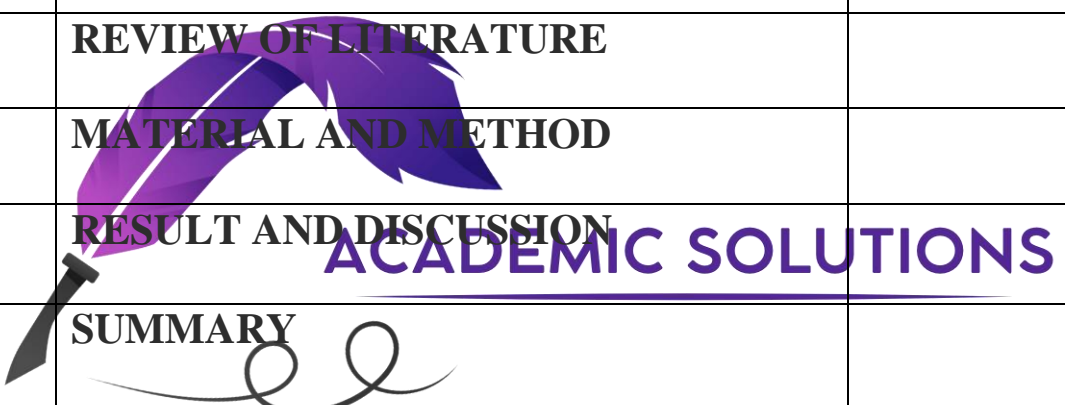


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CHAPTER NO.1

INTRODUCTION

Human food chain includes numerous products which provide variety of nutrients to human body but the milk is most prominent that is secreted through the mammary glands of mammals. During the first several years of life, every individual depends on milk to meet all of his or her nutritional needs. Milk is consumed throughout the lifespan and in all parts of the world regardless of race, age, or colour. Milk is recognized as a nutritious food that provides essential macro-nutrients such as fat, proteins, and carbohydrates (Plant Based Foods Association, 2016). In addition to these macro-nutrients, milk also contains various micro-nutrients, including calcium, selenium, riboflavin, vitamin B12, and pantothenic acid (vitamin B5), which play a significant role in supporting overall body growth and maintenance (Plata-Pérez et al, 2022). Approximately 750 million individuals, primarily small-scale farmers, are involved in milk production to meet their livelihood requirements, enhance food security, and address nutritional needs. Punjab and Sindh provinces play a significant role as the primary milk-producing regions in Pakistan (Nawaz et al, 2022). Food and Agricultural Organization states that the ratio of milk production in Pakistan was 530 million tons in 1998 and 843 million tons in 2018 that reached 914 million tons in 2020 and is predicted about 1020 million tons by 2030 (FAO, 2018 ; Nagy et al., 2022). The production rate of milk is continuously increasing and it is forecasted that this trend will continue moving in the same way going forward (Sattar, 2022). According to the current scenario, Pakistan's position in the multinational milk production industry is impressive, as it ranks fourth due to its admirable ability of milk production. Contribution of the animals is very fascinating as cows produce 81% of the milk, while buffalo contribute 15% and other species including goats, sheep, and camels are contributing less.

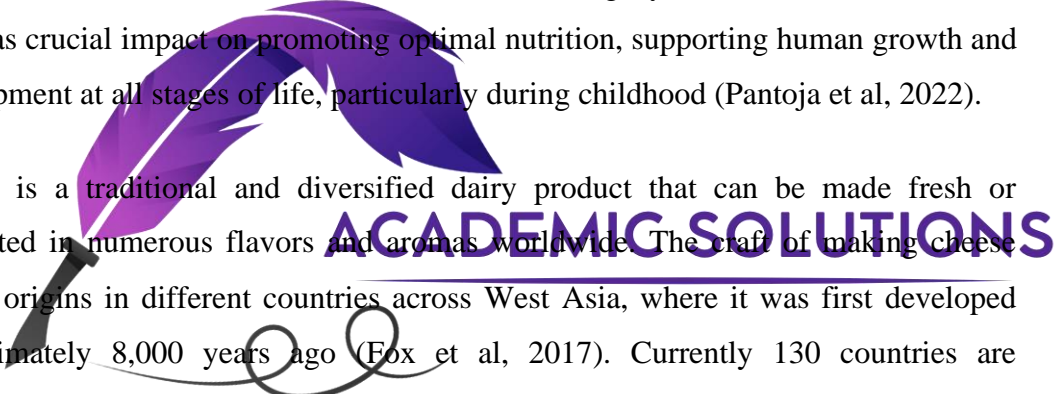
Milk is processed to produce dairy food items such as yogurt, cheese and concentrated milk which are large contributors to human nutrition (Smith et al., 2022). Milk products play a vital role in human nutrition and have been valued for their significant contributions to overall health and well-being. On average, dairy products make up approximately 25-30% of an individual's diet (Stobiecka et al, 2022).

Due to their contribution, the demand for unprocessed, natural food commodities such as fresh milk and milk products is steadily rising on a global scale (Berge and Baars, 2020). In Pakistan, around 97%, of milk consumption is in its fresh form, while a mere 3% undergoes processing, carried out by 20 primary processing plants including Friesland Campina and Nestle. Cheese (3.7%) and milk (3.2%) has the largest share in supplying energy (Varavallo et al, 2022).

Around 11,000 years ago, cattle herders discovered the process of fermenting milk to produce yogurt and cheese. This ingenious technique was developed to effectively break down lactose, a type of milk sugar that adults find difficult to digest (Coelho et al, 2022). These wide variety of milk products have diverse nutritional profiles and unique sensory characteristics that cater to individual preferences and dietary needs. These foods offer vital elements such as proteins, lipids, carbs, vitamins, and minerals that are necessary for a variety of bodily processes. For instance, all of the essential amino acids needed for bodily tissue growth, repair, and maintenance are present in the proteins found in milk products. Milk products' fats act as a concentrated source of energy and help the body absorb fat-soluble vitamins. Additionally, milk products are a notable source of calcium, contributing towards the bone strength and reduce the chances of osteoporosis. The consumption of milk products, particularly fermented varieties like yogurt and cheese, has been associated with potential cardiovascular benefits, such as a lower risk of cardiovascular diseases (Farvid et al, 2017). Overall, milk products offer a valuable and versatile component of a balanced diet, promoting health and well-being through their nutrient content and potential positive effects on various aspects of human health. Milk from a variety of animals, including cows, buffalo, goats, and sheep, can be used to make cheese. Due to its particular flavor, buffalo milk was traditionally the favoured option for Mozzarella. (Shaukat et al, 2022).

Buffalo milk is a nutrient-rich beverage that differs slightly from cow's milk in terms of calorie content, calcium content, protein, fat and mineral content (USDA, 2020). Buffalo milk is typically consist of about 32–49 g/L lactose, 53–90 g/L fat, 22–47 g/L protein, 8–9 g/L ash and 32% calcium of the daily value (Garau et al, 2021). Buffalo milk has long been admired for its advantageous chemical composition which determines its nutritional qualities and adaptability for the manufacture of both

traditional and commercial dairy products especially mozzarella cheese since it contains more fat and less water than cow milk (Pantoja et al, 2022). Diverse range of triglycerides and fatty acids composition found in buffalo milk allows for the separation of milk fat into different fractions based on their unique melting properties. The elevated levels of high-melting triglycerides present in buffalo milk result in a higher density, making it well-suited for cheese production (Khan et al, 2019) (Khedkar et al, 2016). Cheese manufactured from buffalo's milk is highly priced due to its white colour and smooth texture (Becskei et al, 2020). Buffalo milk requires some adaptations of technology and equipment while processing products due to the compositional and physio chemical variations from other ruminant milk . Another major factor that must be kept in consideration is the safety of milk. Milk undergoes various preservative methods such as thermal processing through pasteurization and sterilization, as well as fermentation and culturing during cheese making, which extend its shelf-life and maintains the nutritional integrity of milk. Overall, buffalo's milk has crucial impact on promoting optimal nutrition, supporting human growth and development at all stages of life, particularly during childhood (Pantoja et al, 2022).



Cheese is a traditional and diversified dairy product that can be made fresh or fermented in numerous flavors and aromas worldwide. The craft of making cheese has its origins in different countries across West Asia, where it was first developed approximately 8,000 years ago (Fox et al, 2017). Currently 130 countries are producing various types of cheese, and the total amount produced globally is 2000×10^4 tons. Netherlands and Germany are the biggest cheese exporters globally. Cheese is a very crucial dairy product, but the cheese industry needs more focus of attention (El Sheikha., 2018). In general, the worldwide consumption of cheese is steadily growing and is expected to rise by 13.5% from 2016 to 2025 (OECD/FAO, 2016). At the same time, there has been a rising demand from consumers or end-users for cheese with improved physio-chemical properties, sensory and nutritional quality, and optimal usage characteristics, all while keeping costs reasonable. Dietary similarity, convenience, flexibility of application, and cheese quality, consumption of cheese has expanded the form of pizza toppings, cheese sandwiches, and stuffs. Over the past thirty years, the global production of cheese has increased by around 4% annually. These growing demands of consumers have prompted food researchers and cheese

producers to concentrate on enhancing the quality of current products and creating innovative products (Kisworo, 2022).

Cheese production is involved in two main categories which include acid-set cheese and bacteria-set cheese. Historically, acid-set cheese has been made by direct curdling of the raw milk using an acid (such as lemon juice or vinegar) without any precautionary measures and then separating the whey. The amount of acid required depend on type of acid used, type of milk, and the desired texture of cheese. At present, the majority of artisanal cheeses are still produced using raw (unpasteurized) milk. While sometimes, Cheese makers face challenges in controlling the extent and rate of acidification during cheese making, particularly when using milk that has high levels of lactose-fermenting bacteria. In such cases, pasteurization may be necessary to eliminate most of the bacteria that can ferment lactose. Therefore, cheese milk is pasteurized before being utilized in the cheese-making process. This pasteurization process changes the natural microbiota, resulting in cheese that has a more consistent quality, less intense flavor and ripens more slowly. However, it is crucial to take precautions during pasteurization, as it may negatively affect the milk's ability to form curds with rennet (Tilocca et al, 2020).



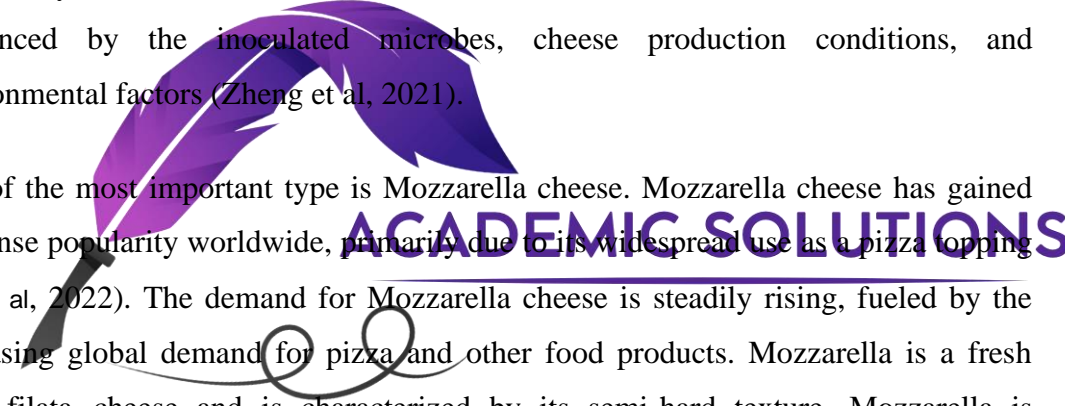
ACADEMIC SOLUTIONS

Whereas, Industrial-scale cheese manufacturing involves processing the milk to alter its characteristics, ensure consistent quality, and decrease bacterial levels. Adding a starting culture of lactic acid producing bacteria (LAB) to raw or pasteurized milk of commercial goods is an accepted procedure. This practice enables consistent acid

production and the whole process is known as acidification. Primitive starter was known as "slop-back" cultures, where sample of whey from the previous day cheese-making incubated overnight and used as a starter culture next day. Some high-cook cheese varieties, such as Parmigiana-Reggiano and Grana Padano are still producing using this type of starter. They work effectively if managed carefully (Fox et al., 2017). Nowadays, defined-strain mesophilic and thermophilic starters are widely used that results in very reproducible rates of acid production if properly selected and maintained (Zdolec et al, 2022).

Numerous dairy products are chemically, biochemically, and physiologically stable as long as they are produced and maintained properly. Cheese, on the other hand, is

physiologically and biochemically active and, as a result, changes with storage in terms of flavor, texture, and activity. The two most important components of milk (fat and protein) are preserved during the manufacturing of cheese by using two conventional preservation techniques such as lactic acid fermentation and a decrease in water activity caused by the removal of water. Microbiological quality of raw milk cheeses can also be improved by providing a minimum aging period of 60 days as mandated by an U.S. Food and Drug Administration (FDA) (Nam et al, 2021). This maturing process enhance the antimicrobial effect of cheese and the microbial community undergo significant changes from the original milk to the final cheese ` factors determine the proper development of cheese such as appropriate ripening conditions that impact its consistency, texture, and shape, water content that determines softness, fat content, protein breakdown capability, properties of the raw milk, curd handling and micro-organisms present in the milk. These factors collectively contribute to the distinctive taste and features of the cheese, which are influenced by the inoculated microbes, cheese production conditions, and environmental factors (Zheng et al, 2021).



One of the most important type is Mozzarella cheese. Mozzarella cheese has gained immense popularity worldwide, primarily due to its widespread use as a pizza topping (Viji et al, 2022). The demand for Mozzarella cheese is steadily rising, fueled by the increasing global demand for pizza and other food products. Mozzarella is a fresh pasta filata cheese and is characterized by its semi-hard texture. Mozzarella is incredibly popular in Italy and accounts approximately 25% of the cheese market. Unique production process of mozzarella such as hot stretching results in its springy texture (Zheng et al, 2018).

In the production of pasta filata cheese, the curd is initially formed through the regular cheese-making process. Then, it is submerged in hot water or whey to solidify into a dense mass. The curd is subsequently changed into a homogeneous and elastic consistency during the stretching process, which is a distinguishing feature of Mozzarella cheese. Stretching is an essential step that gives the cheese the proper flexibility. This distinctive elastic texture including its capacity to melt and expand on heating allows the food industry to use mozzarella cheese as a key component in pizzas, cheese-based products (Vogt et al, 2015; Hammad et al, 2017). The

characteristics of fresh Mozzarella, such as its moistness, softness, quick melting properties, and delicate taste, differ from processed sliced or shredded Mozzarella. However, the optimal freshness of 'real' Mozzarella is typically maintained for only 12-24 hours. According to USDA recommendations, low-moisture mozzarella cheese should have a milk fat percentage of 30% to 45% on a dry weight basis and a moisture level of 45% to 52%. Low moisture part skim Mozzarella, known for its exceptional melt-ability, stretch ability, and elasticity, is widely utilized in the pizza industry. Mozzarella cheese was typically produced using the milk of water buffalo, which is considered to have superior sensory qualities and greater nutritional value (Hebishy et al, 2022).

The functionality of Mozzarella cheese holds significant importance as approximately 75% of the total cheese production. Due to this, it is used as a key ingredient in pizza. Functionality of mozzarella cheese is influenced by a number of variables, such as the cheese's composition, including its fat and moisture content, acidity, pH level, type of starter culture, coagulation of enzymes, stretched process, concentration of salt, and ripening circumstances. Amount of proteolysis, lipid content, and interactions between proteins and water or proteins themselves are other elements that are vital in shaping the cheese's functional qualities. (Vogt et al, 2015).

Fresh mozzarella cheese melts into a tough, excessively elastic texture with a somewhat hard consistency, making it unsuitable as a topping for pizza. It also lacks good water holding capacity and has limited stretchability. On the other hand, if the cheese is aged for too long, it becomes excessively soft and fluid when melted, rendering it unsuitable for pizza. Therefore, achieving desirable functionality requires a precise ripening period for Mozzarella cheese. Microorganisms play a crucial role in the production of various types of cheeses. For the right production of acids as well as the desired texture and flavor profiles, a suitable starter culture must be chosen, especially for high-fat and reduced-fat cheeses that are susceptible to the formation of off flavors. A paired lactic acid bacterium starting culture, specifically *Streptococcus thermophilus*, is used for the creation of mozzarella cheese (Freitas et al, 2020).

There is a need to manufacture unique cheeses having low-fat that bring additional health benefits to the market as customers become more concerned about their diet and health (Guinee et al, 2000). While having nutritional value, fat also adds to the

cheese's sensory and functional qualities. Low moisture mozzarella cheese is frequently chosen for making pizza preparation due to its favorable functionality and capacity to satisfy consumer preferences.

Approximately 5000 tons of cheeses are consumed annually in Pakistan, but only 2500 tons are produced locally. Approximately 90–100 tons of mozzarella cheese are produced in Pakistan each year. The rise in pizza popularity and the opening of international pizza restaurants have increased demand for mozzarella cheese. Locally produced mozzarella, however, does not match the quality of imported cheese. Although the precise amount of mozzarella imported into Pakistan is unknown, information gathered from significant international pizzerias shows that the country imports more than 760 tons of cheese per year (Tirloni et al, 2019).

During the manufacturing process, there are two different acidification methods that may be employed. One is direct acidification, which involves the addition of food-grade organic acids, such as citric acid, to pasteurized milk before the inclusion of rennet. The production of high-quality Mozzarella requires rapid acid production, but too much acidity can lead to the production of inferior cheese therefore, careful management is necessary while processing. Cheese can be utilized immediately after being produced through acidification process. The other is microbial acidification, which is the result of the growth of thermophilic starter cultures but cheese cannot be utilized immediately after processing but undergo storage period (Kindstedt et al, 2004).

In the traditional production of mozzarella cheese, curds are formed after the addition of starter culture. Cut the curd into small pieces with a knife or curd cutter and then drain the whey. After draining the whey, stretching in hot water is commonly done. Stretched cheese has better properties in terms of melting, flavor, and texture (Gonçalves and Cardarelli, 2021). In the past, mozzarella cheese was stretched by hand and this traditional method is still used by small-scale producers. To achieve this, hot water at temperatures between 70 to 80 degrees Celsius is poured into pots containing the cheese curd (Costa et al, 2017). One or multiple screw-based mechanical mixers are utilized to extend the curd in hot water. Steam injection is used to regulate the temperature of water, which typically ranges 82 to 85°C. Benefit for using mechanical processing is that it quickly transforms each curd particle into a

non-uniform but cohesive, flowing substance that is easy to shape (Sharma et al, 2016). After molding into a ball, cheese balls were submerged in a chilled brine solution with a concentration of 20% w/v for two hours, and subsequently, the surface was dried under refrigerated conditions with a temperature range of 7-9°C for six hours. One of the contributing elements to the higher production costs in Pakistan is the use of imported cultures for the production of cheese. But for the past ten years, the National Institute of Food Science and Technology has been committed to creating freeze-dried versions of regional cultures. The objective of this research is to enhance the quality of local Mozzarella cheese and reduce the need for imports, thereby saving foreign exchange. This endeavor requires extensive research work to achieve the desired outcomes (Raquib et al, 2022).

Mozzarella cheese is a delicious and nourishing dairy product containing significant amounts of proteins, vitamins, fat, and carbohydrates. The curds in Mozzarella cheese typically contains high levels of fat and casein but relatively water-miscible components (low amount) such as whey proteins, milk sugar, and water-soluble vitamins mainly found in whey (Jeewanthi et al, 2016). Consuming Mozzarella cheese can provide numerous health benefits, including protection against gout disease, that results in the accumulation of uric acid in the joints (Li et al, 2018). Content of calcium in Mozzarella cheese not only helps with weight loss but also protects against breast cancer and metabolic disorders that increase the chances of developing cardiovascular disease or a heart attack.

The *Syzygiumcumini* (L.) is a member of the Myrtaceae family and is further referred to by a number of other names, including jambolan, jiwat, salam, kerian-duat, and Indian blackberry. It originally comes from the Indian subcontinent, but nowadays, it can be found in various other regions across the globe including Myanmar, and Sri Lanka. The flowers of this tree are white in color, while its fruits start off as green and turn into a light magenta as they mature. Upon ripening, they become dark purple or black. Eating the fruit causes the tongue to turn purple. Jamun is a seasonal fruit that is typically available during the summer months. Despite its popularity, this fruit is highly perishable and can only last for 1-2 days under normal conditions (Nparks, 2021)(Anjum et al, 2021). Industries process the jamun fruit to create products with added value like jam, wine, juice, and jellies. Incorporating JS into different types of

convenient foods such as baked goods (breads, biscuits, cakes, and cookies), extruded snacks, low-calorie foods, beverages, and therapeutic foods could offer opportunities for large-scale utilization of JS. After processing, only the pulp is used, therefore the leftover seeds and skin are thrown away. The majority of the seeds produced during processing are wasted. They are numerous and make up between 10 and 47% of the fruit's overall mass (Al-Dhabi, 2020). The jamun fruit's waste has the potential to be an advantageous byproduct, providing chances for the scientific and research community to help farmers and businesses generate more income.

JS includes nutraceutical ingredients like bioactive phytochemicals, minerals, vitamins, proteins, carbs, and fats. Gallic acid, corilagin, ellagic acid, 3-galloylglucose, 3,6-hexahydroxy diphenoylglucose, 1-galloylglucose, β -sitosterol, quercetin, and 4,6-hexahydroxydiphenoylglucose are the main phytochemicals present in JS and have therapeutic significance (Kumar et al, 2022). Additionally, the seeds are rich in dietary fiber, amino acids, vitamin C, vitamin B complexes (including thiamine, riboflavin, and folic acid), essential minerals, and trace elements like calcium, iron, sodium, magnesium, zinc, phosphorus, chromium, vanadium, and potassium. They additionally include albumin, lipids, and essential oil (Qamar et al, 2022 ; Venu Gopal, 2017). Analysis of the seeds' fatty acid composition indicates the prevalence of lauric, myristic, palmitic, stearic, oleic, linolenic, malvalic, and sterculic acids, with β -sitosterol identified as the primary phytosterols. These distinctive nutritional attributes of the seeds imply that they may be appropriate for use in the pharmaceutical and cosmetic sectors (Ayenampudi, 2022).

In the past, jamun seeds were used to treat diabetes and digestive problems. Several bioactive substances, including phenolics, terpenoids, phloroglucinol derivatives, and saponins, have been discovered as important components in the study being done to confirm their health-promoting effects. Exploring their potential for anti-diabetic, antioxidant, anti-inflammatory, anti-cancer, anti-microbial, cardio-protective, hepatoprotective, and neuroprotective qualities is the focus of ongoing, rigorous research (Singh et al, 2022 ; Kannan et al, 2015). Lauric, myristic, palmitic, stearic, oleic, linolenic, malvalic, and sterculic acids are found in abundance in the seeds' fatty acid makeup, with β -sitosterol being the predominant phytosterol. The seeds contain a considerable amount of iron which aid in boosting hemoglobin levels and function as

a purifier for the blood (Kasthuri et al, 2017; Singh et al, 2022). The seed's iron content assists in fighting against anemia and jaundice (Kshirsagar et al, 2019; Tak et al, 2022). The seed's calcium content has the potential to fulfill the body's dietary need for this mineral and may be incorporated into dietary supplements aimed at supporting pregnant and lactating women (National Institute of Nutrition, 2022). The seed has been used in a variety of food products, including cookies, biscuits, chips, and wine, because of its nutritional and functional qualities (Kasthuri, 2022). Large-scale JS use may be facilitated by including JS in a variety of handy foods, including baked products (bread, biscuits, cakes, and cookies), extruded snacks, low-calorie foods, beverages, and therapeutic foods. The addition of JS powder to products not only enhances their functionality but also improves their preservation properties, thanks to its antimicrobial and antioxidant characteristics. Safety considerations while consuming jamun seeds must be addressed and it must be used till acceptable limits. The prebiotic activity was shown to be significantly increased when JS powder was added to a functional confection (2.16 0.05), comparable to inulin. Additionally, the glycemic index was lowered. Additionally, in vitro, research has shown the functional confection's potential to lower diabetes. Large-scale JS use may be facilitated by including JS in a variety of handy foods, including baked products (bread, biscuits, cakes, and cookies), extruded snacks, low-calorie foods, beverages, and therapeutic foods.



Objectives

- To optimize processing conditions to develop mozzarella cheese.
- To examine the physio-chemical makeup of buffalo milk mozzarella cheese.
- To access the mozzarella cheese made from buffalo milk on sensory basis
- To explore the possibility of mozzarella cheese supplemented with jamun seeds for diabetic patients due to their high jamboline content.

CHAPTER NO 2

REVIEW OF LITERATURE

Milk has been an essential part of human life for thousands of years, from the ancient civilizations to the modern era. Milk has played a significant role in human nutrition, culinary traditions, and cultural practices. Milk is a natural gift from nature, possesses the qualities of an ideal food and abundantly rich in vital nutrients necessary for the growth and well-being of individuals across all age groups. Milk contains energy-providing components such as lactose, fat, bone-strengthening calcium along with various minerals, muscle-building proteins, and health-promoting vitamins (Motshakeri et al. 2022).

Milk is consumed in Pakistan in a variety of ways, including fresh, boiled, and powdered. The World Health Organization (WHO, 2019) recommended 0.5 liters per person every day. Additionally, there are well-known milk-based goods including ice cream, yogurt, ghee, buttermilk, butter, and cheese. It's interesting to note that roughly one-third of Pakistan's food consumption is made up of milk and its derivatives.

More than 750 million people worldwide work in the milk industry, the majority of them are small farmers who depend on it for their subsistence, food security, and nutrition. The amount of milk produced globally in 1987 was 522 million tones, but in 2017 it increased dramatically to 828 million tons. 838 million tons were produced at the end of 2018 (FAO, 2019). Different percentages of milk-producing animals are involved in milk production (Table 1). These percentages can change depending on the location and unique conditions.

Animal	Milk Production Percentage
Cow	82%
Buffalo	14%
Goat	2%
Sheep	1%
Camel	0.3%

Horse	0.2%
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The output of milk increased significantly in South Asian developing nations. The region became a substantial contributor to the world's milk output after 1970. South Asia made up 21% of the world's dairy animal population, with a staggering number of 745 million dairy cows. In addition, 25% of the cattle and buffaloes, 15% of the sheep and goats, and 7% of the camels were found in this area (Siddiqui, 2017).

Pakistan is currently the fourth-largest milk producer in the world (Abid et al., 2022). Approximately 80% of milk is produced on small dairy farms in rural areas, with 15% occurring in peri-urban settings and 5% in metropolitan settings. Taking into account historical patterns, the average annual production of milk was 6.6 million tonnes in the 1960s and 8.1 million tons in the 1970s. Subsequently, there was a significant rise to 12 million tonnes in 1985-86 and a quadrupling to 48 million tonnes by 2018-19, indicating substantial growth over three decades. Between 1985-86 and 2018-19, there have been notable changes in the composition of milk in Pakistan. There was a minor decrease in buffalo milk during this time and a slight increase in cow milk. Approximately 67% buffalo, 31% cow, 2% goat, sheep, and camel milk made up the milk in 1985–1986. According to Nguyen et al. (2022) by 2018–19, the ratio has changed to approximately 60% buffalo, 36% cow, and 4% goat, sheep, and camel.

The majority of milk sold in Pakistan is provided raw, making up around 97% of the country's total milk production. The UHT (Ultra High Temperature) method is mostly used to treat the remaining 3% (Koivusaari et al., 2022). Punjab and Sindh are the two principal milk-producing provinces in Pakistan. Sindh produces about 9.35 million liters of milk per year, compared to Punjab's annual production of approximately 25.62 million liters. Baluchistan generates 0.81 million liters annually, and KPK is anticipated to provide 4.88 million liters (Alam, 2022).

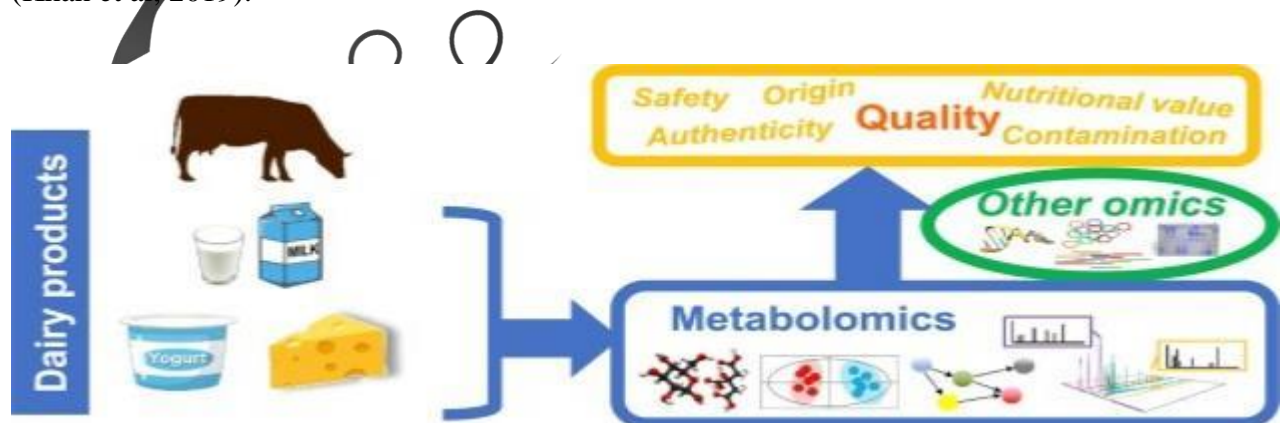
Sindh has the highest annual per capita milk consumption rate in terms of provincial consumption, at 246 kg. It weighs roughly 132 kg in Punjab, 86 kg in KPK, and 108 kg in Baluchistan. Cows typically produce 14 liters of milk per day, whereas buffaloes produce about 10 liters daily. Comparing these yields to milk yields in

wealthy nations, they are still noticeably inferior—about 5–6 times less (Ahmad et al, 2022).

2.1. Milk by products

Dairy products have long been used due to their exceptional nutritional composition and numerous health benefits (Pennington, 2017). Dairy products are an abundant source of essential nutrients, such as proteins, vitamins, minerals, and bioactive compounds required for growth and development. Moreover, they are crucial for maintaining bone health, promoting nerve function, and supporting overall metabolic processes. The presence of bioactive compounds, such as probiotics, prebiotic, and conjugated linoleic acid (CLA), has protective effect against cardiovascular diseases, including hypertension and stroke (Drouin-Chartier et al, 2016).

Dairy products offer an abundance of flavors, textures, and culinary versatility, making them an invaluable tool for enhancing dietary diversity. From creamy yogurts and cheeses to rich butter and wholesome milk, dairy products can be incorporated into a wide range of recipes and preparations. This versatility not only adds depth and dimension to the sensory experience of meals but also enriches their nutritional value (Khan et al, 2019).



(Early, 1998).

2.2. Cheese

The oldest dairy product is cheese, which was created 8000 years ago in the "Fertile Crescent" between the Euphrates and Tigris rivers (Fox and McSweeney, 2017). In Xinjiang, China, cheese making may have begun as early as 1980–1450 BC. However, cheese has become one of the most consumed fermented milk products in modern times, loved by people of all ages. (Yang et al, 2014). Today, cheese production makes up around 40% of the world's milk production, having transitioned from a farm-based to an industrial scale. It is one of the most dynamic food groups, with a phenomenal average annual growth rate of 2.6% from 2013 to 2019 (Bansal and Veena, 2022). The amount of cheese produced worldwide as of 2019 was close to 26 MT. The top three cheese-producing nations are the US (6.3 MT), Germany (3.5 MT), and France (1.9 MT), which together account for 46% of global cheese output (Dairy Industries International, 2020).

Cheese is a nutritional powerhouse because of its high protein percentage, calcium, vitamin, and mineral content and a common part of humans' natural diet due to its wide variety in aroma, texture, shape, and flavor (Bittante et al, 2022). These nutrients contribute to strong bones, teeth, and overall metabolic functions, making cheese an essential component of a balanced diet (Thorning et al, 2016). Cheese also contains bioactive peptides that may have anti-inflammatory and antimicrobial properties, promoting overall health. Additionally, some studies suggest that certain types of cheese, like fermented varieties, contain probiotics that support gut health and improve digestion (Gibson et al, 2017). Its main flaw lies in the length of time it takes to remain stable (Fu et al. 2020).

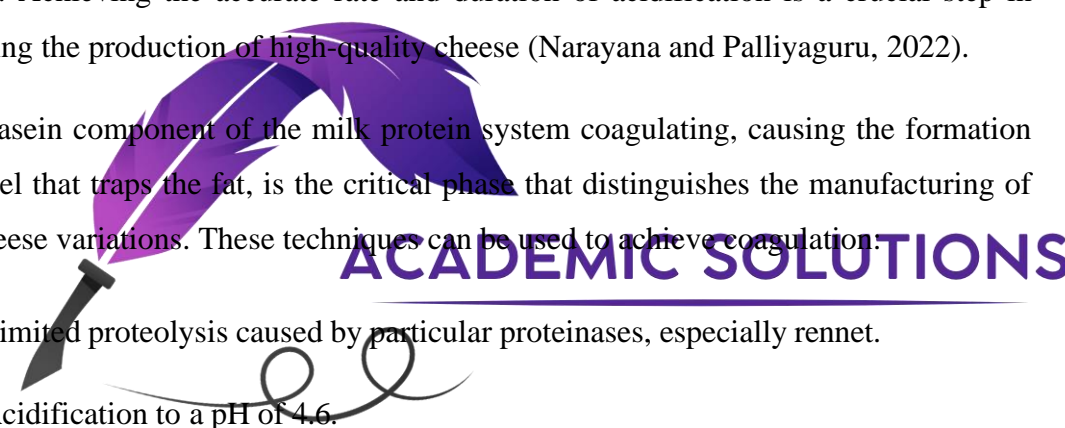
Cheese holds immense cultural significance in various cuisines worldwide. It plays an integral role in traditional dishes and represents the culinary heritage of different regions. From European classics like Swiss Gruyère to Italian Parmigiano-Reggiano, cheese adds depth and flavor to recipes, making it a staple ingredient in diverse culinary creations. The versatility of cheese allows it to be enjoyed in various forms, such as melted, grated, or as a standalone accompaniment (Fox et al, 2017).

2.2.1. Transforming milk into cheese

Casein micelles are recognized as being present in milk's colloidal suspension. Three procedures—acidification, coagulation, and syneresis—convert this suspension into cheese and lead to the development of curd (Fox et al, 2000).

The acidification process plays a fundamental role in the production of high-quality cheese varieties such as Mozzarella, Cottage, and UF Feta-type cheese through the use of starter cultures. Cow Mozzarella and buffalo Mozzarella, both are prepared using commercial cultures such as *S. thermophilus*. *S. thermophilus* has the ability to survive at the elevated temperatures necessary for the production of Mozzarella cheese. Moreover, it plays a crucial role in the acidification process, enabling the curd to be stretched and molded in hot water, while also contributing to the desired flavor and texture characteristics of the cheese (Guidone et al, 2016a, 2016b; Moser et al, 2018). Achieving the accurate rate and duration of acidification is a crucial step in ensuring the production of high-quality cheese (Narayana and Palliyaguru, 2022).

The casein component of the milk protein system coagulating, causing the formation of a gel that traps the fat, is the critical phase that distinguishes the manufacturing of all cheese variations. These techniques can be used to achieve coagulation.

- 
- ◆ Limited proteolysis caused by particular proteinases, especially rennet.
 - ◆ Acidification to a pH of 4.6.
 - ◆ Combining heating to 90°C with acidification to pH 5.2 (Britten and Giroux, 2022).

When milk is coagulated with rennet or acid, the resulting gel experiences syneresis or dehydration when broken, sliced, or put under pressure. The aqueous phase of the gel known as whey is released as a result of this action, which causes the para-casein matrix to constrict. Cheese-makers can control the moisture level of cheese, the actions of microbes and enzymes during cheese manufacturing, the biochemistry of ripening, and the stability of the cheese through the use of syneresis. To improve the flavor and texture of their cheese, cheese-makers take care of these issues (Bansal and Veena, 2022).

2.2.2. Different types of cheese

Cheese is a nutritious food consumed by many advanced nations. Its widespread availability across all demographics and regions of the world means that it is consumed in abundant amount. There are more than 900 different types of cheese in the world, all of which can be neatly categorized according to their method of production, shape, chemical makeup, and state of ripening (Zheng et al, 2021).

Extra-hard cheeses

Extra hard cheeses such as Parmesan and Romano are produced using very hard curds, have a low moisture content of 51% or less, and are made from partially skimmed milk. These cheeses are matured slowly for 1-2 years using bacteria.

Hard cheeses

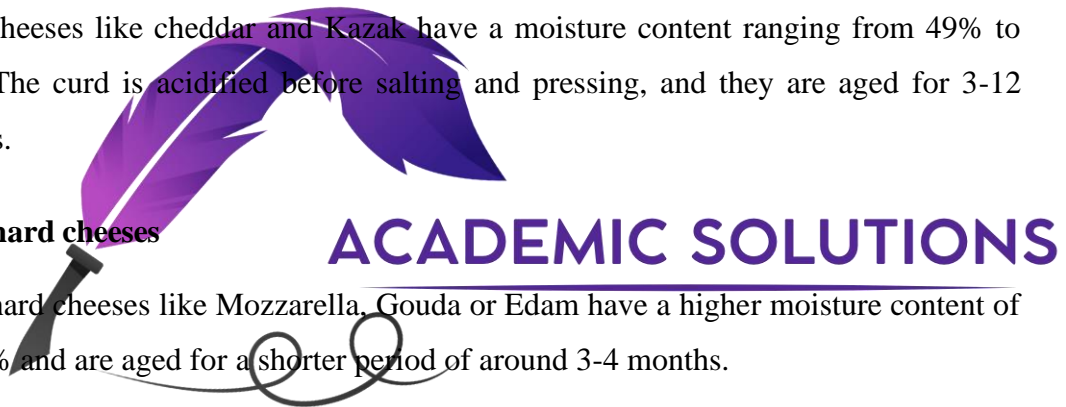
Hard cheeses like cheddar and Kazak have a moisture content ranging from 49% to 56%. The curd is acidified before salting and pressing, and they are aged for 3-12 months.

Semi-hard cheeses

Semi-hard cheeses like Mozzarella, Gouda or Edam have a higher moisture content of 54-63% and are aged for a shorter period of around 3-4 months.

Semi-soft cheeses

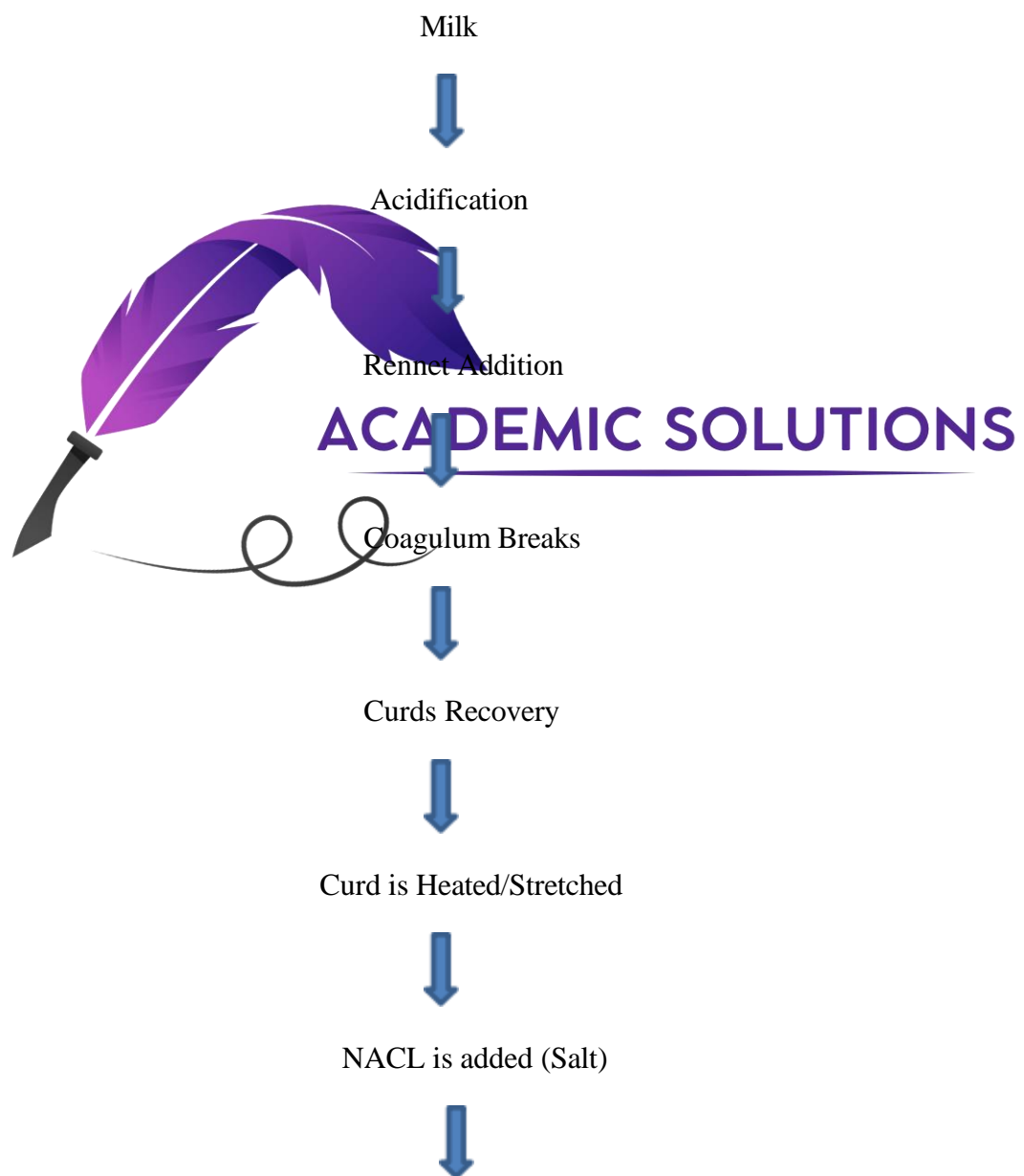
Limburger and blue cheese are semi-soft cheese, have moisture content of 67% or higher and undergo ripening using bacteria like Brevi bacterium and/or mold like Penicillium. It do not provide sufficient protection against the growth of pathogens as its water activity is high and acidity decrease during ripening process, both of which promote the growth of contaminants. Mold is predominantly grown on the surface of some cheeses, like Camembert, while in others, such as blue cheese, it grows beneath the surface (Wei et al, 2022).



2.3. Mozzarella cheese

Mozzarella cheese is among the most consumed cheeses in the United States.. Fresh cheese of this variety is frequently consumed both as a salad topping and on its own. The flavor of freshly made mozzarella cheese is pleasing, much like the taste and texture of milk. Dry and store-able cheese is made by acidifying milk directly with citric acid or vinegar to the desired PH and then adding salt (Minz et al. 2021). Flow chart indicates the steps involved in the preparation of white mozzarella cheese.

Recipe Generation



Shaped Mechanically/Manually



Hardened by Cooling



Dipped into Fresh Water



Placed in Brine

2.3.1. Functional Properties of Mozzarella cheese

The rheological, physicochemical, and micro-structural characteristics of the cheese influence its functional characteristics and are key factors in determining its quality and overall demand (Parafat et al., 2023). The most popular cheese in the world is mozzarella. The composition and structure of mozzarella cheese have a big impact on its functional qualities, which include stretchability, melt ability, browning, and the production of free oil. The composition and structure of the cheese directly influence its ability to stretch and melt properly, achieve desirable browning characteristics, and control the formation of free oil. Various factors including milk pretreatment, pH, moisture content, fat content, mineral content (including salt), and the degree of proteolysis play a vital role in determining the cheese's functionality (Feng et al, 2023). These factors collectively impact the behavior of the cheese. When cheese is used in food products, it enhances the quality and contributes to the sensory attributes of the final dish (Akarca et al, 2023).

Mozzarella cheese is frequently used as an ingredient in pizza and is normally produced in blocks with weights ranging from 2.3 to 9.5 kg. Before being used as an ingredient in pizza, it needs to be shredded or diced. The melt, stretch, and shred properties of this kind make it ideal for use as a pizza topping. Mechanical machines with screws increase the stretching power and are used to stretch mozzarella cheese in hot water during production. Serum and fat globules impede the alignment of proteins

in the fibrous portion (Ah and Tagalpallewar, 2017). However, issues with shreddability can arise when the cheese has a soft, pasty, or wet texture. As a result, the cheese shredding machine may become blocked, producing uneven shreds with sharp edges and twisted shapes. Furthermore, such cheese is difficult to handle, store, and spread evenly on the product due to its propensity for severe matting after shredding (To et al., 2022). On the other hand, if the cheese is hard and dry, it could take longer to shred and have a tendency to break into small pieces that are too small to be seen. These dispersed shreds and particles are challenging to handle and manage (Landin et al, 2022).

2.3.2. Quality of Mozzarella cheese

The physical properties of the mozzarella cheese, particularly the cooking color and oiling, directly affect its quality metrics. These characteristics result from the intricate interaction between casein and fat in the cheese matrix. These physical factors affect the degree of proteolysis stored at a given temperature and time. An investigation shows that some manufacturing changes produce products that are more dependable and have the desired physical features (Serrapica et al, 2022).

2.3.3. Proteolysis of Mozzarella cheese

Cheese's elevated flavour during ripening is a result of proteolysis, while off-flavors are the result of the creation of short peptides that add bitterness. Proteolysis is very important in the evolution of flavor and texture in rennet-curd cheeses. The ripening of cheese results in the release of several bio-active peptides (Pax et al, 2021) that acts as a textural enhancer during cheese maturation. Cheese-making relies heavily on the catabolic byproducts of amino acids, such as thiols and amines, as well as on smaller peptides derived from these compounds (Abdalla et al, 2022).

2.4. Starter culture

Dairy starter cultures play a crucial role in the manufacturing process by introducing specific lactic acid bacteria (LAB) species into milk. These LAB species, intentionally added to milk, primarily ferment carbohydrates, converting them into lactic acid and energy (Akarca et al, 2023). Starter cultures' fermentation activity produces a number of significant effects. First of all, lactic acid production lowers pH,

which helps to promote the emergence of beneficial organoleptic qualities. Second, the starter culture's acidic environment prevents viruses from growing, ensuring the stability and safety of the finished product. In addition, LAB generates organic chemicals that add to the fermented product's aroma and flavor profile (Ahmed et al, 2023).

Starter cultures can be made up of single strains that are combined or utilized singly as well as vague combinations of strains known as mixed strain cultures. These starting cultures are marketed as either lyophilized (freeze-dried containing milk components, nutrients, and energizers) or frozen (liquid nitrogen at -196°C) products (Munekata et al, 2022).

Prior to adding rennet to milk during the cheese-making process, carefully chosen LAB strains from various species are added. These strains' main job is to create lactic acid and, in some cases, flavoring compounds, which adds to the distinctive qualities of different cheese kinds (Narayana et al, 2022).

2.4.1. Cheese starter culture

Lactic acid bacteria (*Streptococcus thermophilus*) are frequently used in the commercial manufacture of cheese. Along with the bacterial strain (*Lactobacillus*), this bacterial species is employed. These are used as starter cultures for several fermented dairy products, including yogurt and fermented milk Swiss and Italian cheese (Silva et al, 2020). Less inoculation was required during cheese production due to *S. thermophilus*. Many types of semi-hard and Argentinean soft cheeses use the widely used strain *S. thermophilus* as a starting culture.

The use of milk products has significantly increased over the past 20 years, which has increased the usage of *S. thermophilus* on a global scale. The global market for items made from bacteria is worth around \$40 billion a year. A new demand for *Streptococcus thermophilus* culture performance is evident when one considers that 1011 bacteria strain cells are needed to create 1 kg of cheese (Padmanabhan and Shah, 2020). Despite this, overuse of LAB in the industrial setting has led to issues with biotechnology, such as phage infection.

2.4.2. Factors affecting the cheese preparation

Coagulants classification

Tofu coagulants include calcium sulfate, gluconolactone, and nigari. Calcium sulfate, a dehydrated form of calcium sulfate, is the most common coagulant used. It is also the oldest coagulant in China. Stone must be pretreated by baking and crushing. Tofu can also be coagulated with transglutamines in addition to the coagulants already mentioned (Mbye et al, 2022).

Concentration of coagulants

when used in sufficient quantities, coagulants produce pale-yellow whey with a mildly pleasant flavor. A larger concentration of the coagulant produces curd with a gritty, hard texture and a bitter flavor. If there isn't enough coagulant, the milk will separate into hazy whey that will effect on the cheese elasticity and increase hardness (Nazish et al., 2022).

Coagulation Temperature

because milk temperature impacts cheese production, texture, and coagulation rate, it should be checked just before adding coagulant. Fast coagulation caused by high temperatures results in a hard, gritty texture, low water-holding capacity, and low yield. But cold weather has the opposite effect. The typical range of temperatures is 70 to 80 degrees Celsius (Laursen et al, 2023).

Coagulation time

Let the milk sit for a bit after adding the coagulant, as the coagulation system needs time to work. Poor coagulation would occur with short stay time, whereas long stay would make molding problematic. To prepare cheese, you should plan on spending anywhere from 10 to 15 minutes (Xing et al., 2019).

2.5. Diabetes Mellitus

One of the most concerning public health problems of the 21st century, especially in underdeveloped regions, is the diabetes epidemic. Cardiovascular disease, renal failure, eye damage, and muscle weakness are only some of the negative health

outcomes that have been linked to diabetes mellitus. The prevalence of non-communicable diseases can be reduced with the use of appropriate treatments (Suryasa et al, 2021). Phytochemicals-rich medicinal plants can lessen the impact of illness and boost resistance. Some of Pakistan's native plants, such as *Gymnemasylvestre*, *Azadirachta indica*, *Aloe vera*, *Momordica charantia*, *Acacia arabica*, *Caralluma fimbriata*, *Allium cepa*, *Allium sativum*, *Althaea officinalis*, *Caesalpinia bonducella*, *Cinnamomum zeylanicum*, *Emblica officinalis*, *E* (Alam et al,2022). The aim of the research is to find out whether *Syzygium-cumini* has any antioxidant or anti-diabetic properties.

2.6. Medicinal Plants

Plants with therapeutic properties were crucial to the development of pharmaceuticals. Traditional medicine has a long history of helping people all around the world. Phytochemicals found in plants are used as starting points for new medication development and discovery. Many synthetic pharmaceuticals are based on substances found in traditional medicine systems, such as lead compounds, bio-active principles, pharmaceutical intermediates, folk medicine, dietary supplements, nutraceutical, and modern medicines (Bouyahya et al, 2021). Ethnobotany and common plants offer a wealth of information that can be applied to the creation of new, all-natural pharmaceuticals. Research into the use of plants in traditional medicine has gained a lot of attention recently. Over 80% of the global population relies on plants for vital medical care, according to a report recorded by the World Health Organization (Awan et al, 2021).

The pharmacological significance of plant metabolites has raised human curiosity for millennia. Many people, especially those living in poor countries, rely on traditional forms of medicine to treat their illnesses (Anand et al, 2021)

In addition to allopathy, many well-established medical traditions rely heavily on plant-based medicines (Rather et al, 2019). These include naturopathy, homoeopathy, yoga, Unani, and Ayurveda.

Scientists have worked at the molecular level to isolate key phytochemicals and better understand their mechanisms of action. Tribal populations in many nations often rely on folk medicine based on herbs. According to the World Health Organization,

millions of people in rural parts of poor nations rely on conventional medications made from plants and animals for their health care needs (Prabhakaran et al, 2011).

2.6.1. Plant based foods

The world's natural resources are being pushed to their limits, according to reports and studies by specialists. As the human population grows, so does the pressure on the planet's limited resources. All of the planet's stressed limits can be traced back to the production and consumption of food (Steffen et al, 2015), highlighting the importance of the food and agriculture industries. A sustainable food system must provide adequate nutrition for everybody while making efficient use of scarce resources. There has been a recent, rising trend towards plant-based diets as a solution to numerous problems. In recent years, there has been a rise in consumer interest in reducing meat consumption and a preference for plant-based diets, as well as novelties in the food industry that capitalize on this trend (Aschemann-Witzel et al, 2020).

Polyphenols, and more specifically, phenolic acids and flavonoids, have been linked to many beneficial effects in plant-based medicine. There has been a recent transaction in interest in the use of all-natural, pharmaceutically sound, and nutritionally sound substances. Phytoconstituents and antioxidants are found in plants, and it has been shown that plants have a wide variety of these compounds (Suh et al, 2017). Antioxidant activity must be evaluated using cell lines when screening medicinal plants for use in the clinic. Antioxidant activity in cells can be measured by cell culture rather than through chemical assays of antioxidant capacity (Chaves et al, 2020).

Natural remedies are currently more promising than completely risk-free man-made pharmaceuticals. According to the World Health Organization, almost 80% of the world's population should receive essential healthcare using herbs (Tran et al, 2022). Many different kinds of plants are employed in traditional medicine because of their healing powers. Recent research has focused on terpenes, alkaloids, and flavonoids produced from plants for their potential anticancer and cytotoxic effects, among other pharmacological benefits (Shaito et al, 2020).

2.6.2. Syzygium-cumini seeds

The tropical fruit *Syzygium-cumini*, a poly-embryonic species in the family Myrtaceae (Qamar et al, 2022), has enormous economic significance. It reaches heights of 30 meters and widths of 15 meters and can live for hundreds of years. Many names, including black plum, Java plum, Indian blackberry, and jamun, are used to refer to this fruit. The Timberland tree's edible fruit is native to and widely grown in Australia, Malaysia, Sri Lanka, Bangladesh, Pakistan, and India. From India and other tropical Asian countries, it was brought to South Africa for the country's enticing and extraordinary health benefits. In folk and traditional medicine, *S. cumini* is used to cure a wide range of conditions. The Unani medical system describes the plant as being used to improve blood quality, as a liver tonic, to strengthen gums and teeth, and as a lotion to get rid of a head ringworm infection (do Nascimento-Silva et al, 2022).

Various medicinal characteristics, including those of a febrifuge, antihelminthic, digestive, diuretic, antibacterial, carminative, refrigerant, and astringent, have been attributed to the plant's bark in previous research. Both the seeds and the fruit are used to treat a wide variety of ailments, including ringworm, urethrorrhea, splenopathy, pharyngitis, and even diabetes. This plant's leaves are used to treat gum disease and tooth decay because of their antibacterial properties. Also, the leaves are effective against a wide range of diseases and conditions, including dermatopathy, gastropathy, fever, colic problems, leukorrhea, constipation, and diabetes (Madani et al, 2021). Anti-diarrheal, antibacterial, and anti-inflammatory properties have been attributed to an extract of *S. cumini* seeds, leaves, and bark. Menorrhagia and diabetes can be treated with the crushed seeds. Its antidiabetic properties have been demonstrated in studies of its pulp, stem, bark, and leaf (Kumar et al, 2022).

2.6.3. Syzygium-cumini seeds

The dark purple, oblong, edible fruit has massive, polyphenol-rich seed located in the centre. Extracts from fruit pulp and seeds have been used medicinally for centuries. Extensive studies on the topic of antidiabetic qualities have been conducted in recent years, yielding a wealth of data. Jamun seed extract has been shown to provide a variety of health benefits, including those related to antioxidants, radio-protection,

gastroprotection, bacteria, allergies, fever, inflammation, and more (Kumar et al., 2022).

Jamboline, also known as antimellin, is a glucoside found in *Syzygium-cumini* seeds that prevents the body from turning starch into sugar, helping to keep glucose levels within healthy ranges (Santos et al., 2022). Anti-diabetic, antibacterial, antioxidant, and anti-inflammatory properties were all discovered in *Syzygium-cumini* seeds. Jamun seed powder has been used traditionally as a natural substance for maintaining a normal blood sugar level. The natural processes of perspiration and urination are supported by this cleansing herb. (Agarwala et al, 2019) noted that it also has blood-cleansing, cooling, liver-stimulating, and digestive effects.

2.6.4. Nutritional profile of *Syzygium-cumini* seed

The proximate makeup and particular phytochemicals of JS is (Kumar et al, 2022).

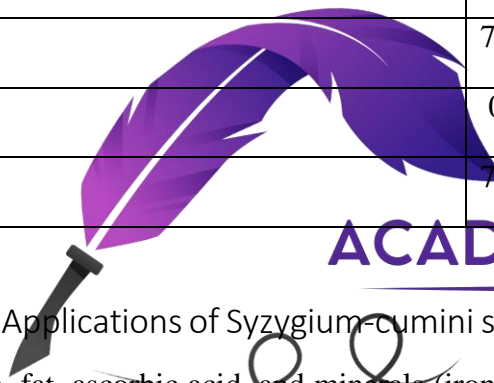
Moisture	9.32–16.35%
Carbohydrates	32.62–41.6%
Total dietary fibers	2.4–17%
Crude fat	0.83–1.19%
Ash	2.19%
Acidity	0.03–0.06
pH	3.79–4.83
Energy	335.66 Kcal
Total soluble solids	3.7 °Brix
Crude protein	1.97–8.5%

Sugars	
Uronic acid	5%
Rhamnose/fuctose	0.9%
Arabinose	6.8%
Xylose	18.8%

Mannose	1.8%
Galactose	2.4%
Glucose	70%

Lipids

Total oil	30 mg/g
SFA	2.91 mg/100 g
MUFA	292.79 mg/100 g
PUFA	7.53 mg/100 g
n-6	0.45 mg/100 g
n-3	7.08 mg/100 g



ACADEMIC SOLUTIONS

2.6.5. Applications of Syzygium-cumini seeds in Foods

Protein, fat, ascorbic acid, and minerals (iron, calcium, and potassium) are all present in sufficient quantities in JS, according to a physicochemical, proximate, vitamin, and mineral analysis. Jamun seeds can therefore be used by food scientists to make functional foods (Singh and Kocher, 2020). Because of the crucial roles that protein, lipids, carbs, and dietary fiber play in the body, their incorporation in JS powder has facilitated the technological advancement of a number of products. The technological properties of a product can be determined by its internal characteristics (calorific value, prebiotic score, glycemic index, and antidiabetic potential), external characteristics (diameter, thickness, spread ratio, uniformity, texture, and aesthetic quality), and shelf life. In addition, JS powder works well as a wheat flour substitute for baking cakes, increasing calorie, mineral, and carbohydrate intake (Sehwag and Das, 2016).

***Syzygium cumini* seeds powder-fortified biscuits**

Addition of the jamun seed powder can raise the level of constituents in the foods that naturally have less protein, fat, ash, and fiber which changes the end product's quality. Significant growth was noted, according to (Priyanka and Mishra, 2015) retaining the diameter, thickness, and spread ratio of the JS powder-fortified biscuits while increasing their nutritious content. High prebiotic activity was found in a functional confection and a low glycemic index containing JS. The functional confection with high amylase inhibitory activity ($IC_{50} = 83.89$ mg) and a high glucose dialysis retardation index also demonstrated its anti-diabetic potential in vitro experiments (Awuchi et al. 2019).

***Syzygium cumini* seeds powder fortified cookies**

Jamun seed powder (JSP) can be used to make herbal multigrain cookies of high nutritional value and excellent quality. The texture, nutrition, and organoleptic quality of the baked cookies were all improved by the addition of 10% (JSP) to the recipe. According to Ayurvedic literature, an average dose of 1-3 g of jamun seed powder per day is recommended for the treatment of diabetes (Shorti et al., 1962). It is uncomfortable to consume jamun seed powder straight up. As a result, this work has been done to create the cookies so that diabetics can consume them simply and receive the required dosage of jamun seed powder (Desai et al, 2018).

***Syzygium cumini* seeds powder fortified chicken chips**

In order to make functional chicken chips and examine their sensory quality, a study was conducted that involved adding a proper amount of jamun seed powder to the standardized functional chicken chips recipe. Sensory scores declined as the level of JSP inclusion rose and were substantially lower for products with 1% JSP added compared to treatments. Therefore, 1% JSP is advised to be added to functional chicken chips (FCC) as a source of naturally occurring antioxidants and preservatives with additional health benefits. Jamun seeds are a natural source of antioxidants and have a variety of beneficial qualities, including antibacterial, antioxidant, cardiac protective, anti-cancer, Anti-diarrheal, and hypoglycemic. According to claims made by Swami et al. (2012), the seeds contain antimellin, an alkaloid and glycoside of jamboline that blocks the diastatic conversion of starch to sugar. Heat treatment does

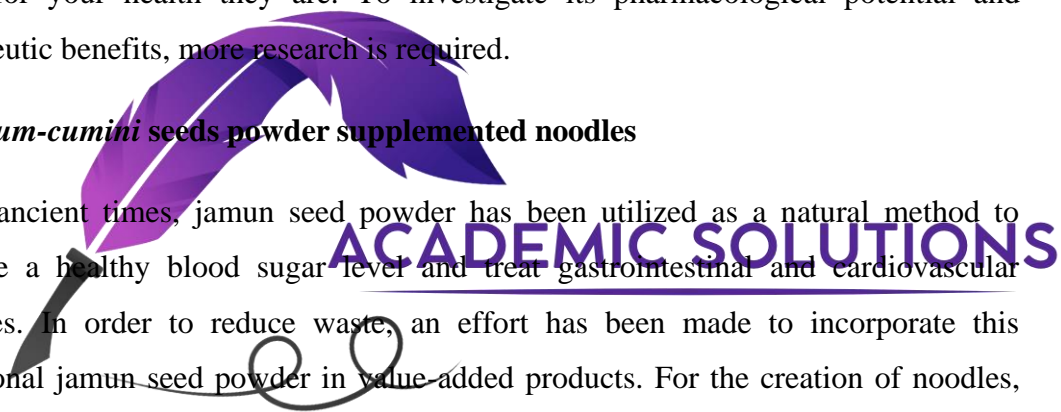
not lessen the anti-oxidative potential of jamun seeds; both heat-treated and untreated samples showed considerable radical scavenging activity on par with BHT, a synthetic antioxidant (Kasthuri et al, 2017).

***Syzygium cumini* seeds powder in composite cake formulation**

A composite cake of great quality and high nutritional content can be made with jamun seed powder (JSP). With additions of 10%, 20%, and 30% (JSP), the cake's weight, ash content, and carbohydrate content increased while its volume, height, specific volume, moisture, protein, and fat content dropped. The crumb and crust's color also became darker. Jamun seed powder is widely used to treat a range of ailments and has a lot of positive health benefits. Understanding the components of the plant that are healthy could help prevent a variety of illnesses and maintain excellent health. This essay will look at its photochemical characteristics and how good for your health they are. To investigate its pharmacological potential and therapeutic benefits, more research is required.

***Syzygium-cumini* seeds powder supplemented noodles**

Since ancient times, jamun seed powder has been utilized as a natural method to balance a healthy blood sugar level and treat gastrointestinal and cardiovascular diseases. In order to reduce waste, an effort has been made to incorporate this nutritional jamun seed powder in value-added products. For the creation of noodles, wheat flour was mixed with jamun seed powder in the following proportions: 2, 4, 6, 8, and 10%. The 90-day storage period of the created items allowed researchers to monitor changes in their sensory, proximate, and cooking properties. The inclusion of jamun seed powder changed the cooking qualities. With the addition of jamun seed powder, cooking time increased from 9.98 to 11.01 minutes, weight increased by 141.42 to 174.52 percent, and volume increased by 140 to 210 percent, respectively. Noodles made from jamun seed powder at an 8 percent concentration were found to have the optimum acceptance and shelf-life, with crude protein, crude fat, crude fiber, and ash contents of 10.89, 0.60, 1.43, and 0.91 percent (Sood et al, 2018).



***Syzygium cumini* seeds powder fortified wine**

Jamun is well-known for its nutritional and medicinal benefits. However, an effective fermentative processing technology to manufacture local Jamun wine is soon to be developed due to its perishable nature and short shelf life of 1-2 days.

In the current study, response surface methodology was used to optimize the concentrations of seed powder and pulp powder for polyphenol and tannins response for the production of Jamun wine. They were kept in storage for 90 days to see how that affected the sensory qualities. Both of these wines scored "very liked" in the sensory assessment of the wines. The jamun wine contained volatile derived from seeds and bark, according to the qualitative analysis (Singh and Kocher, 2020).

***Syzygium-cumini* seeds powder fortified ready-mix powders**

Syzygium-cumini (*S. cumini*) is a tropical and subtropical fruit that is underutilized. It contains a variety of bioactive chemicals that are found throughout the entire plant. The fruit as well as seeds have nutritional and therapeutic value. Fruit and seeds, however, have not yet been completely taken into account as possible functional food ingredients to create dishes with promised health advantages. According to the literature that is currently accessible, fruit and seeds are full of micronutrients and phytochemicals such as myricetin, oxalic acid, gallic acid, citronellol, cyanidin glucoside, tocotrienol, phytosterols, flavonoids, carotenoids, and poly phenols. They also reportedly have a number of advantageous health traits. Additionally, studies in this field would yield useful knowledge for their prospective application as functional food additives. The possibilities for employing *S. cumini* fruit and seeds as functional food ingredients, as well as its bioactive substances and nutraceutical qualities, are all covered in detail in this paper (Harine and Janapriya, 2018).

Sugar Free and *Syzygium-cumini* seeds powder fortified Chocolates

The market now offers sweet snacks and sweets that are low in calories, fat, and sugar due to the rising number of overweight or chronically obese people. Compared to sucrose, sugar substitutes contain fewer calories and a lower glycemic index, which not only aids in weight management but also makes them safe for diabetics. The goal of the study is to create sugar-free chocolate using a combination of different zero-

calorie sweeteners. The rating for sugar-free chocolates as a whole was found to be 8.9. Additionally, fruit powders rich in vitamins and minerals, such as beetroot powder and jamun seed powder (dragon fruit), were used to fortify chocolates. An examination of the vitamins and minerals in the jamun seed powder-fortified sugar-free chocolate was done. A study on the shelf-life of sugar-free chocolate that has jamun seed powder added to it was done. It was observed that chocolates contain low fat and high fiber content. The highest sensory score and increased protein and fiber content were seen in fortified chocolates (Paranjape et al, 2021).

2.7. Phytochemicals analysis

Syzygium-cumini seeds have been extracted using both ethanol and water, and their phytochemicals components have been studied for their potential therapeutic value. Analysis also detected key phytoconstituents in the seeds, including n-alkane, sesquiterpenes, and Polyphenols. Using the agar-well diffusion and minimum inhibitory concentration methods (Tambe et al, 2021), the antibacterial activity of ethanol extracts of *S. cumini* seeds was evaluated against human infections.

The antibacterial activity of the seed extract was found to be quite strong. The Folio-Ciocalteau method was used to determine the total poly-phenolic content of *S. cumini* seed ethanolic extract, and the DPPH assay, total antioxidant capacity, and reducing power assay were used to evaluate antioxidant activity. The high concentration of phenolic components in the seed has been linked to the extract's powerful antioxidant effect. The ethanolic extract of *S. cumini* seeds showed potential cytotoxic effects against cervical cancer cell. The anti-diabetic efficacy of an ethanol extract of the dried seed kernel was also evaluated (Rashid et al, 2022).

Tannins, gallic acid, vitamin C, and anthocyanins (malvidin, glucoside, petunidin, cyanidin, and others) are all important phytoconstituents. Amino acids, flavonoids, sugars, phytosterols, saponins, tannins, and terpenoids are all part of a basic analysis of phytochemicals. (Agarwala et al, 2019) demonstrated that phenol is present in the stem bark of *S. cumini*. The bark of *S. cumini* trees has been found to have butulinic acid, friedelin, sitosterol, and epi-friedelanol. New esters of kaempferol, myricetin, quercetin, Gallic acid, astragalol, and eugenol (eugenin) are also present (Kumar et al, 2022).

2.7.1. Antioxidant activity

Plants can be both mutagenic and poisonous, despite their potential medicinal qualities. Although one might assume plants used in conventional medicine for extended periods to be safe, the toxic effects of most commonly used medicinal plants are not widely discussed in the literature. The toxicity of a substance depends on the duration and magnitude of exposure, as well as the kind of organism and its physiological and developmental state. *Syzygium-cumini*, a member of the family Myrtaceae, has long been used as a medicinal herb in India. The names "Jamun," "Jambul," "Black plum," "Java plum," and "Naaval" all refer to this fruit. The bark, seed, fruits, and leaves of *S. cumini* all have medicinal purposes. The anti-diabetic benefits of the seeds are thought to come from the plant's anti-oxidant characteristics in the leaves and fruits (Rashid et al, 2022) found that seeds had potent anti-cancer, anti-inflammatory, and central nervous system (CNS) actions.

It is hypothesized that elevated oxidative pressure is linked to increased lipid peroxidation, which plays a key role in the amelioration of diabetes problems. Increased production of reactive oxygen species and decreased antioxidant defense cause oxidative stress in tissues and cells. Many hypotheses are proposed to explain the origin of free radicals in diabetes (Qamar et al, 2022). These include glucose autoxidation, progressive and non-enzymatic glycation of proteins, and an increase in the production of glucose-inferred advanced glycosylation end products, as well as an increase in the flux of glucose down the polyol metabolic pathway. Increased production of free radicals causes antioxidant depletion, which can disrupt cellular activities, cause membrane oxidative damage, and make cells more susceptible to lipid peroxidation (Panghal et al, 2019).

The accumulation of hazardous compounds due to oxidative damage could result from decreased GPx activity. *S. cumini* pulp and seed extract treatment significantly restored GSH-dependent enzymes and GSH content, demonstrating their protective effect on antioxidants. It is possible that an increase in the formation of reactive oxygen radicals contributes to the decreased activity of CAT and SOD in the kidney and liver in people with diabetes. Hemoprotein CAT catalyses the decomposition of H_2O_2 , protecting tissues from highly reactive hydroxyl radicals, while superoxide

dismutase (SOD) converts superoxide radicals to H₂O₂. The seed's kernel increases the production of these enzymes, protecting tissues from the damaging effects of free radicals. Similar antioxidant activity and protection from tissue damage were found in the brains of diabetic rats when treated with an aqueous extract of *S. cumini* seeds (Wali et al, 2019). These findings provide the basis for proposing a mechanism to explain the antioxidant protection seen in *S. cumini*.

2.7.2. Nutraceutical Potential

The fruit of *S. cumini* provides a great source of the pigment anthocyanin. Green in appearance while immature, the fruit eventually matures to a deep purple. Until late in fruit development, chlorophyll levels decline from green to white, resulting in an intermediate colour in fruits that accumulate anthocyanins. The fruits' rich crimson or purplish hue is the result of a gradual buildup of pigment. *S. cumini* fruits that start out green fade to a pale green before finally turning white. Initially white, then pink, then red, and finally deep purple when mature, white fruits are described in detail by (Kumar et al, 2022).

The fruit's anthocyanins have long been used in complementary and alternative medicine to treat a wide range of conditions. Fresh fruits, when in season, are harvested for their high nutrient content. In Brazil, people with diabetes drink tea made from the plant's leaves or eat them for their purported anti hyper-glycemic effects. The effects of bark, seed, and fruit extracts have been studied in vivo and in vitro using a variety of animal models and animal cell lines (Gibbert et al., 2021).

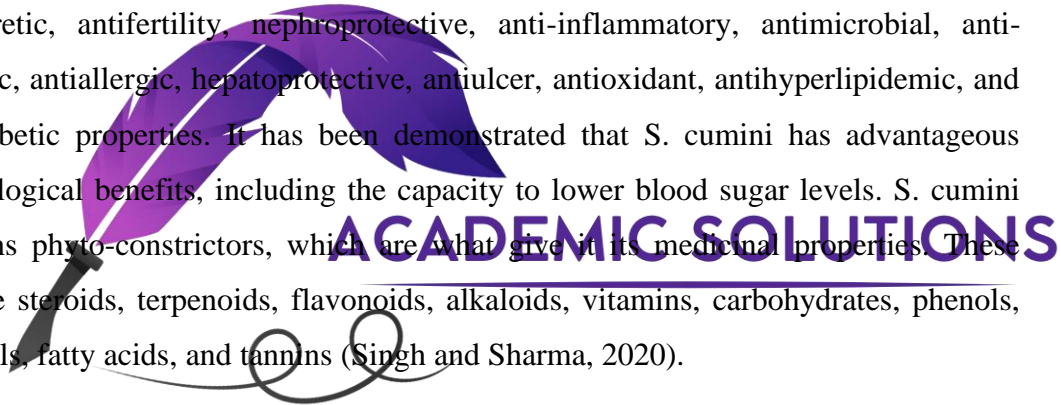
Only a few of the taxonomically identified substances present in the Myrtaceae family include flavonoids, tannins, betulinic acid, ellagic acid, gallic acid, oxalic acid, malic acid, and oxalic acid. There are many trees that contain these chemicals, which can be used singly or in combination to treat a variety of ailments. The use of the plant as a whole for medical purposes has prompted substantial chemical research into the active chemicals inside the plant that are responsible for its therapeutic effects. The *S. cumini* fruit is helpful because it is low in calories and contains no sugar. The two main sugars in fruit are fructose and glucose (Joshi et al, 2019).

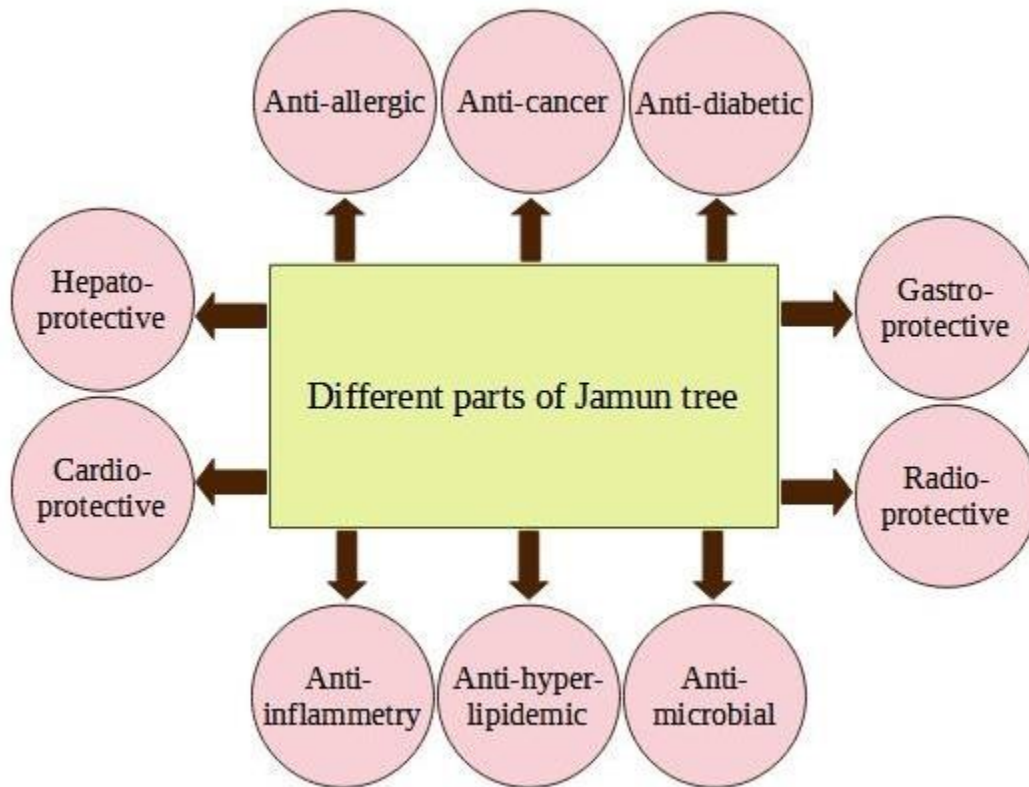
Various portions of *S. cumini* are used as an alternative medicine to treat diabetes. Many recent scientific experiments have confirmed the anti-diabetes effects of stem bark, seeds, and fruits. The powdered seed of *S. cumini* is used to help diabetics

regulate their blood sugar. Seeds' high protein and calcium content can treat number of health issues. The primary ingredient in seeds is jamboline glycoside, which helps regulate blood sugar by inhibiting the conversion of starch to sugar when blood sugar levels are already optimal. It has also been used historically for its medicinal benefits, such as the treatment of conditions like oral illness, enlarged spleen, obesity, dysentery, diarrhea, and so on. Paste, juice, decoction, and powder derived from trees are all used by traditional medicine practitioners to treat illness (do Nascimento-Silva et al, 2022).

2.7.3. Health benefits

Due to their lower risk of adverse effects and their connection to nature, herbal medicines have been rising in popularity in both developed and developing countries in recent years. The fruit of the *Syzygium-cumini* plant has been investigated for its Antidiarrheal, neuroprotective, radioprotection, reduction of plaques formation, antipyretic, antifertility, nephroprotective, anti-inflammatory, antimicrobial, anti-arthritic, antiallergic, hepatoprotective, antiulcer, antioxidant, antihyperlipidemic, and antidiabetic properties. It has been demonstrated that *S. cumini* has advantageous physiological benefits, including the capacity to lower blood sugar levels. *S. cumini* contains phyto-constrictors, which are what give it its medicinal properties. These include steroids, terpenoids, flavonoids, alkaloids, vitamins, carbohydrates, phenols, minerals, fatty acids, and tannins (Singh and Sharma, 2020).





(Anjum et al, 2021)

2.7.4. Anti-Diabetic Effects

Plant extracts have been studied for their potential antidiabetic effects to identify non-traditional therapy options that pose less risk to diabetic patients. Pharmacological studies have shown that components of *S. cumini* exhibit anti-HIV, antimicrobial, and hypoglycemic effects making it a prototypical traditional therapeutic plant. The seeds, fruit, leaves, and bark of plants are used in several traditional medical practices. The seeds are used to cure skin diseases, fever, stomachaches, and leucorrhea, and they also prevent blood from being released in faeces and minimize DNA damage caused by radiation. Chronic diarrhea, spleen enlargement, diabetes, and stomachic disorders are all conditions that can be treated using *S. cumini* seeds. The seed of Jamun can effectively treat liver diseases and bleeding piles (Ayenampudi et al, 2022).

Phytochemical analyses revealed sitosterol, stigmasterol, 12-oleanane-3-ol-3 β -acetate, lupeol, and n-hexane, which are compounds connected to diabetes. Stigmasterol has been shown to have a significant impact on reducing serum glucose concentration and increasing insulin levels, which is indicative of insulin-stimulating and hypoglycemic activity (Aliyar et al, 2021). The sterols in this plant have also been shown to reduce

plasma cholesterol levels because they compete with cholesterol for digestion and absorption. Stigmasterol may lessen cholesterol biosynthesis in humans by blocking sterols 24-reductase (Ponnulakshmi et al. 2019).

Beta-sitosterol has an anti-diabetic effect. Beta-sitosterol and stigmasterol have been shown to be present in the hypoglycemic fractions, although a structural explanation has been proposed by (Ponnulakshmi et al. 2019). These parts have been tested separately, and there is no evidence to suggest that they require each other to produce an inhibitory action against diabetes. Lupirol, a phytoconstrictor, slows the onset of diabetes. When lupirol is administered, serum insulin levels rise. Glycated hemoglobin, serum glucose, and nitric oxide all drop as a result. Therefore, lupeol may be useful as an anti-diabetic drug (Tiwari et al, 2019).

Different enzymes involved in glucose metabolism have been identified to respond to *S. cumini* extracts from both alcoholic and aqueous seed concentrations. Hexokinase activity was increased by 64.7%, 72.7%, and 79.5% after a month of oral glibenclamide administration, seed extract of aqueous *S. cumini* administration, and insulin administration at 6 U/kg BW, whereas glucose-6-phosphatase activity was decreased by 64.7%, 73.50%, and 88.4%, suggesting increased glucose utilization for energy production and decreased gluconeogenesis. People who consumed *S. cumini* seed extract orally had lower blood serum levels of several hepatocellular-toxic enzymes, such as lactate dehydrogenase (LDL), acid phosphatase (ACP), and antacid phosphatase (ALP), than those who did not consume *S. cumini* seeds (Zapater et al, 2021).

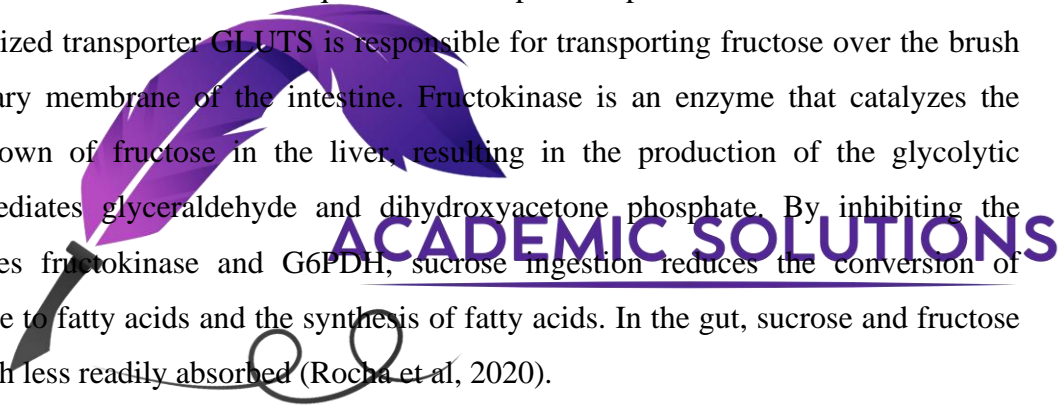
2.8. Alpha-glucosidase inhibition

The anti-diabetic properties of *Syzygium-cumini* were investigated, and it was shown that the plant suppressed the activity of alpha-glucosidase in a non-competitive fashion. Additional evidence for its hypoglycemic effects comes from its stronger antioxidant capability than butylated hydroxyl toluene and curcumin. Lipid peroxidation, protein oxidation, plasma and liver homogenate antioxidant capacity, and glycated hemoglobin were all measured to see how *S. cumini* affect them (Scudino et al. 2023). They hypothesized that *S. cumini* reduces plasma glucose levels to reduce oxidative stress and thus possesses anti-diabetic and antioxidant

characteristics. In addition to its hypolipidemic effect, *S. cumini* has a hypolipidemic effect because it reduces lipid synthesis in the body; however, its ability to restore body weight in alloxan-treated diabetic rats may be due to its hypoglycemic effect. In alloxan-induced diabetes rats, triglycerides and phospholipids are constantly being recycled due to a metabolic abnormality (Khakhariya et al, 2023).

It is thought that *S. cumini* corrects the metabolic imbalance caused by alloxan diabetes by shifting the emphasis from lipid to carbohydrate metabolism in these animals. *S. cumini* seed powder lowered fructose absorption in the intestines and prevented sucrose-induced obesity. The dose-dependent effects of consuming *S. cumini* seeds on plasma and hepatic triacylglycerol concentrations, as well as on intestinal fructose absorption, were observed (Putri et al, 2023).

Obesity could be avoided in those who consume large amounts of sucrose and fructose if the described technique were to be put into practice. It is believed that the specialized transporter GLUT5 is responsible for transporting fructose over the brush boundary membrane of the intestine. Fructokinase is an enzyme that catalyzes the breakdown of fructose in the liver, resulting in the production of the glycolytic intermediates glyceraldehyde and dihydroxyacetone phosphate. By inhibiting the enzymes fructokinase and G6PDH, sucrose ingestion reduces the conversion of fructose to fatty acids and the synthesis of fatty acids. In the gut, sucrose and fructose are both less readily absorbed (Rocha et al, 2020).



CHAPTER NO. 3

MATERIAL AND METHODS

Milk and milk products being a rich source of essential micro and macro nutrients are considered healthy foods for all age group of people. Cheese is a highly nutritious dairy product that offers a wide range of nutrients for its consumers. Being a rich source of fats, proteins, vitamins and minerals, the consumption of this dairy product is increasing by every passing day. Different types of cheese is available today in the markets but among them Mozzarella cheese is considered as a most appropriate type for the diabetic patients. The number of diabetic patients has now reached to more than 33 million. Diabetic patients are offered with a limited type of food, among different types of cheese only mozzarella cheese is recommended for the diabetic patients due to its low fat content. *SyzygiumCumini* is reported to have a number of phytochemicals that help to control the level of blood sugar especially in the diabetic patients. Therefore, the development of *SyzygiumCumini* fortified mozzarella cheese may prove a product of much value especially for the diabetic patients. The effectiveness of *SyzygiumCumini* fortified mozzarella cheese in diabetes management was studied by using alpha amylase inhibition assay. The experimental work for the development of *SyzygiumCumini* fortified mozzarella cheese was performed in the labs of the Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan. Nutritional profile, anti-oxidant, and anti-diabetic properties of *SyzygiumCumini* fortified mozzarella cheese were studied, the methodology adopted and the material used are given below.

3.1. Sample Collection

Syzygium-cumini (seeds) were collected from the local market of Multan as a raw material for cheese preparation. Seeds were rinsed with clean water to get rid of any impurities, sun-dried, and then dried samples were ground in a grinder to a fine powder. The powder was kept in polythene bags at room temperature for further study. This powder was then used for the fortification during cheese preparation.

3.2 Proximate analysis of Syzygium-cumini (seeds)

Using the respective methods (AOAC, 2006) and (AACC, 2000), tests for protein, fat, moisture, ash, fiber and nitrogen-free extract were carried out which are stated below in detail.

3.2.1 Determination of Moisture:

Moisture determination of fortified Mozzarella cheese was performed in accordance with AOAC (2006) regulations. 5 grams of sample, precisely measured, was placed in pre-weighed china dishes and maintained for 24 hours in a hot air oven at 100–105 °C. The sample was then moved to a desiccator, where it was given 30 minutes to cool under controlled circumstances. The sample was weighed again after cooling. The moisture content of the samples was calculated using the formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight (with moisture)} - \text{final weight (moisture free)}}{\text{Initial weight (with moisture)}} \times 100$$

3.2.2 Ash Determination:

ACADEMIC SOLUTIONS

According to AOAC (2006) recommended methodology, an investigation was undertaken to determine the total ash content of the sample. Precisely measured quantity of 5 grams of finely crushed powder of Syzygium-cumini was placed in a crucible and burned until the black smoke vanished and the sample was carbon-free. The charred sample was then carefully transferred into a muffle furnace set at 600 °C for 24 hours before being weighed. The sample was allowed to reside until a distinct gray coloration indicative of ash was achieved. Ash concentration is determined by the weight difference between samples taken before and after. The amount of total ash in the samples was calculated by using formula:

$$\text{Ash (\%)} = \frac{\text{weight of sample after ashing}}{\text{weight of fresh sample}} \times 100$$

3.2.3 Protein Determination:

According to the Kjeldhal apparatus and adhering to AACC (2000) standard protocols, protein content in the sample was determined. A precisely weighed quantity of 5 grams of sample was placed into a separate digestion tube. We add one digestion tablet ($K_2SO_4:FeSO_4:CuSO_4$ ratio 94:5:1) and 25 ml of concentrated H_2SO_4 to each digestion flask to speed up the procedure. To obtain the light green coloration of each sample, place these flasks on the heating mantle for three to four hours. Once the digestive process is over, they may cool to room temperature.

Dilute the digested sample with 250 ml distilled water. To release the ammonia that has been trapped inside the sulfuric acid, add 10 ml of 4% NaOH (a potent alkali) to the distillation assembly. The unconstrained ammonia was added to a solution that also contained 4% boric acid as an indicator and methyl red to create ammonium borate, which serves as a representation of the nitrogen content of the mixture. Finally, the distillate of 0.1N H_2SO_4 was titrated till the color changed, noting the volume employed. Crude protein (%) content present in the sample was calculated using the formula below:


$$\text{Nitrogen (\%)} = \frac{0.0014 \times \text{volume of } 0.1 \text{ N } H_2SO_4 \times 250}{\text{volume of diluted sample} \times \text{original sample weight}} \times 100$$

$$\text{Determination of Crude Protein} = \text{Percentage of Nitrogen} \times 6.25$$

3.2.4 Determination of Fat

According to the well-established AACC (2000) method No. 30-10, an investigation was conducted to determine the crude fat content present within sample. This involved the precise measurement of 5 grams of sample. The samples undergo rigorous extraction process utilizing a Soxhlet apparatus. Petroleum ether was used as a solvent. The extraction procedure was conducted over a period of 2 to 3 hours, allowing sufficient time for the solvent to intimately interact with the samples, facilitating the separation of the crude fat.

$$\text{Fat (\%)} = \frac{\text{weight of sample before extraction} - \text{weight of sample after extraction}}{\text{weight of original sample}} \times 100$$

3.2.5 Crude Fiber Determination

Crude fiber content of the *Syzygium-cumini* was calculated using a protocol. Acid washing and Base washing are the first two steps in this process. 2g sample of moisture- and fat-free powder was transferred to a 500ml beaker for acid digestion. Add 200ml of a diluted acid solution, usually 1.25 percent H₂SO₄. On the heating mantle, boil the solution for about 30 minutes, typically. Filter paper was used to separate the filtrate from the residue after the filtrate had been boiled for 30 minutes. To get rid of any remaining acidic residues, the residues on the filter paper were washed with hot distilled water. The acid-digested sample from the filter paper was put into a 500 ml beaker and 200 ml of a diluted base solution of 1.25% NaOH was added as the next step of base washing. Throughout this process, the boiling went on for 30 minutes. As with acid digestion, the sample's filtration and washing procedures were used once more. After that, the fibre leftovers were placed in a petri dish that had been weighted down before being baked at 110°C to completely dry them off. Weighing the sample on a balance once it has fully dried. The dried sample was then put into a crucible and burned at a high temperature, often between 550 and 600 degrees Celsius, to completely burn off all organic material and leaves only the ash. The crucible was cooled in a desiccator after being burned. After cooling, weight it. Weight will indicate the mass of the ash residue. Percentage of the dry weight of sample will typically indicate the fiber content.

$$\text{Fiber (\%)} = \frac{\text{weight of sample before ashing} - \text{weight of sample after ashing}}{\text{weight of original sample}} \times 100$$

3.2.6 Nitrogen-free extract (NFE):

Using formula, NFE value was calculated

$$\text{NFE (\%)} = 100 - [(\text{Moisture (\%)} + \text{Ash (\%)} + \text{Protein (\%)} + \text{Fat (\%)} + \text{Fiber (\%)})]$$

3.3 Determination of Antioxidants of *Syzygiumcumini*

Using the respective method of (Zahin et al, 2010), antioxidants test such as DPPH, FRAP, and TPC were carried out which are stated below in detail.

3.3.1 Extract preparation of *Syzygiumcumini* (seeds) powder and fortified Mozzarella cheese:

Distilled water, ethanol, acetone, and hexane solvents are combined in a 1:10 ratio with powdered *Syzygiumcumini*(seeds) and enriched cheese. Give it a night to rest, and then use filter paper to purify the samples. The samples were then available for the following antioxidant treatments after being evaporated on a rotary evaporator.

3.3.2 Total Phenolic Contents (TPC):

Total phenolic content of the *Syzygiumcumini* powder sample and fortified Mozzarella cheese was estimated by using the procedure of Ismail et al, 2014 with slight modifications. The Folio-Ciocalteau procedure was used to calculate the total phenolic contents of the samples. The gold standard chemical for phenolic testing is gallic acid. Using a spectrophotometer tuned to a wavelength of 724 nm, the absorbance was measured and total phenolic content was presented as mg considering the gallic acid as standard. Dry equivalent of extracts were used to express the TPC in mg per gram gallic acid.

3.3.3 Free Radical Scavenging Activity (DPPH):

The antioxidant properties of *Syzygiumcumini*(seeds) and fortified Mozzarella cheese were assessed using powdered samples. For the DPPH test, the extract of the solvent was mixed with methanol and mark the volume till 50 micro-liters (having 50 and 100 µg/ml of the sample extract. Following that, 50mM of Tris-HCL buffer (with a pH of 7.4) and 0.1mM of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (1ml solution produced in methanol) was added to each reaction set. Ascorbic acid (C₆ H₈ O₆) was used as a control, and 50 µL methanol solvent as a negative control. The free-radical scavenging potential of DPPH was measured spectrophotometrically at 517 nm wavelength after all sample and standard solutions were kept at 25 °C for 30-35 min (Gülçin et al., 2012).The standard reading was calculated using a blank DPPH sample. They measured the variation between the plant samples and the blank sample by using formula:

$$\% \text{ Radical Scavenging Activity} = \frac{\text{control absorption} - \text{sample absorption}}{\text{Control absorption}} \times 100$$

3.3.4 Ferric Reducing Antioxidant Power:

The method described by Zahin et al. (2010) was modified to test the ferric-reducing antioxidant strength of several fruit extracts of *S. cumini*. 100 l of the test samples were mixed with FRAP working solution (300 uL; contains 300 mmol/L acetate buffer, pH 3.6, 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/L HCl and 20 mmol/L FeCl₃ in a ratio of 10:1:1) contains 300 mmol/L acetate buffer, pH 3.6, and 20 mmol/L FeCl₃ then left the reaction mixture for 10 minutes at 37 degrees Celsius while the absorbance was measured spectrophotometrically (UV-Vis 3000, ORI, and Germany) at 593 nm. To calibrate, ferrous sulphate was utilized. Results were presented as mmol/g and contrasted with quercetin and ascorbic acid.

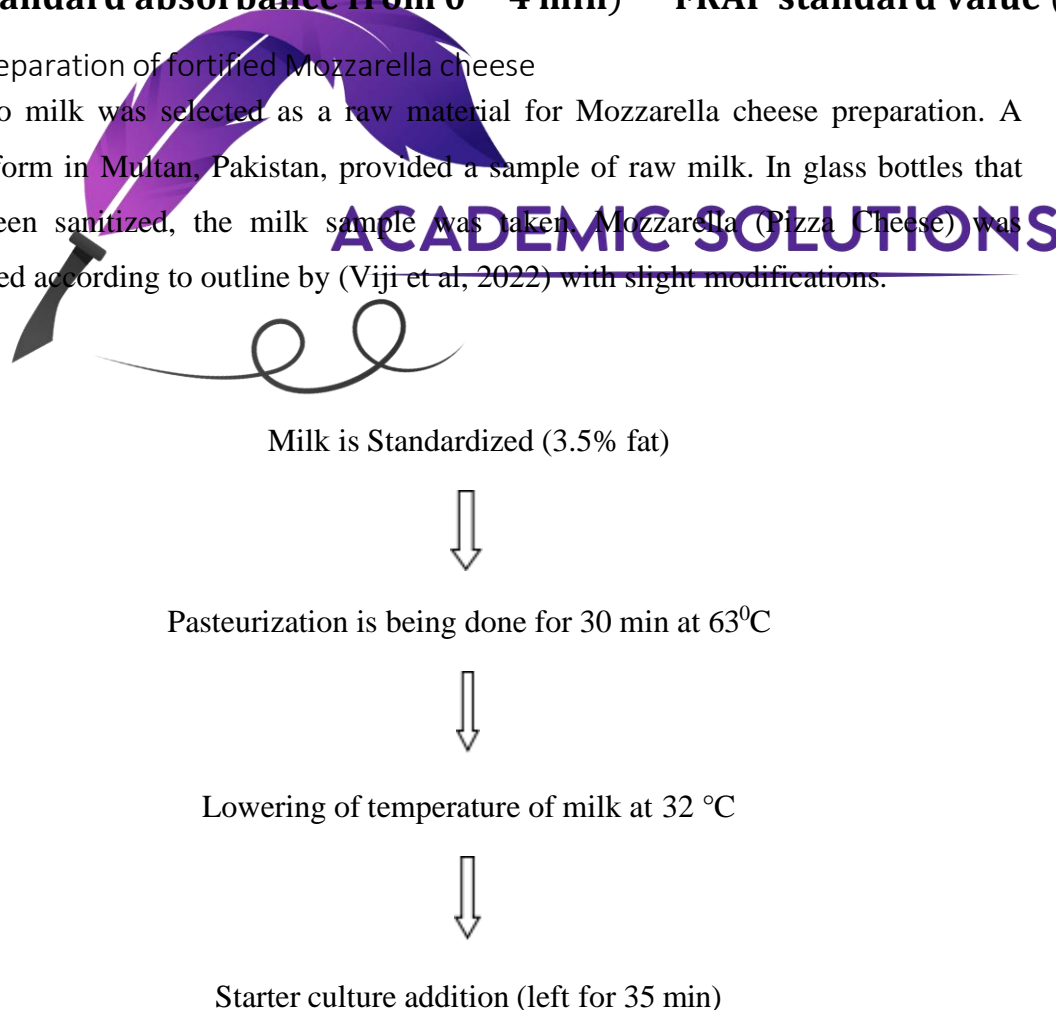
value

$$\text{value} = \frac{\text{sample absorbance from 0 – 4 min}}{(\text{standard absorbance from 0 – 4 min}) - \text{FRAP standard value (1000 } \mu\text{M)}}$$

3.4 Preparation of fortified Mozzarella cheese

Buffalo milk was selected as a raw material for Mozzarella cheese preparation. A dairy farm in Multan, Pakistan, provided a sample of raw milk. In glass bottles that had been sanitized, the milk sample was taken. Mozzarella (Pizza Cheese) was prepared according to outline by (Viji et al, 2022) with slight modifications.

Fig 1:





Rennet addition (left for 45 min)



Piece formation



Cooking at 39 °C for 40 minutes



Half whey drainage



Hold the curd whey for the formation of acids (39 °C)



ACADEMIC SOLUTIONS

Drainage of full whey



Curdling the cheese



Acidification of curd



Kneading a curd



Shaping and stretching



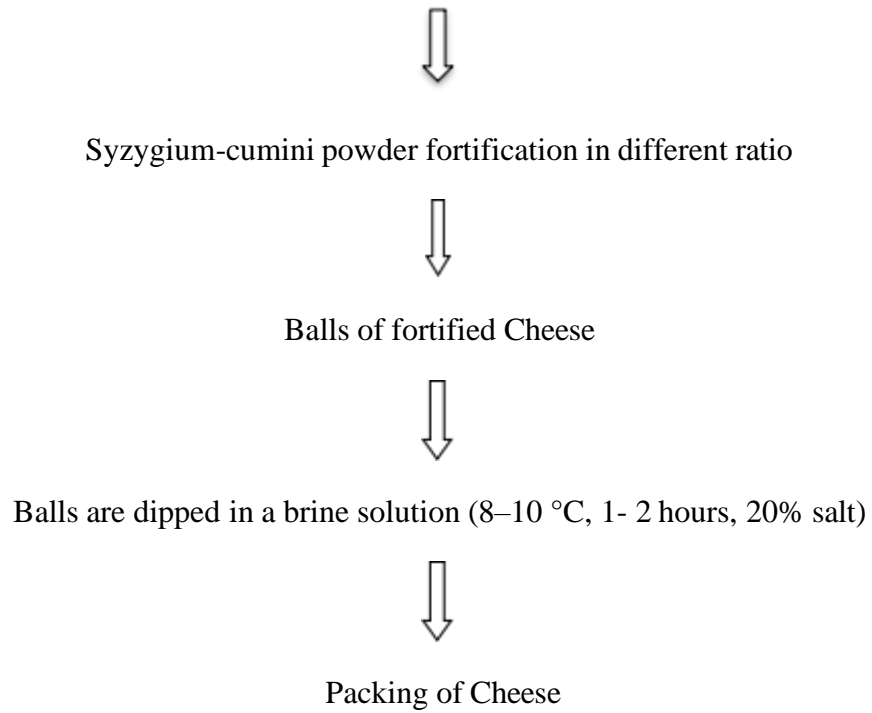


Fig 1: Process flow for making fortified Mozzarella cheese.





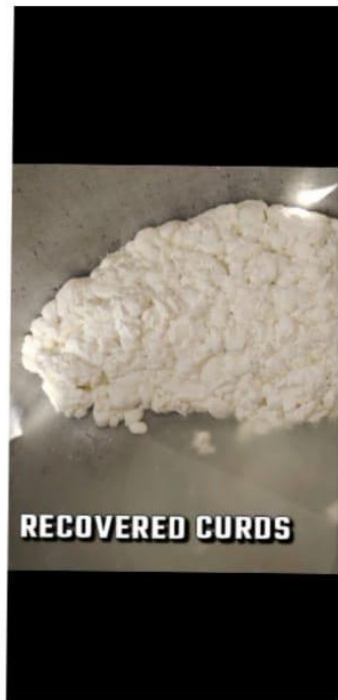
ACIDIFICATION



CUTTING



CURDS RECOVER



RECOVERED CURDS



PILING (SIDE CHANGE)



SALTING AND MOLDING

|S

3.4.1 Experimental plan

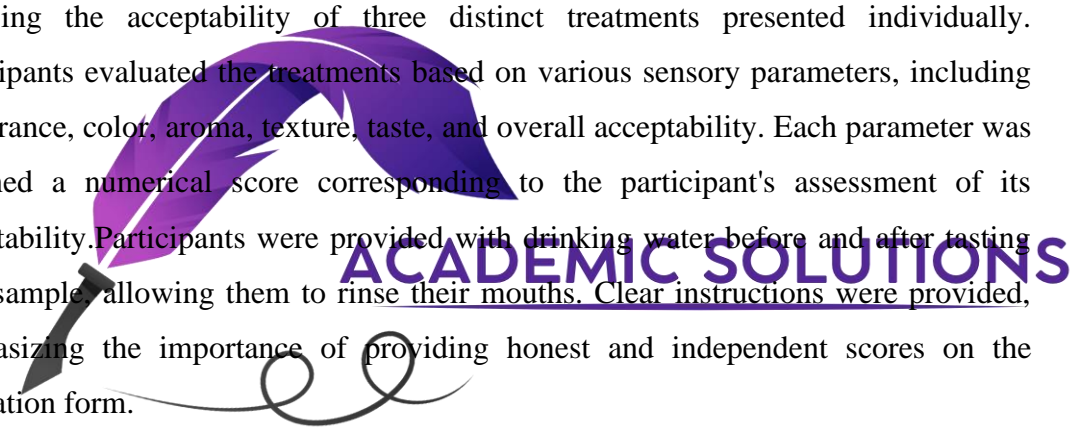
Treatment	Percentages
To (Control)	0%
T1 (<i>Syzygiumcumini</i>)	4% (40 grams)

T2 (<i>Syzygiumcumini</i>)	6% (60 grams)
T3 (<i>Syzygiumcumini</i>)	8% (80 grams)

In my study, Mozzarella cheese was prepared on lab scale using buffalo milk and then fortified with different ratios of *Syzygiumcumini* powder to test its anti-diabetic properties. It was then tested for the different parameters.

3.5 Sensory Evaluation

Using a 9-point hedonic scale, the sensory evaluation of the *Syzygiumcumini* supplemented Mozzarella cheese was conducted. A randomized selection process was employed to choose a diverse group of 20 participants, comprising both males and females, to take a part in a sensory evaluation study conducted at the university. Each participant received a sensory evaluation Performa, which served as a tool for assessing the acceptability of three distinct treatments presented individually. Participants evaluated the treatments based on various sensory parameters, including appearance, color, aroma, texture, taste, and overall acceptability. Each parameter was assigned a numerical score corresponding to the participant's assessment of its acceptability. Participants were provided with drinking water before and after tasting each sample, allowing them to rinse their mouths. Clear instructions were provided, emphasizing the importance of providing honest and independent scores on the evaluation form.





3.6 Sample collection

All of the analytical-grade chemicals used in this investigation, such as sulfuric acid, sodium hydroxide, boric acid, digestion tablets, phenolphthalein indicator, Standardized HCL solution, pH 4 and 7 buffer solution, isoamyl alcohol, hexane, acetone, ethanol, and methanol, were carefully inspected before use.

ACADEMIC SOLUTIONS

3.7 Physicochemical analysis of fortified Mozzarella cheese

Fortified Mozzarella cheese undergo analysis such as

- Moisture content
- Crude fat
- Crude protein
- Ash
- Crude fiber
- NFE content

Moisture, Crude fat, Crude protein, and Ash have been performed according to the procedures described before.

3.8 Determination of antioxidant potential

Fortified Mozzarella cheese undergo the same antioxidants test such as

- DPPH scavenging activity
- FRAP
- TPC

using the respective methods (Zahin et al, 2010) as described before.

3.9 Antidiabetic evaluation

Anti- diabetic activity has been checked according to the method.

- Alpha-glucosidase inhibition test

3.9.2 Alpha-glucosidase inhibition assay (GI):

The glucosidase inhibition (GI) of the fortified Mozzarella cheese was assessed using the methodology described by Apostolidis et al. (2007). The GI analysis involved the addition of 500 μ L (0.1–0.4 mg/mL) of the sample to a solution of 1000 μ L Alpha-glucosidase (1.0 U/L) that was previously prepared in a 0.1 M phosphate buffer (pH 6.9). 500 μ L of a 5 mM nitrophenyl-glucopyranoside solution, also prepared in 0.1 M phosphate buffer (pH 6.9), were added, and the resulting reaction mixture was incubated at 25 °C for 5 minutes before its absorbance was measured at a wavelength of 405 nm (Fapetu et al., 2022). The percentage inhibition was calculated using the formula:

$$\text{Inhibiting ability of Glucosidase} = \frac{\text{Control (A)} - \text{Sample (B)}}{\text{Control (A)}} \times 100$$

- Control (A) is a representation of the control's absorbance.
- Sample (B) is a representation of the sample's absorbance.

CHAPTER 4

RESULT AND DISCUSSIONS

The Mozzarella cheese was produced using buffalo milk according to cheese-making protocol, with the addition of *Syzygium cumini* as a functional ingredient. Different ratios (4%, 6% and 8%) of *Syzygium cumini* seeds powder has been utilized for the fortification. To initiate the cheese-making process, indigenous starter culture was employed. Prior to cheese production, the milk samples undergo standardization. After manufacturing fortified mozzarella cheese samples were utilized to make cheese omelet and used as a topping over the cheese macaroni that is presented to a panel for sensory evaluation. A descriptive sensory analysis was conducted to examine the characteristics and attributes of the Pizza cheese. Subsequently, *Syzygium cumini* powder and the fortified Mozzarella cheese samples undergo proximate analysis for determining the nutritional composition. Analysis of antioxidants was also conducted to assess the stability and shelf life of the product at predetermined intervals. Additionally, the fortified Mozzarella cheese samples undergo alpha-glucosidase inhibition to check the anti-diabetic potential. The outcomes of all the examined parameters are elaborated upon in the subsequent sections.

ACADEMIC SOLUTIONS

4.1 Proximate analysis of *Syzygium cumini* seeds

The nutritional profile of foods, which can be determined from the proximate analysis is essential for the creation of novel food products and the investigation of their therapeutic potential. The ability to identify important nutritional components, including proteins, lipids, moisture, and ash, in raw materials is made possible through proximate analysis carried out in labs. In this research, *Syzygium cumini* (seeds) are investigated by proximate analysis, their antioxidant activity is assessed, a product is developed, and anti-diabetic effects are examined through the α -glucosidase inhibition test in lab settings.

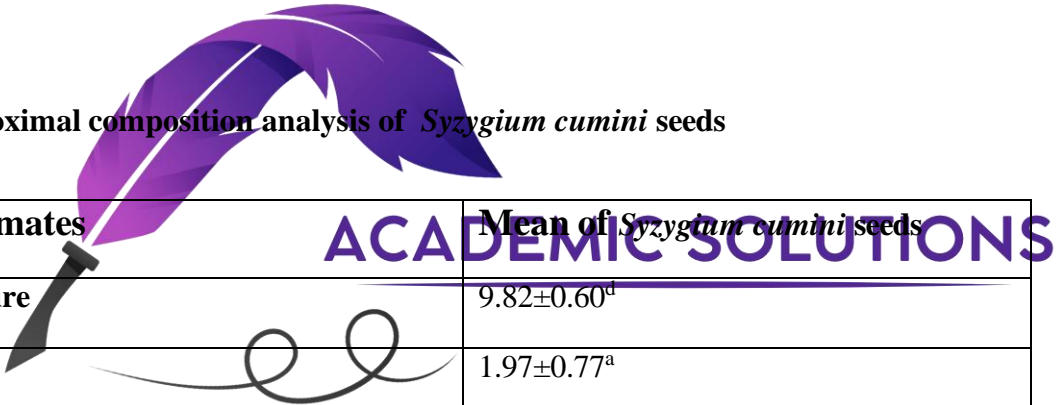
Proximate composition of *Syzygium cumini* (SC) seed powder are presented in Table 4.1.1. Any plant that is needed for research must undergo a close examination in order to better understand the nutrients present in it. Proximal composition of every plant or product differ significantly. *Syzygium cumini* had the lowest moisture content value

(9.82±0.60). Results demonstrate that *Syzygium cumini*(seeds) do not have the ability to accumulate water.

Syzygium cumini had an ash content of (1.97±0.77). The amount of fat in *Syzygium cumini* was (1.51±0.5). *Syzygiumcumini* had a fiber content of (4.18±0.4). The fiber content results were not very significant. When the protein content was examined, it was (2.30±0.55) in *Syzygiumcumini*. SC has lower findings overall for moisture, ash, fat, fiber, and protein. NFE value was determined by subtracting the moisture, fat, ash, protein and fiber sum from the total 100.

The results of the current study's analysis of the *S. cumini* seeds' proximate composition are somewhat consistent with (Kshirsagar, et al, 2019) investigation. Almost all parameters had similar findings with slight differences. These variations could be the result of climatic variations, agricultural techniques, or variety.

4.1 Proximal composition analysis of *Syzygium cumini* seeds



Proximates	Mean of <i>Syzygium cumini</i> seeds
Moisture	9.82±0.60 ^d
Ash	1.97±0.77 ^a
Fat	1.51±0.5 ^c
Fiber	4.18±0.4 ^a
Protein	2.30±0.55 ^d
NFE	80.22±2.77 ^a

4.2. Determination of Antioxidants:

Antioxidants are compounds that hinder the process of oxidation. Many chemical reactions generate harmful free radicals that lead to damage human cells. Oxidation is widely recognized as a significant factor in the development of various disorders within the human body. These reactions are inhibited by a variety of antioxidants, including vitamin C and E. The FRAP, TPC, and DPPH assays are just a few of the techniques used to determine the antioxidant potential.

4.2.1. DPPH Determination

The DPPH assay, which stands for 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay, is utilized to eliminate free radicals by utilizing its ability to donate hydrogen atoms. In this study, *Syzygium cumini* was subjected to analysis using the DPPH assay to assess its antioxidant potential. Statistical analysis of the data indicated that the seeds exhibited a significant capacity for scavenging free radicals. The highest scavenging activity was observed in *Syzygium cumini* when extracted using acetone and ethanol solvents, with values of 81.82 ± 3.43 and 82.13 ± 3.55 . Minimum scavenging activity was evaluated when extracts of the sample were prepared in water solvent. In hexane solvents, 42.84 ± 2.66 was the value of SC, respectively. Overall, *Syzygium cumini* demonstrated scavenging activity against free radicals, exhibiting significant results in all solvents tested, including acetone, ethanol, hexane, and water. It is worth noting that a previous study conducted by (Maheshu et al, 2012) reported even higher scavenging activity results compared to the current research study.

4.2.2. FRAP Determination:

FRAP stands for "Ferric Reducing Antioxidant Power," is a test used to identify antioxidant properties. This method was originally developed to evaluate the antioxidant potential of plasma, but now it can also be used to determine the antioxidant potential of plant extracts. The fundamental concept behind this approach is to measure the ability of a substance, including plant extracts, to reduce ferric ions. In this study, the ferric reducing antioxidant power of extracts from *Syzygiumcumini* was examined. The findings revealed that the choice of solvent for extract preparation had a significant impact on the antioxidant capacity of the plants, and their antioxidant potential varied considerably in the FRAP assay. Due to the existence of numerous phenolic compounds, the antioxidant activity of *Syzygium cumini* seeds varies depending on where they are found. Additionally, it's due to the many mechanisms of action of several tests for antioxidants. The outcome showed that the DPPH and FRAP values in *Syzygiumcumini* seeds were significantly greater than those previously reported by (Ruan, Zhang, and Lin, 2008).

This table presents findings obtained from the FRAP assay, which was employed to measure antioxidant activity. The highest values were observed in SC when acetone was used as the solvent, with values of 433.01 ± 9.23 . When evaluate the antioxidant potential in hexane solvent, a clear difference was observed. SC displayed the lowest value (322.35 ± 14) in the water solvent. Furthermore, when extracts of the sample is used in ethanol and hexane, a decreasing trend was observed in SC, with values of 336.22 ± 13.22 and $365.31 \pm 0.2137.79$, respectively. The acetone extract of SC demonstrated exceptionally noteworthy ferric reducing activity.

Additionally, this test revealed that *Syzygiumcumini*(seeds) possess significant antioxidant potential. In a study conducted by (Maheshu et al, 2012), the antioxidant capacity of SC was assessed using the FRAP assay, and the results obtained were remarkably high. However, in the current study, the results were relatively lower, with a value of $761.57 \pm 0.75 \mu\text{g Fe/g}$.

4.2.3 TPC Determination:

Phenolic compounds originate from plants have the ability to act as antioxidants because of their reducing properties. Total phenolic content (TPC) analysis is a widely employed method for quantifying the phenolic content present in plant samples. This technique measures Polyphenols in various biological matrices, including plasma, organs, and urine. Although the antioxidant activity of *S. cumini* fruit components has been previously observed, these results confirm the variations in potency between investigations. Climate, geographic and soil conditions, irrigation techniques, harvesting window, storage and transportation resources, drying techniques (shade drying, sun drying, oven drying, freeze drying), solvent polarity, extraction techniques, and extraction time are a few factors that must be considered. According to a study by (Lin et al, 2003) the harvesting stage and the type of extraction solvents could alter the number of Polyphenols.

The lowest figure for SC's phenolic content in a water solvent was 27.91 ± 5.1 . The extracts prepared using ethanol solvent have the maximum potential for *Syzygiumcumini* (39.77 ± 2.5). SC in the hexane solvent have the same characteristics as those in the acetone solvent. The SC in the hexane extract was found (31.8 ± 4.99) and in the acetone extract was found (38.53 ± 4.99).

Based on substantial findings in numerous solvents, the current analysis's findings suggested that both samples contained considerable phenolic contents. While ethanol extract had the largest potential for SC, a water solvent had the lowest potential. Ethanol proved to be the most effective solvent, whereas acetone performed well for *Syzygium cumini* extract.

Table 4.4 Antioxidant potential of *Syzygium cumini* seeds powder

Solvents	TPC	FRAP	DPPH
Water	27.91±5.1	322.35±14	20.00±1.26
Hexane	31.8±4.99	365.31±37.79	42.84±2.66
Ethanol	39.77±2.5	336.22±13.23	82.13±3.55
Acetone	38.53±4.99	433.01±9.23	81.82±3.43
Mean	34.52±1.25	364.22±12.99	56.70±2.72



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4.3. Proximate analysis of fortified cheese

Moisture, ash, protein, and fat content in the fortified Mozzarella cheese can be accessed by the proximal composition of cheese. This proximal composition of cheese that has been analyzed in the present research is presented next.

4.3.1 Moisture content

Moisture content in fortified mozzarella cheese is shown in Table. The value of analysis variance for moisture in the same sample is also presented. *Syzygium cumini* seeds powder was used for the supplementation in varied ratios (4%, 6%, and 8%) and then measured the moisture content of the supplemented mozzarella cheese. There are 3 groups (A, B, etc.) in which the means are not significantly different from one another.

Moisture level in the studied sample of supplemented mozzarella cheese lies between 50-55%. It was determined that the moisture content of T3 (50.32 ± 0.97) was lower than those of T2 (51.63 ± 0.60) and T1 (52.72 ± 0.74). The higher mean moisture content was observed in the control sample (54.3 ± 0.72). Minimum reduction in the moisture level of supplemented mozzarella cheese was observed. This little reduction in the moisture content after supplementation is acceptable because mozzarella cheese already contain 55-60% moisture.

Cheese's chemical composition, which includes its moisture content, governs its useful qualities. Therefore, changing the formulation of imitation cheese has an impact on how it functions in real-world settings like lasagne and pizza toppings. For these uses, controlled meltability and predictability of cheese hardness are crucial for slicing, chopping, and shredding the cheese. Moisture has a significant impact on the textural qualities of cheese because it serves as a plasticizer in the network. According to reports, processed cheese with a high moisture content has a weak body and a gritty texture, whereas cheese with a low moisture content is hard, dry, and brittle. Mozzarella cheese's meltability increases and it gets softer and less shredded as moisture levels rise (Hennelly et al, 2005).

4.5 Analysis of Variance Table for moisture content in the control and supplemented Mozzarella Cheese treatments

Source	DF	SS	MS	F	P
treatment	3	20.0377	6.67923	36.68	0.0001
Error	8	1.4567	0.18209		
Total	11	21.4944			



4.3.2 Fat Content

Fat content in fortified mozzarella cheese is shown in Table. Level of fat was highest when the cheese was prepared using the standard method (6.77 ± 0.61), and it was lowest when it was prepared using *Syzygium cumini* seeds powder in ratio 8% (5.53 ± 0.66). The fat content in the 6% supplemented mozzarella cheese is (6.36 ± 0.58) and 4% supplemented mozzarella cheese is (5.96 ± 0.47). The fat content was highest when the seed powder was not supplemented but lower when cheese was fortified using *Syzygiumcumini* seeds powder. This supplementation procedure results in a decrease in fat content because heat causes the phase to change from particulate to soluble. The outcomes are consistent with earlier findings (Rudan, 1999).

Based on scientific data, diets rich in fat increase the chances of coronary heart disease and certain types of cancer. therefore, it's crucial to reduce the amount of fat consumed. The food industry has responded by creating lower-fat items to assist people in eating healthier. 247 new cheese products were developed by the dairy industry in 1994, and many of them were reduced-fat cheeses.

The demand for pizza is increasing sales of mozzarella cheese. Due to its advantageous functionality, mozzarella cheese is frequently used for making pizza. According to its moisture content, mozzarella cheese ranges in fat content from 14 to 22%. There has been interest in creating a lower-fat pizza due to consumers' desire for healthier eating habits and the ongoing demand for the food.

Many crucial tasks are carried out by fat in a diet. Fat improves the flavor, texture, usability, and appearance of the cheese. Pizza uses approximately 75% of all mozzarella cheese, thus appropriate melt (shred fusion) and look (browning and blistering upon heating) are crucial features. When cheese is fat-free, the general standard of cheese decrease.

4.6 Analysis of Variance Table for fat content in the control and supplemented Mozzarella Cheese treatments

Source	DF	SS	MS	F	P
treatment	3	0.85489	0.28496	12.43	0.0022
Error	8	0.18340	0.02293		
Total	11	1.03829			

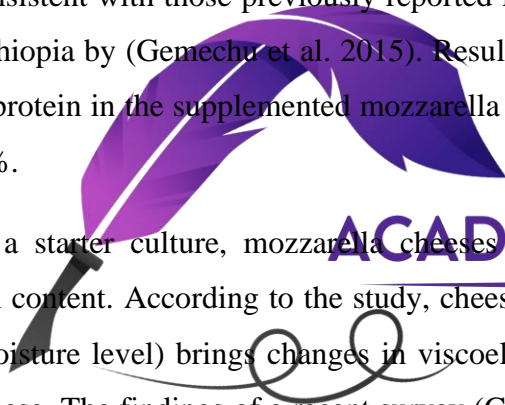


4.3.3 Protein Content

The results of the protein content in fortified mozzarella cheese are shown in Table (4.3.3). Minor variations were found in the protein percentages of fortified cheese samples prepared from the buffalo milk. Cheese containing 4% *Syzygium cumini* seeds powder had a greater protein content (29.70 ± 3.30). Cheese supplemented with 6% *Syzygiumcumini* seeds powder had a protein content of (28.98 ± 3.86) and cheese supplemented with 8% *Syzygium cumini* seeds powder had a protein content of (28.97 ± 3.35).

Due to their great bio-availability, milk proteins are regarded as very nutritive, with casein being the main protein in milk (Hassan, 2005). Whole milk's protein content must meet 2.9% EU requirements (Tamime, 2018). Protein percentages in milk and milk products are significantly important. The protein percentages in the current study are consistent with those previously reported in Pakistan by (Muhammad et al. 2008) and Ethiopia by (Gemechu et al. 2015). Results of the current study indicated that the crude protein in the supplemented mozzarella cheese treatments lies in range between 25-30%.

Using a starter culture, mozzarella cheeses were prepared that varied in Ca and protein content. According to the study, cheese composition (pH, Ca content, protein and moisture level) brings changes in viscoelasticity, and storage-related changes of the cheese. The findings of a recent survey (Guinee et al., 2000a) showed that each of the functional characteristics of commercial Mozzarella lies within a range of values, for example, flowability, 43 to 68%; stretchability, 56 to 134 cm. This Ca content and protein content had a significant impact on each functional feature to reach values typical for commercial Mozzarella. Therefore, it is important to maintain the protein and Ca content to maintain the functional shelf life of Mozzarella cheese.



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4.7 Analysis of Variance Table for protein content in the control and supplemented Mozzarella Cheese treatments

Source	DF	SS	MS	F	P
treatment	3	12.2892	4.09639	19.05	0.0005
Error	8	1.7200	0.21500		
Total	11	14.0092			

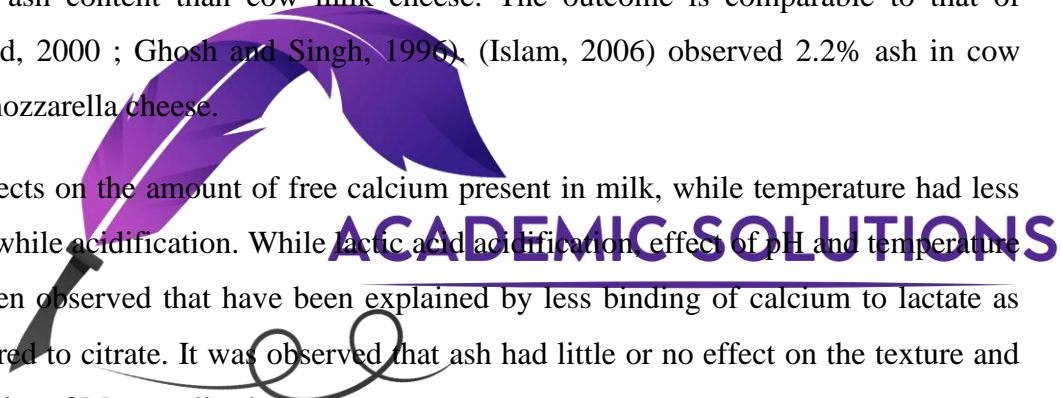


4.3.4 Ash Content

Ash content of the supplemented mozzarella cheese samples was examined in this study. In Table 4.3.4, the ANOVA for the ash content of the supplemented mozzarella cheese is displayed. The ash concentration present in the cheese samples ranged from 1 to 1.5%. The typical ash level in 4%, 6% and 8% fortified cheese was (1.47 ± 0.35) , (1.32 ± 0.27) and (1.26 ± 0.26) respectively. Cheese fortified with 4% *Syzygiumcumini* seed powder had a mean maximum ash percentage while the cheese fortified with 8% *Syzygiumcumini* seed powder had a mean lowest ash content.

The inorganic residues that are left over after the full oxidation and combustion of the organic stuff make up the sample's ash content. It more accurately depicts the food commodity's mineral components. Ash levels was discovered in cheese made from cow and buffalo milk. Studies revealed that buffalo milk cheese had a considerably higher ash content than cow milk cheese. The outcome is comparable to that of (Ahmed, 2000 ; Ghosh and Singh, 1996), (Islam, 2006) observed 2.2% ash in cow milk mozzarella cheese.

pH effects on the amount of free calcium present in milk, while temperature had less effect while acidification. While lactic acid acidification, effect of pH and temperature has been observed that have been explained by less binding of calcium to lactate as compared to citrate. It was observed that ash had little or no effect on the texture and properties of Mozzarella cheese.



4.8 Analysis of Variance Table for ash content in the control and supplemented Mozzarella cheese treatments

Source	DF	SS	MS	F	P
treatment	3	0.27356	0.09119	3.87	0.0560
Error	8	0.18873	0.02359		
Total	11	0.46229			



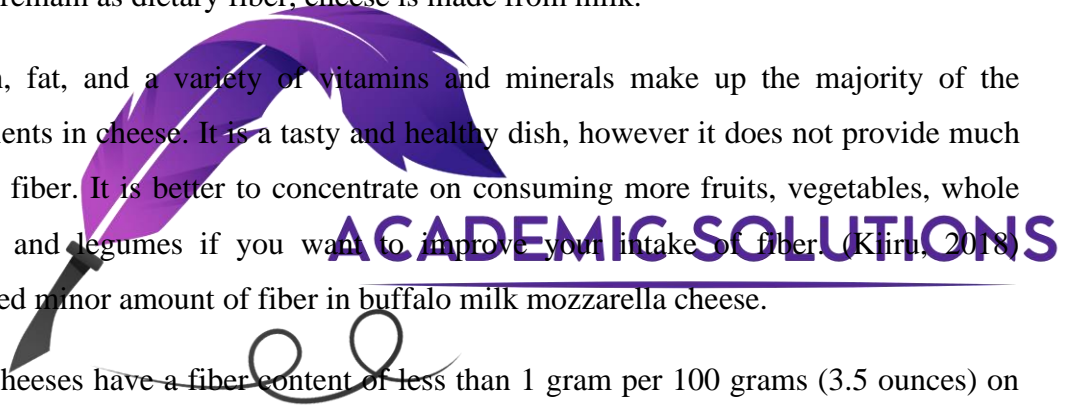
4.3.5 Fiber Content

The results of the fiber content in fortified mozzarella cheese are shown in Table (4.3.5). Minor variations were found in the fiber percentages of fortified cheese samples prepared from the buffalo milk. Cheese containing 8% *Syzygium-cumini* seeds powder had a greater fiber content (0.18 ± 0.1). Cheese supplemented with 6% *Syzygium-cumini* seeds powder had a protein content of (0.16 ± 0.1) and cheese supplemented with 4% *Syzygium cumini* seeds powder had a protein content of (0.14 ± 0.009).

The fiber level of cheese is normally very low. Fruits, vegetables, legumes, whole grains, and other plant-based foods include fiber, which is a form of carbohydrate. While the majority of the milk's carbohydrates, including the naturally existing lactose, are transformed into other compounds during the cheese-making process and do not remain as dietary fiber, cheese is made from milk.

Protein, fat, and a variety of vitamins and minerals make up the majority of the ingredients in cheese. It is a tasty and healthy dish, however it does not provide much dietary fiber. It is better to concentrate on consuming more fruits, vegetables, whole grains, and legumes if you want to improve your intake of fiber. (Kirin, 2018) observed minor amount of fiber in buffalo milk mozzarella cheese.

Most cheeses have a fiber content of less than 1 gram per 100 grams (3.5 ounces) on average. However, when compared to other high-fiber foods like fruits, vegetables, and whole grains, the fiber content of some softer cheeses may be slightly higher than that of harder cheeses.



4.9 Analysis of Variance Table for fiber content in the control and supplemented Mozzarella cheese treatments

Source	DF	SS	MS	F	P
treatment	3	1.28170	0.42723	75.84	0.000
Error	8	0.04507	0.00563		
Total	11	1.32677			



4.3.6 NFE value

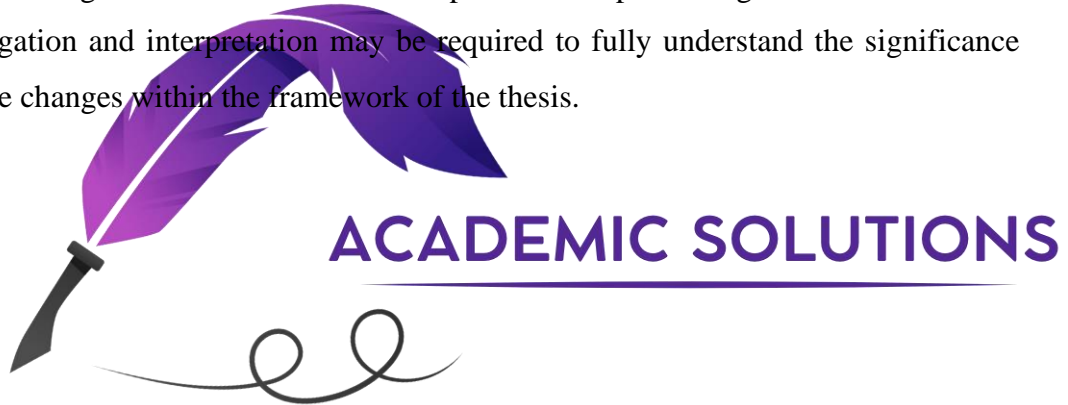
The results of the NFE content in fortified mozzarella cheese are shown in Table (4.3.6). Minor variations were found in the NFE percentages of fortified cheese samples prepared from the buffalo milk. Cheese containing 8% Syzygium-cumini seeds powder had a greater NFE content (13.55 ± 4.8). Cheese supplemented with 6% Syzygium-cumini seeds powder had a NFE content of (11.6 ± 4.8) and cheese supplemented with 4% Syzygium-cumini seeds powder had a NFE content of (10.00 ± 4.7).



4.10 Analysis of Variance Table for NFE content in the control and supplemented Mozzarella cheese treatments

Source	DF	SS	MS	F	P
treatment	3	106.241	35.413	118.75	0.000
Error	8	2.386	0.2982		
Total	11	108.627			

Overall, the proximate analysis's findings indicate that the treatments had different impacts on the composition of the samples. The treatments often result in decreased moisture, ash, fat, and protein contents when compared to the control, which could be due to changes in the nutritional composition or processing effects. Additional investigation and interpretation may be required to fully understand the significance of these changes within the framework of the thesis.



4.11 LSD All-Pairwise Comparisons Test for the control and supplemented Mozzarella cheese treatments

Treatment	Moisture	Ash	Fat	Protein	Fiber	NFE
Control	52.73±0.65 ^c	1.57±0.30 ^b	6.43±0.25 ^c	30.27±0.12 ^c	0.077±0.04 ^c	8.92±0.2 ^a
T1	54.57±0.30 ^b	1.86±0.02 ^a	6.78±0.12 ^b	31.53±0.15 ^b	0.14±0.09 ^c	5.15±0.5 ^b
T2	55.50±0.26 ^a	1.94±0.04 ^a	6.94±0.04 ^{ab}	32.20±0.40 ^{ab}	0.3±0.10 ^b	3.1±0.8 ^c
T3	56.17±0.00 ^a	1.97±0.01 ^a	7.16±0.12 ^a	33.03±0.81 ^a	0.90±0.0 ^a	0.82±0.5 ^d



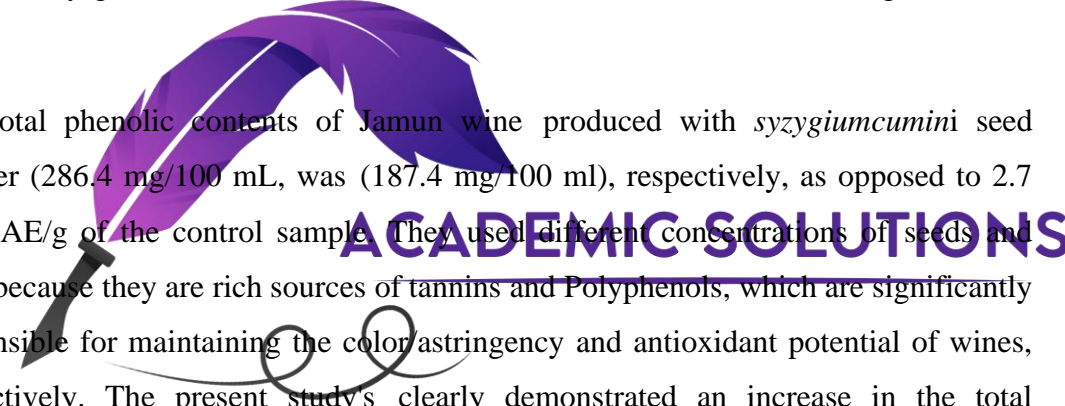
4.5 Anti-oxidant properties of Supplemented Mozzarella cheese (SMC)

The total phenolic content was significantly higher (7.15 ± 0.61) in the mozzarella cheese fortified with 8% *Syzygium-cumini* seeds powder. In comparison to control, ferric reducing antioxidant capacity (34.4 ± 0.96) and DPPH (15.70 ± 2.8) were also higher in the same treatment. Phenolic compounds followed the same trajectory as FRAP and DPPH. By increasing the concentration of *Syzygium cumini* seeds powder compared to the control, phenolic compounds rose in it. These findings are in line with those made public by (O'connel and Fox, 2001), who discovered that cheese was deficient in vitamin C and several significant antioxidants in addition to phenols. The cheese's fortification with powder has made it an antioxidant-rich food.

According to past reports, *Syzygium cumini* seeds have difference in total phenolics, flavonoids, and antioxidant capacity, although various research have also noted this difference. The phenolics recovery and radical scavenging potential may be significantly impacted by variations in climate, soil quality, irrigation, agricultural practices, harvesting times, storage types, and drying techniques such as sun drying and oven drying, as well as the amount of solvents, techniques of extraction, and times of extraction.

According to Lin et al. (2003), the polarity of the solvent used for extraction and the stage of harvesting both have a significant impact on the amount of Polyphenols recovered from seeds. He claimed that as compared to seeds powder extracted with boiling water, seed powder extracted with 75% ethanol demonstrated much higher biological activity. According to the results of another study, the pasteurization process, extraction method, and restoring stages all significantly affect the bioactive compounds of *Syzygium-cumini* seeds and their potential to scavenge free radicals (Qamar et al., 2021). The phenolic recovery and antioxidant potential of Mozzarella cheese was also influenced by biological factors, including type of cheese, ripening and developmental characteristics, environmental factors, and technical aspects, such as the polarity of the extraction solvent.

Cheese may contain some antioxidants, but overall, compared to other food sources like fruits, vegetables, nuts, and seeds, its contribution to the diet's antioxidant intake is typically limited. However, it can be improved through the process of fortification or by adding any substance that is rich in antioxidants. The number of phenolic compounds in supplemented mozzarella cheese has increased by adding *Syzygiumcumini* seeds in different concentrations. (3.4±0.3) mg GAE/100g of total phenolics were found in mozzarella cheese, displaying a linear pattern with a gradual increase in concentration (4–8%) of the seeds powder. In contrast to the *syzygiumcumini* seeds powder that had been supplemented, it was discovered that the standard or control containing 100% mozzarella cheese had 12% DPPH inhibitory activity. The total phenolic content of *syzygiumcumini* seed was previously reported to be 76 mg GAE/100 mg, and the phenolic content of the mozzarella cheese fortified with dehydrated oyster mushroom flour was 2.09 ± 0.68 mg GAE/g DM, which is significantly greater than that of the control mozzarella cheese at 0.01 mg GAE/100 mg.



The total phenolic contents of Jamun wine produced with *syzygiumcumini* seed powder (286.4 mg/100 mL, was (187.4 mg/100 ml), respectively, as opposed to 2.7 mg GAE/g of the control sample. They used different concentrations of seeds and peel, because they are rich sources of tannins and Polyphenols, which are significantly responsible for maintaining the color/astringency and antioxidant potential of wines, respectively. The present study's clearly demonstrated an increase in the total Polyphenols (187.4 mg/100 ml) of jamun wine while maintaining a low concentration of tannins (1.91 mg/100 ml). The goal was to increase Polyphenols while limiting tannins because too much of the latter results in bitter wine. A higher phenolic content in wine may have various advantages since it has a variety of biological effects, including anti-viral, anti-carcinogenic, anti-bacterial, and cardio protective properties, primarily because of its antioxidant and anti-radical activity (Singh and kocher, 2020).

The antioxidant potential of jamun seed powder supplemented noodles was measured as 87% through DPPH, and 80% (SOD-like-activity). In contrast, control sample demonstrated 81% inhibition against free radicals and 15% SOD-like activity. There is a clear correlation between antioxidant potential and the amount of jamun seeds used.

In the current investigation, free radical scavenging activity of *syzygiumcumini* supplemented mozzarella cheese is significantly higher than that reported by (Thongram et al, 2016), who found that cookies containing 10% seed powder with 30% radical scavenging activity. With increasing powder supplementation rates, the free radical scavenging value increased and ranged from 0.39 to 25:56 mol 100g. When compared to Multigrain cookies supplemented with *syzygiumcumini* seeds powder (Desai et al. 2018), the antioxidant profile results for *syzygium cumin* supplemented cookies are suggestive due to higher total phenolic content and antioxidant capacity.



Table 4.12 Total Phenolic Contents (mgGAE) of supplemented Mozzarella cheese

treatment	Mean of supplemented Mozzarella cheese			
	Water	Hexane	Acetone	Ethanol
T0	0.38±0.2 ^a	3.4±0.3 ^a	0.29±0.1 ^a	2.65±0.4 ^a
T1	1.28±0.8 ^b	4.68±0.07 ^b	1.51±0.5 ^a	4.20±0.29 ^a
T2	2.98±0.3 ^c	4.33±0.94 ^{bc}	3.67±0.3 ^b	6.90±0.70 ^b
T3	4.30±0.9 ^c	6.31±0.35 ^c	5.01±1.4 ^b	7.15±0.61 ^c

T0: Mozzarella cheese having 0% *Syzygium cumini*

T1: Mozzarella cheese having 4% *Syzygium cumini*

T2: Mozzarella cheese having 6% *Syzygium cumini*

T3: Mozzarella cheese having 8% *Syzygium cumini*

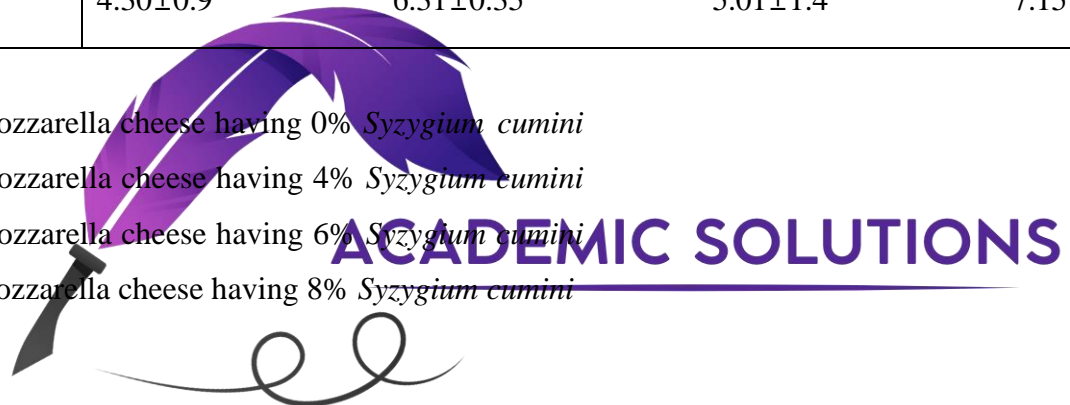


Table 4.13 DPPH Scavenging Activity of supplemented Mozzarella cheese

treatment	Mean of supplemented Mozzarella cheese			
	Water	Hexane	Acetone	Ethanol
T0	9.56±0.7 ^a	10.63±0.8 ^a	7.27±0.5 ^a	12.3±0.6 ^a
T1	9.85±1.3 ^a	12.22±3.3 ^a	12.84±2.8 ^a	13.1±5.7 ^a
T2	10.30±1.1 ^a	12.9±3.1 ^a	14.23±2.9 ^a	13.6±4.2 ^a
T3	8.79±3.7 ^a	11.47±2.1 ^a	15.70±2.8 ^b	15.07±4.2 ^a

T0: Mozzarella cheese having 0% *Syzygium cumini*

T1: Mozzarella cheese having 4% *Syzygium cumini*

T2: Mozzarella cheese having 6% *Syzygium cumini*

T3: Mozzarella cheese having 8% *Syzygium cumini*

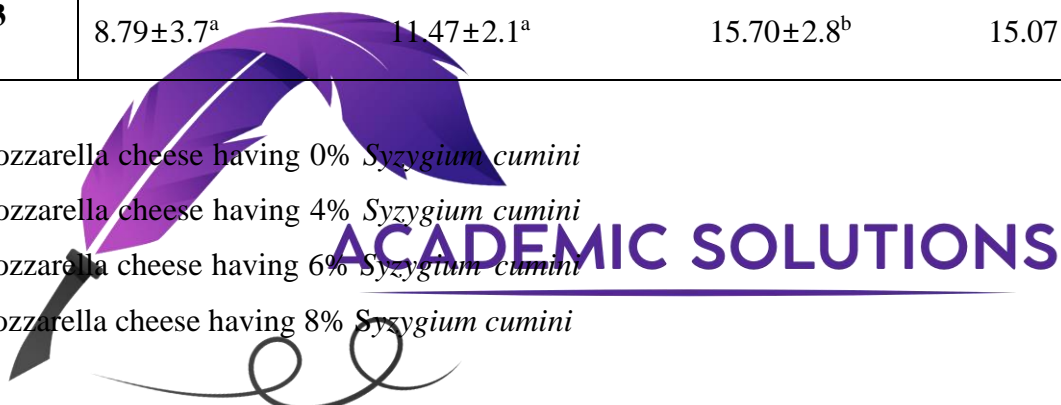


Table 4.14 Ferric Reducing Antioxidant Property (ugFe/g) of supplemented Mozzarella cheese

treatment	Mean of supplemented Mozzarella cheese			
	Water	Hexane	Acetone	Ethanol
T0	0.001±0.0 ^a	0.002±0.0 ^a	0.03±0.04 ^a	0.01±0.01 ^a
T1	12.93±0.6 ^b	14.37±1.5 ^b	17.27±0.56 ^b	13.73±0.85 ^b
T2	19.38±0.8 ^c	21.93±2.1 ^c	26.07±0.6 ^c	20.2±0.62 ^c
T3	25.77±1.1 ^d	29.27±3.0 ^d	34.4±0.96 ^d	26.93±1.0 ^d

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T0: Mozzarella cheese having 0% *Syzygium cumini*

T1: Mozzarella cheese having 4% *Syzygium cumini*

T2: Mozzarella cheese having 6% *Syzygium cumini*

T3: Mozzarella cheese having 8% *Syzygium cumini*

4.6 Alpha-Glucosidase Inhibition for the Evaluation of Anti-Diabetics

Table presents the results of the statistical analysis for the analysis of variance for alpha-glucosidase inhibition. The percentage inhibition of a-glucosidase is used to indicate the anti-diabetic activity of *syzygium cumini* supplemented Mozzarella cheese. The potential of cheese water extracts to inhibit a-glucosidase was assessed using three different dosages of *syzygiumcumini* seeds powder (4%, 6% and 8%). Inhibition of a-glucosidase was seen in all three dosages. The glucosidase inhibitory activity of the Mozzarella cheese supplemented with 8% was by far the highest (WC: 84%). T3 demonstrated the highest level of a-glucosidase inhibition (84%), whereas T2 had a lower level of inhibition (71%). There exist a strong association between the powder used and their ability to block a-glucosidase. As the powder concentration rose, there was a rising trend in the percentage inhibition of the a-glucosidase. Due to this, the cheese that have been fortified with highest amount showed the strongest a-glucosidase inhibitory action.

The key justification for recognizing the enormous potential *syzygium cumini*' phytochemical profile revealed the possible effects that could be brought on by plant metabolites like alkaloids, terpenoids, phenols, flavonoids, tannin, and saponin. A wide range of compounds such as flavonoids present in the seeds stops the breakdown of glucose in addition to avoiding the accumulation of too much fat in our bodies.

The cheese with added cranberries showed the highest a-amylase inhibitory action according to IC50 values. In contrast to feta and Cabbot cheese, all Roquefort cheese extracts showed high a-amylase inhibitory activity based on IC50 values, however the inhibitory activity for o-glucosidase was lower or the same. This could be explained

by the possibility that *Penicillium* species create secondary metabolites with stronger α -amylase inhibitory action but lower α -glucosidase inhibitory activity (Apostolidis et al, 2007).

It was determined that *Eucalyptus camaldulensis* oil had a significant effect on diabetes based on the trend of percentage inhibition of α glucosidase and that the effect of the oil was synergistically enhanced by raising the concentration of aloe vera gel. Anti-diabetic activity decreased as UV light was used to treat the samples. According to the oxidation process that began in the samples, effects on the anti-diabetic action. It was determined that encapsulation and a high aloe vera gel concentration protected the greatest amount of antidiabetic action. Samples were subjected to UV light to examine how the essential oil's antidiabetic properties changed. By looking for a decrease in the proportion of α glucosidase inhibition, the impact of UV radiation was examined. The simple oil treatment (11.20%) demonstrated the biggest percentage reduction when compared to the Treatment: (10%), treatment that was encapsulated. It was determined that oil had a significant effect on diabetes mellitus based on trend of percentage inhibition of α glucosidase and that the effect of the oil was synergistically enhanced by raising the concentration of aloe vera gel. Anti-diabetic activity decreased as UV light was used to treat the samples. According to the oxidation process that began in the samples, effects on the anti-diabetic action. It was determined that encapsulation and a high aloe vera gel concentration protected the greatest amount of antidiabetic action. Linalool, borneol, terpin-4-ol, 2,3-pinanediol, and piperitone are among the several oxygenated mono-terpenoids that may be found in the oil (Bacanl et al., 2017). Linalool has been shown in the past to mimic the effects of insulin and reduce blood sugar levels. Therefore, the oil's heightened activity may be attributed to both the high concentration of linalool and the synergistic effects of the other constituents. The studies show that the active components of aloe vera gel and oil both competitively inhibit α glucosidase. This implies that the bioactive compounds in both components, which are present, may help lower blood glucose levels (Sharma et al, 2022).

A global health concern is type 2 diabetes, according to recent statistics data. Trials showed the overall biochemical basis for diabetes and clinical trials are lying stress on these certain biochemical factors. The findings of this study also point a correlation

between α -glucosidase inhibitory actions and fruit and herb enrichment (in the case of any type of cheese), but not with phenol concentration, suggesting that phytochemical quality and synergy may be more important. This study offers insight that properly optimized phenolic phytochemical-enriched diets could play a role in developing less expensive complementary strategies for type 2 diabetes and hypertension management concurrently combined with other dietary and pharmacological approaches (Shetty, 1997; Shetty and Wahlqvist, 2004). Moreover, Phenolics have antioxidant and antimicrobial potential.



Table 4.15 Analysis of Variance Table for alpha- glucosidase inhibition% of supplemented Mozzarella cheese

Source	DF	SS	MS	F	P
Treatment	3	83.476	27.8254	4.05	0.0504
Error	8	54.914	6.8642		
Total	11	138.390			

Table 4.16 Mean square values for α-glucosidase inhibition% of supplemented Mozzarella cheese

Treatments	Reduction%
T0	45.67±0.58 ^b
T1	51.61±4.37 ^a
T2	44.85±2.11 ^b
T3	46.47±1.89 ^b

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T0: Mozzarella cheese supplemented with 0% *Syzygiumcumini*

T1: Mozzarella cheese supplemented with 4% *Syzygiumcumini*

T2: Mozzarella cheese supplemented with 6% *Syzygiumcumini*

T3: Mozzarella cheese supplemented with 8% *Syzygiumcumini*

CHAPTER 5

SUMMARY

Milk, a substance derived from the mammary gland of female mammals, was initially utilized approximately 11,000 years ago during the Neolithic period. Milk serves as the principal constituent in numerous processed food items, encompassing cheese, ice cream, yogurt, and an extensive array of desserts. Milk, the predominant beverage consumed by infants and toddlers globally. The consumption of milk and milk derivatives is prevalent due to their nutritional benefits and convenience; however, it is important to acknowledge that they also pose potential health hazards. Numerous individuals experience unfavourable responses to milk and milk-derived products as a result of various health hazards, including adulteration, antibiotic resistance, mastitis, lactose intolerance, and allergies to specific proteins.

Syzygiumcumini has been classified as a leguminous plant and are known to possess a significant proportion of protein (1.95-8.6%), carbohydrates (32%-41%), as well as various micro- and macro-nutrients. The composition of jamun seeds has been found to be highly advantageous for the supplementation of cheese. The low cost and simplicity of jamun seeds as a cheese-supplementation ingredient are primary factors contributing to the numerous benefits, such as low rates of CVD, cancer, or diabetes mellitus. While consumers are demanding nutrient-dense, wholesome foods with organoleptic properties and traditional values, *syzygium cumini seeds* are least utilized as a food ingredient in Pakistan despite good production statistics and a suitable cultivation habitat. The study in question was therefore created to investigate the potential of jamun seeds to Mozzarella cheese on account of its palatability, nutritional properties, safety, and sensory acceptability by successive supplementation in baked goods. These facts compel the present study to explore and utilize the locally accessible inexpensive jamun seeds for the development of novel food products.

In this study, buffalo milk was chosen as the subject of investigation due to its cost-effectiveness and nutritional equivalence, despite the availability of milk substitutes derived from various plants and animals. Mozzarella cheese was prepared from it and *syzygiumcumini* seeds were used as a fortifying ingredient to enhance the nutritional properties of the novel product. This study was carried out at the Bahauddin Zakariya

University Multan, Institute of Human Nutrition and Dietetics. The proximal contents, acidity, pH, anti-oxidant ability, sensory assessment of Mozzarella cheese and its anti-diabetic properties were evaluated in samples of raw and fortified mozzarella cheese.

The proximate analysis of *syzygiumcumini* (jamun seeds) yielded the following results: The moisture content lies between 9% and 10%. The ash content exhibits the value of 1-2% in samples. The protein content was found to be 2-3% and fat content found to be 2% only. All of the immediate outcomes yielded statistically non-significant results when evaluating the method. These seeds showed the highest anti-oxidant properties with ethanol (83%) that proved that they are rich in anti-oxidants. However, due to presence of low fat content, seeds are suitable for the fortification in various valuable baked products.

The moisture content of mozzarella cheese produced from buffalo milk exhibited to be higher as observed in the analysis, with a value of 55%. Conversely, the cheese after supplementation displayed the moisture content in T1 (52.72%), in T2 (51.63%) and T3 (50.32%). The controlled mozzarella cheese exhibit the highest protein content at 32.56%, while the supplemented types demonstrates the lowest protein content in T1 (29.70%), in T2 (28.98%) and T3 (28.97%). The fat content of the controlled variety exhibited its value of 6.77% and supplemented types demonstrates the fat content in T1 (5.96%), in T2 (6.36%) and T3 (5.53%). Similarly, the ash content of the controlled variety is 1.57%. Whereas its lowest value of 1.26% was recorded after the supplementation process. The findings demonstrated statistical non-significance ($P \geq 0.05$) solely in relation to the process, while no such association was observed between the method and variety in terms of the ash.

In comparison to mozzarella cheese, the SC seeds employed in the formulation of the supplemented mozzarella cheese that had significantly greater levels of DPPH content (15.70 mg GAE/100g) in acetone, phenolic content (6.31%) in hexane, and ferric reduction antioxidant capacity (26.93 mmol/100g) in ethanol. Total phenolic content of supplemented cheese varied in different solvents. The highest amounts of SC supplementation (8%) in mozzarella cheese demonstrated the highest scavenging activity for free radicals. In comparison, only 7% of the DPPH radicals were scavenged by the mozzarella cheese that had 0% SC added to them. Free radical scavenging values rose with increased supplementation of SC in ferric reducing

antioxidant assay. The SC supplemented mozzarella cheese may help in disease prevention due to their high phenolic content and antioxidant value.

Alpha-glucosidase inhibition test was used to access the antidiabetic properties of supplemented mozzarella cheese. The highest inhibition rate was shown by the sample having highest percentage of *syzygiumcumini* seeds. It has proved that inhibition percentage increased with the increase in the percentage of *syzygiumcumini* seeds powder. Hence, this technology has proved beneficial from the nutritional aspects and plays a key role in controlling the diabetes mellitus. This study presents an alternative approach to cheese manufacturing, aiming to enhance *syzygium cumini* yields and enable Pakistan to diversify its product range, particularly in the domain of cheese. These outcomes are anticipated to yield favourable impacts on consumer well-being and the overall national economy.

