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Kefir: a comprehensive overview of microbiological, physicochemical, and health-promoting properties

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Abstract

Kefir is a naturally carbonated and mildly acidic milk beverage fermented by grains that consist of bacteria and yeast living in a symbiotic relationship. Its unique flavor arises from a blend of lactic acid, ethanol, carbon dioxide, and other compounds produced by the microorganisms within the kefir grains. The microorganisms in kefir are a complex microbial population that varies by geographical region. Kefir is widely acknowledged for its probiotic content, with reported health benefits that include modulation of intestinal microflora, immune system stimulation, and antimicrobial, antineoplastic, hypocholesterolemic, antidiabetic, and antimutagenic effects. It also exhibits β-galactosidase activity and benefits lipid and blood pressure levels. This review focuses on the microbiological aspects, composition, and production of milk kefir, highlighting how its biological properties correlate with the health benefits associated with its consumption.

Keywords: antimicrobial; antineoplastic; antidiabetic; antimutagenic; intestinal; fermented milk, probiotics.

Practical Application: A probiotic-rich, naturally fermented milk drink offering antimicrobial, immune-boosting, and metabolic health benefits through its unique microbial composition and bioactive compounds.

1 INTRODUCTION

Kefir is a slightly acidic, effervescent milk beverage fermented by grains composed of bacteria and yeasts in a symbiotic association (Dertli & Çon, 2017). Its unique flavor results from a mixture of lactic acid, ethanol, carbon dioxide, and other compounds such as acetaldehyde, which are products of the metabolic activity of the microorganisms that make up kefir grains (Guzel-Seydim et al., 2000).

Kefir's microbial composition, particularly its combination of bacteria and yeasts, has been linked to numerous health benefits, with proven inhibitory effects against pathogenic and contaminant microorganisms (Paucean & Carmen, 2008). Known for its probiotic properties (John & Deeseenthum, 2015), kefir is defined as a fermented milk product prepared with lactic acid cultures from kefir grains, which contain beneficial microorganisms including lactose-fermenting and non-fermenting yeasts, as well as bacteria, such as *Bifidobacterium* sp. and *Streptococcus salivarius* subsp. *thermophilus* (Brasil, 2007). According to the Codex Alimentarius International Food Standards (2003), kefir must contain at least 2.7% protein, no more than 10% fat, and a minimum acidity of 0.6%.

The rising popularity of kefir is also due to its antimicrobial, antineoplastic, antioxidant, and anti-inflammatory properties (Liu et al., 2006). Consumers have shown interest in kefir as a potential alternative to conventional therapies in the management of chronic diseases, with probiotic foods being a preferred choice (Ahmed et al., 2013).

This review aims to examine key aspects of the microbiology, composition, and production of milk kefir, linking its biological properties to the health benefits associated with its consumption.

2 MILK KEFIR PRODUCTION

The term "kefir" originates from the Turkish word "keif," meaning "well-being" (Kemp, 1984). Kefir's history traces back thousands of years to the Caucasus Mountains, where it is believed that the local people discovered that fresh milk stored in leather bags would ferment, resulting in an effervescent drink (Duitschaever et al., 1987; Irigoyen et al., 2005). Historically, kefir grains were considered gifts from Allah by the Muslim communities in the region, passed down through generations among Caucasian tribes, and viewed as a symbol of wealth within families (Roberts et al., 2000).

Milk kefir can be produced both traditionally and industrially. Traditionally, kefir is prepared by inoculating kefir grains into milk, and then incubating the mixture at room temperature. After approximately 24 h, the grains are filtered out from

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the fermented milk and then reused in fresh milk for a new fermentation cycle (Semih & Cagindi, 2003). The final beverage is stored at 4°C for preservation (Schwan et al., 2015). Kefir can be made from cow, buffalo, goat, or sheep milk, using whole, semi-skim, or skim milk. Plant-based alternatives, such as soy and coconut milk, may also be used (Farnworth, 2005).

In industrial production, the process utilizes freeze-dried cultures. The type of culture¾ whether traditional grains or commercial cultures—affects kefir's antioxidant capacity during fermentation and storage. Kefir produced from traditional grains has a higher antioxidant capacity, with sheep's milk-based kefir showing higher antioxidant levels compared to cow's milk-based kefir (Yilmaz-Ersan et al., 2018).

3 COMPOSITION OF KEFIR GRAINS

Kefir grains consist primarily of *Lactobacillus kefiri*, bacteria from the genera *Leuconostoc*, *Lactococcus*, and *Acetobacter*, along with both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae*, and *Saccharomyces exiguus*) (Codex Alimentarius International Food Standards, 2003).

These grains are characterized by a symbiotic structure where lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts coexist within a polysaccharide matrix called kefiran. Morphologically, the grains are irregular, cauliflower-like clusters with a white-to-slightly yellowish, gelatinous texture and range from 3 to 35 mm in diameter (Hertzler & Clancy, 2003; Irigoyen et al., 2005; Weschenfelder, 2009).

Studies using scanning electron microscopy show a dense and complex biofilm surrounding the outer layer of the grains, with an unstructured material occupying the interiors (Figures 1 and 2) (Magalhães et al., 2011). The microbial composition within the grains can vary depending on the local microbial community (Magalhães et al., 2011; Wang et al., 2012). The surface of the grains is typically smooth (Figure 1A), while the outer portion is densely populated with microorganisms, forming a biofilm structure comprising bacilli and yeasts (Figure 1B-1D). Fewer cells are observed in the inner portions of the grains (Figure 2), with a fibrillar material present throughout both the internal and external layers (Figure 2B).

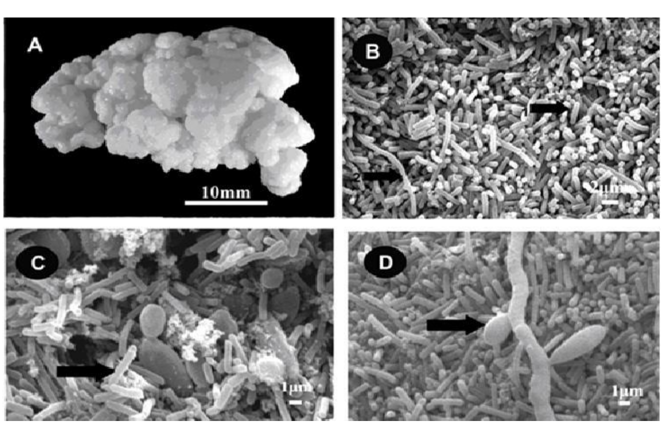
Strains within kefir grains, such as *Lactobacillus kefiranofaciens* and *Saccharomyces turicensis*, exhibit the ability to aggregate cells and form biofilms, particularly with *Lb. kefiri*. A significant co-aggregation has also been observed between *Lb. kefiranofaciens*, *Lb. kefiri*, and *S. turicensis* when co-cultured. It is suggested that grain formation begins with the aggregation of *Lb. kefiranofaciens* and *S. turicensis*, which initiate the formation of small granules. Subsequently, biofilm-producing *Lb. kefiri* attaches to the surface of these granules, further co-aggregating with other microorganisms and milk components to complete grain formation (Wang et al., 2012).

The symbiotic community within kefir grains is so diverse that it has complicated efforts to study and identify the microorganisms present. When separated into pure cultures, these bacteria and yeasts typically fail to grow in milk or display diminished biological activity (Koroleva, 1991). For instance, *Lactobacillus* spp. show enhanced growth when *Candida* spp. yeasts are introduced to the milk substrate (Linossier & Dousset, 1994). Generally, LAB are more numerous than yeasts and AAB within kefir grains, although fermentation conditions may alter this distribution (Farnworth, 2005).

The composition of kefir grains is influenced by several factors, including geographic origin, cultivation methods, handling and storage conditions, and substrate type (Table 1) (Wang et al., 2020; Wszolek et al., 2001).

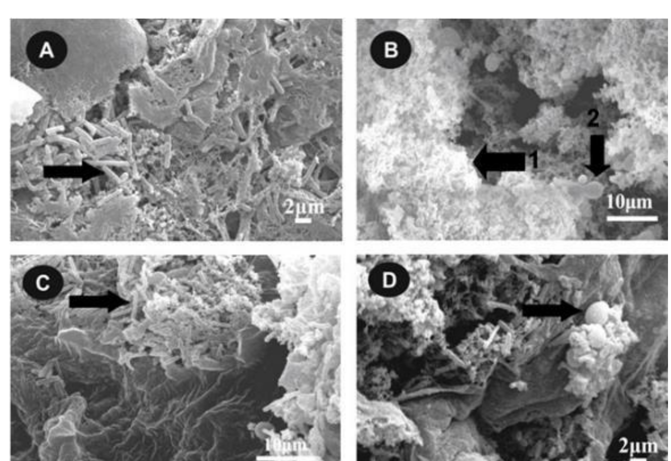
4 CHARACTERISTICS OF MILK KEFIR

The inoculation of kefir grains into milk results in a slightly acidic, effervescent beverage (Guzel-Seydim et al., 2000). The sensory and physicochemical characteristics of kefir primarily depend on the microbiota present in the grains as well as the type of milk used as a substrate (Gao et al., 2012). The microbial species found in the grains are not only crucial for the



Source: Magalhães et al. (2011).

Figure 1. (A) Kefir grain as observed with the naked eye. (B, C, and D) External surface of the kefir grain. Arrows 1 and 2 in Figure 1B indicate bacteria. The arrow in Figure 1C indicates a bacterium. The arrow in Figure 1D indicates a yeast.



Source: Magalhães et al. (2011).

Figure 2. Internal surface of the kefir grain. Arrows in 2A and 2C indicate bacteria. The arrow in 2B indicates polysaccharides. The arrow in 2B also indicates a yeast. The arrow in Figure 2D indicates a yeast.

Table 1. Microorganisms found in kefir and kefir grains across different countries.

Microorganisms	Sampling - Country	References
Leuconostoc sp., *Lactococcus* sp., *Lactobacillus* sp., *Lactobacillus plantarum, Zygosaccharomyces* sp., *Candida* sp., *Candida lambica, Candida krusei, Saccharomyces* sp., *Cryptococcus* sp.	Kefir and kefir grains - South Africa	Witthuhn et al., 2005
Lactobacillus kefiri, Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Lactococcus lactis ssp. *Cremori, Gluconobacter frateurii, Acetobacter orientalis, Acetobacter ovaniensis, Kluyveromyces marxianus, Naumovozyma* sp., *Kazachstania khefir*	Kefir and kefir grains - Belgium	Korsak et al., 2015
Lactobacillus paracasei, Lactobacillus parabuchneri, Lactobacillus casei, Lactobacillus kefiri, Lactococcus lactis, Acetobacter lovaniensis, Kluyveromyces lactis, Kazachstania aerobia, Saccharomyces cerevisiae, Lachancea meyersii	Kefir - Brazil	Magalhães et al., 2011
Acetobacter acetic, Enterococcus faecalis, Enterococcus durans, Lactococcus lactis ssp. *cremoris, Leuconostoc paramesenteroides, Lactobacillus brevis, Lactobacillus acidophilus, Saccharomyces* sp., *Brettanomyces* sp., *Candida* sp., *Saccharomycodes* sp., *Acetobacter rancens*	Kefir - China	Yang et al., 2007
Lactobacillus kefiranofaciens, Dekkera anomala, Streptococcus thermophilus, Lactococcus lactis, Acetobacter sp., *Lactobacillus lactis, Enterococcus* sp., *Bacillus* sp., *Acetobacter fabarum, Acetobacter lovaniensis, Acetobacter orientalis*	Kefir grains - Italy	Garofalo et al., 2015
Lactobacillus kefiri, Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Escherichia coli, Pseudomonas sp., *Saccharomyces turicensis*	Kefir grains - Taiwan	Chen et al., 2008; Wang et al., 2012; Wyder et al., 1999
Lactococcus cremoris, Lactococcus lactis, Streptococcus thermophilus, Streptococcus durans	Kefir - Turkey	Yuksekdag et al., 2004b

Source: adapted from Prado et al. (2015).

fermentation process but also responsible for producing volatile compounds that contribute to the unique properties of kefir (Dertli & Çon, 2017).

The type and concentration of aromatic compounds generated by the microorganisms vary according to the strains of LAB present in the grains (Mauriello et al., 2001). The primary products formed by LAB include lactic acid, acetaldehyde, diacetyl, acetoin, acetone, ethanol, carbon dioxide, and acetic acid. Lactic acid, an odorless, non-volatile compound, imparts acidity to fermented milk. The lactic acid content in kefir typically ranges from 0.80% to 1.15% (Garrote et al., 2001) and is produced through the degradation of lactose by both homofermentative and heterofermentative LAB in the grains, with heterofermentative strains producing lactic acid in greater quantities (Rea et al., 1996).

Lactococcus species are homofermentative and are utilized in fermented dairy beverages due to their acidification capabilities (Chen et al., 2008). The effervescence of kefir arises from carbon dioxide produced during alcoholic fermentation and the heterofermentation of LAB (Liu et al., 2002a).

Acetaldehyde is recognized as the compound responsible for the refreshing taste and yogurt-like aroma of kefir (Ott et al., 2002). It is one of the principal aromatic substances found in kefir, with concentrations ranging from 0.50 to 10 mg/L (Guzel-Seydim et al., 2000).

Diacetyl is a desirable component in many dairy products, imparting a buttery aroma at low concentrations (Guzel-Seydim et al., 2000). In kefir, diacetyl concentrations have been reported between 0.30 and 1.85 mg/L (Aghlara et al., 2009; Beshkova et al., 2003).

Acetoin is typically found in concentrations around 9 mg/L in kefir beverages. Under these conditions, acetoin is generally odorless and tasteless, having no significant impact on flavor (Guzel-Seydim et al., 2000). Acetone, which is naturally present in milk, does not contribute to the sensory characteristics of kefir when present at concentrations below 1 mg/L (Aghlara et al., 2009).

Ethanol concentrations in kefir have been reported to range from 0.01 to 2.5%, depending on the starter culture and method used in the preparation of the beverage (Beshkova et al., 2003; Magalhães et al., 2011). Ethanol is produced through the conversion of acetaldehyde by alcohol dehydrogenase, an enzyme found in LAB and yeasts. Yeasts and *Leuconostoc* bacteria are the primary producers of ethanol; however, these bacteria do not produce ethanol during the metabolism of lactose and citrate, suggesting that yeasts are chiefly responsible for ethanol production (Guzel-Seydim et al., 2000).

Acetic acid imparts a vinegar-like flavor but is not predominant in kefir. It is unlikely that this compound results from lipolysis, as lipases present in milk are typically inactivated by pasteurization (Kondyli et al., 2002). Acetic acid is a volatile short-chain fatty acid found in concentrations ranging from 0.273 to 850 mg/L (Garrote et al., 2001; Guzel-Seydim et al., 2000; Magalhães et al., 2011).

In addition to the microbiota of kefir grains and the quality of the milk, which influence the production of volatile compounds and, consequently, the sensory and physicochemical quality of the beverage, variations in environmental conditions, such as incubation temperature, storage conditions, and preparation methods, also affect the composition of kefir (Farnworth, 2005).

5 KEFIR AS A PROBIOTIC FOOD

Probiotics are live microorganisms that have been demonstrated to confer health benefits to humans and animals when consumed in adequate amounts (Marteau et al., 1995; Plaza-Diaz et al., 2019). They are effective functional foods in treating various ailments, including inflammatory bowel disease and allergies (Sen, 2019). The intestine serves as the primary target for probiotics, functioning as an interface between diet and metabolism while modulating intestinal microbiota (Thakur et al., 2017).

Classified as non-pathogenic, probiotic microorganisms are recognized for their beneficial properties. They possess the ability to survive extreme conditions, such as low pH and the presence of bile salts, enabling them to colonize the gastrointestinal tract (Pringsulaka et al., 2015).

Research has demonstrated the effectiveness of probiotics, including kefir, in addressing gastrointestinal disorders (Reid et al., 2003). In cases of diarrhea, probiotics can aid in prevention and reduce the duration of symptoms (Heyman, 2000). Studies also indicate that kefir consumption can alleviate symptoms associated with chronic constipation (Maeda et al., 2004).

The most common microorganisms in probiotics are LAB, which are abundant in the intestines of healthy animals. The criteria for classifying LAB as probiotics include their ability to promote beneficial effects on the host, maintain high cell counts in food products throughout their shelf life, withstand gastrointestinal transit, adhere to intestinal epithelial cells, produce antimicrobial compounds against pathogens, and maintain a balanced intestinal microbiota (Parvez et al., 2006). The bacterial genera most frequently used in probiotics are *Lactobacillus* and *Bifidobacterium* (Tripathi & Giri, 2014).

Kefir, primarily composed of *Lactobacillus* species, has been recognized as a potential source of probiotics (Zanarati et al., 2015). The consumption of probiotics contributes to a balanced intestinal microbiota by increasing dietary microorganisms and supporting the immune system in combating pathogens (Yerlikaya, 2018).

Kefir's high nutritional value is attributed to its balanced composition of proteins, minerals, and vitamins, alongside the metabolites produced during fermentation. The health benefits of kefir include promoting gastrointestinal proliferation of beneficial microorganisms, antimicrobial properties, anticancer effects, cholesterol-lowering capabilities, antibiotic properties, antimutagenic activity, β-galactosidase activity, and positive impacts on lipid levels and blood pressure, as well as immune system support (Ahmed et al., 2013). Some of these properties will be discussed in detail below.

6 ANTIMICROBIAL ASPECTS

The antimicrobial activity of kefir against various pathogenic bacteria is attributed to the presence of organic acids, hydrogen peroxide, acetaldehyde, carbon dioxide, and bacteriocins (Czamanski et al., 2004). A study involving 21 isolates of LAB from a kefir sample reported that they produced hydrogen peroxide, recognized for its antimicrobial properties (Yuksekdag et al., 2004b). The same research group observed that 11 of these 21 *Lactococcus* sp. isolates generated hydrogen peroxide, and all strains from this genus inhibited the growth of *Staphylococcus aureus*. However, they were less effective against *Escherichia coli* NRLL B-704 and *Pseudomonas aeruginosa* (Yuksekdag et al., 2004a). Lacticin 3147, a bacteriocin produced by *Lactococcus lactis* DPC 3147, has demonstrated antibacterial effects against *E. coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella flexneri*, and *Yersinia enterocolitica* (Santos et al., 2003).

Kefir grains and kefiran have demonstrated antimicrobial activity against specific bacteria and filamentous fungi (Ahmed et al., 2011). Other studies have also observed kefir and kefiran's antimicrobial properties (Kwon et al., 2003; Rodrigues et al., 2005), reporting their efficacy against both Gram-negative and Gram-positive bacteria as well as *Candida albicans*.

Additionally, research involving Caco-2 cells (derived from a colorectal adenocarcinoma) indicated that kefiran had a protective effect against *Bacillus cereus* B10502 and was also able to safeguard cultured enterocytes from the activity of the *B. cereus* B10502 supernatant (Medrano et al., 2008).

7 ANTINEOPLASTIC EFFECTS

The antineoplastic effects of kefir have been linked to cancer prevention and tumor growth inhibition through mechanisms, such as apoptosis induction, immune response enhancement, modulation of gut microbiota, reduction in tumor growth and DNA damage, and antioxidant activity (Sharifi et al., 2017). A selection of *in vitro* and *in vivo* studies illustrating the antineoplastic properties of kefir is presented in Table 2. These studies span various cancer types, including hematologic malignancies (leukemia and lymphoma), breast cancer, gastrointestinal tumors, and sarcomas.

In an *in vivo* study involving mice inoculated with sarcoma cells and treated with oral kefir, an increase in immunoglobulin A (IgA) levels in tissue fractions from the small intestine wall was observed, along with significant tumor growth suppression induced by the lysis of apoptotic tumor cells. It was suggested that kefir exhibits antineoplastic properties and enhances mucosal resistance to gastrointestinal infections following 30 days of consumption (Liu et al., 2002a).

An *in vitro* study observed a reduction in the growth of human breast tumor cells (MCF-7) cultured with cell-free filtered kefir supernatant. The findings suggest that kefir extract contains compounds capable of specifically inhibiting this type of cell, potentially supporting its use in breast cancer prevention or treatment (Chen et al., 2007). These results indicate that kefir

Table 2. Antineoplastic effects of kefir and kefir extracts.

Sample	Biological activity	References
Milk kefir and soy milk kefir	Anti-inflammatory effect in rodent sarcoma	Liu et al., 2002b
Filtered kefir supernatant (*cell free*)	Antiproliferative effect on human breast tumor cells (MCF-7)	Chen et al., 2007
Filtered kefir supernatant (*cell free*)	Antiproliferative effect on human gastric cells (SGC7901)	Gao et al., 2013
Filtered kefir supernatant (*cell free*)	Antiproliferative and apoptotic effects on colorectal tumor cells (Caco-2/HT-29)	Khoury et al., 2014
Filtered kefir supernatant (*cell free*)	Adjuvant antineoplastic effects on multidrug-resistant human colorectal tumor cells (HT-29)	Kim et al., 2021

Source: adapted from Azizi et al. (2021).

may serve as a non-toxic chemotherapeutic adjuvant, warranting further research to explore its antineoplastic potential.

8 EFFECT ON THE IMMUNE SYSTEM

The immune system and nutrition are directly interconnected (Vinderola et al., 2006), and numerous studies have reported that products fermented by LAB have the potential to enhance the immune response in both animal and human models (Ahmed et al., 2013). Studies on the immunomodulatory properties of kefir in rodents indicate that dosage and cell viability can influence the Th1 or Th2 cell-mediated immune response (Vinderola et al., 2005). Consuming kefir may support the activity of peritoneal and pulmonary macrophages, potentially reducing pathogenicity, as well as impacting mucosal immune responses throughout the body. Additionally, kefir microbiota has been shown to induce interleukin-10-producing cells in Peyer's patch-derived cells in rodent models (Vinderola et al., 2006).

Kefir and other fermented dairy products may contribute to maintaining homeostasis and eliciting a robust immune response in intestinal mucosa, increasing IgA production in both the small and large intestines (Vinderola et al., 2006).

9 EFFECT ON LACTOSE INTOLERANCE

A subset of the population is unable to digest lactose due to insufficient production of the enzyme β-galactosidase (Alm, 1982). Research suggests that lactose-intolerant individuals can tolerate yogurt if it contains sufficient viable bacteria to metabolize lactose (Pelletier et al., 2001). The bacteria in yogurt are thought to be protected by yogurt's buffering effect, which preserves bacterial cell viability and ensures intact cell walls, allowing bacterial β-galactosidase to remain active as it transits through the stomach to the small intestine (De Vrese et al., 2001; Montes et al., 1995). Additionally, fermented dairy products exhibit slower gastrointestinal transit than milk, potentially improving lactose digestion (Labayen et al., 2001).

Some kefir grains also exhibit β-galactosidase activity that remains active upon consumption (De Vrese et al., 1992). Studies have shown that pigs fed kefir with fresh grains had significantly higher plasma galactose levels compared to those fed kefir with heat-treated grains. Diets with fresh grains contained active β-galactosidase, facilitating lactose hydrolysis in the intestine, which led to galactose production and subsequent absorption (De Vrese et al., 1992). Notably, kefir beverages are generally free of galactose (Alm, 1982).

10 EFFECT ON CHOLESTEROL REDUCTION

Evidence regarding the cholesterol-lowering effect of kefir consumption remains limited. Some studies suggest that kefir supplementation in cholesterol-rich diets can reduce phospholipid and total serum cholesterol levels in rodents (John & Deeseenthum, 2015). However, other biomarkers, such as high-density lipoproteins (HDLs) and serum triglycerides, showed no significant changes with kefir consumption (Rattray & Connell, 2011). Conversely, another study reported reduced serum triglycerides and total cholesterol levels in hamsters fed with kefir (Liu et al., 2006).

Research suggests that kefir's cholesterol-lowering effect may be linked to bile acid deconjugation by *Lactobacillus* spp. (Brashears et al., 1998; Tamai et al., 1996). Deconjugation of bile acids may stimulate the production of new bile acids needed to replace those lost from the enterohepatic circulation, thereby lowering serum cholesterol levels (Reynier et al., 1981).

The bile salt hydrolase enzyme found in *Lactobacillus* spp. may be responsible for converting conjugated bile acids to unconjugated ones. This process may contribute to serum cholesterol reduction by increasing bile acid synthesis from cholesterol or by lowering cholesterol solubility and absorption in the intestine (Begley et al., 2006).

Studies have also shown that kefir-fed poultry experienced reduced serum cholesterol levels, which may be associated with both reduced cholesterol biosynthesis in the liver and increased bile acid degradation by *Lactobacillus* spp. (Cenesiz et al., 2008). However, further studies are needed to fully understand the role of these bacteria in cholesterol reduction.

11 CONCLUSION

Kefir is recognized as a potential source of probiotics and beneficial compounds of high interest for health. Due to its various health benefits, demand for its consumption has grown, especially among those seeking alternatives to pharmaceutical treatments and healthier lifestyles.

Ongoing research is focused on exploring kefir's properties, which are not yet fully understood. Further studies to better characterize kefir can help unlock new applications, including the development of new products and dietary alternatives to support consumer health.

REFERENCES

Aghlara, A., Mustafa, S., Manap, Y. A., & Mohamad, R. (2009). Characterization of headspace volatile flavour compounds formed during kefir production: Application of solid phase microextraction. *International Journal of Food Properties*, *12*(4), 808-818. https://doi.org/10.1080/10942910802073189

Ahmed, A., Ismaiel, M., Mohamed, F., Ayman, G. K., & Naggar, E. L. (2011). Milk kefir: Ultrastructure, antimicrobial activity and efficacy on aflatoxin B1 production by *Aspergillus flavus*. *Current Microbiology*, *62*(5), 1602-1609. https://doi.org/10.1007/s00284-011-9901-9

Ahmed, Z., Wang, Y., Ahmad, A., Khan, S. T., Nisa, M., Ahmad, H., & Afreen, A. (2013). Kefir and health: A contemporary perspective. *Food Science and Nutrition*, *53*(5), 422-434. https://doi.org/10.1080/10408398.2010.540360

Alm, L. (1982). Effect of fermentation on lactose, glucose, and galactose content in milk and suitability of fermented milk products for lactose-intolerant individuals. *Journal of Dairy Science*, *65*(3), 346-352. https://doi.org/10.3168/jds.S0022-0302(82)82198-X

Azizi, N. F., Kumar, M. R., Yeap, S. K., Abdullah, J. O., Khalid, M., Omar, A. R., Osman, M. A., Mortadza, S. A. S., & Alitheen, N. B. (2021). Kefir and its biological activities. *Foods*, *10*(6), 1-20.

Begley, M., Hill, C., & Cormac, G. M. (2006). Bile salt hydrolase activity in probiotics. *Applied and Environmental Microbiology*, *72*(3), 1729-1738. https://doi.org/10.1128/AEM.72.3.1729-1738.2006

Beshkova, D. M., Simova, E. D., Frengova, G. I., Simov, Z. I., & Dimitrov, Z. H. P. (2003). Production of volatile aroma compounds by kefir starter cultures. *International Dairy Journal*, *13*(6), 529-535.

Brashears, M., Gilliland, S. E., & Buck, L. M. (1998). Bile salt deconjugation and cholesterol removal from media by *Lactobacillus casei*. *Journal of Dairy Science*, *81*(8), 2103-2110. https://doi.org/10.3168/jds.s0022-0302(98)75785-6

Brasil (2007). Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº 46, de 23 de outubro de 2007. Regulamento Técnico de Identidade e Qualidade de Leites Fermentados. *Diário Oficial da União.*

Cenesiz, S., Yaman, H., Ozcan, A., Kart, A., & Karademir, G. (2008). Effects of kefir as a probiotic on serum cholesterol, total lipid, aspartate aminotransferase and alanine aminotransferase activities in broiler chicks. *Medycyna Weterynaryjna*, *64*(2), 168-170.

Chen, C., Hing, M. C., & Kubow, S. (2007). Kefir extracts suppress in vitro proliferation of estrogen-dependent human breast cancer cells but not normal mammary epithelial cells. *Journal of Medicinal Food*, *10*(3), 416-422. https://doi.org/10.1089/jmf.2006.236

Chen, H. S., Wang, S. Y., & Chen, M. J. (2008). Microbiological study of lactic acid bacteria in kefir grains by cultured-dependent and cultured-independent methods. *Food Microbiology*, *25*(3), 492-501. https://doi.org/10.1016/j.fm.2008.01.003

Codex Alimentarius International Food Standards (2003). *Codex Standard for Fermented Milks (Codex Stan CXS 243-2003)*. Codex Alimentarius Commission. Retrieved from https://www.fao.org/fao-who-codexalimentarius

Czamanski, R. T., Greco, D. P., & Wiest, J. M. (2004). Evaluation of antibiotic activity in filtrates of traditional kefir. *Higiene Alimentar*, *18*(1), 75-77.

De Vrese, M., Keller, B., & Barth, C. A. (1992). Enhancement of intestinal hydrolysis of lactose by microbial β-galactosidase (EC 3.2.1.23) of kefir. *British Journal of Nutrition*, *67*(1), 67-75. https://doi.org/10.1079/bjn19920009

De Vrese, M., Stegelmann, A., Richter, B., Fenselau, S., Laue, C., & Schrezenmeir, J. (2001). Probiotics-compensation for lactase insufficiency. *American Journal of Clinical Nutrition*, *73*(2 Suppl.), 421S-429S. https://doi.org/10.1093/ajcn/73.2.421s

Dertli, A., & Çon, A. H. (2017). Microbial diversity of traditional kefir grains and their role on kefir aroma. *Food Science and Technology*, *85*(Part A), 151-157. https://doi.org/10.1016/j.lwt.2017.07.017

Duitschaever, C. L., Kemp, N., & Emmons, D. (1987). Pure culture formulation and procedure for the production of kefir. *Milchwissenschaft*, *42*(2), 80-82.

Farnworth, E. R. (2005). Kefir: a complex probiotic. *Food Science and Technology Bulletin: Functional Foods*, *2*(1), 1-17.

Gao, J., Gu, F., Abdella, N. H., Ruan, H., & He, G. (2012). Investigation on culturable microflora in Tibetan kefir grains from different areas of China. *Journal of Food Science*, *77*(7), 425-433. https://doi.org/10.1111/j.1750-3841.2012.02805.x

Gao, J., Gu, F., Ruan, H., Chen, Q., He, J., & He, G. (2013). Induction of apoptosis of gastric cancer cells SGC7901 in vitro by a cell-free fraction of Tibetan kefir. *International Dairy Journal*, *30*(1), 14-18. https://doi.org/10.1016/j.idairyj.2012.11.011

Garofalo, C., Osimani, A., Milanović, V., Aquilanti, L., De Filippis, F., Stellato, G., Di Mauro, S., Turchetti, B., Buzzini, P., Ercolini, D., & Clementi, F. (2015). Bacteria and yeast microbiota in milk kefir grains from different Italian regions. *Food Microbiology*, *49*, 123-133. https://doi.org/10.1016/j.fm.2015.01.017

Garrote, G. L., Abraham, A. G., & De Antoni, G. L. (2001). Chemical and microbiological characterization of kefir grains. *Journal of Dairy Research*, *68*(4), 639-652. https://doi.org/10.1017/s0022029901005210

Guzel-Seydim, Z. B., Seydim, A. C., Greene, A. K., & Bodine, A. B. (2000). Determination of organic acids and volatile flavor substances in kefir during fermentation. *Journal of Food Composition and Analysis*, *13*(1), 35-43. https://doi.org/10.1006/jfca.1999.0842

Hertzler, S. R., & Clancy, S. M. (2003). Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *Journal of the American Dietetic Association*, *153*(5), 582-587. https://doi.org/10.1053/jada.2003.50111

Heyman, M. (2000). Effect of lactic acid bacteria on diarrheal diseases. *Journal of the American College of Nutrition*, *19*(2 Suppl.), 137-146. https://doi.org/10.1080/07315724.2000.10718084

Irigoyen, A., Arana, I., Castiella, M., & Torre, P. (2005). Microbiology, physicochemical and sensory characteristics of kefir during storage. *Food Chemistry*, *90*(4), 613-620. https://doi.org/10.1016/j.foodchem.2004.04.021

John, S. M., & Deeseenthum, S. (2015). Properties and benefits of kefir: A review. *Songklanakarin Journal of Science and Technology*, *37*(3), 275-282.

Kemp, N. (1984). Kefir, the champagne of cultured dairy products. *Cultured Dairy Products Journal*, *19*(3), 29-30.

Khoury, N., El-Hayek, S., Tarras, O., El-Sabban, M., El-Sibai, M., & Rizk, S. (2014). Kefir exhibits anti-proliferative and pro-apoptotic effects on colon adenocarcinoma cells with no significant effects on cell migration and invasion. *International Journal of Oncology*, *45*(5), 2117-2127. https://doi.org/10.3892/ijo.2014.2635

Kim, D. H., Jeong, C. H., Cheng, W. N., Kwon, H. C., Kim, D. H., Seo, K. H., Choi, Y., & Han, S. G. (2021). Effects of kefir on doxorubicin-induced multidrug resistance in human colorectal cancer cells. *Journal of Functional Foods*, *78*, 104371. https://doi.org/10.1016/j.jff.2021.104371

Kondyli, E., Katsiari, M. C., Masouras, T., & Voutsinas, L. P. (2002). Free fatty acids and volatile compounds of low-fat feta-type cheese made with a commercial adjunct culture. *Food Chemistry*, *79*(2), 199-205. https://doi.org/10.1016/S0308-8146(02)00132-2

Koroleva, N. S. (1991). Therapeutic properties of fermented milks. In: R. K. Robinson (Ed.), *Elsevier Applied Sciences Publishers* (pp. 157-159). Elsevier.

Korsak, N., Taminiau, B., Leclercq, M., Nezer, C., Crevecoeur, S., Ferauche, C., Detry, E., Delcenserie, V., & Daube, G. (2015). Short communication: Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments. *Journal of Dairy Science*, *98*(6), 3684-3689. https://doi.org/10.3168/jds.2014-9065

Kwon, C. S., Park, M. Y., Cho, J. S., Choi, S. T., & Chang, D. S. (2003). Identification of effective microorganisms from kefir fermented milk. *Food Science and Biotechnology*, *12*(5), 476-479.

Labayen, I., Forga, L., Gonzalez, A., Lenoir-Wijnkoop, I., & Martinez, J. A. (2001). Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. *Alimentary Pharmacology and Therapeutics*, *15*(4), 543-549. https://doi.org/10.1046/j.1365-2036.2001.00952.x

Linossier, J. P., & Dousset, X. (1994). Stimulation de la croissance et du métabolisme de *Lactobacillus kefir* par *Candida kefir*. *Microbiologie Aliments Nutrition*, *12*, 341-351.

Liu, J. R., Chen, M. J., & Lin, C. W. (2002a). Characterization of polysaccharide and volatile compounds produced by kefir grains grown in soymilk. *Food Chemistry and Toxicology*, *67*(1), 104-108. https://doi.org/10.1111/j.1365-2621.2002.tb11367.x

Liu, J. R., Wang, S. Y., Chen, M. J., Chen, H. L., Yueh, P. Y., & Lin, C. W. (2006). Hypocholesterolaemic effects of milk kefir and soymilk-kefir in cholesterol-fed hamsters. *British Journal of Nutrition*, *95*(5), 939-946. https://doi.org/10.1079/bjn20061752

Liu, J. R., Wang, S. Y., Lin, Y. Y., & Lin, C. W. (2002b). Antitumor activity of milk kefir and soy milk kefir in tumor-bearing mice. *Nutrition and Cancer*, *44*(2), 183-187. https://doi.org/10.1207/s15327914nc4402_10

Maeda, H., Zhu, X., Omura, K., Suzuki, S., & Kitamura, S. (2004). Effects of an exopolysaccharide (kefiran) on lipids, blood pressure, blood glucose, and constipation. *BioFactors*, *22*(1-4), 197-200. https://doi.org/10.1002/biof.5520220141

Magalhães, K. T., Pereira, G. V. M., Campos, C. R., Dragone, G., & Schwan, R. F. (2011). Brazilian kefir: Structure, microbial communities and chemical composition. *Brazilian Journal of Microbiology*, *42*(2), 693-702. https://doi.org/10.1590/S1517-83822011000200034

Marteau, P., Gerhardt, M. F., Myara, A., Bouvier, E., Trivin, F., & Rambaud, J. C. (1995). Metabolism of bile salts by alimentary bacteria during transit in the human small intestine. *Microbial Ecology in Health and Disease*, *8*(4), 151-157. https://doi.org/10.3109/08910609509140093

Mauriello, G., Moio, L., Moschetti, G., Piombino, P., Addeo, F., & Coppola, S. (2001). Characterization of lactic acid bacteria strains on the basis of neutral volatile compounds produced in whey. *Journal of Applied Microbiology*, *90*(6), 928-942. https://doi.org/10.1046/j.1365-2672.2001.01327.x

Medrano, M., Perez, P. F., & Abraham, A. G. (2008). Kefiran antagonizes cytopathic effects of *Bacillus cereus* extracellular factors. *International Journal of Food Microbiology*, *122*(1-2), 1-7. https://doi.org/10.1016/j.ijfoodmicro.2007.11.046

Montes, R. G., Bayless, T. M., Saavedra, J. M., & Perman, J. A. (1995). Effect of milks inoculated with *Lactobacillus acidophilus* or a yogurt starter culture in lactose-maldigesting children. *Journal of Dairy Science*, *78*(8), 1657-1664. https://doi.org/10.3168/jds.s0022-0302(95)76790-x

Ott, A., Germond, J. E., & Chaintreau, A. (2002). Origin of acetaldehyde production by *Streptococcus thermophilus*. *Applied and Environment Microbiology*, *68*(11), 5656-5662.

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H.-Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, *100*(6), 1171-1185. https://doi.org/10.1111/j.1365-2672.2006.02963.x

Paucean, A., & Carmen, S. (2008). Probiotic activity of mixed cultures of kefir's lactobacilli and non-lactose fermenting yeasts. *Bulletin UASVM*, *65*(2).

Pelletier, X., Laure-Boussuge, S., & Donazzolo, Y. (2001). Hydrogen excretion upon ingestion of dairy products in lactose-intolerant male subjects: Importance of the live flora. *European Journal of Clinical Nutrition*, *55*(6), 509-512. https://doi.org/10.1038/sj.ejcn.1601169

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Mechanisms of action of probiotics. *Advances in Nutrition*, *10*(Suppl. 1), 49-66. https://doi.org/10.1093/advances/nmy063

Pringsulaka, O., Rueangyotchathana, K., & Suwannasai, N. (2015). In vitro screening of lactic acid bacteria for multi-strain probiotics. *Livestock Science*, *174*, 66-73. https://doi.org/10.1016/j.livsci.2015.01.016

Rattray, F. P., & Connell, M. J. (2011). *Encyclopedia of Dairy Sciences* (2ª ed). Fukay, J. W.

Rea, M. C., Lennartsson, T., & Dillon, P. (1996). Irish kefir-like grains: Their structure, microbial composition, and fermentation kinetics. *Journal of Applied Bacteriology*, *81*(1), 83-94. https://doi.org/10.1111/j.1365-2672.1996.tb03286.x

Reid, G., Jass, J., Sebulsky, M. T., & McCormick, J. K. (2003). Potential uses of probiotics in clinical practice. *Clinical Microbiological Reviews*, *16*(4), 658-672. https://doi.org/10.1128/cmr.16.4.658-672.2003

Reynier, M. O., Montet, J. C., Gerolami, A., Marteau, C., Crotte, C., Montet, A. M., & Mathieu, S. (1981). Comparative effects of cholic, chenodeoxycholic, and ursodeoxycholic acids on micellar solubilization and intestinal absorption of cholesterol. *Journal of Lipid Research*, *22*(3), 467-473.

Roberts, M., Yarunin, S., & Danone. (2000). Moves into Russian kefir market. *New Nutrition Business*, *6*(3), 22-24.

Rodrigues, K. L., Caputo, L. R. G., Carvalho, J. C. T., Evangelista, J., & Schneedorf, J. M. (2005). Antimicrobial and healing activity of kefir and kefiran extract. *International Journal of Antimicrobial Agents*, *25*(5), 404-408. https://doi.org/10.1016/j.ijantimicag.2004.09.020

Santos, A., Sanmauro, M., Sanchez, A., Torres, J. M., & Marquina, D. (2003). The antimicrobial properties of different strains of *Lactobacillus* spp. isolated from kefir. *Systematic and Applied Microbiology*, *26*(3), 434-437. https://doi.org/10.1078/072320203322497464

Schwan, R. F., Magalhães-Guedes, K. T., & Dias, D. R. (2015). Kefir - Grains and beverages: A review. *Scientia Agraria Paranaensis*, *14*(1), 1-9. https://doi.org/10.18188/sap.v14i1.11137

Semih, O. E., & Cagindi, C. (2003). Kefir: A probiotic dairy - Composition, nutritional and therapeutic aspects. *Pakistan Journal of Nutrition*, *2*(2), 54-59. https://doi.org/10.3923/pjn.2003.54.59

Sen, M. (2019). Role of probiotics in health and disease - A review. *International Journal of Advancement in Life Sciences Research*, *2*(2), 1-11.

Sharifi, M., Moridnia, A., Mortazavi, D., Salehi, M., Bagheri, M., & Sheikhi, A. (2017). Kefir: A powerful probiotic with anticancer properties. *Medical Oncology*, *34*(11), 183. https://doi.org/10.1007/s12032-017-1044-9

Tamai, Y., Yoshimitsu, N., Watanabe, Y., Kuwabara, Y., & Nagai, S. (1996). Effects of milk fermented by culturing with various lactic acid bacteria and a yeast on serum cholesterol level in rats. *Journal of Fermentation and Bioenergy*, *81*, 181-182.

Thakur, K., Zhang, J. G., Wei, Z. J., Kumar, N., Tomar, S. K., & Pophaly, S. D. (2017). *Nutraceuticals and innovative food products for healthy living and preventive care*. Medical Information Science Reference.

Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, *9*, 225-241.

Vinderola, C. G., Duarte, J., Thangavel, D., Perdigón, G., Farnworth, E., & Matar, C. (2005). Remote-site stimulation and duration of the immune response by kefir. *European Journal of Inflammation*, *3*(2), 63-73.

Vinderola, C. G., Perdigón, G., Duarte, J., Farnworth, E., & Matar, C. (2006). Effects of the oral administration of the products derived from milk fermentation by kefir microflora on immune stimulation. *Journal of Dairy Research*, *73*(4), 472-479. https://doi.org/10.1017/s002202990600197x

Wang, H., Wang, C., & Guo, M. (2020). Autogenic successions of bacteria and fungi in kefir grains from different origins when sub-cultured in goat milk. *Food Research International*, *138*(Part B), 109784. https://doi.org/10.1016/j.foodres.2020.109784

Wang, S. Y., Chen, K. N., Lo, Y. M., Chiang, M. L., Chen, H. C., Liu, J. R., & Chen, M. J. (2012). Investigation of microorganisms involved in biosynthesis of the kefir grain. *Food Microbiology*, *32*(2), 274-285. https://doi.org/10.1016/j.fm.2012.07.001

Weschenfelder, S. (2009). Caracterização de kefir tradicional quanto à composição físico-química, sensorialidade e atividade anti-*Escherichia coli* (Dissertação de mestrado, Universidade Federal do Rio Grande do Sul).

Witthuhn, R. C., Schoeman, T., & Britz, T. J. (2005). Characterisation of the microbial population at different stages of kefir production and kefir grain mass cultivation. *International Dairy Journal*, *15*(4), 383-389. https://doi.org/10.1016/j.idairyj.2004.07.016

Wszolek, M., Tamime, A. Y., Muir, D. D., & Barclayb, M. N. I. (2001). Properties of kefir made in Scotland and Poland using bovine, caprine and ovine milk with different starter cultures. *LWT - Food Science and Technology*, *34*(4), 251-261. https://doi.org/10.1006/fstl.2001.0773

Wyder, M. T., Meile, L., & Teuber, M. (1999). Description of *Saccharomyces turicensis* sp. nov., a new species from kefir. *Systematic and Applied Microbiology*, *22*(3), 420-425. https://doi.org/10.1016/S0723-2020(99)80051-4

Yang, X. J., Fan, M. T., Shi, J. L., & Dang, B. (2007). Isolation and identification of preponderant flora in Tibetan kefir. *China Brewing*, *171*, 52-55.

Yerlikaya, O. (2018). Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. *lactis* strains isolated from raw milk and kefir grains. *Journal of Dairy Science*, *102*(1), 124-134. https://doi.org/10.3168/jds.2018-14983

Yilmaz-Ersan, L., Ozcan, T., Akpinar-Bayizit, A., & Sahin, S. (2018). Comparison of antioxidant capacity of cow and ewe milk kefirs. *Journal of Dairy Science*, *101*(5), 3788-3798. https://doi.org/10.3168/jds.2017-13871

Yuksekdag, Z. N., Beyatli, Y., & Aslim, B. (2004a). Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefirs with natural probiotic. *LWT - Food Science and Technology*, *37*(6), 663-667. https://doi.org/10.1016/j.lwt.2004.02.004

Yuksekdag, Z. N., Beyath, Y., & Aslim, B. (2004b). Metabolic activities of *Lactobacillus* spp. strains isolated from kefir. *Nahrung / Food*, *48*(3), 218-220. https://doi.org/10.1002/food.200300414

Zanarati, D. F., Abatemarco, M., de Cicco Sandes, S. H., Nicoli, J. R., Nunes, Á. C., & Neumann, E. (2015). Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starter or probiotic cultures. *Anaerobe*, *32*, 70-76. https://doi.org/10.1016/j.anaerobe.2014.12.007