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Chemical and microbiological characteristics of kefir grains and their fermented dairy products: A review

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Abstract: Kefir grains are multi-species natural starter culture consisting of lactic acid bacteria, acetic acid bacteria, and yeasts, creating complex symbiotic community and widely used in fermented dairy products. The microbiological and chemical composition of kefir grains indicate that they are very complex probiotic, with lactic acid bacteria, generally the predominant microorganisms. Therefore, kefir grains were usually used the starter in fermented dairy products. Our review provides an overview of microbiological characteristics, microstructure, chemical composition of the kefir grains and their use in fermented dairy products.

Subjects: Dairy Science; Food Microbiology; Product Development

Keywords: kefir grains; kefir; microbiological characteristics; fermented dairy products

1. Introduction

Kefir is a traditional drink obtained via fermentation of milk by kefir grains. The name kefir is derived from the Turkish language word keyif, meaning "good feeling" for the feelings experienced after drinking it (Leite et al., 2015). Kefir grains are white to yellowish, cauliflower-like grains, with a slimy but firm texture (see Figure 1). The grains are composed of an inert matrix made up of polysaccharides and proteins. The matrix is densely populated by lactic acid bacteria species, acetic acid bacteria, and yeasts (Kalamaki & Angelidis, 2016; Macuamule, Wiid, Helden, Tanner, & Witthuhn, 2015).

1.1. Microbiological characteristics

The microflora of kefir grains is remarkably stable, retaining activity for years if preserved and incubated under appropriate cultural and physiological conditions (O'Brien, 2012; Vardjan, Mohar Lorbeg, Rogelj, & Čanžek Majhenič, 2013). The grain-milk ratio, incubation time and temperature, sanitation during separation of kefir grains, washing of grains and cold storage all drastically affect the product

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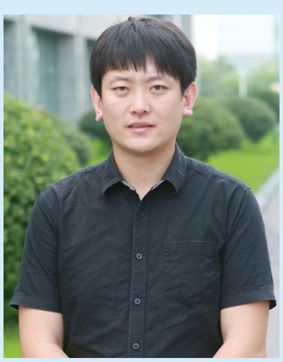

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PUBLIC INTEREST STATEMENT

Kefir grains play a natural starter culture role during the production of kefir and are recovered after the fermentation process by milk straining. These grains are composed of microorganisms immobilized on a polysaccharide and protein matrix, where several species of bacteria and yeast coexist in symbiotic association. In this ecosystem there is a relatively stable microorganism population, which interacts with and influences other members of the community. This review provides an overview of kefir grains' microbiological characteristics, microstructure, chemical composition, and their fermented dairy products, which help industries to develop kefir-like products and also help researchers for further research.

Figure 1. Macroscopic structure of kefir grains (Leite et al., 2013).

quality and the microflora of the kefir grains (Guzel-Seydim, Wyffels, Seydim, & Greene, 2005). However, their complex microbiological association makes them difficult to obtain defined and constant kefir starter culture appropriate for industrial kefir production of conventional properties (Vardjan et al., 2013). The common microorganisms isolated from kefir grains at different regions have differences. The bacteria of the grains are usually various homo- and heterofermentative lactic acid bacteria species of Lactobacillus, Lactococcus, Leuconostoc and Streptococcus; acetic acid bacteria species of Acetobacter. In Taiwanese kefir grains, Lactobacillus was the most frequent genus detected, and *Lb. kefiri* was the most frequently detected species (Chen, Wang, & Chen, 2008). Lactobacilli was present in all kefir grains from Bulgaria indicating the importance of this group of bacteria in the production of the beverage (Simova et al., 2002). Mainville, Robert et al. by polyphasic characterization, identified the species *Lb. heleveticus*, *Lb. kefir*, *Lb. parakefir* in kefir grains from Russia (Mainville, Robert, Lee, & Farnworth, 2006) (see Table 1).

1.2. Microstructure

The exterior surfaces of the kefir grains looked smooth and shiny with the naked eye. However, the grain surfaces, under scanning electron microscopy, were revealed to be very rugged (Mei, Guo, Wu, & Li, 2014). In the inner portion of the grain, a variety of lactobacilli (long and curved), yeasts and fibrillar material were observed. The short lactobacilli and yeast were observed on the outer portion (Zhou, Liu, Jiang, & Dong, 2009). The density of microbial cell on the inner portion was less than that on the outer portion. No lactococcus was found on scanning electron micrographs, which may bedue to the bad attachment of lactococcus (see Figure 2).

1.3. Chemical composition

Kefir grains are soft, gelatinous white biological mass, comprised of protein, lipids and soluble polysaccharide, the kefiran complex. Kefiran is a water souble glucogalactian produced by *Lb. kefiranofaciens*, *Lc. plantarum* (Ahmed, Wang, Anjum, Ahmad, & Khan, 2013; Hamet, Piermaria, & Abraham, 2015; Wang, Xiao, Zheng, Yang, & Yang, 2015), and so on. In general, kefir grains increase their weight with subcultures in milk due to the increase in microorganism biomass together with an increase in amount of the matrix that composed by protein and polysaccharide (Garrote, Abraham, & De Antoni, 2001).

1.4. Fermented dairy products

1.4.1. Cheese

The kefir culture has gained researchers' attention with regarding to cheese manufacturing due to its potential effect on quality, health, and safety properties of the product. Kefir grains or kefir has been

Table 1. Microflora species reported in kefir and kefir grains

Name	Origin of products	Countries or regions	References
Lactobacilli			
Lb. acidophilus	Kefir grains, kefir beverage	Spain, Argentina, Denmark, Turkey	Santos, San Mauro, Sanchez, Torres, and Marquina (2003), Wang, Chen, Liu, Lin, and Chen (2008), Fujisawa, Adachl, Toba, Arihara, and Mitsuoka (1988), Zhou et al. (2009), and Taş, Ekinci, and Guzel-Seydim (2012)
Lb. amylovorus	Kefir grains	Denmark, Brazil	Fujisawa et al. (1988) and Leite et al. (2012)
Lb. brevis	Kefir grains	Spain	Wang et al. (2008)
Lb. buchneri	Kefir beverage	Argentina, Italy	Magalhães, de Melo Pereira, Dias, and Schwan (2010), and Garofalo et al. (2015)
Lb. casei	Kefir grains	Spain, Tibet	Wang et al. (2008), Gulitz, Stadie, Wenning, Ehrmann, and Vogel (2011), and Zhou et al. (2009)
Lb. crispatus	Kefir beverage, kefir grains	Argentina, South Africa, Ireland, Turkey	Garbers, Britz, and Witthuhn (2004) and Taş et al. (2012)
Lb. delbrueckii	Kefir grains	South Africa	Koroleva and Robinson (1991), Santos et al. (2003), and Witthuhn, Schoeman, and Britz (2004)
Lb. fermentum	Kefir grains	Spain, South Africa	Wang et al. (2008) and Witthuhn et al. (2004)
Lb. gasseri	Kefir grains	Spain	Wang et al. (2008)
Lb. gallinarum	Kefir grains	South Africa, Ireland	Garbers et al. (2004)
Lb. helveticus	Kefir beverage, kefir grains	Argentina, Tibet, Turkey	Zhou et al. (2009), and Taş et al. (2012)
Lb. hilgardii	Water kefir, kefir grains	German, Taiwan	Gulitz et al. (2011) and Hsieh, Wang, Chen, Huang, and Chen (2012)
Lb. hordei	Water kefir, kefir grains	German, Taiwan	Gulitz et al. (2011) and Hsieh et al. (2012)
Lb. jensenii	Kefir beverage	Argentina, Tibet	Zhou et al. (2009)
Lb. kefiranofaciens	Kefir grains, kefir beverage	Italian, Belgium, Argentina, Taiwan, Tibet, Denmark, Brazil, Italy, Slovenia, Turkey	Garofalo et al. (2015), Magalhães, et al. (2010), Pintado, Da Silva, Fernandes, Malcata, and Hogg (1996), Chen et al. (2008), Zhou et al. (2009), Fujisawa et al. (1988), Magalhães, et al. (2010), Hamet et al. (2013), Garofalo et al. (2015), Vardjan et al. (2013), and Taş et al. (2012)
Lb. kefirgranum	Kefir grains,	Italian, Belgium, Taiwan	Garofalo et al. (2015), Magalhães, et al. (2010), and Chen et al. (2008)
Lb. kefiri	Kefir grains, milk kefir, kefir beverage	Greece, Tibet, Brazil, Argentina, Taiwan, Italy, Slovenia	Huang et al. (2013), Guzel-Seydim et al. (2005, 2011) Pintado et al. (1996), Chen et al. (2008), Hamet et al. (2013), Garofalo et al. (2015), and Vardjan et al. (2013)
Lb. nagelii	Water kefir	German	Gulitz et al. (2011)
Lb. otakiensis	Kefir beverage, kefir grains	Argentina, Italy	Zhou et al. (2009) and Garofalo et al. (2015)
Lb. parabuchneri	Kefir	Brazil	Magalhães, et al. (2010)
Lb. paracasei	Kefir grains	Argentina	Hamet et al. (2013)
Lb. parakefir	Kefir grains	Argentina, Slovenia	Hamet et al. (2013) and Vardjan et al. (2013)
Lb. plantarum	Kefir grains	Tibet	Huang et al. (2013)
Lb. reuteri	Kefir grains	Turkey	Taş et al. (2012)
Lb. rhamnosus	Kefir grains	Spain	Koroleva and Robinson (1991) and Wang et al. (2008)
Lb. satsumensis	Milk kefir, kefir beverage	Brazil, Mexico	Taş et al. (2012) and Gulitz et al. (2011)
Lb. sunkii	Kefir beverage	Argentina, Italy	Zhou et al. (2009) and Garofalo et al. (2015)
Lb. viridescens	Kefir grains	Spain	Wang et al. (2008)

(*Continued*)

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Table 1. (*Continued*)

Name	Origin of products	Countries or regions	References
Lactococci			
Lc. lactis	Kefir grains, kefir beverage	Taiwan, Argentina, Tibet, Brazil	Chen et al. (2008), Zhou et al. (2009), Pintado et al. (1996), and Leite et al. (2012)
Streptococci			
Leuconostoc lactis	Kefir grains	South Africa	Witthuhn et al. (2004)
Leu. mesenteroides	Kefir grains, kefir beverage	Taiwan, Tibet, South Africa	Chen et al. (2008), Zhou et al. (2009), and Witthuhn et al. (2004)
Streptococcus thermophilus	Kefir grains	Turkey	Taş et al. (2012)
Acetic Acid Bacteria			
A. fabarum	Water kefir	German	Gulitz et al. (2011)
A. lovaniensis	Kefir grains	Belgium	Magalhães, et al. (2010)
A. orientalis	Water kefir	German	Gulitz et al. (2011)
A. pasteurianus	Kefir grains	Argentina	Garrote et al. (2001)
A. syzygii	Kefir grains	Brazil	Miguel, Cardoso, Lago, and Schwan (2010)
Yeast			
Candida albicans	Kefir grains	Spain	Wang et al. (2008)
C. friedricchi	Kefir grains	Spain	Wang et al. (2008)
C. holmii	Kefir grains	Spain	Wang et al. (2008)
C. kefir	Kefir grains	Spain	Wang et al. (2008)
C. lambica	Kefir grains	South Africa, Ireland	Garbers et al. (2004)
Hanseniaospora valbyensis	Water kefir	German	Gulitz et al. (2011)
Kazachstania aerobia	Kefir grains	Italy	Garofalo et al. (2015)
Ka. servazzii	Kefir grains	Italy	Garofalo et al. (2015)
Ka. solicola	Kefir grains	Italy	Garofalo et al. (2015)
Ka. unispora	Kefir grains	Brazil, Italy	Leite et al. (2012) and Garofalo et al. (2015)
Kluyveromyces dobzhanskii	Kefir grains	Turkey	Wang et al. (2008)
Kl. lactis	Kefir	Spain	Latorre-García, del Castillo-Agudo, and Polaina (2007)
Kl. marxianus	Kefir grains, Kefir beverage	Tibet, Brazil, Slovenia	Zhou et al. (2009), Magalhães, et al. (2010), and Vardjan et al. (2013)
Lachancea fermentati	Water kefir	German	Gulitz et al. (2011)
Pichia caribbica	Kefir	Brazili	Miguel, Cardoso, Magalhães, and Schwan (2011)
P. cecembensis	Kefir	Brazili	Miguel et al. (2011)
P. fermentas	Kefir grains	Taiwan	Wang et al. (2008)
Saccaromyces unisporus	Kefir grains	Taiwan, Portuguese	Wang et al. (2008), and Pintado et al. (1996)
S. cerevisiae	Kefir grains, kefir beverage	Tibet, South Africa, Brazil, South Africa, Ireland, Italy	Zhou et al. (2009), Witthuhn et al. (2004), Garbers et al. (2004), and Garofalo et al. (2015)
S. exiguus	Kefir	Spain	Latorre-García et al. (2007)
S. humaticus	Kefir	Spain	Latorre-García et al. (2007)
S. turicensis	Kefir grains	Taiwan	Wang et al. (2008)
Torulospora delbrueckii	Kefir grains	Spain	Wang et al. (2008)
Zygosaccharomyces fermentati	Kefir grains	Brazil	Miguel et al. (2011)

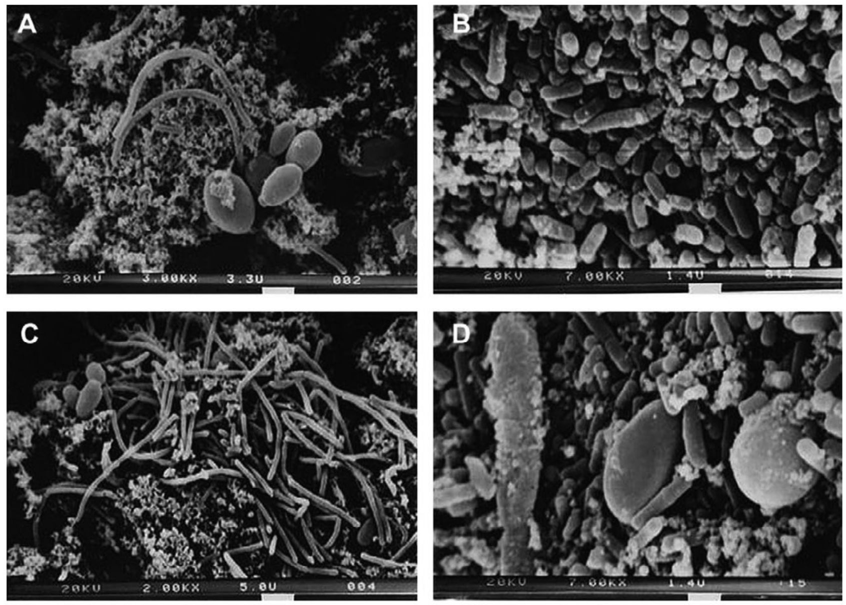

Figure 2. Scanning electron micrographs of Tibetan kefir grains. (A, C) The inside surface of Tibetan kefir grain. (B, D) The outside surface of Tibetan kefir grain (Zhou et al., 2009).

used as a starter in many types of cheese. Goncu and Alpkent used kefir, yoghurt or a commercial cheese culture as a starter in the white pickled cheese production (Goncu & Alpkent, 2005). During 120 days ripenning, scores for appearance, structure, and odour were rated highest in white cheese samples produced by using kefir culture. Kefir can be successfully used as a starter culture in production of white pickled cheese. However, for the commercial production of cheese, direct use of kefir grains is impractical regarding transportation, storage, and cell dosage. Freeze-dried or thermally-dried is a solution for long-term preservation of microorganisms and convenience for shipping (Morgan, Herman, White, & Vesey, 2006). Dimitrellou et al. (2010) evaluated the use of a freeze-dried kefir culture in the production of a novel type of whey-cheese similar to traditional Greek Myzithra-cheese. The use of kefir culture as a starter led to increased lactic acid concentrations and decreased pH values in the final product compared with whey-cheese without starter culture. The degree of proteolysis were significantly higher in cheeses produced by freeze-dried kefir culture during the later stages of ripening. The cheeses produced were characterized as high-quality products during the preliminary sensory evaluation. The freeze-dried kefir culture added in the cheese seemed to suppress growth of pathogens and increased preservation time. Besides, Dimitrellou, Kourkoutas, Koutinas, and Kanellaki (2009), Dimitrellou et al. (2010) also evaluated the use of thermally-dried immobilized kefir on casein as a starter culture for protein-enriched dried whey cheese. Thermally-dried immobilized kefir starter culture resulted in an improved profile of aroma-related compounds. The preliminary sensory evaluation ascertained the soft, fine taste and the overall improved quality of cheese produced with the thermally-dried immobilized kefir. The free or immobilized freeze-dried kefir cells was used as a starter culture in hard-type cheese production. The freeze-dried kefir culture improved aroma, taste, and texture characteristics while increasing the degree of openness in comparison to traditional hard-type cheese products (Katechaki, Panas, Rapti, Kandilogiannakis, & Koutinas, 2008). Then the thermally-dried free and immobilized kefir cells were compared in the hard-type cheese production (Katechaki, Panas, Kourkoutas, Koliopoulos, & Koutinas, 2009). Both free and immobilised cells of kefir culture led to the production of improved cheese products as regards preservation time, sensory and textural characteristics. Thermal drying contributed to the volatile composition of the final product when compared to cheeses made with the alternate method of freeze-drying. The thermal drying process was simple, and of low cost, lower than that of freeze drying. A freeze-dried Tibetan kefir co-culture was used in the Camembert-type cheese production (Mei, Guo, Wu, Li, & Yu, 2015; Mei et al., 2014). A total of 45 compounds were detected during ripenning. Volatile carboxylic acids were abundant in the headspace of the cheese. A total of 147 bacteria and

129 yeasts were obtained from the cheese during ripening. Lactobacillus paracasei represents the most commonly identified lactic acid bacteria isolates, with 59 of a total of 147 isolates.

1.4.2. Kefir

Kefir is a self-carbonated, refreshing fermented yogurt which has a unique flavor due to a mixture of lactic acid, carbon dioxide, acetaldehyde, acetoin, slight alcohol, and other fermentation flavor products (Guzel-Seydim, Seydim, & Greene, 2000; Nielsen, Gürakan, & Ünlü, 2014). Kefir typically contains 89–90% moisture, 0.2% lipid, 3.0% protein, 6.0% sugar, 0.7% ash and 1.0% each of lactic acid and alcohol. Kefir has been reported to contain 1.98 g/L of CO_2 and 0.48% alcohol (Beshkova, Simova, Frengova, Simov, & Dimitrov, 2003), and the content of carbon dioxide (201.7–277.0 ml/L) positively correlated with the concentration (10–100 g/L) of kefir grains (Arslan, 2015). One feature of kefir that differs from other fermented yogurt products is that starter kefir grains are recovered after fermentation. The biomass of kefir grains slowly increases during the process of kefir fermentation (Guzel-Seydim, Kok-Tas, Greene, & Seydim, 2011). Beyond its inherent high nutritional value as a source of protein and calcium, kefir has a long tradition of being regarded as good for health in countries where it is a staple in the diet (Vinderola et al., 2005). Though cow's milk is most common, kefir can be made from any type of milk. For dairy kefir, cow, goat, or sheep milk are all commonly used (Otles & Cagindi, 2003). Tratnik used the goat's milk to produced the kefir. When the goat's milk was fortified with 2/100 g skimmed milk powder, whey protein concentrate and inulin, the acidity level remained very stable in all the samples during the storage period. Goat's samples have significantly lower viscosity and slightly lower sensory profiles (Tratnik, Božanić, Herceg, & Drgalić, 2006). The pasteurised goat milk and goat milk kefir prepared using different amount of Indonesian kefir grains. The best chemical characteristics (pH: 4.37; ethanol content: 0.91%; titratable acidity number: 0.76%; and lactose content: 4.23%) was obtained from goat milk kefir prepared with 7% (w/v) kefir grains and incubation time of 24 h (Chen, Liu, Lin, & Yeh, 2005). Varieties of kefir were made from bovine, caprine and ovine milk, using kefir grains and two direct-to-vat inoculation starter cultures (Wszolek, Tamime, Muir, & Barclay, 2001). Lactic acid bacteria and yeasts were the predominant flora in fresh and stored kefirs. The firmness and all the sensory attributes of the product were influenced by the type of milk used (ovine > bovine > caprine). Storage influenced mouth-feel characters (serum separation, chalky, mouth-coating, and slimy). In general, the type of milk had greater influence on product characteristics than that of starter cultures. Kefir is best made with milk containing fat. As there is an established relationship between many health problems and the consumption of saturated fats and cholesterol, a non-fat choice in kefir is desirable; however, non-fat milk makes a kefir with significantly lower quality (Nielsen et al., 2014). Ertekin and Guzel-Seydim experimented with non-fat milk supplemented with the fat substitutes inulin and Dairy-Lo® to improve the quality of kefir made with skim milk. They found that while kefir grains fermenting whole fat milk resulted in the best quality kefir, Dairy Lo® and inulin could be used without any adverse effect for the production of non-fat kefir (Ertekin & Guzel-Seydim, 2010).

1.4.3. Kefir beverage

Kefir grains successfully ferment the milk from most mammals and continue to grow in such milk. In addition, kefir grains ferment milk substitutes such as soy milk, rice milk and coconut milk, as well as other sugary liquids including fruit juice, coconut water, beer wort and ginger beer (Gaware et al., 2011). Carrot, fennel, melon, onion, tomato and strawberry juices underwent backslopping fermentations, could be carried out by water kefir microorganisms. Results indicated that lactic acid bacteria and yeasts were capable of growing in the juices tested. After fermentation, there was observance of a decrease of the soluble solid content and an increase of the number of volatile organic compounds. The overall quality assessment indicated that carrot kefir-like beverage was the product mostly appreciated by the judges (Corona et al., 2016). Cocoa pulp was also used for new cocoa beverages (Puerari, Magalhães, & Schwan, 2012). A microbial steady structure was detected in the analyzed kefir cocoa beverages and kefir grains. These beverages had the greater acceptance based on taste, odor, and appearance of the beverages. Based on the chemical characteristics and acceptance in the sensory analysis, these results open up perspectives for this innovative application of kefir grains for developing cocoa pulp-based beverages. Cui, Chen, Wang, and Han (2013) experimented with walnut milk to produce kefir beverage. The kefir grains can be used to ferment walnut milk. The suggested optimum fermentation conditions are the following: fermentation temperature of 30°C, fermentation time of 12 h, inoculum size of 3 g of kefir grains (wet weight) and sucrose concentration of 8 g/100 mL.

Cheese whey is the liquid remaining after the precipitation and removal of milk casein during cheese-making. This by-product represents approximately 85–90% of the milk volume and retains 55% of milk nutrients (Rico, Muñoz, & Rico, 2015). Cheese whey represents an important environmental problem because of the high volumes produced and its high organic matter content (Magalhães, et al., 2010). The pressure of antipollution regulations together with whey nutritional value challenges the dairy industry to face whey surplus as a resource and not only as a waste problem (Magalhães, et al., 2010). The production of a functional beverage produced upon whey fermentation by kefir grains could be an interesting alternative for cheese whey utilisation. Cheese whey fermentation by kefir microrganisms could decrease the high lactose content in cheese whey, producing mainly lactic acid and other metabolites such as aroma compounds contributing to the flavour and texture and increasing carbohydrate solubility and sweetness of the end product (Magalhães et al., 2011). Manufacture of beverages through lactic fermentations can provide desirable sensory profiles and have already been considered an option to add value to cheese whey (Magalhães, et al., 2010, 2011; Mazaheri Assadi, Abdolmaleki, & Mokarrame, 2008; Nambou et al., 2014). Magalhães et al. made a tentative and more comprehensive study (including morphological and microbial variations, Chemical composition and sensory analysis) of the kefir grains as a starter culture for cheese whey-based beverages production (Magalhães, et al., 2010, 2011). A steady structure and dominant microbiota, including probiotic bacteria, was detected in the analyzed kefir beverages. Besides, based on the chemical characteristics and acceptance in the sensory analysis, the kefir grains showed potential to be used for developing cheese whey-based beverages. Some researchers prepared fermented dairy-fruits juice beverage making use of juice and whey. The dairyfruits juice beverage provided desirable sensory profiles and uses of whey can be applied to change it from a waste to a delicious beverage (Sabokbar & Khodaiyan, 2015, 2016; Sabokbar, Moosavi-Nasab, & Khodaiyan, 2015).

2. Conclusion

Kefir grains are unique symbiotic associations of different microrganisms, including lactic acid bacteria, yeasts and acetic acid bacteria, cohabiting in a natural polysaccharide and a protein matrix. Kefir is a distinctive fermented dairy product due to the unique, multi-species natural kefir grains used as the starter culture. The microbiological and chemical composition of kefir provide a complex probiotic effect due to the inherent lactic acid bacteria and yeast. Kefir grains ferment the milk from most mammals and will continue to grow in such milk. Now, kefir grains have been widely used in fermented dairy products,including cheese, kefir, whey beverage, as well as other sugary liquids.

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Competing Interests
The authors declare no competing interest.

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