

Review

Kefir as a Functional Beverage Gaining Momentum towards Its Health Promoting Attributes

Vassilios Ganatsios ¹, Poonam Nigam ², Stavros Plessas ³ and Antonia Terpou ^{4,*}

¹ Department of Agricultural Biotechnology and Oenology, International Hellenic University, 66100 Drama, Greece; v.ganatsios@gmail.com

² Biomedical Sciences Research Institute, Ulster University, Coleraine BT52 1SA, Northern Ireland, UK; p.singh@ulster.ac.uk

³ Laboratory of Food Processing, Faculty of Agriculture Development, Democritus University of Thrace, 68200 Orestiada, Greece; splessas@agro.duth.gr

⁴ Department of Agricultural Development, Agri-Food, and Natural Resources Management, School of Agri-Cultural Development, Nutrition & Sustainability, National and Kapodistrian University of Athens, 34400 Psachna, Greece

* Correspondence: aterpou@agro.uoa.gr

Abstract: The consumption of fermented foods posing health-promoting attributes is a rising global trend. In this manner, fermented dairy products represent a significant subcategory of functional foods with established positive health benefits. Likewise, kefir—a fermented milk product manufactured from kefir grains—has been reported by many studies to be a probiotic drink with great potential in health promotion. Existing research data link regular kefir consumption with a wide range of health-promoting attributes, and more recent findings support the link between kefir’s probiotic strains and its bio-functional metabolites in the enhancement of the immune system, providing significant antiviral effects. Although it has been consumed for thousands of years, kefir has recently gained popularity in relation to novel biotechnological applications, with different fermentation substrates being tested as non-dairy functional beverages. The present review focuses on the microbiological composition of kefir and highlights novel applications associated with its fermentation capacity. Future prospects relating to kefir’s capacity for disease prevention are also addressed and discussed.

Keywords: kefir; functional beverage; probiotics; lactic acid bacteria; yeasts; bioactive metabolites; health aspects; antiviral effects



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1. Historical Pathway of Fermented Milk in Human Nutrition

Archaeological evidence indicates that the bioprocess of food fermentation was discovered accidentally thousands of years ago [1–3]. The scientific announcement regarding the importance of microorganisms in food fermentation came more recently, made by Van Leeuwenhoek and Hooke in 1665 [4]. Subsequently, microbes have been applied for food production as one of the most economic methods of food processing and storage. Many different food substrates can be applied to fermentation processes, including milk, meat, seafoods, cereal grains, fruits, vegetables, root crops, and other miscellaneous food compounds. The medicinal and nutritional properties of fermented foods have recently been promoted as more scientific evidence highlights microbial transformation as an approach towards improving food functionality [1,5–7]. Likewise, the role of fermented milk in human nutrition has been well documented, as the virtues of these products have been known to mankind since the time of ancient civilizations [8,9]. However, the scientific community gave impetus to these beliefs almost a century ago, when Élie Metchnikoff suggested the consumption of fermented milk with viable bacteria as a path to prolong life [9,10]. Specifically, Metchnikoff supported that the desirable bacteria in Bulgarian

yogurt could suppress undesirable or disease-causing microorganisms, promoting human health. This observation showed the way for exploring the potential of beneficial bacteria and cultured products in the alleviation of human and animal disorders [1,10]. Likewise, more recent studies have confirmed the ability of probiotic bacteria and their bioactive metabolites to act effectively towards flattening the curve of the COVID-19 pandemic as they exhibit immunomodulatory and antiviral properties [11–14].

There is evidence that fermented milk products can be successfully formulated with probiotic bacteria as key ingredients in developing functional food products that promote health and wellness [1]. The bioprocess of the fermentation raw milk used in the middle ages involved unpredictable and slow souring of milk, achieved through incubation with naturally occurring microorganisms [9]. It is believed that fermented milk products appeared accidentally as milk was stored at room temperature and naturally soured, and there was no knowledge of any exogenous microflora [9,15]. On the other hand, modern bioprocessing involves the manufacturing of various fermented milk products, produced using selected starter cultures [1,16]. Subsequently, novel fermented milk products with enhanced health aspects have overtaken the market through the incorporation of beneficial microbial cultures [1,17]. It is of paramount importance that the microbiota of fermented foods must be kept alive until consumption for the delivery of their health benefits [1]. The main fermented milk products that have overtaken the market may be classified into three different categories: (i) moderately fermented sour types of milk with a pleasant aroma, e.g., cultured milk; (ii) sour and highly sour types of fermented milk, e.g., curds and yoghurt; and (iii) fermented milk enhanced with alcohol or acetic acid in small quantities, e.g., kumiss and kefir [8,9].

Kefir is a fermented milk beverage that has been consumed for thousands of years and has a significant place among functional foods [9,18]. Kefir originates from the northern slopes of the Caucasus Mountains and is believed to date back at least 1000 years [18]. It is believed that kefir grains traditionally passed from generation to generation among the Caucasus tribes and were considered as a source of family wealth [19]. The tribe folk of this region possibly developed kefir through sheer accident and subsequently consumed the nutritious beverage over the subsequent years [19]. Kefir is an acidic, low-alcohol probiotic beverage obtained from the fermentation of milk at ambient temperatures [20]. It is metabolized by a complex mixture of bacteria (including species belonging to *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Acetobacter* genera) and yeasts [18]. In general, we can emphasize the fact that kefir differs from other fermented products as it is produced from kefir grains, which contain a complex symbiotic association (lactic acid- and/or acetic acid-producing bacteria, and lactose-fermenting and non-fermenting yeast), and their microbial ecology depends on the origin and cultivation method of the grains [18,19,21].

2. Health-Promoting Effects of Kefir

Nowadays, consumers are opting for foods with functional properties that may enhance their well-being. These perceptions of consumers towards healthy foods have emerged from the concept of “functional foods” [7]. Public interest towards fermented milk products and beverages containing probiotics has risen over the years along with the increasing demand for safe and quality health-food products, minimizing the demand for expensive food supplements [17]. Following consumer demand, studies have been established aiming to strengthen the fundamental scientific knowledge on kefir and kefir grains and further exploring the possibilities for the production of new consumer-friendly nutritious products [1,18,20]. Moreover, several studies have suggested that beneficial properties can be associated with the regular consumption of kefir. The list of reported health benefits is of high importance, as they include improved digestion, tolerance to lactose, antioxidant activity, antibacterial effects, gut microbiome modulation, control of plasma glucose, alleviation of the effects of obesity, the improvement of cholesterol levels, anti-inflammatory effects, reduced cardiac hypertrophy, and reduced kidney hypertrophy, as well as anti-mutagenic, anti-oxidant, and anti-allergenic activity [19,21,22]. Moreover,

kefir has been reported to provide significant antimicrobial characteristics against various foodborne pathogens, whereas more recently kefir was proven to act as a defence mechanism, sealing the immune system against viral pathogens [1,12,13,21].

2.1. Nutritional Characteristics of Kefir

Kefir is produced traditionally through the fermentation of milk using kefir grains and normally consists of at least 2.7% protein, 0.6% lactic acid, and less than 10% fat, depending on the milk [23]. Kefir beverages can be produced from full-fat, semi-skimmed, and non-fat milk, with low-fat kefir products gaining constant popularity. Kefir was mainly produced via microbial fermentation of cow's milk but currently reports indicate that it can also be prepared using milk from other cattle, such as goats, sheep, buffalo, camels, or even donkeys [19,21]. Small nutritional differences may occur in the final kefir beverage derived from different cattle. In general, the fermentation of milk from any cow results in a slightly acidic, very-low-alcohol milk beverage, which has a distinct flavour, is slightly effervescent, and which can be obtained at ambient temperature [24]. The high nutritional value of kefir beverages occurs as a result of its rich chemical composition that includes proteins, prebiotic oligosaccharides, exopolysaccharides, minerals (Ca, Co, Cu, Fe, Mg, Mo, and Zn), vitamins, fats, and other bioactive metabolites such as bacteriocins [25,26]. In addition, kefir shows an enhanced amino acid profile, being rich in serine, valine, lysine, alanine, phenylalanine, threonine, methionine, tryptophan, and isoleucine in comparison to unfermented milk [21]. Another important aspect is that kefir includes more digestible proteins (e.g., caseins) compared to unfermented milk, whereas hydrolyzation of lactose by β -galactosidase enzymes makes kefir an acceptable product for lactose-intolerant individuals [21,27]. Moreover according to recent studies, the nutritional value of kefir can be enhanced when two different kinds of milk are applied for fermentation, and this technique has been suggested to promote the sensory properties of kefir [21]. Kefir is produced through the fermentative-activity of active "kefir grains" that contain inherent microflora within a polysaccharide matrix of semi-hard granules known as "Kefiran" [18,28]. Kefiran consists of repeated hexasaccharide units and is mainly composed of 85–90% moisture and 10% dry mass. The dry mass includes 57% carbohydrates, 33% proteins, 4% fat, and 6% ash [23,29]. Kefir grains are small, white-to-cream in color, and gelatinous, and particles are usually irregularly shaped (Figure 1). They can be applied as an inoculum or starter culture for milk fermentation [30]. Nowadays, kefir grains are constantly applied in the fermentation of fruits or vegetables, in which grains tend to be colored according to their fermentation substrate [19].

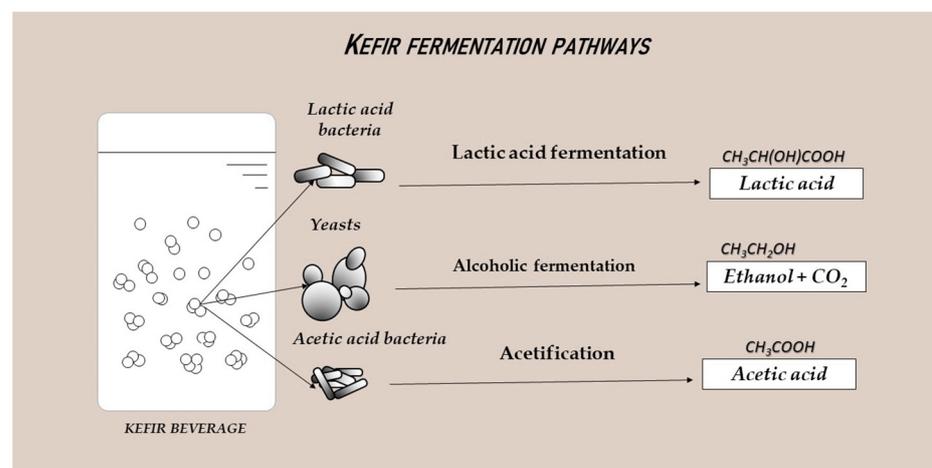


Figure 1. Different fermentation pathways following from the main microbial species (lactic acid bacteria, yeasts, and acetic acid bacteria) contained in kefir and kefir grains.

Kefir grains include a wide range of microflora, mainly bacterial (lactic acid, acetic acid) and yeast strains, which are involved in the milk fermentation bioprocess, being responsible for the product's final quality [6]. The distinctive flavor of the final product has an acidic taste, mainly due to the abundance of lactic acid. The flavor and aroma mainly depend on the microbial group (lactic acid bacteria, acetic acid bacteria, and yeasts) that valorizes the fermentation substrate (milk), providing its primary fermentation metabolites (Figure 1). The abundant lactic acid bacteria contained in kefir grains utilize milk lactose as a fermentable sugar and metabolize it into glucose and galactose. Subsequently, the bacteria metabolize glucose through the homofermentative pathway [25]. In all cases, lactic acid is produced as the primary metabolite, providing a distinctive acidic flavour to the final product (Figure 1). In the cases where acetic acid bacteria are favored during fermentation (e.g., in aerobic conditions), the quality of the final product tends to exhibit a tarter flavor and a slight biting odor due to the accumulation of acetic acid (Figure 1) in these kefir beverages [9,18]. Generally, the final quality of kefir beverages is attributed to the accumulation of primary fermentation components, such as lactic acid, acetic acid, CO₂, ethanol, and secondary metabolites such as acetaldehyde, acetoin, diacetyl, and exopolysaccharides [31].

2.2. Kefir Microbial Diversity and Beneficial Value

Kefir starter cultures are created by growing kefir grains in a strong specific association [20]. The microbiota present in kefir and kefir grains include numerous microbial species, lactic acid and acetic acid bacterial groups, yeasts and filamentous fungi, developing in complex symbiotic associations [23]. Among the bacterial species, lactic acid bacteria predominate over acetic acid bacteria and Bifidobacteria. The predominant *Lactobacillus* species provide significant antimicrobial properties to kefir beverages [1,12,20]. Likewise, it has been reported that the kefir biofilms and their polysaccharide compounds can also be considered as antimicrobial agents [32]. It is not yet clear whether different varieties of kefir originate from a single original starter culture or not, as microbiological studies from products obtained from different locations indicate that microbial populations may differ significantly [20]. The microbial composition of kefir also varies according to the origin of the kefir grains, different cultivation temperatures, different production techniques, and differing types and compositions of the fermentation medium, and even the storage conditions can alter the microbial composition of the final product [20,21].

Another important aspect is understanding the structure and stability of the microbial community of kefir, which is important for the successful production of functional beverages. In kefir products, lactic acid bacteria are mainly responsible for the conversion of lactose to lactic acid, resulting in a pH drop and thus aiding product preservation throughout storage [33]. In addition, lactose-fermenting yeasts convert lactose to ethanol and CO₂ [34]. Other microbial constituents of kefir beverages include non-lactose-fermenting yeasts and acetic acid bacteria [20]. All species co-exist within a synergistic system, and yeasts produce vitamins, amino acids, and other essential growth factors that are important for bacteria [23]. Likewise, the metabolic products of these bacteria can be used as an energy source for yeasts [35]. The microbial diversity of kefir beverages, as described in the literature, varies greatly (Table 1) and the identification of microbiota present in kefir is directly related to the quality of the final product [36]. More importantly, differences in kefir microflora may influence the ability of traditional kefir to exert positive effects on hosts' metabolic effects [37].

Table 1. Variety of microbial species isolated from kefir.

Microbial Species	Reference	Microbial Species	Reference
Lactobacilli		Lactococci	
<i>Furfurilactobacillus rossiae</i>	[36,38]	<i>Lactococcus cremoris</i>	[39]
<i>Lactobacillus acidophilus</i>	[36,38,40,41]	<i>Lactococcus garvieae</i>	[36]
<i>Lactobacillus amylovorus</i>	[36,38,40]	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	[30,33,41–43]
<i>Lactobacillus apis</i>	[31,38,44]	<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	[33,41,45]
<i>Lactobacillus bulgaricus</i>	[38,40]	Streptococci	
<i>Lactocaseibacillus casei</i>	[36,38,40]	<i>Streptococcus durans</i>	[39]
<i>Lactobacillus crispatus</i>	[36,38,40,41]	<i>Streptococcus faecalis</i>	[39]
<i>Lactobacillus delbrueckii</i> subsp.	[36,38]	<i>Streptococcus thermophilus</i>	[30,39]
<i>Lactobacillus fomicalis</i>	[38,40]	Acetic acid bacteria	
<i>Lactobacillus gallinarum</i>	[36,38,40]	<i>Acetobacter aceti</i>	[46]
<i>Lactobacillus gasserii</i>	[38,40]	<i>Acetobacter fabarum</i>	[30]
<i>Lactobacillus gigeriorum</i>	[44]	<i>Acetobacter genera</i>	[30]
<i>Lactobacillus helveticus</i>	[35,38,40,45]	<i>Acetobacter lovaniensis</i>	[30,42]
<i>Lactobacillus intestinalis</i>	[38,40]	<i>Acetobacter orientalis</i>	[30,42]
<i>Lactobacillus jensenii</i>	[38,41]	<i>Acetobacter orleanensis</i>	[44]
<i>Lactobacillus kalixensis</i>	[38,40]	<i>Acetobacter pasteurianus</i>	[44,47]
<i>Lactobacillus kefiranofaciens</i> subsp. <i>kefiranofaciens</i>	[30,31,33,38,40,41,43,44,48,49]	<i>Acetobacter syzygii</i>	[35,43,47]
<i>Lactobacillus kefiranofaciens</i> subsp. <i>kefirgranum</i>	[38,50]	<i>Gluconobacter japonicus</i>	[35]
<i>Lentilactobacillus kefirii</i>	[30,31,38,41,44,50,51]	<i>Gluconobacter morbifer</i>	[44]
<i>Lactobacillus kitasatonis</i>	[38,40]	Yeast	
<i>Lactobacillus mesenteroides</i>	[38,45]	<i>Kazachstania aquatica</i>	[41]
<i>Lentilactobacillus otakiensis</i>	[30,38]	<i>Dekkera anomala</i>	[30]
<i>Lentilactobacillus parabuchneri</i>	[38,50]	<i>Hanseniaspora uvarum</i>	[52]
<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i>	[35,38,47,53]	<i>Issatchenkia orientalis</i>	[52]
<i>Lentilactobacillus parakefirii</i>	[35,38]	<i>Kazachstania aerobia</i>	[30]
<i>Lactiplantibacillus pentosus</i>	[36,38]	<i>Kazachstania exigua</i>	[42]
<i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i>	[35,38,47,54,55]	<i>Kazachstania servazzii</i>	[30]
<i>Limosilactobacillus reuteri</i>	[36,38]	<i>Kazachstania solicola</i>	[30]
<i>Lactocaseibacillus rhamnosus</i>	[36,38]	<i>Kazachstania turicensis</i>	[30,41]
<i>Lactobacillus rodentium</i>	[38,40]	<i>Kazachstania unispora</i>	[30,41,43,45]
<i>Latilactobacillus sakei</i> subsp. <i>sakei</i>	[36,38]	<i>Kluyveromyces lactis</i>	[45,56]
<i>Ligilactobacillus salivarius</i>	[36,38]	<i>Kluyveromyces marxianus</i>	[41–43,56]
<i>Liquorilactobacillus satsumensis</i>	[38,57]	<i>Pichia fermentans</i>	[43]
<i>Lentilactobacillus sunkii</i>	[30,38]	<i>Pichia membranifaciens</i>	[52]
<i>Lactobacillus ultunensis</i>	[38,40,44]	<i>Pichia kudriavzevii</i>	[52]
<i>Levilactobacillus brevis</i>	[36,38,58]	<i>Saccharomyces cariocanus</i>	[30]
<i>Liquorilactobacillus uvarum</i>	[35,38]	<i>Saccharomyces cerevisiae</i>	[30,43,47,52,56]
<i>Lentilactobacillus buchneri</i> subsp.	[30,38]	<i>Saccharomyces servazzii</i>	[41]
Other bacteria		<i>Zygosaccharomyces fermentati</i>	[52]
<i>Pediococcus halophilus</i>	[36]	Other bacteria	
<i>Pediococcus lolii</i>	[36]	<i>Enterococcus</i> sp.	[30]
<i>Pediococcus pentosaceus</i>	[36]	<i>Leuconostoc mesenteroides</i>	[33,52]
<i>Lysinibacillus sphaericus</i>	[52]	<i>Leuconostoc pseudomesenteroides</i>	[43]

The microbial diversity of kefir is still controversial, since in each product the species may vary significantly (Table 1). It is important to note is that most of the microorganisms detected in kefir beverages provide probiotic potential. The probiotic quality of kefir beverages depends on the stability of the microbial population; therefore, an active inoculum for a diverse microflora is important for the successful production of this functional food [1,21]. Numerous bacterial species have been isolated from kefir beverages, including lactobacilli, such as *Lactobacillus acidophilus*, *Levilactobacillus brevis*, *Lactobacillus kefir*, and *Lacticaseibacillus casei*; lactococci, including *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*; *Streptococcus thermophilus*; *Leuconostoc mesenteroides*; *Leuconostoc cremoris*; and a variety of yeast species, such as *Saccharomyces*; *Kazachstania*; and *Kluyveromyces* [59]. Notably, the most detected yeast strains are lactose-fermenting strains such as *S. cerevisiae*, whereas yeast strains more rarely detected in kefir, such as *Kazachstania unispora* and *Pichia fermentans*, cannot provide the appropriate enzymes to ferment lactose [43]. These strains accumulate in kefir, fermenting monosaccharide hydrocarbons (glucose and galactose), which are hydrolyzed beforehand by other lactose fermenting strains with a synergetic effect. This plethora of microbial strains occurring in kefir and kefir grains results in a beverage with a very low hydrocarbon content, as lactose, glucose, and galactose are fermented to organic acids [43]. The accumulated organic acids may act as antimicrobial agents, whereas various bacterial strains present in kefir can produce other antagonistic substances, such as bacteriocins. Bacteriocins have been documented to interfere with the adherence of pathogenic bacteria and potentially contribute to the immune responses of gut health and they are currently being promoted as next-generation antibiotics [12,60].

2.3. Kefir Beverages as Protective Dietary Supplements against Viral Infections

An emerging need has arisen as the current COVID-19 pandemic, caused by SARS-CoV-2, has affected millions people across the globe, with humanity still struggling to provide a cure [61]. As a result, the scientific community has turned to many different pathways to boost the immune system and minimize the effects of the pandemic. A significant pathway involved in sealing the immune system against many different pathogens has been the consumption of functional foods, especially probiotics [12]. Kefir is known to be an optimum product, retaining the viability of microbial strains and providing probiotic characteristics [1,18]. Probiotic strains, contained in adequate amounts in kefir beverages, have been proposed as effective agents which boost the immune system and may contribute as a biological defence weapon to flatten the curve of the COVID-19 pandemic [12,62]. Specifically, various probiotic strains have been proposed as significant dietary supplements against viral infections, as they provide immunomodulating compounds such as lipoteichoic acid, peptidoglycans, and nucleic acids, as well as favoring the development of bacteriocins, lactic acid, and hydrogen peroxide as antiviral agents [12,60].

In the fermentation bioprocess of milk by kefir microorganisms, preliminary components and secondary metabolites are synthesized, including organic acids, antimicrobial peptides, polysaccharides, and CO₂, as well as bacteriocins. Many of these bioactive metabolites have already been proven to suppress the growth of spoilage and pathogenic microorganisms, while acting as natural preservatives during food storage [63]. Another significant outcome provided by these bioactive metabolites is their immunomodulatory and antiviral effects [64]. Particularly, bioactive peptides have been reported to suppress viral activity, boosting the consumer's immune system via the disruption of viral adhesion [12,60,64]. Likewise, bacteriocins produced by probiotic bacteria can be explored as inhibitors of key targets identified for the screening of metabolites for anti-COVID-19 therapeutics [60,65]. For instance, nisin is a well-known bacteriocin that was initially identified in fermented milk and belongs to the group of peptide antimicrobials collectively called type-A (I) lantibiotics [13,66]. Nisin is currently used as a natural GRAS food preservative due to its antimicrobial characteristics and is applied in many different food products, such as cheese, fermented beverages, dairy desserts, milk, and meat [66]. In a recent study, nisin produced by strains of *Lactococcus lactis* showed a significantly effective interaction with

the human angiotensin converting enzyme 2 (hACE2), which can help with the prevention of binding with the SARS-CoV-2 spike protein receptor [13]. Likewise, another recent study showed that bacteriocins from probiotic strains, in particular glycocin F, produced from *Lactococcus lactis* and lactococcine G produced from *Lactiplantibacillus plantarum* subsp. *plantarum* (previously known as *Lactobacillus plantarum*), could provide high affinities in binding SARS-CoV-2 proteins and thus act as anti-COVID agents [65]. As a result, bioactive compounds included in kefir beverages show immense potential as health promoting attributes and further exploration of their immune boosting and antiviral effects is needed.

3. Commercial Kefir Production

Kefir is traditionally produced from cows' milk but it can also be prepared from milk obtained from other species, such as goats, sheep or buffalo, whereas other sources, such as soy, coconut, rice, or almond milk, can also be applied for the production of kefir-like beverages [67]. In general, the production process of milk-based kefir is divided in two main categories: (1) traditional/homemade kefir production, and (2) industrial kefir production (Figure 2).

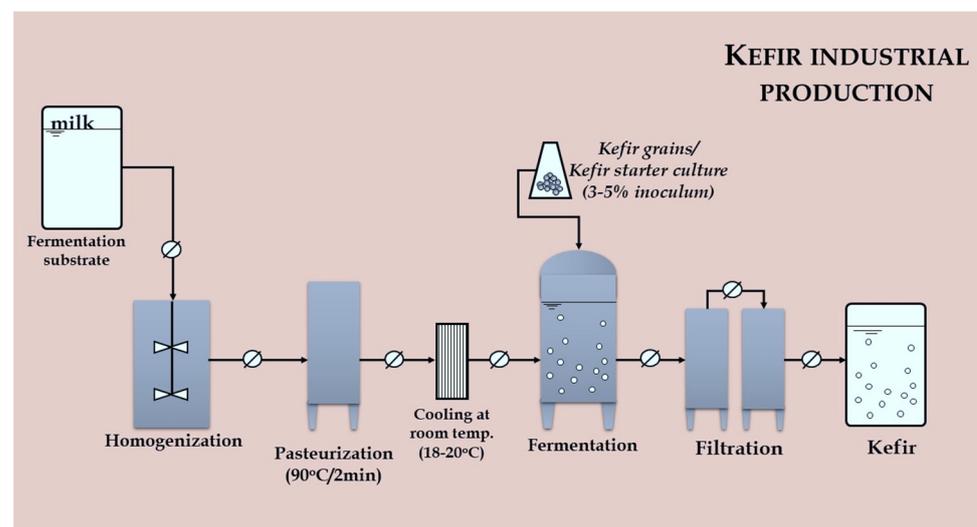


Figure 2. An outline of the main processes involved in industrial kefir production.

Several methods exist for the manufacturing of kefir beverages. Modern techniques have been established targeting the production of beverages with the same characteristics as those of traditional kefir. For the industrial production of kefir, fresh raw milk must be applied to heat treatment, targeting microbiological safety and shelf-life [68]. The combinations of time and temperature applied in the heat treatment of fresh raw milk range between low-temperature, long-time pasteurization (e.g., 85 °C for 30 min, 90 °C for 15 min, 90–95 °C for 2 to 3 min, or 63 °C for 30 min) to ultra-high-temperature (UHT) sterilization (e.g., about 135–154 °C for a couple of seconds) [68]. Subsequently, the pasteurization of milk for kefir production is usually applied at 90 °C for 2 min, with the aim of ensuring sustainable production and maintenance of the milk's nutritional value, although traditional manufacturing techniques suggested no heat treatment [69]. The application of raw milk in fermentation is currently avoided in industrial production, as evidence has shown that pasteurization is an incontrovertible microbial safeguard for milk and milk products. Moreover, low-temperature, short-time pasteurization slightly alters the nutritive and organoleptic characteristics of milk [69] and as a result is currently promoted in industrial kefir production (Figure 2). After milk pasteurization, the fermentation bioprocess is initiated with the addition of the starter culture, either in the form of kefir grains previously recovered from previous batches of milk fermentation, or as an activated selected microbial population, mainly applied in large-scale production processes [9,29].

Milk at the optimum temperature (20–25 °C) is inoculated with active kefir grains at an inoculum which may vary between 2–10% *w/v*. An inoculum between 3–5% *w/v* is usually preferred as a strong and sustainable starter for the industrial production of kefir's [28,70]. In large-scale production, the selected starter is inoculated in the milk at 20 °C for approx. 24 h. In all cases, the fermentation of milk is completed with the formation of a stable coagulate when pH reaches 4.6–4.0 [29,71]. After fermentation, grains are removed by straining, whereas in the case of large-scale production, the fermented milk is directly packed and stored (at 4 °C).

Quality Production Management

The microflora of kefir may vary according to their origin, the applied conditions of fermentation, and the storage and elaboration processes [20]. The exact ratio between the various species of lactic acid bacteria, yeasts, acetic acid bacteria, and other microflora may depend on factors such as fermentation time, temperature, the degree of agitation, and the type of milk or alternative non-dairy substrate of fermentation [20,46]. For example, agitation during fermentation can affect the microbial composition of kefir, favoring the development of homofermentative lactococci and yeasts, as oxygen is enhanced in the fermentation bioprocess [33]. Another aspect that can affect the microbial composition of kefir is the substrate of fermentation. Sugary kefir, for example, favors the development of yeasts, LAB, and acetic acid bacteria, whereas temperature might also alter the culture variety [71,72]. In all cases, following fermentation, kefir grains increase in biomass by approximately 5%, with new florets forming in the process [31,35]. Kefir beverages produced under acidic conditions show a predominance of smaller peptides, suggesting a higher proteolysis level, a characteristic which also influences the nutritional value of the final product [71]. It has been well established that the wide microbial diversity found in kefir can promote its adaptation to different substrates more easily compared to fermentation using single-species starter cultures [18,34]. It is noteworthy that some commercially produced beverages that are labeled as 'kefir beverages' are lacking in microbial diversity compared to traditional kefir products [71]. Another factor worth mentioning is that lactobacilli, lactococci, and leuconostoc species may occur in both commercial and traditional kefir beverages, whereas, on the other hand, most commercial kefir beverages lack acetic acid bacteria [18,20]. Acetic acid bacteria are mainly present in traditional kefir beverages and usually confer beneficial effects in addition to their possible contribution in the manufacturing of a wide range of novel products [18,37,71]. Traditional kefir contains a wide variety of yeast strains, whereas in commercial kefir the yeast population is significantly lower and sometimes only *Saccharomyces cerevisiae* is detected. Likewise, traditional kefir shows a greater variety in the total microflora, providing additional health attributes in contrast to commercial kefir. This outcome highlights the substantial consideration needed in the selection of the commercial kefir starter culture [37,71].

Traditional kefir production results in the production of smaller volumes of final products compared to commercial kefir incubation at ambient temperatures for approximately 24 h [31]. Several batches can be manufactured through traditional kefir production, although this results in a rather shorter shelf-life (2–3 days) compared to industrially manufactured kefir. The sensory properties of traditional kefir show some fluctuations, due to the conditions of handling and storage of fermented beverages, which in many cases cannot be considered equivalent to industrial production (Table 2). Specifically, sensorial variations occur in traditional kefir mainly depending on the origin of the starter culture applied as the inoculum [9,29,72]. In general, all kefir products are characterized as slightly acidic due to the lactic acid produced via lactose fermentation. Furthermore, all beverages are mildly carbonated due to the presence of CO₂, with small amounts of alcohol resulting from yeast accumulation [73]. Other metabolites also included in kefir beverages are bioactive peptides, exopolysaccharides, antibiotics, and bacteriocins [73]. The formed exopolysaccharides of kefir grains are actually hetero-polysaccharides, with glucose and galactose units, and an increase in weight is observed during fermentation due to biofilm

formation, occurring along with the growth of the microbial biomass [29]. This matrix is produced by the lactic acid microflora, which mainly colonize in the form of grains, and its presence in the fermentation medium can subsequently affect the rheological characteristics of the final product (Table 2) [18,31,74]. The concentration of exopolysaccharides is affected by the fermentation temperature and their presence in the beverage has been linked with various health benefits of kefir, such as immunomodulatory, anti-mutagenic, anti-ulcer, anti-allergic, and anti-tumor properties, and it may also act as a prebiotic [75].

Table 2. Effect of different microbial groups on sensory characteristics of kefir beverages.

Microbial Groups	Effect on Kefir Beverage
Mesophilous lactic acid Streptococci (<i>Streptococcus lactis</i> , <i>Streptococcus cremoris</i>)	Development of acidification and coagulate formation.
Lactic acid bacteria (<i>Lactobacillus kefiranofaciens</i> subsp. <i>kefiranofaciens</i> , <i>Lentilactobacillus kefir</i> , <i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i> , <i>Lactobacillus helveticus</i>)	Development of sour taste and lower pH. Stabilization of kefiran structure in kefir grains. Affects the rheological characteristics of the final product. Production of volatile and other compounds with positive effects on texture and flavor.
Lactococci (<i>Lactococcus lactis</i> , <i>Streptococcus diacetyllactis</i> , <i>Leuconostoc dextranicum</i>)	
Yeasts (<i>Saccharomyces cerevisiae</i> , <i>Saccharomyces unisporus</i> , <i>Kluyveromyces marxianus</i>)	Development of a slight alcoholic flavor to the final product. Provide a mildly carbonated effect due to CO ₂ formation.
Acetic acid bacteria	No significant effect. Excessive growth can cause viscous consistency.

In traditional production, kefir grains are re-applied for fermentation in several batches. The re-use of kefir grains results in lower proteolytic activity and a poor reproducibility in the kefir's phosphor-peptide profile [9,71]. These quality variations are not acceptable in industrial production, and led to the development of manufacturing methods with precise control in bioprocessing. Specifically, in order to standardize kefir beverages for marketable production and to facilitate large-scale production, industrial brands use starter cultures consisting of pure strain cultures [29]. This method enables better control of the microorganisms involved and ensures consistent quality in all batches. In addition, the shelf life of the kefir can be extended for up to 10–15 days with refrigerated storage (4 °C) and the modification and improvement in beverages can be facilitated in terms of health-related and nutritional aspects [1,9,29].

4. Modern Biotechnological Processes and Applications

Kefir is considered to be a product with great potential in functional food innovations since it contains a large variety of beneficial microorganisms, along with their bioactive compounds. Dairy kefir products confer a wide spectrum of important health benefits, including physiological, prophylactic, and therapeutic properties [76]. These numerous positive health aspects of kefir have increased the interest of scientists in novel non-dairy substrates for the application of kefir grains. Moreover, the microbial diversity of kefir can contribute to many different fermentation bioprocesses, depending on the fermentation substrate (Figure 1). As a result, scientific research has recently focused on alternative ways to instill kefir's beneficial effects through its adaptation onto non-dairy substrates such as fruits, vegetables, and molasses [18,46].

Water kefir or sugar kefir is a traditional fermented beverage, prepared by adding kefir grains (the inoculum) to a mixture of water, sugar, and sometimes dried or fresh fruits [77]. Traditionally, water kefir is produced by introducing kefir grains into a vessel with water and sugar; subsequently, the vessel is closed and the mixture is left to inoculate at room

temperature for 48–96 h. Sucrose is the main carbohydrate in water kefir fermentation, used as an essential energy source for grain biomass production [20]. The addition of dried or fresh fruit or fruit juices in water kefir fermentation enhances the mixture with different carbohydrates, mainly depending on the chemical composition of the substrate [18]. The names of non-dairy kefir beverages may vary according to the substrate of fermentation and the country of origin. For example, ‘tapache’ is a sugar kefir beverage obtained through the fermentation of pineapple, brown sugar, and cinnamon with kefir grains [78]. Another grape-based kefir beverage is ‘kefir duva’, which is produced through grape must fermentation [79]. In general, in sugar kefir fermentation, final products have an acidic taste and are refreshing, slightly carbonated and lowly alcoholic; sensory properties obtained from the symbiosis between yeast, lactic acid bacteria, and acetic acid bacteria. Sugar kefir beverages can also be enhanced with cereals (oat, maize, and barley), highlighting the ability of cereal ingredients to boost probiotic strains and provide products of enhanced functionality [80]. These results demonstrate the potential industrialization of novel functional probiotic beverages with characteristics similar to traditional sugar kefir beverages.

Fruits and vegetables are strongly recommended by many nutritionists and have been proposed in recent studies as substrates for the adaptation of kefir grains [18,27]. Fruit and fruit juices contain natural sugars and as a result, no sugar supplements are needed when fruits are applied as substrates for fermentation. Vegetable and fruit juices also have a high moisture content and contain carbohydrates, proteins, amino acids, vitamins, and minerals and thus they are considered a suitable medium for microbial growth and the production of fermented beverages, such as kefir, wine, and other products [18,81,82]. Recent studies have reported the successful development of novel non-dairy kefir beverages from fruits and vegetables, such as apples, grapes, strawberries, kiwi, cocoa, pomegranates, prickly pears, and vegetable juices (carrots, beans, fennels, melons, onions, and tomatoes) [18,27,83].

Milk-like substitutes, such as soy milk, coconut, walnut, rice, oat, almond, hazelnut, sesame, tiger nut, hemp, peanut, and cocoa pulp, have great potential as candidates for non-dairy kefir beverage production [84]. However, these substrates need to be supplemented with sugars to stimulate the microbiota of kefir grains as they tend to weaken the inoculum. Finally, recent studies have demonstrated the potential of kefir microbial cultures in the fabrication of novel added-value food products, such as feta-type cheeses [85], whey-based alcoholic beverages [86], vinegars [18,47], potable alcohol, and baker’s yeast [34]. All products include variable microbial strains and highlight microbial transformation as an approach for enhancing bioactive compounds, improving food functionality.

5. Future Prospects towards Health-Promoting Effects

Several scientific studies support the health benefits associated with the consumption of kefir, and its global consumption is constantly rising [87,88]. Furthermore, the recent COVID-19 viral outbreak calls for non-conventional antiviral agents aiming to reduce the risk of infections and promote patient recovery [64]. Scientific research data support the claim that the regular consumption of kefir can be associated with a wide range of health-promoting affects (Figure 3), including anti-carcinogenic [87], anti-oxidant [89], anti-inflammatory [90], antifibrotic [91], anti-microbial [92], and anti-diabetic [93] effects, as well as improved digestion [94] and lactose tolerance [95], and more recent findings support the link between kefir’s probiotic strains and bio-functional metabolites and an enhanced immune system and antiviral activities [65,96].

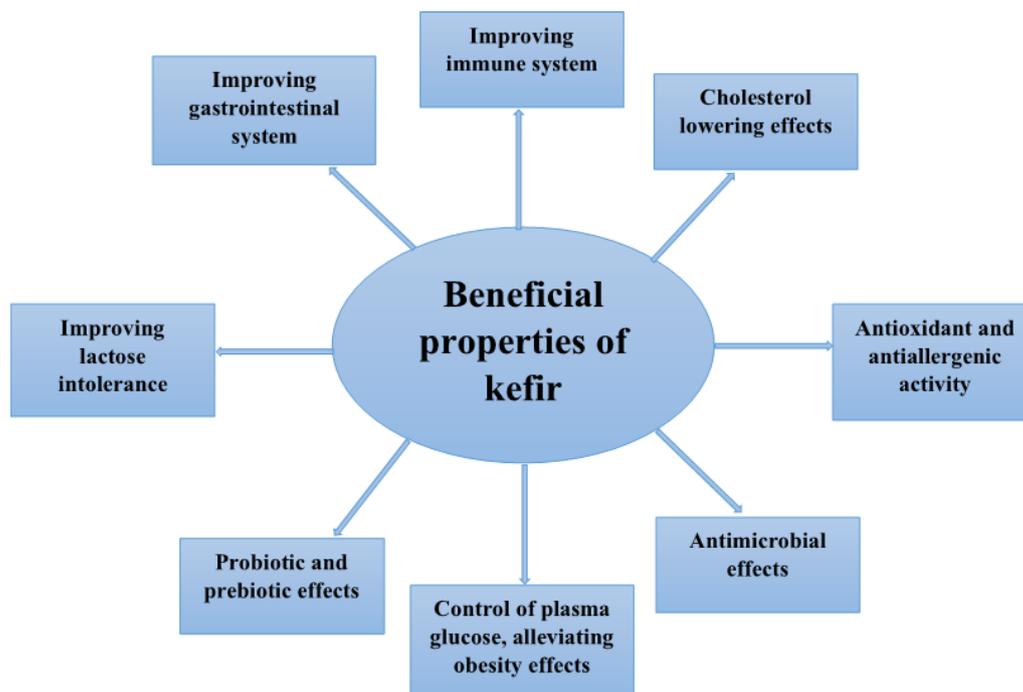


Figure 3. Recapitulated beneficial properties of kefir.

Boosting and stimulating the immune system is one of the most effective strategies to prevent infections caused by many different pathogens, including viruses [1,12]. It has been well-established that probiotic microflora in fermented milk beverages can exert their beneficial effects through two mechanisms—as a direct effect of the live probiotic culture, or through their metabolites [5,64,97]. Due to these numerous health-promoting and disease-preventing effects, kefir is among the most important functional products available. The inhibitory activities of kefir against foodborne pathogens occur as a result of its ability to modulate the intestinal flora by enhancing the number of probiotic bacterial strains [1,23,32,65]. Consumption of kefir can provide the consumer with health-promoting effects produced by probiotics such as lactic acid, acetic acid, and antimicrobial proteins with bacteriostatic effects against various pathogens [23,32,65]. In addition, kefir’s significant content of bio-functional metabolites emphasizes the emerging need to further explore these immune-boosting and antiviral constituents [12,64,65]. In view of these benefits, a demand is emerging for systematic clinical trials to further understand the impact of the regular consumption of fermented functional beverages as a part of the diet or as a food supplement.

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