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REVIEW BASED BOOK CHAPTER

Application of Innovative Technologies in Fermented Dairy Products

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<u>Abstract</u>

Fermentation of dairy products is a way of preserving the nutrients found in milk and this fermentation is performed by diverse microbiota. Probiotic products (yogurt, cheese, and kefir) have been considered beneficial for human health. Their demands have reached a great length by consumers due to their high nutritional value and their enticing sensory characteristics worldwide. Different innovative processing technologies (Non-Thermal Technologies) are in use to further enhance their sensory characteristics such as flavor, texture, and preservation. The insights provided by these studies are summarized in this chapter.

<u>Keywords</u>

Fermentation, Cheese, Kefir, Yogurt, Innovative Methods

1. Introduction

Conventionally, fermentation is a process that is used for the preservation of food products and also ensures food safety. It boosts the nutritional, health-improving qualities of food and also increases its organoleptic characteristics. Many microbes can be used to ferment different dairy products [1]. Lactic acid bacteria (LAB) synthesize exopolysaccharides (EPS) that play a crucial role in the processing of dairy products [2]. A lactic acid bacterium, lactobacillus helveticus is a non-spore-forming gram-positive bacteria, which shows thermophilic, and homo fermentative abilities. This bacterium grows at 42°C temperature, which is specifically safe for the fermentation of dairy products after its isolation from dairy products [3-6].

Fermented dairy products are considered important ingredients in functional foods. Incorporation of probiotics to form high-value products has gained great interest recently. Different dairy products are greatly consumed such as yogurt, cheese, and kefir, etc. Here we will discuss the history, processing, therapeutic potential, value addition, and new innovative technologies used in producing these products [7].

2. Cheese

In the past, cheese was manufactured by Egypt and Summer's people and after that, cheese manufacturing was performed in Rome also. The Middle East and Europe increased the production of cheese and later Oceania, North and South America produced 1000 different varieties of cheese. In the mid-nineteenth century, cheese was produced only at the domestic level but with time new highly developed technologies produced several principal varieties of cheese. About 35% of all milk is specifically used to manufacture cheese. Recently south and North America, Oceania, and Europe have manufactured 19 tons annually [8]. The distinctive nature of cheese results from two important components of milk: its characteristics and composition [9].

2.1. Therapeutic Potential of Cheese

Cheese is one of the healthiest foods in the world, which is available in various shapes, tastes, and textures with a significant nutritional value. Globally, more than 2000 types of cheese are available in specific textures, forms and tastes [10, 11]. Cheddar cheese can be advantageous for those who are hypolactic because it contains a significant level of calcium and negligible amount of lactose [12].

Making cheese involves dehydrating milk and adding additional preservatives including culture, acidity, salting, packaging, and storage. In a procedure known as syneresis, the rennet-induced milk coagulum is chopped and heated to remove moisture. The curds are then packed into fresh cheese after being rinsed and seasoned. For most cheese kinds, the pH gradually decreases to a value between 4.6 and 6.0 during the process [13].

In earlier times, cheese was developed biotechnologically and emerged as a



functional dairy product worldwide. Cheese comprises bioactive compounds such as polysaccharides, fatty acids, and peptides. These substances have several functional attributes such as anti-thrombotic, antitumor, anti-obesity, antioxidant, and anti-hypo-glycemic reactions, and also act as a mineral-binding agent. Lactic acid bacteria also act as probiotics, which is very helpful in increasing the good digestion and absorption of nutrients to improve gut health [14, 15].

2.2. Value Addition in Cheese

Value addition/fortification is a method to improve the nutritional value of food products. Cheese is fortified by the addition of essential micro-nutrients (vitamins, carotenoids, polyphenols, and polyunsaturated fatty acids), the addition of minerals (selenium, zinc, and iron), and also fortified by agrifood-industry by-products. Allogenic microbes that are from entomological origin can be used to increase the technological, organoleptic, and nutritional value of cheese [16]. In the earliest days of calving, mammary glands of mammals secrete milk, which consists of important substances such as minerals, vitamins, lipids, and proteins. This initial milk contains a good level of lysozymes, oligosaccharides, and lactoferrin substances with anti-oxidant, and anti-inflammatory qualities, and also strengthens the immune system of humans. These are also present in mature milk but in limited amounts [17, 18]. A starter culture of lactic acid bacteria, essential oils, and extracts of plants are used in the processing of cheese which is conveniently found and applied directly in milk and curd to make cheese with higher nutritional value [19, 20].

Colostrum is used as a functional ingredient to develop novel FPC (probiotic cream cottage cheese). For this purpose, *Acidophilus-bifidus thermophilus* culture and BCP (bovine colostrum powder used 1, 2, and 3 percent. Then the antioxidant properties, antibacterial activity, fatty acid profile, and physicochemical properties of food products were analyzed. By increasing the amount of colostrum powder in all samples of cheese during its manufacturing, antioxidant activity was increased by (P < 0.05) and the storage time of cheese also increased. 2% bovine colostrum powder is used for the fortification of cheese and it shows a higher value of benefits than other treatments. So,



the result is cheese which contains 2% of bovine colostrum powder shows greater health and nutritional effects [21].

The quality of food products has been greatly improved with advancement in the field of biotechnology and the microbial strains produced by biotechnological approaches also proved to be helpful in the designing of cheese with enhanced nutritional value. Bioactive peptides are released due to the hydrolysis of enzymes, which occur during process of fermentation & digestion of dairy [14, 15].

3. <u>Yogurt</u>

Yogurt is an industrial food product that has been used around for ages. Numerous studies have been conducted since yogurt's discovery in an attempt to understand better and enhance the taste and texture to benefit human health. Commercially, yogurt and other dairy food products are thought to have originated from the Middle East [22]. Initially, yogurt was synthesized at a small level without any standardized methods till the twentieth century. But after the twentieth century, it became more commercial and its production started at a high level [23].

Yogurt is derived from the bacterial fermentation of milk by two species of bacterial cultures, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus* [24]. Along with these two bacteria, other species of bacteria are also present in yogurt. The presence of these additives in fermented food products either enhances their probiotic nature (living microbes) or taste and texture [25]. During the manufacturing of yogurt, lineal strains of *lactobacillus bulgaricus* genetically produce acetaldehyde in dairy products [26]. Lactic acid bacteria are one of the major constituents of yogurt because they are used and transform sugars into lactic acid. These lactic acid bacteria are also helpful in the reduction of harmful substances and in the prolonging of the shelf life of dairy food products [25].

3.1. Therapeutic Potential of Yogurt

Globally, the demand for yogurt is increasing tremendously by consumers after knowing its therapeutic properties and its great nutritional value. These beneficial effects include the strengthening of the immune system, increasing HDL cholesterol levels, and controlling blood sugar levels, also used in the treatment of gut-related diseases like constipation, inflammation of the stomach, and diarrhea. Additionally, it prevents various death-taking diseases like colorectal cancer and dangerous bacterial infections [27-30].

3.2. Value Addition in Yogurt

To increase health benefits and nutrient contents of foods, vegetables, and fruits are used to add value to food products because they are rich in vitamins, organic compounds, polyphenols, and dietary fiber. Clinically pulp of Annona species, oleracea, carrots, and strawberry juices are added to yogurt for the improvement of flavor and nutritional status [31-35]. Especially, for the enrichment of fermented dairy products such as yogurt, fruit juices are the best options. For this purpose, passion fruits are considered suitable because they are rich in many different organic compounds that inhibit the proliferation of infectious bacteria by decreasing the pH of fermented foods [36].

Malnutrition is a major problem all over the world, to overcome this problem food is enriched with essential nutrients. Due to the high consumption of yogurt, it is enriched with micronutrients like vitamin D₃, B₉, and B₇, minerals, bioactive compounds, and dietary fibers [37]. A Fortified puree of pineapple and coconut milk is used for the enrichment of yogurt because pineapple is rich in (bromelain enzymes, antioxidants, phenols, and organic substances) that lower the fat content in yogurt, and the addition of coconut milk (enriched in phenols, organic compounds, and proteins) which are found to be beneficial for human health [38].

4. <u>Kefir</u>

Kefir is an acidic and less alcoholic drink produced by the fermentation of milk or glucose water with kefir grains. Its origins may be traced to the Caucasus, Eastern Europe, and the Balkans. Because of its health-promoting qualities, its use has spread throughout the world throughout time. People in the US, Japan, France, Brazil, and



other nations have grown to love this tangy, viscous beverage [39-42]. Kefir word is derived from the Turkish language "kef" which means delight sensation. The people of the Middle East use this word commonly [43].

Chemically, kefir consists of different kinds of yeast and bacteria and is helpful in the enhancement of its sensory attributes [44]. Different kinds of drinks and foods are synthesized by using kefir grain to add additional characteristics like pleasant smell and taste to the final product [45]. Kefir grains are considered to be a hub of beneficial microorganisms. These grains consist of about thirty varieties of lactic acid bacteria that include Lactotococcus, Lactobacillus, leuconostic species and yeast includes *Torsulaspora, Saccharomyces, Candida, Kluyveromyces, Mycotorula* species [46].

These microbes develop, spread, and impart their distinctive traits to the subsequent generations of grain. There exists a mutualistic symbiotic relationship between these microorganisms and are also present in an inactivated form inside of a protein-polysaccharides pattern [47-49]. These grains of kefir are considered irregular, lightly yellow, or gelatinous lumps and also have an elastic texture. They vary in size and range from 0.3-3.5 cm in diameter. Chemically, kefir grains consist of about water (83%), proteins (4-5%), and kefiran (9-10%) [50]. Researchers suggested that excellent quality of kefir is obtained by incubation of homogenized milk with 3 percent (V/V)) bacteria (*streptococcus* and *lactococci* species) and yeast (*streptococcus* and *lactococci* species) at 258 degrees temperature for 24 hours [51].

4.1. Therapeutic Potential of Kefir

Kefir shows exceptional therapeutic potential because of the presence of bioactive substances such as carbon dioxides, hydrogen peroxides, organic acids, ethanol, and exopolysaccharides and antibiotics like bacteriocins work together to enhance the health benefits of kefir [49, 52]. Kefir also provides a healing effect against several diseases such as diabetes, cancer, high blood pressure, and high cholesterol levels which cause heart attacks, different allergies, microbial disorders, and other inflammatory diseases [53-55].



4.2. Value Addition in Kefir

Kefir presents an advanced level of physiochemical and biological properties, which is why different nutritious food additives and juices are added to increase its nutritional value. Recently, many researchers have worked on fortification and enrichment of kefir, to increase shelf life, health benefits, and nutrient contents. Encapsulated blackberry juice is considered a successful additive for the enrichment of kefir because of its exceptional morphological, physiochemical, and microbial safety behavior [56]. Recent research shows that the addition of hazelnut milk in kefir dairy products surprisingly increased the nutrient contents, shelf life, antioxidant potential, and phenolic substances in the final product. It is also observed that after fortification of kefir beverage organic and bioactive properties also improved [57]. Da Costa studied that People who are allergic to dairy foods can drink a kefir beverage, which is a combination of fermented extracts of beans, sesame seeds, and yam [58].

5. Innovative Technologies

New innovative methods are applied for the fermentation of dairy products. Recently, non-thermal technologies have been applied in the dairy industry other than thermal technologies because of their high potential for properties in fermented dairy products.

Non-thermal technology involves the processing of food in a limited time while keeping the temperature lower. These conditions ensure the safety of food and enhance the shelf life of dairy products. Non-thermal technologies (high pressure, ultrasound, and pulsed electric fields) provide high-quality dairy products with fresh-like characteristics [59, 60].

5.1. <u>Ultrasound Technology</u>

New technologies are developed to get safe, nutritious, energy-efficient, and costeffective food products with a high acceptance level from consumers. Ultrasound technology proved to be one of the most effective technologies to meet the increasing demands of consumers [61, 62]. Ultrasound is waves that exhibit a frequency higher than the human hearing range (>18 kHz), produced by an instrument called

ultrasonic transducers, that convert electric energy to vibration form. Although the frequency of the spectrum of ultrasound waves ranges from 20kHz-1MHz, in food industries 20-40kHz frequency range is applied [63]. Ultrasounds are categorized into two types based on their frequency range.

i. <u>High-frequency and Low-intensity</u>

These waves frequency ranges at 100kHz and its intensity is less than 1Watt cm². These waves are also considered low-power and energy waves [64]. Soria and Villamiel studied low-frequency ultrasound waves used to check the acidic behavior, interaction of proteins, ripeness, and firmness of dairy products [65].

ii. Low-frequency and High-intensity

These ultrasound waves use a frequency that ranges from 20-500k Hz and its intensity is greater than 1W cm² [64]. Psiyasena, Mohareb and McKellar researched the applications of these waves on fermented dairy products and found that they are involved in the inactivation of microbes during fermentation because improving the viability of microbes is very important to get high-quality final product [66]. Soria and Villamiel, also proved that high-intensity ultrasound waves alternate the chemical and physical properties of dairy products [65].

Recently Galvan and his colleagues found that ultrasound technology is greatly used in the fermentation of dairy products because fermentation is a slow process that needs more time for the conversion of organic substances to simple form during the action of enzymes and microbe enzymes [67].

In fermented dairy industries ultrasound technology is applied to reduce the viscosity, for extraction of novel nutrients, increase ultrafiltration of whey protein, and is used in fat homogenization [68]. Pitt and Rose researched the effect of ultrasound on the cell growth of microbes by protecting the transfer of waste products and enhancing the transport of nutrient contents and oxygen to cells which proved to increase the viability of microbes by increasing their growth [69].



5.1.1. Application in Yogurt Processing

Sfakianakis and Tzia research that ultrasound waves in the production of yogurt are a new innovative method for better sensory and physiochemical characteristics of the final product. When ultrasound intensity increased and its exposure time on milk also increased, it was observed that yogurt showed greater viscosity and water-holding capacity [70]. Clinically milk treated with ultrasound treatment (25 kHz, 400 W, 45 or 75 °C for 10 min) produces high-quality yogurt. Processing innovations in the cheese industry have been proven to be of great importance in improving the functional attributes, flavors, and textures and also in prolonging the shelf life of food products [71].

5.1.2. Applications in Cheese Processing

In the cheese industry, ultrasound technology is used in the cutting of cheese. Highenergy acoustic vibrations of ultrasound instruments do not damage the structure and shape of cheese, work efficiently, and reduce the loss of the final product [68, 72, 73]. High-intensity ultrasound is used to cut all types of cheese such as Swiss, mozzarella, and cheddar cheese. These high-intensity sound waves do not affect the quality of cheese during storage of cheese after it is cut for 21 days and stored at 4°C [72].

5.1.3. Applications in Kefir Processing

Düven, Kumcuoğlu and Kışla [74] researched the impact of ultrasound waves during the fermentation of kefir by applying 24kHz frequency, 22millimeter prob with 30 percent amplitude for 5 minutes and analyzed different parameters such as acidity, microbial activity, and pH. Their research results show that after the ultrasound application exopolysaccharides were produced and the time of fermentation for kefir production decreased by 1 hour and this technology positively improved the quality of kefir.

5.2. Pulsed Electric Field

In ancient times thermal processes were mostly used for the safety of food from harmful microbes but high temperature reduces the nutritional value of products by changing



their flavor, taste, and physiochemical properties that's why new technology which is pulsed electric field used recently to reduce the loss of nutrients and sensory characteristics of the final product [75, 76]. Application of different voltage pulses ranges from 1 to 40 kilo volt/centimeter for micro-milli seconds time in solid, liquid food products that are present between electrodes which produces an electric field, commercially used for the preservation of foods from harmful microbes is termed a pulsed electric field [77, 78].

5.2.1. Application in Cheese and Kefir Processing

Peng researched the effect of a pulsed electric field on the proliferation of microbes, and lactic acid bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus*) during the process of fermentation of dairy products like cheese and kefir. Different parameters such as pH, acidification rate, and microbial growth during the fermentation of dairy products are measured and analyzed. Different ranges of pulse from 60 to 428 V/cm or above were applied in microbial culture and observed their effects on these parameters. Peng and his colleagues observed that the application of electric field pulses sowed promising results reducing the pH, and acidity in and controlling the growth of microbes in the food product [79].

5.2.2. Application in Yogurt Processing

The inactivation of microbes is a very important factor in the manufacturing of yogurt, a new technique that uses pulsed electric field technology and produces high-quality final products. High-pressure electric pulses immobilized the cells of microbes, their cells burst and release their contents. Pulsed electric fields are applied to treat milk and inactivate the microbes. Milk is exposed to electric pulses in the presence of a probiotic culture, and the intensity of pulses ranges from 15-50 kV/cm for some seconds, finally, this process enhances the sensory characteristics of final products [70].

5.3. High-Pressure Technologies

This method is an advanced technological process to increase the shelf life of fermented products by applying pressure ranges from above 1000 M pascal. This



technology is not only used in preservation but also to maintain the nutrition of food without changing flavor, color, and aroma [80, 81]. High-pressure technology, highpressure homogenization, and high hydrostatic pressure are all considered highpressure treatments, and their effects on the dairy industry have been studied recently [82]. Serra and her colleagues studied that high-pressure homogenization technology increases the volatile acids (lactic acid and pyruvic acid), non-volatile acids (butyric and acetic acid), and these acids greatly increase aromatic compounds (diacetyl and acetaldehyde), and also sensory characteristics of the final product [83].

5.3.1. Application in Yogurt Processing

Mota studied that during the fermentation of milk for yogurt production high-pressure method greatly increases the viscosity of yogurt, denatures the whey proteins, and disturbs the casein micelles by reducing syneresis. When pressure between 100-300 M Pascals is applied in a starter culture, protein split into simple amino acids and peptides, which increases the growth of probiotics because pressure affects the metabolic process and activates stress response that improves the survival of probiotics during the process of fermentation [84].

5.3.2. Application in Cheese Processing

In the cheese industry, this technology helps to increase the process of salting, improve the texture also upgrade the coagulation of rennet and curd production [85]. Trujillo's [86] research shows that the application of High-pressure technology in cheese increases the number of amino acids, moisture, and salt contents in cheese made from pasteurized milk. Butz and his colleagues [87] & Iwanczak and Wisniewska [88] investigated that high hydrostatic pressure is also an innovative method that helps to control proteolysis during the ripening of cheese and also helps to reduce its maturation time. This technology is also applied to packaged cheese products because high pressure effectively controls the growth of pathogenic microorganisms like *Listeria monocytogenes*. When we apply pressure of 600 M pascals in fresh cheese, this pressure efficiently decreases the detection limit of bacteria (*Listeria monocytogenes*) which is 0.91 log colony forming unit per gram [89].



5.3.3. Application in Kefir Processing

According to Mainville [90], while some earlier research has examined the effect of high hydrostatic pressure (HHP) technology in kefir processing, it has always been with a particular emphasis on the microbial activity and preservation of this fermented dairy product. To the best of our knowledge, nevertheless, there is no evidence of how HHP processing affects other aspects of kefir quality. While concerning the impact of HHP processing on additional kefir quality parameters, no information is currently available. This is a crucial consideration for the creation of innovative dairy products as well as safe, marketable goods with acceptable sensory attributes [91].

6. In-line and On-line Analysis of Fermented Dairy Products

6.1. By Using Focused Beam Reflectance Measurement

In fermented dairy products, particle size greatly influences the relationship of viscosity during processing and sensory properties of fat-free fermented milk products. Heck, Nöbel and Hinrichs [92] work on the analysis of particle size inline by using focused beam reflectance measurement to get actual-time data of fermented dairy products, especially fresh cheese. They do the comparison of in-line and off-line particle sizes of fermented dairy products (fresh cheese) and found that this analysis helps to control different parameters like consistency, product flow rate and pressure, and downstream processing used to improve the viscosity of product after fermentation in fresh cheese processing. Further studies work to use artificial intelligence (AI) to collect correct data of different parameters by using inline sensors during the process of fermentation which will help to improve the quality and texture of cheese.

6.2. By Using the Multiple Light Scattering Method

Ramezani [93] looks at the monitoring of milk fermentation using multiple light scattering. The studies were conducted on milk that had undergone fermentation at temperature ranges (36-44°C) and with starter concentrations ranging from 0.05% to 4.5% (w/w). Additionally, antibiotics were present in quantities of up to 100 μ g/kg. Multiple light scattering was used to continually monitor the fermentation process, and



a pH meter, rheometer, and texture analyzer were used concurrently. Changes in pH, rheological parameters, and sample hardness were shown to be associated with the backscattering signal captured by several light scattering measurements during the fermentation process. The elevated starter culture concentration of about 4.5% (w/w) with incubation temperature (44°C) resulted in a gelation period of 120 minutes. The pH, texture, and rheological monitoring supported these findings. Even at low dosages (1.3 μ g/kg), the impact of antibiotics on gel formation could be detected using backscattering spectra analysis. Overall, the findings demonstrated the benefits of employing multiple light scattering as a quality control instrument for real-time milk fermentation monitoring.

Conclusion

Fermentation of foods by the action of microbes plays an essential role in the production of high-quality, nutritious food products. Nowadays, the consumption of yogurt, cheese, and kefir has greatly increased and is considered a main part of their diets. These products show a significant therapeutic potential. Also, different scientific research suggested that different extracts of fruit or essential oils can be used as an additive to improve the nutritional value of fermented dairy products. In ancient times thermal processes were used to preserve food products but these processes disturb the texture, color, and flavor of dairy products, that's why non-thermal technologies (ultrasounds, pulsed electric field, and high-pressure) have been developed to improve both sensory and nutritional profile of fermented dairy products. However new research is needed to analyze the different parameters that are involved in the processing of controlled fermentation of dairy products by using in-line and on-line techniques like multiple light scattering methods or focused beam reflectance measurement. Based on the reviewed scientific research, it can be concluded that innovative processing technologies and other techniques can significantly improve the nutritional and sensory properties of fermented dairy products.

Author Contributions

All authors equally contributed for manuscript preparation and editing.

Conflicts of Interest

The authors declare no conflict of interest.



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