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Milk *kefir*: nutritional, microbiological and health benefits

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Abstract

Kefir is fermented milk produced from grains that comprise a specific and complex mixture of bacteria and yeasts that live in a symbiotic association. The nutritional composition of *kefir* varies according to the milk composition, the microbiological composition of the grains used, the time/temperature of fermentation and storage conditions. *Kefir* originates from the Caucasus and Tibet. Recently, *kefir* has raised interest in the scientific community due to its numerous beneficial effects on health. Currently, several scientific studies have supported the health benefits of *kefir*, as reported historically as a probiotic drink with great potential in health promotion, as well as being a safe and inexpensive food, easily produced at home. Regular consumption of *kefir* has been associated with improved digestion and tolerance to lactose, antibacterial effect, hypocholesterolaemic effect, control of plasma glucose, anti-hypertensive effect, anti-inflammatory effect, antioxidant activity, anti-carcinogenic activity, anti-allergenic activity and healing effects. A large proportion of the studies that support these findings were conducted *in vitro* or in animal models. However, there is a need for systematic clinical trials to better understand the effects of regular use of *kefir* as part of a diet, and for their effect on preventing diseases. Thus, the present review focuses on the nutritional and microbiological composition of *kefir* and presents relevant findings associated with the beneficial effects of *kefir* on human and animal health.

Key words: **Milk *kefir*: Probiotics: Fermented milk: Health benefits**

Introduction

Kefir has its origin in the Caucasus, Tibetan or Mongolian mountains, where before 2000 years BC the grains were already being traditionally passed from generation to generation among the Caucasus tribes, being considered a source of family wealth. The name *kefir* originates from the Slavic *Keif*, meaning 'well-being' or 'living well', due to the overall sense of health and well-being generated in those who consume it[1]. *Kefir* differs from other fermented products because it is produced from *kefir* grains that comprise a specific and complex mixture of lactic acid- and acetic acid-producing bacteria, and lactose-fermenting and non-fermenting yeast, which live in a symbiotic association[2].

Kefir grains, when inoculated into a culture medium such as milk, produce acidified fermented milk that is slightly carbonated and contains small amounts of alcohol. During fermentation, lactic acid, bioactive peptides, exopolysaccharides, antibiotics and numerous bacteriocins are produced[1,3]. According to the *Codex Alimentarius* (Codex Stan 243-2003)[4], a typical *kefir* (fermented milk obtained from *kefir* grains) should contain at least 2·7 % of protein, 0·6 % of lactic acid, and less than 10 % of fat. The percentage of alcohol is not established. The total number of micro-organisms in the fermented milk produced should be at least 10^7 colony-forming units (CFU)/ml and the yeast number not less than 10^4 CFU/ml[4].

The micro-organisms present in *kefir* possess probiotic potential. Numerous bacterial species isolated from *kefir* demonstrate high resistance to the low pH and bile salts in the gastrointestinal tract, and are able to adhere to the intestinal mucus[5]. Additionally, the microbiota present in *kefir* can produce antagonistic substances, such as organic acids and bacteriocins[6], and interfere with the adherence of pathogenic bacteria in the intestinal mucosa[7], potentially contributing to the improvement of gut health.

Kefir has raised interest in the scientific community due to its suggested beneficial properties, including improved digestion and tolerance to lactose[8], antibacterial effect[9], hypocholesterolaemic effect[10], control of plasma glucose[11], anti-hypertensive effect[12], anti-inflammatory effect[9,13], antioxidant activity[14], anti-carcinogenic activity[15] and anti-allergenic activity[13]. Therefore, the present review focuses on the nutritional and microbiological composition of *kefir* and presents relevant findings associated with the beneficial effects of *kefir* on human and animal health.

Characteristics of *kefir* grains

Kefir grains have a similar shape to the cauliflower. They are elastic, irregular, gelatinous, with an ivory or white colour, and variable size, from 0·3 to 3·5 cm in diameter[16,17] (Fig. 1). In general, *kefir* grain consists of 4·4 % fat, 12·1 % ash, 45·7 % mucopolysaccharide, 34·3 % total protein (27 % insoluble, 1·6 % soluble and 5·6 % free amino acids), vitamins B and K, tryptophan, Ca, P and Mg[18].

Abbreviations: ACE, angiotensin-converting enzyme; CFU, colony-forming units; LAB, lactic acid bacteria.

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Fig. 1. Appearance of *kefir* grains.

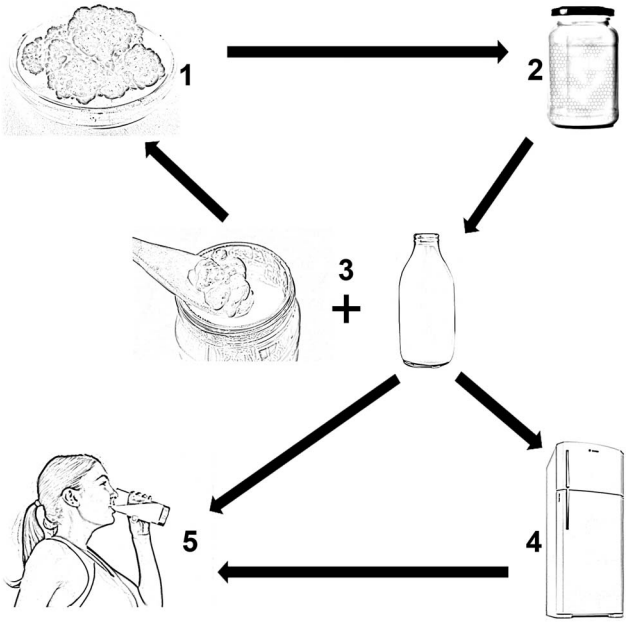

Fig. 2. Domestic production of *kefir*. (1) Separation of *kefir* grains. (2) Addition of milk to the *kefir* grains in a half-open container at room temperature to ferment for 10 to 24 h. (3) Filtration and separation of *kefir* grains. Possible addition of the *kefir* grains to fresh milk to start a new fermentation. The *kefir* is adequate for consumption. (4) The *kefir* can be refrigerated (4°C). (5) The *kefir* is safe and ready to drink.

The presence of D-glucose and D-galactose in a 1:1 ratio in the complex structure of polysaccharides (kefiran) is responsible for the connection between the micro-organisms in *kefir* grains[19]. Kefiran features include viscosity, water solubility and resistance to bowel enzymic hydrolysis. The production of kefiran is mainly related to the presence of *Lactobacillus kefiranofaciens* and *Lactobacillus kefiri* in the grains[2,20].

In *kefir* grains, the peripheral portion is composed almost exclusively of bacteria, predominantly *Bacillus*, whereas the inner portion of the grain contains yeasts, and the interface of the inner and outer portions has a mixed composition, where bacteria with long polysaccharide filaments, yeasts and fungi are found[2,19].

The grains can be stored in different ways. When stored at 4°C, they are active for only 8 to 10 d. Lyophilisation or drying at room temperature for 36 to 48 h allows maintenance of the activity for 12 to 18 months[16]. Wszolek *et al.*[21] proposed a conventional method of drying at 33°C or vacuum drying to preserve the grains. However, Garrote *et al.*[22] observed that freezing at −20°C was the best method for grain preservation. *Kefir* grains remain stable for many years without losing their activity, if stored under favourable conditions. The process of reconstitution consists of performing successive incubations in milk. The grains slowly re-establish their structure and, subsequently, new *kefir* grains are formed[23].

Production of *kefir*

Kefir can be produced from whole, semi-skimmed or skimmed pasteurised cow, goat, sheep, camel or buffalo milk[24]. *Kefir* from cows' milk is the most common. The *kefir* grains can be added to the fermentation substrate as a starter culture[24].

Although there is an ideal relationship between the grains and the fermentation substrate (1:30 to 1:50 (w/v) in the case of animal milk), in practice, the measures are made empirically[1]. Fermentation typically occurs at temperatures ranging from 8 to 25°C, in a partially closed container, at a variable time from 10 to 40 h. However, the most common incubation time is 24 h[25–27].

After fermentation, the grains are separated from the fermented milk by filtration using a sieve[2]. When milk is used as a substrate, the *kefir* is similar to yogurt. The higher the fat content in the milk, the thicker and creamier the *kefir*[24]. *Kefir* grains may increase in size by up to 2 % of the original to form a new biomass, which allows continuous production, since the grains can be further added to a fermentation substrate[1,16,17]. Pure starter and lyophilised culture can be used, eliminating the step of recovering the *kefir* grains. *Kefir* can be consumed immediately after grain separation or can be refrigerated for later consumption. During the cooling step, alcoholic fermentation leads to the accumulation of CO_2, ethanol and vitamin B complex[1,24]. This maturation step reduces the lactose content, making the product desirable for consumption by individuals with lactose intolerance and diabetes[1] (Fig. 2).

Nowadays, micro-organisms isolated from *kefir* grains or starter cultures containing freeze-dried lactic acid bacteria (LAB) and *kefir* yeasts are being used in *kefir* production. However, the composition of the final fermented milk presents a lower number and variety of micro-organisms than the fermented milk produced from *kefir* grains[28].

Nutritional composition of *kefir*

The nutritional composition of *kefir* varies widely and is influenced by milk composition, the origin and composition of the grains used, the time/temperature of fermentation and storage conditions. However, the nutritional composition of *kefir* is still not well described in the literature.

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Regarding the chemical composition, moisture is the predominant constituent (90 %), followed by sugars (6 %), fat (3·5 %), protein (3 %) and ash (0·7 %)[23]. During fermentation, proteins become easily digestible due to the action of acid coagulation and proteolysis. *Kefir* shows a similar profile of amino acids to the milk used as the fermentation substrate[29]. The levels of ammonia, serine, lysine, alanine, threonine[14], tryptophan, valine, lysine, methionine, phenylalanine and isoleucine are higher in *kefir* compared with unfermented milk[30]. According to Liutkevičius & Šarkinas[31], the essential amino acid contents in *kefir* are in descending order: lysine (376 mg/100 g); isoleucine (262 mg/100 g); phenylalanine (231 mg/100 g); valine (220 mg/100 g); threonine (183 mg/100 g); methionine (137 mg/100 g); and tryptophan (70 mg/100 g).

The lactose from milk is degraded to acid during the fermentation process, which causes pH reduction and increase in consistency. Approximately 30 % of milk lactose is hydrolysed by the β-galactosidase enzyme, turning lactose into glucose and galactose. Furthermore, bacteria present in *kefir* convert glucose into lactic acid[29]. In this context, *kefir* is a good option for lactose-intolerant individuals.

The lipid content (monoacylglycerols, diacylglycerols and TAG, NEFA and steroids) in *kefir* can vary depending on the type of milk used in the fermentation. In the fermented milk, the presence of NEFA contributes to the improvement of digestibility[20].

Kefir contains a rich vitamin composition, when it is ready for consumption. The vitamin content depends on the quality of the milk used, micro-organisms present in the *kefir* grains, and the way of preparation. *Kefir* presents vitamins B_1, B_2, B_5, C[23], A and K, and carotene in its composition. According to Liut Kevičius & Šarkinas[30], the concentration of pyridoxine, vitamin B_{12}, folic acid, biotin, thiamin and riboflavin increase during the fermentation process.

Among the minerals, *kefir* is a good source of Mg, Ca and P[20]. Additionally, minerals such as Zn, Cu, Mn, Fe, Co and Mo are found in milk *kefir*.

Lactic acid, CO_2 and ethanol are the main products that originate from the lactic fermentation process. *Kefir* also contains formic, propionic and succinic acids, aldehydes, traces of acetone and isoamyl alcohol, and a variety of folates[25]. The pH of *kefir* varies between 4·2 and 4·6, ethanol content between 0·5 and 2·0 % (v/v), lactic acid between 0·8 and 1·0 % (w/v) and CO_2 between 0·08 and 0·2 % (v/v)[32]. Biogenic amines such as putrescine, cadaverine, spermidine and tyramine are also found in *kefir* samples as a consequence of the LAB activity[33]. The high levels of biogenic amines are related to the depreciation of the sensorial properties of fermented milk, and are considered to be an important indicator of quality and acceptability. The high concentration of bioactive amines in fermented products, especially putrescine, cadaverine, agmatine and *N*-methylputrescine, as well as monoamines such as penicillamine and histamine are positively correlated with inharmonious bitter taste[34]. Özdestan & Uren[35] reported total biogenic amines contents in *kefir* samples between 2·4 and 35·2 mg/l, with tyramine being the most abundant bioactive amine. These values, however, are far below the recommended limits.

Finally, several compounds that are generated during fermentation exert a direct influence on the aroma and taste of *kefir*, such as lactic acid, acetic acid, pyruvic acid, hippuric acid, propionic acid, butyric acid, diacetyl and acetaldehyde[36].

Microbiological composition of *kefir*

The microbiota present in *kefir* and its grains include numerous bacterial species from lactic acid and acetic acid groups, yeasts and filamentous fungi, which develop complex symbiotic associations[3]. In this relationship, yeasts produce vitamins, amino acids and other essential growth factors that are important for bacteria. Likewise, the metabolic products of bacteria are used as an energy source for the yeasts. This symbiosis allows the maintenance of stability, so that throughout the fermentation cycle, the microbiological profile of *kefir* grains and *kefir* remains unaltered, despite variations in the quality of the milk, microbial contamination, presence of antibiotics and other inhibitory substances[24].

The identification of microbiota present in *kefir* and its grains is important since it is directly related to the quality of the probiotic product[37]. Different methodologies have been applied to study the microbiota of *kefir*; however, the classical approach of culturing micro-organisms in nutrient media (universal and selective) and identification of isolated cultures is still being performed[38]. Nowadays, the understanding of microbial ecology of foods has dramatically changed. The use of a combined approach using culture-dependent and culture-independent methods, such as functional genomics, transcriptomics, proteomics and metabolomics, are encouraged to understand the behaviour of micro-organisms in foods[39]. Using culture-independent methods, including metagenomics, has allowed characterisation of a number of previously unknown micro-organisms in *kefir*[40]. In particular, analysis of the 16S rRNA gene libraries and/or molecular techniques such as denaturing gradient gel electrophoresis are very useful to evaluate and understand the complex microbial populations and diversity of strains from the probiotic *kefir*[41].

The microbial diversity of *kefir* described in the literature varies greatly. In our review, we present a complete description of the bacteria and yeasts that have been identified in *kefir* to date (Table 1). The number of different microbial species in *kefir* is estimated to be more than 300. The microbial composition of *kefir* also varies according to microbiological culture medium, origin of *kefir* grains, different techniques employed during processing, different room temperatures, type and composition of milk used, storage conditions of *kefir* and *kefir* grains. Additionally, the amount of grain added to the milk, agitation and incubation temperature can influence the extent of acidification and consequently the microbiological composition of the final fermented milk. Witthuhn *et al.*[42] observed that the population of bacteria in *kefir* may vary from $6{\cdot}4\times10^4$ to $8{\cdot}5\times10^8$ CFU/g and yeasts from $1{\cdot}5\times10^5$ to $3{\cdot}7\times10^8$ CFU/g. After 24 h of fermentation, *kefir* presented 10^8 CFU/ml of *Lactobacillus*, 10^5 CFU/ml of *Lactococcus*, 10^6 CFU/ml of yeasts and 10^6 CFU/ml of acetic acid bacteria[43].

According to Lopitz-Otsoa *et al.*[2], the microbial composition of *kefir* grains comprised 65 to 80 % of *Lactobacillus* and *Lactococcus* and the remaining portion was completed by yeasts.

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Table 1. Species found in the microbiota of *kefir* and its grains

Species	References
Lactobacilli	
Lactobacillus acidophilus	Santos *et al.* (2003)[93]; Taş *et al.* (2012)[94]
Lactobacillus brevis	Simova *et al.* (2002)[26]; Mobili *et al.* (2009)[95]
Lactobacillus bulgaricus	Yuksekdag *et al.* (2004)[96]
Lactobacillus casei	Zhou *et al.* (2009)[79]; Magalhães *et al.* (2011)[97]
Lactobacillus crispatus	Garbers *et al.* (2004)[37]
Lactobacillus delbrueckii	Santos *et al.* (2003)[92]; Simova *et al.* (2002)[26]
Lactobacillus fermentum	Angulo *et al.* (1993)[98]; Witthuhn *et al.* (2005)[99]
Lactobacillus fructivorans	Delfederico *et al.* (2006)[100]
Lactobacillus gallinarum	Garbers *et al.* (2004)[37]
Lactobacillus gasseri	Angulo *et al.* (1993)[98]
Lactobacillus helveticus	Simova *et al.* (2002)[26]; Valasaki *et al.* (2008)[101]
Lactobacillus hilgardii	Yoshida *et al.* (1994)[102]
Lactobacillus kefir	Magalhães *et al.* (2011)[97]
Lactobacillus kefiranofaciens	Wang *et al.* (2008)[46]; Leite *et al.* (2012)[45]
Lactobacillus kefirgranum	Takizawa *et al.* (1994)[103]
Lactobacillus mesenteroides	Garbers *et al.* (2004)[37]
Lactobacillus paracasei	Magalhães *et al.* (2011)[97]
Lactobacillus parakefiri	Garrote *et al.* (2001)[104]; Leite *et al.* (2012)[45]
Lactobacillus reuteri	Santos *et al.* (2003)[93], Gao *et al.* (2013)[40]
Lactobacillus reuteri	Taş *et al.* (2012)[94]
Lactobacillus rhamnosus	Delfederico *et al.* (2006)[100]
Lactobacillus viridescens	Angulo *et al.* (1993)[98]
Lactococci	
Lactococcus lactis subsp. *lactis*	Yuksekdag *et al.* (2004)[96]
Lactococcus lactis subsp. *cremoris*	Yuksekdag *et al.* (2004)[96]
Lactococcus lactis subsp. *lactis* biovar diacetylactis	Garrote *et al.* (2001)[104]
Streptococci	
Streptococcus cremoris	Ergullu & Ucuncu (1983)[105]
Streptococcus durans	Yuksekdag *et al.* (2004)[96]
Streptococcus faecalis	Ergullu & Ucuncu (1983)[105]
Streptococcus thermophilus	Yuksekdag *et al.* (2004)[96]; Taş *et al.* (2012)[94]
Acetic acid bacteria	
Acetobacter aceti	Koroleva (1991)[106]
Acetobacter lovaniensis	Magalhães *et al.* (2011)[97]
Acetobacter syzgii	Miguel *et al.* (2010)[107]
Other bacteria	
Bacillus sp.	Angulo *et al.* (1993)[98]
Bifidobacterium bifidum	Taş *et al.* (2012)[94]
Enterococcus durans	Yuksekdag *et al.* (2004)[96]
Escherichia coli	Angulo *et al.* (1993)[98]
Micrococcus sp.	Angulo *et al.* (1993)[98]
Leuconostoc mesenteroids	Witthuhn *et al.* (2005)[99]
Pediococcus acidilactici	Sabir *et al.* (2010)[108]
Pediococcus dextrinicus	Sabir *et al.* (2010)[108]
Pediococcus pentosaceus	Sabir *et al.* (2010)[108]
Yeast	
Brettanomyces anomalus	Wyder & Puhan (1997)[109]
Candida albicans	Angulo *et al.* (1993)[98]
Candida friedricchi	Angulo *et al.* (1993)[98]; Wyder & Puhan (1997)[109]
Candida lipolytica	Witthuhn *et al.* (2005)[99]
Candida holmii	Kumura *et al.* (2004)[110]
Candida inconspicua	Simova *et al.* (2002)[26]
Candida kefir	Wyder (2001)[111]
Candida krusei	Witthuhn *et al.* (2005)[99]
Candida lambica	Witthuhn *et al.* (2005)[99]
Candida maris	Simova *et al.* (2002)[26]
Candida pseudotropicalis	Ottogali *et al.* (1973)[112]
Candida tannotelerans	Dousset & Caillet (1993)[113]
Candida tenuis	Ottogali *et al.* (1973)[112]
Candida valida	Dousset & Caillet (1993)[113]
Cryptococcus humicolus	Witthuhn *et al.* (2005)[99]
Debaryomyces hansenii	Loretan *et al.* (2003)[114]
Issatchenkia occidientalis	Latorre-García *et al.* (2007)[115]
Kazachstania aerobia	Magalhães *et al.* (2011)[97]
Kluyveromyces lactis	Latorre-García *et al.* (2007)[115]
Kluyveromyces marxianus	Taş *et al.* (2012)[94]
Kluyveromyces lactis	Magalhães *et al.* (2011)[97]
Lachancea meyersii	Magalhães *et al.* (2011)[97]
Pichia fermentas	Wang *et al.* (2008)[46]
Saccharomyces cerevisiae	Zhou *et al.* (2009)[79]
Saccharomyces delbruecki	Pintado *et al.* (1996)[116]
Saccharomyces exiguus	Latorre-García *et al.* (2007)[115]
Saccharomyces fragilis	Motaghi *et al.* (1997)[117]
Saccharomyces humaticus	Latorre-García *et al.* (2007)[115]
Saccharomyces lactis	Motaghi *et al.* (1997)[117]
Saccharomyces lipolytic	Garrote *et al.* (1997)[22]
Saccharomyces turicensis	Wyder (2001)[111]; Wang *et al.* (2008)[46]
Saccharomyces unisporus	Wyder (2001)[111]; Latorre-García *et al.* (2007)[115]
Torulopsis holmii	Wyder (2001)[111]
Torulospora delbrueckii	Loretan *et al.* (2003)[114]
Zygosaccharomyces sp.	Wyder (2001)[111]; Witthuhn *et al.* (2005)[99]
Weissella	Gao *et al.* (2013)[40]
Yarrownia lipolytica	Wyder (2001)[111]; Angulo *et al.* (1993)[98]

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Hallé *et al.*[44] found that 80 % of *Lactobacillus* belonged to *Lactobacillus kefiri* and the remaining 20 % belonged to *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum* and *Lactobacillus kefiranofaciens*.

The diversity of yeasts present in *kefir* can also be assessed using culture-dependent and culture-independent methods. Yeast species such as *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Candida kefyr*, *Kluyveromyces marxianus* subsp. *marxianus*, *Torulaspora delbrueckii*, *Pichia fermentans*, *Kazachastania aerobia*, *Lachanceae meyersii*, *Yarrowia lipolytica* and *Kazachstania unispora* are present in *kefir* and *kefir* grains in greater numbers[45,46].

Kefir consumption

In Russia, USA, Japan and Central and Northern Europe, *kefir* has been used in the control of many diseases due to its nutritional and therapeutic aspects. Recently, a new formulation of *kefir* with the addition of enzymes such as lipase or α-amylase to prevent and control obesity was patented in Japan. In the former Soviet Union countries, the consumption of *kefir* has been informally recommended for healthy individuals to reduce the risk of chronic diseases and also for patients with gastrointestinal and metabolic disorders, hypertension, IHD, weight control and allergies[47].

The industrial production of *kefir* is large in Germany, Austria, France, Luxembourg, Norway, Switzerland, Czech Republic, Slovakia, Poland and Israel. In Brazil, the consumption of *kefir* occurs domestically with spontaneous fermentation of *kefir* grains in milk, without the control of the time or temperature of fermentation. The consumption and industrial production of *kefir* on a larger scale are lacking so far.

Health effects of *kefir*

Kefir has a wide spectrum of important health benefits, including physiological, prophylactic and therapeutic properties. These effects are a result of a wide variety of bioactive compounds produced during the fermentation process and the highly diverse microbiota, which act either independently or synergistically to influence these health benefits[48]. Therefore, the main studies reporting the beneficial effects of *kefir* on animal models and human subjects in the last 10 years, besides those described throughout this review, are presented in Tables 2 and 3. A schematic diagram of the potential beneficial effects of *kefir* on human physiology and health is shown in Fig. 3.

Effect of kefir *on lactose intolerance*

Milk and dairy products contain high concentrations of lactose. Intestinal absorption of lactose requires hydrolysis of this disaccharide and its subsequent absorption in the small-intestinal mucosa. However, a significant proportion of the world population demonstrates limitations in the digestion of lactose due to insufficient activity of intestinal β-galactosidase[49]. This enzyme, naturally present in *kefir* grains, reduces lactose content of the *kefir* during fermentation, which in turn makes the final product suitable for individuals with lactose intolerance[36]. Moreover, fermented products such as *kefir* are characterised by a delayed gastric emptying, which helps in lactose digestion. Hertzler & Clancy[8] found that the consumption of *kefir*, which is similar to yogurt, was able to improve lactose digestion and tolerance in healthy adult subjects clinically diagnosed with lactose intolerance. In this study, yogurt and *kefir* were similarly able to reduce the severity of flatulence related to milk by 54 % to 71 %. According to Alm[50], after the fermentation period, *kefir* has a reduction of 30 % in the content of lactose, compared with the unfermented milk, providing better comfort for individuals with lactose intolerance. In addition, enzymes released from the lysed micro-organisms may aid in lactose digestion in the gut in a similar manner to most probiotic preparations containing LAB. It is important to note that there are only a few studies on *kefir* concerning lactose intolerance, and more work is needed to better understand the effects of *kefir* consumption and its possible effectiveness in reducing the unpleasant symptoms of lactose intolerance in humans. The amount and regularity of consumption of *kefir* to perform these desirable effects should also be studied.

Antimicrobial properties of kefir

Studies of the early twentieth century observed that the positive effect on life expectancy of regular consumption of yogurt containing lactic acid-producing micro-organisms was due to the existing competition between LAB and harmful pathogens. Since then, antifungal and antibacterial activities of probiotics like *kefir* have been extensively studied[2].

Antibacterial properties of *kefir* are related to a combination of several factors, including competition for available nutrients and the inherent action of organic acids, H_2O_2, acetaldehyde, CO_2 and bacteriocins produced during the fermentation process[51]. These substances also exhibit some effects similar to those of nutraceuticals, preventing gastrointestinal disorders and vaginal infections[36].

Kefir exerts bactericidal effects on Gram-negative bacteria; however, it is more potent against Gram-positive bacteria[52]. This antagonistic action has been observed against bacteria, such as *Salmonella*[53], *Shigella*, *Staphylococcus*[9,53], *Helicobacter pylori*[54], *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Bacillus subtilis*, *Micrococcus luteus*[55], *Listeria monocytogenes*, *Streptococcus pyogenes* and also against the yeast *Candida albicans*[9].

Silva *et al.*[6] reported antimicrobial activity of *kefir* against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella sonnei*. Ulusoy *et al.*[56] observed that *kefir* produced from lyophilised commercial grain (PROBAT KC3; Danisco) presented antibacterial effect against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Escherichia coli*. The results were comparable with the antibacterial action of ampicillin and gentamicin.

Kefir grains have shown to have higher antibacterial activity than *kefir*. This is observed especially against Gram-positive cocci, including staphylococci and Gram-positive bacilli[1]. The antifungal and antibacterial activities may explain the wide use

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Table 2. Health benefits of milk *kefir* in animal studies

Animals	Research purpose	Dose	Duration	Effects	Reference
Swiss mice	To evaluate the immunomodulatory properties of *Lactobacillus kefiri* strains isolated from *kefir* grains	10^8 CFU/d	1 and 3 weeks	*L. kefiri* was able to down-regulate expression of proinflammatory mediators and increase anti-inflammatory molecules in the gut immune system	Carasi *et al.* (2015)[118]
Wistar rats	To investigate the protective effect of *kefir* milk on ethanol-induced gastric ulcers in γ-irradiated rats	5 ml/kg wt per d	18 d	The *kefir* milk prevented ethanol-induced gastric ulcer in γ-irradiated rats. This effect could be attributed to its antioxidant, anti-apoptotic and radioprotective activities	Fahmy & Ismail (2015)[119]
Sprague–Dawley rats	To investigate the effect of *kefir* on ischaemia–reperfusion injury in an animal model	2 x 10^9/kg wt per d	60 d	The *kefir* showed a protective effect in tissue and serum functions	Yener *et al.* (2015)[120]
Sprague–Dawley rats	To investigate the effect of *kefir* on osteoporosis prophylaxis in an ovariectomised rat model	164, 328 and 656 mg/kg wt per d	12 weeks	*Kefir* consumption by female ovariectomised rats prevented oestrogen deficiency-induced bone loss	Chen *et al.* (2015)[121]
Wistar rats	To evaluate the effects of *kefir* on oxidative stress in diabetic animals	1·8 ml/d	8 weeks	*Kefir* reduced the progression of hyperglycaemia and oxidative stress in rats	Punaro *et al.* (2014)[122]
Wistar rats	To evaluate the effects of *kefir* on nicotine cessation-induced anxiety, depression and cognition impairment, in an animal model	5 mg/kg wt per d	7 d	*Kefir* had a potential effect on the treatment of nicotine cessation-induced depression, anxiety and cognition impairment in rats	Noori *et al.* (2014)[123]
Sprague–Dawley rats	To evaluate the effect of low-dose aspirin and *kefir* on arterial blood pressure and renal apoptosis in rats fed with a salt diet	10·0 % (w/v)	4 weeks	*Kefir* and low-dose aspirin used independently protected renal function in rats fed with the salt diet	Kanbak *et al.* (2014)[124]
Ob/Ob mice	To evaluate the effects of *kefir* on the hepatic lipid metabolism of mice	140 mg/kg wt per d	4 weeks	*Kefir* consumption resulted in inhibition of the hepatic lipogenesis pathway rather than the promotion of lipid utilisation. *Kefir* had a protective effect in fatty liver models	Chen *et al.* (2014)[125]
Sprague–Dawley rats	To evaluate the potential use of *Lactobacillus plantarum* (Lp09 and Lp45), obtained from *kefir* grains, as cholesterol-reducing probiotics in rats	2 ml/d (10^9 CFU/ml)	4 weeks	The strains presented the potential for the management of hypercholesterolaemia	Huang *et al.*(2013)[126]
Sprague–Dawley rats	To evaluate the hypocholesterolaemic activities of *Lactobacillus plantarum* (Lp27) isolated from *kefir* grains in rats fed with a high-cholesterol diet	2 ml/d (10^9 CFU/ml)	4 weeks	The strain exhibited efficient cholesterol-reducing ability *in vivo*	Huang *et al.*(2013)[127]
Sprague–Dawley rats	To evaluate the functional properties of *Lactobacillus acidophilus* LA15, *L. plantarum* B23 *and L. kefiri* D17 isolated from *kefir* grains in rats fed with a high-cholesterol diet	2 ml/d (10^9 CFU/ml)	4 weeks	The strains could be used in cholesterol control	Zheng *et al.*(2013)[128]
Germ-free mice	To investigate the effects of *Lactobacillus kefiranofaciens* M1, isolated from *kefir* grains, in mice	2 × 10^8 CFU/mouse	14 d	*L. kefiranofaciens* M1 was able to act directly on the mouse host without intestinal microbiota and enhance immunoregulation and intestinal functionality	Chen & Chen (2013)[129]
BALB/c mice	To investigate the effects of *Lactobacillus kefiranofaciens* M1, isolated from *kefir* grains, on enterohaemorrhagic *Escherichia coli* infection	2 × 10^8 CFU/mouse per d	7 d	*The L. kefiranofaciens* M1 was able to enhance mucosal immunity and prevent or reduce the infection severity	Chen *et al.* (2013)[130]
C75BL/6 mice	To study the ability of *kefir* to protect mice from *Giardia intestinalis* infection	*Kefir*:drinking water 1:100 *ad libitum*	14 d	*Kefir* reduced *G. intestinalis* infection and promoted the activation of different mechanisms of humoral and cellular immunity that are down-regulated by parasitic infection	Franco *et al.* (2013)[131]
C57BL/6 mice	To evaluate the effect of *Lactobacillus kefiranofaciens* M1 from *kefir* in dextran sodium sulfate-induced colitis mice	10^7–10^8 CFU/d	14 d	*L. kefiranofaciens* M1 showed anticolitis and anti-inflammatory effects	Chen *et al.* (2012)[132]
db/db mice	To investigate the effect of *kefir* on blood glucose levels in diabetic mice	10 ml/kg body wt	45 d	*Kefir* decreased blood glucose, serum TAG and NEFA concentrations in diabetic mice after 45 d of administration	Lee *et al.* (2011)[133]
BALB/c mice	To evaluate the effect of the oral administration of kefiran on the balance of immune cells in mice	300 mg/l	0, 2 and 7 d	Kefiran can modify the balance of immune cells in the intestinal mucosa	Medrano *et al.* (2011)[134]
BALB/c mice	To evaluate the oral administration of *kefir* and a *kefir* cell-free fraction in a breast tumour model	3·1 ml/d	2 or 7 d	The anti-tumour effect was observed in mice administered *kefir* or the *kefir* cell-free fraction	de Moreno de LeBlanc *et al.* (2007)[87]
Wistar rats	To determine the effects of *kefir*-supplemented diet on enzymes and proteins present in the rat intestine	4·2 g/kg wt	22 d	The addition of *kefir* into a normal diet was able to increase the activity of the intestinal dipeptidase and decrease the intestinal sugar Na^+-dependent uptake. Thus, *kefir* could benefit protein digestion and reduce glycaemic index	Urdaneta *et al.* (2007)[27]
BALB/c mice	To characterise the immunomodulating capacity of two fractions of *kefir*	200 ml/d	2, 5 or 7 consecutive days	*Kefir* induced a mucosal response, maintaining the intestinal homeostasis	Vinderola *et al.* (2006)[135]
Wistar rats	To evaluate the antimicrobial and cicatrising activities of *kefir* and kefiran	70 % *kefir* gel	7 d	Both *kefir* and kefiran showed antimicrobial activity and enhanced wound healing	Rodrigues *et al.* (2005)[9]
BALB/c mice	To determine the immunomodulating capacity of *kefir* on the intestinal mucosal immune response	*Ad libitum*	2, 5 or 7 consecutive days	*Kefir* and pasteurised *kefir* were able to modulate the mucosal immune system in a dose-dependent manner	Vinderola *et al.* (2005)[81]

CFU, colony-forming unit.

Table 3. Health benefits of milk *kefir* in human studies

Volunteers	Research purpose	Dose	Duration	Effects	Reference
Diabetic patients (*n* 60)	To determine the effect of *kefir* on glucose and lipid profile control in patients with type 2 diabetes mellitus	600 ml/d	8 weeks	The *kefir* decreased the fasting blood glucose and HbA1C levels and can be useful as a complementary or adjuvant therapy for the prevention of diabetes. *Kefir* consumption did not result in lowered plasma lipid concentrations	Osdrahimi *et al.* (2015)[73]
Healthy overweight or obese premenopausal women (*n* 75)	To compare the potential weight-reducing effects of *kefir* and milk in a dairy-rich non-energy-restricted diet in overweight or obese premenopausal women	Two servings/d of milk or *kefir*	8 weeks	The *kefir* drink led to a similar weight loss compared with low-fat milk	Fathi *et al.* (2015)[136]
Patients with functional constipation (*n* 20)	To evaluate the effects of *kefir* on the symptoms, colonic transit and bowel satisfaction scores of patients with chronic constipation	500 ml/d	4 weeks	*Kefir* had positive effects on the constipation symptoms	Turan *et al.* (2014)[137]
Healthy volunteers (*n* 22)	To evaluate if *kefir* can be considered an alternative to fluoride rinse	100 ml/d	2 weeks	The *kefir* drink could inhibit salivary mutans streptococci as well as the sodium fluoride rinse. *Kefir* may be used in caries control strategies adjunctively	Ghasempour *et al.* (2014)[138]
Healthy volunteers (*n* 18)	To evaluate the influence of *kefir* consumption on inflammatory markers in healthy adults	200 ml/d	6 weeks	*Kefir* was able to control the inflammatory response	Adiloğlu *et al.* (2013)[80]
Patients with dyspepsia (*n* 82)	To evaluate the effect of *kefir* conjugated triple therapy for *Helicobacter pylori* eradication	250 ml of *kefir* twice daily	2 weeks	*Kefir* improved the efficacy and tolerability of triple therapy in eradicating *Helicobacter pylori*	Bekar *et al.* (2011)[139]
Healthy children (*n* 125)	To examine the role of *kefir* in preventing antibiotic-associated diarrhoea	150 ml/d	2 weeks	*Kefir* did not prevent antibiotic-associated diarrhoea	Merenstein *et al.* (2009)[140]
Patients with colorectal cancer (*n* 40)	To investigate the effect of *kefir* consumption on mucositis induced by fluorouracil-based chemotherapy	250 ml twice per d	On the first 5 d of each chemotherapy cycle	*Kefir* consumption had no effect on serum proinflammatory cytokine levels and on the incidence of mucositis development in cancer patients	Topuz *et al.* (2008)[141]
Healthy mildly hypercholesterolaemic male subjects (*n* 13)	To determine whether *kefir* supplementation would alter plasma lipids in mildly hypercholesterolaemic men		4 weeks	*Kefir* consumption did not result in lowered plasma lipid concentrations	St-Onge *et al.* (2002)[72]

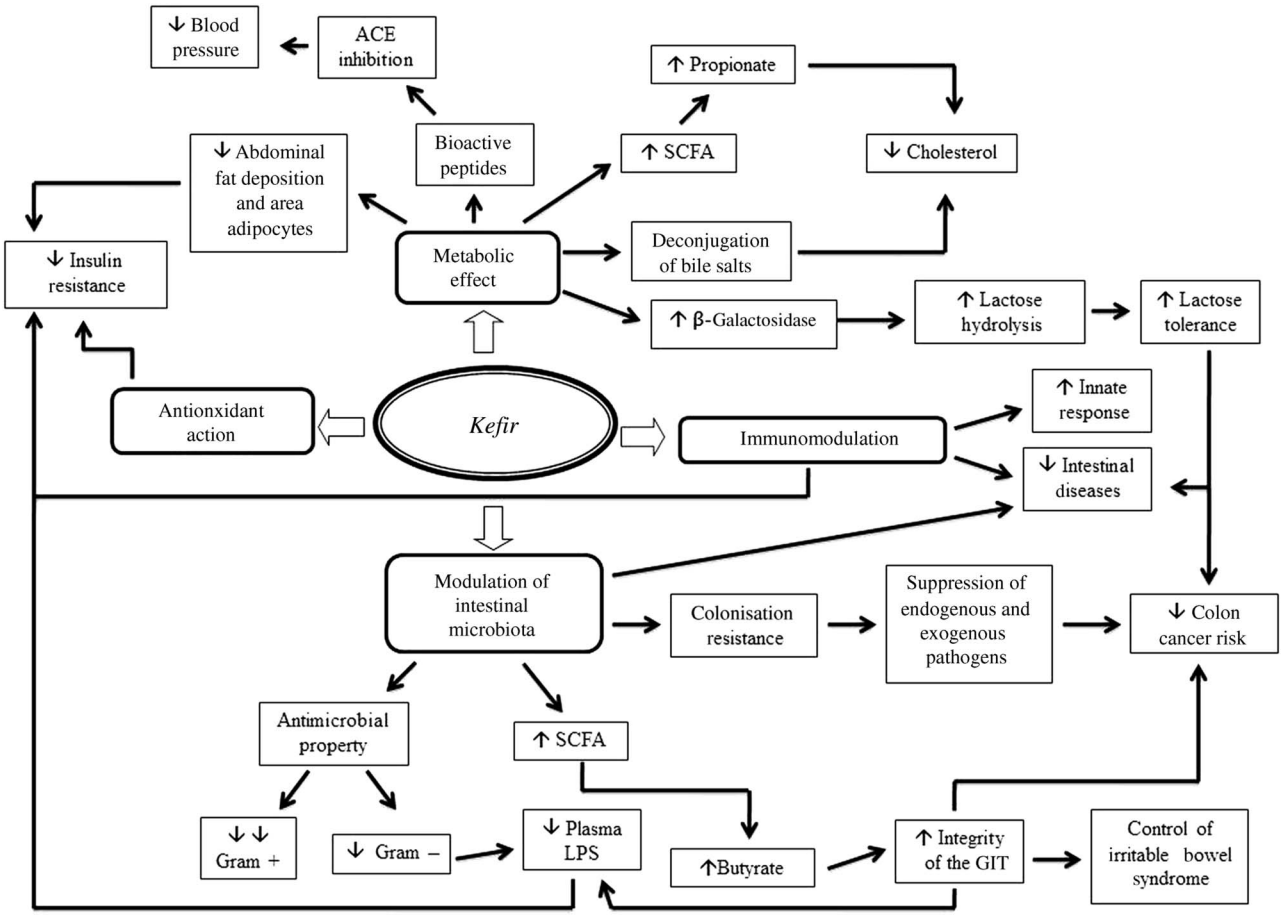

Fig. 3. Schematic diagram of the beneficial physiological effects of *kefir* on human health. ACE, angiotensin-converting enzyme; LPS, lipopolysaccharide; GIT, gastrointestinal tract.

of *kefir* in the prevention of infectious diseases and tumour development[57].

According to Brialy *et al.*[58], fresh *kefir* presented an intrinsic inhibitory potential against *Staphylococcus aureus*, *Kluyveromyces lactis* and *Escherichia coli*. However, this effect was not verified against *Saccharomyces cerevisiae* and *Candida albicans*. *Kefir* has been shown to lose its intrinsic inhibitory effect after lyophilisation and re-constitution in distilled water or milk.

In an *in vitro* study, Ismaiel *et al.*[59] tested the antimicrobial activity of *kefir* grains and *kefir* suspension against several species of bacteria and fungi and observed higher inhibitory action against *Streptococcus faecalis* and *Fusarium graminearum*. The concentration of *kefir* from 7 to 10% (w/w) was able to completely inhibit the sporulation of *Aspergillus flavus*, and consequently the production of aflatoxin B1, which demonstrates the antifungal properties of *kefir* against filamentous fungi. The organic acids produced during fermentation of *kefir* can change the molecule aflatoxin B1, by converting it into less toxic forms such as aflatoxicol aflatoxin B and B2a[60] In this context, *kefir* appears as a safe alternative for food preservation, providing protection against poisoning from aflatoxin B1.

Additionally, *kefir* was able to increase the population of LAB and reduce the levels of *Enterobacteriaceae* and *Clostridium* in the intestinal mucosa of mice[61]. Oral administration of milk *kefir* or soya milk *kefir* in mice over a period of 28 d was able to significantly increase *Lactobacillus* and *Bifidobacterium* while reducing *Clostridium perfringens* in animal faeces[62].

Thus, the antimicrobial activity of *kefir* is differentiated when the fermented milk is used in the reconstituted or liquid form, and also when *kefir* grains are used. However, it is noted that both *kefir* grains and fermented milk constitute interesting alternatives that can be used for the prevention of some infections, especially those involving the gastrointestinal tract. We emphasise that it is necessary to perform *in vivo* studies to investigate the antimicrobial properties of *kefir*, especially with regard to the reduced infection rates and severity of symptoms with *kefir* consumption in animal and human studies, since the results of *in vitro* studies conducted until now show promising results.

Hypocholesterolaemic effect of kefir

The consumption of probiotic dairy products has been proposed as a strategy to reduce levels of circulating cholesterol. Guo *et al.*[63] in a meta-analysis with thirteen trials, including 485 participants with high, borderline high and normal cholesterol levels, observed that the consumption of probiotic dairy products was able to lower serum cholesterol (mean net change of 6·40 mg/dl; 0·17 mmol/l), LDL-cholesterol (mean net change

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of 4·90 mg/dl; 0·13 mmol/l) and TAG (mean net change of 3·95 mg/dl; 0·04 mmol/l) concentrations. Some mechanisms are proposed to justify these findings:

(1) The LAB inhibit the absorption of exogenous cholesterol in the intestine due to binding and incorporation of cholesterol by the bacterial cells. The high count of LAB present in *kefir* may directly or indirectly reduce cholesterol in the medium by up to 33 %[64]. Vujičić *et al.*[65] verified that after 24 h of fermentation, *kefir* cultures were able to absorb from 28 to 65 % of the cholesterol present in the culture medium.
(2) Probiotic bacteria increase the production of SCFA. Among the different SCFA produced, propionate reduces the production of cholesterol by inhibiting hydroxymethylglutaryl CoA (HMG-CoA) reductase activity. Additionally, plasma cholesterol is redistributed to the liver, where the synthesis and secretion of bile acids are increased, since the activity of the 7α-hydrolase enzyme is stimulated. Moreover, propionate inhibits the intestinal expression of genes involved in the biosynthesis of cholesterol[66].
(3) Another possible pathway involves the deconjugation of bile acids, which may be increased in the large intestine, caused by the bile salt hydrolase (BSH) enzyme. The BSH enzyme catalyses the hydrolysis of glycine and/or taurine conjugated to the bile salts in residual amino acids and free bile salts, increasing excretion. With the increasing excretion of bile salts, fewer of them are carried back to the liver by the enterohepatic circulation, which increases the demand for cholesterol for *de novo* synthesis of bile salts in the liver. Thus, the liver increases the hepatic uptake of LDL from the circulation[67] which leads to the reduction of serum LDL-cholesterol concentrations.

Some animal studies have demonstrated the hypocholesterolaemic effect of *kefir*[12,68]. Hamsters fed a hypercholesterolaemic diet supplemented with freeze-dried *kefir* (milk or soya milk) showed a significant reduction in TAG concentration and in the atherogenic index[69]. In this study, the effects were partially related to increased faecal excretion of neutral sterols and bile acids. Also, *Lactobacillus plantarum* MA2 isolated from *kefir* grains originated from Tibet was effective in reducing plasma and liver cholesterol and TAG concentrations. This micro-organism was also able to increase faecal excretion of cholesterol and TAG in mice fed a high-fat diet[70].

Animals that consumed hyperlipidaemic diets associated with kefiran showed a reduction in serum total cholesterol, LDL-cholesterol and TAG concentrations, as well as a reduction in liver cholesterol and TAG concentrations compared with controls[12]. Uchida *et al.*[71] evaluated the anti-atherogenic effect of kefiran in rabbits fed a high-cholesterol diet and observed lower atherosclerotic lesion in the abdominal aorta and lower concentrations of hepatic cholesterol and lipid peroxidation in the animals fed kefiran in comparison with the control group.

The consumption of *kefir* (0·5 litres/d) by hypercholesterolaemic adult men for 4 weeks did not affect the circulating concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol or TAG; however, it increased the SCFA concentrations in their faeces[72]. Ostadrahimi *et al.*[73] conducted a double-blind randomised placebo-controlled clinical trial with diabetic patients, who consumed 600 ml of *kefir* daily for 8 weeks. *Kefir* consumption was not able to influence serum TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol levels compared with the control, showing that *kefir* was unable to reduce plasma lipids in diabetic patients.

The benefits of *kefir* in lowering cholesterol have shown conflicting results. Such inconsistent results may be due to different experimental protocols used, origin of the grains, and fermentation conditions of *kefir*, and consequently the variety of *kefir* composition. This scenario may be related to lack of standardisation in nutritional and microbiological composition of *kefir* used in scientific research.

Control of plasma glucose by kefir

The regular consumption of probiotics has the ability to improve blood sugar levels. This effect has been attributed mainly to the probiotic ability to positively modulate the composition of the intestinal microbiota and hence reduce intestinal permeability, oxidative stress and inflammation[74].

Similar effects can be observed with regular consumption of *kefir*. Hadisaputro *et al.*[11] evaluated the effect of *kefir* consumption for 30 d in controlling glycaemia in Wistar rats induced to diabetes mellitus by administration of streptozotocin. *Kefir* supplementation was able to reduce plasma glucose compared with the control group.

In a clinical trial, diabetic adults that consumed 600 ml/d *kefir*, for 8 weeks, showed a significant decrease in fasting glucose levels and glycosylated Hb compared with baseline. Additionally, these same parameters were found to be significantly reduced in individuals who consumed *kefir* compared with control subjects who consumed a conventional fermented milk[73].

The regular intake of probiotics can reduce the amount of Gram-negative bacteria in the intestinal lumen, and therefore lower the amount of lipopolysaccharide (LPS). In addition, probiotics can improve intestinal barrier function leading to the reduction of intestinal permeability. The absorption of lower amounts of LPS may therefore diminish the low-grade chronic inflammatory process characteristic of diabetes. Moreover, lower LPS may restore the function of insulin receptors leading to a better control of blood glucose. We can thus conclude that *kefir* might be used in the prevention of diabetes; however, more studies are needed to demonstrate such effects.

Anti-hypertensive effect of kefir

Some evidence indicates that probiotic bacteria or their fermented products play an important role in controlling blood pressure. The anti-hypertensive effects have been observed in experimental and clinical studies[75], although the data are limited and controversial.

Quirós *et al.*[76] found that *kefir* is able to inhibit the activity of angiotensin-converting enzyme (ACE) through the action of bioactive peptides generated from casein during the milk fermentation process. According to Maeda *et al.*[12], the antihypertensive activity observed in their study was due to the ability of kefiran to inhibit ACE activity.

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The ACE-inhibitory peptides inhibit the production of the vasoconstrictor angiotensin I, and consequently the production of aldosterone, a hormone that stimulates the increase of serum Na concentration, causing an increase in blood pressure. Additionally, ACE-inhibitory peptides also inhibit the breakdown of bradykinin, a hormone that has vasodilating action, contributing to the decrease in blood pressure[77].

Experimental and especially clinical studies that have evaluated the antihypertensive effect of milk *kefir* are rare in the literature to date. Furthermore, the milk *kefir* peptides that exhibit the ability to inhibit ACE action have not yet been identified.

Anti-inflammatory properties of kefir

The inflammatory state is associated with the development of some chronic diseases such as obesity, diabetes and cancer[78]. Therefore, the number of studies that have evaluated the immunomodulatory properties of probiotics is increasing.

The immunomodulatory properties of *kefir* may result from direct action of the microbiota or may be indirect, through different bioactive compounds produced during the fermentation process[79]. The bioactive peptides, produced during milk fermentation by the microbiota present in *kefir*, are able to activate macrophages, increase phagocytosis, suppress the Th2 immune response, increase the production of NO and cytokines, and stimulate the secretion of IgG and IgA by B lymphocytes in the intestinal lumen[80]. According to Vinderola *et al.*[81], these bioactive compounds are able to promote the cell-mediated immune response against infections and intracellular pathogens[57].

The anti-inflammatory potential of *kefir* was evaluated in an animal model of asthma, sensitised with ovalbumin. The administration of *kefir* (50 mg/kg) was found to significantly inhibit the total number of inflammatory cells and eosinophils in the bronchoalveolar fluids. In addition, *kefir* administration decreased IL-4, IL-13 and IgE to a normal level[13]. Thus, *kefir* has therapeutic potential for the prevention of allergic bronchial asthma.

Rodrigues *et al.*[9] evaluated the anti-inflammatory action of *kefir* in rats using a protocol of oedema and granuloma induction. In this study, water *kefir*, milk *kefir* and kefiran extract inhibited the inflammatory process by 41, 44 and 34 %, respectively. The treatments also significantly reduced oedema in the animals. The results demonstrate the presence of anti-inflammatory compounds in the symbiotic cultures of *kefir*.

The immunomodulatory effect of *kefir* can be attributed to the ability of this probiotic to decrease or restore intestinal permeability. Thus, the contact between the host and the antigens present in the intestinal lumen is decreased, which in turn can reduce the inflammatory response.

In this situation, *kefir* may be able to reduce intestinal permeability against food-borne antigens. Liu *et al.*[62] observed that animals treated with ovalbumin and consumed milk and soya milk *kefir*, during 28 d, exhibit lower concentrations of IgE and IgG than the control animals. These results suggest the potential of *kefir* in the prevention of food allergy and in the improvement of mucosal resistance against pathogen infection.

The effect of *kefir* is not restricted to the modulation of the immune system in the gastrointestinal tract but goes far beyond it. Such effect is a consequence of the micro-organisms and bioactive compounds present in *kefir*, which positively modulate the composition of the intestinal microbiota and consequently the immune system of the host.

Antioxidative activity of kefir

Harmful biological effects of reactive oxygen species *in vivo* are controlled by a broad spectrum of antioxidant defence mechanisms, including dietary compounds and enzymes with antioxidant activity.

According to Güven *et al.*[25], in a toxicity test with carbon tetrachloride (CCl_4) in rodents, *kefir* exerted a higher antioxidant effect than vitamin E. Additionally, Ozcan *et al.*[82] evaluated the effect of *kefir* supplementation in rodents induced to oxidative stress by the use of Pb. After 6 weeks of treatment, the consumption of *kefir* increased glutathione peroxidase and reduced malondialdehyde to levels comparable with those of the non-induced group. The results support the hypothesis that *kefir* is a potential tool in the control of oxidative stress.

Liu *et al.*[83] evaluated the antioxidant activity of *kefir* prepared from goat and cow milk. The authors reported the great ability of *kefir* to bind the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and superoxide radicals, besides the inhibition of linoleic acid peroxidation. In this situation, the antioxidative activity of *kefir* can reduce DNA damage, which explains its anticarcinogenic potential[84].

It is known that the increased concentration of free radicals has a strong relationship with an increased risk of chronic diseases. Therefore, *kefir* consumption should be encouraged since it is a natural source of antioxidant compounds and also stimulates the activity of enzymes of the antioxidant system.

Anticarcinogenic activity of kefir

The regular consumption of *kefir* is able to positively modulate the composition of the intestinal microbiota and immune system of its host. Therefore, it is believed that this fermented milk may play an important role in the modulation of carcinogenesis.

Hosono *et al.*[85] observed that all bacterial strains isolated from *kefir* had a remarkable ability of binding to mutagens (>98·5 %), which could be further eliminated with the faeces, protecting the colonocytes from damage. Also, Khoury *et al.*[86] showed the ability of *kefir* to inhibit proliferation and induce apoptosis in HT 29 and Caco 2 colorectal cancer cells. Here, *kefir* was able to induce cell cycle arrest at the G1 phase, decrease mRNA expression of transforming growth factor-α (TGF-α) and transforming growth factor-β1 (TGF-β1) in HT 29 cells, and to up-regulate protein expression of Bax:Bcl-2 ratio and p53-independent-p2, indicating its pro-apoptotic effect *in vitro*. Therefore, the regular consumption of *kefir* can decrease the risk for colon cancer development; however, more studies are needed to understand the mechanisms of action involved in this process.

Also, in a murine breast cancer model, de Moreno de Leblanc *et al.*[87] showed that the antitumour effect of *kefir* was related

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to the immune response in mammary gland of mice. Additionally, oral administration of milk and soya milk *kefir* in mice inoculated with sarcoma 180 ascites tumour resulted in inhibition of 64·8 and 70·9 % of tumour growth, respectively, compared with administration of unfermented milk. Furthermore, *kefir* was able to induce cell lysis by apoptosis and increase levels of IgA in the intestinal mucosa of animals after 30 d of consumption. These data suggest that *kefir* is a promising probiotic in cancer prevention[57].

The reduction in cancer risk can also be attributed to the presence of some polysaccharides and bioactive compounds, such as specific proteins and peptides, present in *kefir*. In this way, water-soluble polysaccharides of *kefir* grains showed a protective effect against pulmonary metastasis, whereas the water-insoluble polysaccharide fraction inhibited melanoma metastasis in mice[2]. Additionally, the bioactive compounds of *kefir* can prevent cancer initiation or suppress the initiated tumour growth by hindering certain enzymes, avoiding the conversion of procarcinogens to carcinogens[36].

The antimutagenic activities of milk, yogurt and *kefir* were compared using the Ames test. *Kefir* showed a significant reduction in the mutagenicity induced by methyl methanesulfonate, sodium azide, and aflatoxin B1, while yogurt and milk reduced mutagenicity to a lesser degree. In *kefir*, higher levels of conjugated linoleic acid isomers and butyric, palmitic, palmitoleic and oleic acids were found in relation to milk and yogurt, factors that may have contributed to the reported outcomes[88].

Moreover, *kefir* has shown protective effects against radiation-induced gastrointestinal damage in mice. Diluted *kefir* solutions were able to protect the crypts from radiation and promote crypt regeneration[89]. Matsuu *et al.*[90] also verified that *kefir* protected colonic crypt cells against radiation-induced apoptosis and reduced active caspase-3 expression. Thus, the use of *kefir* may be an alternative to help cancer patients who undergo radiotherapy.

The possible anticancer effect of milk *kefir* can be considered systemic, since its regular consumption has a potential in cancer prevention to influence both the gastrointestinal tract and other organs, such as breasts and lungs. This beneficial effect can be a result of the improvement of gut microbiota and immune system associated with the increased consumption of bioactive compounds produced by the *kefir* microbiota.

Healing action of kefir

Recent studies have explored the beneficial effects of probiotics far beyond the intestine. Some of these novel benefits include healthier skin, improvement of eczema, atopic dermatitis and burns, healing of scars, and rejuvenation[91].

Rodrigues *et al.*[9] tested the scar ability of a 70 % *kefir* and kefiran gel in skin wounds infected with *Staphylococcus aureus* from Wistar rats. The treatment with *kefir* and kefiran for 7 d showed a protective effect on connective tissue, greatly improving tissue healing compared with treatment with 5 mg/kg neomycin–clostebol emulsion.

The healing properties of *kefir* were tested in an animal model with experimental burn and contamination with *Pseudomonas aeruginosa*. *Kefir* grains and gels prepared with *kefir* culture after 24, 48 and 96 h of incubation were evaluated. After 2 weeks of treatment, the wound area and the percentage of inflammation were reduced in animals treated with *kefir* grains and gel compared with those treated with silver sulfadiazine cream, used for the topical treatment of burns of second and third degrees. In addition, the percentage of epithelialisation and healing in animals treated with *kefir* was also improved. The authors concluded that treatment with *kefir* gel was effective in improving outcomes from a severe burn compared with conventional treatment[92].

The ability of *kefir* to heal wounds can result from its antimicrobial and anti-inflammatory activities, which may act synergistically contributing to the healing[92]. Thus, the beneficial health effects provided by *kefir* go far beyond the gastrointestinal tract, contributing to wound healing.

Conclusion

Kefir contains a large variety of beneficial micro-organisms and bioactive compounds, being considered a product with a great potential as a functional food. *Kefir* could be an interesting alternative as a probiotic drink, since it is safe, can be produced at home, has a low production cost, and can be easily incorporated in the diet. The numerous physiological effects described in the literature and highlighted in the present article support the health-promoting benefits of *kefir*. However, many questions still need to be answered. The methodological standardisation of studies constitutes an important step to better understand the physiological benefits of *kefir*. First, it is worth mentioning that the detailed knowledge of *kefir* composition is still scarce, and needs to be characterised for the understanding of the *in vivo* physiological effects and for finding new possibilities for *kefir* application. Second, more animal and human studies demonstrating clear cause and effect of *kefir* consumption and the reduction of disease risk must be performed. Unfortunately, numerous human studies with *kefir* and other probiotics have often been poorly designed, frequently driven by costs rather than scientific need. The sample size and period of time of experiment usually are not coherent with the objectives of the study and the analyses performed to verify changes in the metabolic parameters. Therefore, different study designs of experimental and clinical trials with the use of *kefir* cause difficulties in drawing clear conclusions. There is also a need for good clinical studies targeting specific mechanisms of action to better evaluate and understand the physiological effects of *kefir* as part of a diet. Third, different manufacturing conditions of *kefir* may alter the original characteristics of micro-organisms, which therefore may influence their effects on health. The methods of producing *kefir*, time and temperature of fermentation, type of milk used, different origin of grains, ratio of grains:milk (w/v) and cooling time of the product after fermentation may influence the chemical and microbiological composition of the fermented milk. In this context, it is necessary to better understand the mechanisms of action of *kefir* in oxidative stress, immune-modulatory action, anti-inflammatory properties, modulation of gut microbiota and maintenance of gut integrity, which can have a beneficial effect on attenuation

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or delay of the progression of chronic diseases, and thus positively affect human health.

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