CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Formulation and Evaluation of Low Fat Mozzarella Cheese Using ***Hibiscus rosa sinensis*** Mucilage

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2025

I dedicate this thesis to my loving and supportive family and teachers who have fully helped me in achieving my life goals.

CERTIFICATE OF APPROVAL

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Acknowledgement

All praise and thanks to the Supreme Allah to whom we only bow down. I would also like to express my gratitude to my family and friends for their continuous mental and physical support and prayers. I would also wholeheartedly say a big thank you to my supervisor Dr. Rizwan ur Rehman (Associate professor , Department of Bioinformatics and Biosciences, CUST) for his support.

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Abstract

Mozzarella cheese is a fermented dairy product with a milk fat content ranging from 30% to 45% on a dry basis. This fat content plays a crucial role in providing the cheese with its characteristic stretchability, making it highly desirable in the food processing industry. Plant mucilages have several uses in the food, pharmaceutical and cosmetic sectors. Belonging to the Malvaceae family, *Hibiscus rosa-sinensis* Linn is a source of mucilage and subtropical as well as an ornamental plant. The principal aim of this research is to extract mucilage from Hibiscus rosa sinensis and to analyze its physicochemical characteristics and quantification. This research also intended to realize the low-fat mozzarella cheese by utilizing the mucilage of *Hibiscus rosa-sinensis* as a carbohydrate-based fat substitute. The sensory qualities evaluation of *Hibiscus rosa-sinensis* mucilage was done by an expert panel on the 9-point hedonic scale and a complete physicochemical investigation of *Hibiscus rosa-sinensis* the mucilage at different concentration has been carried out (0.625% to 2.5% v/v). It is shown in the results that Hibiscus rosa sinensis mucilage keeps the positive sensory features unchanged while adding to its nutritional area, thus, it is the best choice for those who want to keep a healthy lifestyle. As explained in the paper, the various research results show how plant-based substitute fat is effectively replacement of the fat in low-fat mozzarella cheese for people who are interested in healthy diet but are not willing to give up texture and flavor. The results demonstrated an important reduction in the fat content of all the produced recipes; besides the T1 (0.625%) giving the consumers the best experience of texture taste, T3 (2.5%) also contributed to the crustiness and functionality improvement of the low-fat mozzarella cheese.

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Abbreviations

AOAC	Association of Official Analytical Collaboration
CHD	Congenital heart disease
CLA	Conjugated linoleic acid
CVD	Cardiovascular disease
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTGS	Distributed Temperature Gradient Sensing
EPS	Exopolysaccharide
F-C	Folin-Ciocalteu
FRAP	Fluorescence recovery after photobleaching
FTIR	Fourier Transform Infrared
GA	Gallic acid
HFD	High Fat Diet
HLC	High lactose content
LDL-C	Low-density lipoprotein Cholesterol
LF	Low Fat
LLC	Low lactose content
MLC	Medium lactose content
MWPs	Micro particulated whey proteins
MetS	Metabolic syndrome
PAGE	Polyacrylamide gel electrophoresis
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
S.D	Standard deviation
SCFAs	Short-chain fatty acids

TNF-α	Tumor necrosis factor-alpha
TPC	Total polysaccharide content
USD	United States dollar
UV	Ultraviolet
WEL	Water extract of leaf
aw	Water availability
ppm	Parts per milion

Chapter 1

Introduction

1.1 Background

Cheese is a highly nutritious and flavorsome food and is a vital source of vitamin D and calcium, as well as a big dietary source as it is almost full of the vitamins, fats, proteins, minerals, and other nutrients. Cheese is commonly appreciated for its sensory qualities, and it also functions as a nutritional booster that can be added to various dishes. In food systems, it is meant to exhibit specific functional properties in both its raw form (e.g., grateability, shredability, sliceability) and cooked form (e.g., flavor, mouthfeel, flowability). The requirement for cheeses and cheese toppings having specific functional properties is still on the increase, especially in pizza, burger, and sandwich industries. As an incredibly versatile product, cheese comes with a very large range of textures, flavors, and applications. Not only cheese made up of casein gets affected by the casein content and its quantity, but also the kind and the strength of casein interactions in cheese, as well as the cheese's composition and ripening condition, play significant roles in determining its macro and microstructure [1].

Mozzarella cheese, which is referred to as pasta filata (stretched-curd) in the family of cheese, has a distinguishing fibrous texture that is obtained by means of the stretching procedure. At the curd production stage, the cheese is taken out of the hot water and then cooled before stretching it. It is not enough to consider pH correctly, but also the regulated. The functional properties mainly including elasticity, fluidity, viscoelasticity, and oil release upon heating are altered by the process conditions that determine the cheese microstructure and composition [2].

Fast food has become an increasingly prominent part of the American diet, with its consumption rising significantly since the early 1970s. In 1970, there were around 30,000 fast-food outlets, which grew to 140,000 by 1980, accompanied by a 300% surge in sales. By 2001, the number of fast-food locations had reached approximately 222,000, generating over $125 billion in revenue. Projections for 2002 anticipated a 4.1% increase in outlets and an estimated $130.1 billion in sales. Additionally, the report revealed that 30% of consumers considered restaurant or fast-food meals essential to their lifestyle, and 60% indicated they planned to maintain the same frequency of fast-food consumption in 2002 as they did in 2001 [3].

Cheese is consumed by 74% of all children aged 2 years to 19, only 75% by males, and 73% by females. There appears to be a general decrease in cheese consumption among different age groups, as younger eaters are found to consume more than their older counterparts implying an inverse linear relationship of reduction in cheese consumption with age. Furthermore, there is a greater number of females aged 6 to 11 that consume cheese than there are aged 12 to 19 [4]. New data on food consumption by 10.1% of the survey participants was analyzed. Their analysis showed that a part of them 6.3% were those who ate it regularly once a month, and 2.7% at least one time every week, and the remaining 1.1% led the race with them being the most frequent eaters. It is noted from the research that fast-food intake was mainly a problem for the younger people but according to the survey, it was gradually becoming an issue for the elderly ones as well ($P < 0.001$).Young fast-food comers were the people who also smoked the most and who had higher school degrees opposite to the naysayers. Further, they have indicated to have more energy intake than the recommended level and were more likely to surpass the recommended energy limits [5].

Fatty foods with cheese components have several adverse health consequences. A lot of cheese are already prepared that is widely used in burgers, pizzas, and sandwiches. The high sodium and saturated fat in it are directly linked to serious problems such as obesity, hypertension, and cardiovascular disease. Studies show that cheese significantly reduces the consumption of low-density lipoprotein cholesterol (LDL-C) in the case of other animal fats, so it can be a healthy alternative. However, in the long run, too many pieces of that cheese from fast food restaurants can raise the cholesterol level and add more calories overall that will eventually lead to obesity. Oversupply of salt in fast food cheese products can provoke hypertension thus leading to heart health deterioration. Cheese, indeed, is the storehouse of minerals, evaporating and vitamins such as the notice of calcium, but it's equally important to be cautious and use moderation when consuming too much of it, especially fast food because it's likely to increase the risk of metabolic disorder like insulin resistance and Type 2 diabetes [6].

Hibiscus rosa sinensis L., popularly known as Chinese *hibiscus* or shoeblack plant, is an often grown plant belonging to Malvaceae, which is native to tropical and subtropical areas. This evergreen shrub with above-ovoid leaves has one large picture-perfect flower with five petals of different colors, such as red, pink, yellow, and white. The flowers of plant are majorly attractive to the pollinators such as bees and butterflies. Optimum growth is achieved in well-drained and full-sun soil, but still the plant is extremely sensitive to frost. The phytochemical analyses confirm that *H. rosa-sinensis* contains high levels of anthocyanins, quercetin and other flavonoids, tannins, saponins, and sterols, such as β-sitosterol, as well as sterols like β-sitosterol and stigmasterol. Along with this, it provides the necessary vitamins i.e thiamine, riboflavin, and ascorbic acid and minerals i.e calcium and iron which results in its connection to traditional medicine to cure respiratory diseases and skin diseases and for hair health as well [7].

The plant's extracts also demonstrate antioxidant, anti-inflammatory, and antimicrobial effects, which shows that they could be used in the pharmaceutical industry. Another item to discuss is that the extract can produce a slimy solution, about the same thickness of petroleum jelly when it is combined with water, in a solution with a pH of 7.1. Therefore, this makes the extract a contender for drug formulations because of its low levels of microbial contamination and acceptable heavy metal concentrations [8].

These are the plants whose mucilage is normally the most popular fat replacers in different types of food:

Okra (*Abelmoschus esculentus*) [9] ,Cactus (*Opuntia species*, e.g., prickly pear) [10],Guar (*Cyamopsis tetragonoloba*) [11],Psyllium (*Plantago ovata*) [12],Flaxseed (*Linum usitatissimum*) [13] ,Chia seeds (*Salvia hispanica*) [14],Icelandic moss (*Cetraria islandica*) [15],*Hibiscus* (*Hibiscus sabdariffa*) [16],Tamarind (*Tamarindus indica*) [17],Mallow (*Malva species*) [18].

Carbohydrate-Based Fat replacents can be achieved with a combination of maltodextrin. It bears a creamy mouthfeel full of full-fat cheese and is found in the present of trans-fat and cholesterol mainly [19]. Guar gum doing two things, by increasing viscosity and reducing syneresis, guar gum is a texture modifier used to improve the quality of low-fat cheese [20].Protein based fat replacements are those that whey protein concentrate are usually used to make cheese recipes better textures and mouthfeels by the way of replacing fat [21].

Mucilage, a natural polysaccharide found in various plants, has diverse applications across multiple industries, particularly in food for thickening and stabilizing. It aids digestion, promotes skin tissue repair, and enhances wound healing by improving cell function and collagen synthesis. Additionally, its low toxicity and adaptability make it valuable in nanomedicine and biotechnology, with potential to replace synthetic materials sustainably, prompting further research into its scalability and functionality [22].

The prevalent nature of the mucilage derived from the *Hibiscus rosa sinensis* leaves is the presence of the type of carbohydrates and polysaccharides, more precisely. The sugar test and uronic acid identification techniques are involved in the process. Molecular tests of mucilage showed a definite amount of carbohydrates and uronic acids, but no tannins, sulfates, and chlorides were observed. Optically, it shows a greenish color with a peculiar smell and when dissolved in water, it forms a bulky, highly viscous solution. The mucilage is an excellent candidate for the pharmaceutical industry, given its great gel formation, low water content, and the almost provided neutral pH of 7.1. Also, it stands satisfactory by being a still effected bacterial count, a lead concentration that is just above detection level and an adequate increase in the concentration of arsenic in heavy metals over the control sample [23].

Under the study's findings, an argument is made for the hypothesis that *Hibiscus rosa sinensis* leaf mucilage can be used as a pharmacological adjuvant for the administration of medications. It is one of the acceptable, appropriate, and well-matched formulations in the applications of novel drug delivery systems, especially controlled release applications, that can be seen among numerous physicochemical evaluations [24].The *Hibiscus rosa sinensis* leaves were separated from mucilage using the solvent precipitation method. The mucilage's antioxidant activity was evaluated for different assays including Hydrogen peroxide, hydroxyl, superoxide, nitric oxide, and FRAP. Even in the event of mucilage that shows at every test, it was noticed that its capacity to scavenge radicals was not as effective as that of the relevant standards. The over-agar diffusion method-based antibacterial assay also performed well against the gram-positive Streptococcus pyogenes and the gram-negative Klebsiella pneumoniae [25].It was discovered that the polysaccharide-rich *Hibiscus* mucilage has superior physicochemical qualities neutral pH, a greater swelling index, and favorable, lower particle size and flow characteristics [26].

1.2 Problem Statement

Traditional low-fat mozzarella cheese poses serious threats to its functional attributes including inferior flavor, texture, stretchability, fat leakage and meltability, which decreases customer appeal, while using synthetic additives may pose health risks.

1.3 Aim

This research aims to develop low-fat mozzarella cheese using *Hibiscus rosa sinensis* mucilage as a fat replacer to improve meltability, texture, flavor and the cheese's physicochemical, and sensory properties to meet consumer expectations.

1.4 Objectives

The main objectives of this study are as follows:

i To formulate low fat mozzarella cheese using different concentration of *Hibiscus rosa sinensis* mucilage.

ii To investigate the functional groups of mucilage through FTIR analysis and antioxidant properties of whey proteins and cheese.

iii To evaluate the cheese's physicochemical and sensory attributes and comparing these with market control.

1.5 Scope of Study

This study focuses on formulating and evaluating low-fat mozzarella cheese using *Hibiscus rosa-sinensis* mucilage as a natural fat replacer. It aims to develop a healthier alternative while preserving key sensory, textural, and functional properties. The research involves extracting and characterizing mucilage, assessing its physicochemical properties, and testing its suitability in various cheese formulations. Key evaluations include pH, moisture, fat content, texture (firmness, stretchability, meltability), and consumer acceptability. Additionally, microbial stability and shelf-life are analyzed to ensure product safety. By exploring *Hibiscus rosa-sinensis* mucilage as a fat replacer, this study contributes to healthier dairy alternatives and innovations in food technology.

1.6 Impact on Society

Low-fat mozzarella cheese can have a meaningful impact on society, particularly in health and nutrition. Consuming it encourages consuming less calories and fat, which helps control weight and lowers the risk of cardiovascular disease and obesity. Additionally, it accommodates a range of dietary requirements, making it appealing to people who are health-conscious as well as those who have certain limitations, like lactose intolerance. More broadly, the support for low-fat mozzarella by the government and public health programs to encourage healthy eating behavior will also be coherent. Moreover, the increasing interest of consumers in nutrient-rich products would induce the food business to innovate, inspiring individuals to choose wholesome choices and laying the foundation for the general change in food culture.

Chapter 2

Literature Review

2.1 Cheese

Cheese, being an integral part of a diverse array of global diets, is milk protein coagulated, mostly casein then allowed to ferment and age to provide the right aromas and textures. The production process of curd starts with the boiling of milk and its followed by the adding of the culture and the starter for rennet. After that, slices, draining, and making the molds are carried out. The nutritional content of cheese is boosted not only by its high protein and fat content but also by its great amounts of calcium, phosphorus, vitamin A, and B12 [22]. Beyond just being a high-protein dish, it is also culturally significant in many different cuisines, which adds to its global appeal. The global cheese market is expected to reach approximately USD 182.5 billion by 2025 [27]. However, cheese consumption raises some health concerns, such as lactose intolerance and cardiovascular issues linked to its saturated fat content, which can increase cholesterol levels [28]. One of the most popular fermented dairy products is cheese. Its quality and health advantages are greatly enhanced by the aging process. Due to intricate biochemical and metabolic processes, cheese acquires a wide variety of unique flavors and textures as it ages, resulting in a dynamic change in the microbiological composition. Numerous useful bioactive substances may be released throughout the ripening process. Various tactics are used to quicken this process, with the main goal being to raise the rates of lipolysis and proteolysis. However, aging can result in microbiological spoiling, including early and late blowing. Thus, to maintain ideal circumstances, smart ripening rooms with computerized monitoring systems such as sensors, software platforms, and temperature and humidity data loggers are crucial [29].

2.2 Mozzarella Cheese

A popular cheese, mozzarella is known for its delicate flavor, soft texture, and distinctive stretchiness, which results from the pasta filata method. In order to curdle the milk proteins, rennet is added after milk has been fermented with thermophilic lactic acid bacteria. Rennet is a crucial enzyme for making cheese; it is often made from the abomasum of unweaned calves and contains chymosin, which causes milk to coagulate. Alternative sources including microbial rennet from fungus (*Mucor miehei, Rhizomucor pusillus*) and recombinant chymosin from genetically modified organisms (*Aspergillus niger, Kluyveromyces lactis*) are now commonly used due to ethical and industrial demands. Rennet plays a crucial role in the development of curd in low-fat mozzarella cheese by dissolving kappa-casein and creating a gel matrix that enhances texture and helps preserve moisture. Additionally, it helps the cheese stretch during the pasta filata process and, by limiting the degradation of proteins, may subtly improve flavor [30]. The distinctive elasticity and smooth surface of mozzarella are achieved by heating and stretching the curd in hot water [31].

2.3 Physiochemical Attributes of Mozzarella Cheese

2.3.1 Chemical Attributes

2.3.1.1 Water Activity and Moisture Content

The quality of mozzarella cheese is significantly affected by its moisture content and water activity. Moisture content is a necessary factor in giving the cheese its particular texture, melting abilities, as well as its lifespan. Mozzarella comes in two major types: low-moisture mozzarella with 45-52% moisture used for the most part in the making of the pizza, and high-moisture mozzarella with 52-60% moisture that is usually not used in recipes but is eaten as it is [31].The higher moisture levels facilitate the smoothness of the cheese by allowing more protein molecules to bond with water; however, there can be a reduction in the durability due to the fact that it becomes easier for the pathogen to grow on a highly humid cheese. Successful freezing also can be referred to as an increase of the shelf life of fresh cheese [32].

Water activity (aw) is another key factor that decides the availability of water for biochemical reactions and microbial activity. It is very critical in the sense of not only the healthiness of the products but also proliferative properties such as the stretchability of some foods and its ability to hold back during melting in the case of cooking. To put it another way, reduced-fat mozzarella usually suffers the excess loss of water and still have unsatisfactory texture and melting properties if specific adjustments are not made. Careful manipulation of these three factors namely time of production, duration of conservation, and the alignment of customer expectations in food preparation with culinary uses, leads to less variation in the characteristics of cheese compared over time [33].

2.3.1.2 pH

Mozzarella cheese's pH value is very essential in determining its quality, texture, and flavor profile. In general, the pH of regular mozzarella ranges from 5.1 to 5.4, and this is the result of the factors that are part of the cheese production process. Firstly, the pH of milk that will be used for mozzarella cheese production is a key factor here, as this can directly indicate the quality and freshness of the milk. A higher pH (above 6.8) might be the result of bacterial contamination, for example, mastitis, the treatment of which could negatively affect the final product [34].

Throughout the cheese making process, the pH decreases as lactic acid bacteria convert lactose into lactic acid. This development of "wet acid," which primarily occurs before whey drainage, is crucial for calcium solubilization, affecting the curd's texture and moisture content. Maintaining a controlled pH during this stage can result in a smoother, less curdy texture in the final cheese. Moreover, the pH also affects flavor generation by means of proteolysis where proteins are generally broken down into smaller portions like peptides and amino acids [35].

2.3.1.3 Acidity

An important factor that is an issue about mozzarella cheese is the acidity which is the reason for that as it affects the flavor, texture, and overall quality. Normally, the pH level of fresh mozzarella ranges between 5.1 and 5.4, that trend shows it is somewhat acidic. This acidity basically comes from the lactic acid bacteria doing fermentation on lactose and turning it into lactic acid, lastly the cheese making process. When pH is lower during the production process, more calcium is released into the solution, resulting in a smoother texture and higher water content.

On the other hand, if pH is too high, the cheese will look hard and will have an unpleasant taste that is often perceived as ”acidic” and ”sour”. Equally, the acidic balance also influences the cheese's properties such as melting capacity and stretching, which are very important properties for pizza and pasta [34].

2.3.1.4 Protein and Casein Content

The majority of the fat in mozzarella is composed of saturated fatty acids, which comprise all mozzarella cheese has always been a well-loved food and it should be noted that it is high in protein content which is very important for many people who follow different diets. A case to illustrate is the fact that usually an ounce of part-skim mozzarella supplies around 6 to 7 grams of protein. Most of the protein in mozzarella is casein which happens in the form of a hydrolyzate in the stomach during the digestive process. The protein ensures a gradual release of amino acids [2].

The protein structure of mozzarella cheese is mainly composed of casein, which makes up about 80% of its total protein content. Casein exists in micelles, which are aggregates of casein molecules that give the cheese its distinctive texture and functional properties. These micelles trap fat globules and water, contributing to mozzarella's characteristic moisture retention and elasticity. The structure is influenced by factors such as pH, temperature, and calcium presence, all of which affect the stability and interactions within the casein network [35].

Moreover, casein is also important for cheese to be melted and stretched which are needed for cooking purposes, particularly in pizza and pasta. The different types of casein, such as alpha-casein and beta-casein, can further influence the cheese's texture and flavor. The cheese's water-insoluble and water-soluble calcium content is the most essential part, however, since it is responsible for the functionality of flavor and texture during storage and processing [36].

2.3.1.5 Lipid and Fat Content

Mozzarella cheese is well-known for its smooth and genuine taste and creamy texture, as well as low lipid content in the structure of fat which improves nutritional value. The fat content varies depending on the type; as such, whole-milk mozzarella usually contains about 22 grams of fat per 100 grams, whereas part-skim mozzarella is around 15 grams. On top of that, mozzarella is of lower total fat than numerous hard cheeses. Thus, it is one of those options, which if it is taken by the people who are worried about fat, does not affect the flavor of the meal. With total fats lower than many hard cheeses, mozzarella is a popular choice for those who want to cut fats in their diet but still enjoy flavor.

Saturated fatty acids are mostly found in mozzarella cheese, and they make up about 60% to 70% of its total fat content. For example, you will find around 6 grams of fat per one-ounce serving (about 28 grams) of whole milk mozzarella cheese, with about 4 of those grams being saturated fat. Examples of saturated fatty acids include myristic acid, stearic acid, and palmitic acid. Saturated fatty acids occupy a large part of the lipid profile of cheese, but by including low amounts of saturated fats, mozzarella is distinguishable among the highest levels of fats. It is thus also recommended to eat it in moderation. Furthermore, mozzarella can be eaten due to its good amount of conjugated linoleic acid (CLA), which is a naturally occurring fatty acid in dairy products. CLA has been associated with various health benefits such as anti-cancer properties and improved metabolic health [37].

2.3.2 Lactose Content

The lactose amount in the mozzarella cheese can be different because of the type of milk and the process of production. Because lactose is not completely eliminated during the fermentation process, traditional mozzarella prepared from cow's milk still contains some of it. However, the bacteria that keep on working during storage is what causes the lactose to be reduced thus the process is a slower continuous loss of lactose. Furthermore, lactic acid bacteria are encouraged to further lower the lactose concentration during manufacturing by procedures such hot water stretching [38].

Researchers evaluated the impact of varying lactose-to-casein ratios in cheese milk by preparing Mozzarella cheese using high (HLC), medium (MLC), and low (LLC) lactose levels. Their findings showed that reducing lactose content in the milk (as in LLC and MLC) resulted in lower residual lactose, galactose, and lactic acid compared to cheeses made with higher lactose content (HLC). The adjustment of lactose also influenced the texture and functionality of the cheese—LLC variants were firmer and had less stretchability than others. Furthermore, the reduction of lactose content was slower in the low-lactose cheeses, which resulted in a better balance and control of the pH during ripening, the reduction of meltability, and the formation of blisters in the crust during baking. The research emphasizes a different treatment of feeding cows sweet dairy milk, as a mechanism to control the stretchiness, firmness, and melting of low-moisture, part-skim Mozzarella that is non fermented [39].

2.3.2.1 Enzymatic Activity

Mozzarella cheese is affected by three elements that are mentioned in the text, they are the texture, the taste, and the functions in the development of the enzyme content of the cheese. This revealed that the changing in trash and flowing of cheese with time is highly affected by the digestion of protein that uses proteolytic enzymes. It was found that the amount of lactose in cheese is irrelevant to enzyme activity, as the degree of proteolysis varied little among the cheeses with differing lactose-to-casein ratios. Caseins are the most attacked part of the stabilized culture of enzymes, who assist the cheese in getting its structural identity. This research uses proteomic techniques, such as mass spectrometry and electrophoresis, to assess the protein degradation. It also emphasizes the important part that fresh milk and proper methods of cheese manufacturing play. Only the predicted byproducts, para- -casein (from -casein via chymosin) and -caseins (from β-casein by plasmin), are created under these circumstances. However, the presence of aberrant fragments such as degraded β-casein and αS1-casein suggests production shortcuts and the use of inferior materials like stored milk or repurposed curd. The study also looks at the effects of storage, emphasizing how prolonged storage at different temperatures can cause unwanted protein changes and lower the quality of the final product. Analytical techniques like sodium dodecyl sulfate-PAGE assist in uncovering such inappropriate practices, thus helping to maintain product integrity and safeguard consumer trust [40].

2.3.3 Physical Attributes

2.3.3.1 Texture

The important factors such as the stretching process, pH, and calcium levels will determine the texture and the hardness of mozzarella. Aligned casein fibers connect the fibrous texture of the cheese with water and fat while stretching, thus, creating the cheese's flexibility. The pH range is 5.2–5.3, which is best for calcium-casein shaking and for a smoother texture. Pasteurization and milk composition also affect the production of firmness; maturation with slightly higher pH levels resulted in a more structured texture [2].

2.3.3.2 Meltability

The distinctive meltability of mozzarella cheese, which is regulated by a number of important elements, makes it highly valued. The moisture content is important because softer cheese melts more readily when it has a higher moisture content. Fat content is an essential factor that has to be taken into account; cheeses with higher content of fat, tend to melt more easily, because fat contributes to a smoother structure. The melting depends on protein aggregation, which is primarily casein. The way proteins are linked together and denatured under different temperatures is important to getting the proteins to melt best. Moreover, a pH range of 5.0 and 5.5 is a must to reach stretch and melt perfection; the acid level should also be considered. The texture and flavor of cheese change with age, and fresh mozzarella usually melts more readily than aged cheeses. Additionally, the temperature at which mozzarella is heated is crucial; it melts best at 130°F to 150^{o}F. Lastly, the ultimate melting characteristics of the cheese are also greatly influenced by the production processes, such as pasteurization and curd treatment. To achieve the appropriate melt and stretch in culinary applications, it is imperative to comprehend these aspects [41].

2.3.3.3 Stretchability

The researchers set out to understand how various factors, such as temperature and the cheese's composition, impact its ability to stretch. To conduct the test, mozzarella samples were placed in a water bath, ensuring precise temperature control for accurate stretch measurements. The findings revealed that stretchability improves with higher temperatures, peaking around 60-70^{o}C.

More precisely, the cheeses that are higher in fat and moisture demonstrate more elasticity. For one, these physical quality attributes are gained by the production of cheese. By the way of a note, at the neuro-technological level, that we are able to use the same physical characteristic (the elasticity of the cheese) as the food parameters used for the cheese quality improvement. In addition to that, this examination highlights the fact that cheese safety can be improved if so desired; alternatively, it might also be compromised by the inattentive manufacturing process. This shortage research delivers some important proof of the physical properties of mozzarella cheese, which will enrich our knowledge and therefore will help us to control the quality of cheese production better. As a result, the study shows that it is mostly the temperature and the composition that outline the stretch-ability of the Mozzarella cheese [42].

2.3.3.4 Oil separation

The main reason for oil separation in mozzarella cheese is the change in the cheese matrix and the storage process. The cheese during the storage process and the cheese before stretching totally differ and the main reason is the cheese matrix degradation. Mozzarella stretching is a primary cause of fat migration to the surface and thus a major promoter of oil separation because it creates channels for both water and fat. This is due to the fact that as the cheese stretches, a gap is created, and fat migrates to the surface thus, separation occurs. Various irregularities are responsible including pH value, calcium concentration, and the degree of development of proteolysis satisfactions during aging with this process. pH value, salt concentration, calcium concentration and the degree of proteolysis are determinant factors. In the event that these factors are closely regulated it can effectually lead to prevention of oil separation and to preservation of the required texture and quality of the product. Oil is responsible for the texture of the cheese, which was discovered recently. It was found that the fat distribution in the cheese matrix that in turn influenced both the melting behavior and the melting resistance of the cheese were affected by the homogenization process, which is a technology that reduces the size of fat globules in milk. A study was conducted in which two types of cheese were produced mozzarella with homogenized milk and mozzarella without homogenized milk. It can be said that the cheese made with homogenized milk had better elasticity and meltability because it is the result of the study that directly compares the two cheese types. Fat can eventually damage the collagen matrix, but the collagen network reformulation due to stretching of curd alleviates that to an extent- cheese made from such technological approach would experience less oil leakage [43].

2.4 Impact of High Fat Food on Health

The effects of high-fat diets (HFD) on oxidative stress, chronic inflammation, and cognitive impairment are examined in this article. Reactive oxygen species (ROS) and free fatty acids have been found to rise in the body when dietary fat intake is excessive. These dangerous chemicals cause inflammation, especially in important parts of the brain like the hypothalamus. This inflammation contributes to long-term neurological impairment, which also hinders normal brain function to a great extent. The malfunctioning of neuronal system due to oxidative stress is one of the major alarming consequences of high-fat diets. Oxidative stress is a type of damage that causes the brain cells to fail to communicate, which leads to loss of cognitive abilities, such as memory loss, mood problems, and learning deficit. Chronic inflammation and oxidative stress can amplify the chances of neurodegenerative diseases from Parkinson's and Alzheimer's, which could in turn, bring about the cognitive impairment of the patient.

In the context of this matter, the study bears witness to the fact that concentrating fat ingestion for the sake of releasing its weakening impact on brain function is of most importance. Synapses protection and mental activities that in fact maintain the everyday activities can be brought through a on-time diet that embeds in the menu those nutrients that control synthesis of free radicals, inflammation suppressors and, of course, fat. Sustaining well-functioning brain and avoiding cognitive decline associated with oxidative stress and chronic inflammation calls for sticking to a balanced diet and healthy lifestyle [44].

Analysis has provided the fact, that meals that are high in the amount of fats such as saturated fats, those are very likely to provoke both the metabolic changes of a body and excessive fat accumulation, a key reason of obesity. In comparison to the unsaturated fats, saturated fats are not only easily stored in adipose tissue but are not as fuel-efficient. Therefore, weight gain and metabolic equilibrium are achieved by this progress of fat retention, which also leads to complications of serious nature due to obesity, for instance, insulin resistance, type 2 diabetes, cardiovascular diseases and many others. Conversely, the intake of PUFA (polyunsaturated fatty acid) foods and fats, such as omega-3 and omega-6 fatty acids, has been confirmed to efficiently process the metabolism of nutriment by reducing adipose tissues, therefore, vastly improving metabolic status. PUFAs (polyunsaturated fatty acids) are indispensable elements in the process of fat decomposition and spending energy increases, both processes which are vital for the achievement of a healthy mass. These fats contribute to the reduction of body fat by increasing the transformation of stored fat into energy. The activity of key enzymes that bring about fatty acid metabolism is also increased by PUFAs, thereby, aiding in the establishment of metabolic efficiency. A very efficient mechanism that this enzyme has is it ensures that fat is utilized, instead of being retained in excess, in the process of disease prevalence treatment. PUFAs preventing weight gain from the other side are also verified by the studies that show their possibility in lowering adverse events inducing from a high-fat diet like oxidative stress and chronic inflammation. The effect of dietary fat quality on oxidative processes and liver mitochondrial activity is another important factor to take into account. The kind of fat that is consumed has a big impact on how the body produces energy and handles oxidative stress. Saturated fats have a tendency to raise oxidative stress, which damages cells and causes liver mitochondrial malfunction. The organ's capacity to control lipid metabolism may be compromised by this failure, which could exacerbate fat storage and lead to metabolic diseases. However, it has been discovered that unsaturated fats in particular, polyunsaturated fats (PUFAs) improve mitochondrial performance, lowering oxidative damage and promoting general metabolic health. Therefore, a diet high in healthy fats is essential for preserving liver function and avoiding metabolic disorders.

Therefore, metabolism, fat reserve, and the general state of health are the main things that the quality of dietary fat in a human body depends on. From the circulation to the inflammation, sun resistivity also protects the skin. PUFA has the valuable quality of enabling fat to be burned, of course without it we will be about 20 pounds heavier a month, increasing space between pleasure, and instead, it is the short-term consuming of the obesity-related foods, which makes a difference. The consumption of nonfermented foods during this period was shown to stimulate the microflora of the colon, which leads to a good metabolic state besides other positive effects. In this fashion unsaturated fats are important to the metabolical function and also beneficial for one to choose eating habits that lower the risks of developing obesity [44].

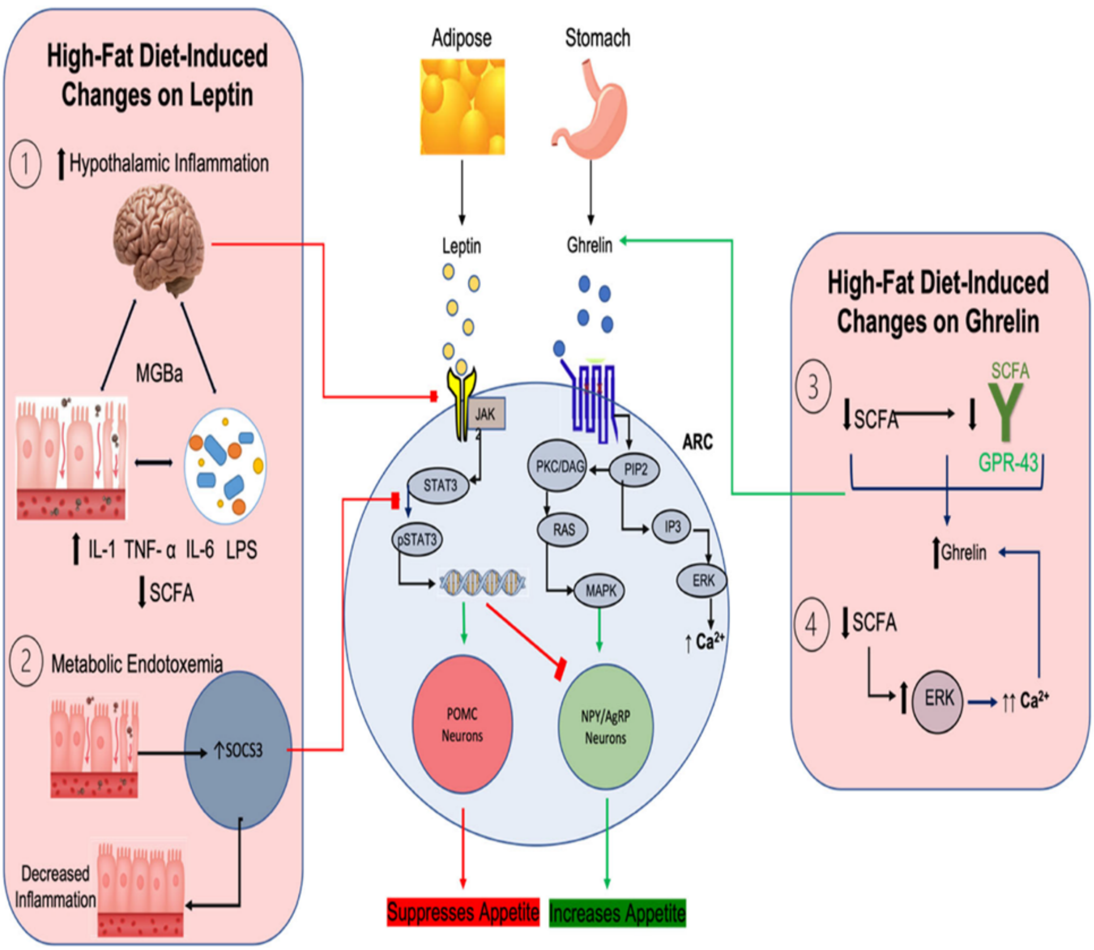


Figure 2.1: Dietary Fat Modulation of Gut Microbiota and Impact on Regulatory Pathways Controlling Food Intake [45]

The findings have shown that high-fat diets impact the bones through various physiological and biochemical pathways. HFD causes the immune system to be disordered, and that is low-intensity chronic inflammation, which is the main pathway of bone fragility. Through eating more fat than the body needs, particularly from what is considered bad like trans-fat and saturated fat, you become inflamed. In response to this, more pro-inflammatory cytokines, the main triggers of inflammatory reactions-incorporating interleukin-6 (IL-6), and tumor necrosis factor-alpha-(TNF-α)-are released. As a result of this it is the production of these cytokines that is being elevated causing the unbalancing of bone metabolism due to the stimulation of osteoclast activity, thus, the resorption of bone and the inhibition of osteoblast function, which is fundamental for the bone creation unbalanced bone metabolism that overstims bone resorption leads to bone modeling to be blocked, which ultimately increases bone deterioration and may cause osteopenia. In addition to causing inflammation abnormalities, a HFD also changes the microbiota of the gut to the extreme that gut dysbiosis accompanies the alterations. Since the role of the intestinal microbiota is immunity, energy metabolism, and food absorbtion, they are crucial in maintaining bone health.

A high-fat diet via non imbalancing of the gut biofilm, especially after the consumption of trans fat and saturated fat, can create an environment in which the repression of good bacteria by these harmful bacteria takes over. As a result of the malodors, the intestines are not able to uptake calcium and thereby, the bones no longer receive the mineral which is needed for them to remain resilient and be built. Over the time of the occurrence, worse and weaker bone structures are easier to become brittle and more fracture-prone since calcium malabsorption has arisen. Effects of poor calcium absorption are additionally demonstrated by reduced bone formation being softer and more inclined to snap.

Also, the support of gut microbiota is the factor that reflects the direct dependence of bone turnover on gut bacteria, as one of the important regulatory signals. In this way, the microbiota of the gut by the process of fermentation of dietary fibers supports the production of SCFAs, thus, the latter increases the absorption of calcium in the bone tissue and reduces inflammation. When the high-fat diet is ingested, the SCFA production is reduced, which in turn worsens the process of bone loss. That is why it is of vital importance to have a proper ratio of gut flora to human well being since the relation of two is pretty complex.

Moreover, interference in the imbalance of gut flora occurs which leads to increased oxidative stress if following a lipid-rich diet, too. Consumption of high-fat diet increases the rate of oxidative stress by promoting reactive oxygen species (ROS) which thus, lead to oxidative damage of the bone cells. In addition to the fact that ROS mainly triggers the osteoclast-driven bone damage, the presence of ROS is the disruptor of osteoblast activity. This, in turn, decreases their capability to produce new bone matrix. Oxidative stress, inflammation as well as, low calcium uptake are all facilitators of the mechanism that leads to bone loss and reduces the overall density of the bone.[46].

Oxidative stress, which is the accumulation of fat due to excessive formation of free radicals in the body, is one of the primary reasons for bone cell and bone metabolism impairments. Racketing of the osteoblasts (the rate at which they function) is a common way the disease functions to deteriorate bone structure. Osteoblasts, which are cells forming bones, require utmost activity in order to protect their strength and density. The upsurge of Reactive oxygen species (ROS) as an answer to oxidative stress leads to the deterioration of cells and the decrease in the ability of the osteoblasts to produce new bone formation. As the slowing down of this process, by which bone is created using calcium and phosphorus, happens, bone not being replaced and new bone not being produced, resorption and regeneration become imbalanced. This imbalance is the beginning of bone mass reduction, the ultimate stage of which is the occurrence of the risk for fractures and osteoporosis.

In addition to oxidative stress, insulin resistance induced by excessive fat intake also hampers the bone health by disrupting the normal function of essential hormones and metabolic processes. Through the mechanism of osteoblast activation and bone building induction, insulin not only exerts a regulatory role on bone metabolism but is also a promoter of these processes. However, the role of insulin to the bone is diminished or even inhibited when there is insulin resistance that arises from prolonged exposure to high-fat diets. In line with this, there is a resultant disarray of the overall bone strength that is accompanied by an impaired bone mineralization that follows this interaction. Insulin resistance is, moreover, linked to chronic inflammation which, in turn, makes osteoclasts, the cells that resorb bone, more active. As a consequence, people with insensitivity to the hormone get weaker bones and are more susceptible to bone diseases.

Leptin is a hormone, principally regulating hunger and energy, which is similarly affected by insulin resistance. Leptin, however, is also one of key metabolic drivers integrating skeletal growth and the remodeling process. Leptin being a bone growth promoter normally operates through the mechanism of instructing osteoblasts to create new bone tissues, which not only keeps the body in a state of balance but also prevents bone loss. In contrast, in overweight and insulin-resistant subjects, the mechanisms that underpin the stability of leptin level rather run amock. In such instances, leptin is found to be deficient of a specific species of the hormone, occasionally referred to as leptin resistance, which in turn makes the hormone's function in promoting proper bone formation suffer. Instead, osteoclast activity and osteoblast inhibition, due to the malfunctioning signaling machinery, occur, leading to the loss of bone mass. Accordingly, the function of the bone, its mass and structure becomes anomalous and, therefore, the risk of fractures of the bone being high.

Besides, the hormonal disorders stemming from insulin resistance and leptin malfunction hold sway over the whole body rather than merely the bone renewal process. It is a complex affair, the correlation between the metabolism of the bones and the hormonal regulation and although the insulin and leptin levels are the culprits, they can cause the whole bone health to be jeopardized. One of the reasons for bone formation inhibition is that the affected bone does not get fully mature thus a gradual decrease in bone density is noticeable over time. This continuous cycle of bone regeneration fails in case of the metabolic disturbances persist, thus it adds to the decline in skeletal health and results in age-related bone diseases to become more common.

In summary, the creation of fat and the resulting oxidative stress, insulin resistance, and hormonal disorders heighten the probability of the decrease in the activity of bone building cells. Such cells are then prevented from the new bone-making process by oxidative stress, an ailment that is further compounded by insulin resistance that leads to the crucial metabolic pathways disruption necessary for bone strength. Variants of leptin that work the other way round also excite the problem by hyper-actively controlling the resorption of bone thus decreasing the passage of bone allocates. On the whole, people who consume fat-rich diets have a high chance of getting bone diseases such as fractures and osteoporosis. On the other hand, a diet that ensures metabolic and hormone regulation is supported while the required nutrients are also supplied for the bones to be firm and healthy is the right way to prevent these adverse effects [47].

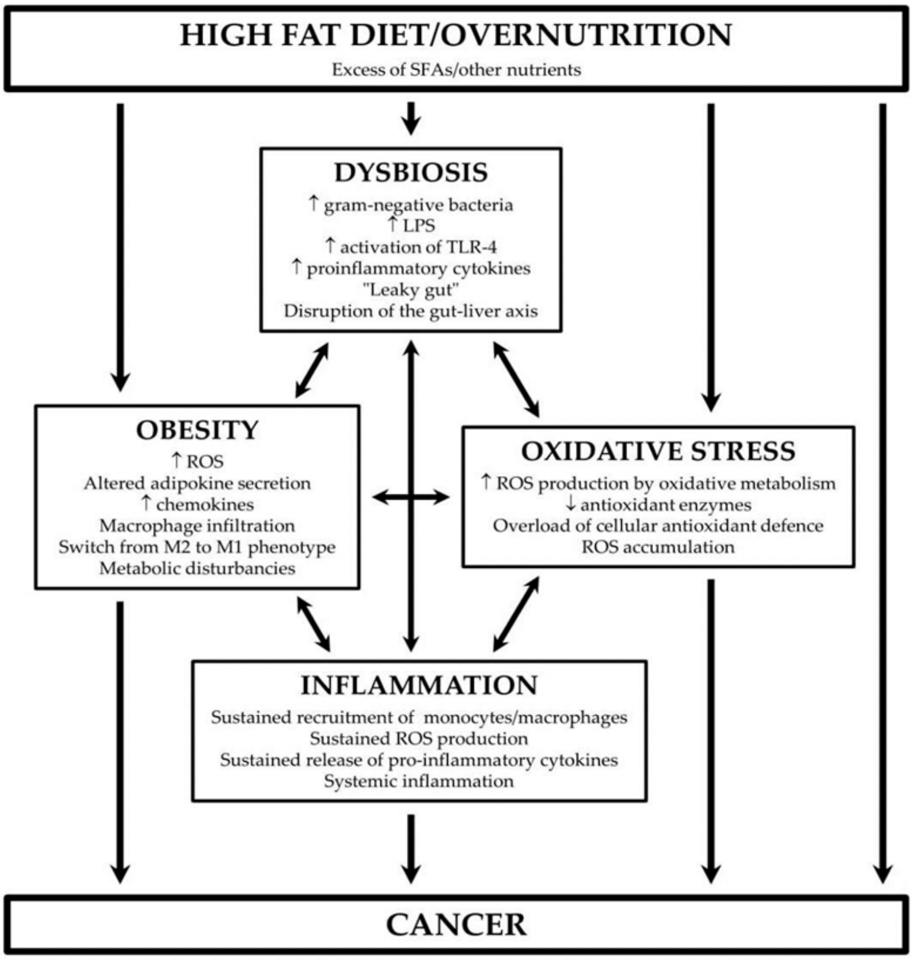


Figure 2.2: Interplays between disturbances arising from a deficiency in nutrients

2.5 Role of High-Fat Diet in the Development of Atherosclerosis

Evidence from epidemiological, clinical, and experimental research consistently demonstrates that excessive consumption of a high-fat diet (HFD) is detrimental to health. It is well-established that dietary fat, especially saturated fat primarily found in animal sources, plays a crucial role in elevating plasma low-density lipoprotein (LDL) levels and is closely linked to the development of atherosclerosis. Epidemiological studies have indicated that reducing dietary fat intake leads to lower LDL levels, subsequently decreasing the incidence of atherosclerosis [47].

Furthermore, an increased consumption of HFD, combined with other factors like physical inactivity and genetic predisposition, can disrupt the balance between energy intake and expenditure, resulting in obesity and metabolic syndrome (MetS). This, in turn, heightens the risk of cardiovascular disease (CVD) and mortality. While the relationship between HFD, obesity, and CVD risk is widely accepted, it remains uncertain whether prolonged consumption of a normal- calorie HFD, such as on a daily basis throughout one's lifetime, has adverse effects on metabolism and promotes atherosclerosis [48].

Obesity is connected to adverse effects on major CHD risk factors, including raised cholesterol and triglyceride levels, decreased high-density lipoprotein (HDL) cholesterol, elevated blood pressure, and an increased risk of developing diabetes. Some studies, like the Framingham study, suggest that obesity carries an independent risk for CHD beyond the influence of conventional risk factors. Even a minor weight gain can elevate an individual's risk of suffering a heart attack [48].

2.6 Fat Reduction Strategies in Cheese Making

Reducing the fat content in cheese poses considerable challenges, as fat significantly influences the texture, flavor, and overall sensory qualities of the final product. There have been many recipes created that counteract these difficulties and can ultimately lead to very close imitations of full-fat cheeses in terms of fat content. WHO, what, when, where, and how are some auxiliary questions that can make a summary descriptive. In comparison to full-fat, these dairy products usually contain a high amount of fat. The addition of fat substitutes like buttermilk and microparticulated whey proteins (MWPs) to cheese milk is one such strategy to reduce cheese milk. These substances make it possible for people to feel the fat-like sensation that they are used to experiencing and meanwhile expanding the moisture-retention capabilities of low-fat varieties so that the texture of the cheese is not compromised. In fact, the addition of MWPs and buttermilk to a 10 percent fat Dutch cheese provides the same sensory qualities found in full-fat cheese as indicated by research studies. [49].

Another effective method in cheese production via the utilization of cultures that generate exopolysaccharides (EPS). Particular lactic acid bacteria transduce EPS, a type of polysaccharides, that enhance the texture and wetness of low-fat cheeses. According to a study, the addition of EPS-producing cultures in the production of low-fat Cheddar cheese resulted in increasing the yield and moisture content of the product. Thus, a smooth texture with less hardness was achieved as it ripened [50].

In addition, other hydrocolloids such as inulin, guar gum, xanthan gum, and resistant starch were also studied as viable ingredients to be added to low-fat cheeses that would improve their properties. Fat mimetics, which operate by thickening the cheese matrix and growing it in their presence, are provided by these components, and that is why they function as fat replacers, thus, making the texture softer and the moisture content higher. The inclusion of these hydrocolloids in the recipe of low-fat Cheddar cheese resulted in a significant improvement in their textural properties along with an increase in their sensory acceptability as was concluded in Sustainable Food Systems study [51].

Furthermore, making use of their benefits, nuclear satellites can withstand the harsh conditions of outer space so that exploration of other planets and deep space is not hampered. One of the advantages it offers over other methods is its increased resistance to the harsh conditions of outer space, making it a solid solution for deeper space exploration. In the words of the study of powdery cheese derivatives, which as a result of the homogenization process become functional materials, homogenization performed at 20 MPa led to 20 mV reduction in the zeta potential of the fat globules in milk, and thus to; and production of a very soft, consistent, smooth and devoid of extraneous particles texture pattern according to Quark-type cheese was achieved [52].

2.7 Potential Industrial Applications and Market Feasibility

Using plant-origin mucilages, like the fat replacers in low-fat mozzarella cheese, presents a competitive product and about the sustainability of the market. This paves a way for these plant-derived substances to act as alternatives to the traditional fat replacers of some foods. A biodeterministic research has confirmed that the inclusion of aloe vera in the process can significantly increase the overall quality of these products [9]. Similarly, a study of the use of aloe vera mucilage in place of fat in low-fat mozzarella cheese was conducted, and it was determined that the quality of cheese was markedly improved. The obtained outcomes implied that plant mucilages were effective enough to produce cheese products that are much healthier while they do not lose their quality [53].

Adoption of biopolymers of this nature is in agreement with consumers' increasing craving for pure-label and health-related food articles. As a result, plant mucilages are sustainable and distributed ubiquitously; they are the affordable source. Besides just the consumers' demands for low-fat products, this is also a possibility for the producers to find a less expensive way to produce low-fat cheese that still tastes good. To sum up this discussion, the utilization of plant mucilages as fat replacements in cheese production has amazing technological prospects as it accommodates the compromise between good quality and good nutrition, thus being welcomed by both the producer and the consumer.

2.8 *Hibiscus rosa sinensis*

2.8.1 Composition

Hibiscus rosa sinensis, popularly known as *Hibiscus* is rich in lots of bioactive compounds such as flavonoids, polyphenols, and organic acids thus it has strong medicinal properties. The review underscores the different pharmacological effects of *Hibiscus*, specifically the antioxidant, anti-inflammatory, antimicrobial, and antihypertensive properties. Furthermore, the plant is a potential treatment for diseases like diabetes, heart diseases, and infections. Somehow, the piece also proves how *Hibiscus* can be used in the traditional treatments and be a part of the modern healthcare. On the whole, the review points out the *Hibiscus rosa sinensis* as a very useful source of natural compounds, which have a great number of health benefits, and it calls for research in the field of natural products development to achieve the maximum therapeutic potential and clinical applications [48].

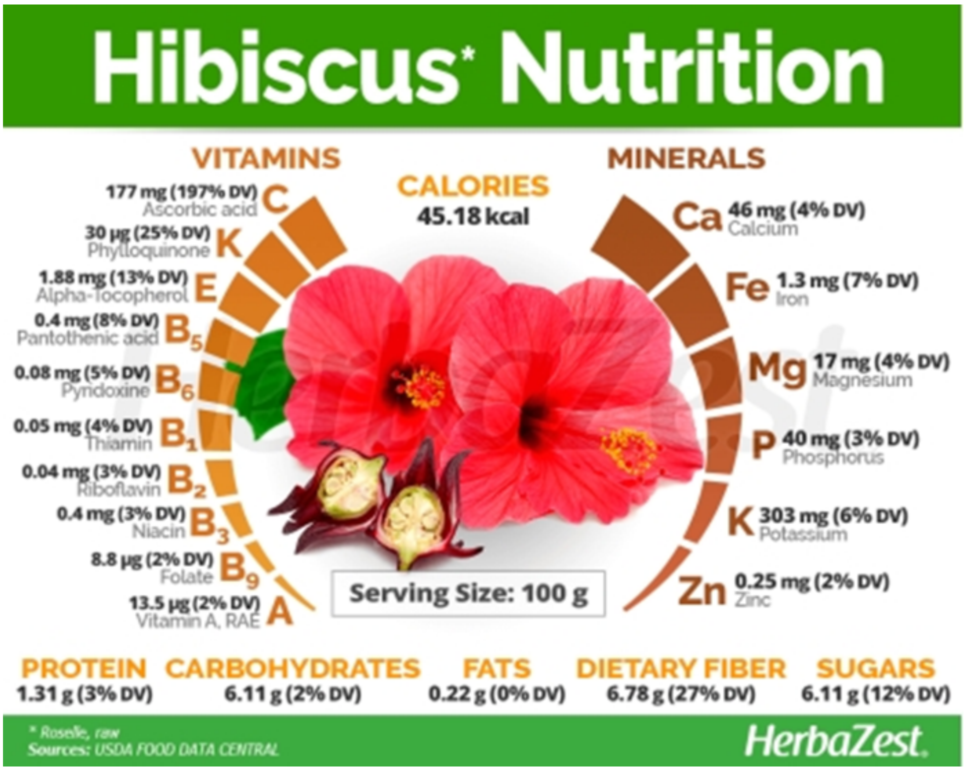


FIGURE 2.3: *Hibiscus rosa sinensis* Nutrient composition [49]

2.8.2 *Hibiscus rosa-sinensis* Mucilage

Because of its unique functional qualities, *Hibiscus rosa-sinensis* mucilage has attracted a lot of interest from a variety of businesses. *Hibiscus rosa-sinensis* mucilage is often extracted by soaking plant portions in water, then filtering and precipitating them with ethanol or other solvents. This technique produces a mucilage that is rich in polysaccharides.

According to studies, galactose and arabinose are the main components of acidic polysaccharides, which are important ingredients. The study's outcomes highlight the structural complexity of mucilage in terms of its carbohydrates, which lend it the attributes of functionality [55]. Pharmaceutical formulants as *Hibiscus rosa-sinensis* mucilage stabilizers have been studied by their physicochemical characteristics as pH, swelling index, and flow behavior. Mucilage, undoubtedly, is crucial with its unique physicochemical and pH features that allow to use it in a great variety of applications [56].

The thickening, emulsifying, and stabilizing features inherent in plant-derived mucilages make them very much sought-after in the food industry as they are not only sustainable but also healthy surrogates for artificial additives. With the help of these ecologically friendly and harmless biomaterials, the food industry can use them in food applications, that is the natural thickeners and emulsifiers, which shall increase their use. *Hibiscus rosa-sinensis* Mucilage being an appealing organic ingredient for applications like replacing fat in the production of low-fat mozzarella cheese when we talk about their beneficial properties [57].

2.8.3 Functional Attributes

2.8.3.1 Medicinal properties of *Hibiscus rosa sinensis*

Hibiscus rosa sinensis is the most popular herbal solution which can be used in both modern and traditional herbal therapy based on its plethora of health purposes. The existence of these health-promoting compounds in this plant is among the reasons for its effectiveness as an antioxidant. These compounds include flavonoids, polyphenols, and organic acids. Its ability to decrease oxidative stress and inflammation is one of the fundamental advantages of it; this helps deal with conditions like arthritis. Moreover, a recent study has revealed that *Hibiscus rosa sinensis* has antihypertensive actions that are responsible for lowering blood pressure in hypertensive individuals. In addition, the plant also has antibacterial properties, which facilitates the treatment of diseases caused by pathogens. It has been demonstrated that the plant has an additional benefit to weight loss and that it carries out this process through the promotion of the metabolism of fats in the body. All things considered, *Hibiscus rosa sinensis* has a variety of therapeutic benefits, which indicate its importance [50].

2.8.3.2 Antioxidant Properties

Hibiscus rosa sinensis is a highly effective emulator of oxidation processes. The main powerhouses behind these activities are the prolonged list of the bioactive substances produced by *H. rosa-sinensis* which it includes among them flavonoids, phenolic acids, and also anthocyanins. Research shows that the derivatives of the main component of the *Hibiscus rosa sinensis* leaves may be efficiently scavengers of free radicals and thereby reducing oxidative stress in cells. Antioxidant replacements, according to the same trial, are capable of preventing cancer and cardiovascular diseases through free radical scavenging, thus cellular damage is thus reduced. The paper also highlights the immune-boosting benefits of the hibiscus plant extracts, thereby proving *Hibiscus rosa sinensis* to be an incredible component in functional foods that are meant to improve one's health and well-being [8].

2.8.3.3 Antimicrobial Properties

The research proved by proving that *Hibiscus rosa sinensis* extracts display efficient antibacterial activities against common pathogens counts for Escherichia coli and Staphylococcus aureus. The justification relates to the fact that antmicrobial

FIGURE 2.4: Health benefits of *Hibiscus rosa sinensis* [51]

effects are suitable with the drug used and environmental conditions. *Hibiscus* extracts also contain some antimicrobial activity as well. The results of the experiment showed that treatment with *Hibiscus* extracts at different levels, 0.1, 0.5, 1.0, 2.0 micrograms/ml of the extracts, treated *S. aureus*, and the experiments results demonstrated that for each dose the extract was able to completely inhibit the bacteria growth. The article also highlights *Hibiscus rosa sinensis*'s potential as a natural substitute for artificial antibacterial agents, emphasizing its uses in traditional medicine and its function as a food preservation element. These results demonstrate *Hibiscus*' medicinal potential in preventing microbial infections and improving general health [52].

2.8.3.4 Nutritional Properties

Hibiscus rosa sinensis is a really good on top of a perfectly healthy diet. One of the main vitamins that this plant is rich in is vitamin C, which is an absolute necessity for healthy skin, and improving the immune system. Besides, it consists of important minerals like calcium, iron, and phosphorus all which are main components for healthy bones and generally body processes. Conversely, *Hibiscus* is packed with dietary fiber and is also low in calories, which in turn, supports good digestion and a fuller feeling. Along with the addition of better taste, *Hibiscus rosa sinensis* is also associated with a number of nutritional benefits that can stimulate your physical health and wellbeing [53].

2.8.3.5 Anticancer Properties

Studies have found that the extracts from *Hibiscus* flowers have the potential to be powerful antioxidants and anti-inflammatory agents. This is important because they help in counteracting the oxidative stress and inflammation that comes with the progress of cancer. *Hibiscus* contains anthocyanins, flavonoids, and polyphenols which are the phytochemicals that possess the property of inhibiting the proliferation of cancer cells and thus the promotion of apoptosis in all kinds of cancer like breast, liver, and colon cancers. Furthermore, the anti-inflammatory nature of *Hibiscus* may also prevent the growth of tumors by eliminating the supportive environment that they require [54].

2.8.3.6 Antidiabetic Properties

Hibiscus rosa sinensis that has been experimentally used in the treatment of diabetes and has confirmed its antidiabetic properties, making it a potential natural treatment for diabetes management. *Hibiscus* is discovered by the research must go through it. According to the results of numerous studies, the extracts from the leaves and the flowers of *Hibiscus* can efficiently reduce the blood sugar level and thus can improve the insulin sensitivity as well.

This actually is due to the bioactive compounds like anthocyanins, flavonoids, and phenolic acids that are the ones that improve glucose metabolism and protect the body from the oxidative stress glycaemia, which is closely linked to diabetes. The other advantage of *Hibiscus* is that the herb is able to delay carbohydrates from being digested by hindering amylase activity, thus lessening glucose absorption in the intestines, in addition to causing the pancreas to maintain blood sugar homeostasis.

At the same time, the anti-inflammatory properties of *Hibiscus* have been largely shown by this herb as a key element of the process of reversing the complications in the context of diabetes. Hypothetically, the research identifies *Hibiscus rosa sinensis* as a potential treatment for diabetes and metabolic health [48].

2.8.3.7 Neuroprotective Properties

Hibiscus rosa sinensis is the name that has gotten attention in the new field of research due to its neuroprotective properties which have the ability of giving more health in the brain and prevention of neurodegenerative diseases as a part of neurogenic damage. Some studies have been made that have shown that the *Hibiscus* oxidative stress and thus work as a free-radical terminus in the process of neural degeneration. The flavonoids and phenolic acids in the herbal *Hibiscus* which the bioactive compounds are made of, are playing a critical role in the activity of reducing inflammation and support of neuronal survival. Overall, current evidence positions *Hibiscus rosa sinensis* as a promising natural option for enhancing neuroprotection and improving cognitive health [55].

2.8.3.8 Wound Healing Property

Hibiscus rosa sinensis has been demonstrated through clinical trials to have healing properties, and thus has become a useful tool in traditional medicine and natural treatments. The reports claim that the *Hibiscus* flower and leaf extracts exhibit strong antimicrobial properties, thus helping to prevent infections in wounds and expedite the healing process. The bioactive compounds in *Hibiscus* are the ones that are essential in the healing process due to their effect on cell proliferation and tissue regeneration. In addition, *Hibiscus* is a component of the whole process of healing as it stimulates collagen synthesis, and without it, skin wouldn't regenerate. Equally, *Hibiscus* extracts also have anti-inflammatory effects that can ease the pain and the swelling of wounds, along with the healing process [56].

2.9 Impact of Hibiscus Mucilage on Cheese Characteristics

It has been investigated whether adding *Hibiscus rosa-sinensis* mucilage to cheese formulations can improve and alter a number of physicochemical and textural characteristics. The effects of adding *Hibiscus* extract to white soft cheese and found that it significantly changed the cheese's structure and content. According to the research, not just the *Hibiscus* extract concentration was associated with a reduction in the total solids, protein content, and lipid content but significant decreases in pH levels too. These developments offer signs that *Hibiscus* mucilage binds with cheese components and thus may be the reason for the overall cheese balance decrease due to changes in its shelf life.

Moreover, the results of the research demonstrated that cheese enriched with *Hibiscus* extract at the level of 10% was much less tough, with the data being 11.8 g in the control sample to 2.1 g only. These data are affirming that the mucilage from *Hibiscus* does its thing and gives cheese a soft texture. *Hibiscus* extract did not only alter the cheese's hardness but it was able to lessen its gumminess and chewiness as well, thus it became more suitable for immediate use without being crushed with a light bite. These modifications could result in increased patronage, especially for those who prefer softer cheeses [64].

2.10 Use of *Hibiscus rosa sinensis* Mucilage in Food Products

Due to its special functional features such as gelling, thickening, emulsifying, and stabilizing, the *Hibiscus rosa-sinensis* mucilage, a natural polysaccharide derived from the plant, has been the subject of numerous investigations in alternative food applications. Its potential as a natural, healthy-ingredient in the food business was the subject of the research publications that have documented its application in various food products. The list below represents some of the most common uses of *Hibiscus rosa-sinensis* mucilage, which is backed up by the results of research:

2.10.1 Food Stabilizer and Thickening Agent

A study conducted showed the mucilage of *Hibiscus rosa-sinensis* is one of the best natural stabilizing and thickening agents in culinary products such as sauces, soups, and beverages. The mucilage can function as a gelling agent, which can accordingly be used as the key ingredient to improve the consistency and texture of mentioned goods, and thus it can be a natural alternative for artificial thickeners. As the demand of consumers for a product with clearer labels increases, the study stresses its role in product quality improvement without the need of artificial additives, which is the food industry is becoming increasingly crucial [57].

2.10.2 Gluten-Free Baked Goods

Hibiscus rosa-sinensis the mucilage's wonderful utility was displayed by its potential in gluten-free baking where it was employed to improve the texture and structure gluten-free cakes and bread. In a study that was into the use of *Hibiscus* mucilage in gluten-free flour blends, it was revealed that it could be used as an improver of bread volume and dough rheology. It appears that it should be possible to utilize this mucilage in the making of a new gluten-free product that is as elastic and textured as is the one made with wheat [58].

2.10.3 Dietary Fiber Source

According to research, *Hibiscus* mucilage have been used in fiber-enriched foods including cereals and snack bars. Health benefits of consuming dietary fiber are innumerable, and it is the most essential requirement for the health of the gut. The product's total nutritional value increased, thanks to the-now higher fiber it contained due to the presence of the mucilage [59].

2.10.4 Emulsifying Agent in Plant-Based Foods

A research study consists of the mucilage of *Hibiscus rosa-sinensis* that was evaluated as an emulsifying agent for plant-based food compositions, including plant-based dressings and yogurts. Typically Starch is used to thicken food that is not made from animal by-products, Drug delivery is the main product of *Hibiscus rosa sinensis*. Now, the mucilage is generally thought to be able to stabilize emulsions by forming small droplets and blocking direct contact between oil and water, thus, plant-based substitutes which usually face the difficulty to maintain the texture and consistency of texture and consistency can take advantage of its stabilizing power.

2.10.5 Encapsulation for Nutrient Delivery

The experimental procedure was conducted to reveal the bulk of *Hibiscus rosa sinensis* mucilage bioactive compounds for the controlled release system of functional foods. The study found out that the mucilage is a very good encapsulating matrix giving maximum protection to the vitamins and antioxidants thereby enhancing their stability in food products. In that respect, the mucilage can be a good choice in the functional foods segment, including fortified snacks or nutraceutical drinks. This is because it has the ability to encapsulate the bioactive chemicals in the foods and beverages [59].

2.10.6 Use in Beverages

Hibiscus rosa-sinensis refers to the fact that mucilage is one of the few gelling agent that can give the mouthfeel and texture of the food items a more palatable set of attributes without adding any outside flavors to the mix. That is why this mucilage was used in the production of functional beverages such as smoothies and health drinks. This was shown in a study on the use of mucilage in fruit-based beverages, in which the component added to the drink's viscosity and sensory attractiveness turning it into a wanted component for the beverage manufacturers looking to make more palatable and wholesome products.

2.10.7 Confectionery Products

Within the confection field, the mucilage was also examined for its possible use in marmalades and gum-based sweets. Due to its jellification and stabilization features, it can be used as a gelatin substitute and can provide vegan and vegetarian customers with ecofriendly plant-based products. The revenues of a trial marketing study revealed that it could be employed to produce plant-based gummies by adopting new natural technologies that would be appropriate for children and seniors as well as other dietary groups seeking to avoid high intakes of gelatin [60].

In other words, trials have been conducted to show that the mucilage of *Hibiscus rosa-sinensis* has multifunctional properties and can be used in a large number of food commodities except for cheese, for instance, it can act as a dietary fiber source, thickener, emulsifier, and stabilizer. It has become a valuable ingredient in the production of plant-based, sustainable, and healthier food items which are demanding due to the perfect texture, enhanced nutrient content, and the absence of artificial substances. Thus, the direction of the food industry of tomorrow might be influenced on a grand scale by *Hibiscus* mucilage due to future research being able to fully grasp its potential.

2.11 Other Fat Replacers in the Formulation of Low Fat Mozzarella Cheese

Recently, there has been quite a buzz around the investigation of plant-derived mucilages as fat impostors in low-fat mozzarella cheese. Mucilages have been used in dairy products to forestall the societal need for fat by complying with and reproducing fats texture in hydrocolloids because of their hydrophilic and gel-forming qualities. Nevertheless, a few studies are available with respect to *Hibiscus rosa-sinensis* mucilage's utilization in mozzarella cheese, although studies both mucilages offer valuable inputs. E.g. looked explored the addition of okra mucilage to low fat mozzarella cheese. According to the study, the addition of okra mucilage at different concentrations (0.625% to 2.5% v/v) improved the textural parameters and functional properties like meltability and stretchability while the fat content had dropped greatly. Notably, the 1.0% concentration was the most effective on the texture, while the 0.25% concentration was the best on the organoleptic aspect [9].

The studies on aloe vera mucilage as a fat replacement also gave good results for the texture and sensory characteristics of low-fat mozzarella cheese; the best organoleptic findings were made at a 2.5% (v/v) concentration. These results suggest that mucilages can be successful imitators of fat role in cheese, by enhancing texture and all at the same time by moisture retention [61].

Due to its physico-chemical characteristics similar to those of other plant mucilages, *Hibiscus rosa-sinensis* mucilage lawfully is a fat substitute in low-fat mozzarella. Enhanced texture, retention of moisture, and overall sensory acceptability may occur because of its hydrophilic character and gel-forming capacity. Further research is necessary, however, to get a clearer understanding of the precise effects of *Hibiscus rosa-sinensis* mucilage on cheese functional properties, while the chemical makeup and consumer acceptability have to be studied as well. Asbestos examination and evaluation and efficacy of Century Argentina will be more refined with the help of such panel studies.

2.12 Potential Benefits of *Hibiscus rosa sinensis*

Using *Hibiscus rosa-sinensis* mucilage in low-fat mozzarella cheese compositions offers some advantages:

2.12.1 Texture Enhancement

The basic hardening capability of *Hibiscus* mucilage appears as an obstacle towards the textural and sensory appeal of low-fat cheese. It increases the product to the preferred higher standard which in turn enhances the adaptability of the low-fat dairy product. One of the main drawbacks is the loss of smoothness, elasticity, and creaminess that the cheese undergoes when the fat content is reduced; most of the time the result is a crumbly, dry, or rubbery product. The high-molecular-weight polysaccharides–rich *Hibiscus* mucilage through its job in thickening and solidifying the cheese matrix (the classification of ingredients) is helping undo these [62].

Water absorbing agents have been used for a long time in food industry for the reasons of their capacity to bind water, create gels, and to increase viscosity so that they might substitute fat in a variety of food products. The most malleability, adhesiveness, and thermal quality of cheese are the basic requirements for the prevention of its extremal harshness and fragility when activities like processing and storage are undergone. Exudate moulding is a process that involves removing fat from make-called no-fat cheese whereas at the same time bulking it with vivacious and super-sensitive textures that will permeate sensors with a coiled aesthetic signalling real fatty feel. Because of the fat-like feeling of the structured liquified gels, down-fat cheese products are delivered to the palate smooth and pleasant, and the enriched taste comes from upping the fat functionality with mucilage.

It is a familiar fact that taking plants' mucilages can be a viable alternative to fats in dairy foods. E.g., chia seed mucilage is used as a fat-substitute in cheesemaking and yogurt making. Thus, it makes the cheese or yogurt more palatable, better textured, and enhances moisture retention. Moreover, the Made by chia mucilage that is serving as a gel in the cheese the basic of the cheese product is the stable one that holds the fat loss and gives it a creamy texture always. The up-sizing of big parts of the *Hibiscus* mucilage molecules could bring about those advantages mentioned above in the case of mozzarella cheese with no fats.

2.12.2 Moisture Retention

One of the essential properties of hydrophilic *Hibiscus* mucilage is its ability to keep a sufficient amount of water for the appropriate texture and mouthfeel while the low-fat cheeses are still able to be consumed, but without having the fluffy taste. The high dryness and crumbliness of the end product as a consequence of the absence of fat, the ingredient that makes it smooth and soft, indeed, is one of the main drawbacks to the reduction of the fat content of cheese. *Hibiscus* mucilage, being a water molecule binding agent, can be used in cheese to limit vapor loss thereby ensuring better mouthfeel and structural stability [9].

Multiple recent studies have brought to light the excellent performance of mucilages derived from plants as functional food additives. Thus, to illustrate, another mucilage, the okra one which is very effective, has helped lift the fat-writing out of the low-fact mozzarella cheese, thus, it has resulted in more moisture along with textural quality improvement such as good pliability and meltability.

Plant mucilages' ability to form a hydrocolloid gel matrix is an important factor in mimicking the fat sensation in the mouth, thus preventing the cheese from getting stiff and fragile.

Similarly, *Hibiscus* mucilage is expected to act as a natural hydrocolloid, which in turn may facilitate the low-fat mozzarella to retain water, and it, at the same time, may increase viscosity and spreadability. Natural mucilages act as clean-label alternatives which are not only safer but also do not contain synthetic compounds, thus guaranteeing healthiness as well as satisfaction of customers who demand healthier and more natural ingredients.

To a greater extent, the nutritional value of the finished cheese product can be enhanced by the presence of *Hibiscus* mucilage, which will also have nutritional and ecological benefits such as the existence of bioactive components that have antioxidant characteristics.

2.12.3 Natural Ingredient

Given that the *H. rosa-sinensis* mucilage is a hydrocolloid accounting for a distinct part of the plant structure, it blends in well with the clean-label trend and therefore appeals to health-conscious consumers to a large extent being a natural, minimally processed food ingredient that is adored by them. The last couple of years have seen the rise of this movement, with a vegetarian doing all that pertains to vegetable instead of meat. He has since become the protagonist of the tale known as the Salvin Surveyor for collecting marine life in a scientific way. The law passed during the inquiry prohibits the new book's publication in the pleased member's library wave of consciousness and the necessity for another type of food that would be healthy as well as transparent which are the main driving forces behind the Organic Movement. The clean-label concept stimulates the use of artificial additives, preservatives, and synthetic chemicals in a few situations only where there is some health risk of the latter and on the other hand, it supports the use of natural, organic, and sustainably sourced foods that have a lower impact on human health and the environment [63].

Producers should meet the changing means of producing goods and present the information about the goods in a way customers understand. Specifically, consumers today are very informed and selective about the products they get and hence use of only those products which are endorsed for them by the doctor according to their need and consumption of the product that have no effect on their diet or body. They avoid meat or obtain the restricted intake. They carefully examine the labels to look for natural alternatives that do miracles for their health and at the same time bring multiple functional benefits. This chemical is well-suited to customer's preference as a food constituent, namely low-fat cheese, as it functions as a natural stabilizer, texturizer, or fat substitute.

It is of great use in this instance as previously chemically treated starches or synthetic emulsifiers would be used, when despite this the food would still be changed without a change in the product's integrity the water and moisture retention will be guaranteed. Moreover, the *Hibiscus* mucilage being botanicals is furthered in physical attractiveness by the clean-label happening because this brand stands for sustainability. The removal of the Mucilage from *Hibiscus* plants through the pressing method is the most environmentally suitable route as it is the least processed and environmentally benign with some stabilizers being treated very severely or being produced from animals.

The beneficial characteristics of the *Hibiscus* mucilage so far are not only due to the possible presence of bioactive substances having anti-inflammatory and antioxidant properties. To the industry that is inclined to follow the trend of the increasing natural food formulations and circular economy application, the usage of *Hibiscus* mucilage could become an effective solution for them because the products of high-quality, good texture, and tasty food can be made freely signed using this ingredient. *Hibiscus* mucilage is very neat, it is also multifunctional and can be used in plant-based protein products that are clean-label-oriented, sustainable, and health-conscious and is becoming more important the food industry continues to go towards the transparent mode of operation.

2.13 Impact of Low Fat Mozzarella Cheese on Human Health

The food science and human nutrition aspect requires a study of the connection between the construction geometry of the various cheese types and their respective influences on the heart performance structure. Because saturated fat has a role in setting high LDL cholesterol levels affecting heart diseases for which you usually hear doctors advising you to instead limit its intake this is the normal saying according to the health guidelines. On the other hand, recent research revealed that this opinion may be too simple as it does not take into consideration the synergism of nutrients in whole diets [64].

The term ”cheese matrix” actually refers to the complicated structure of cheese which includes interactions between various proteins, lipids, minerals, and vitamins. This matrix is capable of not only changing the way nutrients are absorbed and digested at the same time as the adverse effects of saturated fats are lowered. For instance, the combination of calcium and fat found in the cheese matrix may lead to insoluble soaps production in the intestines, resulting in a decrease of cholesterol levels in the blood by decreasing fat absorption.

Furthermore, the fermentation process used to make cheese may increase the release of bioactive peptides that are demonstrated to have lipid-lowering and antihypertensive properties. These peptides' bioavailability is involved in cheese structure, and the release of advantageous substances is also conditioned. Hence, the entire matrix of cheese is a major factor in its metabolic effects, while its saturated fat concentration alone is not the factor that would define its health impact.

Chapter 3

Methodology

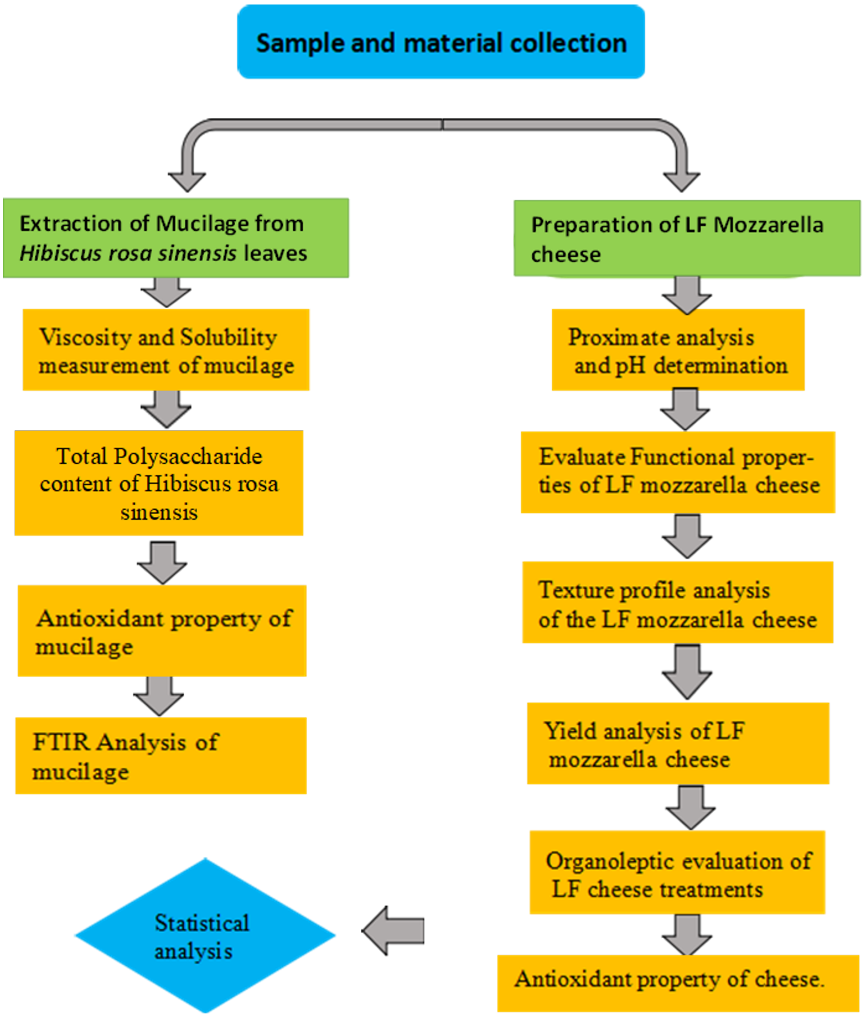


FIGURE 3.1: Research plan concept

3.1 Sample and Material Collection

The leaves of *Hibiscus rosa-sinensis* and fresh buffalo milk samples was purchased from the local market. Skimmed milk (1.5%) was acquired from the local food market. The ingredients that is be used to prepare mozzarella cheese included fresh buffalo milk, distilled water, vinegar (for acidification) and dried rennet. All the chemicals that was used for the qualitative testing of mucilage and low-fat mozzarella cheese analysis is of analytical grade [66].

FIGURE 3.2: Leaves of *Hibiscus rosa sinensis*

3.2 Extraction of Mucilage from Leaves of *Hibiscus rosa sinensis*

The mucilage from fresh *Hibiscus rosa-sinensis* Linn leaves was extracted by following the method outlined by [65] with minor modifications. The leaves was collected, thoroughly washed to remove impurities, and dried. The dried leaves were grounded and soaked in water for 5-6 hours, followed by boiling for 30 minutes. The mixture is then be left undisturbed for 1 hour to ensure complete mucilage extraction into the water. The solution was filtered through an eight-layer muslin cloth to separate the residue. To precipitate the mucilage, acetone was added in an amount three times the volume of the filtrate. The separated mucilage was dried at a temperature below 50°C, ground into a fine powder, passed through a #80 sieve, and stored in desiccators for future use.

FIGURE 3.3: Mucilage of *Hibiscus rosa sinensis* leaves

3.3 Solubility Measurement of *Hibiscus* Mucilage

The solubility of the isolated mucilage was evaluated by dissolving it in various solvents. For this purpose, 1 gram of *Hibiscus rosa-sinensis* mucilage was sequentially dissolved in 10 ml of both polar (water) and nonpolar (ethanol) solvents at room temperature [26].

FIGURE 3.4: Checking the solubility of *Hibiscus rosa sinensis* mucilage by dissolving in different solvents

3.4 Total Polysaccharide Contents of *Hibiscus rosa sinensis* Mucilage

The total polysaccharide content of the extracted *Hibiscus rosa-sinensis* mucilage was determined following the method of [66], with minor adjustments. The phenol-sulfuric acid colorimetric method was employed, using glucose as the standard. The optimal absorbance of the resulting solutions were measured at 490 nm with a UV/Vis spectrophotometer.

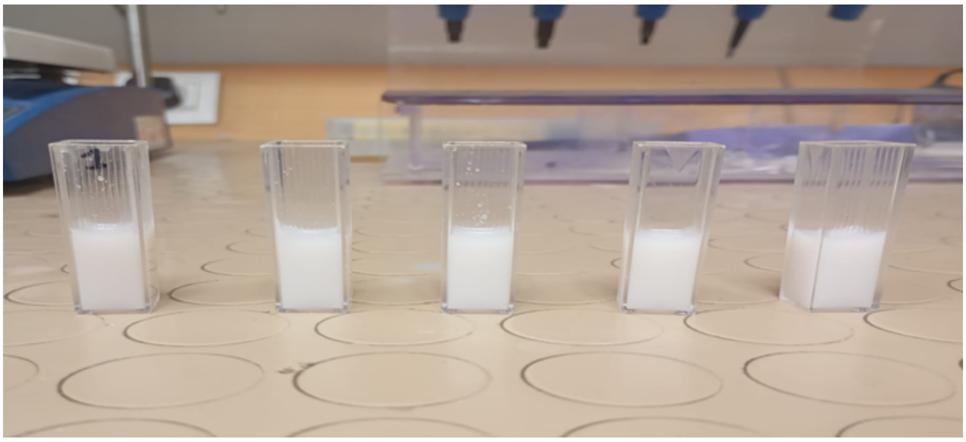


FIGURE 3.5: Measuring the absorbance of different glucose concentration by spectrophotometer

3.5 Total Polyphenolic Content of *Hibiscus rosa sinensis* Mucilage

TPC was calculated using the previously published spectrophotometric F-C reagent method [67]. 270 μl of the ethanolic extract of various strengths (25ppm, 50ppm, 100ppm, 150ppm and 200ppm) and 1.36 ml of the 10% F-C reagent were added in the aluminum- foil-covered falcon tubes. After 5 minutes, 1.36 ml of 7.5% sodium carbonate was added, and the mixture was then mixed and allowed to sit at 45^{o}C in an incubator with distilled water for 45 minutes. There were three solutions of each sample. Experiment was performed in triplicates.

The absorbance was determined with a spectrophotometer set at 765 nm. TPC was determined using gallic acid concentrations (0 - 120 ppm) as the standard and a calibrated curve (R2 = 0.895).

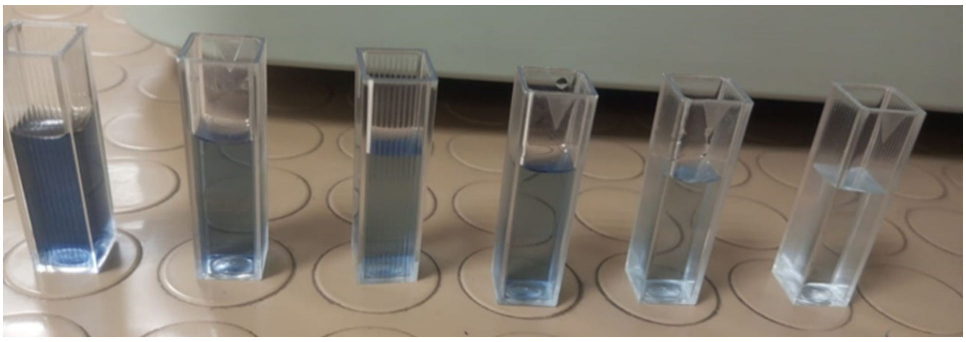


FIGURE 3.6: Gallic acid concentrations

3.6 Antioxidant Profile of Mucilage

With just minor adjustments, the DPPH test, which was previously reported by [68], was utilised to assess the extracts' anti-oxidant activity. The experiment was conducted by using 0.004% (w/v) DPPH solution prepared in ethanol. Solutions of 1ppm, 5ppm, 50ppm, and 100ppm were prepared to note absorbance at 0 and after 30 minutes. There were three samples of each concentration.

Experiment was performed in triplicates. A sample of extracts (25μl) was mixed with 0.1 molar tris base HCL buffer (250μl) and then the DPPH reagent (1 ml) was added. At 517 nm, the absorbance was determined with a spectrophotometer. This formula was utilised to determine the activity of scavenging free radicals:

$$Scavenge\ percentage = \frac{A\ blank\ -\ A\ sample}{A\ blank} \times 100\%$$

A sample of the treatment with plant extract absorbance is at 517 nm. A blank is the sample's absorbance at 517 nm without plant extract.

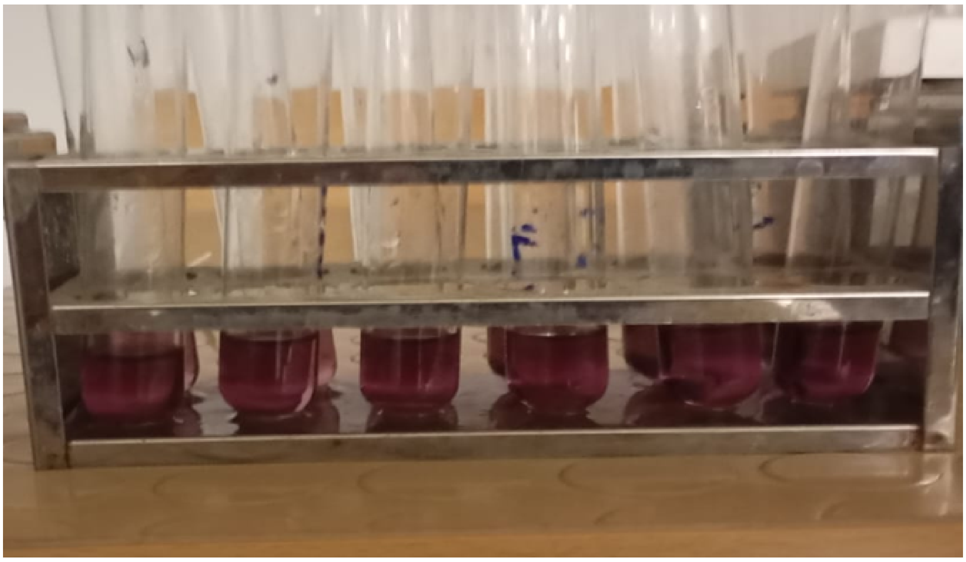


FIGURE 3.7: Check the antioxidant profile of *Hibiscus rosa sinensis* Mucilage different concentration in triplets by DPPH

3.7 FTIR Analysis of *Hibiscus rosa sinensis* Mucilage

According to the instrument handbook, *Hibiscus rosa-sinensis* leaf mucilage was used for FTIR analysis at Parkin Elmer. model no. L1600401 Spectrum Two DTGS Serial Number 107435, UK, having a scan range of 400 to 4000cm^{-1}.

3.8 Preparation of Low Fat Mozzarella Cheese

Mozzarella cheese was prepared following the method described by [69] with slight modifications. The standardized version with 3.5% fat was served as the negative control, while the reduced-fat variant with 1.5% fat was used as the positive control. Additional cheese treatments were prepared by incorporating varying concentrations of *Hibiscus rosa-sinensis*: 0.625% (T1), 1.25% (T2), and 2.5% (T3). The milk used for these treatments was pasteurized at a low temperature of 62^{o}C for 10 minutes. Then it was be cooled to 40^{o}C with continuous stirring. Once the temperature reaches 40^{o}C, 5 mL of 4.5% vinegar and 0.05 ppm rennet were added to each treatment. After 30 minutes, the curd was cut into small squares and left to settle for 10 minutes. Then the curd was slowly heated from 40^{o}C to 42^{o}C over 30-40 minutes during further cooking in whey. Once scalding is complete (after 40 minutes), the whey was drained using a fine mesh, and 1% (w/w) NaCl was added. The curd was stretched in hot water at 80^{o}C, shaped into the desired form, and stored at 4^{o}C for further analysis.

FIGURE 3.8: Different Milk samples for making Mozzarella cheese by using different concentrations of *Hibiscus rosa sinensis*

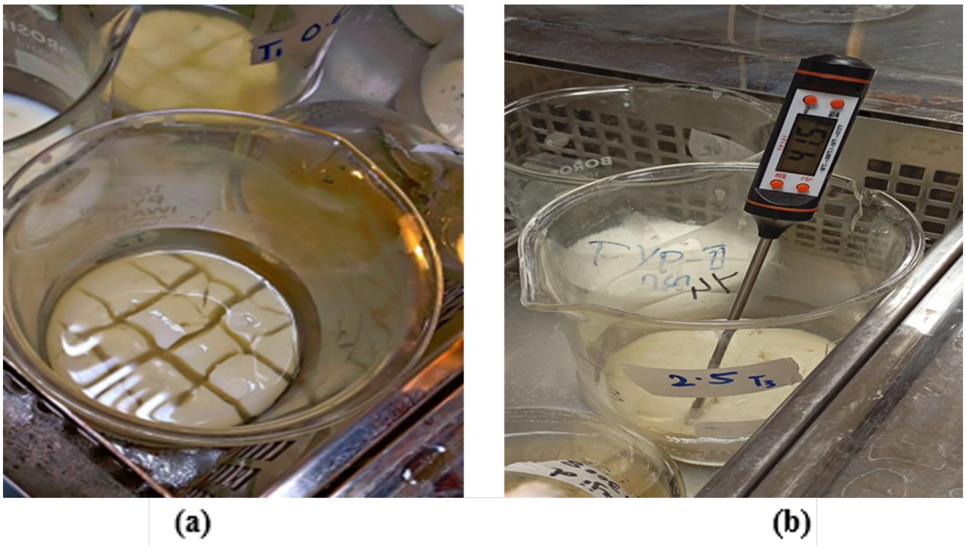
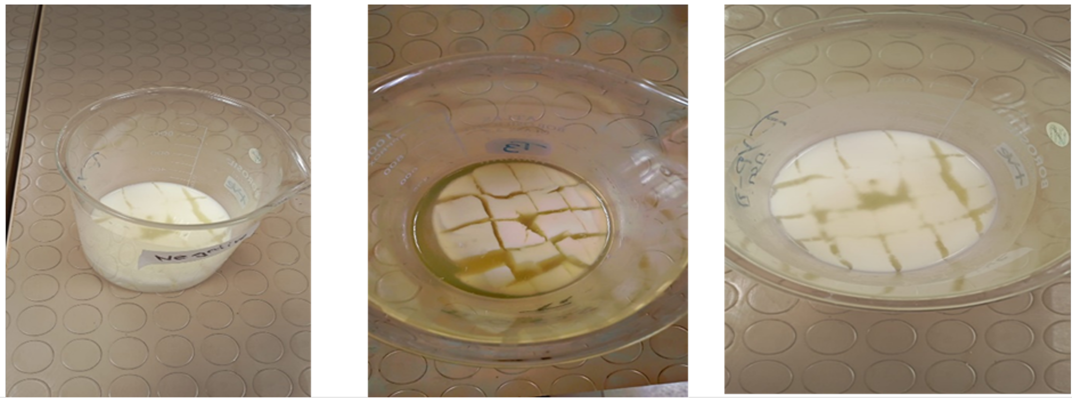


FIGURE 3.9: (a) Curd is cut into small squares (b) Maintain the temperature during processing



(a) (b) (c)

FIGURE 3.10: a,b and c Shows that different samples of Mozzarella cheese is left undisturbed to settle it down for separation of cheese from whey

3.9 Proximate Analysis and pH Determination of LF Mozzarella Cheese

Proximate analysis of the low-fat mozzarella cheese treatments containing *Hibiscus rosa-sinensis* mucilage was performed to determine moisture, crude fat, crude protein, and total ash percentages, following AOAC official methods (AOAC, 1999). The fat content in the cheese samples were measured using the Gerber centrifuge method for fat extraction, as described by [70].

3.10 Functional Properties of LF Mozzarella Cheese

The functionality of low-fat mozzarella cheese at various concentrations (Negative, Positive , T1, T2, and T3) were evaluated using the method outlined by [1], with minor modifications. The functional properties to be assessed was include meltability and fat leakage.

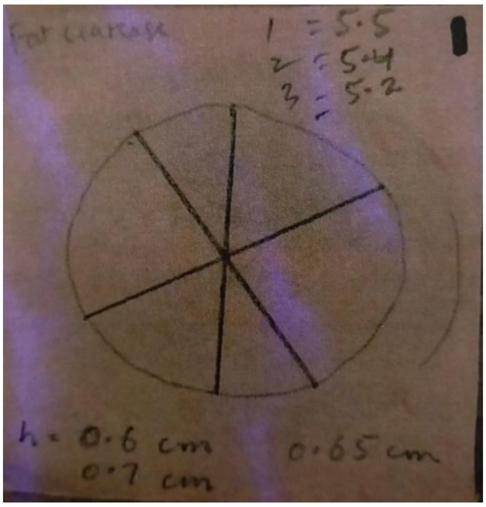


Figure 3.11: Fat leakage

3.11 Texture Profile Analysis of The LF Mozzarella Cheese

The texture profile analysis of the low-fat mozzarella cheese was determined by following the method explained by [71] with slight modifications. The firmness of the low-fat mozzarella cheese was assessed using a texture analyzer capable of handling samples weighing approximately 3-7g. Various probes designed for measuring texture, hardness, shear, and puncture was utilized. For analysis, a piece of low-fat mozzarella cheese (64 x 32 cm) was cut, and texture was measured on days 0, 3, 5, 7 and 9.

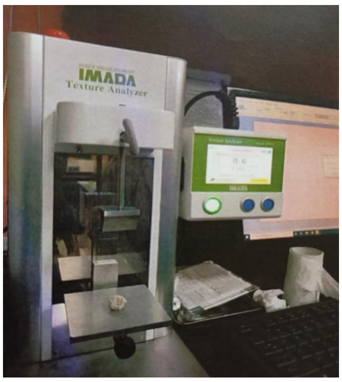


Figure 3.12: Texture Profile Analysis of samples

3.12 Antioxidant Profile of Whey and Mozzarella Cheese

The antioxidant activity of the samples was assessed using the DPPH (1, 1 - diphenyl - 2 - picryl - hydrazyl radical) method, as described [72] for milk and cheese, and [73] for Ricotta cheese with minor adjustment. A 5 g sample of mozzarella was mixed with 10 mL distilled water then 2ml of sample was added in 8 ml of 0.11 mM DPPH ethanolic solution. As a control, 2 mL of ethanol replaced the sample and was combined with 8 mL of 0.11 mM DPPH solution. The mixtures were thoroughly stirred, reacted at room temperature in the dark for 20 minutes, and then centrifuged for 10 minutes at 9000 g and 22^{o}C. Absorbance at 517 nm was measured on the supernatant using a double beam UV-VIS spectrophotometer. Antioxidant activity was calculated using the following Formula:

$$\%\ antioxidant\ activity\ =\ (A0\ \ As)/A0\ \times\ 100$$

where A0 represents the absorbance of the control, and As represents the absorbance of the tested sample. The decrease in absorbance indicates the reduction of the DPPH radical by the antioxidant molecules in the sample, reflecting its ability to inhibit the DPPH radical.

3.13 Yield Analysis of LF Mozzarella Cheese

The theoretical yield of the low-fat mozzarella cheese was calculated using a modified version of the Van Slyke yield equation, with the result expressed as a percentage.

$$Theoretical\ yield\ (g) = \frac{[(0.859\ \times\ \%milk\ fat)\ +\ (\%milk\ casein\ -\ 0.1)\ \times\ 1.13]}{1\ -\ (\%cheese\frac{moisture}{100})}$$

The actual yield (%) were determined by weighing the curd after proper pressing, following the method of Khan and Masud [74].

3.14 Organoleptic Evaluation of LF Cheese Samples

A panel of nine trained judges were evaluated all mozzarella cheese treatments made from low-fat buffalo milk for sensory attributes, including color, appearance, texture, flavor, and stringiness, using a 9-point hedonic scale. The scale will range from 9, indicating maximum liking, to 1, indicating maximum dislike, as described in [75].

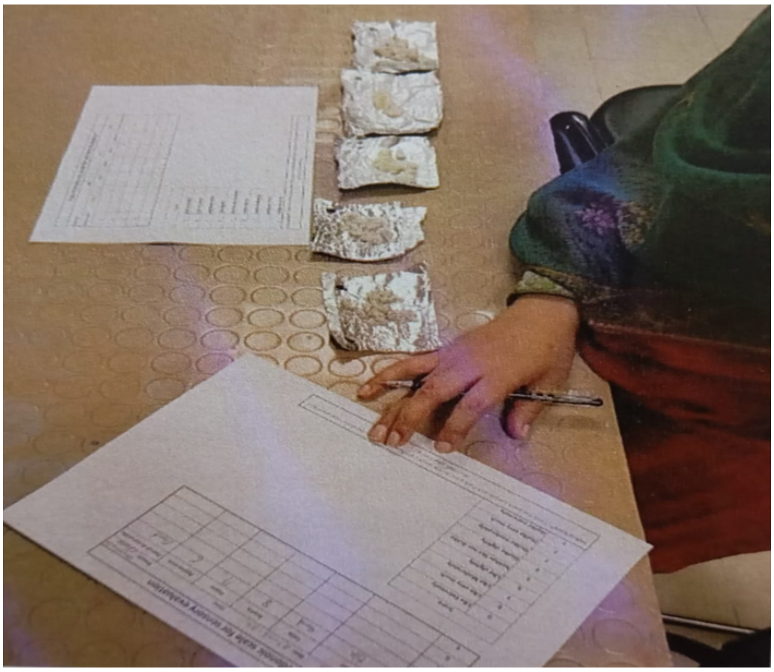


FIGURE 3.13: Organoleptic evaluation of Low Fat Mozzarella Cheese by Panel

3.15 Statistical Analysis

The collected data was be statistically analyzed using IBM SPSS Statistics 21, with the results presented as mean values and standard deviations. Tukey's test was be applied to assess significance at a 95% confidence level ($p \leq 0.05$). To ensure statistical accuracy, all trials was be conducted in triplicate using the batch flow production method.

Chapter 4

Results and Discussion

4.1 Solubility Measurement *Hibiscus* Mucilage

The solubility test for *Hibiscus* mucilage was performed to evaluate its ability to dissolve in water and different organic solvents. A 1% sample of the extracted mucilage was tested in various polar and non-polar solvents, such as distilled water and ethanol. The mucilage showed good solubility in water but formed a precipitate in ethanol. These results align with prior studies, which suggest that the mucilage is more soluble in warm water than in cold and is entirely insoluble in ethanol.

4.2 Total Polysaccharide Content of *Hibiscus rosa sinensis* Mucilage

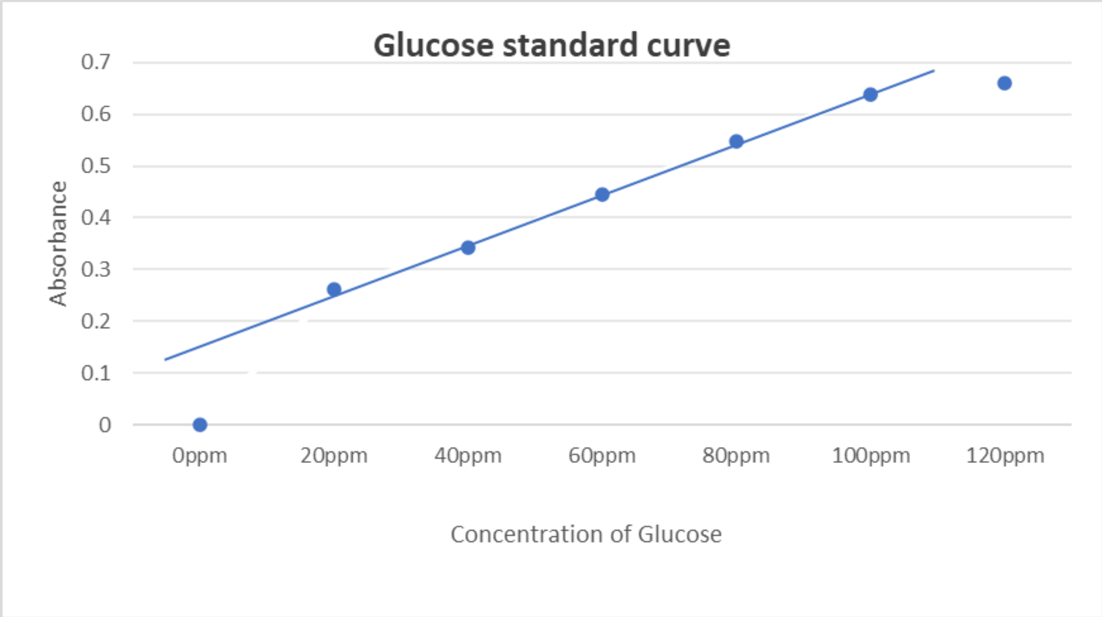


FIGURE 4.1: Glucose standard curve

TABLE 4.1: Absorbance at 490nm of different Glucose concentrations

Glucose concentration	**Absorbance at 490nm**
0ppm	0.000
20ppm	0.262
40ppm	0.343
60ppm	0.446
80ppm	0.547
100ppm	0.637
120ppm	0.660

The table presents the absorbance values at 490 nm for varying glucose concentrations (0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm, and 120 ppm). This method is typically used to determine the polysaccharide content in a sample by measuring its glucose concentration, as glucose is a major component of polysaccharides. The absorbance values correlate directly with the concentration of glucose in the sample, with higher concentrations resulting in higher absorbance readings.

At 0 ppm, the absorbance is 0.000, which is expected as there is no glucose present, serving as the baseline measurement. As the glucose concentration increases, the absorbance values also increase, indicating a proportional relationship between glucose concentration and absorbance.

For example, at 20 ppm, the absorbance is 0.262, at 40 ppm the absorbance increases to 0.343, and at 60 ppm, the absorbance rises further to 0.446. This trend continues with 80 ppm at 0.547, 100 ppm at 0.637, and the highest concentration of 120 ppm with an absorbance of 0.660.

The increasing absorbance with increasing glucose concentration suggests that the method is reliable for measuring glucose levels in the sample. The glucose concentration is directly related to the polysaccharide content because polysaccharides are polymers made up of glucose units. This absorbance data can be used to quantify the polysaccharide content in unknown samples by comparing their absorbance values to a standard curve generated from known glucose concentrations.

The technique is predicated on the idea that when chemicals interact with the glucose molecules in the sample, a color shift that can be measured at 490 nm results. Analyzing glucose which is a polysaccharide through a colorimetric reaction is a new useful opt method.

Definitely, the absorbance's constant growth caused by glucose concentration admits a method that uses this technique to accurately determine the polysaccharide content.

Under the condition that polysaccharides are widely applied in different industries, food, and medicine are included, the exact estimation of glucose has to be the primary concern. This method contributes to finding out correct polysaccharide content for a wide range of applications and is in line with standard tests in biochemical.

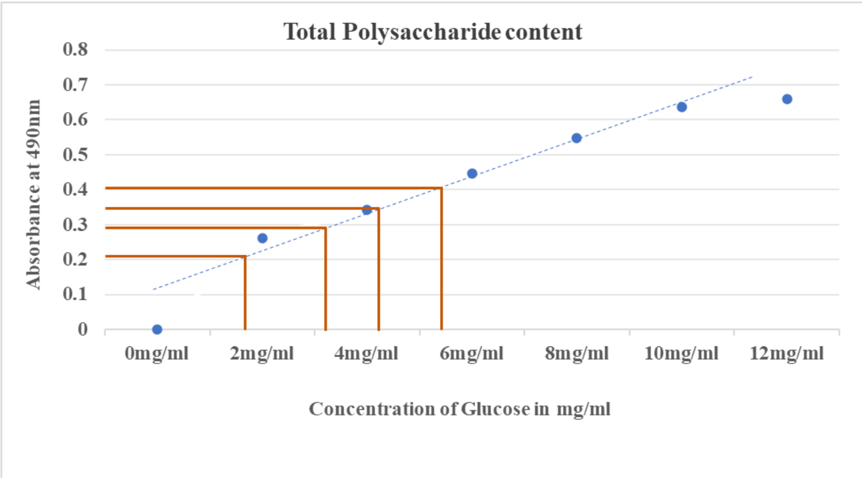


Figure 4.2: Total Polysaccharide content in *Hibiscus rosa sinensis* Mucilage

Table 4.2: Total polysaccharide content in *Hibiscus rosa sinensis*

Sample	Absorbance at 490nm	Polysaccharide content mg/ml
25ppm	0.201	2.6
50ppm	0.295	4.2
75ppm	0.354	5.2
100ppm	0.412	6.4

In addition to the matching polysaccharide content in milligrams per milliliter (mg/ml), the table displays the absorbance values at 490 nm for the following sample concentrations: 25 ppm, 50 ppm, 75 ppm, and 100 ppm.

The relationship between absorbance and polysaccharide content indicates a colorimetric method being employed to determine the concentration of polysaccharides in the sample. As the concentration of polysaccharides increases, so does the absorbance, which is typical in colorimetric assays based on the interaction between polysaccharides and the reagents used in the assay.

At 25 ppm, the absorbance is 0.201, corresponding to a polysaccharide content of 2.6 mg/ml. As the concentration increases to 50 ppm, the absorbance rises to 0.295, and the polysaccharide content increases to 4.2 mg/ml, showing a direct proportionality between the two parameters.

Similarly, at 75 ppm, the absorbance value is 0.354, and the polysaccharide content is 5.2 mg/ml, while at 100 ppm, the absorbance increases to 0.412, with the polysaccharide content reaching 6.4 mg/ml.

The proficiency of the assay to analyze the amount of polysaccharides in the sampled material is mirrored by the positive correlation between the absorbance and the concentration of polysaccharide in the sample. Greater quantities of polysaccharides in the test produce more pronounced changes in color, which can be measured at the 490 nm.

This characteristic of the colorimetric reaction with regard to concentration is displayed by the increase in the absorbance rate with a higher concentration. The foundation of this technique is that the observable alteration of the color is caused by polysaccharides; for instance, some of the indications can be measured by a spectrometer.

Polysaccharides with higher concentrations lead to the existence of more intense absorbance, which constitutes an argument for the method's accuracy in ascertaining the quantity of polysaccharides that are present. It is common that this method is used in both analytical chemistry and biochemistry processes to measure the level of the polysaccharide content in different materials especially in food science, biotechnology, and pharmaceutical industry studies.

4.3 Total Phenolic Content of *Hibiscus rosa sinensis* Mucilage

The study discovered a high correlation between the amount of phenolic compounds in plant materials and their antioxidant activity [89]. Consequently, it is essential to consider the impact of total phenolic concentration on extracts' antioxidant activity. According to certain theories, polyphenols are important phytochemicals that have potent antioxidant and other medicinal qualities. It has been demonstrated that phenolic compounds are the primary plant chemicals with antioxidant activity, and that their redox properties are what enable them to do so. Phenolic chemicals are one class of antioxidants that have the ability to both adsorb and neutralize free radicals [90].

The FC method was used to determine the total phenolic content of the plant extract, and a calibration curve was created using gallic acid. The standard curve was used to create a regression equation, which was then used to quantify the amount of gallic acid in the *Hibiscus rosa sinensis* ethanol extract: y= 0.0054x+0.1183, R2 = 0.9759, where x is the equivalent gallic acid (mg/ml) and y is the absorbance. The absorbance values (0.21, 0.322, 0.335, 0.579 and 1.248) increase as the concentration of Gallic acid increases (0 to 200 μg/ml). This implied that the amount of light absorbed at 765 nm and the concentration of gallic acid in the solution were directly correlated. Gallic acid curve shown in figure 4.3.

TABLE 4.3: Absorbance of different Gallic acid concentrations

GA conc.(μg/mL)	**Absorbance**
12.5	0.21
25	0.322
50	0.335
100	0.579
200	1.248

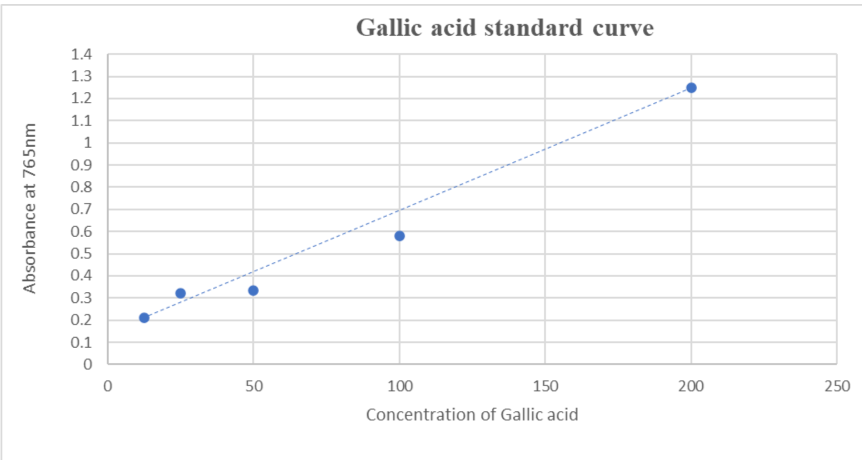


FIGURE 4.3: Graph plot of Gallic acid results

The *Hibiscus rosa sinensis* mucilage showed phenolic contents. The total phenolic contents of plant extracts from *Hibiscus rosa sinensis* depend on the kind of extract, or the polarity of the extraction solvent. Since phenols are highly soluble in polar solvents, the extracts that are produced when these chemicals are extracted include large amounts of them [91]. At 100ppm concentration, the of *Hibiscus rosa sinensis* leaf extract exhibited absorbance up to 0.809. At 50ppm concentration, the of *Hibiscus rosa sinensis* leaf extract exhibited absorbance up to 0.718. At 200ppm concentration, the of *Hibiscus rosa sinensis* ethanolic leaf extract exhibited absorbance up to 0.328. As the extract content grew, the absorbance values increased as well, as seen in table. This implied a favourable relationship between the extract's phenolic component concentration and absorbance measured at the specific wavelength used (typically around 765 nm for phenolic compounds).

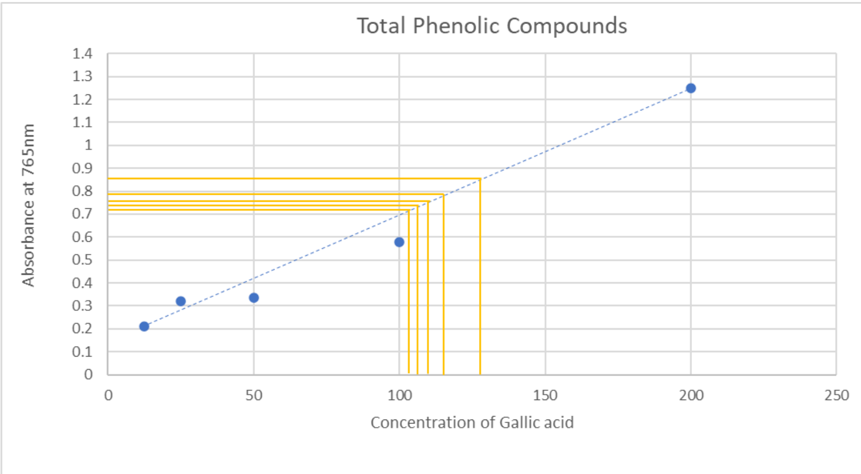


FIGURE 4.4: Total Phenolic compounds in *Hibiscus rosa sinensis* mucilage

TABLE 4.4: Phenolic concentration in *Hibiscus rosa sinensis* mucilage

Sample	**Absorbance at 765nm**	**Phenolic concentration μg/ml**
25ppm	0.719	106
50ppm	0.733	109
100ppm	0.757	112
150ppm	0.792	120
200ppm	0.861	130

The table provides data on the relationship between absorbance values at 765 nm and the phenolic concentration (μg/ml) for different sample concentrations (25 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm). Absorbance at 765 nm is commonly used in colorimetric assays to quantify phenolic compounds, as phenolics typically absorb light at this wavelength due to their chemical structure.

The increasing trend in both absorbance and phenolic concentration suggests a direct correlation between the concentration of the sample and the amount of phenolic compounds present.

For the sample with a concentration of 25 ppm, the absorbance value is 0.719, corresponding to a phenolic concentration of 106 μg/ml. As the concentration increases, the absorbance values also rise, with 50 ppm showing an absorbance of 0.733 and a phenolic concentration of 109 μg/ml.

This pattern continues for higher concentrations, with 100 ppm having an absorbance of 0.757 and a phenolic concentration of 112 μg/ml, 150 ppm having an absorbance of 0.792 and a phenolic concentration of 120 μg/ml, and 200 ppm showing the highest absorbance of 0.861 with a phenolic concentration of 130 μg/ml.

The data clearly indicate that as the sample concentration increases, the phenolic concentration also increases, reflecting the typical behavior observed in assays measuring phenolic content. This trend can be attributed to the increased availability of phenolic compounds in the solution, which directly influences the absorbance value at 765 nm.

The findings align with those in similar studies, which show that absorbance is proportional to phenolic concentration within a certain range of sample concentrations. This is in accordance with the Beer-Lambert law, which states that absorbance is directly proportional to the concentration of the absorbing substance, assuming the path length and the molar absorptivity are constant.

Thus the data in the table given stand as the strong evidence that the method is reliable for the determination of phenolic content in a sample and show that the absorbance measurements at 765 nm can make it possible to estimate phenolic concentration of a whole set of concentrations.

This methodology is commonly exploited in research endeavors directed to evaluate antioxidant making from phenolics, since phenolics are very popular in this type of investigation as these natural products posses many beneficial effects, especially attaining to acknowledgement of free radicals and oxidative stress which will be the result of certain phenolic compounds.

4.4 Antioxidant Profile of Mucilage

The scavenging of free radicals is the known method by which antioxidants stop oxidation. The scavenging of stable DPPH free radicals approach allows for a quick evaluation of a compound's or extract's antioxidant capability [81]. The technique used to measure the antioxidant activity of the extract of *Hibiscus rosa sinensis* leaves is called the DPPHH (2, 2 diphenyl-1-1 picrylhydrazyl) test. Methanol was used as control.

Scavenging activity is shown in (Table 4.5). Leaf extract demonstrated the ability to scavenge free radicals. Scavenging activity is shown in (Table 4.5). Leaf extract demonstrated the ability to scavenge free radicals.

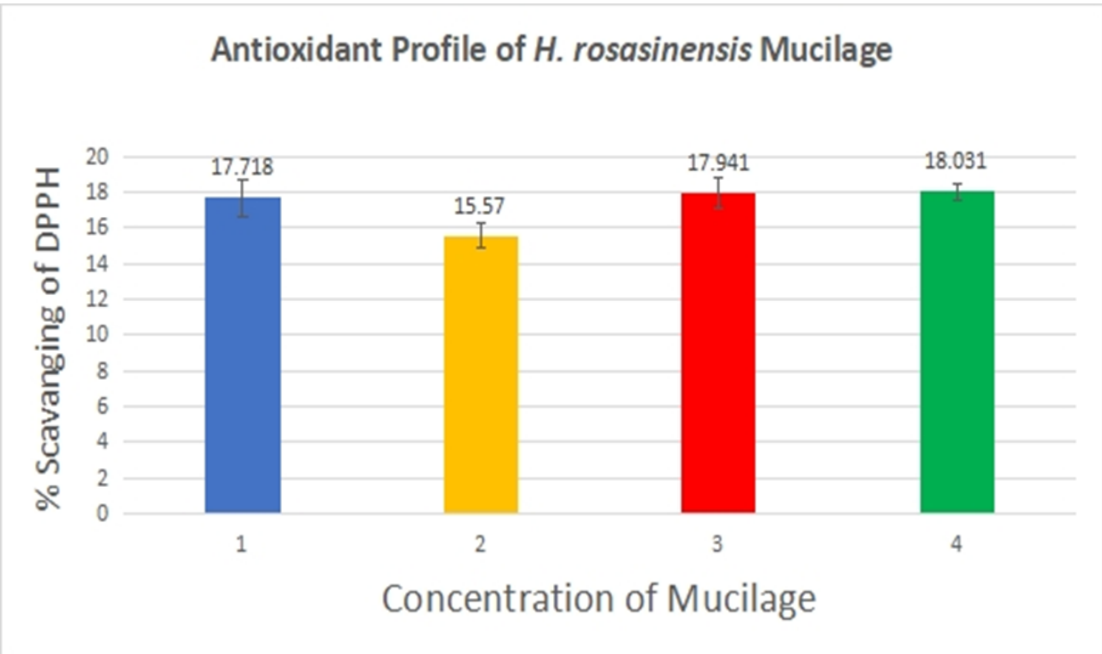


FIGURE 4.5: Antioxidant property of *Hibiscus rosa-sinensis* Mucilage

TABLE 4.5: %age scavenging of *Hibiscus rosa sinensis* leaf extract after 30 minutes

Extract concentrations(ppm)	Scavenging activity after 30 minutes
25	17.718 ± 1.05
50	15.57 ± 0.67
100	17.941 ± 0.89
150	18.031 ± 0.47

The scavenging activity of *Hibiscus rosa sinensis* mucilage increases as the concentration of mucilage increases. Scavenging %age at 25ppm is 17.718%, 50ppm is 15.57%, 100ppm is 17.941% and 150ppm is 18.031%. The data are shown as mean ± S.D. and each value in the table was derived by averaging the results of three tests. The antioxidant activity of the leaf of *Hibiscus rosa sinensis* was calculated using DPPH.

The DPPH stable free radical assay is a simple, rapid, and sensitive method to evaluate the antioxidant activity of a specific medication or plant extracts [82]. When free-radical DPPH interacts with an odd electron at 517 nm, the highest absorption occurs (purple hue). DPPH and an antioxidant that scavenges free radicals combine to form DPPHH, which has less hydrogen than DPPH and is hence less absorbent. In contrast to the DPPH-H state, this radical form decolorizes, or turns yellow, as it accumulates more electrons.

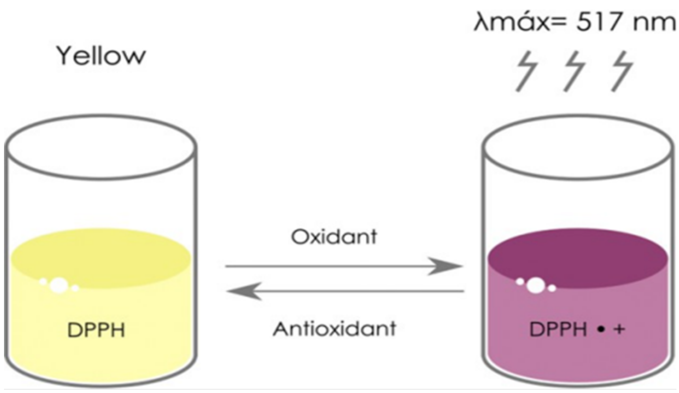


FIGURE 4.6: Demonstrating how an antioxidant and free-radical scavenger combine to produce DPPHH from DPPH

The antioxidant effect is indicated by the test samples' decreased purple DPPH color. Therefore, antioxidant molecules can neutralize DPPH free radicals by providing a hydrogen atom or electrons. This results in the formation of the colorless, stable molecule 2, 2 - diphenyl - 1 - hydrazine, which lowers the solution's absorbance at 517 nm. To confirm the stability of the DPPH solution, it was left undisturbed for 10 minutes. To confirm the stability of the DPPH solution, it was left undisturbed for 10 minutes. The color of the solution did not change during the experiment, suggesting that DPPH had reached its maximum stability. To confirm the stability of the DPPH solution, it was left undisturbed for 10 minutes. The color of the solution did not change during the experiment, suggesting that DPPH had reached its maximum stability. The intensity of absorption was measured at 517 nm. When *Hibiscus rosa sinensis* leaf extract was gradually added to the DPPH solution, the absorption peak strength at 517 nm steadily decreased, and the color of the solution gradually changed from deep violet to pale yellow.

The antioxidant activity percentage was calculated as [**?**] $\times$ 100.

According to [83] the ability of phenolic compounds in plant extracts to donate hydrogen is a major factor in their scavenging activity. *Hibiscus rosa sinensis* extract seems to donate a great amount of hydrogen, which is supported by the increased scavenging activity over time. The antioxidants DPPH radicals seem to have a greater effect on peroxidation of lipids because of their ability to give hydrogen atoms [84]. It is important to scavenge radicals because it causes the harmful effects to stop. The DPPH free radical scavenging method by antioxidants resulted in reduced lipid peroxidation. This method is very popular for determining the antioxidant capacity of a compound. The purple-colored DPPH+ radical cation is most seen at 517 nm.

When the DMPD+ donor is in the presence of electrons it causes decoloration of the solution. The extent of the reaction is directly related to the size of the sample's antioxidant capacity. The study's results were found to be compatible with previous research on phenolic compounds' behaviors and DPPH radicals in the ways they interact. The conclusion of the current work brought out the potential applicability of *Hibiscus rosa sinensis* extract in health-related sectors and, at the same time, the importance of time in the enhancement of its antioxidant properties was stressed.

4.5 FTIR Analysis of *Hibiscus rosa sinensis* Mucilage

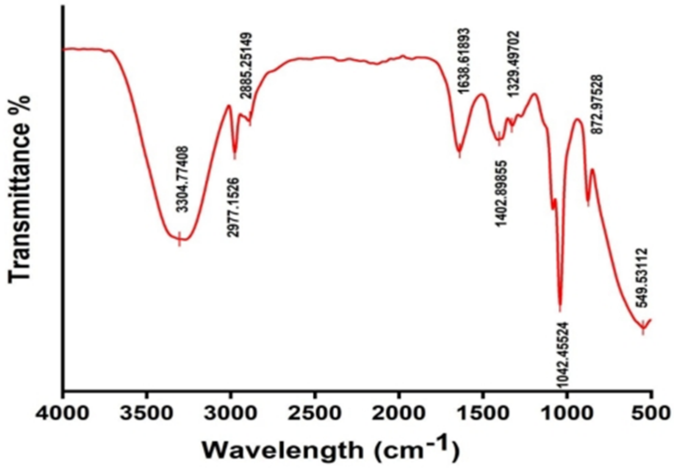


FIGURE 4.7: FTIR Analysis of *Hibiscus rosa sinensis* Mucilage

TABLE 4.6: Analysis of Functional groups according to the peak values of FTIR

Peak values in WEL	Type of functional groups
3304.77408	O-H stretch
2977.1526	Asymmetric C-H vibrations
2885.25149	O-H stretch
1638.61893	Aromatic C=C bending
1402.89855	C-CHO bending
1329.49702	O-H stretch
1042.45524	C-O stretching vibrations
872.97428	O-H stretch
549.53112	O-H stretch

The FTIR spectroscopy is used to scrutinize the chemical structure of *Hibiscus rosa-sinensis* mucilage as part of the composition and functional characteristics study. *Hibiscus rosa-sinensis* mucilage FTIR spectrum, in which there are multiple prominent peaks, matching different functional groups. The peak values (in cm^{-1}) from a Wavelength-Dependent Spectroscopy (WEL) examination are shown in this table, and each peak corresponds to a distinct functional group. Moreover, alcohols or phenols groups can be observed because they are distinguishable due to the peaks at 3304.77, 2885.25, 1329.50, 872.97, and 549.53 cm^{-1} that are the symmetrical stretch of the OH (hydroxyl) groups. The signal at 2977.15 cm^{-1} is related to C-H bonding in which it is likely to be seen in alkyl groups. The peak at 1638.62 cm^{-1} is attributed to C=C bonds, which although they are identified in conjugated compounds or alkenes. The presence of aldehyde functional groups can be inferred from the peak at 1402.90 cm^{-1}, which basically is associated with a C-CHO (aldehyde) group. The signal at 1042.46 cm^{-1} is responsible for the S=O(sulfonyl) group, which informs us about the sulfur-containing compounds.

On the whole, this spectrum is the one to distinguish the clusters of functions found in the sample. Because it has gel-forming and binding qualities, the presence of these functional groups promotes the prospective use of *Hibiscus rosa-sinensis* mucilage as a natural, bio-based ingredient for fat substitution in food items, such as in the formulation of low-fat mozzarella cheese. Mucilage's many hydroxyl (-OH) groups help it hold onto moisture, which enhances its suitability as an emulsifier, thickening agent, and stabilizer in food, medicine, and cosmetics. Carboxyl (-COOH) groups enhance the mucilage's solubility and gel-forming capacity, which is crucial for regulating viscosity and producing smooth textures in low-fat formulations like reduced-fat mozzarella cheese.

Besides, phenolic groups have been found to be both antioxidant and antibacterial, and this is of great significance in enhancing the shelf life and stability as well as the promotion of product growth. Else, amino (-NH2) groups present in thermoplastic agents may be responsible for the bioactivity based on *Hibiscus* as well as the rewarding multiply medicinal advantages. Thus, *Hibiscus rosa-sinensis* mucilage with the help of these functional groups is a beneficial, natural replacement of synthetic additives, so over the long run it becomes a more eco-friendly and safer option for a variety of applications [85]. Besides this, fat substitutes contain different functional groups like those which are originated from proteins, polysaccharides, or synthetic chemicals, which affect their performance in cooking. The main hydroxyl (-OH) and carboxyl (-COOH) groups to be seen in polysaccharide-based fat substitutes, for example, guar gum, xanthan gum, and *Hibiscus rosa-sinensis* mucilage, are able to absorb moisture, solidify gels, and control viscousness by mimicking fat structure and the mouthfeel.

Fat substitutes that are protein-based, like whey and soy proteins, have carboxyl (-COOH) and amino (-NH2) groups that lead emulsification and stabilize the emulsions but at the same time provide them with a creamy consistency and fat-like quality [86]. In contrast, the substitutes like the fat-based one, which is Olesta, achieve this effect using the -COO- functional group to copy the chemical structure of triglycerides and imitate the taste of fat without adding calories, and even though they may irritate the digestive system, they are still as harmful as the real fat [87].

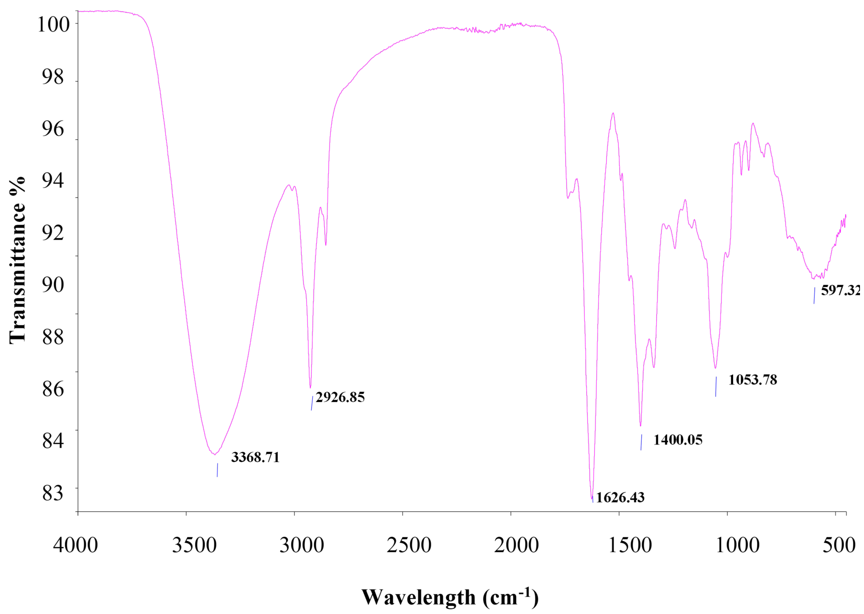


FIGURE 4.8: FTIR Analysis of *Hibiscus rosa sinensis* leaf extract [89]

Bands at 3368.71 cm^{-1}, 2926.85 cm^{-1}, 1626.43 cm^{-1}, 1400.05 cm^{-1}, 1053.78 cm^{-1}, and 597.32 cm^{-1} (Unknown) were observed when leaf extract was observed in water

(WEL). These bands indicated the existence of amines (N-H) group, aldehyde (C-CHO) group, and sulfoxide (S=O) group [89].

Simplesse and other synthetic fat substitutes incorporate hydroxyl (-OH) and ester (-COO-) groups to simulate the sensation and consistency of fat that can be achieved with lower calorie content. The efficiency of every fat substitute for different functions is identified by these active groups; Nutritional benefits are brought by proteins, polysaccharides offer natural, biocompatible replacement, and synthetic substitutes offer low-calorie options with varying appeal to consumers [88].

4.6 Proximate Analysis of LF Mozzarella Cheese

Table 4.7: Proximate Analysis of LF Mozzarella cheese samples

Treatments	**Moisture (%)**	**Total Solids (%)**	**Fat (%)**	**Protein (%)**	**Carbohydrate (%)**	**Ash (%)**
T1	52.57	47.53	11.63	28.2	3.8	3.9
T2	53.1	46.9	11.7	27.2	4.02	3.98
T3	53.1	46.9	11.75	26.9	4.15	4.1
Positive	52.2	47.8	11.85	27.15	4.8	4
Negative	47.17	52.83	24.05	24.5	3	1.28

Due of variances in formulation and chemical mix, we noticed discrepancies between the treatments. Compared to T2 and T3, which contained 53.10% moisture and 46.90% total solids, respectively, T1 had slightly less moisture (52.57%) and more total solids (47.53%). A somewhat denser matrix is suggested by T1's reduced moisture content, which could be the result of variations in how protein and water-binding components interact. A larger concentration of non-water components is indicated by higher total solids in T1, which may be the result of a higher protein or ash content.

T1, T2, and T3 all had fat contents that varied from 11.63% to 11.75%, indicating that the distribution of fat was comparatively consistent among these treatments.

The addition of varying amounts of *Hibiscus rosa-sinensis* mucilage, however, might have had an impact on fat retention and caused slight variations. With a protein concentration of 28.20%, T1 was the highest, followed by T2 (27.20%) and T3 (26.70%). The slow decrease in protein concentration from T1 to T3 may be a sign that other factors, like moisture or carbs, are gradually replacing protein-rich components. Protein distribution may also be influenced by the interaction between proteins and mucilage, since some hydrocolloids can modify the development of protein networks, resulting in minute changes.

From T1 (3.80%) to T2 (4.02%), the amount of carbohydrates increased marginally, reaching its greatest in T3 (4.15%). This rise may be the result of increased quantities of mucilage, which contains polysaccharides, and indicates a growing enrichment of carbohydrate-based components in T2 and T3. Mucilage may have indirectly increased the carbohydrate content in T2 and T3 by promoting greater moisture retention due to its hydrophilic nature.

From T1 (3.90%) to T2 (3.98%), the ash content a measure of the overall mineral content also rose, peaking in T3 (4.10%). The added mucilage's natural mineral content or other formulation modifications that impact mineral solubility and retention could be the cause of this tendency. The treatments with somewhat lower moisture (52.20%) and greater total solids (47.80%) were not the same as the Positive Control. This implies that its formulation, which may have been caused by the presence of additional stabilizers or the lack of mucilage, encouraged water loss or decreased water-binding capacity. At 11.85%, the Positive Control had the greatest fat percentage of any treatment or control, suggesting that its composition was designed to maximize fat retention, most likely by using conventional fat substitutes. Although it kept a balanced protein structure, its protein concentration (27.15%) was marginally lower than T1 but greater than T2 and T3, suggesting that formulation changes may have caused the modest decrease.

At 4.80%, the Positive Control also had the greatest carbohydrate content, presumably as a result of the addition of more stabilizers or fillers based on carbohydrates. Although it was lower than T2 and T3, its 4.00% ash level was higher than T1, indicating a distinct mineral composition. The On the other hand, Negative Control had the highest total solids (52.83%) and the moisture content is the lowest (47.17%), composition is suggesting more concentrated with the minimum water retention. This is probably because there are no hydrocolloids or fat substitutes, which often aid in moisture retention. Since the Negative Control most likely comprised full-fat ingredients without fat substitutes, its abnormally high fat level of 24.05% was much higher than that of any other treatment. At 24.50%, its protein level was decreased, possibly as a result of the high fat content diluting it. Furthermore, the Negative Control had the lowest carbohydrate content (3.00%), indicating that components high in carbohydrates contributed very little. The compositional variations between the Negative Control and the other treatments demonstrate how mucilage-based fat substitutes can be used to adjust nutritional and functional properties. These results shows that adding *Hibiscus rosa-sinensis* mucilage as a fat substituent to formulate low-fat mozzarella cheese affected the cheese's overall composition, moisture retention and protein distribution. The observed changes are consistent with the anticipated functional characteristics of hydrocolloids, which have the ability to modify the structural, nutritional, and textural characteristics of cheese.

Hibiscus rosa-sinensis and Aloe vera mucilage have different chemical compositions, emulsification qualities, water-holding capacities, and interactions with the cheese matrix, and their different proximate analyses of low-fat mozzarella cheese. The high acemannan content of aloe vera mucilage helps it retain more moisture, which makes the cheese richer and softer in carbohydrates. *Hibiscus* mucilage, on the other hand, has a decreased affinity to bind water, which results in cheese that is harder and retains more protein and total solids. While *Hibiscus* mucilage increase structural integrity and mineral content, aloe vera mucilage adds a smoother texture and greater meltability. Mucilages of both plants work well to replace fat in cheese, the choice of which one to use relies on the ideal ratio of moisture, texture, and nutritional makeup; *Hibiscus* promotes firmness and aloe vera promotes softness [61].

Individual properties of okra include high viscosity and radical emulsification, which is due to the substantial content of galacturonic acid and pectin. All these result in high moisture retention and cheese with a relatively soft texture. *Hibiscus* mucilage, however, has a less value in water binding, making the cheese hard and with more total solids. *Hibiscus* mucilage, by the way, increases the thickness providing a little less fat retention while okra mucilage cheese is stirred to fine emulsification for better fat distribution. The former simply has a diluting effect on protein assimilation, but the latter has so much more protein that it easily outnumbers the former.

Okra mucilage is more soluble in water than *Hibiscus* mucilage, hence okra mucilage cheese has a higher carbohydrate content. Conversely, *Hibiscus* mucilage typically has a richer mineral profile than okra mucilage with ash percentage being high. The two mucilages are similar as fat alternatives but the disparities are big. Okra mucilage enhances moisture retention and softness whereas *Hibiscus* mucilage adds to a firmer texture with better mineral retention and protein. Hence, the choice of mucilage lies in the desired technological functions of low-fat mozzarella cheese. [9].

The results obtained in the study carried out on the texture, moisture content, fat distribution and general characteristics of low-fat mozzarella cheese product made by different fat substituents in the presence of different hydrocolloids are presented. The minerals' content is influenced by murcilage from *Hibiscus rosa-sinensis*, Aloe vera, and okra which the article states also that no specific effects on the fat globular distribution was observed on the protein and carbohydrates.

Hibiscus mucilage yields a firmer and more protein retentive texture, but aloe vera mucilage tenders a smoother texture and increases the moistness. Being low in fat, okra mucilage still retains the creaminess as it is the substance that makes the fat dispersion and emulsification work better. According to the study, it was concluded that each fat replacer brought benefits in its unique way and the decision which one to select for low-fat mozzarella cheese can be based on the nutritional composition, texture desired and practical features [76].

4.7 pH of LF Mozzarella Cheese

Table 4.8: pH of all samples of LF Mozzarella cheese after 5 days

Sample	**Ph after 5 days**
Negative control	5.55
Positive control	5.50
T1	5.49
T2	5.52
T3	5.55

The table reveals the pH values of mozzarella cheese samples after the storage of five days and the differences between the experimental groups T1, T2, and T3 that were exposed to varying quantities of *Hibiscus rosa-sinensis* mucilage, as well as the control groups including the positive and negative controls. The negative control, which contains no *Hibiscus* mucilage, has the pH of 5.50, which is slightly higher than the pH of the positive control, indicating a possible traditional fat substitute or an alternate formulation. Depending on the addition of *Hibiscus rosa-sinensis* mucilage, the pH of mozzarella cheese testing samples T1, T2, and T3 may differ.

The pH measurements show that the mucilage from *Hibiscus rosa-sinensis* indeed does not alter the acidity of mozzarella cheese in general over a span of 5 days. T1, which had a pH of 5.49, making it the lowest pH, was the test sample that had been exposed to the lowest level of mucilage. T2's pH of 5.52, meaning a very similar value was obtained as for the positive control, indicates a good pH stability of a cheese with a moderate mucilage content. T3's pH, which is the maximum mucilage, is 5.55, thus similar to that of the negative control, leading to the implication that there is no increase in acidity upon increasing the mucilage amount.

The pH of mozzarella cheese is a remarkable aspect that influences its texture, sensory traits, and microbiological stability. Mozzarella cheese pH values should normally be slightly acidic with a range of 5.0 to 5.5 which helps maintain its adaptable characteristics. The small differences in pH among the samples illustrate that the *Hibiscus rosa-sinensis* mucilage did not substantially acidify or alkalize the cheese. Consequently, the cheese retains its desired quality. Mucilage's core functions might be as a buffer and neutralizing of acids in the cheese matrix possibly causing T3's higher pH than T1 and T2. Furthermore, *Hibiscus rosa-sinensis* also contain active substances that are capable of inhibiting bacterial growth, therefore, may play a role in proper storage of the productions.

In general, the results can be taken to mean that the pH of mozzarella cheese is not considerably affected by the use of *Hibiscus rosa-sinensis* mucilage. In contrast, it regulates the average pH to a value that is entirely within the acceptable range and this contributes to the impression of the cheese. The slight acidification, that is probably caused by the combined action of the mucilage and the cheese matrix, can be inferred from the saw-like decrease in pH for T1 and T2 as compared to that of the control. However, the pH of T3 is higher, which may indicate that the larger amount of mucilage in relation to the cheese might act as a buffer, thus slowing down the acidification process. Further studies will have to test the impact of mucilage on the physicochemical and microbiological features of the cheese over longer periods of time.

Fat substitutes mixed with low-fat mozzarella in storage can lead to changes in its pH which will affect its physicochemical and functional properties. A study by [92] was carried out to investigate the effect of pre-acidification and whey protein-based fat replacers on low-fat Mozzarella cheese. A result of the dairy protein casein was the decrease in pH when the milk was acidified. The casein micelles that are involved in the cheese matrix dissociate from the calcium salts releasing them, thus increasing hydration. Nevertheless, pre-acidification with pH 5.5 or less is a disadvantage to the cheese's baking quality and foaminess. Basically, the cheese got softer with the aging period but its melting and stretching performances were greatly enhanced. The obtained results suggest that the pH of low-fat Mozzarella can be changed by altering the milk pH through pre-acidification and adding whey protein-based fat substitutes to its textural and functional features [93].

A study was carried out to analyze the influence of pH on the rheological and physical features of simulated pizza cheese. Higher pH values (6.2) were associated with the enhancement of the said cheese's rheological properties such as increased apparent viscosity participating in better melting and stretching. However, when a study was carried out examining the four pH values such as 5.8, 6.0, 6.2, and 6.4, this assumption was not true. Conversely, the melting and stretching of the said cheese were hindered at the beginning of the study; however, as the pH was reduced, the said features started improving. According to this work, the desired rheological and physical properties of analogue pizza cheese need to be met with the higher pH levels of 6.2 or above [94].

It is advised to use fat substitutes with different sources that are either plant-based or animal-based when you want to produce low-fat mozzarella cheese that has better quality and performance. Fat substitutes through fatty characteristics the fat imitations can upgrade the cheese's meltability, texture, and just the beauty in generally [69]. Taking into account all the necessary steps, one of the most serious aspects influencing the quality of mozzarella cheese during storage is the pH following the addition of fat substitutes. The development of low-fat white cheese with the best physico-chemical and functional characteristics can be given by the right choice of suitable fat substitutes and pH control.

4.8 Functional Properties of LF Mozzarella Cheese

4.8.1 Fat Leakage

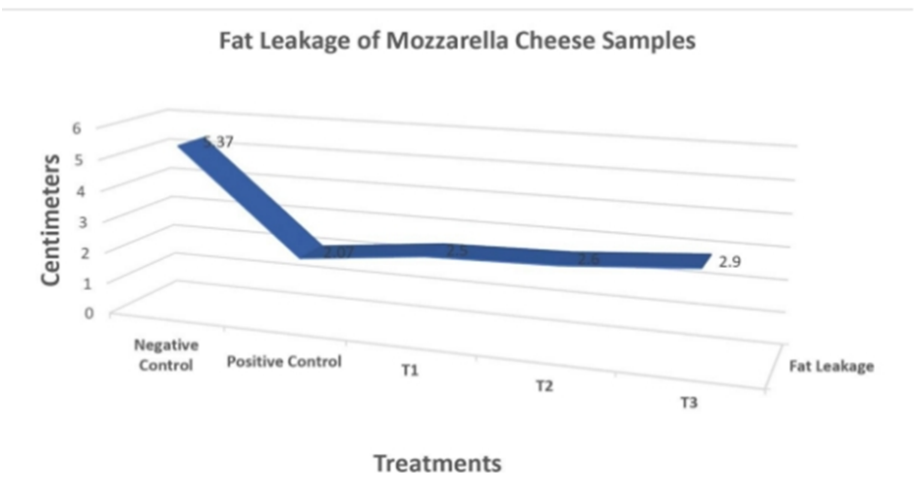


Figure 4.9: Fat Leakage in different cheese samples such as negative control, positive control, T1, T2 and T3 is 5.37cm, 2.07cm, 2.5cm, 2.6cm and 2.9cm

The graph shows the effect of several interventions on fat retention by clearly visualizing the fat leakage in mozzarella cheese samples under varied settings.

The greatest amount of fat leakage is seen in the negative control, which is untreated or modified. This suggests that mozzarella is extremely vulnerable to fat loss in the absence of any fat preservation techniques. For comparison with the other samples, this acts as a baseline.

A reference point for usual fat retention in mozzarella is established by the moderate amount of fat leakage shown by the positive control, which most likely contains standard fat retention agents or formulations.

The degrees of fat leakage vary among the different treatment (T1, T2, and T3), which use different formulations or fat-replacement techniques. Out of all the treatments, Treatment T3 shows the highest increase in fat leakage, which may indicate that its formulation was less successful in preserving fat retention. Treatments T1 and T2, on the other hand, show significantly less fat leakage than T3, which shows that they contain minimum fat content.

All treatments are significantly more successful in preventing fat loss than the negative control, because negative control shows a high leakage level, in comparison to the positive control they all cause some increase in fat leakage.

The treatments potential is highlighted by this to control fat leakage in mozzarella cheese, resulting in a more acceptable product with enhanced texture and less fat loss a major component in preserving the cheese's quality and attractiveness to consumers.

4.8.2 Meltability

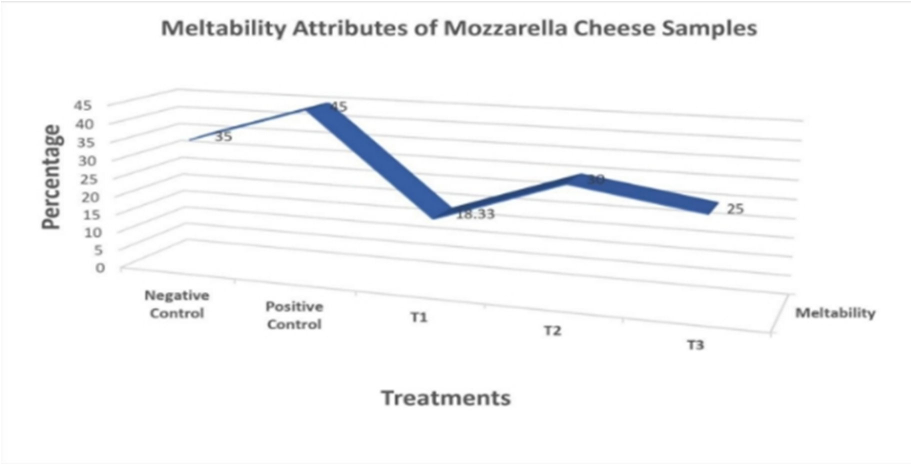


Figure 4.10: Meltability

The chart that shows the meltability properties of different samples of low fat mozzarella cheese gives important information that how different formulations by using different concentrations of *Hibiscus rosa sinensis* affect the cheese's capacity to melt when heated. Meltability is an important mozzarella quality factor, particularly for its uses like in pizza where the cheese's capacity to melt and stretch is highly considered. The meltability of the negative control, which most likely reflects a formulation with no fat replacer, was about 35%. Although it is less than what is thought to be optimum for mozzarella cheese, this finding implies that the negative control still has some typical meltability.

On the other hand, the positive control showed the maximum meltability, approaching 45%, which is consistent with the typical properties of typical mozzarella cheese. This is significant because it shows that the traditional stretchability and meltability were successfully maintained in the formulation of the positive control that customers anticipate from mozzarella. It gives information about the performance of regular mozzarella cheese and develop a standard by which different treatments can be measured. T2 have a meltability of about 30%, that stood out among the treatments as a formidable rival to the positive control. This is especially noteworthy because T2 was able to attain about 80% of the meltability of the positive control, maybe by utilizing *Hibiscus rosa sinensis* mucilage. This implies that the fat replacer did not materially alter the cheese's functional qualities and without compromising the essential meltability feature *Hibiscus* mucilage might be a good substitute for customers seeking lower-fat choices.

Treatment T3 shows a 25% meltability was marginally less successful than treatment T2 in maintaining meltability. With a meltability of only 18.33%, Treatment T1 fell behind, suggesting that this formulation would have had difficulty in preserving the desired functional qualities of mozzarella while utilizing the chosen fat substitute.

As used in T1, T2 and T3 treatments all the three showed good meltability which is crucial for consumer acceptance. Despite this, T1 could be formulated in a way other than the best. For instance, the maintaining feature of differently substituted low-fat mozzarella with meltability was confirmed by the findings indicating that *Hibiscus rosa sinensis* can bring beneficial functional properties to such cheeses. This allows for the development of sub-standard products that still meet the necessities of the consumer with respect to the senses.

On the balance of these things, the table illustrates the option of altering cheese production thus producing cheese that has the nutrients that are required nowadays and customers, still respond to the product's melting capacity showing it as a good choice for such people who are cautious about health and to those who want to eat healthier without sacrificing the taste.

According to the research, the differences in the fat substitute used had various effects on cheese meltability, with some methods proving to be better than others. For example, treatments T2 displayed quite good meltability, while the meltability of the fat replacer was not worse off as compared to the latter and that no the functional property was decreased too much. As an illustration about T1, a group of other treatments reported less meltability of which the substitution of fat was the key factor.

The general outcomes of the investigation showed fat replacement diminished meltability which in comparison to the full-fat mozzarella but some of the treatments still presented the desired meltability, hence they are viable alternatives when it comes to the production of low-fat mozzarella cheese and nutrition [69].

As the research, the application of hydrocolloids as fat substitutes can significantly affect the meltability of cheese. Hydrocolloids added, far from the full-fat mozzarella, reduced the meltability. On the other hand, it was found out that some of the hydrocolloids provided accepted meltability levels. They were also quite a bit better than some other hydrocolloids that either lacked texture or had poor meltability, which is a sign that if fat substitutes such as hydrocolloids slightly reduce meltability, they can still be used to make low-fat mozzarella with the needed functional properties. When compared to full-fat mozzarella, moved to basically full-fat mozzarella would be the first option for comparison on average [78].

4.9 Texture Profile Analysis of LF Mozzarella Cheese

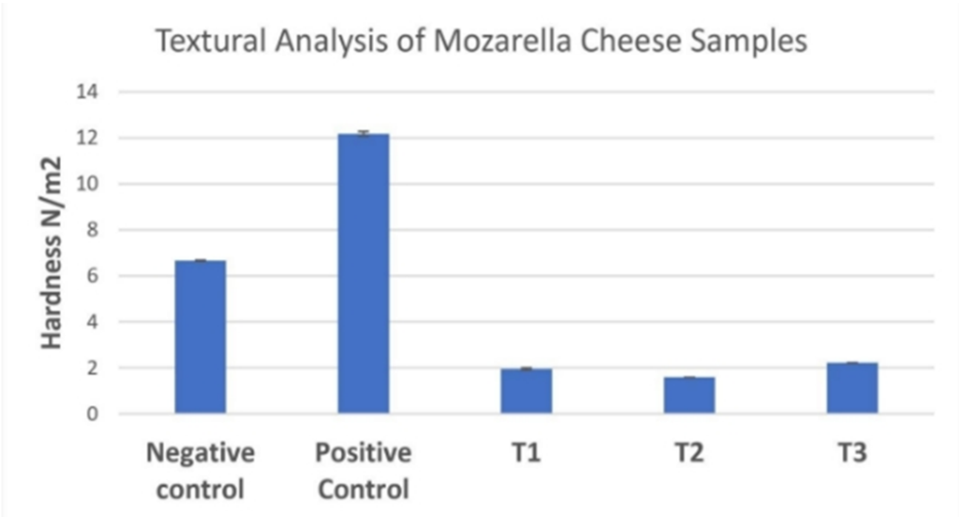


FIGURE 4.11: Texture profile analysis of LF mozzarella cheese samples. The value of hardness of negative control is 6.8 N/m^2, positive control is 12.2 N/m^2, T1 is 2N/m^2, T2 is 1.7 N/m^2 and T3 is 2.2 N/m^2

Significant differences are found between the treated samples (T1, T2, and T3), the Negative Control, and the Positive Control when the hardness values of the mozzarella cheese samples are analyzed. With the exception of the positive control, the Negative Control, which represents the cheese that has not been treated and acts as a baseline for comparison, has the greatest hardness value. Given that untreated cheese is typically assumed to have a somewhat softer texture, this finding may appear paradoxical. But this might be because of the cheese's inherent structure and composition, which could give it a harder texture when it's raw and unprocessed.

Conversely, the Positive Control sample, which is the one that is given the standard treatment in order to change cheese hardness, is a good example that is a lot harder than the Negative Control. The result infers that the cheese was undergoing a more rigid texture and was fully transformed by the treatment given to the Positive Control. The increase of hardness indicates that the Positive Control has been remodeled in a way that has made it more firm, perhaps by modifying the moisture content or the size of protein networks that eventually results in a firmer cheese texture. T3, the cheese meal that had the highest hardness among all the treated samples, comes up on top, which undoubtedly shows that this special treatment was the most successful in rendering the cheese harder. This might have been so because T3 was subjected to a specific blend of fat substitutes, hydrocolloids, or creation parameters, which, most likely, fortified the matrix of the cheese. The production of cheese, such as the one as this kind, is the most desirable because it hardens the cheese, making it suitable for such culinary applications wherein one desires a firmer one. Despite its hardness still being less than that of the hard controls, T2's value is lower than T3's, which tell us that while the treatment was successful, T2, the hardness of which is still less than that of T3, did not see as much hardness increase as T3.

The impact of T2's fat substitute or the addition of other ingredients to the protein structure or moisture retention of the cheese may not have been as noticeable as of T3, which might have caused the texture to turn out softer. T1's hardness, which is slightly smaller than T3's, is indicative that through the treatment, the cheese's hardness in the same manner as with T3 was also increased. The difference between T1 and T3 shows how similar treatments produce different effects on the mozzarella texture. That notwithstanding, T1 as well as T3 show that the treatments are able to increase the hardness level of the low-fat mozzarella cheese in comparison to the controls. Eventually, this leads to a harder texture without sacrificing some other sensory qualities.

The general discovery in this regard shows that with the exception of the treatment of the Negative and Positive controls, the treatments T1, T2, and T3 all of them were able to increase the hardness of mozzarella cheese. The treatment that had the highest success in making mozzarella cheese harder than ever before is T3, meaning that it was the best among the treatments. Therefore, one gets the idea that good knowledge in making low-fat cheese is not only important for the texture but also it is important to make a decision on the right treatment which should give you a high-quality cheese. It is mentioned in the experiment that by adding tragacanth gum to the recipe for low-fat mozzarella cheese as a fat substitute with no fat replacer becomes harder than the control cheese. This means that tragacanth gum improves bond stability between the protein and that it helps to retain water, thus, the cheese structure and firmness are affected as a result. On the other hand, tragacanth gum is effective in making up for the lost fat, which is usually the case in mozzarella cheese and can be seen from the increased firmness of the treated samples. The treated cheese with tragacanth gum had a higher hardness value than the control, but it was still within the desired range for mozzarella cheese and it was still in the order of firmness and texture. This retaining of the characteristic firmness and textture made it an ideal material for many cooking processes [79].

The results of the experiment revealed that the hardness of the mozzarella cheese increased noticeably when tragacanth gum replaced fat in comparison to the control. The authors explained that the saving nature of the gum in the cheese compound, which, in turn, resulted in the material becoming harder. The substitution of okra mucilage for fat, however, gave a little bit of hardness to the cheese, but not as much as the tragacanth gum did. It was noted that while the structural features of okra mucilage were underscored, tragacanth was the most effective fat replacer given its potential to firm up the cheese. On the other hand, the incorporation of *Hibiscus rosa sinensis* mucilage into the cheese product will result in a softer cheese than the positive control, indicating that this fat substitute was less successful in increasing hardness [80].

4.10 Antioxidant Profile of Whey

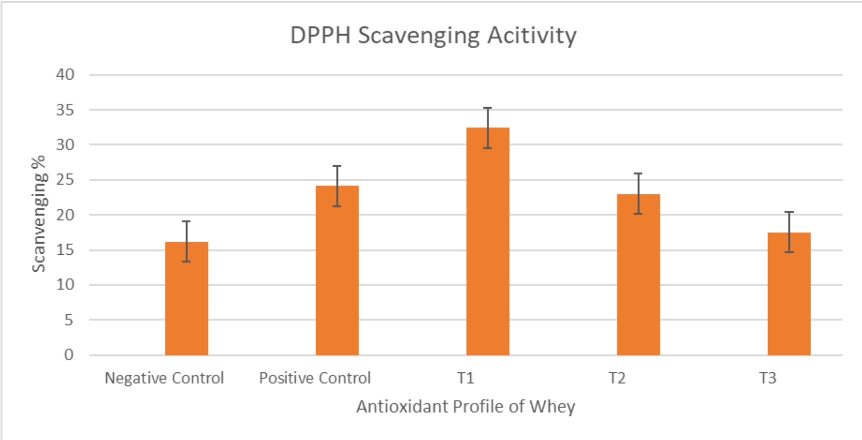


FIGURE 4.12: Antioxidant Profile of Different samples of Whey

TABLE 4.9: Scavenging activity of different samples of Whey

Extract concentrations(ppm)	**Scavenging activity after 30 minutes**
Negative control	16.18 ± 1.37
Positive control	24.16 ± 2.72
T1	32.4 ± 13.87
T2	23.02 ± 10.18
T3	17.54 ± 5.17

The table presents scavenging activity data after 30 minutes for T1, T2, and T3 samples, plus the negative and positive controls. The level of oxidative stress (a product or ROS) or free radicals that are neutralized by the antioxidants in the samples is one of the most common measures for determining the scavenging activity of the products. Also, the concentration (ppm) readings are given along with uncertainties, which indicate the accuracy of the measurements. Such a degree of uncertainty provides a more accurate understanding of the results as inconsistency and reliability are shown through it.

The negative control has the scavenging activity of 16.18 ± 1.37 ppm, and this is the initial point of the system's scavenging in the absence of active chemicals. The fact that the negative control's relative scavenging activity was poor might suggest that the sample did not produce an important amount of regular reactive species that the scavenger was involved in. This problem occurs mainly because the control sample is theoretically supposed to be free from the synthetic antioxidant agent, meaning that it has to be used only for internal detection of inherent system activity as well as external factors which might affect the measurement. A lower numerical value of uncertainty in a given case leads to a higher period of consistency in the negative control's readings.

The question addressed by the positive control, which is an indicator of the presence of materials that are shown to be efficient in scavenging reactive species, was a pertinent one: to which the answer was that the active compound in the positive control specifically reduces reactive oxygen species or free radicals, and thus increases the scavenging activity above the one in the negative control, the value of which is 24.16 ± 2.72 ppm. Cellular damage may occur if cellular antioxidants do not quench the reactive oxygen species causing free-radiation species such as hydrogen peroxide (H2O2), superoxide anion (O2−), and hydroxyl radicals (OH•). The higher level of uncertainty in the positive control compared to the negative control is indicative of the lower reliability of the positive control measurements, which is likely related to some experimental variations that were not taken into account or to the preparation of sample materials that were not done on time. Nevertheless, the scavenging ability of the positive control is still clearly seen and should be handled with care because of its inherent antioxidant virtues.

For T1, the scavenging activity is recorded as 32.4 ± 13.87 ppm, which is the highest among all the samples tested. This means that T1 has a strong scavenging effect, which presumably is brought about by a potent component interacting with free radicals or other oxidizing species. A striking feature of T1 is its impressive scavenging activity, which indeed suggests that T1 may be a very effective ROS scavenger. At the same time, the high level of uncertainty, 13.87 ppm, related to T1 signals that the measurements carried out were affected by a high degree of variability. This may be due to a number of reasons such as inconsistent experiment conditions, varying concentrations of the sample, and erroneous measurements. It could also imply that T1 is a compound whose defense mechanism against free radicals is very responsive to the prevailing conditions. T2's scavenging activity, measured at 23.02 ± 10.18 ppm, is marginally lower than that of the positive control but closer to it. This implies that while T2 is not as efficient as T1, it may have some scavenging potential. T2's comparatively high uncertainty number raises the possibility that the measurement was more variable than other samples, either as a result of variations in sample preparation or the variable behavior of T2's active ingredients under testing circumstances. Although it might not be as strong as T1, the value suggests that T2 could still be a useful scavenger of reactive species.

At 17.54 ± 5.17 ppm, T3 has a slightly higher scavenging power than the negative control, but it is lower compared to the positive controls and T2. The result of T1 compared to the positive control suggests a mild level of scavenging activity, which could be due to a smaller amount of the molecule involved or a weaker scavenger. In contrast to T1 and T2, the uncertainty in the case of T3 is low, so it stands as a sign that T3 measurements were more correct and constant. It might mean that the activity of T3 is rather stable but its general efficacy is less than that of T1 or the positive control.

In a word, it can be concluded that the diverse samples exhibit varied degrees of scavenging potential, whereas T1 shows the greatest scavenging activity. These differences can be attributed to the type, extent, and content of the ingredients that are active in each of the samples, i.e., they are due to the varied scavenging ability of the samples. It is suggested that in the case of the T1 sample a strong antioxidant or some other substance that can effectively reduce the percentage of reactive oxygen species might be present. Conversely, the positive control, which acts as a benchmark for the antioxidant capability of the samples, confirms the reliability of the scavenging activity measurement technique.

In a number of the samples there is more lack of clarity as far as T1 and T2 are concerned, this may suggest that they are not compatible in the readings, maybe it is due to inconsistent preparation and/or testing conditions. These particular experimental conditions would also be technical sample preparation, testing environment, or any other impurities that could have affected the results. These results stress the importance of testing for both the scavenging activity as well as the appropriate random error, as this gives information about the strength and reliability of the samples' antioxidant capacity. A better understanding of the antioxidant potential of these samples might be achieved by more thorough investigation, and by complementing extra testing and methodological improvements.

4.11 Antioxidant Profile of Mozzarella Cheese

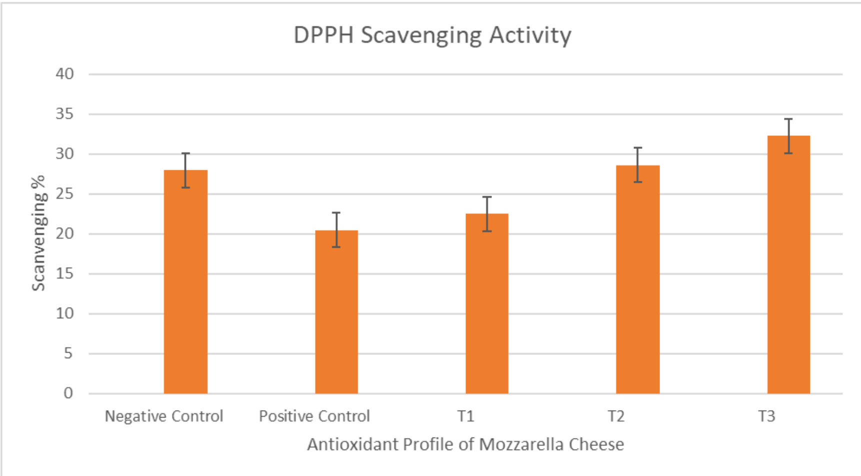


FIGURE 4.13: Antioxidant Profile of Different samples of Mozzarella cheese

TABLE 4.10: Scavenging activity of Different samples of Mozzarella Cheese

Samples	**Scavenging activity after 30 minutes**
Negative control	27.98 ± 4.18
Positive control	20.49 ± 5.24
T1	22.5 ± 1.88
T2	28.63 ± 0.81
T3	32.27 ± 11.24

The findings from a 30-minute scooping activity of mozzarella cheese samples are presented here. Scavenging potential is expressed in parts per million (ppm). In the case of the development of foods, such as mozzarella cheese, the scavenging activity of the substance the ability of a substance to neutralize the free radicals and reactive oxygen species (ROS) has the key role. Besides the positive control, which did not have any scavenging agents, a control with a negative scavenging rate was the initial stage that presented the base level of scavenging activity in the place of scavenging agents and demonstrated a scavenging rate of 27.98 ± 4.18 ppm. This number means that the mozzarella cheese itself, without extra antioxidants, can mean that there are naturally present vitamins, minerals, and other bioactive elements in dairy products which mozzarella cheese has a moderate baseline potential to neutralize free radicals.

The scavenging activity of the positive control, which represented a known antioxidant or scavenging agent, was less at 20.49 ± 5.24 ppm. This finding is counterintuitive because, according to the fact that the positive control was an antioxidant, it should have had higher scavenging activity than the negative control. This incongruity can be ascribed to many variables. For instance, the positive control can be constituted of an antioxidant that has less strength or a component that interacts with the free radicals in a way that the scavenging would become less effective. The poorer scavenging capability is also because the content of the positive control's antioxidant is diluted or not fully optimal. This outcome leads to the view that the total scavenging capability of mozzarella is interfered with by the antioxidant agent and the food matrix.

Based on the data, T1 exhibited a scavenging activity of 22.5 ± 1.88 ppm in the mozzarella cheese samples, which was a bit more than the positive control and a little less than the negative control. It implies that though the component included in T1 possessed some potential in terms of the enhancement of the scavenging ability of the mozzarella, it was not fully effective as the mozzarella itself. A natural antioxidant or a substance that subtlely raises the cheese's overall antioxidant profile could be the addition. The observation that T1 still showed less scavenging activity than that of the negative control makes it possible that either the ingredient's concentration was not so high as to greatly increase scavenging activity or other factors might significantly have counteracted it.

Conversely, T2 was more successful than the negative and positive controls and showed a scavenging activity of 28.63 ± 0.81 ppm. It is an obvious and clear conclusion must being made which can be the scavenging activity of the mozzarella enhanced dramatically due to the presence of the component in T2. The presence of potent antioxidant substances that are properly integrated into the cheese structure could be the cause of the constancy of the value (low standard deviation), which is an indication that the ingredient performed its action effectively and stably. Plant-based antioxidants with high scavenging activity, like flavonoids, polyphenols, and other bioactive substances.

The highest scavenging activity (32.27 ± 11.24 ppm) was recorded by T3 although the result showed even a greater spread, indicating thereby the unequal ability of the treatment to scavenge. The high standard deviation opens up the possibility that other non-uniform aspects of the component such as cheese composition and testing environment may have been the culprit of the scavenging effect in T3. This difference may have arisen from the ingredient being potentially unstable or it may have been the way it interacted with the other cheese ingredients. A more consistent level of scavenging activity in T3 can be achieved through further treatment process improvement measures including addressing this inconspicuous element of fluctuation.

Consequently, their outcomes refer to the fact that that treatment (T1, T2, and T3) was not capable of the same scavenging activity of the cheese. T1 had a little half-farther scavenging ability than the positive control, T3 although it was the best, had the most serious deviation, and T2 showed the best-sustained and most powerful scavenging activity, thereby strengthening the supplementary mode of action. These results are in line with the preceding studies which show that the addition of natural antioxidants to dairy products will increase their antioxidant activity. It also implies that ingredient concentration and choice are the most crucial factors in improving mozzarella cheese's scavenging activity. In addition, the performed research could provide more information on the exact substances that were responsible for the increased scavenging activity in T2 and T3.

4.12 Yield (%) Analysis of LF Mozzarella Cheese

TABLE 4.11: Theoretical and actual yield % of LF Mozzarella Cheese

Samples	**Theoretical Yield**	**Actual Yield**	**Recovery data**
Negative	12.95 ± 0.39	11.8 ± 0.36	1.15 ± 0.13
Positive	10.25 ± 0.67	9.76 ± 0.38	0.46 ± 0.05
T1	10.33 ± 0.20	9.8 ± 0.30	0.53 ± 0.02
T2	10.45 ± 0.22	9.132 ± 0.32	1.318 ± 0.10
T3	10.45 ± 0.22	9.192 ± 0.10	1.258 ± 0.14

The table contains the theoretical yield, actual yield, and recovery data, with uncertainty of each sample noted in the corresponding cells. Actual yield is the observed value in the trials, which is the true data that comes from a real-world trial, while the theoretical yield indicates the value that would be obtained if the experiment can be fully carried out or a perfect experiment be performed. The percentage of the complete substance or material is determined by the actual yield divided by the theoretical yield, so the recovery data stands for the amount of recovery.

The recovery value for the negative sample is 1.15 ± 0.13, with the actual yield being 11.8 ± 0.36 and the theoretical yield being 12.95 ± 0.39. This points out that the actual yield is very near to the expected value (the nearby recovery rate is more than 100%), meaning that the efficacy of the procedure is three-fourths. The very low values that represent the uncertainty indicate that highly precise measurements of the theoretical and actual yields were kept throughout this process.

The recovery value in the positive sample is 0.46 ± 0.05, with a predicted yield of 10.25 ± 0.67 and a real yield of 9.76 ± 0.38. A considerable loss or inefficiency in the recovery process is implied by this low recovery value. The theoretical and actual yields are different, but this is certainly due to some certain factors like the experimental and/or methodological limitations, whereas the theoretical yield's uncertainty is relatively high so the original estimate might have been quite wide-ranging.

T1's recovery value is 0.53 $\pm$ 0.02, and the theoretical yield is 10.33 $\pm$ 0.20, and the actual yield is 9.8 $\pm$ 0.30. This time, the recovery of the drug sample is higher than that of the positive sample, but the recovery still remains under 100%, which can be interpreted as a slower process of healing.

In Relation to the Positive sample, the measurements have appeared to be less variable and lengthy as evidenced by the relatively low uncertainty of the values.

In T2, the recovery is 1.318 $\pm$ 0.10, and the actual yield is 9.132 $\pm$ 0.32, while the theoretical yield is 10.45 $\pm$ 0.22. The statement that the recovery number is above 100% provides an interpretation that says the recovery process was more successful than initially introduced being the nice weather, experimental conditions, or a systematic measurement error. The increased level of uncertainty in the recovery data is an indication of the greater accuracy of the measurement.

The final paragraph of the section is about T3 which has the highest recovery ratio of 1.258 $\pm$ 0.14, a predicted yield of 10.45 $\pm$ 0.22 and a crude yield of 9.192 $\pm$ 0.10. As was the case with T2, the recovery number above 100% can be due to either incorrectly taken measurements or simply the process being very successful. Although a slight higher level of uncertainty, especially in the recovery value is observed, the results lay in a reasonable precision interval.

In short, the table clearly demonstrates the success of recovery as well as the differences between the designed and actual yield for each sample. Changes in the experiment, method, or even the possible errors in the measurements of the experiment will result in the recovery data being different.

The uncertainty of each value also shows how precise the measurements were, which is essential for evaluating the accuracy of the experiment results.

4.13 Organoleptic Evaluation of LF Cheese Samples

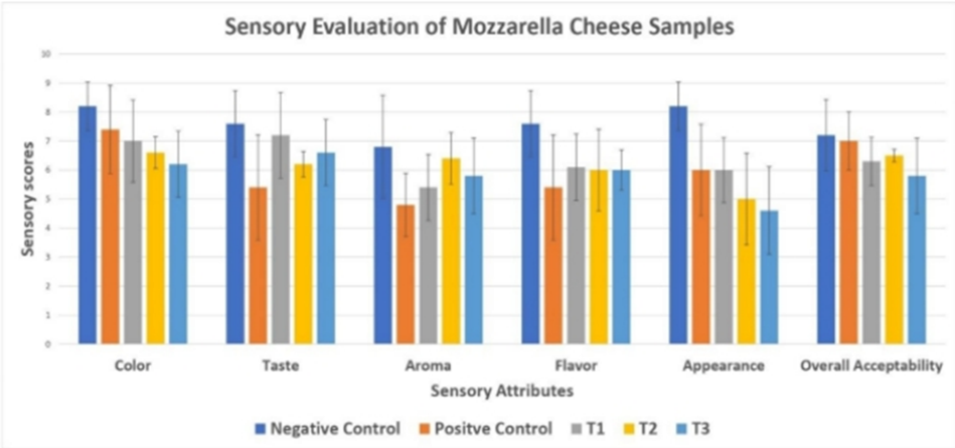


FIGURE 4.14: The sensory evaluation of Low-Fat Mozzarella cheese samples

A complete sensory analysis of several mozzarella cheese samples is shown in the graph, along with a positive control, a negative control and three treatment groups (T1, T2, and T3. Color, taste, aroma, appearance, flavor and overall acceptability were the six main sensory aspects that were evaluated using a 9-point hedonic scale, where 1 denotes ”dislike extremely” and 9 denotes ”like extremely.” Not only by its nutritional makeup the cheese quality is evaluated by its sensory qualities also, which have a great impact on customer preference, these sensory qualities are essential in evaluating consumer acceptability.

The results showed that the positive control had the lowest ratings for every attribute, which indicate that the panelists did not find it acceptable. This can be the result of poor formulation or processing conditions that adversely affected its sensory qualities, including an uneven flavor, an undesirable fat content or an inappropriate texture. The negative control, on the other hand, scored best in many areas, suggesting higher consumer acceptability. This is probably because the fat protein, and moisture balance was favorable, creating a pleasing taste, texture and overall sensory attributes.

With the greatest evaluations for color, taste, aroma, and general acceptance across the treatment groups, T1 was the most favored. The good ratings for T1 predict that its formulation was able to preserve the desired sensory qualities of traditional mozzarella cheese, maybe as a result of a well-balanced mix of protein interactions, moisture retention, and fat replacent. Despite some shortcomings in comparison to T1, T2 came in second place and excelled in flavor and look. With the exception of appearance, it is noted that T2 and T3 generally scored lower than T1. This suggests that even though T3's presentation was visually appealing, the panelists didn't think highly of its flavor, texture, or odour.

The panelists' extreme distaste for the positive control was indicated by sensory scores ranging from 1 to 6, which were probably caused by its poor meltability, bland or off flavors, and unappealing texture. With scores between 6 and 8, the negative control demonstrated moderate to high preference, indicating an ideal ratio of protein interactions, fat content, and moisture retention that produced a rich, creamy texture and a well-developed flavor. T1 received the highest ratings and T3 the lowest, suggesting less desirable sensory qualities. The treatments (T1, T2, and T3) scored between 4 and 7, indicating moderate preference. These results demonstrate how the formulation process and kind of fat substitute had a major influence on the low-fat mozzarella cheese's sensory qualities. Among the treatments, T1 was the most successful in maintaining good sensory qualities, followed by T2, while T3 had the least appealing profile. However, the negative control was the most liked. This emphasizes how crucial it is to choose the right fat substitute and processing method to preserve the quality of low-fat mozzarella cheese without sacrificing its flavor.

While salt levels impact the strength of flavor, fat concentration has a major impact on the creaminess and texture of both heated and unheated cheeses. Although it may affect meltability, higher calcium levels often increased the cheese's hardness, especially when heated. The results show that the key to maximizing the sensory qualities of mozzarella-style cheeses is the balance of these elements [77].

Chapter 5

Conclusion and Future Prospects

5.1 Conclusion

Fat had to be substituted by adding the *Hibiscus rosa-sinensis* mucilage material from different concentrations, to the low-fat (LF) mozzarella cheese made through buffalo milk. The study aimed at examining the applicability of the mucilage in the carbohydrate form of fat substitute and its impact on the nutritional, sensory, and functional properties of the cheese. The discoveries brought to light that a certain percentage of mucilage, ranging between 0.625% and 2.5%, is one of the chief modifiers of the cheese properties, which is spelled out as the part of the formulation and production processes where even a small change can make the biggest difference in the end result of the cheese.

Compared to the low percentages like T2 and T3, 2.5% (T3) mucilage was found to be the most influential factor in improving the functional and textural qualities of LF mozzarella cheese at various concentrations. This addition, in general, transformed the dairy product's structural integrity, flexibility, and melting properties which made it a viable option for using in the preparation of low-fat cheese. However, at the same time, T2 (0.625% mucilage) was most highly rated by the assessors using the organoleptic analysis which involves an evaluation of taste, fragrance, and mouthfeel. This means that the lower mucilage concentrations were better for the sense of taste rather than the larger ones that might have caused some imperceptible changes in flavor and texture which are not liked by customers.

Besides, the investigation demonstrated that a 1% mucilage solution could indeed produce some textural and functional properties that equaled those of low-fat mozzarella cheese, which is available on the market. This new finding suggests the feasibility of using *Hibiscus rosa-sinensis* mucilage as a fat replacement naturally, which in turn can reduce the fat content along the proper properties of the cheese. One of the possible ways to harmonize the sensations caused by mucilage alongside other conventional additives is by using particular food conditioners such as natural flavors and colors which in turn will help increase the end product sensory attributes.

5.2 Future Prospect

The invention of the mucilage, made from *Hibiscus rosa-sinensis* as a natural low-fat cheese replacement, offers many exciting research lines. The ability to substitute for other dairy products with hibiscus in the form of cheddar, processed cheese, yogurt, and ice cream by its effect on texture and functionality may be one of the main important discovery. In addition, the use of advanced food formulation technologies to optimize the mucilage concentration can greatly help to achieve the right balance between fat reduction and the preservation of sensory quality. The extensive customer acceptability research would be the fundamental part to identify the potential of the product and choose the right strategies for the commercialization process. The rise of plant-based and functional foods allows a possible study of *Hibiscus rosa-sinensis* mucilage wellness that could include its alleged health benefits for the gut and digestion. Its character to boost texture through the mucilaging process turns it into a wonderful component to make vegan cheese substitutes, which could be a catalyst for the dairy-free goods to be even better.

Scaling up the manufacturing of mucilage-enriched cheese and guaranteeing its storage stability so that it maintains consistency throughout time should be the key goals of future research. Furthermore, investigating the synergistic effects of *Hibiscus rosa-sinensis* mucilage with hydrocolloids and other natural fat substitutes may result in the creation of novel, nutrient-dense food formulations. Lastly, to ensure that the product complies with international food safety standards, obtaining regulatory permits and carrying out safety evaluations will be crucial for widespread commercialization. As a result, the dairy and functional food industries may both benefit from the development of healthier, more environmentally friendly, and consumer-friendly dairy substitutes.

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