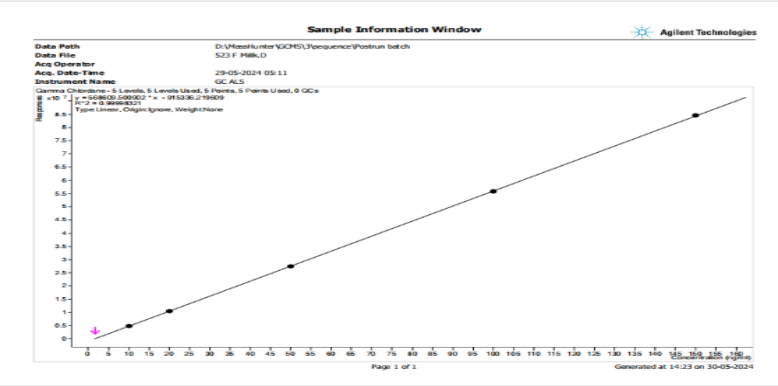

Figure 27:Standard calibration graph of Gamma chlordane

i)Calibration plot for Alpha Endosulfan (10, 20,50,100,150ppb)

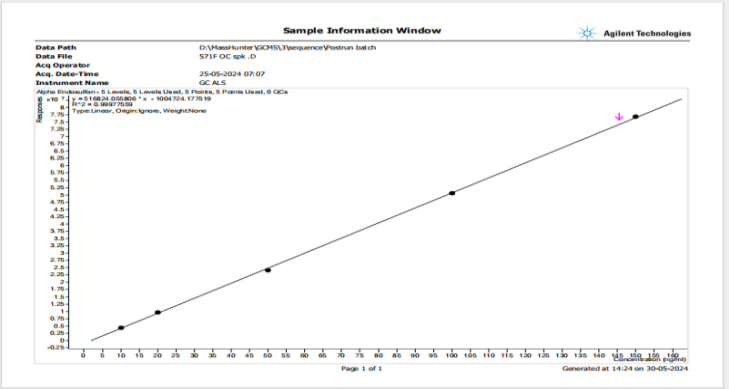

Figure 28: Standard calibration graph of Alpha Endosulfan

j) Calibration plot for Alpha chlordane (10, 20,50,100,150ppb)

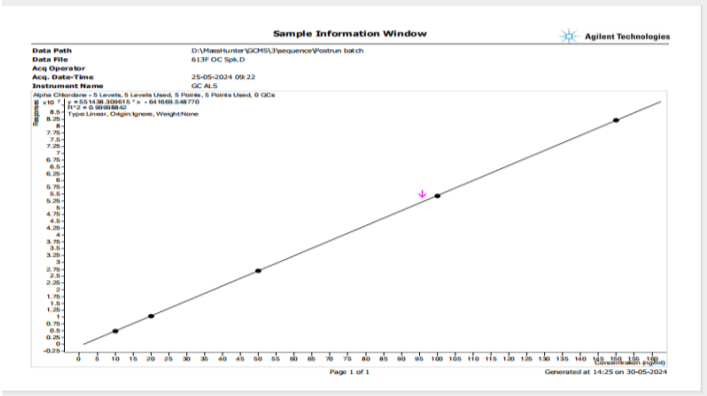

Figure 29 ; Standard calibration graph of Alpha Chlordane

k) Calibration plot for DDE (10, 20,50,100,150ppb)

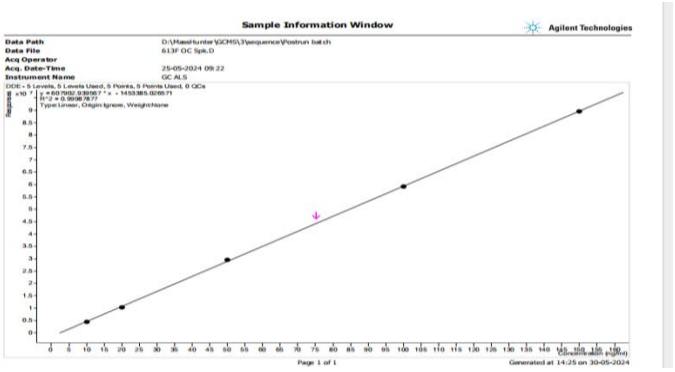

Figure 30: Standard calibration graph of DDE

l) Calibration plot for Dieldrin (10, 20,50,100,150ppb)

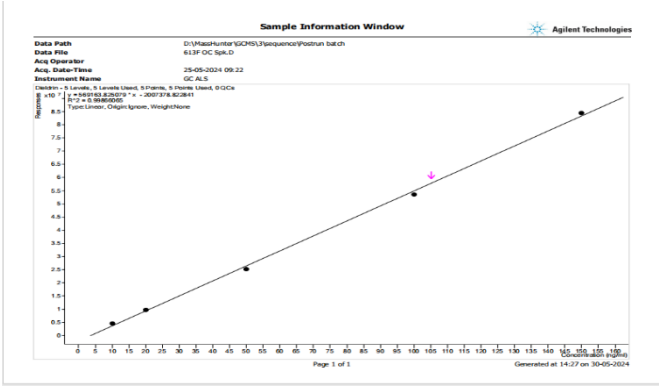

Figure 31:Standard calibration graph of Dieldrin

m) Calibration plot for Endrin (10, 20,50,100,150ppb)

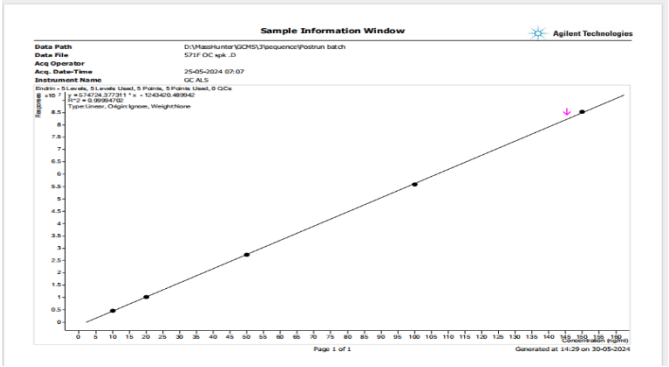

Figure 32: Standard calibration graph of Endrin

n) Calibration plot for 4,4’-DDT (10, 20,50,100,150ppb)

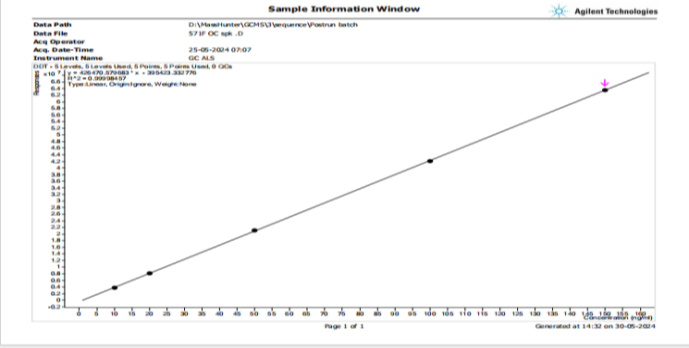

Figure 33: Standard calibration graph of 4,4’DDT

o) Calibration plot for Beta Endosulfan (10, 20,50,100,150ppb)

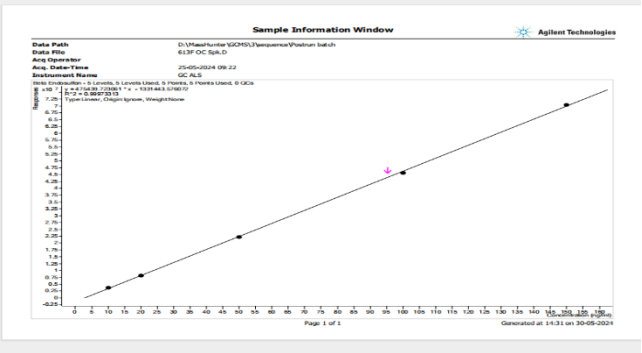

Figure 34: Standard calibration graph of Beta endosulfan

p) Calibration plot for Endrin aldehyde (10, 20,50,100,150ppb)

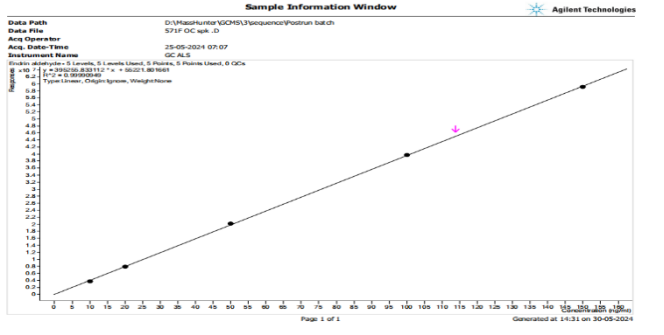

Figure 35: Standard calibration graph of Endrin aldehyde

q) Calibration plot for Endosulfan sulfate (10, 20,50,100,150ppb)

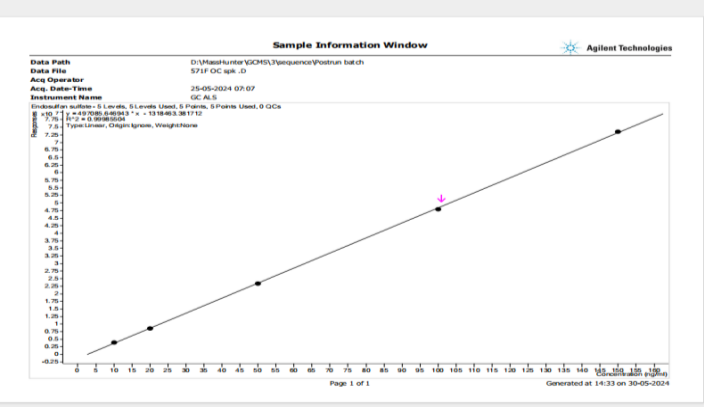

Figure 36:Standard calibration graph of Endosulfan sulfate

r) Calibration plot for Endrin ketone (10, 20,50,100,150ppb)

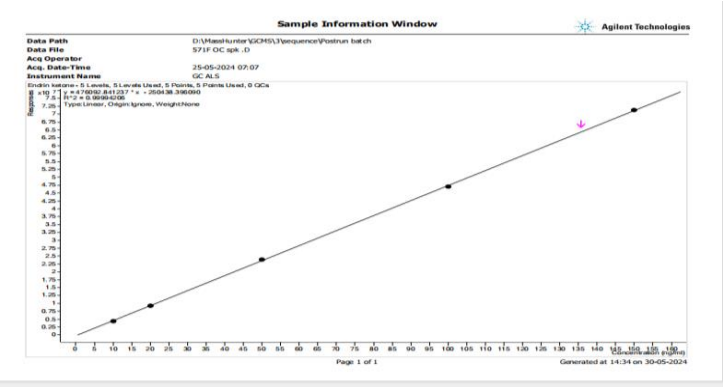

Figure 37: Standard calibration graph of Endrin ketone

s) Calibration plot for Methoxychlor (10, 20,50,100,150ppb)

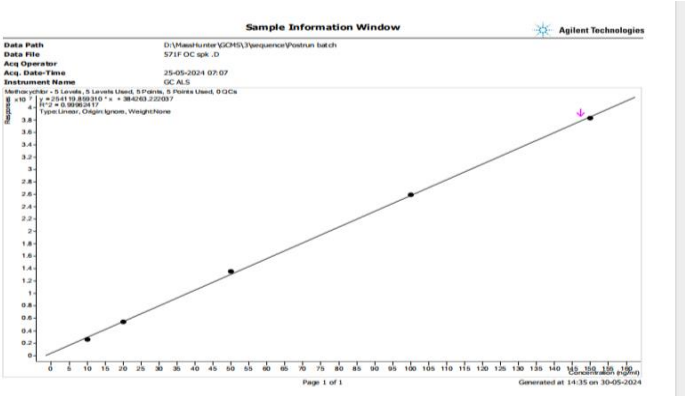

Figure 38:Standard calibration graph of Methoxychlor

t) Calibration plot for 4-4’-DDD (10, 20,50,100,150ppb)

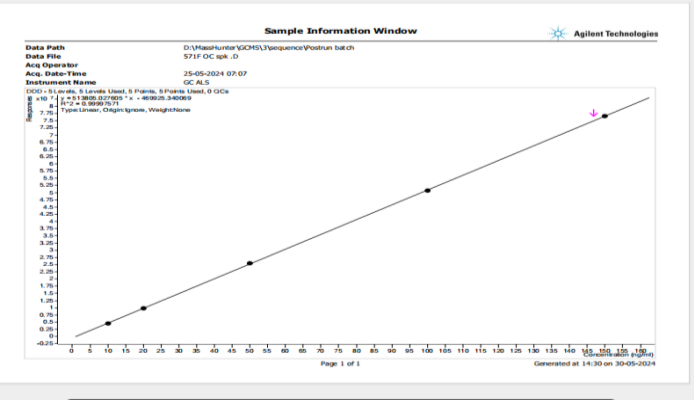

Figure 39: Standard calibration graph of 4-4’DDD

4.9.1 Recovery studies

The optimization of extraction procedure plays an important role in quantification of Pesticides. Recovery experiments provide information on both precision and trueness, thereby the accuracy of the method. Recovery determination is done by spiking the known concentration of Pesticides in the sample matrix and then it is extracted and cleaned up by QuEChERS method. The mean recovery should be in the range of 70-120%. The recoveries were calculated using calibration curve constructed using spiked samples.

The identification of pesticide residue can also be performed by comparison of peak retention time in the samples to those of peak in the pure analytical standards. Each peak was characterized by its specific retention time and area. From this value, the actual amount of Pesticide residue presents in the sample (in mg/kg) was determined by the following formula:

$$\text{Pesticide Con.} = \frac{\text{Sample area} \times \text{Con. of std} \times \text{Dilution factor} \times \text{Final volume}}{\text{Std area weight of sample} * \text{Vol for evaporation}}$$

$$\text{Recovery (\%)} = \frac{\text{Concentration (mg/kg)} * 100}{\text{Spiking level (mg/kg)}}$$

Figure 41 shows the chromatogram for the mixed milk sample spiked with 0.1ppm OC pesticide mix.This peaks acts as a reference point to identify the unknowm compound,measue their quantities accurately.

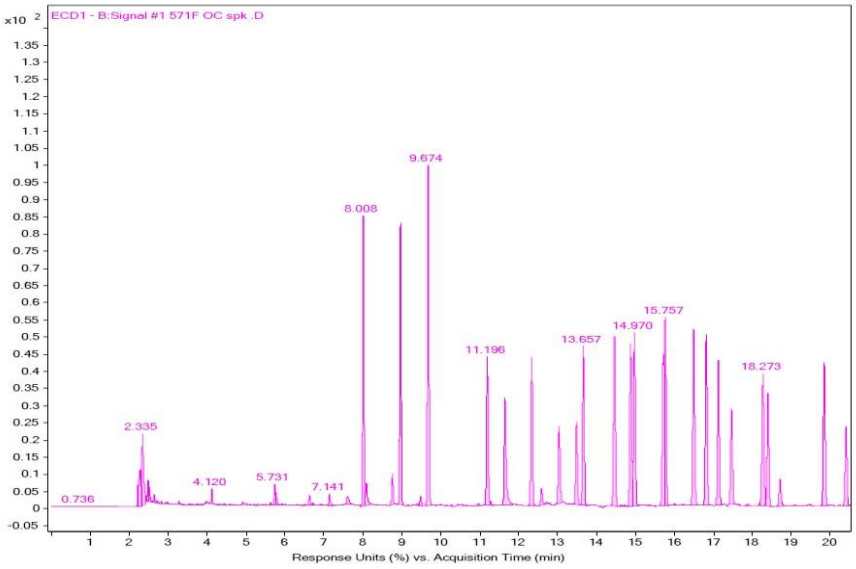

Figure 40 :Chromatogram of spiked OC mix in mixed milk

The recovery studies for 10 OC pesticides were calculated and is given in the Table 26 The recovery percentage calculated for the pesticides was found to be in the range 70-120%.

Sl.no.	Pesticides	RT	Obtained concentration (mg/kg)	Calculated concentration (mg/kg)	Recovery %
1	Alpha BHC	8.01	143.2	0.070	70.6
2	Beta-BHC	8.76	167	0.082	82.3
3	Gamma -BHC	8.96	151.6	0.075	74.8
4	Delta - BHC	9.68	177.6	0.088	87.6
5	Alpha endosulfan	14.87	145.4	0.072	71.7
6	Dieldrin	15.76	148	0.073	73
7	Endrin	16.50	145.4	0.072	71.7
8	4,4’ -DDD	16.82	146.8	0.072	72.4
9	4-4’ - DDT	18.27	149.8	0.074	73.8
10	Methoxychlor	20.41	147.2	0.073	72.6

Table 27: Recovery studies of 10 OC pesticide

4.10 Analysis of Pesticide in milk samples

4.10.1 Pesticide analysis in Mixed milk

Gas chromatogram of the sample was compared with standard pesticide and interpretion was done. By comparing the overlaid chromatograms the peaks of OC standards not match with the peaks of sample.The peaks shown on the chromatogram may be matrix interference peak.So there is no pesticide present in the sample. Figure 42 shows the overlayed GC chromatogram for the mixed milk sample and the OC standards.

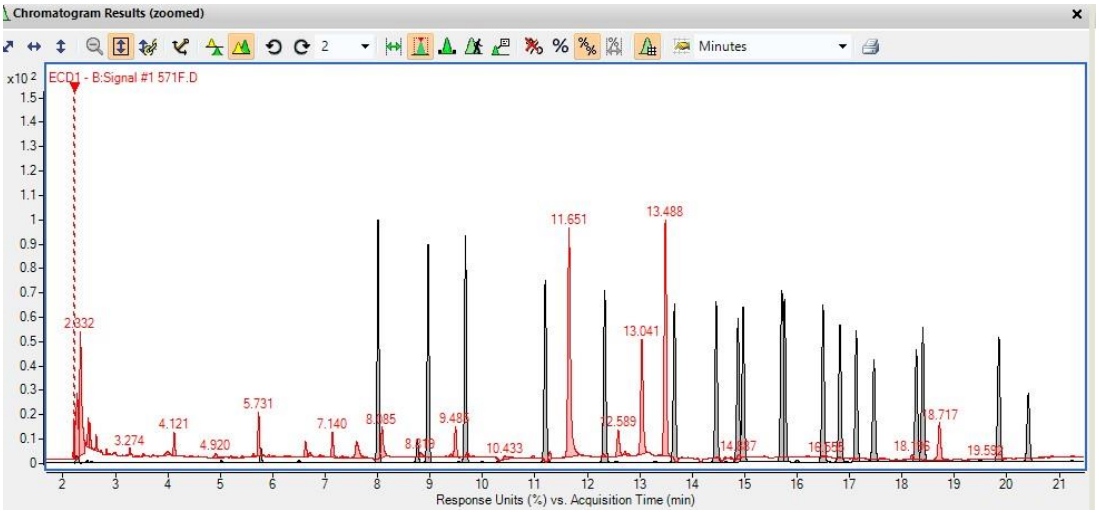

Figure 41: Gas Chromatogram profile for Mixed milk and standard (overlayed)

4.10.2 Pesticide analysis in Cow milk

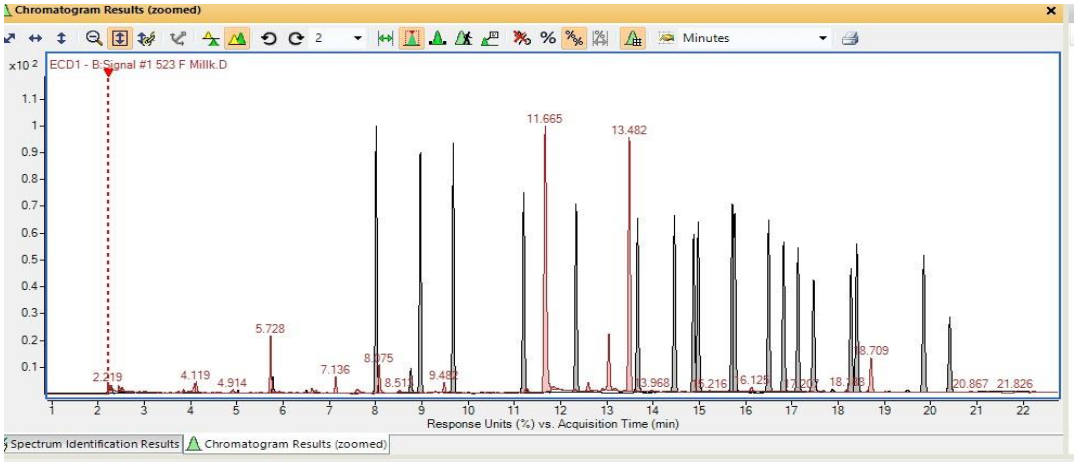

Figure 42 : Gas Chromatogram profile for Cow milk and standard (overlayed)

Chromatogram for the sample was overlayed with the standard chromatogram and RT's were compared they are not matching to each other, so the present cow milk sample not contain pesticide.

4.10.3 Pesticide analysis in Buffalo milk

Chromatogram for the sample was overlayed with the standard chromatogram and RT's were compared they are not matching to each other, so the present buffalo milk sample not contain pesticide.

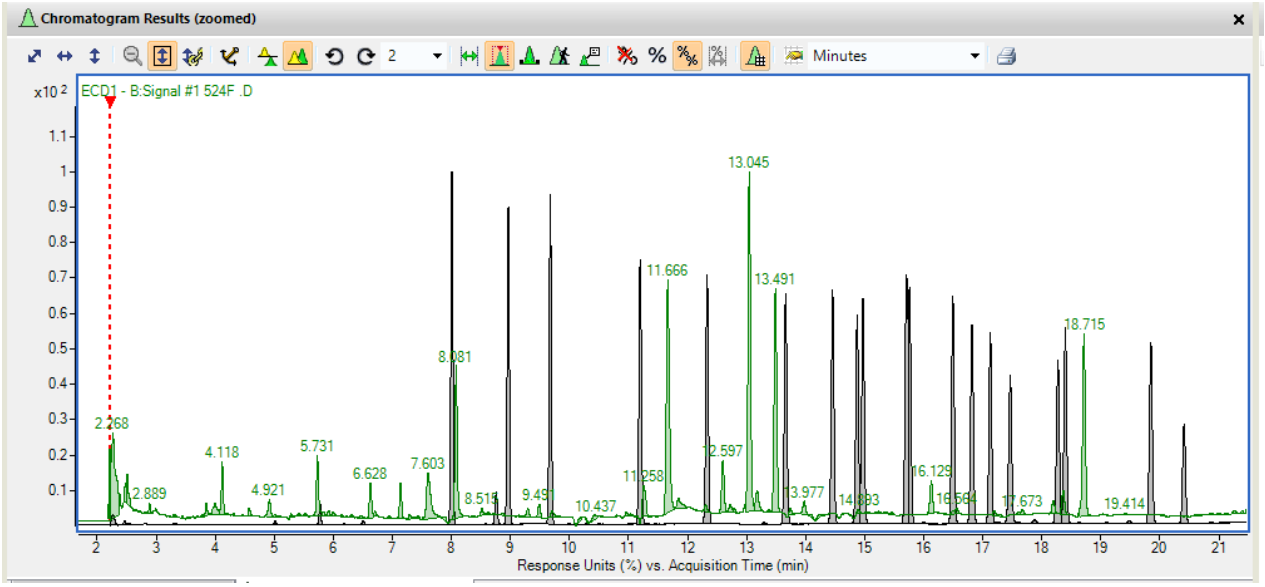

Figure 43: Gas Chromatogram profile for buffalo milk and standard

4.10.4 Pesticide analysis in Toned milk

Chromatogram for the sample was overlayed with the standard chromatogram and,the peaks appearing at the same retention time will indicate the presence of pesticide, here no peaks are present at the retention time of standards.so it confirms that present toned milk sample not containing pesticide.

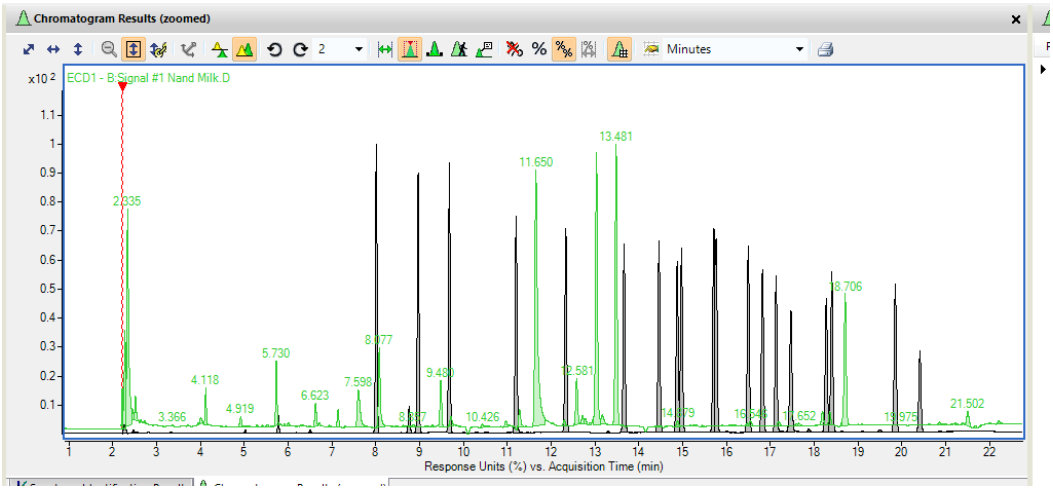

Figure 44 : Gas Chromatogram profile for toned milk and standard

4.10.5 Pesticide analysis in Pineapple Flavoured milk

Chromatogram for the sample was overlayed with the standard chromatogram and RT's were compared they are not matching to each other ,so the present pineapple flavoured milk sample not contain pesticide.

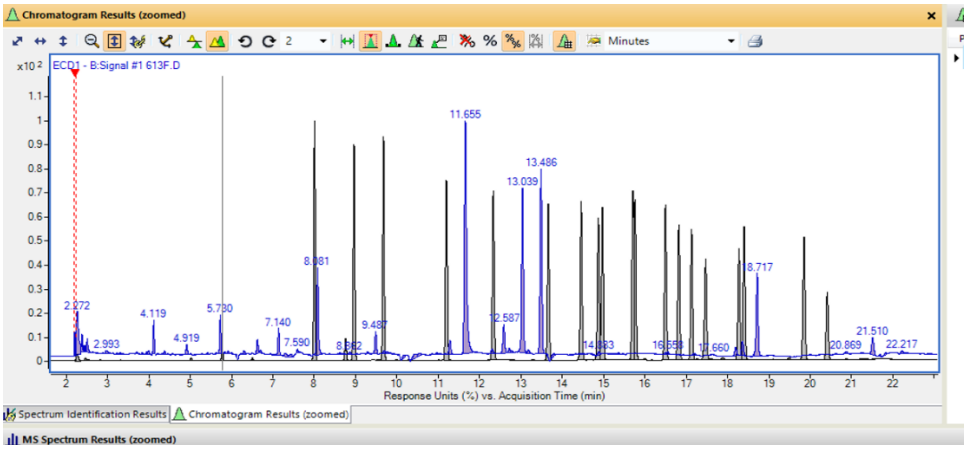

Figure 45: Gas Chromatogram profile for pineapple flavoured milk and standard.

Five samples of milk were analysed for pesticides. Samples includes cow milk, mixed milk, buffalo milk, toned milk and pineapple flavoured milk.In that OC pesticide mix were spiked and analysed the pesticide residue through GC-ECD.By analysing the gas chromatogram of these sample and standard peaks of OC mix it confirms that there is no presence of pesticide in that milk samples.

Chapter 5

CONCLUSIONS

The population growth in megapolises stimulates an increase in dairy products demand, so milk quality has become a global concern particularly in developing countriesand underdeveloped countries. It is often exposed to highest level of fraudulent activity. Mil kadulteration done for economical gain that ultimately led to decrease in its nutritional value and increase safety hazards. Thus, a reliable and efficient quality control system is needed to monitor the quality of milk and milk products, which requires collaboration with regulatory authorities like Food safety and standard authority of India. It is imperative that consumers remain cognizant of the frequent adulterants and quality of milk and milk products. Producers of milk and dairy products should also understand the importance of inspection of their product to make sure they adhere to basic quality standard. In conclusion, the comprehensive assessment of milk and milk products,incorporating both quality parameters and pesticide residue analysis via gas chromatography, yields significant insights into product safety and quality. Through the meticulous evaluation of quality parameters such as fat content, protein content, lactose content, total solids,Titratable acidity, moisture content, ash content, RM value etc, nutritional adequacy are ensured, contributing to consumer confidence and satisfaction. Among 75 samples collected from different states of India, 22.5 % of samples met the required quality standards. The chemical components such as fat, total solids, milk solid not fat were also below the standards set by FSSAI. The practice of adulteration by sugar also was confirmed by this study. This analyses carried out brings more awareness to general public about the malpractices in dairy products. Furthermore, the analysis of pesticide residues using gas chromatography provides crucial information regarding food safety, allowing for the detection and quantification of pesticide contaminants in milk and milk products. This aspect of the study is vital for safeguarding public health and mitigating potential risks associated with pesticide exposure.The findings of this project underscore the importance of adherence to FSSAI regulations in maintaining the integrity and safety of dairy products. By integrating advanced analytical techniques, producers can enhance quality control measures, ensuring that productsmeet or exceed regulatory thresholds for consumer safety. Moving forward, continued collaboration between researchers, industry stakeholders, and regulatory authorities will be essential for further refining quality assessment methodologies and ensuring ongoing

compliance with FSSAI standards. Through such efforts, the dairy industry can continue to provide safe and high-quality milk and milk products that meet the expectations of consumers while prioritizing public health and regulatory compliance.

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