Traditional Bulgarian Dairy Products

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Definition

Dairy products are nutritionally indispensable, consumed daily and one of the most desired foods by a large part of the world population.

1. Introduction

Fermented milk is very useful for many reasons. The related products have a prolonged shelf life; they are safe because lactic acid bacteria (LAB) act as preservatives and inhibit the development of pathogenic microflora, they are extremely suitable for the absorption of nutrients from milk, and they are beneficial due to the health impact of lactic acid bacteria on various body functions [1].

According to Rul (2017), traces of fermented milk products (milk lipids discovered on clay vessels) have been found as early as 8000 B.C. in Asia Minor and Eastern Europe, soon after the domestication of milk-producing animals (cows, sheep, and goats) [2]. Evidence of kefir consumption was found in China in a Bronze Age tomb [3]. The first dairy products resembling yoghurt were invented around 5000–6000 B.C. in Mesopotamia [1]. Located on the Balkan Peninsula, between Western Europe and the Middle East, at this time the Bulgarian lands were inhabited by various ancient communities, with their typical material culture including fermented food traditions. The climate in Bulgaria is especially suitable for animal dairy husbandry. Bone fragments, collected from Azmashka Neolithic village (near Stara Zagora, 6000 B.C.), belonged to 118 cattle, 73 sheep, and 27 goats [4]. Historians believe that the Thracian tribe Bizalti (who inhabited today’s lands of Shumen, Targovishte and Varna) were the first to start purposefully preparing fermented dairy products [5]. Another direction in the search for the origin of lactic acid dairy fermentation is offered by the descriptions of the Greek historian Herodotus, according to whom the Scythians (nomadic tribes living between the rivers Dnieper and Don) consumed sour milk. Mare’s fermented milk was also used by the proto-Bulgarians for food, and stored in leather bags made of stomachs. The resulting product was called koumiss and was a staple food during military campaigns. The Slavs are known to have consumed sura, a product obtained by placing yoghurt in wooden barrels in the summer and consuming it in the winter by liquefying it with drinking water. When the ancient Bulgarians rediscovered sheep’s yoghurt used by the Thracians and Slavs, it became preferable to mare’s milk. The observation that fermented dairy products are beneficial for human health dates back to their invention since it is described in Indian Ayurvedic scripts from about 6000 B.C. [6]. In Europe, the healing effects of Bulgarian yoghurt have been known since at least 1542, when the French King Francois I was cured of chronic diarrhoea by a simple yoghurt diet [2]. However, the discovery of yoghurt microbiota (as a cause of yoghurt fermentation) happened only in the 20th century. In 1905, Stamen Grigorov, a Bulgarian medical student in Geneva, Switzerland, was the first to describe the rod-shaped lactic acid bacterium (named Bacillus bulgaricus Grigoroff), accompanied by a spherical Streptococcus, in Bulgarian yoghurt [7]. Based on Grigorov’s findings, in 1909 the Russian biologist and Nobel Prize winner Elie Metchnikoff, developing his theory about the prolongation of life, was the first who proposed that daily yoghurt consumption engenders the longevity of the Bulgarian peasant population, especially in the mountainous regions. Metchnikoff suggested that there is a connection between the consumption of yoghurt and the number of Bulgarian centenarians. He further proposed the hypothesis that the inhibition of harmful food fermentation in the gut can delay the process of ageing. At the heart of his research is lactic acid, which reduces the number of putrefactive microorganisms [7][8]. Then, the benefits of yoghurt consumption were widespread in Europe by doctors, pharmacologists and journalists, which led to a general demand for yoghurt as a medicine in the first third of the 20th century, for instance, against “food neophobia” and other gastrointestinal disorders. In the period 1909–1912, physicians and bacteriologists Guéguen, Bulloch, Vaughan, Hertz, and Lane independently of each other discussed the therapeutic nature of the "lactic acid bacillus", and as a result, Danone's company distributed its yoghurt through the city pharmacies of Barcelona in 1912, followed by entering the French yoghurt market in 1923 [7]. Today, more than 45 bln metric tons (MT) of fresh dairy products are consumed annually in Europe. In 2019, 6.4 bn MT of cheese and 6.1 bn MT of yoghurt were produced by the countries of the European Union. Bulgarian dairies processed 663,644,000 L of raw milk in 2020, 94.3% of which was cow's milk. In comparison to the other EU countries, the obtained genuine yoghurt (156,610 MT) and white brined cheese (899 MT) in Bulgaria were in limited quantities, but they are known for their very high quality [9].

2. Microbial Content of Traditional Bulgarian Dairy Products

Genuine Bulgarian yoghurt contains a starter culture consisting of L. delbrueckii ssp. bulgaricus and S. thermophilus. Their proto-cooperation was comprehensively reviewed in several studies [10][11][12][13]. Briefly, L. bulgaricus, which is a bacterial species with high proteolytic activity, releases free amino acids that favour the growth of S. thermophilus. On its own, S. thermophilus engenders rapid acidification and diminishes the levels of dissolved oxygen in milk, thus improving the culture conditions for its symbiotic partner. Lactose is the main carbon source for LAB growth in milk, and different species have developed various mechanisms to transport and utilize it. Figure 1 shows the routes of lactose conversion to lactic acid and other volatile compounds contributing to the unique flavour of dairy foods.

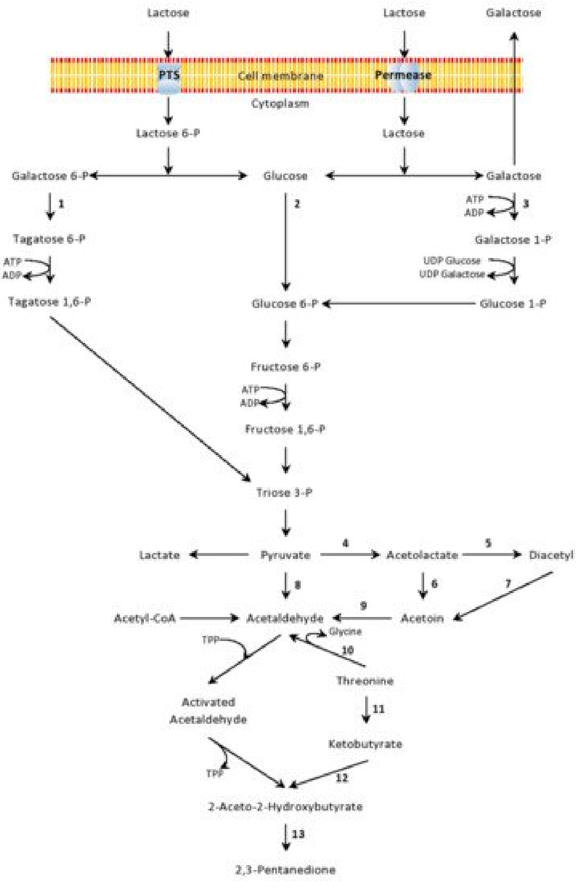

Figure 1. Schematic presentation of lactose transport, metabolization routes and formation of volatile compounds by lactic acid bacteria (LAB) in dairy foods, according to Rul (2017), and Solopova et al. (2012) [2][14]. Designations: 1, tagatose 6-phosphate pathway; 2, glycolysis; 3, Leloir pathway; 4, acetolactate synthase; 5, oxidative decarboxylation; 6, acetolactate decarboxylase; 7, diacetyl reductase; 8, pyruvate decarboxylase; 9, acetoin dehydrogenase; 10, threonine aldolase; 11, threonine deaminase; 12, acetolactate synthase; 13, oxidative decarboxylation.

Two forms of lactose can be introduced into the cell, unphosphorylated or phosphorylated. The first engages the transporter lactose permease LacS. Inside the cell, the enzyme β-galactosidase hydrolyses lactose to glucose and galactose [15]. Depending on the LAB species, lactose transport is coupled to proton symport or galactose antiport [2]. L. bulgaricus and S. thermophilus release the galactose moiety of lactose into the medium, whereas Leuc. lactis, Lactococcus ssp., and many Lactobacillus ssp. metabolize it. Glucose is metabolized via glycolysis, whereas galactose, depending on the particular LAB, follows either the tagatose 6-phosphate or the Leloir pathway [16][17]. Other LAB, such as starter lactococci, L. paracasei, L. casei, and L. plantarum, import lactose exclusively by lac-PTS—the phosphoenolpyruvate (PEP)-dependent phosphotransferase system. In this way, lactose is translocated and phosphorylated simultaneously with PEP as the first phosphoryl donor. After translocation, lactose is hydrolysed to glucose and galactose-6-P; glucose enters the glycolytic pathway through phosphorylation by glucokinase, whereas galactose-6-P is further metabolized via the tagatose-6-P pathway. However, pyruvate, aspartate and aromatic amino acids are the key metabolites needed for the production of the wide spectrum of volatile compounds contributing to the specific aroma of dairy foods [18][19]. As for Bulgarian yoghurt, its sour smell is associated with high amounts of lactic acid, but the overall flavour is also influenced by acetaldehyde, acetoin, diacetyl, and 2,3-pentanedione [20].

When artisanal yoghurt is prepared, it contains rich autochthonous LAB microflora. According to Velikova et al. (2018), 53% of the LAB strains isolated from homemade yoghurts belong to L. bulgaricus, 14% to other lactobacilli, and 32% to lactic acid cocci (S. thermophilus, Pediococcus acidilactici, Lactococcus lactis, Enterococcus faecium). In Table 6 are shown the most common lactobacilli in Bulgarian yoghurt, which are L. helveticus, Lacticaseibacillus paracasei, Limosilactobacillus fermentum, and Lacticaseibacillus rhamnosus; several strains of Leuconostoc mesenteroides, Leuc. pseudomesenteroides, and Weissella confusa were isolated from yoghurt as well [10].

Regarding white brined cheese, the starter culture usually contains Lc. lactis subsp. lactis and Lacticaseibacillus casei, L. bulgaricus and S. thermophilus. The majority of non-starter LAB are mesophilic lactobacilli such as Lactiplantibacillus plantarum, L. paraplantarum, L. pentosus, L. paracasei subsp. paracasei, Lentilactobacillus hilgardii, and L. brevis (Table 1). Most of them are salt- and acid-tolerant facultative anaerobes, which grow well in cheese as their number reaches up to 10^9 CFU/g during ripening [21]. Other authors report an extremely large amount of LAB, up to 3.7×10^{10} CFU/g, observed in white brined cheese produced by a small family farm near Dryanovo, Bulgaria [22]. Four different strains of L. plantarum, Pediococcus spp., Enterococcus spp., and Leuconostoc spp. with probiotic properties have been isolated from Bulgarian home-made brined cheese [23]. Four bacteriocinogenic strains of Ent. faecium have been isolated and identified from Bulgarian home-made white brined cheese, and the authors propose to use them as preservatives in the production of dairy products [24]. The species Ent. faecium and Ent. durans are used as adjunct culture in the manufacture to accelerate the proteolysis of β-casein and αs1-casein [21].

Kashkaval microflora was recently described by Teneva-Angelova et al. (2018) [25]. The main starter cultures used in its production include the mesophilic Lc. lactis ssp. lactis and Lc. lactis ssp. cremoris, and the thermophilic L. bulgaricus, L. helveticus, and S. thermophilus. A broad spectrum of other species has been isolated from artisanal samples contributing to their flavour: Leuc. mesenteroides, L. lactis ssp. lactis biovar. diacetylactis, and Enterococcus spp. [26][27].

The study of Tserovska et al. (2002) showed that 18 different LAB strains can be isolated from home-made katak. Nine of them belonged to the lactic acid cocci (P. acidilactici, P. pentosaceus); the others—to L. delbrueckii ssp. bulgaricus, L. delbrueckii ssp. delbrueckii, and L. delbrueckii ssp. lactis) [28]. The predominance of lactic acid cocci in homemade cheese and katak was reported by Kirilov et al. (2011). From 110 identified LAB strains, 58 belonged to genus Enterococcus, 20 to Streptococcus spp., 11 to Lactococcus spp., and only 21 were identified as Lactobacillus spp. [29]. Typical for kefir are LAB species, L. kefiranofaciens, Lentilactobacillus kefiri [30], Lentilactobacillus buchneri [31], L. plantarum [30], and the yeast species Kluyveromyces marxianus [32], Kazachstania unispora [33], Dekkera anomala [34], and several species of the genus Saccharomyces [35]. Koumiss is fermented mainly by LAB species, L. acidophilus, L. helveticus, Ligilactobacillus salivarius, L. buchneri, L. plantarum [36], S. thermophilus, and Leuconostoc spp. [37], and yeasts Torula kumiss, Saccharomyces lactis, Sacch. unisporus, and

Kluyveromyces lactis [38][39].

Table 1. The microbial content of traditional Bulgarian dairy foods and beverages.

Product	Starter Strains	Accompanying Microflora	Reference
Yoghurt	L. delbrueckii ssp. bulgaricus, S. thermophilus	L. helveticus, L. paracasei, L. fermentum, Lacticaseibacillus rhamnosus, Leuc. mesenteroides, Leuc. pseudomesenteroides, W. confusa, P. acidilactici, Lc. lactis, Ent. faecium	[10][11][12][13]
White brined cheese	Lc. lactis ssp. lactis, L. casei, L. delbrueckii ssp. bulgaricus, S. thermophilus	L. plantarum, L. paraplantarum, L. pentosus, L. paracasei ssp. paracasei, Lentilactobacillus hilgardii, L. brevis, Leuconostoc spp., Ent. faecium, Ent. durans	[21][22][23][24]
Kashkaval	Lc. lactis ssp. lactis, Lc. lactis ssp. cremoris, L. delbrueckii ssp. bulgaricus, L. helveticus, S. thermophilus	Leuc. mesenteroides, L. lactis ssp. lactis biovar. diacetylactis, Enterococcus spp.	[25][26][27]
Katak	L. delbrueckii ssp. bulgaricus, S. thermophilus	L. delbrueckii ssp. delbrueckii, L. delbrueckii ssp. lactis, P. acidilactici, P. pentosaceus, Enterococcus ssp.	[28][29]
Kefir	L. kefiranofaciens, Lentilactobacillus kefiri, Lentilactobacillus buchneri, L. plantarum, L. amylovorus, Levilactobacillus brevis, L. casei, L. paracasei, L. crispatus, Lactobacillus delbrueckii subsp. bulgaricus, L. helveticus, L. parakefiri, L. satsumensis, L. uvarum, S. thermophilus, Lc. lactis ssp. cremoris, Lc. lactis ssp. lactis, Kluyveromyces marxianus, K. lactis	Leuc. lactis, Leuc. mesenteroides, Acetobacter fabarum, A. lovaniensis, A. syzygii, Ent. faecium, Gluconobacter japonicus, Weissella spp., Halococcus spp., Candida inconspicua, Dysgonomonas spp., Geotrichum candidum, Kazachstania aerobia, Kz. exigua, Kz. unispora, Lachancea meyersii, Pelomonas spp., Pichia fermentans, P. guilliermondii, P. kudriavzevii, Sacch. cerevisiae, Sacch. martiniae, Sacch. turicensis, Sacch. unisporus, Shewanella spp.	[30][31][32][33][34]
Koumiss	L. acidophilus, L. helveticus, Ligilactobacillus salivarius, S. thermophilus, K. lactis	L. buchneri, L. plantarum, Leuconostoc spp., Sacch. lactis, Sacch. unisporus, Torula kumiss	[36][38][39][35]

References

1. Tribby, D. Yogurt. In The Sensory Evaluation of Dairy Products, 2nd ed.; Clark, S., Costello, M., Drake, M., Bodyfelt, F., Eds.; Springer Science & Business Media: New York, NY, USA, 2009; pp. 191–224.
2. Rul, F. Yogurt: Microbiology, organoleptic properties and probiotic potential. In Fermented Foods, Part II: Technological Interventions; CRC Press: Boca Raton, FL, USA, 2017; pp. 419–450.
3. Yang, Y.; Shevchenko, A.; Knaust, A.; Abuduresule, I.; Li, W.; Hu, X.; Wang, C.; Shevchenko, A. Proteomics evidence for kefir dairy in Early Bronze Age China. J. Archaeol. Sci. 2014, 45, 178–186.
4. Kostov, D. Domestic and wild animals, from the Neolithic period, in the “Azmashka” settlement hill, near Stara Zagora. Thrakia J. Sci. 2006, 4, 55–60. (In Bulgarian)
5. LB Bulgaricum, Yoghurt History. Available online: (accessed on 10 January 2021).
6. Fisberg, M.; Machado, R. History of yogurt and current patterns of consumption. Nutr. Rev. 2015, 73, 4–7.
7. Stoilova, E.R. Producing Bulgarian Yoghurt: Manufacturing and Exporting Authenticity, 1st ed.; Amsterdam University Press: Amsterdam, The Netherlands, 2014; pp. 38–45.
8. Mackowiak, P.A. Recycling Metchnikoff: Probiotics, the Intestinal Microbiome and the Quest for Long Life. Front. Public Health 2013, 1, 52.
9. Activity of the Dairies in Bulgaria in 2020: Official Bulletin of Bulgarian Ministry of Agriculture, Foods and Forests. Available online: (accessed on 15 January 2021).
10. Velikova, P.; Petrov, K.; Lozanov, V.; Tsvetanova, F.; Stoyanov, A.; Wu, Z.; Liu, Z.; Petrova, P. Microbial diversity and health-promoting properties of the traditional Bulgarian yogurt. Biotechnol. Biotechnol. Equip. 2018, 32, 1205–1217.

11. Hao, P.; Zheng, H.; Yu, Y.; Ding, G.; Gu, W.; Chen, S.; Yu, Z.; Ren, S.; Oda, M.; Konno, T.; et al. Complete Sequencing and Pan-Genomic Analysis of Lactobacillus delbrueckii subsp. bulgaricus Reveal Its Genetic Basis for Industrial Yogurt Production. PLoS ONE 2011, 6, e15964.
12. Liu, M.; Siezen, R.J.; Nauta, A. In Silico Prediction of Horizontal Gene Transfer Events in Lactobacillus bulgaricus and Streptococcus thermophilus Reveals Protocooperation in Yogurt Manufacturing. Appl. Environ. Microbiol. 2009, 75, 4120–4129.
13. Ivanov, I.; Petrov, K.; Lozanov, V.; Hristov, I.; Wu, Z.; Liu, Z.; Petrova, P. Bioactive Compounds Produced by the Accompanying Microflora in Bulgarian Yoghurt. Processes 2021, 9, 114.
14. Solopova, A.; Bachmann, H.; Teusink, B.; Kok, J.; Neves, A.R.; Kuipers, O.P. A Specific Mutation in the Promoter Region of the Silent cel Cluster Accounts for the Appearance of Lactose-Utilizing Lactococcus lactis MG1363. Appl. Environ. Microbiol. 2012, 78, 5612–5621.
15. Aleksandrzak-Piekarczyk, T. Lactose and β-Glucosides metabolism and its regulation in Lactococcus lactis: A Review. In Lactic Acid Bacteria—R & D for Food, Health and Livestock Purposes; Kongo, J.M., Ed.; InTechOpen: London, UK, 2013; pp. 467–486.
16. Vaughan, E.E.; van den Bogaard, P.T.; Catzeddu, P.; Kuipers, O.P.; de Vos, W.M. Activation of silent gal genes in the lac-gal regulon of Streptococcus thermophilus. J. Bacteriol. 2001, 183, 1184–1194.
17. Tenea, G.N.; Suárez, J. Probiotic Potential and Technological Properties of Bacteriocinogenic Lactococcus lactis Subsp. lactis UTNGt28 from a Native Amazonian Fruit as a Yogurt Starter Culture. Microorganisms 2020, 8, 733.
18. Cheng, H. Volatile Flavor Compounds in Yogurt: A Review. Crit. Rev. Food Sci. Nutr. 2010, 50, 938–950.
19. Pastink, M.I.; Teusink, B.; Hols, P.; Visser, S.; De Vos, W.M.; Hugenholtz, J. Genome-Scale Model of Streptococcus thermophilus LMG18311 for Metabolic Comparison of Lactic Acid Bacteria. Appl. Environ. Microbiol. 2009, 75, 3627–3633.
20. Ott, A.; Hugi, A.; Baumgartner, M.; Chaintreau, A. Sensory investigation of yogurt flavor perception: Mutual influence of volatiles and acidity. J. Agric. Food Chem. 2000, 48, 441–450.
21. Özer, B. Cheese: Microflora of White-Brined Cheeses. In Encyclopedia of Food Microbiology, 2nd ed.; Batt, C.A., Tortorello, M.-L., Eds.; Academic Press: London, UK, 2014; pp. 402–408.
22. Nemska, V.; Lazarova, N.; Georgieva, N.; Danova, S. Lactobacillus spp. from traditional Bulgarian dairy products. J. Univ. Chem. Technol. Metall. 2016, 51, 693–704.
23. Georgieva, R.; Iliev, I.; Chipeva, V.; Dimitonova, S.; Samelis, J.; Danova, S. Identification and in vitro characterization of Lactobacillus plantarum strains from artisanal Bulgarian white brined cheeses. J. Basic Microbiol. 2008, 48, 234–244.
24. Favaro, L.; Basaglia, M.; Casella, S.; Hue, I.; Dousset, X.; de Melo Franco, B.D.G.; Todorov, S. Bacteriocinogenic potential and safety evaluation of non-starter Enterococcus faecium strains isolated from homemade white brined cheese. Food Microbiol. 2014, 38, 228–239.
25. Teneva-Angelova, T.; Balabanova, T.; Boyanova, P.; Beshkova, D. Traditional Balkan fermented milk products. Eng. Life Sci. 2018, 18, 807–819.
26. Begovic, J.; Brandsma, J.; Jovcic, B.; Tolinacki, M.; Veljovic, K.; Meijer, W.; Topisirovic, L. Analysis of dominant lactic acid bacteria from artisanal raw milk cheeses produced on the mountain Stara Planina, Serbia. Arch. Biol. Sci. 2011, 63, 11–20.
27. Dimitrov, D.; Simov, Z.; Dimitrov, Z.; Ospanov, A. Improving of the microbiological and proteolytic profile of kashkaval cheese by modification in heat treatments of cow’s milk and cheddared curd. J. Microbiol. Biotechnol. Food Sci. 2015, 4, 546–549.
28. Danova, S.; Nemska, V.; Tropcheva, R. Bulgarian yogurt-like product “Katak”. In Yoghurt in Health and Disease Prevention, 1st ed.; Shah, P.N., Ed.; Academic Press: London, UK, 2017; pp. 307–329.
29. Kirilov, N.; Dimov, S.; Dalgalarrondo, M.; Ignatova, T.; Kambarev, S.; Stoyanovski, S.; Danova, S.; Iliev, I.; Haertle, T.; Chobert, J.; et al. Characterization of enterococci isolated from homemade Bulgarian cheeses and katuk. Eur. Food Res. Technol. 2011, 233, 1029–1040.
30. Chen, H.; Wang, S.; Chen, M. Microbiological study of lactic acid bacteria in kefir grains by culture-dependent and culture-independent methods. Food Microbiol. 2008, 25, 492–501.
31. Garofalo, C.; Osimani, A.; Milanović, V.; Aquilanti, L.; De Filippis, F.; Stellato, G.; Di Mauro, S.; Turchetti, B.; Buzzini, P.; Ercolini, D.; et al. Bacteria and yeast microbiota in milk kefir grains from different Italian regions. Food Microbiol. 2015, 49, 123–133.
32. Wang, J.; Zhao, X.; Tian, Z.; Yang, Y.; Yang, Z. Characterization of an exopolysaccharide produced by Lactobacillus plantarum YW11 isolated from Tibet Kefir. Carbohydr. Polym. 2015, 125, 16–25.
33. Chang, J.; Ho, C.; Mao, C.; Barham, N.; Huang, Y.; Ho, F.; Wu, Y.; Hou, Y.; Shih, M.; Li, W.; et al. A thermo- and toxin-tolerant kefir yeast for biorefinery and biofuel production. Appl. Energy 2014, 132, 465–474.
34. Pogačić, T.; Šinko, S.; Zamberlin, S.; Samaržija, D. Microbiota of kefir grains. Mljekarstvo 2013, 63, 3–14.
35. Guzel-Seydim, Z.; Koktas, T.; Greene, A.K. Kefir and Koumiss: Microbiology and Technology. In Development and Manufacture of Yogurt and Other Functional Dairy Products; Yildiz, F., Ed.; CRC Press: Boca Raton, FL, USA, 2019; pp. 143–163.

36. Danova, S.; Petrov, K.; Pavlov, P.; Petrova, P. Isolation and characterization of Lactobacillus strains involved in koumiss fermentation. Int. J. Dairy Technol. 2005, 58, 100–105.
37. Savijoki, K.; Ingmer, H.; Varmanen, P. Proteolytic systems of lactic acid bacteria. Appl. Microbiol. Biotechnol. 2006, 71, 394–406.
38. Montanari, G.; Zambonelli, C.; Grazia, L.; Kamesheva, G.K.; Shigaeva, M.K. Saccharomyces unispora as the principle alcoholic fermentation microorganism of traditional koumiss. J. Dairy Res. 1996, 63, 327–331.
39. Behera, S.K.; Panda, S.K.; Kayitesi, E.; Mulaba-Bafubiandi, A.F. Kefir and Koumiss: Origin, Health Benefits and Current Status of Knowledge. In Fermented Food—Part II: Technological Interventions, 1st ed.; Ray, R.C., Montet, D., Eds.; CRC Press: Boca Raton, FL, USA, 2017; pp. 400–417.

Keywords

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