Chapter

From Traditional Bulgarian Dairy Products to Functional Foods

Dilyana Nikolova, Ramize Hoxha, Nikola Atanasov, Elena Trifonova, Lili Dobreva, Veronica Nemska, Yana Evstatieva and Svetla Danova

Abstract

Production of the traditional yoghurt, white-brined cheese, and yellow cheese, named "kashkaval", in the Bulgarian region determines everyday consumption and health benefits for the local population. Artisanal dairy products and their autochthonous microbiota are a promising source for the research and creation of new minimally treated, but safe, functional and delicious food. The species from *Lactobacillaceae* are used in different fermentation technologies, improving the structure, taste, and aroma of the final products. These products possess a prolonged shelf life due to the biopreservative capabilities of the lactic acid bacteria (LAB) strains, their positive health impact, and many physiological functions in the body. This chapter examines the traditional and modern technologies for the production of typical Bulgarian dairy products. Based on the studies of artisanal products, different LAB species from non-starter microbiota are presented, which contribute to the organoleptic qualities of the products and their beneficial properties. The research focus is aimed at the evaluation of various functional characteristics of non-starter strains, such as metabolic activity and food biopreservation. The long-term goal is to study the tradition to create new functional formulas that are the desired and effective factors for health and longevity.

Keywords: Bulgarian yoghurt, white-brined cheese, lactic acid bacteria, antifungal activity, antibacterial activity, *Lactiplantibacillus plantarum*

1. Introduction

Different types of lactic acid fermentation are the base of more than 3500 traditional fermented foods found worldwide [1]. After sourdough products and vegetables, dairy products are the third most desired and consumed products in Bulgaria (**Table 1**). Yoghurt and yoghurt-like products are the most famous of all dairy products. Their consumption is the highest in the countries of the Mediterranean, Asia, and Central Europe. The consistency, taste, and aroma differ from region to region and from that of other fermented products. In some areas, yoghurt is produced in the form of a very thick liquid, while in other countries, it is in the form of a softer gel.

Dairy products	Year									
	2012	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
Pasteurized milk (L)	19.6	20.1	18.8	18.5	17.3	17.2	16.8	16.9	18.0	19.2
Yoghurt (kg)	29.0	28.1	26.9	25.8	27.5	27.6	29.3	29.2	29.6	28.9
Cheese (kg)	12.4	12.7	12.1	11.7	11.8	11.5	11.8	12.1	11.8	12.0
"Kashkaval" (kg)	3.5	3.7	3.7	3.9	3.9	4.0	4.2	4.5	4.6	5.1
Other dairy products (kg)	2.1	2.2	2.3	2.4	2.3	2.8	2.8	2.8	2.8	3.4
Overall	66.6	66.8	63.8	62.3	62.8	63.1	64.9	65.5	66.8	68.6

**Data from the National Statistical Institute (NSI) of the Republic of Bulgaria.*

Table 1.
*Average dairy product consumption per family member in Bulgaria, 2012–2021.**

Yoghurt and yoghurt-like products can also be prepared in the form of a dessert or as a drink.

The wide consumption of fermented milk products in our country is not only the result of a centuries-old tradition. They are widely accepted as a native food with a naturally balanced composition of essential nutrients (proteins, carbohydrates, fats, mineral salts, vitamins, and enzymes), which are easily digestible and necessary, especially for growing and aging organisms [2]. In addition, lactic acid fermentation with selected bacteria repeatedly increases the functional and biological significance of milk-based foods.

1.1 Traditional Bulgarian yoghurt

Bulgarian yoghurt is a traditional food produced by microbial lactic acid fermentation of pasteurized milk. Its traces date as early as 8000 BCE when the domestication of milk-producing animals like sheep, cows, and goats began [3]. Before the commercialization of dairy production in Bulgaria, yoghurt was mainly for family use and mostly made from ewe milk.

Nowadays, both industrial and artisanal technologies of yoghurt production are similar. The processes start with the filtration of the raw milk for the removal of solid particles (**Figure 1**). A common "technological" step is heating the milk for some time and subsequent storage at a suitable temperature. Regardless of the way of production (**Figure 1**), the process is carried out by inoculation with two symbiotically connected bacterial species – *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. The symbiotic relationship of the starter results in the shortened fermentation process and stable characteristics of the final product [4].

The main difference between the artisanal and industrial processes is the step of homogenization (**Figure 1**). It affects the texture and body of the product by reducing whey syneresis (whey separation) and densification of the coagulum, resulting in a thicker yoghurt with improved consistency. In homemade and artisanal yoghurts, the milk fat floats to the surface, forming a creamy layer, called "kajmak". The taste and aroma of Bulgarian yoghurt are specific and also dependent on the raw milk used and the flavoring properties of the starter cultures [5, 6].

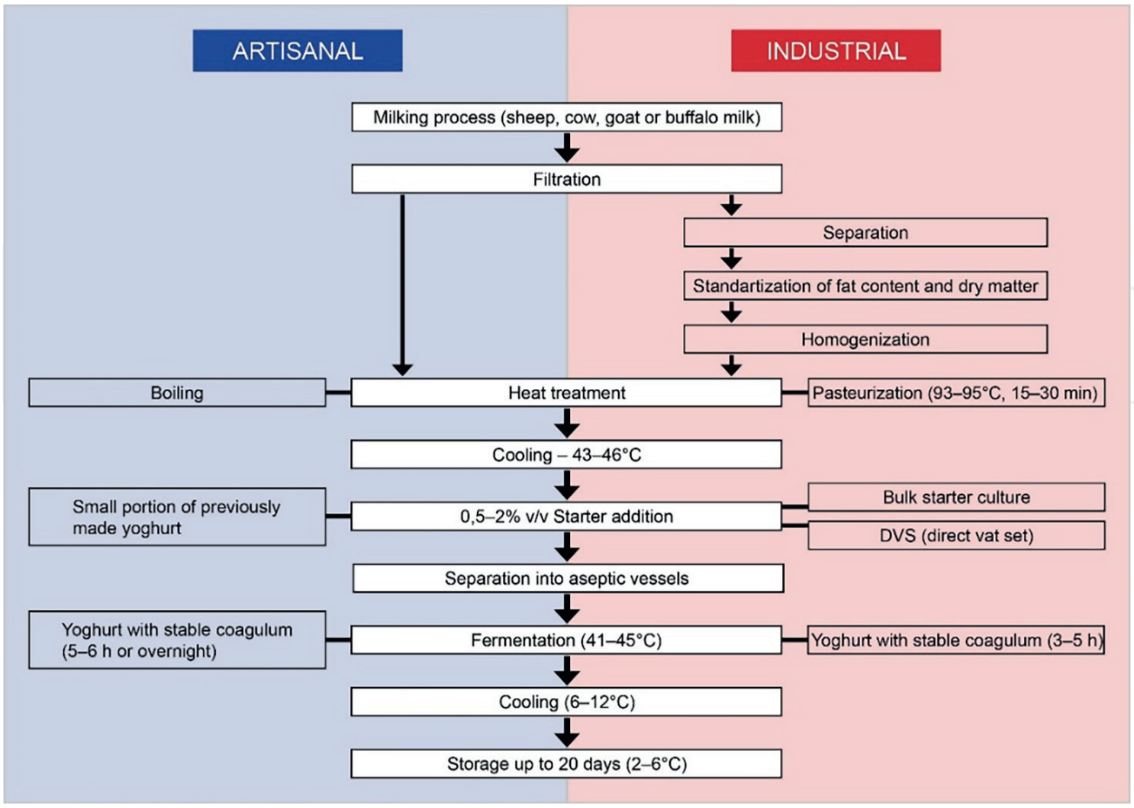

Figure 1.
Scheme of artisanal and industrial technology of Bulgarian yoghurt production.

Yoghurt is evaluated by five organoleptic qualities – appearance, color, aroma, consistency, and taste, according to the International Dairy Federation (IDF) Standard 99A (1987) [7] – and some extra qualities for Bulgarian yoghurt include consistency after stirring, body, and texture, according to the Bulgarian National Standard (12:2010) [8].

One of the rarest and almost forgotten Bulgarian dairy products is called *brano mliako*. "It is made in the region of the Rhodope Mountains by an ancient recipe that has remained unchanged for centuries. It is known to be one of the most unique organic foods in the world because of its qualities. "*Brano mliako*" is made from ewe milk and very much resembles yoghurt. The specificity is that it is made only at the end of summer. The technology starts by collecting raw milk in wooden containers, where it is filtered and thickened. The dehydrated milk then ferments spontaneously, or a starter culture can be added to it, mainly sheep yoghurt. After that, a thin layer of goat or sheep tallow is poured on top, so that the product can be "sealed". This anaerobic preservation makes "*brano mliako*" "suitable to consume for 3 to 4 months.

Gruev (1970) [9] developed a laboratory technology and obtained the same final dairy product. The raw milk is twofold concentrated at reduced pressure at 45–50°C, sterilized by the Koch method for 30 min, and cooled to 45°C. Then, the milk is inoculated with 1% yoghurt starter culture and left for the fermentation process until achieving approximately 190°T acidity. The addition of a 2% yeast-based starter culture, which has been isolated from "*brano mliako*" and cultured in grape must, continues the fermentation. At the end of the yeast fermentation, the obtained dairy product is put in glass containers, hermetically sealed, and stored at 8–10°C for 4–5 months (Gruev, 1970) [9].

1.2 Traditional cheese

- *White-brined cheese:* After yoghurt, the second most widespread and consumed dairy product in Bulgaria is white-brined cheese. It is prepared from cow, goat, sheep, and buffalo milk as well as from mixed milk. Cheese made from a mixture of different types of milk usually possesses improved nutritional value [10]. The production technology of white-brined cheese is specific and involves fermentation and storage in brine (**Figure 2**).

Traditionally at the beginning of the process, the raw milk is preheated, cooled, and fermented by using an enzyme mixture that curdles the casein in the milk, called rennet. Rennet contains the endopeptidases pepsin, lipase, and chymosin [11]. It is originally isolated from the abomasum of new-born ruminants but in industrial processes is mainly biotechnologically obtained by fermentation with recombinant microorganisms, including *Escherichia coli*, *Kluyveromyces lactis,* or *Aspergillus niger* var. *awamori* [12]. Then, the coagulate must be strained in order to separate the aqueous phase from the solid matter. The cheese is later put to ripen for at least 45 days at 15°C, a lengthy process of intensive acidification, lipolysis, and proteolysis. After that,

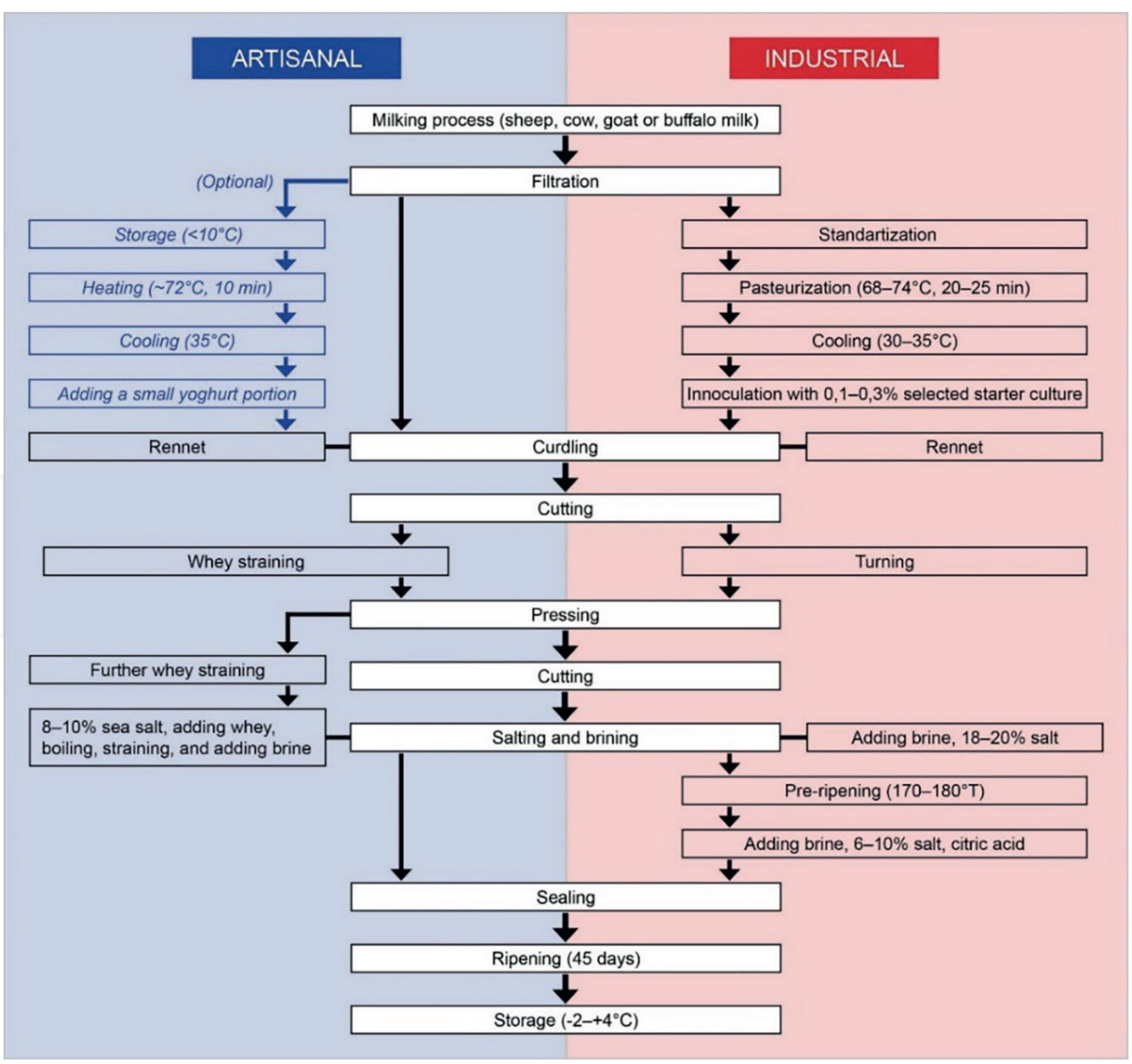

Figure 2.
Scheme of artisanal and industrial technology of Bulgarian white-brined cheese production.

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the product is salted and packaged in containers with brine. The brine should comprise 6–10% sodium chloride, and the acidity of the final product must be 160–180°T. The shelf life of the white-brined cheese is 12–18 months. The final product is a hard cheese with low water and high solid content. Its quality is determined by microbiological, physical, and chemical characteristics and evaluated by its organoleptic qualities, which guarantee the safety, prolonged shelf life, and high nutritional value of the product. The high energy and nutrition of the cheese are mostly due to the high fat and protein content as well as easily digestible peptides; essential free amino acids; minerals, including zinc, magnesium, phosphorus, and calcium; and vitamins, including vitamin A, B, E, and D [13].

- "*Kashkaval*" is the most widespread yellow cheese in Bulgaria and is also from the group of semi-hard cheese. This dairy product can also be made from various kinds of milk, like cow, sheep, goat, and mixed milk, according to the National Bulgarian Standard 14:2010. The production technology of "*Kashkaval*" involves a mandatory cheddarization process, which takes a period of 2 to 6 months for the final product to develop its characteristic aroma. The technology starts with the filtering and centrifugation of raw milk. The milk is then heated to 60–63°C for 20 s to decrease the number of potential spoilage microorganisms and at the same time to preserve the LAB strains. After that, it is cooled and poured into a large vessel, then rennet is added, and the curdling process takes place for 30–40 min. The coagulated mass is cut into small pieces so that the whey can be easily released and stirred for 20 min, followed by another stirring process at 38–39°C, the so-called "cheese baking" process. In some artisanal technologies, weak acids, like acetic acid or citric acid, can be added at this stage to "boil" the cheese. This step is completed when the cheese grains become hard but elastic. The whey is then drained, and the cheddarization takes place at 35–37°C for 2–3 h. Upon this process, a rapid lactic acid fermentation starts, where LAB transform lactose to lactic acid, the pH decreases to 5.2–5.4, and calcium P-caseinate breaks down to monocalcium P-caseinate, achieving a soft and easy-to-manipulate final product. Before getting ready for the next stage, the cheese needs to harden for some time. Then, it is cut into thin slices and placed in a concentrated saline solution for water to be removed and for the cheese to become more firm. Further, the cheese is boiled to obtain a slurry consistency, which is then kneaded and placed in vessels. On the next day, the product is separated from the vessels and left to ripen at 8–12°C for around 55 days. Ultimately, to prevent it from drying out, the ripened "*Kashkaval*" can be covered with melted wax or paraffin. The LAB starter culture determines the specific taste of the final product [14].

Different "*Kashkaval*" products are being made across Bulgaria, and all are in the group of semi-hard cheese. The hardness, texture, flavor, and aroma of the final products depend on the moisture and the aging period. The quality during storage can be evaluated by physical parameters, such as water content, dry matter, total fat content, total protein content, and total salt content [15].

2. Microbiota of the unique Bulgarian dairy products

The diversity of milk, selected starter and nonstarter cultures, and technological treatments give exclusively heterogeneous products with various organoleptic, texture, and nutritional qualities [16]. The uniqueness of presented traditional dairy products is a complex result of the climate conditions, the composition, and activities of milk microbiota [17]. With an understanding of its important role, several studies aimed to characterize LAB diversity, not only in Bulgaria. Discovery of rod- and globular-shaped bacteria (cocci), named initially *Thermobacterium bulgaricum* and *Streptococcus thermophilus,* respectively, started in 1905 with the work of Bulgarian scientist Dr. Stamen Grigorov. The cooperation between the two microorganisms was considered to be one of the most important characteristics of the typical Bulgarian yoghurt. In 1938 Dr. K. Popdimitrov considered that using only the two bacterial species is proper for the production of Bulgarian yoghurt and classified all other microorganisms as undesirable. According to the national standard, the starter cultures for Bulgarian yoghurt include strains of Bulgarian origin from species *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. At least 10^7 CFU/g in a ratio of 1:2–1:5 is needed as a yoghurt starter, according to the definition of FAO/WHO (2003) [1]. LAB reach 10^7–10^8 CFU/g for *L. bulgaricus* and 10^8–10^9 CFU/g for *Str. thermophilus* in the final product. However, homemade and artisanal yoghurts contain different non-starter species that add to the different sensory qualities of the final products. The biodiversity of autochthonous microbiota and its use as starter cultures make Bulgarian dairy products exceptional. Species from the family *Lactobacillaceae* and genera *Lactococcus, Leuconostoc, Streptococcus, Enterococcus*, *Pediococcus*, and especially the new genera of previously determined genus *Lactobacillus* have been isolated throughout the years from different homemade and artisanal dairy products [18–21]. The autochthonous microbiota of "*brano mliako*" is comprised of yeast species as well, including *Kl. lactis* and *Saccharomyces cerevisiae* [2]. Our research proved that such products contain more diverse microbiota (**Figure 3**).

A special protocol with an overnight enrichment step, however, was needed. More than 90 artisanal samples from yoghurt, white-brined cheese, and "kashkaval", made at home or small farms in different rural regions of Bulgaria, have been collected. The combination of classical phenotypic and microbiological with molecular and typing methods, according to the polyphasic taxonomic approach applied, allowed us to establish the biodiversity of their LAB microbiota (**Figure 3**). The most persistent was the species *Lactiplantibacillus plantarum.*

Multiplex PCR analyses (according to Torriani et al. [22]) allowed to identify closely related *Lactiplantibacillus paraplantarum*, *Lactiplantibacillus pentosus*, and *L. plantarum* and revealed their presence in homemade yoghurt and white-brined cheese [23, 24]. By the gold standard, 16S rDNA sequencing, *L. bulgaricus* was identified in yoghurt samples. In addition, *L. plantarum* presence in yoghurt with a dominance in ripened samples of cheese was confirmed. In the early stage, however, significant number of enterococci and lactococci have to be pointed for white-brined cheese samples. Originally, *lactobacilli* have a low density in cheese (<50 CFU/g). During the ripening period, they significantly increase and become the dominating microbiota in the final product (10^7–10^8 CFU/g). Therefore, an enrichment step with pre-culturing of collected samples allowed us to establish LAB diversity.

Nemska et al. [20] isolated 74 pure cultures from different dairy products, made by traditional recipes without the addition of industrial starters. Using classical phenotypic tests, 45 out of them were identified as lactobacilli [20]. The group of lactobacilli from white-brine cheese was the most numerous (23 strains from cow, sheep, buffalo, goat, and a mixture of cow and buffalo milk), followed by yoghurt isolates (14 strains from cow, sheep, and buffalo milk), 1 strain from curd (from goat

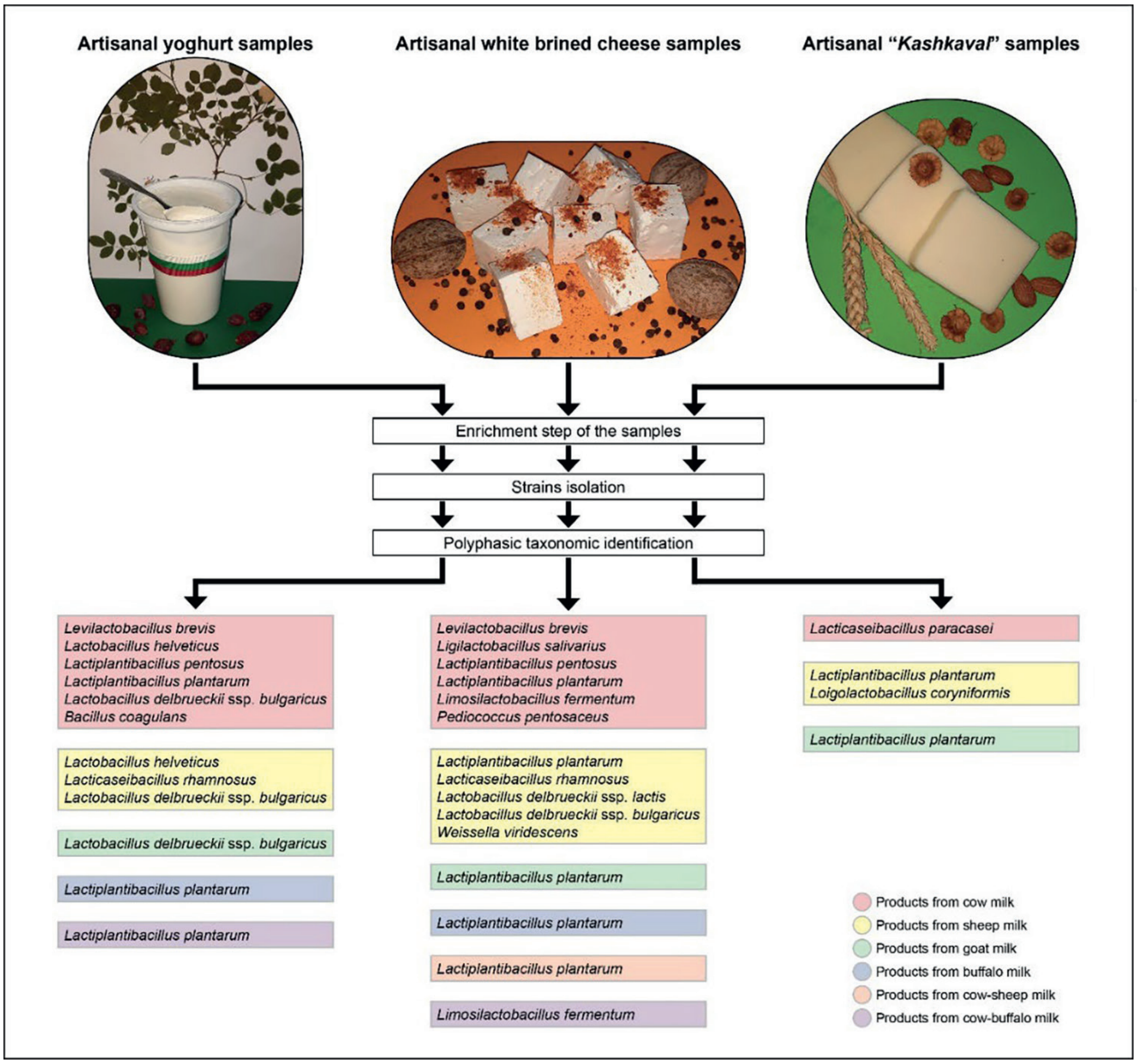

Figure 3.
Biodiversity of LAB microbiota in artisanal Bulgarian dairy products, studied during a period of 10 years.

milk), and 4 strains from yellow cheese (from goat and cow milk) [20]. Twenty-two strains were identified as *L. plantarum*. A predominance of *L. plantarum* in the non-starter microflora of Bulgarian white-brined cheese has been reported [23, 25]. This species is also reported to be present in high density in many different types of cheese [26–28]. *Ligilactobacillus acidipiscis* was also identified in a homemade ripened sample of cheese, made from raw ewe milk, in the region of nuclear power plant (Kozloduy, Bulgaria) (Danova, 2015, unpublished data). The tendency of rich LAB microbiota in samples made from non-pasteurized milk was proven in several samples from sheep, cow, and mixed milk [19, 20]. Most traditional cheese achieve their specific taste and aroma due to the natural microflora of raw milk. The raw milk that is used impacts the unique organoleptic and sensory qualities of the final product. Different cheese products can be made with rennet and natural microflora, with or without starter cultures.

Today, the wide variety of cheese with specific organoleptic properties is due to the different combinations of LAB species. It is important to maintain an optimal strain balance in the starter because of the complex microbial interactions. Even small differences in the microbial composition can lead to surprising or unwanted effects on the quality of the cheese product.

According to Bulgarian National Standard for white-brined cheese, a mesophilic and/or thermophilic starter is used in a ratio of 2:1. The mesophilic starter is predominant and contributes to the ripening processes and developing the taste and aroma of the final product. The mesophilic LAB for white-brined cheese and "*Kashkaval*" are represented by *Lactococcus lactis* subsp. *lactis* and *Lacticaseibacillus casei*. The thermophilic starter includes *L. bulgaricus* and *Str. thermophilus.* For yellow cheese "*Kashkaval*", in addition to *L. bulgaricus* and *S. thermophilus*, the thermophilic *Lactobacillus helveticus* is also present. For all Bulgarian dairy products, the strains in the starters must be isolates of Bulgarian origin.

3. Functional characteristics of the autochthonous microbiota in Bulgarian dairy products

3.1 Metabolic activity as a factor of organoleptic properties of fermented dairy products

In the manufacturing process, starters are added for lactose fermentation, lipolysis (fat degradation), and proteolysis [29]. LAB that are naturally found in milk have a major role in the fermentation processes alongside the intentionally added starter cultures. The fermentation improves the general characteristics of the end product including texture and consistency, aroma, flavor, and the development of color. The intricate biochemical processes during fermentation involve many different enzymatic reactions. With an understanding of the strain/species specificity, a large screening for metabolic activity of newly isolated Bulgarian strains was carried out. Results with identified *L. plantarum* strains from cheese (unpublished data) and [20, 23, 24, 30] are summarized (**Figure 4**).

The produced LAB enzymes responsible for lipolysis and proteolysis in milk are among the key factors for the sensory qualities of taste and texture of cheese [31]. LAB possess different degrees of lipolysis, which is important for the selection of strains that can be used for starter cultures. During lipolysis, triglycerides hydrolyze into mono- and diglycerides, free fatty acids, and glycerol. The reduced glycerides participate in coagulation processes with different components of dairy foods that lead to the texture development of the final product [32] (Esteban-Torres et al., 2014). This characteristic is related to the flavor development of fermented dairy products [33].

During proteolysis, hydrolysis of the protein peptide bonds occurs and transforms them into peptides and free amino acids. Although many LAB are considered to have weak proteolytic activity, they possess complex proteinase/peptidase systems comprising peptidases on the cell wall initiating the degradation processes, peptide transporters, and intracellular peptides that break down peptides into shorter molecules and free amino acids [34]. Thus, essential amino acids may have accumulated in fermented products, due to the high peptidase activity (**Figure 4**) estimated for *L. plantarum* strains. For 8 of tested L. plantarum strains from white-brined cheese, accumulation of free amino acids from 0.170 to 0.609 mMGly/L was shown. The accumulated free amino acids are involved in reactions of deamination, transamination, decarboxylation, and desulfurization with an impact on the flavor profile of the end product [34]. At the same time, these 8 strains were characterized by low proteolytic activity. With a sample screening protocol [35], using milk agar and calcium caseinate agar (Fluka), we differentiated with low proteolytic activity all strains generating clear zone 1–8 mm, moderate zone 8-13 mm, and high zone >13 mm.

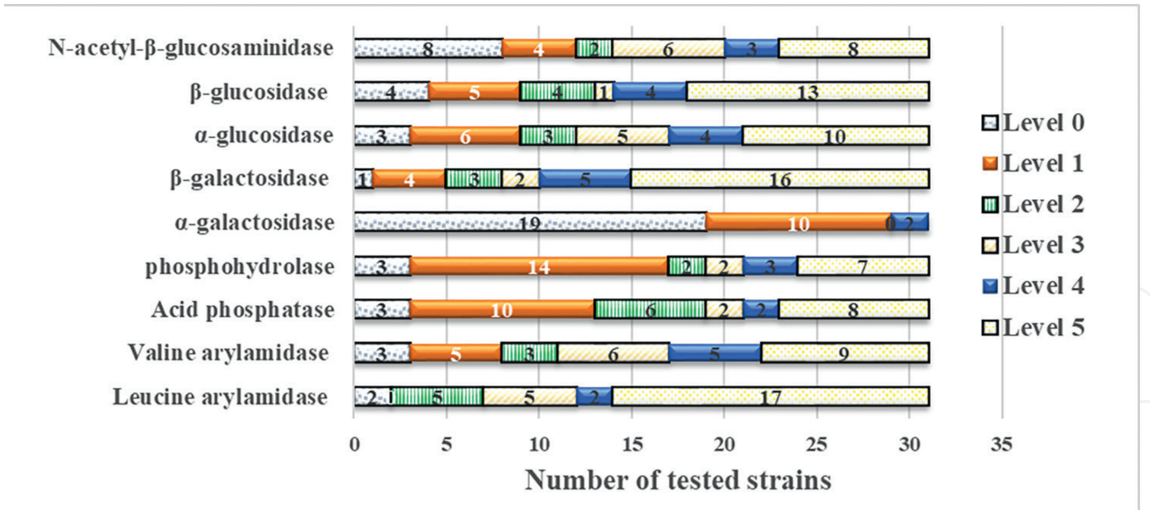

Figure 4.
Enzymatic activity of 31 L. plantarum strains isolated from artisanal Bulgarian dairy products (semi-quantitative analysis with the API ZYM test strips, bioMérieux, France).

Despite high peptidase activity (**Figure 4**), only 2 out of the 31 tested exponential *L. plantarum* cultures possessed high activity. The best results were obtained with 72 h cell-free supernatants (CFS) of *Lactobacillus* cultures. *L. bulgaricus* with yoghurt origin showed fast milk coagulation in combination with moderate to high proteolytic activity. Peptidase as well as β-galactosidase activity is promising for candidate probiotics, while other enzyme activities may contribute to organoleptic characteristics and stability of the products.

3.2 Antagonistic LAB activity and food preservation

It is well known that contamination of different types of foods with filamentous fungal microorganisms, yeast, and bacteria is the main reason for food spoilage. The presence of unwanted species in dairy products can cause different types of deterioration of organoleptic properties [36]. Also, molds such as *Aspergillus* and *Penicillium* can produce mycotoxins [37]. More than 60 yeast species have been identified as spoilage agents of dairy products, represented most frequently by a high diversity of *Candida* spp., as well as genera *Kluyveromyces*, *Geotrichum*, and *Yarrowia lipolytica* and phylum *Basidiomycota*, mainly *Cryptococcus* and *Rhodotorula* species [38]. Dairy product spoilage by yeasts results in visible alteration due to growth on the surface of the product, unpleasant changes in odor, flavor, or texture and production of different metabolites such as CO_2, alcohols, aldehydes and esters, proteolytic and lipolytic enzymes [36], and biogenic amines [39].

LAB can be used not only as starter cultures but also as protective cultures to improve the safety and/or shelf life of the product [40]. Their preservative action is due to the combined action of a number of antimicrobial metabolites produced during the fermentation process. LAB produce a large range of antimicrobial substances, including organic acids (lactic, acetic, etc.), fatty acids, antifungal peptides, reuterin, and bacteriocins [41–44]. Formally, the metabolites produced by LAB can be divided into two main groups: substances with a low molecular mass < 1000 Da and substances with a high molecular mass > 1000 Da, such as bacteriocins/bacteriocin-like inhibitory substances (BLIS). To be defined as food-grade bioprotective cultures, LAB strains are selected according to their antimicrobial properties.

As bioprotective cultures, LAB are expected to possess antibacterial/antifungal activity that is exhibited and maintained throughout the manufacturing process and storage time, to have no impact over the functions of the starter cultures, not to modify the organoleptic properties of the final product, to be used with the lowest possible inoculum that maintains the same activity to reduce the cost value, and to have easy propagation and resistance to technological processes [38]. In the later stages of ripening, *lactobacilli* are well adapted to the environment inside the cheese, withstanding the low pH, high salt concentration, absence of sugars, and anaerobic conditions, and they may produce BLIS.

Lactobacillus, as well as *Leuconostoc, Lactococcus, Pediococcus,* and *Weissella*, are the most frequently cited genera to possess antifungal properties and have been the most evaluated *in situ* in recent years as well [45]. Many *Lactobacillus* species, including *L. plantarum, Levilactobacillus brevis, L. casei,* and *Lacticaseibacillus paracasei*, were shown to exhibit antifungal activity against a large spectrum of fungal representatives, including *Penicillium, Kluyveromyces, Candida, Rhodotorula*, *Aspergillus,* etc., which are among the most common spoilage microorganisms in dairy products [46–52]. Other lactobacilli, such as *Limosilactobacillus fermentum, Lacticaseibacillus rhamnosus, Schleiferilactobacillus harbinensis, L. helveticus,* and *Lactobacillus amylovorus*, were shown to be able to extend the shelf life when added as adjunct cultures in yoghurt and cheese [49, 53–55].

The well-expressed profile of antagonistic activity of the autochthonous LAB microbiota of traditional fermented foods against a number of pathogens and food-associated contaminants is established. Our strains, isolated from yoghurt, white-brined cheese, and "Kashkaval," show a strain-specific broad spectrum of activity against Gram (+) and Gram (−) food spoilage microorganisms and clinical pathogens (**Figure 5**).

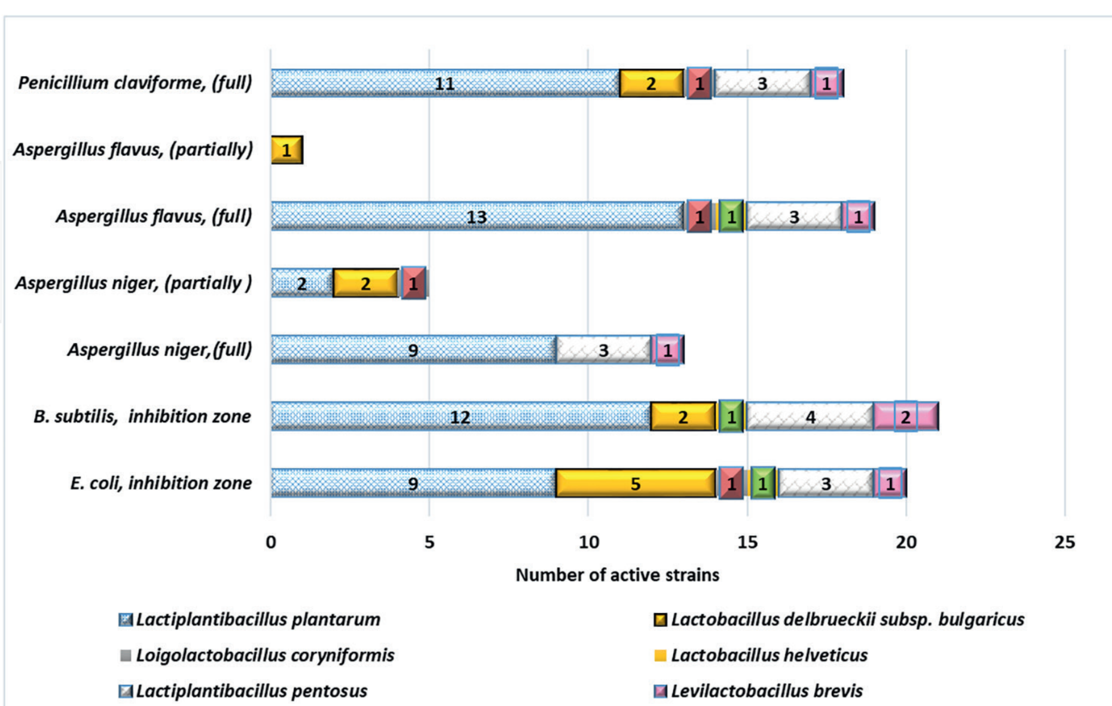

Figure 5.
Antagonistic activity of strains isolated from artisanal Bulgarian dairy products against food-associated pathogens and contaminants.

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Initial results with *L. plantarum* strains from white-brined cheese were highly promising [23]. In the presence of 5% (v/v) CFS from 12 newly isolated strains from cheese and yoghurt cultures, the growth of *Pseudomonas aeruginosa* PA01 (from a patient with cystic fibrosis) was significantly inhibited up to 50–80% (unpublished data). A group of active strains belonging to the species *L. plantarum* and *L. rhamnosus* from dairy products inhibited *Staphylococcus aureus* MRSA as well as out-patient antibiotic-resistant strains [56]. In situ results of antibacterial/antifungal activity of whey fractions, skimmed milk, cow milk, and soya milk are also important [24]. For the strains of LAB, which form the microbiota of traditional fermented foods, a well-expressed profile of antagonistic activity against a number of pathogens and food-associated contaminants is established. Our isolated strains from yoghurt, white-brined cheese, and "Kashkaval" show antibacterial activity against Gram (+) and Gram (−) bacterial representatives, but their antifungal activity is also well expressed (**Figure 5**).

4. Bulgarian dairy products as functional foods

For more than a decade, the focus of nutritional science aims toward optimal nutrition, the objective of which is the optimization of the daily diet regarding nutrients, non-nutrients, and other food ingredients that favor the maintaining of good health [57]. The increased lifestyle diseases, in combination with the high healthcare costs, are the reasons for the rising research to formulate and produce foods with functions that could improve health and well-being and lower the risk of or delay ongoing major diseases [58]. Within this context, the concept of functional foods has arisen.

A functional food must have health benefits and can be classified as one if it enhances target functions or reduces the risk of specific diseases, has to provide benefits beyond the basic nutritional functions, should be or look like a traditional food, and should have a dietary pattern and be a part of the normal daily diet. From this baseline, the most complete definition of functional foods was proposed by the EC Concerted Action on Functional Food Science in Europe (FUFOSE). It states that a functional food is one that beneficially affects one or more target functions in the human body beyond normal nutritional effects, relevant to improved health state and well-being and/or reduced risk of disease, and it is consumed as a normal food pattern, not in the form of a pill, capsule, or any other dietary supplement (European Commission, 2010) [59].

Dairy products have a special place in Bulgarian diet. After Stamen Grigorov's discovery, extensive research has begun on the unique nutritional characteristics of Bulgarian yoghurt. In 1909, the Russian biologist and Nobel Prize winner Elie Metchnikoff developed a theory regarding the prolongation of life. He proposed that there is a relationship between the increased life expectancy of Bulgarians and the daily consumption of yoghurt [60]. Then, it was suggested that the consumption of yoghurt is connected with the increased number of Bulgarian centenarians. Metchnikoff's main research was on lactic acid, which is proven to reduce the number of putrefactive microorganisms [61, 62]. Then, he further proposed another hypothesis that the inhibition of pathogens and the harmful fermentation of food in the gut can slow down the process of aging.

Studies report that *L. bulgaricus* possess high antimicrobial properties and are able to colonize the human intestines, which suggests its probiotic functions [63, 64].

More studies describe that regular consumption of yoghurt that contains viable *L. delbrueckii* and *Str. thermophilus* can improve decreased lactose intolerance and the overall digestion of lactose [65, 66]. From intensive research, Bulgarian yoghurt could be considered a dairy product with functional characteristics, as scientific results state that functional probiotic foods can modulate the microbial composition in the gut, thereby improving intestinal health [67, 68].

Our achievements clearly showed that products with a century-old tradition of production and consumption in the Balkans are a promising source of beneficial LAB microbiota with the capacity to transfer them into new safety food with functional properties.

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Conflict of interest

The authors declare that there is no conflict of interest.

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