FOOD SCIENCE & TECHNOLOGY | REVIEW ARTICLE

Chemical and microbiological characteristics of kefir grains and their fermented dairy products: A review
Xin Gao1* and Bo Li1

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

ABOUT THE AUTHOR
Xin Gao is an assistant in Shanghai Urban Construction Vocational College. He received his Ms in Biochemistry and Molecular Biology from Liaoning Normal University in China (2008). He works in the area of Food Technology, with an emphasis on Dairy Science. His research focuses on traditional fermented dairy products. He is currently exploring the lactic acid bacteria strains with high yield extracellular polysaccharide that can be used in the low fat yogurt.

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.
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 be due 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 soluble glucogalactan produced by *Lb. kefirana-faciens, 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                                                                 |
|--------------------|-------------------------------------|-----------------------------------------------|----------------------------------------------------------------------------|
| 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, Adachi, Toba, Arihara, and Mitsuoka (1988), Zhou et al. (2009), and Taş, Ekin, 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), Gultiz, 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                             | Zhou et al. (2009), and Taş et al. (2012)                                  |
| Lb. hilgardii      | Water kefir, kefir grains           | German, Taiwan                               | Gultiz et al. (2011) and Hsieh, Wang, Chen, Huang, and Chen (2012)         |
| Lb. hordei         | Water kefir, kefir grains           | German, Taiwan                               | Gultiz 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. kefranorum     | Kefir grains                        | Italian, Belgium, Taiwan                     | Garofalo et al. (2015), Magalhães, et al. (2010), and Chen et al. (2008)     |
| Lb. kefri          | 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                                        | Gultiz 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 Gultiz et al. (2011)                                |
| Lb. sunkii         | Kefir beverage                     | Argentina, Italy                             | Zhou et al. (2009) and Garofalo et al. (2015)                              |
| Lb. vinidescens    | Kefir grains                        | Spain                                        | Wang et al. (2008)                                                        |
| 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                                     | Wittthuhn et al. (2004)                                                     |
| Leu. mesenteroides        | Kefir grains, kefir beverage | Taiwan, Tibet, South Africa            | Chen et al. (2008), Zhou et al. (2009), and Wittthuhn et al. (2004)          |
| Streptococcus thermophilius | 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, Logo, and Schwan (2010)                                    |
| Yeast                     |                    |                                                  |                                                                             |
| Candida albicans          | Kefir grains       | Spain                                            | Wang et al. (2008)                                                         |
| C. friedrichii            | 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. (2006)                                                      |
| Hanseniaspora 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 dozhanskii  | 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              | Brazil                                           | Miguel, Cardoso, Magalhães, and Schwan (2011)                               |
| P. cecembensis            | Kefir              | Brazil                                           | 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. cerevisae              | Kefir grains, kefir beverage | Tibet, South Africa, Brazil, South Africa, Ireland, Italy | Zhou et al. (2009), Wittthuhn et al. (2004), Garbers et al. (2004), and Garofalo et al. (2015) |
| S. exigus                 | 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)                                                         |
| Torulopsis delbrueckii    | Kefir grains       | Spain                                            | Wang et al. (2008)                                                         |
| Zygosaccharomyces fermentati| Kefir grains      | Brazil                                           | Miguel et al. (2011)                                                        |
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 ripening. 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 CO₂ 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 microorganisms 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 microorganisms, 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.
Chen, H. C., Wang, S. Y., & Chen, M. J. (2008). Microbiological study of lactic acid bacteria in kefir grains after culture-dependent and culture-independent methods. Food Microbiology, 25, 492–501. http://dx.doi.org/10.1016/j.fm.2008.01.003

Chen, M. J., Liu, J. R., Lin, C. W., & Yeh, Y. T. (2002). Study of the microbial and chemical properties of goat milk kefir produced by inoculation with taiwanese kefir grains. Asian-Australasian Journal of Animal Sciences, 18, 711–715. http://dx.doi.org/10.5713/ajas.2005.711

Corona, G., Randazzo, W., Miceli, A., Guarcello, R., Francesca, N., Erten, H., ... Settanni, L. (2013). Characterization of kefir-like beverages produced from vegetable juices. LWT-Food Science and Technology, 66, 572–581. http://dx.doi.org/10.1016/j.lwt.2015.11.014

Cui, X. H., Chen, S. J., Wang, Y., & Han, J. R. (2013). Fermentation conditions of walnut milk beverage inoculated with kefir grains. LWT-Food Science and Technology, 50, 349–352. http://dx.doi.org/10.1016/j.lwt.2012.07.043

Dimitrellou, D., Kandylis, P., Mallouchos, A., Komaitis, M., Gaware, V., Kotade, K., Dolas, R., Dhamak, K., Somwnshis, S., Garbers, I. M., Britz, T. J., & Witthuhn, R. C. (2004). PCR-based molecular identification of yeasts in milk kefir grains and selected single strains of lactic acid bacteria with lactic acid bacteria and yeasts. In R. K. Robinson (Ed.), Therapeutic properties of fermented milks. Bioresource Technology, 95, 284–288. http://dx.doi.org/10.1016/j.biortech.2009.02.061

Eretkin, B., & Guzel-Seydim, Z. B. (2010). Effect of hot replacers on kefir quality. Journal of the Science of Food and Agriculture, 90, 543–548.

Fujisawa, T., Adachi, S., Toba, T., Arihara, K., & Mitsuoka, T. (1988). Lactobacillus kefiranofaciens sp. nov. isolated from kefir grains. International Journal of Systematic and Evolutionary Microbiology, 38, 12–14.

Garbers, J. M., Birtz, T. J., & Witthuhn, R. C. (2004). PCR-based denaturing gradient gel electrophoresis identification and characterization of the microbial consortium present in kefir grains. World Journal of Microbiology and Biotechnology, 20, 687–693. http://dx.doi.org/10.1023/B:WJMB.0000013249.34050.1e

Garofalo, C., Osmani, A., Milanovic, V., Aquilanti, L., De Filippis, F. D., Stellato, G., ... Clementi, F. (2013). Bacteria and yeast microbiota in milk kefir grains from different Italian regions. Food Microbiology, 49, 123–133. http://dx.doi.org/10.1016/j.fm.2015.01.017

Gazotte, G. L., Abraham, A. G., & De Antoni, G. L. (2001). Chemical and microbiological characterisation of kefir grains. Journal of Dairy Research, 68, 639–652.

Gaware, V., Kotade, K., Dolas, R., Dhamak, K., Somwnshis, S., Nikam, V., ... Kashid, V. (2011). The magic of kefir: A review. Pharmacology Online, 1, 376–386.

Goncu, A., & Alpkent, Z. (2009). Sensory and chemical properties of white pickled cheese produced using kefir, yogurt or a commercial starter culture. International Dairy Journal, 15, 771–776. http://dx.doi.org/10.1016/j.idairyj.2009.10.008

Guiliz, A., Stodie, J., Wenning, M., Ehrmann, M. A., & Vogel, R. F. (2011). The microbial diversity of water kefir. International Journal of Food Microbiology, 151, 284–288. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.09.016

Guzel-Seydim, Z., Seydim, A. C., & Greene, A. K. (2000). Organic acids and volatile flavor components evolved during refrigerated storage of kefir. Journal of Dairy Science, 83, 275–277. http://dx.doi.org/10.1038/jds.20022–0302(2000)374874–0

Guzel-Seydim, Z., Wyczalski, J., Seydim, A. C., & Greene, A. K. (2005). Turkish kefir and kefir grains: Microbial enumeration and electron microscopic observation. International Journal of Dairy Technology, 58, 25–29. http://dx.doi.org/10.1111/j.1111.2005.58.issue-1

Harianto, D., Hamet, M. F., Londero, A., Medrano, M., Vercammen, E., Van Hooide, K. V., Gorroto, G. L., ... Abraham, A. G. (2013). Application of culture-dependent and culture-independent methods for the identification of Lactobacillus kefiranofaciens in microbial consortia present in kefir grains. Food Microbiology, 36, 327–334. http://dx.doi.org/10.1016/j.fm.2013.06.022

Harnett, M. F., Pierronia, J. A., & Abraham, A. G. (2013). Selection of EPS-producing Lactobacillus strains isolated from kefir grains and rheological characterization of the fermented milks. LWT-Food Science and Technology, 63, 129–135. http://dx.doi.org/10.1016/j.lwt.2015.03.097

Hsieh, H. W., Wang, S. Y., Chen, T. L., Huang, Y. L., & Chen, M. J. (2011). Effects of the anti-listerial activity of feta-type cheese produced using lactobacilli isolated from Tibetan kefir grains: A potential probiotic bacterium with cholesterol-lowering effects. Journal of Dairy Science, 94, 2816–2825. http://dx.doi.org/10.3168/jds.2012-6371

Kalpana, M. S., & Angelidis, A. S. (2016). Isolation and molecular identification of yeasts in Greek kefir. International Journal of Dairy Technology. doi:10.1111/1747-0312.12329

Katechaki, E., Panas, P., Rapti, K., Kandilogiannakis, L., & Kotinas, A. A. (2008). Production of hard-type cheese using free or immobilized freeze-dried kefir cells as a starter culture. Journal of Agricultural and Food Chemistry, 56, 5316–5323. http://dx.doi.org/10.1021/jf070358y

Korate, N. V., & Robinson, R. K. (1999). Products prepared with lactic acid bacteria and yeasts. In R. K. Robinson (Ed.), Therapeutic properties of fermented milks (pp. 159–179). London: Elsevier Applied Science.

Latorre-Garcia, L., del Castillo-Aguado, L. D., & Polaina, J. (2007). Taxonomical classification of yeasts isolated from kefir based on the sequence of their ribosomal RNA genes. World Journal of Microbiology and Biotechnology, 23, 785–791. http://dx.doi.org/10.1007/s11274-006-9298-y

Leite, A. M. O., Mayo, B., Rachid, C. T. C. C., Peixoto, R. S., Silva, J. T., Paschoalin, V. M. F., & Delgado, S. (2012). Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. Food Microbiology, 31, 215–221. http://dx.doi.org/10.1016/j.fm.2012.03.011

Leite, A. M. O., Miguel, M. A. L., Peixoto, R. S., Ruas-Madiedo, P., Paschoalin, V. M. F., Mayo, B., & Delgado, S. (2015). Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. Journal of Dairy Science, 98, 3622–3632. http://dx.doi.org/10.3168/jds.2014-9265

Macuanma, C. I. S., Wild, I. J., Helden, P. D. V., Tanner, M., & Witthuhn, R. C. (2015). Effect of milk fermentation by kefir grains and selected single strains of lactic acid bacteria on the survival of Mycobacterium bovis BCG. International Journal of Food Microbiology, 217, 170–176.

Magalhães, K. T., Dias, D. R., de Melo Pereira, G. V., Oliveira, J. M., Domingues, L., Teixeira, J. A., ... Schwan, R. F. (2011). Chemical composition and sensory analysis of cheese whey-based beverages using kefir grains as starter culture. International Journal of Food Science & Technology, 46, 871–878. http://dx.doi.org/10.1111/j.1111.2011.16.issue-4
Magalhães, K. T., de Melo Pereira, G. V., Dias, D. R., Schwan, R. F. (2010). Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. World Journal of Microbiology and Biotechnology, 26, 1241–1250. http://dx.doi.org/10.1007/s11274-010-0668-9

Mainville, I., Robert, N., Lee, B., & Farnworth, E. R. (2006). Polyphasic characterization of the lactic acid bacteria in kefir. Systematic and Applied Microbiology, 29, 59–68. http://dx.doi.org/10.1016/j.syapm.2005.07.001

Mazaheri Assadi, M., Abdolmaleki, F., & Makramme, R. R. (2008). Application of whey in fermented beverage production using kefir starter culture. Nutrition & Food Science, 38, 121–127.

Mei, J., Guo, Q., Wu, Y., & Li, Y. (2014). Microbial diversity of a camembert-type rye using freeze-dried tibetan kefir co-culture as starter culture by culture-dependent and culture-independent methods. PLoS ONE, 9, e111648. http://dx.doi.org/10.1371/journal.pone.0111648

Miguel, M. G. D. C. P., Cardoso, P. G., Lago, L. D. A., & Schwan, R. F. (2010). Diversity of bacteria present in milk kefir grains using culture-dependent and culture-independent methods. Food Research International, 43, 1523–1528. http://dx.doi.org/10.1016/j.foodres.2010.04.031

Miguel, M. G. D. C. P., Cardoso, P. G., Magalhães, K. T., & Schwan, R. F. (2011). Profile of microbial communities present in tibico (sugary kefir) grains from different Brazilian States. World Journal of Microbiology and Biotechnology, 27, 1875–1884. http://dx.doi.org/10.1007/s11274-010-0646-6

Morgan, C. A., Herman, N., White, P. A., & Vesey, G. (2006). Preservation of micro-organisms by drying: A review. Journal of Microbiological Methods, 66, 183–193. http://dx.doi.org/10.1016/j.mimet.2006.02.017

Nambou, K., Gao, C., Zhou, F., Guo, B., Ai, L., & Wu, Z. J. (2014). A novel approach of direct formulation of defined starter cultures for different kefir-like beverage production. International Dairy Journal, 34, 237–246. http://dx.doi.org/10.1016/j.idairyj.2013.03.012

Nielsen, B., Gürakan, G. C., & Ünlü, G. (2014). Kefir: A multifaceted fermented dairy product. Probiotics and Antimicrobial Proteins, 6, 123–135. http://dx.doi.org/10.1007/s12602-014-9168-0

O’Brien, K. V. (2011). The effect of frozen storage on the survival of probiotic microorganisms found in traditional and commercial kefir. Retrieved from http://etd.lsu.edu/docs/available/etd-04252012-155457/

O’Toole, S., & Cagindi, O. (2003). Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. Pakistan Journal of Nutrition, 2, 54–59.

Pintado, M. E., Da Silva, J. A. L., Fernandez, P. B., Malcata, F. X., & Hogg, T. A. (1996). Microbiological and rheological studies on Portuguese kefir grains. International Journal of Food Science & Technology, 31, 15–26. http://dx.doi.org/10.1111/j.1365-2621.1996.tb03186.x

Puerari, C., Magalhães, K. T., & Schwan, R. F. (2012). New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. Food Research International, 48, 634–640. http://dx.doi.org/10.1016/j.foodres.2012.06.005

Rico, C., Muñoz, N., & Rico, J. L. (2015). Anaerobic co-digestion of cheese whey and the screened liquid fraction of dairy manure in a single continuously stirred tank reactor process: Limits in co-substrate ratios and organic loading rate. Bioresource Technology, 189, 327–333. http://dx.doi.org/10.1016/j.biortech.2015.04.032

Sabokbar, N., & Khodaiyan, F. (2015). Characterization of pomegranate juice and whey based novel beverage fermented by kefir grains. Journal of Food Science and Technology, 52, 3711–3718. http://dx.doi.org/10.1007/s13197-015-2029-3

Sabokbar, N., Moosavi-Nosab, M., & Khodaiyan, F. (2015). Preparation and characterization of an apple juice and whey based novel beverage fermented using kefir grains. Food Science and Biotechnology, 24, 2095–2104. http://dx.doi.org/10.1007/s10068-015-0278-6

Santos, A., San Mauro, M., Sanchez, A., Torres, J. M., & Marquino, D. (2003). The antimicrobial properties of different strains of lactobacillus spp. isolated from kefir. Systematic and Applied Microbiology, 26, 434–437. http://dx.doi.org/10.1010/j.1742-7889.2003.00796.x

Simova, E., Beshkova, D., Angelov, A., Hristova, T., Frangova, G., & Spasov, Z. (2002). Lactic acid bacteria and yeasts in kefir grains and kefir made from them. Journal of Industrial Microbiology and Biotechnology, 28, 1–6. http://dx.doi.org/10.1007/s10295-001-00186-8

Taş, T. K., Ekinli, F. Y., & Güzel-Seydim, Z. B. (2012). Identification of microbial flora in kefir grains produced in Turkey using PCR. International Journal of Dairy Technology, 65, 126–131. http://dx.doi.org/10.1111/j.1111.2011.65.issue-1

Tratnik, L., Božanč, R., Herceg, Z., & Drgalić, I. D. A. (2006). The quality of plain and supplemented kefir from goat’s and cow’s milk. International Journal of Dairy Technology, 59, 40–46. doi:10.1111/j.1471-0307.2006.00236.x

Vardjan, T., Mohar Lorberg, P., Rogelj, I., & Čanžek Majhenič, A. (2013). Characterization and stability of lactobacilli and yeast microbiota in kefir grains. Journal of Dairy Science, 96, 2729–2736. http://dx.doi.org/10.3168/jds.2012-5829

Vinderola, C. G., Duarte, J., Thangavel, D., Perdigón, G., Vardjan, T., Mohar Lorbeg, P., Rogelj, I., & Čanžek Majhenič, A. (2006). The effect of frozen storage on the survival of probiotic microorganisms found in traditional and commercial kefir. Retrieved from http://etd.lsu.edu/docs/available/etd-04252012-155457/

Wang, J., Xiao, Z., Zheng, T., Yang, Y., & Yang, Z. (2013). Characterization of an exopolysaccharide produced by Lactobacillus plantarum WW11 isolated from Tibet kefir. Carbohydrate Polymers, 125, 16–25.

Wang, S. Y., Chen, H. C., Liu, J. R., Lin, Y. C., & Chen, M. J. (2008). Identification of yeasts and evaluation of their distribution in Taiwanese kefir and viili starters. Journal of Dairy Science, 91, 3798–3805. http://dx.doi.org/10.3168/jds.2007-0468

Witthuhn, R. C., Schoeman, T., & Britz, T. J. (2004). Isolation and characterization of the microbial population of different South African kefir grains. International Journal of Dairy Technology, 57, 33–37. http://dx.doi.org/10.1111/j.1111.2004.57.issue-1

Wszolek, M., Tamime, A. Y., Muir, D. D., & Barclay, M. N. I. (2001). Identification of kefir. Journal of Dairy Research, 72, 195–202. http://dx.doi.org/10.1017/S002202990000828

Zhou, J., Liu, X., Jiang, H., & Dong, M. (2009). Analysis of the yeast microbiota in kefir grains. International Journal of Food Science and Technology, 44, 123–135. http://dx.doi.org/10.1111/j.1365-2672.2008.01927.x

Zhou, J., Liu, X., Jiang, H., & Dong, M. (2009). Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis. Food Microbiology, 26, 770–775. http://dx.doi.org/10.1016/j.fm.2009.04.009
