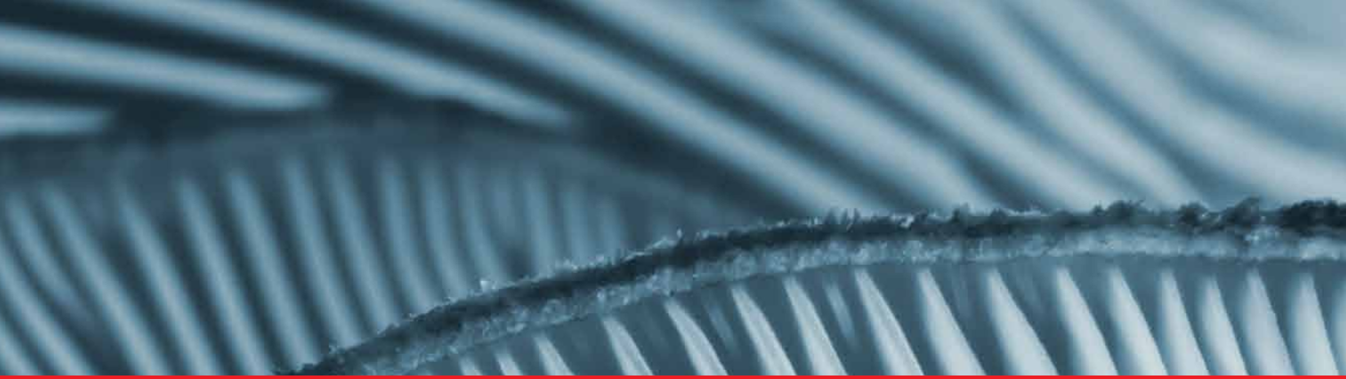

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Food Science and Nutrition, Volume 4

# Recent Trends on Cheese as Functional Food with Great Nutritive and Health Benefits

*Edited by Adham M. Abdou*

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# Food Science and Nutrition

Volume 4

## Aims and Scope of the Series

The significance of food is undeniable, especially in light of the impending challenge facing humanity: ensuring there will be enough food to meet the basic needs of a population expected to reach approximately 10 billion by 2050. These food-related challenges align with some of the United Nations' sustainable development goals, with a target to achieve them by 2030. One thing is certain: food should be not only nourishing and safe but also tailored to the diverse needs of individuals throughout their lifetimes, all while meeting consumers' sensory expectations. Understanding the diverse chemical composition of food, often referred to as biodiversity, and how these components can contribute to human health by considering factors like bioaccessibility, bioavailability, and bioactivity at the organ level, is crucial for grasping and promoting a healthy diet. Thanks to the continuous evolution of analytical methods and interdisciplinary research, significant strides have been made in the field of food science and nutrition.

# Meet the Series Editor

Maria Rosário Bronze has been working in Analytical Chemistry since 1986. Her Ph.D. in 1999 contributed to the study of food products using capillary electrophoresis. The main goal of her research since 1999 has been focused on Analytical Chemistry applied mainly to the analysis of foods and by-products of food industry. She conducted research in collaboration with national and international research groups, at iBET and ITQB Technology Division. From 2017 until 2021 she was head of Food & Health Division at iBET and head of the Food Functionality and Bioactives Laboratory. MR Bronze has been an Associate Professor at the Pharmacy Faculty of Lisbon University and head of the Structural Analysis Laboratory since 2012. As a researcher, MR Bronze is a Senior Scientific Advisor at Food & Health Division at iBET and Head of Food Functionality and Bioactives Laboratory at the same Institute, Collaborator at iMED and Researcher at ITQB NOVA. Her current research is focused on quality and beneficial health effects of food components. Gas and liquid chromatography associated with mass spectrometry are used by MR Bronze in the characterization of samples. Sensory evaluation is also an important area of her research. The main food products studied by her are olive tree products (olive, olive oil, leaves), cereals such as maize, legumes (faba bean, pea, chickpea, lentils) fruits (apple, grapes, opuntia ficus), fruit juices and wine, among others. More recently her interests have also involved biodiversity, bioaccessibility, and bioavailability studies on food products and their components, mainly phytochemicals as phenolic compounds, using different analytical tools such as mass spectrometry. As a senior scientific advisor at Food & Health Division at iBET she is involved in different areas: (i) isolation, characterization and formulation of bioactive and functional compounds or extracts from natural sources and wastes from food and other related industries; (ii) pre-clinical assays to provide support to understand health claims related with the beneficial effects of food nutrients/bioactive components; (iii) establishment of analytical methodologies including mass spectrometry state-of-the-art to fully characterize different matrices, from food products, natural extracts or biological fluids (Food Functionality and Bioactives Laboratory).

# Meet the Volume Editor

Dr. Adham M. Abdou received a bachelor's degree in veterinary sciences from Cairo University, Egypt, in 1986. He obtained a master's degree and Ph.D. in Food Hygiene (Dairy Science) from Benha University, Egypt, in 1990 and 1997, respectively. Dr. Abdou is a faculty member at the same university. He carried out his Ph.D. research work at Miyazaki University, Japan, during 1994–1996. He worked at Pharma Foods Institute (PFI), Kyoto, Japan from 2002 to 2006. He is currently a scientific consultant at PFI. Since 2007, he has been a full professor at Benha University. In 2008, he was appointed as a scientific reviewer for the Ministry of Higher Education and became a board member of the committee for promoting professors in Egypt (2012 to 2015). Currently, Dr. Abdou is an Emeritus Professor, Benha University, Egypt.

# Contents

# Preface

Cheese is one of the oldest fermented and preserved foods in the world. More than 1500 cheese varieties have been classified to date.

Cheese has great nutritional value, which may be attributed to the elaboration of several bioactive peptides during its ripening. Moreover, cheese contains beneficial fatty acids such as conjugated linoleic. Therefore, cheese is considered a functional food. Recent trends to develop functional cheese varieties are ongoing. Several studies have reported the potential for using the cheese matrix as an excellent delivery vehicle for bioactive peptides, vitamins, minerals, and other innovative functional ingredients. Cheese varieties can respond quickly to consumer's evolving needs for functional foods, creating some exciting opportunities for cheese manufacturers. Some novelty versions of functional cheese have been recently launched, including snacks to provide satiety and support fitness and weight; snacks containing billions of live and active probiotics; cheese treats fortified by vitamins A, B, and D, as well as with prebiotics and postbiotics. Other cheese snacks are fortified with micronized iron, zinc, selenium, PUFA, and polyphenols.

This book provides insight into cheese ripening and its impact on the health benefits of cheese. It also discusses cheese's bioactive peptides and fatty acids, the hidden functional benefits of cheese, and recent trends in camel milk cheese and Indian cheese. This volume is a useful resource for food scientists, food chemists, researchers in human nutrition, functional food and/or cheese manufacturers, and a cheese lovers worldwide.

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Section 1

# Introduction

Chapter 1

# Introductory Chapter: Cheese as a Natural Functional Food

*Adham M. Abdou*

## 1. Introduction

Cheese is one of the oldest fermented and preserved foods. It was found in early civilizations. The world's oldest cheese was recently found in an Egyptian tomb, in 2018 researchers published proteomic analyses of the solid white mass as the ancient cheese.

Today, more than 1500 varieties of cheese are classified by milk source, coagulation method, moisture content, texture, ripening or aging, and probiotic content. Cheese has great nutritional value with a good source of proteins, fats, vitamins, and minerals. The health benefits of cheese can be attributed to the formation of several bioactive peptides during its ripening or aging. In addition, cheeses contain healthy fatty acids, such as conjugated linoleic acid and phytanic acid. Therefore, cheese can be considered an optimally functional food. According to the EU, functional foods are foods that contain biologically active components that can improve human health or reduce the risk of disease. Recent trends to develop functional and healthier cheese varieties continue. In addition, cheese would be an ideal choice of functional foods. Several studies have reported the possibility of using the cheese matrix as a carrier for bioactive peptides, vitamins, minerals, and other innovative functional ingredients. In addition, the cheese matrix can act as a potential environment for probiotics because its matrix has a high buffering capacity and a dense protein network that protects probiotics from the harsh stomach environment. The cheese category can quickly respond to the changing needs of consumers for healthy food. This would create exciting opportunities for cheese producers. Recently, some innovations have been introduced to the market regarding cheeses as health food snacks, treats, and other forms. Cheese snacks that support satiety, fitness, and weight; snacks containing billions of live and active LGG probiotics; cheese treats enriched with vitamins A, B, and D; cheese forms supplemented with prebiotic oligosaccharides; and other cheese snacks with added liposomal micronized iron and zinc, selenium, and polyphenol (green tea catechins).

## 2. Cheese as a functional food with great health benefits

Cheese is a crucial food for bone health, dental and oral health, and weight management. It contains essential nutrients such as calcium, protein, magnesium, zinc, and vitamins A, D, and K, which are essential for maintaining healthy bones. Cheese proteins and their derived peptides also positively enhance bone health by regulating cellular markers and signaling of osteoblasts and osteoclasts. Cheese-eating can protect against cavities by increasing saliva production during chewing


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and preventing erosion. Cheese has the highest anticariogenic properties among dairy products studied. Cheese consumption can also help maintain body weight due to its fat, protein, and various vitamins and minerals. Consuming full-fat cheese was linked with a lower risk of obesity compared to low-fat cheese. Balancing cheese intake with low-energy-dense foods, such as fruits and vegetables, is wise. Cheese can also promote satiety as it enhances the feeling of inhibition over further eating and between meals. High protein content in cheese enhances satiety regardless of fat content, providing the potential for decreased energy intake when included as part of a diet.

Low-sodium cheeses, such as Swiss, mozzarella, ricotta, feta, cottage, parmesan, goat cheese, and low-fat cream cheese, can help lower blood pressure. Cheese proteins are rich in ACE inhibitory peptides that can significantly lower blood pressure. Some research has found a link between a diet containing cheese and lower blood pressure, with studies showing that adding certain cheeses to a diet may lead to a decrease in blood pressure.

Cheese and other dairy products are rich sources of antioxidants, which are essential for brain health and neurodegeneration. Cheese's antioxidant properties are attributed to the degradation of casein during ripening, and probiotic cultures can increase its antioxidant activity. Consuming cheese may protect against sodium-rich foods as it contains proteins and peptides that protect blood vessels from short-term adverse effects.

Cheese found to promote gut health by containing probiotic bacteria, which are necessary for maintaining cholesterol levels. Studies have shown that fermented dairy products can change the intestinal microbiota in favor of the host, promoting the growth of *Lactobacillus* and *Bifidobacterium* bacteria. Additionally, cheese may contain natural bioactive peptides that regulate the gut microbiota.

Cheese can help to prevent common cancers, such as colon and bladder cancer. The calcium content of cheese can help prevent these cancers, and cheese may contain specific anticancer peptides produced during cheese processing. Milk proteins are promising candidates for developing anticancer drugs, and peptides derived from dairy products can be detected in fermented milk products, such as cheese.

Immunity support is another benefit of cheese as it contains conjugated linoleic acid (CLA), which may help reduce inflammation and protect against coronary heart disease and obesity. Full-fat dairy products can be healthy when eaten in moderation, and cheese enriched with probiotic microorganisms can strengthen the immune system and prevent immune aging.

Muscle health is also improved by cheese consumption as it increases muscle protein synthesis. Ingestion of cheese protein leads to increased plasma amino acid concentration and subsequently increases muscle protein synthesis.

## 3. Cheese as a vehicle for functional ingredients (cheese fortification)

Cheese fortification increases the number of essential micronutrients in food while improving its nutritional value and providing health benefits with little to no risk. Fortification is the addition of a nutrient, known as a fortifier or additive, to a cheese variety. This nutrient is either absent or present in low concentrations and acts as a carrier within the meal. Fortification aims to eliminate nutrient intake deficiencies and the resulting deficiencies. It aims to achieve a balanced overall nutrient profile, compensate for nutrient losses during processing, and meet the needs of consumers looking to supplement their diet. As a result, cheese fortification is used as a public health practice to increase the consumption of essential nutrients.

### 3.1 Minerals fortification

#### *3.1.1 Iron*

A crucial component of human nutrition is iron (Fe). Iron insufficiency is a prevalent and pervasive issue in both industrialized and developing nations. The addition of iron derivatives to food, such as cheese, can be considered a potential strategy for the prevention of iron insufficiency. The nutritional content of dairy products can be enhanced through the fortification of iron (Fe). However, there is a potential for a negative impact on the appeal of cheese. The organoleptic alterations and bioavailability of iron are influenced by various factors, including the physical qualities of the added iron (such as valence, solubility, and degree of chelation), as well as the physical features of iron after processing or storage. The incorporation of iron in Fe-fortified cheese is achieved through the formation of iron-polyphosphate/whey-protein complexes, ferric casein complex, and the addition of ferric chloride (FeCl3). The process of fortifying cheeses with iron enhances their role as primary sources of dietary iron, hence decreasing the prevalence of iron insufficiency. If all cheeses were fortified, there would be a 14% increase in the average intake of Fe. Research conducted on Cheddar cheese indicates that it is feasible to fortify the cheese with iron without adversely affecting its quality; cheddar cheese remains unaffected by any iron supply, even after a 12-month aging period. Recently, a micronized, dispersible ferric pyrophosphate (Sunactive Fe) has been used in Japan to fortify milk and milk products. Milk products have previously been shown to be difficult to fortify with readily absorbable iron due to organoleptic problems. Published studies showed that iron absorption from Sunactive Fe is similar to that of ferrous sulfate from a fortified infant cereal, as well as from fortified dairy products. The high relative bioavailability is presumable due to the very small particle size. Micronized dispersible ferric pyrophosphate can be expected to provoke fewer unacceptable sensory changes than water-soluble iron compounds in different food vehicles; therefore, some cheese varieties fortified with Sunactive Fe have been launched in the Japanese market.

#### *3.1.2 Zinc*

Zinc is a vital micronutrient that is naturally occurring in various food sources. The human body cannot store zinc and hence necessitates regular consumption of this mineral to sustain overall well-being and preserve optimal health. Foods that are consumed in significant quantities, such as cheese, are regarded as preferred vehicles for fortifying food products with low zinc concentrations. Hence, the fortification of cheese with zinc presents a commendable opportunity to enhance the nutritional value of the diet for individuals who are susceptible to certain health risks. Zinc supplementation has been found to offer a modest level of antioxidant protection throughout the maturing process of Cheddar and Edam cheeses. Additionally, it has been shown that the inclusion of zinc-fortified Edam cheese has the potential to enhance the maturation process and enhance the sensory characteristics of the product. In Japan, there has been a recent utilization of a micronized and dispersible form of Zinc oxide known as Sunactive Zn to fortify various food products, including select varieties of cheeses. SunActive Zn fortified cheese offers enhanced bioavailability, prevention of precipitation, increased stability, and elimination of any undesirable taste associated with zinc.

#### *3.1.3 Selenium*

Milk proteins can effectively retain selenium, resulting in its subsequent transfer during the cheese-making process. Selenium-containing amino acids, such as Se-methionine, can be readily integrated into milk proteins, so rendering cheese a favorable dietary source of selenium. Caciocavallo cheese, which is produced using milk that has been enriched with Se. This particular cheese variant exhibits an increased concentration of linoleic acid and conjugated linoleic acid (CLA). Also, it has been reported that fortification of Turkish white cheese with Se through fortification of brine solution during the ripening process, yielded the highest selenium recovery, this resulted in a recovery rate of 70.91%.

### 3.2 Vitamins fortification

Vitamin D is frequently utilized as a fortifying agent in cheese, whereas vitamins A, C, and E are commonly found in other sectors of the food industry. The compounds C and E have been found to possess properties that stabilize fat and may potentially be utilized in the production of cheese. Ricotta cheese, a well-known and widely consumed Italian fresh whey cheese, is traditionally made from cow's milk. The implementation of food fortification has proven to be an effective approach in mitigating the widespread occurrence of vitamin D insufficiency on a global scale. A published study provides evidence that ricotta cheese serves as a suitable substitute dairy medium for the fortification of vitamin D3. The incorporation of emulsified flaxseed oil enables the fortification of vitamin D3 in cheese. The addition of vitamin D3 and PUFA to cheese resulted in favorable effects on the composition, yield, and chemical stability of the final product. Obtaining vitamin D from dietary sources poses challenges due to its limited natural occurrence in food. The cheese matrix is an effective medium for delivering vitamin D. Hence, the utilization of microencapsulation is essential to maintain the functionality of vitamin D. This technique has significant advantages such as enhanced stability against mechanical and photochemical stress, higher oral bioavailability, and enhanced organoleptic qualities. The thermal stability of the fat-soluble vitamins A, D, and E is noteworthy as they do not undergo degradation with the storage time. The addition of vitamins A and E has been found to offer enhanced protection against lipid peroxidation throughout the three-month aging process of Cheddar cheese, and the incorporation of lecithin has been found to result in a 15% increase in the retention of vitamin A and a 26% increase in the retention of vitamin E. Cottage cheese, which has been enriched with vitamins C and A, has a retention rate of 70% and 75%, respectively, over 2 weeks of refrigeration at a temperature of 3°C. Notably, this retention occurs without any discernible alterations to the cheese's flavor, aroma, or appearance. To develop a fortified cheese with vitamin B12, the use of encapsulation vitamin B12 within double emulsions demonstrated an efficiency exceeding 96% and effectively prevented the loss of vitamin content during simulated stomach digestion in laboratory settings. The double emulsion stabilized with sodium caseinate exhibited a release efficiency of less than 5% for encapsulated vitamin B12. Specifically, the encapsulation process boosted the retention rate from 6.3% to over 90% in fortified cheese.

## 3.3 Addition of bioactive materials

### *3.3.1 Omega 3 and polyunsaturated fatty acids (PUFAs)*

The incorporation of polyunsaturated fatty acids (PUFAs) into cheese occurs during the cheese-making process. The oxidation of these highly unsaturated fatty acids leads to the production of distinct odors such as oxidized, rancid, or fishy odors. Microencapsulation is employed as a technique to conceal undesirable sensory attributes and safeguard polyunsaturated fatty acids throughout their processing. The use of flaxseed oil, which is abundant in alpha-linolenic acid, enables the enhancement of cheese with omega-3 and other polyunsaturated fatty acids (PUFAs). The utilization of calcium caseinate for stabilizing flaxseed oil particles has resulted in enhanced resistance against lipid peroxidation and improved chemical stability in fortified cheese. The addition of omega 3 and PUFAs to cheese resulted in favorable effects on the composition, yield, and chemical stability of the resultant fortified cheese. A recent study reported successful trials to fortify food with nanoliposomal encapsulated omega-3 and PUFAs, and another study showed the increase of the bioavailability of omega-3 in fortified dairy products by nanoemulsion of algal oil rich in omega 3.

### *3.3.2 Conjugated linoleic acid (CLA)*

Conjugated linoleic acid (CLA) refers to a group of isomers that are found in meat and dairy products obtained from ruminant animals. The cis-9/trans-11 and trans-10/cis-12 isomers of CLA are considered to be bioactive. Several investigations have identified alternative CLA isomers in aged cheeses. In the field of fortification, the incorporation of CLA poses a challenge due to its hydrophobic nature. Hence, in aquatic environments, it is more advantageous to introduce the product in the form of an emulsion. Moreover, it is imperative to homogenize the fortified milk before the cheese production process to provide a uniform dispersion and stabilization of fatty acids.

### *3.3.3 Gamma amino butyric acid (GABA)*

GABA is an amino acid that works as a neurotransmitter inhibitor inside the central nervous system of mammals. GABA has been demonstrated to have significant impacts on brain function, including the potential to mitigate or prevent conditions such as anxiety, depression, insomnia, and memory impairment. Additionally, GABA has been found to boost the immune system, inhibit inflammation processes, and potentially offer protective effects against hypertension and diabetes. GABA is found naturally in trace amounts in several plant-based meals and is particularly abundant in fermented items, such as fermented dairy products, such as cheese. Several studies have documented the capacity of specific strains of lactic acid bacteria (LAB) and bifidobacteria to synthesize GABA. It has been reported the capacity of *Lactobacillus brevis* BT66 (referred to as DSM 32386) and *Streptococcus thermophilus* 84C to generate significant levels of GABA. These strains can create GABA in cheese during the ripening process. Recently, a natural GABA has been produced by natural fermentation in Japan and added at a standardized dose to enrich some kinds of functional foods, including some varieties of cheese. The addition of natural GABA allows customers to get the health benefits of GABA in their functional cheese.

#### *3.3.4 Coenzyme Q10 and ubiquinol*

Coenzyme Q10 (CoQ10) is a potent natural antioxidant that plays a crucial function in cellular bioenergetics and has been associated with a wide range of established health advantages. The fortification of food products has the potential to substantially boost intake. However, achieving this goal has been challenging, especially with low-fat, water-based products, mostly due to their lipophilicity. Various forms of coenzyme Q10 (CoQ10) with enhanced water solubility or dispersibility have been formulated to enable the enrichment of aqueous products and enhance their bioavailability. The bioactivity of CoQ10 can be maintained when it is integrated into cheese. The encapsulation of coenzyme Q10 in a simple emulsion could improve the stability of CoQ10 in cheese.

Ubiquinol, a bioactive form of Q10, has enhanced absorption properties and is commonly utilized in the production of dietary supplements. In Japan, ubiquinol serves as an effective fortifying agent for functional foods, including several forms of functional cheese.

#### *3.3.5 Collagen peptides*

Collagen peptides, found in dairy products such as cheese, have various applications in addressing skin aging, managing conditions, such as osteoarthritis, and improving nail strength. They are particularly useful in cheese production, where hydrolyzed collagen can enhance structural, mechanical, and health-related characteristics, and play a crucial role in the management and prevention of osteoporosis and related ailments.

#### *3.3.6 Carotenoids*

Lutein-fortified cheeses provide daily carotenoid intake and can help prevent oxidative stress-related illnesses. Lutein-enriched cheeses can preserve physical-chemical, microbiological, and sensory attributes without significant changes. Lutein exhibits favorable stability characteristics during storage, rendering cheeses a suitable carrier for delivery.

#### *3.3.7 Polyphenols*

Polyphenols, such as green tea polyphenols, have antioxidant properties and can interact with protein, carbohydrates, and lipids, affecting the nutritional composition of cheese. The cheese curd matrix exhibits a significant capacity for the retention and preservation of polyphenols that are incorporated into it.

#### *3.3.8 Other additives*

Various additives as plant and animal by-products have been reported in many published researches. The fortification aimed to enhance cheese organoleptic, sensory, and health benefits of the fortified cheeses. Ingredients, such as tomato, cranberry, green tea, broccoli, grape, Morinaga, asparagus, saffron, hibiscus, and others, have been published.

### 3.4 Probiotics fortification

Probiotics are live microorganisms that can improve the health of a host when administered in adequate quantities. Cheese is an ideal carrier for probiotics due to its protective barrier against acidic conditions in the gastrointestinal tract (GIT) and its high lipid content, which provides additional safeguarding during transportation. Probiotics can be used in cheese fortification with other strategies, such as iron or zinc fortification, to enhance sensory attributes and make "multi-fortified" cheese a commercially viable product. Cottage cheeses offer several advantages over other dietary options, including pH, fat content, mechanical consistency, low oxygen level, less demanding technological requirements, non-matured state, cold storage, and short shelf-life. Probiotics compete with pathogenic bacteria within the gastrointestinal tract, producing peptides called bacteriocins with antibiotic-like properties. These enzymes inhibit the growth of pathogenic bacteria, such as *Listeria monocytogenes*. Probiotics can incorporate enzymes into the host organism, exhibiting metabolic activities such as protease, lipase, esterase, amylase, and other enzymatic functions. They also facilitate the enzymatic conversion of indigestible carbohydrates into short-chain fatty acids, which have therapeutic properties for gastrointestinal disorders and decrease the pH of the intestinal environment. Probiotics synthesize vital vitamins, bioactives, and antioxidant enzymes and have favorable characteristics in cheese processing and preservation. The selection of strains plays a crucial role in probiotic development. Techniques used in cheese manufacturing significantly impact the viability of probiotic microorganisms, including inoculation methods, flavorings, competition and antimicrobial presence, pH and temperature conditions, preservation methods, salt, and packing materials.

### 3.5 Prebiotics fortification

Prebiotics are nondigestible food components that give probiotic microorganisms nourishment, increasing their chances of survival and implantation in the host digestive system. Inulin and fructooligosaccharides (FOS) are significant prebiotic components in foods, providing nourishment to probiotic microorganisms and enhancing their survival and implantation in the host's digestive system. Inulin and FOS have been used in cheese making to create reduced-fat cheese additives with prebiotic properties and dietary fiber. Incorporating inulin into soft cheese and cream cheese yielded lower fat content and elevated moisture levels, with a satisfactory resemblance to the control cheeses. However, the retention of inulin was inadequate to achieve the necessary functional properties. Galactomannans, hydrophilic polysaccharides obtained from legume seeds, have nontoxic characteristics and emulsifying and gelling qualities, facilitating the creation of a protective coating. Chitosan, a biopolymer derived from crustacean exoskeletons, has inhibitory properties against bacteria and fungi proliferation and can be enhanced by fatty acids. Its desirable attributes include biodegradability, biocompatibility with human tissues, and non-toxicity. Incorporating FOS in reduced-fat formulations indicates a potential resemblance to the structure and overall qualities of the original full-fat cheese. However, achieving an exact replication of full-fat cheese after fat reduction is challenging, and the inclusion of FOS presents a technological obstacle.

## 4. Cheese novelties as healthy snaking trends

As a result of its natural nutritional advantages, such as its high protein and calcium content, the recent trend to develop functional and/or functional cheese, therefore, cheese is becoming more and more popular as a snack food. Cheese is a product that offers opportunities for creativity in terms of taste, format, texture, and flavor, while also being considered luxurious. Cheese has the potential to rival other widely consumed protein snacks, such as nutrition bars and jerky, in terms of its ability to promote satiety and contribute to fitness and weight management goals as a nutritious protein snack, particularly when consumed in a single-serve portion.

The impetus behind advancements in cheese production is driven by the growing inclination toward nutritious snacking, a phenomenon that is propelled by the demands of fast-paced lifestyles and the increasing urbanization of society. In the context of smaller sizes, cheese producers prioritize the examination and promotion of the nutritional advantages associated with their products.

Cheese snacks are a suitable choice for health-conscious individuals seeking nutritious snacking alternatives as they offer a natural source of high protein and low-fat milk along with additional health advantages. A recently emerged category within the cheese snack market is that of solid shelf-stable cheese snacks (SSSCs). These snacks are comprised solely of dehydrated natural cheese, rendering them both nourishing and satisfying, while also possessing a convenient and enticing texture. To enhance their attractiveness, SSSCs are available in a variety of dimensions, configurations, arrangements, and tastes. The user did not provide any text to rewrite. Nevertheless, it is imperative to conduct additional studies to enhance nutrition and intestinal health, decrease sodium levels, and advocate for sustainable manufacturing methods. The exploration of innovations in novel packaging materials is equally deserving of attention.

The opportunities to innovate with cheese snacks are infinite, as cheese pairs well with salty, savory, and sweet accompaniments. Cheese also may be cut and formed into many shapes and sizes. Moreover, cheese can be loaded with nutrients, namely protein and calcium, which appeal to health- and wellness-conscious consumers.

Cheese snack innovations are on the rise. Cheese may be cut and formed into many shapes and sizes healthy and functional cheese varieties would be available in the form of small portions, bites, balls, slices, sachets, and others to offer a dose-controlled fortified and/or functional cheese, which appeals to health- and wellness-conscious consumers.

## 5. Cheese as a rich source of valuable materials

### 5.1 Calcium

Cheese is a great dietary source of calcium, essential for sustaining life and regulating vascular function, neuronal transmission, muscle function, and hormone secretion. Only 1% of the total calcium is needed for specific physiological processes, while the remaining 99% is primarily sequestered within the skeletal system. Calcium intake is around 1000 mg for the average adult.

### 5.2 Proteins

Cheese is also a great source of protein, essential for the body's formation, regulation, repair, and protection. Approximately 1–2 servings of protein-rich foods per day are sufficient for most adults. Parmesan cheese is the most protein-rich option, with one ounce providing seven grams.

### 5.3 Vitamin K2 and B12

Cheese is rich in essential vitamins K2, B12, and B12, which are crucial for hemostasis and brain function. Vitamin K2 is less emphasized than K1, but it interacts with calcium and vitamin D. Hard cheeses, such as gouda and brie, have higher levels of vitamin K2, with gouda and brie having the highest levels. Vitamin B12, the largest and most complex vitamin, is essential for erythrocyte synthesis, protein synthesis, and cognitive processes. Cheeses, such as Swiss cheese, have the highest concentration of B-12, providing 39% of the recommended daily intake.

### 5.4 Healthy fats

Cheese, in moderation, can help you get these necessary fats into your diet. Try choosing aged cheeses, such as parmesan, and using it as a garnish for salads. The fats in the cheese will help keep you full and help your body absorb the vitamins in your vegetables.

### 5.5 Conjugated linoleic acid (CLA)

Conjugated linoleic acid (CLA) is a complex molecule often underappreciated due to low-fat and no-fat dietary patterns. CLA is a vital component of a healthy diet, often found in dairy and meat products derived from grass-fed ruminant animals. It aids in reducing adipose tissue, promoting lean muscle mass, and supporting immune and inflammatory systems. Cheese derived from grass-fed cows typically contains high levels of CLA, which is positively correlated with fresh grass consumption.

### 5.6 Gamma amino butyric acid (GABA)

GABA would occur naturally in various types of cheese as a byproduct of certain starter cultures. The concentration of GABA found in 22 varieties of Italian cheese ranged from 0.260 to 391 mg/kg, and from 320 to 6773.5 mg/kg in Cheddar cheese. The GABA concentration detected in the latter studies ranged between 15 and 5000 mg/kg, even though L-glutamate was added to milk before starting the fermentation process. Also, it has been reported that *Lactobacillus brevis* and *Streptococcus thermophilus* isolated from traditional alpine cheeses were capable of producing high concentrations of GABA.

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Section 2

# Cheese Ripening and Bioactive Peptides

Chapter 2

# Cheese Ripening: Impact on Cheese as a Functional Food

*Dina A.B. Awad and Adham M. Abdou*


## Abstract

One of the most popular types of fermented dairy products is cheese. The process of cheese aging is essential for improving cheese quality, and health benefits. Ripened cheese at different times acquired wide diversity of characteristic aromas and textures due to establishing a cascade of intrinsic complex biochemical and metabolic outcomes, resulting in a dynamic shift in microbial flora. Various functional bioactive compounds could be released during the cheese ripening process. Many strategy approaches are employed to accelerate cheese ripening based on increasing lipolysis and proteolysis rate. During cheese aging, microbial spoilage as early and late blowing may occur so, designing smart ripening rooms are very essential equipped with computerized monitoring systems including sensors, software platforms, temperature, and humidity data loggers.


**Keywords:** cheese ripening, cheese peptides, factors affecting cheese ripening, cheese microbiota, bioactive ingredients in cheese

## 1. Introduction

Cheesemaking depends on the concentration of milk proteins with or without milk fat. After the salting and packing processes, cheese acquired a longer shelf life. Around the world, cheeses can be produced using a variety of milk sources, processing methods, starter culture, coagulants, and ripening conditions, giving rise to a large number of variants with a vast diversity in terms of texture, flavor, and shape [1]. Cheese ripening (aging or maturing) is the most crucial industrial stage in cheese technology, which establishes a cascade of complex biochemical steps, producing a wide variety of microbial flora and different volatile compounds. This is the critical stage during which the cheese's firmness, aroma, flavor, and other specific cheese characteristics are acquired. Ripening occurs under temperature and humidity circumstances that differ depending on the type of cheese. The longer the cheese ripens, the less moisture it retains and the firmer and stronger-tasting it becomes. Cheese kinds are actually defined based on how they ripen; Brie, Camembert, and Roqueforti are examples of mold-ripened cheese, while Limburger and Tilsit are examples of surface-ripened cheese. Internally-ripened cheeses fall into six categories: semi-hard cheeses like Monterey Jack, hard cheeses like Cheddar and extra-hard cheeses like Parmesan and Asiago; pasta filata, which includes mozzarella and provolone; high-salt cheeses like Feta; cheeses with eyes like Dutch types (Gouda and Edam) and Swiss types (Emmental and Gruyere) [2]. The curd's remaining citrate is metabolized by some citrate-positive lactic acid bacteria (LAB), like *Lactococcus lactis subsp. Cremoris,* and *Lactococcus lactis subsp. lactis biovar. diacetylactis,* producing a variety of taste compounds, including acetoin, acetate, diacetyl, 2-butanone, and 2,3-butanediol, which are linked to the development of Dutch cheeses [3].

The development of flavor and sensorial characteristics in cheese is significantly influenced by ripening conditions, especially the time factor. Each variety of cheese has a distinct volatile chemical at a variable concentration, and flavor which could be measured by a diverse series of methodologies, and computational and descriptive approaches [4, 5]. Several bioactives and healthy peptides are produced from milk components during ripening as a result, mainly, of its degradation by starter cultures endo and exo-enzymes [6].

## 2. Factors affecting the cheese ripening process

### 2.1 Cheese microbiota

In the cheese industry, characterization of the cheese microbiota is crucial, since some specific microorganisms improve cheese characteristics while others may decrease quality [7]. The cheese microbiota is a sophisticated ecosystem that can develop from raw milk, acidifying starters, and adjunct cultures, as well as a group of prokaryotic, eukaryotic, and viral populations that may arise from machinery and the environment of the cheese manufacturing plant.

LAB are the majority microbiota with a significant role during cheese production and ripening. The shape, numbers, and proportions of LAB are then shaped by cheese making and ripening which exert a selection pressure on microorganisms. Diverse microbiota members may interact cooperatively and competitively, which could have an impact on ripened cheese's rheological, organoleptic, and safety properties [8, 9]. The progression of various microbial species and their interaction during the cheese-making and -ripening processes, in particular, are crucial for the formation of the distinctive sensory characteristics of each cheese variety [10, 11].

Notably, LAB are a kind of Gram-positive bacteria with the ability to withstand acidic pH, and they are primarily cocci or rods at the microscopical examination. The LAB can convert milk carbohydrates to lactic acid. There are more than sixty genera of LAB, the most common genera found in food for fermentation are *Lactobacillus*, *Leuconostoc, Pediococcus, Enterococcus, Lactococcus, Streptococcus*, and *Weissella* [12, 13]. The natural bacterial population of LAB in milk is the fundamental basis for the manufacture and technology of raw milk cheeses. A recent study conducted in Italy indicates that the microbial population of milk, particularly that used to produce Grana Padano PDO cheese, may be impacted by the multifaceted nature of farming practices. The implementation of stringent hygiene measures and management levels in the dairy farm affects negatively the presence of diverse milk microbiota and decreases bacterial load [14].

LAB play a significant role in raw milk cheeses like Parmigiano Reggiano (the long-ripened hard cheeses) both as starter cultures (SLAB) during curd acidification and as non-starter cultures (NSLAB) during cheese-ripening [15–17].

The majority of facultative heterofermentative lactobacilli, or other terms called NSLABs, are frequently isolated from cheese [18]. For instance, one common NSLAB isolated from long-ripened, hard-cooked cheeses is the *Lactobacillus casei* group, which includes *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus paracasei*, and *Lacticaseibacillus casei* species. These species contribute to the formulation of the distinctive cheese flavor during the ripening process [19].

During cheese ripening, a complicated sequence of activities happens, resulting in a series of biological reactions. Proteolysis is one of the most critical processes, which is launched by the starter, followed by non-starters, and completed by proteolytic enzymes secreted by the bacterial population. These mechanisms cause conformational changes in the particular peptides and amino acid composition, which continually alters during the aging process. The author Sforza et al. [20] discovered a high association between peptide evolution and enzyme activity, allowing for the classification of cheeses based on their ripening circumstances.

Another earlier study established a relation between the hard cheese Parmigiano Reggiano microbiota with the proteolysis rate that takes place during ripening, which is the cause for the valuable and high-quality characteristics of long-ripened cheeses. The study found the raw milk microbiota for different samples from various dairies were quite similar on the curd level. Once ripening time progressed the microbial composition changed, revealing significant differences between cheese samples at early ripening storage time (1 and 2 months) and at late ripening storage (time 7 and 9 months old). These were attributed to NSLAB species, which are more correlated with different peptide profiles and the significant difference in kinetics and activities of the proteolytic enzymes. This study underlines the critical role of NSLAB in ripened cheese proteolysis. Additionally, the potential use of several peptides as indicators of a specific microbial composition allows for the preservation and valorization of cheese's specificity and connection to its production location [21].

The reaction between propionic acid bacteria and LAB influences the organoleptic properties of the produced final cheese product as texture, flavor, and ripening stability. Specific inoculation doses from *Propionibacterium freudenreichii* strains, one of the propionic acid bacteria spp., usually chosen, are utilized for the manufacture of Emmental cheeses and other Swiss-type cheeses which under propionic acid fermentation produce characteristic taste with distinctive eye formation [22].

A study investigated that the amount of free lysine, glycine, and methionine that is available in cheeses is influenced by propionibacteria and LAB. The highest content of free amino acids was presented in the mature cheeses that were produced by combining mesophilic LAB *Propionibacterium* and *L. casei* strains. Compared to the mesophilic LAB starter culture or the addition of *L. casei*, it was clear that the addition of *Propionibacteria* had a greater impact on the free amino acid content in matured cheese samples. Notably, this study demonstrates that the growth of *Propionibacteria* in milk is exaggerated by mesophilic LAB, resulting in the production of a significant amount of free amino acids [23]. Not only changing the cheese's body and texture and forming the flavors that give it its distinct character, chemical reactions that take place on the surface of the cheese during ripening also provide an extra protection layer against dry storage conditions [24]. Furthermore, during maturation, the surface microbiota grows quickly, and the residing microorganisms have inhibitory characteristics that hinder pathogenic foodborne bacteria or molds that produce mycotoxin from colonizing the cheese [25, 26]. Numerous enzymes can initiate chemical reactions which may originate from natural milk enzymes, rennet extract utilized in manufacture, or produced by bacteria that survive pasteurization or are added during the process of manufacture or ripening. For instance, three distinct strains of *Brevibacterium linens* were used as surface inoculants to initiate the formation of red smears during the fermentation of Munster cheese [27]. Environmental pathogens pose a significant risk to the ripening and manufacturing processes of bloomy rind cheeses, such as Camembert, Brie, and related varieties. Due to the product's exposure to the open air during ripening, this risk is especially elevated [28]. The most growing fungi include *Penicillium candidum*, *Kluyveromyces marxianus*, and *Geotrichum candidum* [29]. All predominantly used fungi have a great role in ripening and characteristic organoleptic properties [30].

Cheeses that have been ripened by mold may be distinguished into two main types: those with blue veins and the other with surface mold-ripened cheeses or bloomy rinds [31]. The development of a particular mold called Penicillium roqueforti gives Roqueforti, also referred to as blue-veined cheeses, characterized by unique flavor and appearance. Roqueforti cheese is produced in several cities across the globe. Each of these countries' distinct blue cheese varieties has its own characteristics and methods of manufacture [32].

The microbiota of cheese has been shown to have anti-cancer and cholesterol-lowering capabilities in addition to participating in the enhancement of cheese flavor through the synthesis of volatile molecules [33–35]. The cheese microbial dynamic is affected by the interactions among some factors as LAB used as SLAB or NSLAB, cheese-making processes, and some storage conditions [36–38]. Owing to the cheese microbiota being correlated to the quality and physicochemical properties of cheese, it became critical to understand the cheese microbial properties. In a study reported by Choi et al. [39], the post-inoculation cheese microbiota was found to be dominated by SLAB, and the authors observed that the addition of SLAB resulted in modifications to the microbial community structure, microbial diversity, biomarkers, and predicted functional qualities. Additionally, 105 and 119 days after age, undefinable *Lactobacillus*, or NSLAB, were found.

### 2.2 Enzymes

The primary factor in turning milk into cheese is the presence of enzymes, which can be found in the milk itself or introduced as rennet or microbial enzymes. The disintegration of caseins is by far the most significant of the enzymatic processes. Rennet, native milk proteinase, and peptidases generated by starter cultures, enzymes of secondary starters, and enzymes of non-starter cultures are the five primary systems that aid in the hydrolysis of casein [40].

Depending on the cheese type, the ripening time for cheeses made with rennet might range from a few weeks to several years. Microbiological and biochemical alterations take place during ripening, giving the variety's distinctive flavor and texture their development. Primary (lipolysis, proteolysis, and metabolism of residual lactose, lactate, and citrate) or secondary (metabolism of fatty acids and amino acids) processes can be used to categorize biochemical changes in cheese during ripening. Early in the ripening process, lactate is quickly produced from residual lactose. An essential precursor for several processes, such as racemization, oxidation, and microbial metabolism, is lactate. In certain types, the metabolism of citrate is quite important. Cheese's lipolysis is aided by lipases derived from several sources, especially milk [5].

Cheese ripening can be accelerated by increasing the concentration of important enzymes used in cheese production. Plasmin, chymosin, and intracellular and/or cell wall proteinases and peptidases of the LAB and NSLAB are among the proteinases and peptidases found in cheese. Proteases and lipases from animals or fungi are frequently utilized in the production of enzyme-modified cheese. It is uncommon, though, for these enzymes to be used directly to enhance the flavor and ripening of cheese. The primary disadvantages of this approach are the limited availability of commercial enzymes that have been approved for use in cheese ripening and the incapacity to blend the enzymes into the cheese matrix. Exogenous enzymes can be either single enzymes or commercial enzyme combinations. Exogenous enzymes can be added directly into cheese blocks, in combination with starter cultures or coagulants, with cheese-milk, or at the stage of dry salting. When making cheddar cheese, the latter process is employed [41]. Exogenous enzymes were supposed to accelerate cheese ripening and aid in the production of unique tastes in specific cheese varieties. In that regard, a few examples of distinct enzymes or their mixes were reviewed and documented [5].

### 2.3 Dairy animal feeding

It was worth noting that dietary supplementation of lactating dairy cows may change the quality of dairy products, especially cheese products. The characteristic volatile substances that contribute to cheese flavor were attributed to the aromatic qualities of milk obtained from lactating dairy ruminants. Extensive research has been conducted concerning lactating dairy ruminants fed specific experimental diets, such as those distinguished, for example, by the addition of trace elements, natural supplements, or agricultural byproducts rich in bioactive compounds. Cheese contains a variety of volatile substances, such as carboxylic acids, lactones, ketones, alcohols, and aldehydes. The relative amounts of each substance are determined by the biochemical processes that take place during ripening. These processes are primarily mediated by endogenous enzymes and elements of bacterial origin whose function can be greatly influenced by the bioactive substances consumed by animals in their diet and released in milk through the mammary gland. According to Ianni et al. [42] there was a significant correlation between the quality of the biochemical changes in cheese products throughout ripening and the various dairy animal feeding practices.

### 2.4 Level of sodium chloride

It is well-recognized that consuming too much sodium raises the risk of hypertension, cardiovascular disease, and even stomach cancer [43, 44]. The issue for the food industry is reformulating food products that contain less sodium aiming to offer low-salt food in the human diet is currently one of the top priorities for public health organizations [45]. To comply with the World Health Organization's (WHO) recommendation of 2 g/day, it has been suggested by the World Health Organization that sodium intake be reduced by 30% [46].

Food businesses must carefully re-evaluate the composition and processing of high-sodium foods considering public awareness of excessive sodium intake and nutrition claims connected to salt content. Although it is usual practice to replace some ingredients in products through reformulation, it is still difficult to reduce salt in cheese due to sodium chloride's various functions and essential activities in cheese making. Salt improves the taste and fragrance profile, controls the texture, final pH, and water activity, and influences microbiological growth. It also favors the drainage of remaining whey. In the end, salt concentration affects the shelf-life of cheese by regulating the activity of starter and non-starter LAB during cheese production and ripening. Any adjustment to the salting process, such as lowering the sodium chloride (NaCl) amount or substituting alternative salting agents, could upset the delicate balance within the parameters, changing the cheese's quality. Depending on the kind of cheese and manufacturing method (for example, soft, semi-hard, hard, and mold-ripened cheeses), the decrease of NaCl content may be treated differently. As a result, specific tactics could be implemented to preserve the general quality and safety of various cheese types [47].

As a result, making cheeses with less NaCl content is becoming increasingly popular in the dairy business. Since NaCl is essential for textural qualities, microbial development, autolysis, enzyme activity during ripening, and ultimately cheese flavor, hence, reducing NaCl in cheese-making poses a number of technological, sensory, and microbiological issues [48]. Reduced NaCl semi-hard cheeses typically have enhanced cohesiveness, adhesiveness, acidity, bitterness, and an unpleasant aftertaste along with a decrease in salt and hardness as their sensory defaults [49, 50]. Additionally, reducing the NaCl concentration of cheese affects product safety due to the possibility of germs like *Listeria monocytogenes* growing in the product [51].

According to popular perception, cheese contains varying quantities of salt, depending on the type of cheese. NaCl content for soft, semi-hard, and hard cheeses ranges from 0.5% to 2.5%, whereas it ranges from 3% to 5% for blue-type cheeses. In cheese, NaCl performs a variety of crucial tasks, including modifying the curd and rind's physical characteristics, regulating the microbiota's growth as the cheese ripens, preventing the formation of infections or spoilers, and enhancing the flavor [52, 53].

In a study followed the strategy of reduction of NaCl or its partial substitution with other salts such as potassium chloride in a semihard cheese (Reblochon), it was reported that lowering the salt level in the semi-hard cheese samples caused spoiler growth to accelerate, as seen by increased Pseudomonas species formation and increased cheese proteolysis and lipolysis [45].

## 3. Bioactive components produced during cheese ripening

Bioactive peptides are released during the ripening process of cheese and are protein fragments in the form of short amino acid sequences. During cheese maturation, various functional bioactive compounds could be generated such as volatile fatty acids, exopolysaccharides, vitamins, organic acids, peptides, and amino acids (γ-aminobutyric acid and conjugated linolenic acid). Many laboratory and animal research studies demonstrated that most of these generated bioactive compounds exhibited different biological activities including antihypertensive, antioxidant, anticancer, and antimicrobial activities [54–56]. The above bioactivities lead to health-protective effects associated with a reduced incidence of cardiovascular disease risk factors, such as obesity, dyslipidemia, and type 2 diabetes [57], as well as reduced incidence of metabolic syndrome [58, 59]. The artisanal methods employed for the manufacturing of Mexican cheeses, depending on natural milk indigenous microflora, may offer many potential health benefits. A previous study [60] demonstrated that peptides derived from both fresh cheese and a model cheese (laboratory scale) had an antihypertensive peptide through suppressing ACE-inhibitory effect. Although artisanal cheese is under storage conditions, producing different product varieties such as (Crema de Chiapas, Cocido, and Fresco of Sonora). Few limited studies are available for artisanal cheeses in Mexico and the various derived bioactive compounds in artisanal cheeses, and those available focus primarily on the antioxidant and ACE activity of water-soluble extracts (WSE) obtained from different types of artisanal cheese varieties such as (Crema de Chiapas, Cocido, and Fresco of Sonora) from different storage conditions [61, 62].

Distinct peptide sets found in different cheese varieties contribute to their distinctive flavors and possible health benefits. These bioactive molecules support the body's defense against potentially harmful substances by acting as antioxidants, anti-inflammatory agents, and even antimicrobial activities. Angiotensin-converting enzyme (ACE) inhibitory characteristics have been demonstrated by some cheese peptides, indicating that they may lower blood pressure by preventing the angiotensin-converting enzyme from acting [63].

Protein breakdown is typically linked to biological functional qualities and is aided by the action of milk-specific enzymes. Furthermore, the microorganisms used or added during cheese production may produce bioactive peptides [64]. Milk proteases and peptidases produce large and intermediate-sized peptides, which are then hydrolyzed by enzymes from the cheese SLAB and NSLAB strains. These are known as primary proteolysis reactions [65].

Both the body's digestive processes and the fermentation procedures used to produce fermented dairy products produce functional peptides. They are produced by the proteins; casein and whey. When liberated from their original proteins, these peptides take on an active role. When they enter the circulatory system, they may have a systemic effect, or they can be absorbed in intact form and have different physiological effects locally in the gut. It is well known that lactoferroxins and casooxins function as opioid antagonists, while casomorphins and lactophorins generated from milk proteins are opioid agonists. The opioids' analgesic qualities are comparable to aspirin. The actions of casokinins (which lower blood pressure), casoplatelins (which reduce blood clotting), immunopeptides (which boost immune function), and phosphopeptides (which carry minerals) are all examples of casokinin action. Casein phosphopeptides may improve calcium, phosphorus, and magnesium bioavailability for better bone health. They may also aid in the prevention of dental cavities and play a function in the secretion of enterohormones and immunological boosting. The involvement of casein peptides in blood pressure regulation looks promising. Certain casein and whey protein hydrolyzates prevent the conversion of angiotensin I to angiotensin II. Because angiotensin II elevates blood pressure by constricting blood vessels, inhibiting it causes blood pressure to fall. Dairy foods would thus be a natural functional food for managing hypertension due to their ACE inhibitory action. There are several commercially marketed whey products that include discrete bioactive peptides. The glycomacropeptide (GMP) is produced by proteolysis from kappa-casein [5, 63].

Proteins and lipids are the sources of the most common and extensively researched bioactive molecules. Cheese proteins are broken down to form bioactive peptides and amino acids such as gamma-aminobutyric acid and ornithine, while fats are hydrolyzed to produce and be an origin of bioactives like conjugated linoleic acid, carotenoids, fat-soluble vitamins, and sphingolipids, among other things. Depending on their nature, these chemicals exhibit various bioactivities [6, 35].

High quantities of lactic acid and other organic acids are produced by LAB during the fermentation of lactose [66]. The ripening process is a complicated process in which several milk enzymes participate, including rennet and enzymes from LAB, which leads to successive transformations that aim to affect the various curd ingredients [40, 67].

Proteinases found in LAB contribute to the proteolysis of cheese proteins, converting them into oligopeptides that can either be further degraded into shorter peptides and amino acids through the synergistic action of various intracellular peptidases produced by specific LAB. These peptides, amino acids, and their derivatives help the final cheese to develop its texture, flavor, and health benefits. The generated peptides were subjected to both *in vitro* laboratory experiments and *in vivo* animal models, which confirmed that they possessed a variety of biological activities, including the ability to scavenge free radicals (antioxidants), inhibit microbial growth (antimicrobial), fight inflammation (anti-inflammatory), immunity enhancer (immunomodulatory), opioid blocker receptors activity (analgesic), and lower blood pressure by suppressing the angiotensin-converting enzyme producing antihypertensive effect. Furthermore, some LAB produce vitamins, some antimicrobials, conjugated linoleic acid, and other functional lipids with anti-inflammatory and anticarcinogenic properties, as well as bioactives that contribute to physiological processes like neurotransmission and hypotension induction with diuretic effects [63].

During the early weeks of cheese ripening, NSLAB proliferate at a very slow rate, but eventually take control of the cheese microbiota following the death phase of the starter culture [68, 69]. The process of cheese ripening is sophisticated and a dynamic process. The variety of proteolytic enzymes naturally found in milk and the remaining coagulants, as well as the enzymatic metabolism of LAB, are crucial to this process [70]. Peptides are continuously released during ripening by the action of plasmin and LAB's enzymes; some of these peptides are then digested, while others accumulate throughout storage [71, 72].

One of the major milk groups that can enhance the sensory qualities, shelf life, and microbial safety of cheese as well as the nutritional content and functional features of the finished products is NSLAB. It includes; *Pediococcus*, *Enterococcus*, *Lactobacillus*, and *Leuconostoc* genera. Due to the creation of certain metabolites throughout the ripening process, ripened cheese develops a diversity of flavors, nutritional qualities, and rheological characteristics [73–75]. Peptides, amino acids, biogenic amines, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids, minerals, and

| Type of bioactive component | Type of ripened cheese | Reference |
|---|---|---|
| 1. Antioxidant peptides | Cheddar | [76, 77] |
| | Coalho | [78] |
| | Fresco | [79] |
| | Parmigiano-Reggiano | [80] |
| | Cottage cheese | [81] |
| 2. ACE-inhibitory activity | Mozzarella, Italico | [82] |
| | Red cheddar, Camembert | [83] |
| | Emmental | [84] |
| | Manchego, Ronca, and goat cheeses | [85] |
| | Gouda | [33] |
| 3. Gamma amino butyric acid (GABA) | Artisanal Spanish cheese | [86] |
| | Italian Pecorino Marchigiano and Pecorino Filiano cheeses | [87] |
| | Lighvan cheese (traditional semi hard Iranian cheese) | [88] |
| 4. Organic acids | Ossalano cheese | [89] |
| | Cheddar cheese | [90] |

**Table 1.**
*List of some published bioactive compounds found in ripened cheese varieties.*

any other molecules that can alter the sensory and rheological properties, as well as the nutritional value and health benefits of the finished products, are examples of metabolites [73].

The aforementioned functional biological activities have been linked to a decreased incidence of most common coronary artery disease risk factors, such as dyslipidemia, diabetes type 2, and overweight [57], as well as different metabolic syndromes [59].

As shown in **Table 1** various bioactive components in different ripened cheese.

## 4. Ripened cheese additives

One of the simplest and most traditional methods for preserving food to increase its shelf life is the direct addition of additives which may be chemical or naturally derived preservatives. These ingredients are added to cheese in order to prevent spoilage and pathogenic microbial growth, increase shelf life, enhance organoleptic and sensory characteristics, and maintain nutritional value [91, 92].

Food preservatives permitted for use in matured cheeses in the EU are classified into three functional groups: antimicrobials, antioxidants, and antibrowning

| Cheese additive | Character | Function | Reference |
|---|---|---|---|
| Nisin | A bacteriocin with the designation E234 has been accepted as a food additive by the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA). Nisin is a 34-residue protein that is mostly generated by *Lactococcus lactis* strains | Nisin has an antibacterial action against a variety of Gram-positive foodborne and spoilage bacteria | [93–96] |
| Natamycin | A bacteriocin that is produced by aerobic fermentation of *Streptomyces natalensis* and its related species More than 40 nations have approved it as a food additive, and the FDA regards it as a GRAS (generally recognized as safe) substance | commonly employed antifungal to prevent yeasts and molds contamination in dairy products (hard and semi-hard cheese) | [97] |
| Lysozyme | A naturally occurring enzyme that is prevalent in egg whites, from which it is typically separated for industrial preservation | It has some bactericidal properties against Gram positive bacteria like LAB and clostridia but less effective against Gram negative bacteria. Lysozyme (E-1105) has been employed to prevent the "late blowing" problem in hard and semi-hard cheeses | [98–100] |
| Ripening enzymes | Like proteinases, peptidases, and lipases produced by *Penicillium candidum* | Strategies for accelerating the cheese ripening process by addition of liposome-encapsulated enzymes to cheese milk provide some definite advantages | [101, 102] |

**Table 2.**
*Additives to cheese milk and its role in cheese ripening.*

chemicals. During cheesemaking, these compounds are added to the milk vat as antimicrobials and antioxidants, or to the cheese as surface defenders against unwanted agents. Lysozyme, sorbic acid/sorbates, nisin, natamycin, hexamethylene tetramine (HTM), nitrates/nitrites, and propionic acid/propionates are authorized additions for matured cheeses. As illustrated in **Table 2** different cheese additives are added to cheese milk for enhancement of the cheese ripening process.

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Chapter 3

# Cheese's Bioactive Peptide Content and Fatty Acids Profile

*Ilyes Dammak and Carlos A. Conte-Junior*


## Abstract

This chapter provides an in-depth review of the latest research developments in cheese's bioactive peptides and fatty acid profiles, emphasizing their potential health benefits, particularly in managing obesity and hyperlipidemia. It delves into the generation of bioactive peptides during cheese fermentation and maturation, their potential health-promoting effects, and the factors influencing their content. The chapter also offers a comprehensive analysis of the fatty acid profile in cheese, discussing the impact of various cheese-making processes on this profile and the subsequent implications for human health. Furthermore, it explores innovative strategies for enhancing the bioactive peptide content and optimizing the fatty acid profile in cheese. These strategies include using bioactive edible films, which have shown promise in improving the microbial quality of cheese and reducing lipid oxidation, thereby extending its shelf life. The chapter also investigates the encapsulation of bioactive compounds, a technique that has been used to enhance the stability and functionality of these compounds. Through this comprehensive review, the chapter offers valuable insights into the potential of cheese as a source of health-promoting bioactive peptides and fatty acids and the various strategies for optimizing their content and functionality.


**Keywords:** bioactive peptides, fatty acids, cheese, health benefits, cheese processing

## 1. Introduction

Cheese, an extensively consumed dairy product, is highly regarded for its abundant flavor, pleasing texture, and significant nutritional content. The above substance is a notable reservoir of proteins, fats, vitamins, and minerals. Cheese is not only rich in conventional nutrients, but it also contains bioactive peptides and a distinctive composition of fatty acids. These components have garnered significant attention in scientific research due to their potential positive effects on human health [1, 2].

Bioactive peptides are specific segments of proteins that have advantageous effects on different physiological functions or states, potentially influencing overall well-being. The peptides are encoded within the primary structure of the parent protein and can potentially be released through various mechanisms, such as digestion or food processing. Peptide formation is a prevalent process observed in cheese production, primarily facilitated by fermentation and maturation. Recent studies have provided evidence suggesting that bioactive peptides can significantly impact health conditions such as obesity and hyperlipidemia [3, 4].

In contrast, the fatty acid composition in cheese is primarily determined by factors such as the origin of the milk, the feeding habits of dairy animals, and the methodologies utilized during the cheese manufacturing processes. The association of various health benefits with fatty acids, particularly unsaturated ones, has been documented [5, 6].

In addition to these naturally occurring compounds, innovative strategies are being developed to preserve cheese's bioactive peptide content and fatty acid profile. One such strategy is the use of bioactive edible films, which have shown promise in improving the microbial quality of cheese and reducing lipid oxidation, thereby extending its shelf life [7, 8]. Another promising approach is the encapsulation of bioactive compounds, a technique that has been used to improve the stability and functionality of these compounds [9].

The cheese's bioactive peptides are influenced by the cheese-making process and the type of milk it produces. An investigation on goat milk cheese revealed a higher concentration of bioactive peptides than cow milk cheese [10]. Moreover, certain probiotic strains in cheese-making have been shown to enhance the bioactive peptide content. A study on cheddar cheese showed that using *Lactobacillus casei* 300 resulted in the formation of peptides with antihypertensive activity [11]. Moreover, the utilization of specific enzymes, such as proteases, in the cheese production process has demonstrated the ability to augment the presence of bioactive peptides. The investigation conducted on Gouda cheese demonstrated that utilizing proteases derived from *Lactobacillus helveticus* led to the production of peptides exhibiting antihypertensive properties [12]. In addition, a study on blue cheese showed that the ripening process led to the formation of peptides with antioxidant activity [13]. Furthermore, a study on Camembert cheese showed that the ripening process led to the formation of peptides with antithrombotic activity [14]. Finally, a study on Roquefort cheese showed that the ripening process led to the formation of peptides with anti-inflammatory activity [15].

For cheese processing, reducing salt content in cheese through methods such as decreasing the brine soaking time has not impacted the bioactive peptide formation or fatty acid bioaccessibility in cheese [15]. The ripening process of cheese also plays a significant role in forming bioactive peptides. For instance, a study on Mexican goat cheese showed that the ripening process led to the formation of peptides with antioxidant activity [13]. Furthermore, an investigation conducted on Dutch-style cheese revealed that the presence of fatty acids and conjugated fatty acids not only plays a role in the overall nutritional profile of cheese but also, when combined with chemometric techniques, can serve as chemical biomarkers for evaluating the source and maturation status of cheeses, as well as verifying their authenticity [8]. Additionally, a research investigation on Parmigiano–Reggiano cheese revealed that the defatting procedure could retain the entire cheese's nutritional characteristics, encompassing bioactive peptides. This was accomplished through high-performance liquid chromatography (HPLC) [16].

The incorporation of plant-based diets, such as flaxseed, in dairy animals has been demonstrated to enhance the nutritional profile of cheese [10, 11]. Flaxseed is recognized as a substantial reservoir of α-linolenic acid, classified as an omega-3 fatty acid, and widely acknowledged for its advantageous impact on cardiovascular well-being. Research has indicated that the inclusion of flaxseed in the diet of dairy animals results in an elevated presence of omega-3 fatty acids in their milk. Consequently, this dietary modification contributes to the resulting cheese's enhanced fatty acid composition [10, 11].

Similarly, certain forages in dairy animals' diets can enhance cheese's bioactive peptide content. A notable example is sulla, a leguminous forage, which has been shown to increase the bioactive peptide content in cheese [17]. Bioactive peptides refer to fragments of proteins that can positively impact bodily functions or conditions, potentially influencing overall health. Moreover, the germination of certain seeds, such as black soybeans, has been found to enhance their anti-Alzheimer activity [14]. This suggests a potential application in cheese production, where these germinated seeds could be used as an ingredient or supplement. The resultant cheese could have enhanced neuroprotective properties, offering a novel approach to preventing or managing neurodegenerative diseases like Alzheimer's. Later, dairy animals' diet and specific ingredients or supplements can significantly influence cheese's nutritional and bioactive properties. This opens up exciting possibilities for the production of functionally enhanced cheese products, contributing to the field of functional foods.

This chapter aims to comprehensively review recent research developments on cheese's bioactive peptides and fatty acid profiles. It will delve into their health-promoting potential, the factors influencing their content, and the innovative strategies for enhancing their presence and functionality in cheese. The chapter is designed to be accessible to readers from various fields, not just those who are dairy science or nutrition experts.

## 2. Bioactive peptides in cheese

In the realm of functional foods, cheese holds a unique position due to its rich content of bioactive peptides and specific fatty acid profiles. This section delves into the intricate processes that lead to the generation of these bioactive peptides during cheese production and the subsequent health benefits they confer.

### 2.1 Generation of bioactive peptides in cheese

Producing bioactive peptides in cheese is a complex procedure that necessitates a comprehensive comprehension of the biochemical and microbiological mechanisms at play for its thorough analysis. Hence, acquiring a thorough comprehension of these factors can facilitate the optimization of the proteolysis process and augment the production of bioactive peptides. These peptides are primarily produced due to gastrointestinal digestion, a multifaceted physiological process characterized by the enzymatic degradation of proteins into smaller peptides and amino acids. Simultaneously, the process of milk processing, which involves pasteurization and homogenization, has the potential to generate these bioactive peptides. Moreover, enzymatic hydrolysis, which involves the enzymatic degradation of proteins into smaller peptides, constitutes another noteworthy factor. Indeed, many digestive enzymes, including pepsin, trypsin, and chymotrypsin, are responsible for the cleavage of proteins at specific locations. Microbial fermentation, a biological process in which microorganisms such as bacteria and yeast metabolize organic substances, frequently produces peptides. The various processes, both independently and in combination, play a significant role in producing bioactive peptides. These peptides have garnered considerable attention due to their potential to promote health (**Figure 1**).

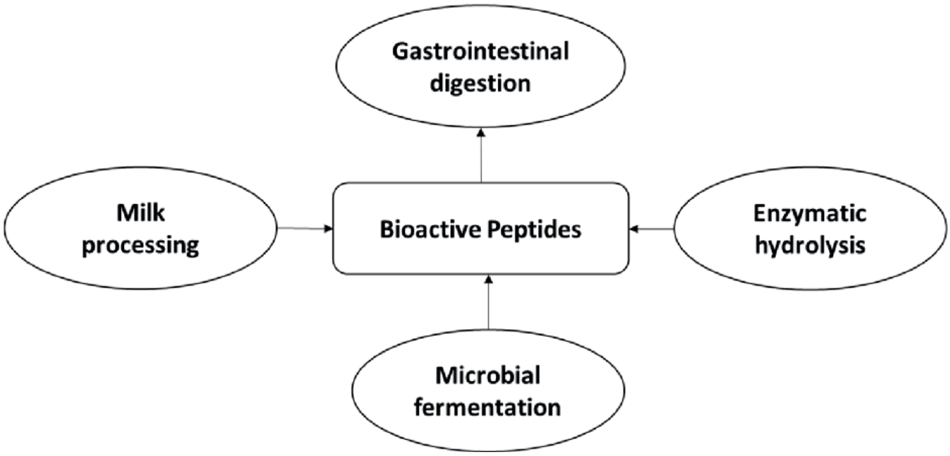


**Figure 1.**
*Production mechanisms of bioactive peptides [18].*

The enzymatic degradation of proteins by starter cultures plays a crucial role in this process [19]. Nevertheless, it is crucial to consider the involvement of nonstarter cultures, as they can play a significant role in the proteolytic activity throughout the ripening phase and influence the cheese's ultimate peptide composition. Within the given framework, nonstarter cultures, called secondary or adjunct cultures, encompass bacteria deliberately introduced into the milk used for cheese production alongside the primary starter cultures. Nevertheless, their contribution to the acidification of the cheese curd, the primary function of starter cultures, is not substantial. In contrast, cultures that do not initiate fermentation play a significant role in shaping cheese's flavor, texture, and various attributes as it undergoes the ripening phase. Nonstarter cultures encompass various microorganisms, including lactobacilli, propionibacteria, and specific fungal species. These organisms can synthesize enzymes that catalyze the hydrolysis of proteins, fats, and carbohydrates in cheese, forming flavor compounds and other substances, such as bioactive peptides [20].

The research conducted by Kurbanova et al. offers significant insights into the contribution of distinct starter cultures in producing bioactive peptides [21]. However, it would be interesting to investigate further the potential influence of the interaction between various starter and nonstarter cultures on the peptide profile. For example, what impact would the inclusion of additional strains of lactobacilli or other bacterial species have on the production of bioactive peptides? Furthermore, it would be advantageous to investigate the impact of fermentation conditions, including temperature and duration, on the efficacy of these cultures and the consequent production of peptides.

The significance of proteolysis in milk conversion into cheese, as emphasized by Lepilkina and Grigorieva [22], is undeniably pivotal. Nevertheless, it is important to acknowledge that proteolysis is a multifaceted phenomenon that can be affected by various factors, such as the specific enzymes utilized, the properties of the milk proteins, and the parameters of the cheese production procedure. Hence, acquiring a thorough comprehension of these factors can facilitate the optimization of the proteolysis process and augment the production of bioactive peptides.

The research conducted by Helal et al. [23] offers a noteworthy viewpoint on the temporal aspects of proteolysis and the production of bioactive peptides in whey fermentation. However, further investigation into optimizing the fermentation regime is warranted to maximize the production of specific bioactive peptides. For example, could an extended duration of fermentation lead to an increased concentration of specific peptides? Alternatively, could it result in the deterioration of these peptides and the formation of additional compounds?

The study conducted by Araújo-Rodrigues et al. highlights the potential of utilizing autochthonous starter cultures to improve cheese's sensory attributes and safety characteristics [24]. Nevertheless, it is crucial to consider the potential obstacles linked to this methodology, including the fluctuation in microbial populations within unpasteurized milk and the possible impact of environmental factors on the functionality of these microorganisms.

Furthermore, it is crucial to emphasize the research conducted by Sturova et al. [25], which involved the development of a distinctive approach to cheese maturation. This method involved the utilization of a noble mold derived from a combination of whole milk and secondary protein-carbohydrate raw materials. Their research findings indicated that the proteolysis and lipolysis processes exhibited higher-intensity levels in the experimental cheeses manufactured using a noble mold. Consequently, the final product displayed enhanced organoleptic qualities. Utilizing this methodology can potentially augment the production of bioactive peptides within the context of cheese manufacturing.

In addition, Samelis et al. conducted a study to assess the efficacy of a blended thermophilic and mesophilic starter culture comprising *Streptococcus thermophilus* ST1 and the Greek autochthonous nisin-A-producing *Lactococcus lactis* in the production of the traditional Galotyri Protected Designation of Origin (PDO) cheese [26]. According to their study, it has been indicated that the distinctive characteristics of cheese, including its fermentation techniques and the type of milk employed, have the potential to affect the behavior of bacteria and the subsequent production of bioactive peptides.

In addition, a research study conducted by Moiseenko et al. examined the capacity of *Lacticaseibacillus paracasei* strains, which were obtained from the traditional South African fermented beverage mahewu and kefir grains, to generate bioactive peptides possessing antioxidant and angiotensin I-converting enzyme inhibitory (ACE-I) characteristics during the process of milk fermentation [27]. The current research study provides valuable insights into the exploration of unconventional isolation sources for synthesizing bioactive peptides in the domain of cheese production. To summarize, the generation of bioactive peptides in cheese is a complex occurrence that is influenced by various factors, including the use of diverse starter and nonstarter cultures, the intricacies of the fermentation process, and the unique characteristics specific to the type of cheese being produced. In order to optimize the generation of bioactive peptides and enhance the health-promoting properties of cheese, future investigations should prioritize a thorough analysis of these factors.

In summary, the generation of bioactive peptides in cheese is a complex process influenced by various factors, including the use of diverse starter and nonstarter cultures, the intricacies of the fermentation process, and the unique characteristics specific to the type of cheese being produced. Understanding these factors can help optimize the proteolysis process and enhance the production of bioactive peptides.

Transitioning to the health benefits of these peptides, it is important to note that the bioactive peptides produced in cheese have been associated with various health-promoting effects.

## 2.2 Health-promoting effects of bioactive peptides in cheese

Bioactive peptides in cheese have been associated with various health benefits. These peptides are thought to exert their beneficial effects through various mechanisms, including inhibiting enzymes involved in disease processes, modulation of the immune system, and scavenging free radicals (**Table 1**). These studies present supplementary evidence to substantiate that cheese can produce bioactive peptides that confer various health benefits, including antithrombotic and antidiabetic properties.

In a recent study conducted by Helal et al. [23], the researchers examined six distinct types of cheese to assess the concentrations of bioactive peptides and their corresponding physiological effects. The cheeses examined in this study consisted of three distinct types of Egyptian cheese, namely Karish, Domiati, and Ras, and three internationally popular cheese varieties, including Feta-type, Gouda, and Edam. The study's results revealed that a significant portion of the bioactive peptides identified exhibited inhibitory effects on crucial enzymes associated with the advancement of cardiovascular diseases, specifically angiotensin-converting enzyme (ACE), as well as diabetes, particularly dipeptidyl peptidase-IV (DPP-IV). It is worth mentioning that Gouda cheese exhibited the most significant ACE inhibitory and DPP-IV-inhibitory activities, in addition to displaying the highest level of antioxidant activity. Further investigation has clarified the involvement of bioactive peptides in the composition of cheese and other edible substances. The study conducted by Helal et al. [28] contributes additional knowledge to the ongoing scholarly conversation regarding synthesizing bioactive peptides in cheese. The primary objective of this study was to investigate the effects of spontaneous fermentation and the inclusion of natural whey starters on the peptidomics profile and biological activities of cheese whey. The study revealed spontaneous fermentation incorporating natural whey starters considerably deteriorated whey proteins. The breakdown process facilitated the subsequent release of peptides with biological activity and enhanced the digestibility of the protein content. The researchers conducted an investigation involving identifying more than four hundred peptides. The peptides being examined were primarily derived from β-casein, κ-casein, and α-lactalbumin. Out of the identified peptides, a collective count of 49 exhibited bioactive characteristics, with 21 of these peptides demonstrating inhibitory effects on the angiotensin-converting enzyme (ACE).

| Cheese type | Bioactive peptides | Bioactivities | References |
|---|---|---|---|
| Cheddar | $\alpha_{S1}$- and β-CN fragments | Angiotensin-converting enzyme (ACE) inhibitory<br>Antioxidative | [21] |
| Mozzarella, Crescenza, Italico, Gouda | β-CN f(58–72) | ACE inhibitory | [22] |
| | $\alpha_{S1}$ -CN f(1–9) | ACE inhibitory<br>Cytomodulatory | [23] |
| | β-CN f(60–66) | | [24] |
| Festivo | $\alpha_{S1}$-CN f(1–9), f(1–7), f(1–6) | ACE inhibitory | [25] |
| Emmental | Peptides not identified | ACE inhibitory | [18] |
| Manchego | Ovine $\alpha_{S1}$-, $\alpha_{S2}$- and β-CN | ACE inhibitory | [26] |

**Table 1.**
*Biologically active peptides isolated from several cheeses.*

Moreover, the study additionally revealed that the lactotripeptide isoleucine-proline-proline (IPP) exhibited higher levels compared to valine-proline-proline (VPP), with the most significant concentration observed during the period of spontaneous fermentation after 24 hours. The results of this study indicate that the fermentation process, regardless of whether it occurs spontaneously or is initiated using natural whey starters, plays a significant role in producing bioactive peptides in cheese. Rehman et al. [29] conducted an independent investigation to evaluate the therapeutic efficacy of water-soluble peptides (WSPs) derived from probiotic cheddar cheese produced from buffalo milk. The study focused specifically on the antithrombotic properties of these peptides [29]. The study's results demonstrate a significant increase in antithrombotic activity as the ripening period progressed for both the control and probiotic cheddar cheese samples.

Incorporating a probiotic adjunct in cheddar cheese production led to a significant improvement in its antithrombotic activity. Pontonio et al. conducted an independent study to increase the worth of whey obtained from ricotta cheese. Their approach involved the development of a biotechnological method for generating bioactive peptides that demonstrate inhibitory properties against angiotensin-I-converting enzyme (ACE) [30]. The study utilized a methodology that incorporated the combination of membrane filtration and fermentation techniques. The fermented R-UF demonstrated a notable anti-angiotensin-converting enzyme (ACE) activity. The purified active fractions of the fermented R-UF displayed sequences that partially or fully coincided with κ-casein antihypertensive fragments that have been previously reported. The ricotta cheese, which was enriched with a fortification level of 5%, demonstrated a concentration of approximately 30 mg of bioactive peptides. The investigation conducted by Vázquez-García et al. involved the assessment of peptide fractions found in Mexican goat cheeses during different ripening periods [13]. An examination was conducted to explore the correlation between the peptide fractions and the antioxidative properties demonstrated by the cheeses [13]. The study findings indicate that the observed DPPH radical scavenging activity in ripened Mexican goat cheese can be attributed to the peptides that naturally occur in the milk or are produced as a result of the action of starter cultures during the cheese ripening process.

In addition, Martini et al. undertook a research investigation aimed at assessing the impact of different stages of ripening in Parmigiano–Reggiano (PR) cheese peptide fractions on the enzymatic activity of α-glucosidase, α-amylase, and dipeptidyl peptidase-IV (DPP-IV), as well as the formation of fluorescent advanced glycation end-products (fAGEs) [31]. The PR peptide fractions exhibited inhibitory properties against the specific enzymes and the formation of fAGEs. These studies present supplementary evidence to substantiate that cheese can produce bioactive peptides that confer various health benefits, including antithrombotic and antidiabetic properties. Bioactive peptides demonstrate diverse pharmacological characteristics, encompassing opioid activity, anti-tumor effects, anti-lipidemic properties, and immunomodulatory activities. As depicted by Shafique et al. [32], cheddar cheese-derived bioactive peptides have the potential as functional foods, affecting chronic diseases like obesity, cardiovascular, and diabetes. These peptides have multifunctional therapeutic potentials, including antimicrobial, immunomodulatory, antioxidant, enzyme inhibitory, antithrombotic, and phytopathological effects. They regulate immune, gastrointestinal, hormonal, and neurological responses, which are crucial in disease prevention and treatment. **Figure 2** illustrates the depiction of the action mechanism of bioactive peptides.

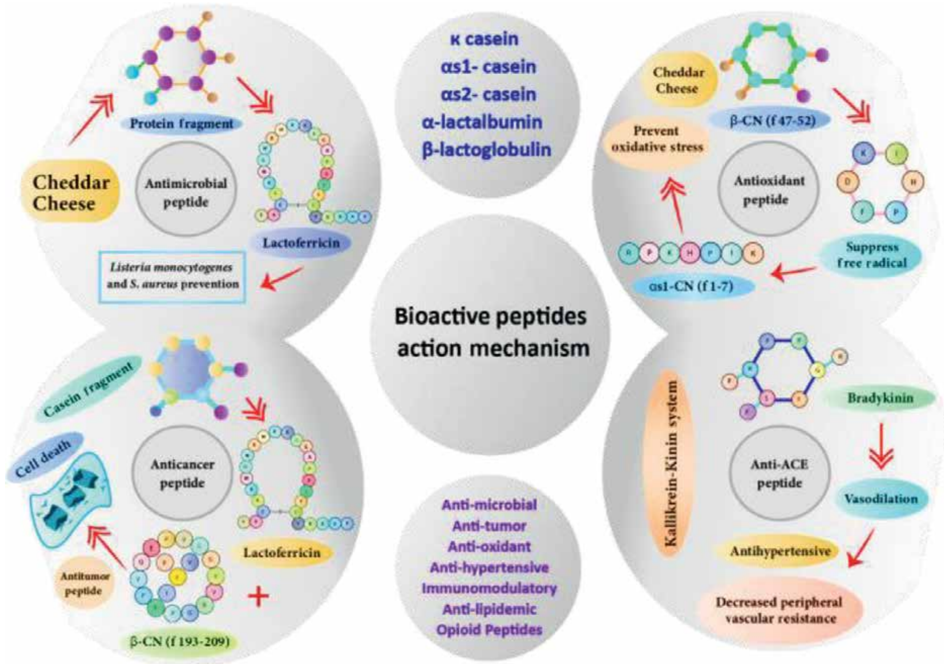


**Figure 2.**
*Action mechanism of bioactive peptides [32].*

In summary, bioactive peptides in cheese have been linked to a range of health benefits, including antithrombotic and antidiabetic properties. These peptides have multifunctional therapeutic potentials, including antimicrobial, immunomodulatory, antioxidant, enzyme inhibitory, antithrombotic, and phytopathological effects. They regulate immune, gastrointestinal, hormonal, and neurological responses, which are crucial in disease prevention and treatment.

In the next section, we will delve into the fatty acid profile of cheese and how it contributes to the overall nutritional value and health benefits of this popular dairy product.

## 3. Fatty acid profile in cheese and its health implications

The fatty acid composition of cheese is primarily influenced by the origin of the milk, as well as the feeding practices of dairy animals and the methods employed during the cheese manufacturing process. The consumption of unsaturated fatty acids has been linked to numerous health advantages. Gas chromatography (GC) is a widely recognized analytical method that provides high precision and accuracy in the quantification of fatty acid composition in cheese. The findings are commonly reported in milligrams of fatty acids per gram of cheese (mg FA/g cheese), as illustrated in **Table 2**. The obtained data comprehensively analyze the fatty acid composition of the cheese, encompassing saturated, monounsaturated, and polyunsaturated fatty acids. The provision of this information is of utmost importance in comprehending the nutritional characteristics of cheese, as distinct fatty acids exert varying impacts on human well-being. For instance, it is widely acknowledged in the academic literature

| | **Samples of cheese** |
|---|---|
| C8:0 | 4.49 ± 1.55 |
| C9:0 | 0.04 ± 0.07 |
| C10:0 | 14.97 ± 4.97 |
| C11:0 | 0.13 ± 0.05 |
| C12:0 | 8.04 ± 1.90 |
| C13:0 | 0.18 ± 0.08 |
| C14:1 | 0.96 ± 0.56 |
| C14:0 | 23.07 ± 4.69 |
| C15:0 | 1.35 ± 0.61 |
| C16:1 | 2.50 ± 0.67 |
| C16:0 | 73.75 ± 19.02 |
| C17:1 | 0.50 ± 0.21 |
| C17:0 | 0.96 ± 0.33 |
| C18:2 | 6.15 ± 1.71 |
| C18:1 cis | 38.62 ± 7.93 |
| C18:1 trans | 5.57 ± 3.95 |
| C18:1 total | 50.40 ± 11.29 |
| C18:0 | 27.36 ± 7.38 |
| C19:0 | 0.06 ± 0.08 |
| C20:0 | 0.29 ± 0.14 |
| ∑SFA | 154.69 |
| ∑UFA | 54.35 |

**Table 2.**
*Cheese's fatty acid profiles (mg FA/g cheese) as determined by gas chromatography [33].*

that monounsaturated and polyunsaturated fatty acids are generally regarded as advantageous for maintaining cardiovascular health.

The fatty acid composition of cheese exhibited notable variations throughout the production season, potentially influenced by many factors, including the grazing patterns of cows, moisture content in their feed, ambient temperature, lactation stage, and the overall health condition of the animals [34].

Recent studies have investigated dietary factors' influence on dairy products' fatty acid composition. For example, a research investigation on Kivircik ewes revealed that the incorporation of 3% palm oil into their dietary regimen resulted in a notable elevation in the concentration of palmitic acid in their milk, escalating from 28% to 36% [35]. The observed rise in palmitic acid concentration in the milk corresponds to a 29% increase, assuming that the palm oil utilized contained 80% palmitic acid. The research study additionally demonstrated that the incorporation of palm oil into the dietary regimen resulted in elevated levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), primarily attributable to heightened concentrations of palmitic acid (C16:0) and oleic acid (C18:1), respectively.

The ramifications for human health resulting from alterations in fatty acid composition are multifaceted and intricate. Based on a specific perspective, there exists evidence indicating a potential association between certain saturated fatty acids, namely lauric (C12:0) and myristic (C14:0) acids, and a decreased risk of developing coronary heart disease [36]. On the other hand, incorporating palm oil into one's dietary intake leads to an elevation in the collective levels of saturated fatty acids, which have been established to have adverse effects on human well-being [37].

Their fatty acid profile determines the nutritional quality of dairy products, and various indices are employed to evaluate the diet's nutritional value and its impact on consumer health. The research presented empirical findings that the inclusion of 3% rumen-protected palm oil in the dietary regimen enhanced ewe milk's health characteristics, particularly in relation to the optimal levels of desirable fatty acids (DFA) for the n-6/n-3 ratio. Despite the lack of observed improvement in the atherogenicity index (AI), thrombogenicity index (TI), health-promoting index (HPI), and h/H (hypocholesterolemic/hypercholesterolemic acids) indices, the values obtained in this study align with those documented in prior scholarly works. This discovery suggests that the milk examined in the current investigation does not harm consumers' health [35].

The research conducted by Zajác et al. examined the fatty acid composition of traditional Slovak cow's lump cheese, as depicted in **Figure 3** [38]. The cheese, which a nearby local farmer manufactures, exhibited diverse levels of fatty acids. The primary fatty acids identified in cow's lump cheese were palmitic acid (C16:0) with a concentration range of 29–37 g/100 g, oleic acid (C18:1) (n-9) with a concentration range of 19–26 g/100 g, myristic acid (C14:0) with a concentration range of 10–13 g/100 g, and stearic acid (C18:0) with a concentration range of 8–10 g/100 g. The investigation recorded noteworthy alterations in the concentrations of particular fatty acids throughout the production duration. The research determined that the fat content of cow's lump cheese was 23%, with a standard deviation of ±1.5%. The lowest observed fat content was 21%, whereas the highest recorded fat content was 27%. The cheese

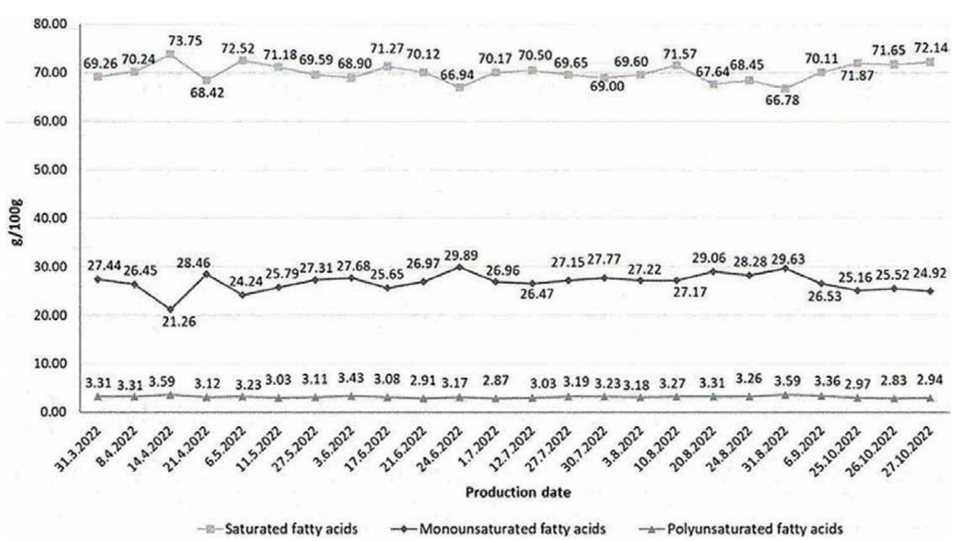


**Figure 3.**
*Cow's lump cheese combines saturated, monounsaturated, and polyunsaturated fatty acids [38].*

sample exhibited a composition of 70 g/100 g for saturated fatty acids, 26 g/100 g for monounsaturated fatty acids, and 3 g/100 g for polyunsaturated fatty acids.

The sensory attributes of cheese, including color, texture, flavor, and nutritional value, are notably impacted by the composition of fatty acids in the cheese. Various types of fatty acids have the potential to impact human health, with certain ones offering benefits while others may pose potential risks. The fatty acid composition can also function as a discriminative characteristic for different types of cheese, depending on the milk utilized during their manufacturing process. In their investigation, González-Martín et al. utilized Near-Infrared Spectroscopy (NIRS) technology to determine the presence of 19 fatty acids in cheese, ranging from C8:0 to C20:0. The evaluation involved the analysis of both the aggregate amount of saturated fatty acids ($\sum$SFA) and the aggregate amount of unsaturated fatty acids ($\sum$UFA). The research study substantiated the feasibility of Near-Infrared Spectroscopy (NIRS) as a prompt, dependable, and effective technique for obtaining data on the lipid composition of cheese samples [33].

Furthermore, it is important to exercise caution when interpreting the use efficiencies of diet ingredients in production systems that employ varying ingredient inclusion rates. The organic production systems demonstrated higher efficiency in nongrazing and concentrated utilization, underscoring the significance of incorporating pasture in cows' diets. This approach reduces the reliance on costly ingredients that compete with human food sources and presents opportunities to enhance profitability and sustainability within the system by promoting greater pasture intake. The existing data do not provide sufficient evidence to support the notion that incorporating higher levels of concentrate or nonpasture feeds in the diets of organic cows is justified. This is because the data does not demonstrate that organic herds possess a greater capacity to utilize concentrate or nonpasture ingredients more effectively than conventional herds.

The current study's findings suggest that organic herds demonstrated a reduced occurrence of mastitis cases, expressed as a proportion of the overall herd, compared to conventional herds. This observation is consistent with the findings documented by Ellis et al. in their study [39]. In contrast, Stergiadis et al. [40] observed no significant variation in mastitis occurrences across herds with varying levels of production intensity, ranging from organic to highly intensive. Nevertheless, in terms of numerical data, it was observed that the higher-intensity systems exhibited a greater incidence of mastitis cases, even in the presence of preventive antibiotic measures. Nevertheless, the Redundancy Analysis (RDA), as determined in the present study, aligns with the findings of Stergiadis et al., as it reveals the presence of adverse correlations between grazing practices and incidences of mastitis [40]. Hence, the elevated levels of pasture consumption within organic systems may have limited significance for mastitis. In their study, Ellis et al. [39] and Ward et al. observed a correlation between enhanced cow cleanliness on farms and decreased mastitis cases and somatic cell count (SCC). However, the authors reported no significant disparity in cow cleanliness during outdoor grazing between organic and conventional systems [41]. The study provides no documentation regarding the cleaning and milking strategies employed.

Consequently, it is not feasible to make any assertions regarding the potential influence of these strategies on mastitis cases. Nevertheless, previous research has established a correlation between genetic selection for a high milk yield, particularly observed in the Holstein breed, which was more prevalently utilized in the conventional herds examined in this current study, and somatic cell count (SCC) levels, consequently leading to mastitis [42]. The present study observed that the organic herds exhibited reduced milk yields and somatic cell counts (SCC), alongside a greater proportion of lower-yielding breeds such as Ayrshire and Shorthorn. This particular breed composition could potentially account for the decreased incidence of mastitis.

Previous research has demonstrated that low-input and organic milk display reduced levels of saturated fatty acids (SFA) compared to high-intensity and conventional milk [43, 44]. In contrast to prior research, the current study reveals divergent outcomes. Prior studies have demonstrated a reduced concentration of saturated fatty acids (SFAs) in organic milk compared to milk derived from intensively managed herds [45]. The observed disparity has been ascribed to the elevated intake of fresh herbage within organic farming systems. However, no notable differences were observed between the organic and conventional systems regarding pasture intake. The variation in intake was minimal, measuring 123 g/kg dry matter (DM) [46]. The present study reveals a comparable pattern, wherein the disparity in the proportion of total forage and pasture between the conventional and organic herds was found to be less than 131 g/kg and 166 g/kg DM per day, respectively. The results derived from the RDA demonstrate a significant association between the consumption of whole crops and grass silage and the saturated fatty acid (SFA) content of milk compared to grazing. As mentioned earlier, the observation is supported by the research conducted by Ellis et al., wherein their investigation revealed that incorporating whole crops and grass silage into the diet increased saturated fatty acids (SFA) levels in milk [47].

Furthermore, Ormston et al. [48] discovered a positive correlation between saturated fatty acid (SFA) consumption and grass and maize silage intake. The observed disparity in organic diets, with average values of 778 g/kg DM and 920 g/kg DM for whole crop and grass silage, respectively, compared to conventional diets, may have played a significant role in the elevated levels of saturated fatty acids (SFA) found in organic milk during the present investigation. Furthermore, substantial evidence supports that breed is crucial in determining the fatty acid (FA) profile. This is evident from studies that have observed lower concentrations of saturated fatty acids (SFA) in Holstein-Friesian cows' milk than in other breeds [48, 49]. The present study's findings also demonstrated a positive association between certain individual fatty acids (C6:0, C8:0, C10:0, C12:0, and C14:0) and non-Holstein breeds, as indicated by the results obtained from RDA. However, the disparities in the breed systems were not found to be statistically significant. Conventional herds exhibited a slight numerical advantage of approximately 14 Holstein cows per 100 cows compared to crossbreeds and organic herds. The observed variation in the composition of the herd may have played a role in the observed rise in the levels of specific saturated fatty acids (SFAs) in the milk, including C6:0, C8:0, and C14:0. It is worth mentioning that the somatic cell count (SCC) in organic milk exhibited a higher value exclusively during September to December, a period characterized by minimal or zero pasture intake in both agricultural systems. This finding is consistent with the research conducted by Butler et al. [43], which observed a higher level of saturated fatty acids (SFA) in milk from organic farms compared to nonorganic low-input farms during the indoor period in August and October.

In summary, the fatty acid composition of cheese has complex and multifaceted implications for human health. While certain saturated fatty acids have been linked to an increased risk of cardiovascular disease, other fatty acids, such as monounsaturated and polyunsaturated fatty acids are generally regarded as beneficial for cardiovascular health. The fatty acid profile of cheese can also be influenced by various factors, including the grazing patterns of cows, the moisture content in their feed, ambient temperature, lactation stage, and the overall health condition of the animals. Therefore, understanding these factors and their impact on the fatty acid profile of cheese is crucial for optimizing the health benefits of cheese consumption.

In the following section, we will delve into the innovative strategies aimed at enhancing the health benefits of cheese consumption. Specifically, we will focus on methods designed to increase the production of bioactive peptides and optimize the fatty acid profile in cheese, as these components play a crucial role in promoting health and preventing disease.

## 4. Innovative strategies for enhancing bioactive peptides and optimizing fatty acid profile in cheese

In conjunction with naturally existing compounds, novel methodologies are being devised to augment the bioactive peptide composition and optimize the fatty acid configuration in cheese. One strategy that has been explored is the utilization of bioactive edible films. These films have demonstrated the potential to enhance the microbial characteristics of cheese and mitigate lipid oxidation, consequently prolonging its shelf life [50, 51]. In cheese preservation, Aloe-based bioactive edible films have been observed to enhance the cheese's lipid stability and microbial quality (**Figure 4**). *Aloe vera*, a botanical species widely recognized for its therapeutic attributes, encompasses diverse bioactive constituents, such as polysaccharides, vitamins, enzymes, and antioxidants. Including these bioactive compounds in an edible film can provide advantages to the packaged food item [7]. The Aloe film has been found to contain bioactive compounds that can inhibit lipid oxidation, which significantly contributes to the degradation of cheese quality [7].

Consequently, lipids' stability is improved, maintaining the cheese's sensory attributes and nutritional composition throughout its shelf life. In addition, the antimicrobial properties of *Aloe vera* have been found to contribute to the preservation of the microbial quality of cheese [7]. The bioactive compounds present in the Aloe film exhibit inhibitory effects on spoilage microorganisms and pathogens, thereby effectively prolonging the cheese's shelf life and ensuring its safety for consumption.

Encapsulation of bioactive compounds is a technique employed to improve the stability and functionality of these compounds [52], presenting itself as a promising method. Pop et al. [53] thoroughly examined the encapsulation process for *Moringa oleifera* bioactive compounds in their study. The authors emphasized the significant potential of encapsulation techniques in facilitating the incorporation of bioactive molecules into various food products.

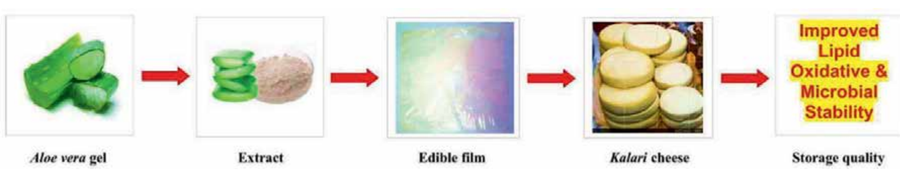


**Figure 4.**
*A bioactive edible film derived from Aloe improved the lipid stability and microbial quality of cheese [7].*

Furthermore, the utilization of whey proteins in cheese manufacturing is gaining traction owing to their advantageous effects on human health. Whey proteins are a significant provider of essential amino acids, including leucine, isoleucine, and valine, classified as branched-chain amino acids [50]. Rapidly digestible proteins offer older individuals enhanced nutritional advantages compared to casein and other protein sources. As a result, their extensive integration into clinical nutritional products has been observed [50].

Nevertheless, the general reception of whey protein-fortified products among consumers tends to be low owing to unfavorable flavor and aroma characteristics and negative mouth-feel attributes. These include the accumulation of mouth-drying, mouth-coating, chalky, metallic, and filming sensations associated with repeated consumption, as indicated by previous research [50]. Hence, further investigation is necessary to improve the sensory characteristics of these products, thereby augmenting consumer acceptance and appropriateness. Concerning the composition of fatty acids, the utilization of distinct starter cultures and adjuncts has the potential to yield cheese products that exhibit an elevated content of conjugated linoleic acid (CLA) and omega-3 fatty acids, which are known to possess health-promoting properties [45].

Furthermore, it should be noted that specific processing techniques, such as high-pressure processing (HPP), have the potential to modify the fatty acid composition of cheese. The study conducted by Inácio et al. [54] proved that subjecting cheddar cheese to high-pressure processing (HPP) resulted in an elevation of unsaturated fatty acid levels, particularly oleic acid.

In summary, pursuing novel approaches to augment the bioactive peptide composition and optimize the fatty acid makeup in cheese represents a vibrant and auspicious realm of scholarly investigation. The abovementioned strategies are designed to enhance cheese's nutritional composition, prolong its shelf life, and enhance its sensory characteristics.

## 5. Conclusions

Exploring cheese as a source of bioactive peptides and fatty acids has opened new avenues in functional foods. This chapter has provided a comprehensive overview of the current knowledge and recent advancements in this area. Bioactive peptides generated during cheese fermentation and maturation have been associated with various health benefits, including managing obesity and hyperlipidemia. They exert their beneficial effects through various mechanisms, such as inhibiting enzymes involved in disease processes, modulating the immune system, and scavenging free radicals. The content of these peptides in cheese is influenced by several factors, including the type of milk used, the cheese-making process, and the specific strains of bacteria involved in fermentation.

The fatty acid profile of cheese, particularly the unsaturated fatty acids, also plays a crucial role in human health. The type of milk used, the diet of the dairy animals, and the cheese-making processes largely determine this profile. Recent research has shown that specific starter cultures and adjuncts can produce cheese with a higher content of health-promoting fatty acids, such as conjugated linoleic acid (CLA) and omega-3 fatty acids.

Innovative strategies are being developed to enhance the bioactive peptide content and optimize the fatty acid profile in cheese. These include the use of bioactive edible films and the encapsulation of bioactive compounds, which have shown promise in improving the microbial quality of cheese, reducing lipid oxidation, extending its shelf life, and improving the stability and functionality of these compounds.

In conclusion, cheese, a staple food item consumed worldwide, holds significant potential as a source of health-promoting bioactive peptides and fatty acids. The ongoing research and development in this field are expected to produce cheese varieties with enhanced health benefits, thereby contributing to the growing functional foods market. However, further research is needed to fully understand these bioactive compounds' mechanisms of action and optimize the cheese-making processes for their production. The potential of these findings extends beyond the cheese industry, offering insights that could be applied to the broader field of food science and nutrition.

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## Conflict of interest

The authors declare no conflict of interest.

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Section 3

# Functional Cheese

**Chapter 4**

# Perspective Chapter: Beyond Delicious – The Hidden Functional Benefits of Cheese

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## Abstract

Cheese; a diverse and healthy milkproduct with a long history that stretches back thousands of years. It is available worldwide in varying forms and is valued for its delicious taste and superior nutritional content. Classification of cheese is dependent on texture or moisture content, method of coagulation or coagulating agent, maturation or ripening, type of milk and manufacturing techniques. Cheese is comprised of macronutrients, micronutrients and functional nutrients; major macronutrients in cheese are proteins and fats, major micronutrients in cheese include vitamins and minerals and functional nutrients in cheese include cheese bioactive peptides, polyphenols, probiotic, prebiotic, conjugated linoleic acid, sphingolipids, phytanic acid, lactoferrin, γ aminobutyric acid and organic acids. Other than its great taste and flavor cheese is responsible for providing many health benefits i.e. gut protecting activity, antioxidative activity, anticariogenic activity, antihypertensive, antihyperglycemic, cardioprotective and osteoprotective activity to the body. This chapter will focus on the classification, nutritional composition and health benefits of cheese.

**Keywords:** cheese, nutritional content, macronutrient, macronutrient, health benefits

## 1. Introduction

Cheese is a soft solid mass of milk comprised of water, protein, fatty acids, minerals, and vitamins. It is considered a high-quality product due to its high biological value and nutritional composition, produced via fermentation, coagulation, separation, and maturation of milk components [1, 2]. Around 8000 years ago, during the agricultural revolution, cheese first developed in the areas of Iraq between the Euphrates River and Tigris known as the Fertile Crescent. Since Mesopotamian times, cheese production has played a significant role in the history of humans due to the domestication of various plants and animals as a source of food. Milk from several domesticated animals such as goats, sheep, and cattle was used as a growing medium for bacteria to produce different milk products such as cultured milk, yogurt, and cheese [3–5].

Despite the decrease in per capita consumption of dairy items, cheese consumption remains high throughout the world. It is projected that by 2032, the global processing of milk into various milk products, especially cheese, is to increase by 30%. European Union, United States, and New Zealand are expected to be the major exporters of cheese that will fulfill around 65% of the cheese demand globally. However, Saudi Arabia, Russia, Japan, and the United Kingdom are forecasted as the main importers by 2032 [6].

The nutritional composition of cheese is influenced by numerous factors including animal, breed, stage of lactation, and fat content in the milk. It is a nutrient-rich product; abundant in casein, fatty acids, fat-soluble vitamins, and minerals that are retained in curd during processing [2]. The widespread acceptance of cheese is attributed to several factors i.e. nutritional composition, health benefits, variety, and compatibility [7].

Owing to the recent increasing trends of functional foods, cheese manufacturers are exploring the possibility of using cheese as a functional food and have also produced reduced-fat cheese as a result [7]. Food biotechnology has played a significant role in the cheese industry, leading to the development of a wide range of cheese. Besides the fact that cheese was discovered unintentionally, it is regarded as an exceptional product that possesses various health benefits [3].

## 2. Classification and types of cheeses

Cheese is a concentrated form of milk that has been manufactured and used by humans since the dawn of civilization. Till now, there are more than 1500 different variants of cheese that have been discovered and each variety possesses unique properties [8]. There are no specific criteria for the classification of cheese. However, some integrated approaches have opted to represent the varieties of cheese.

Types of cheese are classified on the basis of [9, 10]:

- Texture or moisture content
- Method of coagulation or coagulating agent
- Maturation or ripening
- Type of milk
- Manufacturing techniques

### 2.1 Classification based on texture or moisture content

Classification of cheese based on texture primarily relies on the total moisture content. Moreover, moisture level directly imparts the firmness or texture of cheese as higher water content results in a softer texture. **Table 1** represents the categories of cheese based on moisture content [8, 10–12].

| Category | Moisture | Example |
|---|---|---|
| Very Hard Cheese | <25% | Grana Padano and Parmigiano Reggiano |
| Hard Cheese | 25–35% | Cheddar, Cheshire, Gloucester, Cantal, and Leyden |
| Semi-Soft/Semi-Hard Cheese | 36–40% | Colby, Monterey, Lancashire, and Wensleydale |
| Soft Cheese | >40% | Cottage, Mozzarella, Brie, and Camembert |

**Table 1.**
*Classification of cheese based on texture or moisture content.*

## 2.2 Classification based on the method of coagulation or coagulating agent

Coagulation of milk means the conversion of liquid milk into a semi-solid mass or coagulum. Different coagulating agents/methods including enzymes, acid, heat/acid, and concentration/crystallization can be used for milk coagulation [8, 10, 13, 14].

### *2.2.1 Enzymatic coagulation*

Enzymatic coagulation of milk is the most preferable method adopted by cheese manufacturers for cheese production. This process involves a proteolytic enzyme known as chymosin or renin, which is synthesized by the chief cells of the stomach. Previously, it was extracted from the dried stomach of milk-fed calves. However, due to the advancement in the field of biotechnology, a genetically engineered product rennet is developed. Rennet coagulated cheeses are the most abundantly produced as they account for more than 75% of total cheese production, and they also incorporate all the ripened cheeses. The production of rennet-coagulated cheese is divided into two stages (**Figure 1**). The first stage involves the conversion of milk particles into para-casein micelles. In the second stage, micelles aggregate in the presence of calcium ions and at a temperature of >20°C to form a gel-like structure (coagulum). Parmesan, gouda, and cheddar are some examples of rennet-coagulated cheeses [11, 15, 16].

### *2.2.2 Acid coagulation*

The isoelectric pH for the milk to curd is 4.6 and it is achievable by adding acids such as vinegar (acetic acid) and lemon juice (citric acid) to the milk. Acid triggers the partial unfolding of casein molecules and creates a mesh-like structure by interlinking. These interconnected micelles transform the milk into a semisolid state. Acid-coagulated cheeses like cottage, quarg, cream cheese, and some types of Queso Blanco account for more than 25% of the total cheese produced. Acid-coagulated cheeses are usually consumed fresh because of high contain high moisture content [10, 16–18].

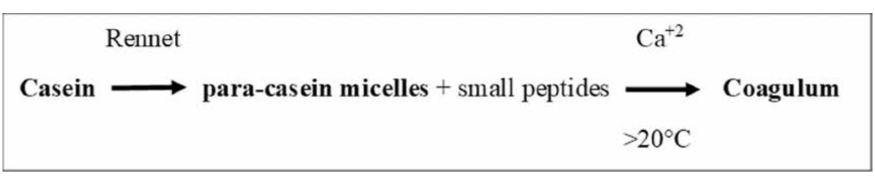


**Figure 1.**
*Stages of rennet coagulated cheese production.*

*2.2.3 Heat/acid coagulation*

Milk can coagulate at pH > 4.6, but this requires an elevated temperature of >80°C. Before the addition of acid, milk is boiled to obtain soft curds such as ricotta, mascarpone, and impastata. Heat/acid-coagulated cheeses are mostly a mixture of whey and milk but can also be made by using whey solely [10, 16, 19].

*2.2.4 Concentration/crystallization*

The concentration/crystallization method of coagulation is used to produce whey cheese by crystallizing lactose and concentrating the whey. Whey cheese like mysost, mysusostur, brunost, mesost, myseost, and braunkäse exhibit a smooth, creamy texture and a luscious caramel-like flavor. Such cheeses are predominantly prepared in northern European countries like Germany, Norway, Sweden, Iceland, and Denmark [8, 10].

### 2.3 Classification based on maturation or ripening

Maturation or ripening is a complicated procedure that can enhance the sensory properties of cheese, it involves various metabolic, biochemical, and microbial changes. Primary changes involve processes like proteolysis, glycolysis and lipolysis, and glycolysis. Whereas secondary changes include catalytic activities such as decarboxylation, esterification, amino acid desulfurization, deamination, and fatty acid oxidation. These changes are influenced by several factors including coagulating agents, enzymes (lipases and proteinases), starter and non-starter cultures and their enzymes, and secondary microbial cultures and their enzymes. Numerous factors including the type of cheese, moisture level, and the desired flavor determine the duration of ripening ranging from weeks to years. However, the ripening process can be accelerated by genetically modifying the starter culture, increasing the concentration of the enzymes, and elevating the temperature and pressure of the system [10, 16, 20–22].

### 2.4 Classification based on milk type

Milk is a lacteal secretion that varies in composition from one species to another. However, the main components such as carbohydrates, proteins, water, fats, vitamins, and minerals remain consistent. The variation in nutrient composition among the milk of distinct species contributes to unique flavors, textures, and aromas in cheese [10, 13, 23]. Milk with elevated total solids and casein levels with a smaller casein micelles size and the BB variant of kappa-casein exhibit better cheese quality [24]. The preferable milk sources for cheese production include cattle, buffalo, goats, and sheep [25].

*2.4.1 Cattle milk*

The cheese industry predominantly relies on cow milk accounting for more than 85% [10, 26]. The nutrient composition of cattle milk represents that it contains 3.7% fat, 3.4% protein, 4.8% lactose, and 0.4% ash (minerals and vitamins). Additionally, it yields a flavor-dense and creamy-textured cheese. A wide range of cheeses is produced from cow milk ranging from soft to extra hard and not ripened varieties.

Cheeses like Cheddar, Brie, Feta, Mozzarella, and Gouda are prepared by rennet coagulation of heat-treated cattle milk [23, 27].

#### *2.4.2 Buffalo milk*

Unlike cattle milk, buffalo milk has a high protein and fat content, 4.7% and 6.7%, respectively. However, both contain similar levels of ash (0.8%) and lactose (4.8%). It also has an enhanced casein-to-protein ratio and calcium content than other varieties. Buffalo milk cheeses such as Mozzarella di bufala and Campana represent about 11% of the total cheese production. Italian cuisine frequently uses buffalo milk cheese, especially in dishes such as caprese salad, pizza, and pasta. Owing to the savory flavor and creamy texture, buffalo milk cheeses are more expensive than others [10, 23, 26, 28].

#### *2.4.3 Goat milk*

Since ancient times, goat milk has been used to prepare cheese. The nutrition profile of goat milk reveals that it contains 4.5% fat, 2.9% protein, 4.1% lactose, and 0.8% minerals and vitamin content. Goat milk cheese like Chevre, Feta, and Bûcheron has a soft and creamy texture because of its high moisture content (more than 70%). Additionally, such cheeses feature a distinctive flavor featuring earthy, nutty, and mildly sweet characteristics. Goat milk cheeses are rich in calcium, easy to digest, and do not require a long maturation duration. Besides this, cheeses made from goat milk account for only 2% of all production [10, 23, 26, 27].

#### *2.4.4 Sheep milk*

Sheep milk has higher consistency than goat and cow milk because of its high-fat content (~7.4%). Furthermore, it is more nutrient-dense than other milk varieties, comprising 4.5% protein, 4.8% lactose, and 1.0% ash. Cheese made from sheep milk represents only 2% of the total cheese manufactured. Goat milk cheeses have a distinct flavor and rich, creamy texture. Owing to the low moisture content, the coagulum is firm leading to rapid synesis and a slower diffusion of sodium chloride. Feta, Roquefort, and Pecorino are among the well-known varieties of sheep milk cheeses [10, 23].

#### *2.4.5 Mixed milk*

Cheeses made from a combination of milk from various species are known as mixed milk cheeses. The nutrient composition, flavor, and texture of cheese vary with the type of milk. Notable varieties include Ossau-Iraty Valdeon and Gorgonzola. Ossau-Iraty and Gorgonzola comprise cow and sheep milk, and Valdeon is a cow and goat milk cheese [10, 13].

### 2.5 Classification based on the manufacturing techniques

Cheese making is a complex process involving the selection, pretreatment, standardization, acidification, and coagulation of milk, dehydration of coagulum, and the shaping along with salting of cheese curds. Moreover, some varieties necessitate the maturation/ripening of cheese as an additional step. Dehydration is a prime step in the cheese manufacturing process as it concentrates the milk and enhances the shelf life of the cheese. Numerous factors, including milk type, pH, cultures, moisture, and salt concentration, generate distinct flavors and textures of cheese. These distinctive characteristics primarily enhance during ripening/maturation. Therefore, manufacturing steps largely influence the nature and quality of the final cheese product and result in the emergence of a new classification system [3–5, 13, 29, 30]. Cheeses with different manufacturing techniques are as follows:

#### *2.5.1 Brined cheese*

Brined cheeses also referred to as pickled cheeses, are those that mature in brine water. Ripening in brine solution preserves the cheese by reducing the moisture content. A high concentration of salt enhances microbial stability by increasing the osmotic pressure. The texture of pickled cheeses like Feta and Domiati varies from semi-hard to soft. Fresh brined cheeses have an acidic and salty flavor and develop a piquant flavor upon ripening. During the maturation process, the cheese is immersed in a 14% salt (NaCl) solution and allowed to mature for a minimum of seven days at a temperature of 14–16°C. Once the pH level drops to 4.5, it is stored for at least two months, at a temperature of 3–4°C [14, 31].

#### *2.5.2 Surface-ripened cheese*

Surface-ripened cheeses are a diverse class characterized by the development of microbial or fungal growth during ripening. These cheeses are further categorized based on microbial growth as smear-ripened or washed-rind and mold-ripened cheeses. The distinctive characteristics of surface-ripened cheeses rely on the microbial (bacteria or fungal) growth on the surface of the cheese.

Smear-ripened cheeses like Brick, Beaufort, Comté, Italico, and Butterkase develop a thick layer of bacterial growth on the surface during maturation after washing with a salt solution. These are also known as bacterial surface-ripened or red-smear cheeses because of the red smear. *Brevibacterium linens, Brevibacterium aurantiacum Arthrobacter arilaitensis, Arthrobacter bergerei, Agrococcus casei, Microbacterium gubbeenense, Staphylococcus succinus* subspecies *Casei, Staphylococcus equorum* subspecies *linens*, and lastly *Corynebacterium casei* are a few well known bacterial species involved in the production of smear-ripened cheese.

Mold-ripened cheeses like Camembert and Brie develop a layer of fungi. Such cheeses create a compound ecosystem consisting of filamentous, acid, and salt-tolerant species of fungi such as *Debaroymces hansenii, Geotrichum candidum,* and *Penicillium camemberti).* Furthermore, mold-ripened cheeses have a soft texture [10, 14, 32–34].

#### *2.5.3 Blue cheese*

Blue cheeses or blue-vined cheeses belong to the non-surface ripened cheeses. Inculcation of *Penicillium Roquefort* (a fungus) in milk creates unique blue vines varying from whitish and pale green to dark blue-green. The aging of cheeses encourages the mold to grow under specific humidity and temperature conditions. Blue cheeses have a soft texture and a piquant flavor due to the n-methyl ketones produced by extensive lipolysis. Some well-known types of blue cheese include Bleu d'Auvergne, Cabrales, Gorgonzola, Danablu (Danish Blue), and Stilton, and Roquefort [10, 14, 35–37].

#### *2.5.4 Nutritional composition of cheese*

In addition to its delicious flavors and textures, cheese is a fantastic dairy product with exceptional nutritional makeup. Understanding cheese's potential as a food with considerable health benefits requires a thorough comprehension of its nutritional composition. Cheese is a prime example of the nutritional power of dairy products, because of its high protein content, different fat content ranges, and a wide variety of crucial vitamins and minerals. Understanding cheese's macronutrient and micronutrient makeup reveals the breadth of its nutritional value and emphasizes the importance of cheese as an important part of a balanced diet. By delving into the nutritional details of cheese, cheese also provides many functional nutrients important for human health [38, 39].

### 2.6 Macronutrients

#### *2.6.1 Proteins*

Cheese is a great source of high-quality proteins providing many physiological functions and contains all the essential amino acids except methionine and cysteine. [38]. Major proteins present in cheese include casein 97–98% and 2–3% whey. The digestibility of proteins in cheese is increased with the ripening of cheese because as the cheese ages the casein in protein is further broken into free amino acids and water soluble peptides. The high amino acid content in cheese promotes human growth and development. As the protein from cheese gets bioavailable to the body it provides anti-antibacterial, antioxidative, antihypertensive, antithrombotic, cholesterol lowering, and immune-modulating activities [39].

#### *2.6.2 Fat*

The fat content of cheese varies between 20 and 35% of its dry mass, of which 66% are saturated fatty acids (SFA), 30% are monounsaturated fatty acids (MUFA), and 4% are polyunsaturated fatty acids (PUFA) including omega-3 and omega-6 fatty acids. The fat content of cheese is dependent on the type of cheese, animal nutrition, manufacturing processes, and some other factors [40, 41]. Oleic acid is the most prevalent unsaturated fatty acid found in milk fat. The concentration of Tran's fatty acids in cheese is varied as per the season. In comparison to winter, the cheese composition in the summer is lower in saturated fatty acids (SFA), and higher in cis-monounsaturated fatty acids (MUFA) and total trans fatty acids. Trans-18:1 and − 18:2 isomers exhibit seasonal fluctuation as well [42]. Cheese's flavor, texture, and nutritional value are all influenced by these fatty acids.

Fatty acids in cheese also provide many health benefits i.e. saturated fatty acids have a variety of physiological functions and are crucial for controlling cell functioning, gene expression, genetic regulation, PUFA bioavailability, and fat synthesis [39]. However unsaturated fatty acids present in cheese support the health of the brain, heart, and eye, as well as promote anti-inflammatory properties. Particularly among unsaturated fatty acids; omega-3 fatty acids have been linked to brain and cardiovascular health [43].

#### *2.6.3 Carbohydrate*

Cheese contains carbohydrates in the form of lactose; as it ripens most cheese varieties become lactose-free, advantageous for individuals with lactose intolerance.

People on low-carb or ketogenic diets benefit from its low carbohydrate content and low glycemic index, which helps to regulate blood glucose levels. [39, 44, 45].

## 2.7 Micronutrients

### *2.7.1 Minerals*

An abundant quantity of calcium is present in cheese in the form of colloidal calcium phosphate (CCP) which is essential for healthy bones, adequate neuron functioning, and muscular contraction. The concentration of calcium in cheese is varied on the basis of the type of cheese, the manufacturing process, and the aging of cheese. As compared to soft and fresh varieties of cheese, hard and aged cheeses usually have greater calcium content. Hard cheese contains around 800 mg of calcium per 100 grams. Due to the acidification of the milk in the containers, soft cheese has a lower calcium concentration than semi-hard and hard cheeses [38, 46]. Absorption of calcium in the body is affected by various factors i.e. pH, calcium-binding proteins, and interactions with other nutrients in the digestive system [39, 47]. In addition to calcium, cheese is also a good source of phosphorus, magnesium, and zinc, important for overall bone and dental health [7].

### *2.7.2 Vitamins*

The concentration of fat soluble vitamins is dependent on the fat content of the cheese. During cheese processing, the majority of fat-soluble vitamins are retained whereas some water-soluble vitamins are lost as a result of losses in whey. Vitamin A, B2, B12, and B9 are often found in cheese and remain stable after pasteurization and ripening. Hard cheese contains 10% of daily calories, 15% vitamin A, 10% vitamin B2, 20% vitamin B6, and 40% vitamin B12. Although the concentration of vitamins in cheese might vary depending on farming practices, nutrition, mastitis, and milk heating [38, 39].

Cheddar cheese has been consumed by many people as a vitamin D substitute. It has been recommended to fortify low-fat cheeses with vitamin A and D to compensate for the loss of these fat soluble vitamins during milkfat separation. Vitamin A and E are present in the form of β- carotenes, α-tocopherol, and γ-tocopherol; aid in providing antioxidant properties and help to regulate normal physiological functions of the body. However, B vitamins in cheese have a number of health advantages i.e. helps in supporting the immune system, aids in managing nervous system health, and helps to regulate energy metabolism [39, 48].

### *2.7.3 Functional nutrients*

In addition to its basic nutritional composition, cheese also contains functional nutrients that offer potential health benefits beyond basic nutrition. These functional nutrients include bioactive compounds (polyphenols), peptides, probiotics, and other components that may positively impact human health.

### *2.7.4 Cheese bioactive peptides*

Cheese bioactive peptides are protein fragments in the form of small amino acid sequences released during the ripening of cheese. Different cheese varieties contain distinct sets of peptides, contributing to the unique flavors and potential health effects. These bioactive compounds function as antioxidants, and anti-inflammatory agents, and even provide antibacterial properties; aiding the body's defense against potentially dangerous substances. Some cheese peptides have been shown to exhibit Angiotensin converting enzyme (ACE) inhibitory properties, which means they can help control blood pressure by blocking the angiotensin-converting enzyme [41].

#### *2.7.5 Polyphenols*

Polyphenols are naturally present in cheese and during the process of cheese making they interact with milk proteins; essential for cheese production and human nutrition. They improve the nutritional value and functionality of cheese i.e. they serve as antioxidative, anticancerous, and anti-inflammatory agents. Polyphenols also aid in the prevention of, cardiovascular disorders, diabetes mellitus, and neurological illnesses [49].

#### *2.7.6 Probiotic*

Probiotics are living organisms that work with the objective of immunological, antagonistic, and competitive forces to promote the host's health. The major naturally occurring gut florae are *Bifidobacterium* and *Lactobacilli*, which are intentionally added to milk products like cheese and yogurt during the fermentation process. Important health benefits of these microbes include antimicrobial activity, improved gastrointestinal and immune health, and antimutagenic and anti-carcinogenic activities [50, 51].

#### *2.7.7 Prebiotic*

Prebiotics are indigestible carbohydrates that provide sustenance for healthy microbes in the stomach, fostering their development and activity. Some percentage of prebiotics is present in some varieties of cheese i.e. Galactooligosaccharides, synthetic fructooligosaccharides (FOS), oligofructose, inulin, and lactulose. These prebiotics influence physiological and metabolic functions i.e. they help to improve health and decrease the likelihood of various diseases. They serve a significant role in improving renal functional tests, they provide cardiovascular support, manage gastrointestinal health, improve bone mineral density by increasing the absorption of calcium, magnesium, copper, and, zinc and also strengthen the immune system [52].

#### *2.7.8 Conjugated linoleic acid (CLA)*

CLA is a fatty acid found in the milk of ruminant animals, it is a class of linoleic acid (C18:2) isomers containing conjugated double bonds. Cheese made from the milk of grass-fed animals may contain higher levels of CLA. It has been associated with several potential health benefits i.e. it helps in reducing high body mass, helps in muscle coordination, and prevents cellular inflammation. CLA's antioxidant activity promotes anti-carcinogenic effects and also helps in reducing the risk of atherosclerosis. It also exhibits antiadipogenic, anti-diabetogenic, and anti-inflammatory effects [41].

#### *2.7.9 Sphingolipids*

The second-best source of sphingolipids after soybeans is milk and its products, especially cheese. Sphingolipids are made up of a sphingoid base, a fatty acid, and numerous head groups providing distinct biological roles. The concentration of sphingolipids in cheese is modified by many factors i.e. animal breed, food, and cheese-making procedures. Sphingomyelin content in cheese per kilogram is 115 mg. Sphingolipids are responsible for providing many health advantages i.e. cardiovascular health support, immune system modulation, prevention against cancer, and helps in proper brain functioning [39].

#### *2.7.10 Phytanic acid*

Another important functional nutrient present in cheese is Phytanic acid; (C20) fatty acid. Phytanic acid is a C20 saturated fatty acid with four methyl branches which has been documented to provide anti-carcinogenic properties, improve glucose homeostasis and increase hepatocyte glucose absorption [41].

#### *2.7.11 Lactoferrin*

Lactoferrin is a glycoprotein present in milk and dairy products. It yields microbicidal peptides such as lactoferricin and lactoferrampin. Lactoferrin is beneficial for the human body i.e. it possesses antibacterial, immune modulating, and anti-inflammatory properties [53].

#### *2.7.12 γ aminobutyric acid*

γ aminobutyric acid (GABA) is a 4-carbon nonprotein amino acid; produced during the fermentation process during cheese making. The total content of GABA in cheese is dependent on the kind of cheese, ripening stage, amount of protein, natural microbiota, and activity of proteolytic enzymes. All cheeses contain a certain amount of GABA but commercial French cheeses contain great concentrations. GABA in the human body works as an antihypertensive, immunomodulatory, antidiuretic, and tranquilizing agent. GABA can manage diabetes mellitus, treat depression, and alcoholism, control pain, prevent anxiety, and boost the concentration of growth hormones [41].

#### *2.7.13 Organic acids*

Organic acids are naturally occurring compounds that form during the fermentation and aging process of cheese, providing distinct flavor and a variety of health benefits. Organic acids produced during cheese fermentation and processing include lactic acid, acetic acid, butyric acid, propionic acid, and hippuric acid. These acids serve many health benefits i.e. they serve as antibacterial, anti-inflammatory, and gut-producing healthy bacteria [41].

### 2.8 Health aspects

Cheese is a fermented dairy product that contains beneficial microorganisms and health promoting components that help to maintain a good nutritional status [54].

#### *2.8.1 Gut protective activity*

Chief microorganisms found in cheese include Probiotic *Lactobacillus spp.* and *Bifidobacterium spp*. These microorganisms help in forming healthy gut microbiota and are thus beneficial in suppressing the growth of harmful bacteria. According to a study published in the Journal of Applied Microbiology, Cheese contains particular probiotic strains that help to form short-chain fatty acids which are beneficial for gut health. The presence of probiotic strains in cottage cheese leads to excellent probiotic microbe survival, forms good metabolic behavior, and also leads to the production of potential antioxidant peptides and antilisterial substances in the gastrointestinal tract [55].

Fortification of cheese with *L. helveticus SBT2171*; a microorganism that helps in regulating T cells (Treg) and also prevents in controlling & preventing inflammatory response against IL-17, IL-4, and IL-10 production in gut Peyer's patch cells in healthy mice. This fortified cheese also helped in improving colitis symptoms in rats with Dextraran Sulphate Sodium-induced colitis. Fortification of Italian hard cheeses with *L. helveticus R0389* leads to the reduction of intestinal epithelial cell chemokine production [56].

#### *2.8.2 Anti-oxidative activity*

Cheese contains sulfur amino acids, whey proteins (especially -lactoglobulin), vitamins A, E, and C, and -carotene all of these contribute to the antioxidative activity. Fermentation and cheese maturation is linked to the formation of bioactive peptides, which are responsible for enhancing antioxidative properties. The use of probiotic strains significantly boosts antioxidant levels. Antioxidants in cheese can scavenge superoxide, hydroxyl, and peroxide radicals, as well as reactive oxygen species. Cheese fermented with *Lactobacillus acidophilus* has significantly improved antioxidant activity. Cheeses are fortified with *L. acidophilus 2499;* a microorganism that contains a good percentage of L-carnosine and anserine [57, 58]. L-carnosine has a role in chelating metal ions and possesses antiglycating, antioxidative, and anti-crosslinking characteristics. Carnosine plays a great role in muscular health and has a role in preventing neurodegenerative illnesses [59].

#### *2.8.3 Anti-cariogenic activity*

Cheese contains an abundant amount of calcium, and plays an important role in increasing the pH of the oral cavity thus preventing the process of demineralization. Other than calcium, *Casein*; an anti-cariogenic phosphoprotein found in cheese creates a protective layer on the tooth enamel decelerating the process of caries development [60]. Consumption of cheese after the consumption of fermentable carbohydrates prevents the decline in pH and promotes anticariogenic activity [61]. Cheese has a pH that is near neutral or slightly alkaline, which helps to balance the acidic conditions in the mouth. Its high calcium and phosphorus content contributes to the cariostatic mechanism. Cheese also helps in reducing radiation caries caused by head and neck cancers. According to certain research, consumption of cheese among both non-irradiated and irradiated people showed surface enamel rehardening; promoting saliva production through mastication [62, 63].

#### *2.8.4 Antihypertensive activity*

Cheese contains two well-known antihypertensive bioactive peptides i.e. *VPP (Val-Pro-Pro)* and *IPP (Ile-Pro-Pro)* present in a variety of cheeses (Gouda, Domiati, and Edam) [64]. Other than these bioactive peptides cheese also contains

(RPKHPIKHQ9, YPFPGPI) which plays a role as an antihypertensive agent and as an Angiotensin Converting Enzyme (ACE)-inhibitor [65]. In another study, a decrease in total cholesterol and low-density lipoprotein was seen after three weeks of following a hypocaloric diet accompanied by probiotic cheese containing *L. plantarum TENSIA*; a microorganism. This probiotic cheese has great potential for decreasing metabolic syndrome symptoms and arterial blood pressure [66]. Cheddar cheese fortified with *Lactobacillus casei 279*, *Lactobacillus casei LAFTI L26, Lactobacillus acidophilus LAFTI L10, Lactobacillus rhamnosus 6134,* and *Lactococcus lactis* showed enhanced bioactivity for ACE inhibition and high blood pressure control [67].

#### *2.8.5 Anti-cancerous activity*

Cheese made from the milk of ruminant animals such as cow and sheep contains a fatty acid known as *conjugated linoleic acid* [49] and possess anti-carcinogenic qualities. Contents found in CLA are *cis*-9, *trans*-11 CLA, and *trans*-11 C18:1 all are responsible to suppress cancer cell development and are responsible to promote malignant cell death. CLA has been found to prevent a variety of health conditions responsible for causing cancer i.e. obesity, inflammation, atherogenicity, and immunomodulation [68, 69]. CLA suppresses the growth of human malignant melanoma and colorectal cancer cells and is cytotoxic to MCF-7 cells; causing breast adenocarcinoma [70].

Cheese is high in calcium, which has been linked to lower the risk of colon cancer. Some studies have shown that consuming more calcium, notably from dairy products like cheese, may protect against the development of colorectal adenomas and colorectal cancer [71]. *Lactobacillus plantarum Lb41*; a microorganism has been used in the fortification of cheese as it possesses anticancer properties [72].

#### *2.8.6 Anti-hyperglycemic activity*

Cheese is very much beneficial in maintaining high blood sugar levels due to the presence of fats proteins, calcium, and bioactive peptides. Proteins have a role in delaying carbohydrate digestion thus slowing the surge in blood sugar level. Fats slow digestion and also help to bind carbohydrates, thus preventing them from being absorbed rapidly into the bloodstream. Calcium keeps the body's cells open to insulin and thus prevents insulin resistance and enhances insulin sensitivity [73]. Bioactive compounds present in cheese (di-peptidyl-peptidase-IV, DPP-IV) have a role in preventing the development of diabetes mellitus [74].

#### *2.8.7 Cardioprotective activity*

Cardiovascular Disease (CVD) is a broad term that includes a variety of conditions such as heart failure, atherosclerosis, cerebrovascular disease, peripheral vascular disease, and other cardiac abnormalities. Bioactive compounds i.e. *VPP* and *IPP* are the most prevalent cardio-protective peptides found in hard cheese and provide cardio protective activities i.e. ACE inhibitory action. The primary isomer of CLA found in cheese is *Cis9 trans11 C18:2* [49]. It is responsible for anti-atherosclerotic, antioxidative, and anti-inflammatory activities. Isolation of oleic acid [cis9trans11 C18:2 [49]] from hard cheeses and blue cheeses had shown to have cardio protective properties [75, 76]. Cheese has a high concentration of vitamin K2; which functions in lowering vascular calcification [77].

#### *2.8.8 Osteoprotective activity*

Cheese is a good source of calcium, vitamin D, and proteins; all of these are the three primary nutrients that govern bone structure and maintenance. Bone growth and development is a very major concern in infancy and adolescence; its consumption should be according to the recommendations in these lifespans for healthy bone development. Parmesan cheese a type of cheese is a "functional food" for bone health and osteoporosis prevention because it includes high biological value protein and calcium that is easily accessible [78]. Phosphate is essential for the mineralization of cartilage and osteoid tissue, found in high concentrations in protein-rich foods such as Swiss cheese (500 mg/100 g). Calcium obtained from cheese has a role in enhancing bone mass more than calcium tablets. The mass of cortical bone tissue has been raised by cheese consumption because of its high calcium content. According to the study, consumption of 20 g of cheese have a role in lowering the risk of hip fractures in both men and women by 10–15% [79].

Certain cheeses, such as Gouda and Brie, contains vitamin K2, a type of vitamin K. Vitamin K2 is associated with improved bone health because it improves calcium metabolism and directs calcium to the bones rather than soft tissues [80]. Vitamin K is also required as a cofactor in the carboxylation of glutamic acid important to control vascular calcification and bone metabolism [81].

## 3. Conclusion

Cheese is a premium food product manufactured from fermented milk that is rich in proteins, vitamins, minerals, and milk fat. It is made through whey separation, maturation, and the coagulation of milk proteins. Since ancient civilizations, cheese has been a common dietary item. Its nutritional benefit is related to the healthy milk components that it contains. There are over 1500 kinds of cheese that are well recognized, each with its distinct characteristics. Cheese has a protein content that ranges from 4–40%, making it a rich source of high-quality proteins. Its fat content ranges from 20 to 35% of its dry mass, with the summer months having greater levels of fatty acids than the rest of the year. Cheese is advantageous for lactose-intolerant people and those following low-carb or ketogenic diets because it ripens by partly eliminating lactose with whey and fermenting the remaining lactose to form lactic acid and other compounds. Numerous micronutrients, including as calcium, phosphorus, zinc, magnesium, and vitamins, are present in it. Functional elements including polyphenols, peptides, and probiotics found in cheese enhance human nutrition and calcium absorption. GABA, organic acids, and Lactoferrin all improve flavor and quality. It has a long shelf life, beneficial bacteria, antioxidant and anti-cariogenic effects, and antihypertensive and anti-cancerous characteristics. Overall, cheese is a nutritious and healthful dairy food that supports general health and wellbeing.

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Section 4

# Camel Milk's Cheese and Paneer Cheese

Chapter 5

# Recent Trends on Camel Milk Cheese Processing: Nutritional and Health Value

*Zeineb Jrad, Olfa Oussaeif and Halima El-Hatmi*


## Abstract

The capacity of dairy components to prevent chronic diseases has piqued researchers' interest in the role they play in the creation of functional meals. In this regard, the demand for camel milk has increased dramatically due to its outstanding therapeutic properties and health-promoting effects. Ever since ancient times, camel milk has only ever used unprocessed for the consumption of the nomads and their own families. The limited use of camel milk is due to its manufacturing difficulties. For a long time, cheese-making from camel milk was considered a challenge, due to its unique composition. However, due to the development of processes, and enzymatic and microbial technologies, the dairy sector is now able to offer consumers camel cheese with improved functionality and nutritional advantages. The current chapter highlights the recent processing opportunities regarding the cheese-making from camel milk and summarizes existing knowledge on the nutritional value of camel milk cheese.


**Keywords:** camel milk, cheese, nutritional value, dairy processing, biological activities

## 1. Introduction

Currently, great importance is given to nutrition as a vector of health. Milk is one of the food substances considered very nourishing and necessary for the growth of young children. Over the years, a particular attention has been paid to milk from non-bovine species such as goat, ewe, mare, and camel. The camel milk sector is gaining importance due to the particular composition of this milk and its potential value in improving the consumer health. Indeed, some people start consuming camel's milk because they think that camel's milk can cure various diseases, such as jaundice, diabetes, ulcers, autism, asthma, allergies, and cancer [1]. Several studies have provided an opportunity to understand the potential benefits of camel milk through the production of bioactive peptides. Thus, numerous such peptides are identified such as angiotensin-converting enzyme inhibitors, antimicrobial, anti-inflammatory, anti-obesity, antioxidant as well as antidiabetic and anti-proliferative peptides [2–5]. In recent years, the research on camel milk transformation has gradually increased. Much of the work in this field has focused on making yogurt, butter, ice cream, and cheese from camel milk. However, one of the major issues confronting the camel milk cheese-making is the low yield, the long coagulation time, the watery consistency, and the fragile and poor structure, affecting both product performance and customer sensory perception [6]. This behavior is probably due to the large size of casein micelles [7], the low amount of κ-casein to β-casein ratio and its specific enzyme cleavage site, and the lack of β-lactoglobulin in camel milk [8]. The small size of camel milk fat globule could be another cause of poor gel structuring in camel milk [9].

Many attempts have been made to solve this problem including the use of enzymes as coagulants and/or the addition of acidification of the milk with different starter cultures or acids [10–15]. Some researchers mixed camel milk with other milk in order to enhance the yield and organoleptic properties of camel cheese [16–18], whereas others used plant protease [19–22] or microbial transglutaminase [22–24]. Additionally, it could be possible to overcome the associated difficulty regarding camel milk cheese-making through the application of new technologies [8, 25] or optimization of processing conditions [7, 26–28].

In the current chapter are highlighted advanced approaches used in camel cheese-making process steps. In addition, selected camel cheese varieties' specific characteristics and their typical nutritional value and functionalities are also described.

## 2. Constraints associated with camel's milk cheese-manufacturing

Cheese is one of the most consumed dairy products worldwide due to its tremendous nutritional benefits such as richness of proteins, fatty acids, calcium, vitamins A and $B_{12}$, and bioactive peptides coming from milk fermentation with lactic acid bacteria [29]. Cheese is usually produced from casein coagulation and precipitation following acidifying or/and fermentation.

The main factors responsible for crud formation are listed as follows:

- Whey proteins (β-lactoglobulin (β-LG) and α-lactalbumin (α- LA)) and their thermal stability,
- Characteristic of casein micelle (size and mineralization),
- Ratio κ-casein/β-casein content,
- Total solids content and
- Size of the fat globules.

Moreover, these different components have particularities in camel milk. It is found that whey proteins in camel milk account for 20–25% [30]. As in human milk, the major and most allergenic protein of bovine milk, β-lactoglobulin (β-LG), is not detected in camel milk, but it is the α-lactalbumin (α-LA) which is the most abundant protein [31, 32]. The absence of β-LG in camel milk presents itself as an obstacle in the camel cheese-making. In fact, the implication of β-LG on milk transformation is important mainly through its heat-induced association with κ-CN.

Since heat treatment is necessary for eliminating harmful bacteria, extending the shelf life of milk, and guaranteeing its safety for human consumption, it is a crucial component of the dairy processing sector [33]. It is known that the manufacture of cheese is always preceded by a step of milk heat treatment (pasteurization). Differing from cow milk, camel milk is less stable and more sensitive to various heat treatments and this instability provides a crud with a weak structure [34]. It is important to note that casein distribution and micelle size are limiting factors in cheese coagulation. The primary structure of the four caseins was elucidated by Kappeller et al. [35] deducing that camel caseins are less phosphorylated and less rich in micellar calcium phosphate than their bovine counterparts. The camel casein micelles are also larger in diameter than bovine milk casein, this character associated with reduced surface area provided a long coagulation time and weak cheese coagulum [36]. Camel's milk caseins, on the other hand, are richer in proline residues (particularly β-casein), residues known by their stereo-chemical rigidity, thus explaining the destabilization of the secondary structures of these proteins in a more pronounced way than occurs in bovine caseins. Camel κ-casein represents 3.5% of total camel caseins and it contains two phosphate residues present in two positions: $Ser^{141}$ and $Ser^{159}$. Only κ-casein is a glycoprotein with amphiphilic properties. Camel and bovine κ-casein do not have the same affinity for camel and bovine rennet calf [35].

The cleavage site of camel κ-casein by chymosin is different from that of bovine κ-casein, the hydrolysis taking place at the $Phe^{97}$-$Ile^{98}$ bond splitting a macro-peptide of 65 amino acids. A classification into two groups of κ-caseins of different species has been proposed by Nakhasi et al. [37]. These two groups differ in the site of cleavage by chymosin: Phe-Met bond for group I (ewe, buffalo, goat, and cow) and Phe-Ile or Phe-Leu for group II (Camel, woman, rat, mouse, and sow). This difference probably reflects differences in the ability of ruminant and non-ruminant milk to coagulate.

The protein κ-casein is considered the constituent limiting the growth of submicelles as well as the size of the micelles [35]. It is also the stabilizing factor of the micelle thanks to the hydrophilic C-terminal groups of this protein which are responsible for the steric repulsive forces, which oppose the flocculation of the micelles [38]. For these reasons, κ-casein is most likely localized to the periphery of the casein micelle.

On the other hand, the high amount of β-casein seems to have an important implication in the softer aspect of camel cheese compared to bovine one. It is the major protein in camel milk and represents more than 65% of total caseins. As compared to the other milk proteins, CN-β is more hydrophobic and exhibits more chaperone-like behaviors, which prevents protein aggregation [35]. Moreover, CN-β with its amphiphilic nature rises for non-polar residues to adsorb at hydrophobic surfaces, resulting in good emulsifying properties that are responsible for the smoothness of cheese [39]. Apart from this, milk fat also plays an important role in cheese quality and yield. Camel milk fat globules, surrounded by thick membranes, are small in size ranging from 1 to 9 μm in diameter according to Mehaïa et al. [40]. Camel milk fat is also distinguished by high levels of phospholipids [41], which are good emulsifiers, that give camel milk cheese its soft texture and great water retention.

## 3. Camel milk cheese historical background and recent insights into cheese-making

In the past, people used the process of creating cheese to preserve milk. The first cheese was made in the United States. There were more than 45 million kg of cheese manufactured in the United States in 1849. Raw milk was used to make the majority of the cheese. Despite being a novel technology, pasteurizing milk for making cheese was not widely practiced in 1914. The most popular cheeses today are Parmesan and

Gorgonzola from Italy, Emmental from Switzerland, Roquefort and Camembert from France, and Edam from Holland. Since then, cheese has gained enormous popularity throughout the world. Research on cheese from minor milk species has increased in the last 30 years, especially for camel milk. This interest was initially influenced by the difficulties of camel's milk coagulation. Furthermore, its technologies that are currently being applied for the cow's cheese-making are not successfully applicable to turn camel milk into cheese. Earlier attempts at creating camel cheese involved combining camel milk with goat and sheep milk and the use of bovine rennet, but the obtained coagulum was extremely soft and crumbly [42, 43]. After that, the difficulty of camel milk coagulation and the low cheese's yield has been confirmed by the author's observations [44, 45] who are into the idea of overdosing the rennet concentrations about four times more than that used for cow milk. The used rennet was that of the calf combined with a coagulant preparation derived from a mold conventionally used in the dairy industry named *Rhizomucor miehei*. Additionally, they demonstrated how the camel milk may be supplemented with calcium chloride or calcium phosphate to somewhat sidestep this challenge. In fact, salts modify the ionic environment of the casein micelle and bring about a lowering of the pH value of the milk, which promotes the activity of the rennet and the process of aggregation of the casein leading to the coagulation. Unlike, the obtained cheese turned out to be fragile and crumbly, the cheese's yield was low with high loss in fat-which led to not creamy product.

More recently, many researchers used Camifloc—a specific rennet used to coagulate camel milk—as a coagulant [28, 46]. Later, some authors used Camifloc to coagulate camel milk either by adding sheep's milk at 50 and 75% levels [47] or by varying the levels of added salts [28]. In concert, these results demonstrate a reduction in coagulation time and an improvement of the texture of cheese as well as sensory appreciation. Other alternative has been considered such as introducing the camel chymosin synthesis gene into *Aspergillus niger* to obtain a specific recombinant enzyme for camel milk coagulation named Chymax-M1000® produced by Ch. Hansen©. Hence, the camel cheese qualities were enhanced [10, 26]. Then, several studies have demonstrated that the association of camel chymosin with starter cultures improves the camel cheese yields. Different starters were added like thermophilc, mesophilic or blended strains, and yogurt starters [10, 19, 21, 48, 49]. Walle et al. [50] reported that cooking camel milk coagulated with camel chymosin combined with starter cultures improved the hardness of cheese.

The production issues related to camel cheese were resolved by mixing the milk of other bovines such as cow, buffalo, sheep, and goat milk in order to boost the casein concentration in camel milk. According to the research of Shahein et al. [17], the 30% (w/w) addition of buffalo milk to camel milk improved the rennet's ability to coagulate, increased curd yield, improved curd hardness, reduced weight loss, and improved the sensory and microbiological quality of the finished product [17]. However, Saadi et al. [18] produced soft cheese by adding sheep's milk to camel milk, and discovered a significant improvement in the yield and cheese quality.

Moreover, processing conditions could affect greatly the quality and the nutritional value of produced cheese. For instance, raising the total solid content in camel milk using the ultrafiltration process was found to increase the cheese yield, firmness, and nutritional value due to the end product's higher protein and fat, in comparison with conventional processing [8, 51]. Furthermore, the addition of *Allium roseum* powder to UF camel milk produces a camel cheese with higher anti-oxidant activities [8]. Likewise, it has been documented that cheese hardness is significantly influenced by the milk pasteurization temperature, high-pressure treatment, and pre-acidification. Mbye et al. [25] showed that camel milk pasteurization at temperatures not exceeding 65°C for 30 min or high-pressure processing at 350 MHz for 5 min at 4°C are more effective in providing cheeses from camel milk with a semi-hard texture.

In fact, the most notable modifications during cheese-making are observed after high-pressure processing or homogenization resulting in the reduction of the size of the native milk fat globule. In addition to that, high-pressure processing alters the conformational shape of casein micelles by reducing electrostatic and hydrophobic interactions, which causes micellar fragments to disaggregate and improves milk's physico-chemical and technological applications. Casein micelles are broken apart, increasing the surface area and hastening the rennet coagulation process. Consequently, this treatment can lead to the formation of new clusters of fat and protein, providing the opportunity for many different cheese textures. Some research has come out and has been studied the effect of pre-acidification technology on camel cheese aspect. The pre-acidification of the milk before adding the enzyme can be done by adding directly acids or by using bacteria that can metabolite lactic acid. In both cases, the presence of acid in the milk attributes to the decrease of its pH value and decreases the coagulation time of camel milk [10–15].

Other trials have been tested by various authors in order to improve camel milk cheese's textural and sensorial qualities. These include the use of rennet substitutes of animal origin such as bovine pepsins and pepsins extracted from poultry proventriculi such as chicken and duck [18, 52]. Recently, a great deal of research has been undertaken in order to find effective and competitive coagulants using plant extracts such as ginger (*Zingiber officinale*) [53], *Moringa oleifera* L. [20], Withania (*Withania coagulans*) [21], and nettle (*Urtica dioica*) leaves [19]. The results of these studies showed that the ability of partially purified plant extracts to coagulate camel milk and form a firm curd. It has also been demonstrated that camel milk cheese produced using a combination of withania extract and camel chymosin exhibited a higher quality when compared to chymosin alone [21]. At the same time, other studies have been devoted to improving camel milk coagulation using microbial transglutaminase (MTGase). Abou-Soliman et al. [23] reported that adding MTGase to camel milk at a concentration of 80 U after 20 min of renneting is recommended to improve the yield, textural, and some sensory properties of soft cheese made from camel milk. Alia et al. [22] found that MTGase and the *Cynara cardunculus* L. flower extracts showed excellent coagulating properties and immense potential as coagulants for cheese production using camel milk.

## 4. Camel cheese nutritional value and bioactive components

### 4.1 Substances composition of camel cheese

The variation of composition observed in the camel cheese may be due to the original milk composition and cheese-making processing circumstances. The total solids components, including protein and fat, progressively concentrate into the cheese curd depending on how the cheese is prepared and how the whey is drained. Additionally, the kind, amount of ash, and salt addition can all affect the minerals in the cheese during the cheese-making process. In addition to its impact on milk clotting, the acidification process is essential for the removal of colloidal minerals from casein micelles, coagulant retention in the curd, coagulum strength, and cheese yield. The chemical composition of the different camel cheeses is detailed in **Table 1**.

| Cheese variety | Total solids | Protein | Fat | Yield | Reference |
|---|---|---|---|---|---|
| | | | Soft unripened Cheese | | |
| | | | With whole camel milk | | |
| | | | Enzymes used as coagulant | | |
| Without any addition | 28.38–39.90 | 15.62–22.32 | 0.88–20.21 | 11.4–13.07 | [11, 13, 49, 51, 53–55] |
| With different level of salts | 31.10–42.92 | 15.29–21.59 | 15.69–18.51 | 13.45–13.95 | [18, 26, 28, 55] |
| With processed milk | 29.54–40.9 | 13.2–21.90 | 0.15–22.2 | 11.7–17.0 | [8, 16, 25] |
| With pre-acidification | | | | | |
| Direct acidification | 34.0–41.28 | 8.56–35.55 | 3.85–16.50 | 9.86–25.56 | [11, 13, 14, 56] |
| Starter cultures | 30.90–44.36 | 11.12–21.30 | 13.14–20.91 | 13.22–18.10 | [13, 15, 23, 49] |
| | | | Plant protease as coagulant | | |
| | 35.40–40.76 | 10.16–16.40 | 10.71–19.7 | 8.70–11.73 | [14, 25] |
| With other milks | | | | | |
| Cow milk | 32.51–38.58 | 25.70–31.55 | 3.73–3.97 | — | [56] |
| With buffalo milk | 41.24–45.33 | 13.10–14.03 | 17.50–20.50 | 14.7–20.1 | [17] |
| With sheep milk | 42.92–53.26 | 21.59–25.27 | 18.51–24.9 | — | [18, 57] |
| | | | Domiati-Type cheese | | |
| | 29.4–46.5 | 13.51–20.61 | 1.0–26.2 | — | [12] |
| | | | Mozzarella cheese | | |
| | 46.3 ± 0.42 | 29.0 ± 0.59 | 13.9 ± 0.44 | — | [58] |
| | | | Akawi-cheese | | |
| | 37.6–38.1 | 23.3–23.7 | 9.8–9.8 | — | [58] |
| | | | Brined cheese | | |
| | | | Soft brined cheese | | |
| Day of ripening | | | | | |
| 0–60 | 45.02–36.46 | 20.37–14.39 | 26.0–22.75 | 9.43–13.44 | [48, 59] |
| 0–30 | 35.39–36.68 | 14.44–12.67 | 13.20–14.25 | 13.22 | [60] |
| 0–7 | 48.17–49.07 | 29.25–40.48 | 1.30–1.17 | 65.57 | [61] |
| | | | Dry brined cheese (Feta-type) | | |
| | 48.68 ± 5.65 | 30.64 ± 3.90 | 57.4 ± 2.70 | | [62] |

**Table 1.**
*Composition and yield of different types of camel milk cheese (%).*

## 4.2 Proteins

Protein is one of the main nutritional substances in camel milk cheese. The protein content in camel milk directly affects the nutritional value of camel dairy products. The protein content in camel milk cheese is affected by several factors, such as the type of starter culture used for cheese-making, meaning that a camel cheese made with thermophilic (STI-12) and blended (RST-743 and XPL-2) cultures had a significantly higher protein value [15]. The variation of protein content into the categories of camel cheese obtained might be attributed to the processing condition of cheese manufacture. The ultrafiltration process and cheese fortification enhanced significantly the protein content of soft camel cheese [8]. Moreover, the protein content into the cheese curd depends on how the whey is drained. Besides mixing camel milk with other dairy animal milk has a substantial effect on the protein amount in camel cheese [58].

### 4.3 Fat

Fat is an important factor that may be responsible for cheese quality. Fat contents are progressively concentrated into the cheese curd according to cheese processing and the method used to drain the whey. Castillo [63] reported that the rheological and micro-structural properties of gels' strength and the higher curd loss from the cheese vat resulted in excess whey fat loss. A huge variation of fat content in camel mozzarella cheese blends of bovine milk and 30% of camel milk [58]. A significant difference in fat cheese was observed in soft white cheese with different starter cultures [15], whereas the variation of percentages of salt to make Domiati-type camel cheese has no effect on fat content [12]. El-Hatmi et al. [8] reported a low content in soft camel cheese due to a loss of fat in permeate during the ultrafiltration process.

### 4.4 Total solids

According to the studies focused on camel cheese characterization, there is a variation in total solids observed, and this variation might be attributed to the original milk composition on protein and fat, the processing condition of cheese manufacturing and the method used in why draining. In fact, total solids are concentrated into the cheese curd. The acidifying process of milk during cheese-making, is a determining factor in the dry matter content of cheese, and this is due to its important role in the removal of colloidal minerals from casein micelles, the retention of coagulant in the curd, syneresis of the gel, coagulum strength, and cheese yield [64, 65].

### 4.5 Biological properties

The bioactive peptides derived from camel milk proteins and products, particularly fermented milk, have received much attention during the last decade. However, limited studies have been done with camel cheese as a source of bioactive peptides. There are mainly two approaches used to produce bioactive peptides from camel milk, that is, bacterial fermentation and enzymatic hydrolysis. Identified camel peptides of single and/or multiple functions have been reported as follows:

- *Anti-oxidant peptides:*

Several anti-oxidant peptides have been obtained from the action of digestive enzymes or lactic acid bacteria proteinase on camel milk casein. These peptides have been identified as fragments of a camel β-CN, α-CN, GlyCAM-1, and PGRP-1 [2, 66, 67]. To our knowledge, antioxidant peptides of camel cheese have never been identified, but the anti-oxidant activity of camel milk cheese was well documented. El-Hatmi et al. [8] reported that UF camel milk cheese exhibited antioxidant activity and this power was improved after cheese fortification with *Allium roseum* powder [8] or quinoa flour [68].

Whereas Abou Soliman et al. [23] showed that the cross-linking between camel-milk proteins caused by MTGase negatively influenced the antioxidant activity of cheese.

- *Antimicrobial peptides:*

The presence of antimicrobial activity in the camel whey protein digests has also been reported [2]. Jrad et al. [69] identified cationic peptides from the peptic digests of camel lactoferrin (LF). They found these peptides to have antimicrobial activities against *Listeria innocua*. Digestion of camel LF with pepsin resulted in an antimicrobial peptide homologous to cow lactoferrampin B. The camel lactoferrampin represents LF fragment f 271–284 with the sequence LVKAQEKFGRGKPS [69]. In addition, other antibacterial peptide derived from a camel β-CN was identified and presented high homology with casesidin bovine peptide [70]. Low molecular-identified antimicrobial peptides were generated from fermented camel milk with *Lactobacillus plantarum* [71].

- *ACE-inhibitory peptides:*

The ACE-inhibitory peptides found in hydrolyzed camel proteins and in products of camel milk have received much attention. The ACE-inhibitory peptides isolated from the digests of camel milk casein with potential activity are dipeptides ("AI," "IY," "VY," "LY," "TF") and tri-peptides ("IPP," "LHP") [72]. The angiotensin-converting enzyme inhibition of camel cheese was also investigated, but there is no information about derived peptides [73].

- *Anti-diabetic peptides:*

Camel milk constitutes a center of interest for scientists due to its known beneficial impact on diabetes. Identification of camel milk-derived peptides and their structure-activity relationship study and characterization in the context of molecular markers related to diabetes are studied. The main targeted enzymes for their inhibition by camel milk proteins/peptides are carbohydrate digestive enzymes, specifically intestinal α-glucosidase and pancreatic α-amylase, and the dipeptidyl peptidase IV (DPP-IV), an enzyme that breaks down major incretin hormones that stimulate the release of insulin in response to glucose [3, 74]. It is now obvious that identified anti-diabetic peptides that target the key molecular pathways involved in overall glucose homeostasis liberated from both camel milk whey and casein [75]. Moreover, camel cheese exhibited α-amylase and α-glucosidase inhibition activity [73].

Extensive other *in vitro* studies have found that camel milk and its derived products possess anti-obesity, anti-biofilm, anti-cancer, anti-inflammatory, anti-hemolytic, and anti-hyperpigmentation activities. This provides potential for the development of functional products using camel milk.

## 5. Conclusions

Worldwide, camel milk and its derivative products production and consumption have increased due to its medicinal and health-promoting potential, which makes it the best choice as a substitute of cow's milk. However, the processing methodology of camel milk into dairy products is facing several difficulties. In fact, making cheese from camel milk using the same conventional methods used for cheese manufacture from cow's milk is challenging and occasionally impossible because of a number of issues, such as prolonged coagulation times, weak curd formation, and ultimately lower cheese yield. Therefore, with current advancements in dairy technology, the processing of camel milk into cheese become now possible. Camel milk cheeses made with camel chymosin, with starting cultures and through technological processing, have significantly improved in some cheese-making qualities. Moreover, it was discovered that clotting camel milk with plant protease and chymosin is a successful method to make cheese from camel milk. Additionally, camel milk has significant enhancement in various features of cheeses when combined with other milk species. Some studies have focused on the functional characteristics and nutritional quality of camel milk cheeses, but additional clinical studies are required to confirm the therapeutic effects of these functionalities in the human body.

## Conflict of interest

The authors declare that they have no conflict of interest.

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**Chapter 6**

# Indian Cheese Revolution: *Withania coagulans* in Dairy Industry

*Mayur Ram, Bimal S. Desai and Sumankumar S. Jha*

## Abstract

Commonly called as Indian Cheese Maker, Paneer dodi, Paneer phool and Vegetable rennet, *Withania coagualans* belongs to family Solanaceae and widely used in Indian System of Medicines due to its anti-diabetic, anti-microbial and immune modulator properties. The specific epithet coagulans reveals its coagulating properties and hence used in Punjab and parts of Northern India in cheese and paneer industries. The plant is rich in enzyme Withanin which is responsible for the coagulating properties. Many herbal prescriptions like Liv 52, (Liver Health Support Supplement) which is an Ayurvedic poly herbal formulation consists of extracts of both *Withania somnifera* and *Withania coagulans.* Commercial cultivation of this plant is in its initial phase in parts of Punjab, Haryana and also in neighboring countries as it has wide distribution extending up to South Asia. Plant is also rich in Withanolide contents and can be a future prospect for South Gujarat region, as coagulating agent for dairy industries and rennet enzyme production. Seeds are available in local markets of Surat and Navsari, routinely used for controlling diabetes. The chapter aims at the possibilities of cultivating this plant in South Gujarat conditions in India, since the other species *Withania somnifera* is also available and has naturalized in AES Zone III of South Gujarat.

**Keywords:** rennet, Withanin, withanolides, poly herbal, anti-diabetic

## 1. Introduction

South Asia is home to many rich Traditional Systems of Medicine (TSM) including Ayurvedic, Unani, Siddha and Tibetan systems, which have been helpful in sustaining healthy life of tens of millions of people for centuries. India possesses abundant reserves of medicinal and aromatic plants (MAPs) across a vast territory characterized by diverse environmental conditions. The strategic geographical location, unique geomorphology, the existence of ancient flora from geological eras, and the harmonious interplay between biotic and non-biotic factors have collectively contributed to India being recognized as a region of remarkable plant diversity and endemism. These factors directly influence the wide range of medicinal and aromatic plant species found within the country. Himalayan sage scholars of TSM have said "*Nanaushadhi Bhootam Jagat Kinchit*" i.e., there is no plant in the world, which does not have


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medicinal properties.' These venerable scholars possessed extensive knowledge about the medicinal properties of numerous plant species, with estimates suggesting their understanding extended to hundreds of plants. It is not an overstatement to assert that the utilization of plants for enhancing human health dates back to the very origins of human existence [1].

The genus Withania, belonging to the Solanaceae family, holds a prominent position in the Indian Ayurvedic system of medicine due to its remarkable pharmaceutical and nutraceutical properties. Out of the 26 identified species within the genus Withania, only two species, namely *Withania somnifera* (L.) Dunal and *Withania coagulans* (Stocks) Dunal, have garnered significant economic importance [2]. Withania has been widely utilized in traditional folk medicine for treating a wide range of ailments. Additionally, one particular species, *W. coagulans*, is commonly referred to as the Indian cheese maker or Vegetable rennet. This species has been traditionally employed in various regions of India for the preparation of rennet ferment used in cheese production from vegetables. Different parts of this plant have been reported to possess a variety of biological activities [3].

Since decades ago, fruits of this plant were used widely in the production of traditional cheese from raw cow's milk. It is believed that the milk coagulation ability corresponds to the presence of an enzyme in the berries of the plant as it is related to the pulp and husk of the berry. Cottage and Cheddar cheese can be made using fruit extracts of *W. coagulans* as a good alternative to animal rennet, though the cheese produced with the described extract has a notable bitter flavor that can be reduced by increasing the ripening time [4].

### 1.1 Indian cheese: a wealth of health benefits

Indian cheese, particularly varieties like paneer has been a staple in Indian cuisine for centuries. Beyond its culinary appeal, Indian cheese offers a plethora of health benefits, making it a popular choice not just for its taste, but for its nutritional value as well.

- *Rich protein source*: One of the standout features of Indian cheese is its high-quality protein content. Protein is the building block of the body, essential for repairing tissues, building muscles, and maintaining a strong immune system. For vegetarians, in particular, Indian cheese provides a crucial source of this vital nutrient.

- *Calcium and bone health*: Indian cheese is a rich source of calcium, a mineral critical for bone and teeth health. Adequate calcium intake is essential throughout life to prevent osteoporosis and ensure the structural integrity of bones. Along with calcium, it also provides phosphorus, further enhancing bone health.

- *Weight management and satiety*: Despite being calorie-dense, Indian cheese can aid in weight management. Its high protein and fat content provide a sense of fullness, curbing hunger and reducing overall calorie intake. This makes it a valuable addition to diets aimed at weight control.

- *Vitamins and minerals*: Indian cheese contains an array of vitamins and minerals, including B-vitamins such as B12 and minerals like zinc. Vitamin B12 is crucial for nerve health and red blood cell production, while zinc supports the immune system and promotes wound healing.

- *Digestive health*: Some Indian cheeses, through their fermentation process, contain probiotics. These beneficial bacteria are excellent for gut health, aiding digestion, and promoting a balanced intestinal environment. A healthy gut is linked to improved overall well-being.

- *Versatility in diets*: For lacto-vegetarians, Indian cheese provides an excellent source of protein, ensuring that they meet their nutritional needs without relying on meat-based protein sources. Its versatility in Indian cuisine means it can be included in various dishes, making it easier to incorporate into different diets.

- *Heart and brain health*: Indian cheese contains healthy fats, including omega-3 fatty acids, which are beneficial for heart health and cognitive function. These good fats contribute to overall cardiovascular health and support brain function.

## 2. Distribution

*W. coagulans* is distributed in the East of the Mediterranean region extending to South Asia i.e., Iran, Afghanistan, Pakistan (Sind and Baluchistan), Nepal and India, up to 1700 m. In India, it is found in (North-West India) Himachal Pradesh, Punjab, Uttarakhand and Rajasthan. In Rajasthan it is sporadically distributed in Barmer, Jaisalmer and Jodhpur districts of Western Rajasthan desert and it is not common, categorized as "vulnerable species" by Pandey *et al.* [5]. It's important to note that *Withania coagulans* has also been introduced and cultivated in other parts of the world, including certain regions of the United States, Australia, and Europe. However, its natural distribution is primarily cantered in South Asia. Overall, *Withania coagulans* is adapted to grow in arid and semi-arid environments and is commonly found in the regions mentioned above.

## 3. Cultivation

*W. coagulans*, commonly known as Indian Rennet or "Paneer Doda," is primarily cultivated in specific regions where it is native or adapted to grow. The main cultivation areas for *Withania coagulans* include:

- *India*: *W. coagulans* is extensively cultivated in various parts of India. It is particularly grown in the arid and semi-arid regions of Rajasthan, Gujarat, Punjab, and Haryana. These regions provide the suitable climate and soil conditions for its growth.

- *Pakistan*: *W. coagulans* is also cultivated in Pakistan, primarily in areas with similar climatic conditions to those found in India. It is grown in regions like the Thar Desert and other arid parts of the country.

- *Afghanistan*: *W. coagulans* is known to be cultivated in certain regions of Afghanistan, particularly in areas with dry and arid conditions.

Outside of its native range, *W. coagulans* has also been introduced and cultivated in some other countries, such as the United States, Australia, and parts of Europe. However, the cultivation in these areas might be limited and less widespread compared to its native regions. It's worth noting that successful cultivation of *W. coagulans* requires specific growing conditions, including well-drained soil, warm temperatures, and a semi-arid climate. The plant is well-adapted to arid and dry regions, and its cultivation is typically focused in areas that provide the necessary environmental factors for its growth.

*W. coagulans* cultivation is primarily concentrated in India. India is one of the main countries where *Withania coagulans* is cultivated due to its suitability for the plant's growth requirements and its traditional use in Ayurvedic medicine. In India, the cultivation of *W. coagulans* is particularly prominent in regions with arid and semi-arid climates. States like Rajasthan, Gujarat, Punjab, and Haryana are known for their significant cultivation of *W. coagulans*. These areas provide the necessary conditions,

**Figure 1.**
*Morphology of Withania coagulans.*

| | |
|---|---|
| Kingdom | Plantea, Plants |
| Subkingdom | Tracheobionta, vascular plants |
| Super division | Spermatophyte, seeds plants |
| Division | Angiosperma |
| Class | Dicotyledons |
| Order | Tubiflorae |
| Family | Solanaceae |
| Genus | Withania |
| Species | Coagulance |
| *Deshmukh et al. [6].* | |

**Table 1.**
*Taxonomical classification.*

| | |
|---|---|
| Sanskrit name | Rishyagandha |
| Hindi name | Punir, Punir bandh, Akri, Binputakah, Paneer doda |
| English name | Indian cheese maker, Indian organic compound, Vegetable organic compound |
| Trade name | Paneer dodi, panner, doda, panir bed, Paneer dhodi |

**Table 2.**
*Synonyms.*

including dry and warm climates, well-drained soil, and limited rainfall, which are favorable for the growth of *W. coagulans*.

Farmers in these regions have been cultivating *W. coagulans* for generations and have developed knowledge and expertise in its cultivation practices. The plant is typically grown as a cash crop by farmers who recognize its value in traditional medicine and Paneer Industries demand in the market. Overall, India is a major contributor to the cultivation and production of *W. coagulans*, meeting both domestic demand and supplying it to various other countries for its medicinal and therapeutic applications (**Figure 1**, **Tables 1** and **2**).

## 4. Botanical description

*W. coagulans* is a sturdy gray undershrub, reaching a height of 60–120 cm. Its flowers from November to April, while the berries ripen from January to May. The natural regeneration is from the seed. The flowers dioceous, in auxiliary clusters; pedicils 0.6 mm long, Deflexed, slender. The calyx of *W. coagulans* is campanulate, measuring 6 mm in length and covered with a delicate stellate gray tomentum. Its teeth are triangular, approximately 2.5 mm long. The corolla of *W. coagulans* measures 8 mm in length and is covered with a stellate mealy texture on the outside. It is divided approximately one-third of the way down, and its lobes are ovate-oblong with a sub-acute shape. Male flowers stamens about level with the top of the corolla -tube; filament 2 mm long, glabrous; anthers 3–4 mm long. Ovary ovoid, without style or stigma. Female flowers stamens scarcely reaching 1/2 way up the corolla-tube; filaments about 0.85 mm long; anther smaller than in the male flowers, sterile. Ovary ovoid, style glabrous; stigma mushroom-shaped, 2-lamellate. Berry 6–8 mm globose, smooth, closely girt by the enlarged membranous calyx, which is scurfy -pubescent outside. Seeds 2.5–3.0 mm diameter, somewhat ear shaped, glabrous [7, 8].

## 5. Phytochemistry

Different chemical components such as alkaloids, hormones, tannins, saponins, carbohydrates, protein, amino acids and organic acid are shown by aqueous and methanolic extracts from the *W. coagulans*. Seeds have 17.8% free sugars, maltose, fatty oil, D-galactose & D-arabinose. The fruit contains a milk-coagulating enzyme, two esterases, free amino acids, essential oil and fatty oil and alkaloids, triacontaine hydrocarbon, dihydrostigmasterol sterol. Proline, hydroxyproline, valine, tyrosine, aspartic acid, glycine, asparagine, cysteine, and glutamic acid are amino acids. Alkaloidal fractions were isolated from the fruit's alcoholic extract. Leaves contain four withanolides-called steroidal lactones, Withaferin-A, 5, 20α(R)-dihydroxy-6α,7αepoxy-1- oxo-(5α)-with a-2,24-dienolide and two minor withanolide, of which one is probably 5α, 17α-dihydroxy-1- oxo-6, 7α-epoxy-22R-witha-2,24- dienolide [9].

The term "withanolide" is a structural designation coined by combining "withan" from the genus Withania with "olide," which is the chemical term for a lactone. Major bioactive phytoconstituents isolated from *W. coagulans* are lactone steroids called withanolides. A new group of steroidal lactones called withanolides has been recently isolated from different species of the solanaceae family, mainly *Withania Somnifera*. The whole plant of *W. coagulans contains* various withanolides, including coagulin F, coagulanolide, withacoagulin, and coagulin G. Additionally, the roots, leaves, and fruits of this plant have been reported to contain four, two, and two withanolides respectively, which serve as important biogenetic precursors of withanolides, withanolides shows antitumurous, anti-inflammatory, antibacterial, immunosuppressive activities [10]. A new withanolide, with a unique chemical structure similar to the aglycones of the cardiac glycosides, was isolated from the fruits of *W. coagulans*, and was screened for cardiovascular effects. The diverse therapeutic applications of withanolides found in *W. coagulans* have garnered significant interest in the scientific community. A new Withanolide isolated from *W. coagulans* have been found to be active against several potentially pathogenic fungi. Withanolides have been reported to possess both immunostimulating and immunosuppressive effects in different studies. Withanolides have been reported to have effect on haemopoietic system and bone marrow. Glycowithanolides have been found to have effects on CNS [11].

Withanolides, which constitute a major component of *W. coagulans*, can be classified chemically into the following groups [6].

a. Withaolide glycoside

b. Withaphysalin

c. Physalin

d. Nicadrenons or ring D aromatic withanolides

e. Acnistins

f. Perculactones

g. Withajardines

## 6. Medicinal use of different parts of *Withania coagulans*

Different parts of *W. coagulans*, including the leaves, roots, seeds, and fruit, have been traditionally used for their medicinal properties. Here are some of the medicinal uses associated with each part [12].

- *Leaves*: The leaves of *W. coagulans* have been used in traditional medicine for their anti-inflammatory and analgesic properties. They are often used topically to alleviate pain, reduce inflammation, and promote wound healing. The leaves may also be used in poultices or as a paste for skin conditions and joint ailments.

- *Roots*: The roots of *W. coagulans* are highly valued in traditional medicine systems like Ayurveda. They are known for their adaptogenic properties, meaning they help the body cope with stress and promote overall well-being. The root extracts are used to support the immune system, improve vitality, and enhance mental clarity. *W. coagulans* root extracts are also used for their potential anti-inflammatory, anti-diabetic, and anti-cancer effects.

- *Seeds*: *W. coagulans* seeds are used for their medicinal properties, particularly in the management of diabetes. The seeds contain compounds that have blood sugar-lowering effects and may help regulate blood glucose levels. They are used in traditional medicine as a natural remedy for diabetes and to support healthy blood sugar control [13].

- *Fruit*: The fruit of *W. coagulans* is traditionally used for its digestive and laxative properties. It is believed to aid in digestion, alleviate constipation, and promote regular bowel movements. The fruit is also used as a natural coagulant or rennet substitute in cheese making, as mentioned earlier.

## 7. Pharmacological properties

The plant's berries are used to coagulate milk. It has always played a significant role in the Ayurvedic, Unani, and traditional Indian medical systems. Numerous studies have shown that the isolated withanolides from *W. coagulans* have interesting biological properties. The plant's sweet fruits are also said to have sedative, emetic, alterative, and diuretic properties. They are helpful for liver complaints that are persistent. They have occasionally been employed as blood purifiers. Additionally, they are used to treat other intestinal infections, flatulent colic, and dyspepsia. These are used to treat asthma, biliary conditions, and stuttering Maurya [11].

- *Anti-inflammatory*: *W. coagulans* exhibits anti-inflammatory properties, which can help reduce inflammation in the body. These effects are attributed to the presence of bioactive compounds like withanolides, flavonoids, and other phytochemicals.

- *Antioxidant*: *W. coagulans* possesses antioxidant activity, meaning it can help neutralize harmful free radicals and reduce oxidative stress. The plant contains withanolides, flavonoids, and other compounds that contribute to its antioxidant potential.

- *Immunomodulatory*: *W. coagulans* has immunomodulatory properties, meaning it can modulate or regulate the immune system. It may help enhance immune function, support immune responses, and exert immunomodulatory effects. These properties are attributed to various bioactive compounds present in the plant.

- *Anti-diabetic*: *W. coagulans* has been studied for its potential anti-diabetic properties. There is evidence suggesting that Withania contribute to the regulation of blood sugar levels, enhancement of insulin sensitivity, and mitigation of complications associated with diabetes. The seeds of *W. coagulans*, in particular, have shown promising effects in managing diabetes [14].

- *Anti-cancer*: *W. coagulans* exhibits potential anti-cancer properties. Research suggests that its bioactive compounds, such as withanolides, may have cytotoxic effects on cancer cells, inhibit tumor growth, and induce apoptosis (programmed cell death) in certain cancer types. However, further studies are needed to fully understand its mechanisms and potential applications in cancer treatment.

- *Adaptogenic*: *W. coagulans* is considered an adaptogen, a category of natural substances that help the body adapt to stress and promote overall well-being. It may help reduce stress, improve resilience, and support the body's ability to cope with physical and mental challenges [15].

## 8. Significance of *W. coagulans* in dairy industries

The primary step in cheese production is milk coagulation. For thousands of years, coagulating enzymes, which are preparations of proteins that break down other proteins, have been utilized in the process of making cheese. Interestingly, this practice of using enzymes in cheesemaking is believed to be one of the earliest known applications of enzymes. The first evidence of cheesemaking can be traced back to cave paintings dating around 5000 BC [16]. Over time, scientists have developed alternatives to animal rennet, such as rennet substitutes produced by microorganisms and genetically engineered microorganisms. However, there is a growing interest in vegetable coagulants, which are milk-clotting enzymes extracted from plants. Cheeses made with vegetable coagulant can be found mainly in Mediterranean, West African, and southern European countries. Spain and Portugal have the largest variety and production of cheeses. *Withania coagulans*, commonly known as Indian Rennet or "Paneer Doda," has traditional uses in the dairy industry. It is used as a natural coagulant or rennet substitute in the process of cheese and paneer (Indian cottage cheese) production.

In traditional cheese making, rennet is commonly used as a coagulant to separate milk into curds and whey. However, *W. coagulans* offers an alternative source of coagulant enzymes that can achieve similar effects. The ripe fruit of *W. coagulans* contains enzymes known as milk-clotting proteases or chymosin-like proteases. These enzymes have the ability to coagulate milk proteins, resulting in the formation of curds [17]. This property makes *W. coagulans* a natural substitute for animal-based rennet in cheese making, particularly in vegetarian or vegan cheese production. In the dairy industry, *W. coagulans* is sometimes used to produce a variety of cheeses, including traditional Indian paneer. Paneer is made by coagulating milk with an acid or coagulant, and *W. coagulans* can be used as a natural coagulant in this process. The concentrated aspartic protease can be used instead of rennet for cheese preparation especially "cheddar". Cheese can be prepared by lyophilised extract has highest content of fat, total solids, crude protein, and ash which result in highest cheese yield as compared to pure chymosin and fungi rennet [18].

It's important to note that while *W. coagulans* can be used as a rennet substitute in cheese making, the specific techniques and processes may vary depending on the cheese recipe and desired outcome. Industrial cheese production often utilizes standardized rennet sources, but *W. coagulans* may find applications in small-scale or artisanal cheese production, especially in regions where it is traditionally used [19].

Overall, *W. coagulans* offers a natural alternative for coagulating milk in the dairy industry, particularly for those seeking vegetarian or vegan options in cheese making.

### 8.1 Some key points highlighting the importance of *W. Coagulans* in the dairy industry

Importance in the dairy industry due to its potential applications as a natural coagulant or rennet substitute in cheese and paneer production.

- *Vegetarian and vegan cheese production*: *W. coagulans* offers an alternative source of coagulant enzymes for cheese making, particularly for those seeking vegetarian or vegan options. Traditionally, animal-based rennet derived from the stomach lining of young calves is used as a coagulant in cheese production.: *W. coagulans* provides a natural, plant-based substitute, allowing for the production of vegetarian and vegan cheeses.

- *Cultural significance*: *W. coagulans* has historical and cultural significance in regions where it is traditionally used. In India, it is often employed in the production of paneer, a popular cheese-like dairy product widely consumed in Indian cuisine. By preserving traditional cheese-making practices and utilizing *W. coagulans*, the dairy industry can maintain cultural authenticity.

- *Market demand*: The demand for vegetarian and vegan cheese products has been growing globally, driven by consumer preferences for plant-based alternatives. *W. coagulans* enables dairy manufacturers to cater to this expanding market segment by offering cheese products that align with vegetarian and vegan dietary choices.

- *Sustainability and animal welfare*: *W. coagulans* contributes to sustainable and ethical practices in the dairy industry. By reducing reliance on animal-based rennet, the industry can minimize its impact on animal welfare and promote more sustainable production methods.

- *Traditional medicine integration*: *W. coagulans* is not only relevant to the dairy industry but also holds significance in traditional medicine systems like

Ayurveda. The integration of traditional medicinal knowledge and practices into the dairy industry allows for a holistic approach to product development, incorporating both nutritional and therapeutic aspects.

While it has potential advantages, industrial-scale cheese production often utilizes standardized rennet sources. However, *W. coagulans* finds value in small-scale or artisanal cheese production and meets the demand for vegetarian and vegan options.

## 9. The significance of *W. coagulans* in food industries

### 9.1 Nutritional profile

Both macro and micronutrients are abundant in *W. coagulans*. The *W. coagulans* mineral constitution. In addition to being an excellent source of carbohydrates, it also contains a small amount of hydration, protein, fat, and fiber. Research studies have revealed that *W. coagulans* exhibits comparatively higher levels of essential minerals like magnesium (greater than *Alhagi maurorum*, *Berberis lyceum*, and *Tecomella undulate*), calcium (greater than *Dature alba*, *A. maurorum*, *Chenopodium album*, *B. lyceum*, *T. undulate*), potassium (greater than B. lyceum and T. undulate), and iron (greater than *D. alba*, *B. lyceum*, and *T. undulata*) [20]. The composition of roots, leaves, and fruit varies in terms of their nutrient content. Roots predominantly consist of carbohydrates (75.71%), followed by fiber (5.76%), lipids (5.5%), protein (2.95%), and ash (1.92%). In comparison, leaves contain carbohydrates (65.31%) as the major component, accompanied by fiber (11.76%), lipids (5%), protein (2.95%), and ash (3.26%). As for fruit, it consists of carbohydrates (60.14%) as the primary constituent, along with protein (4.65%), lipids (5%), and ash (4.21%). Hameed and Hussain [21]. The berries of *W. coagulans* include two esterases, free amino acids, milk coagulating enzymes, and essential oil. The primary amino acids found in the plant are proline, tyrosine, valine, hydroxyproline, glycine, cysteine, asparagine, glutamic, and aspartic acids. The major fatty acids found include arachidonic acid, stearic acid, palmitic acid, linoleic acid, and oleic acid [22]. Similarly, research found 20 constituents in the essential oil of the fruit of *W. coagulans* is primarily composed of sesquiterpenes (54%) and esters (21.50%), which are the dominant compounds. Additionally, fatty acids (5.5%) such as nonanoic acid, hexanoic acid, methyl ester of hexadecanoic acid, methyl ester of nondecanoic acid, methyl ester of 8,11-octadecadienoic acid, methyl ester of 9-octadecenoic acid, and ethyl ester of linoleic acid are present. Alkanes (9.11%) and aldehydes (0.32%) are present in smaller percentages Shahnaz *et al*. [23]. In addition, it was shown that a de-fatted meal made from *W. coagulans* seeds included free sugar (17.8%) in the form of D-galactose and D-arabinose (1:1), with trace amounts of maltose also present. Additionally discovered and reported for the hypocholesterolemic impact of corn oil combined with *W. coagulans* were higher concentrations of -sitosterol and linoleic acid. Maurya [11].

The fruit of the plant is known to contain certain constituents that may contribute to its potential health benefits. Here are some components found in *W. coagulans* fruit:

- *Withanolides*: *W. coagulans* is known to contain bioactive compounds called withanolides. These are steroidal lactones that are believed to have various pharmacological activities, including anti-inflammatory, antioxidant, and immune-modulating properties [24].

- *Flavonoids*: Flavonoids are a group of plant compounds known for their antioxidant activity. *W. coagulans* fruit may contain flavonoids, which contribute to its potential antioxidant effects.

- *Alkaloids*: *W. coagulans* may also contain alkaloids, which are natural compounds found in various plant species. Alkaloids can have diverse physiological effects and may contribute to the overall chemical composition of the fruit [4, 25].

- *Antioxidant compounds*: *W. coagulans* is known to contain bioactive compounds, such as withanolides and flavonoids, which possess antioxidant properties. These compounds help neutralize harmful free radicals in the body and protect against oxidative stress [26].

While detailed information on the specific nutrient composition of *W. coagulans* is limited, the following nutrients are commonly found in similar plants within the same family or genus:

- *Carbohydrates*: *W. coagulans* likely contains carbohydrates, which are the primary source of energy in plants. The specific types and amounts may vary.

- *Protein*: *W. coagulans* is likely to contain proteins, which are important for various physiological processes in the body.

- *Fiber*: Like many plant-based foods, *W. coagulans* may contain dietary fiber, which can contribute to digestive health and help regulate blood sugar levels.

- *Vitamins*: *W. coagulans* may contain various vitamins, although specific quantities are unknown. Common vitamins found in similar plants include vitamin C, vitamin A, and certain B vitamins.

- *Minerals*: Similar to other plants, *W. coagulans* may contain minerals such as potassium, calcium, magnesium, and iron. The exact mineral profile can vary (**Table 3**).

The coagulating properties of *W. coagulans* berries on milk are widely recognized. Additionally, the milk coagulating potential of *W. coagulans* fruit extract was evaluated. *W. coagulans* fruit, an enzyme called aspartic protease was isolated using fractional ammonium-sulfate precipitation and cation-exchange chromatography. Casein was used to test the protease enzyme's proteolytic activity. Using skim milk, the ability of *W. coagulans* crude fruit extract to cause milk to coagulate was evaluated [17]. Therefore, it was discovered using mass spectrometry and inhibitory experiments that aspartic protease is the sole enzyme responsible for milk coagulation. Additionally, the activity of the enzyme was steadily decreased by the rising salt concentrations (NaCl, $CaCl_2$). As a result, it was determined that this enzyme would be suitable to create reduced salt cheese [6].

Buffalo milk mozzarella cheese was made using the fruits of *W. coagulans*, which served as milk coagulants. Therefore, using an aqueous fraction of *W. coagulans* to make cheese might be an option. Buffalo milk cheese was made using an extract from the fruit of *W. coagulans*, and its storage capabilities were examined (5 months). Cheese produced using lyophilized berry extract showcased the highest

| Macro-minerals | Minerals (mg/kg) |
|---|---|
| Calcium | 9260 |
| Magnesium | 35,280 |
| Potassium | 2450 |
| Sodium | 125 |
| **Micro-Minerals** | **Minerals (mg/kg)** |
| Iron | 98.8 |
| Copper | 2.2 |
| Zinc | 40.2 |
| Chromium | 0.6 |
| Cadmium | 1.4 |
| Lead | 1.9 |
| Nickel | 1.8 |
| *Khan et al. [27].* | |

**Table 3.**
*Mineral composition of W. coagulans.*

concentrations of ash, fat, crude protein, and total solids. To create the cheese, *W. coagulans* alcoholic and aqueous fractions containing plant proteinase were utilized at varying concentrations (0.5, 1, and 1.5%) [28].

Additionally, cottage cheese made with an aqueous plant fraction had a significantly higher moisture content and pH, whereas cheese made with calf rennet and *W. coagulans* had the same levels of ash, fat, and crude protein. a white cheese of acceptable grade can be produced using a 0.5% alcoholic plant extract. Tofu made with calcium sulphate and *W. coagulans* extract were compared in terms of how well they produced coagulation of soy milk. The two types of tofu could not be distinguished based on sensory evaluation, while the tofu produced by *W. coagulans* had a lower yield and more moisture.

## 10. Future prospects and potential of *W. coagulans*

*W. coagulans*, also known as Indian rennet or vegetable rennet, is a medicinal plant that has been traditionally used in Ayurvedic medicine. General insights and potential avenues for exploration.

- *Medicinal applications*: *W. coagulans* has been studied for its potential health benefits, including anti-inflammatory, antioxidant, anti-diabetic, and anti-cancer properties. Future research could focus on investigating the specific bioactive compounds responsible for these effects and their mechanisms of action. This could lead to the development of novel therapeutic agents or natural remedies for various ailments.

- *Functional food and nutraceuticals*: *W. coagulans* has been explored for its potential as a functional food ingredient or nutraceutical. It contains high levels of bioactive compounds like withanolides, flavonoids, and saponins, which could contribute to its health-promoting properties. Future studies may investigate the formulation of *W. coagulans* extracts or derivatives into various food products or supplements, evaluating their efficacy and safety profiles.

- *Agricultural applications*: *W. coagulans* is a perennial shrub that is drought-tolerant and adaptable to arid environments. It may have potential for cultivation in regions where other crops struggle to thrive, thus offering economic opportunities for farmers in such areas. Additionally, research efforts could focus on improving cultivation practices, optimizing yield, and enhancing the quality of harvested plant material.

- *Phytochemical exploration*: *W. coagulans* is known to contain various bioactive compounds, such as withanolides, which have been of interest in pharmaceutical and herbal medicine research. Further exploration of the phytochemical profile of *W. coagulans*, including isolation and characterization of novel compounds, could open up new avenues for drug discovery, natural product synthesis, or plant breeding programs.

It's important to note that these prospects are speculative, and future research and developments will determine the actual potential and applications of *W. coagulans.*

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Cheese is a palatable and dense model food with great nutritional value. Cheese lovers all over the world have access to an almost overwhelming variety of cheeses. Cheese has many health benefits that go beyond its nutritional and flavor properties. The health benefits are due to the presence of unique bioactive peptides and fatty acids in cheese. Furthermore, cheese is an excellent tool for making functional foods because it can serve as an excellent delivery vehicle for bioactive peptides, vitamins, minerals, probiotics, postbiotics, prebiotics, and other novel bioactive substances. This book offers opportunities for cheese manufacturers, cheese researchers, nutritionists, and even cheese lovers to learn more about the hidden health and nutritive benefits of cheese. The book reflects the trends and innovations in the development of cheese as a functional food.

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