



## Therapeutic and Dietary Support for Gastrointestinal Tract Using Kefir as a Nutraceutical Beverage: Dairy-Milk-Based or Plant-Sourced Kefir Probiotic Products for Vegan and Lactose-Intolerant Populations

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Review

# Therapeutic and Dietary Support for Gastrointestinal Tract Using Kefir as a Nutraceutical Beverage: Dairy-Milk-Based or Plant-Sourced Kefir Probiotic Products for Vegan and Lactose-Intolerant Populations

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**Abstract:** Kefir is a fermented milk beverage different in consistency and taste from other popular milk-product yogurt. Unlike yogurt prepared using lactic acid bacteria in fermentation, milk is fermented for kefir production using preculture in the form of kefir grains. Therefore, the metabolic activities of a mixed culture, including strains of bacteria and yeast, contribute to the probiotic characteristics in kefir. This article is based on the review of published studies on the functionality and nutraceutical properties of kefir. The therapeutic and dietary properties of kefir beverage and its probiotic strains have been discussed for their several health benefits. Concise selected information mostly from recent reports has been presented for two categories of kefir products: milk used for the production of dairy-based traditional kefir beverages for the lactose-tolerant population, and the plant-sourced substrates used for the production of dairy-free kefir beverages for lactose-intolerant and vegan consumers.

**Keywords:** kefir; gut; microbiota; inflammation; probiotics; lactic acid bacteria; milk; beverage; vegan; lactose

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## 1. Introduction

The therapeutic and dietary properties of fermented foods and beverages have been studied and reported as evidence for the activity and suitability of specific microbial strains. Such strains can be employed in the fermentation of dairy-based or plant-sourced substrates as a targeted approach to produce fermented products with enhanced functionality possessing nutraceutical properties [1]. Consumers are considering the consumption of health foods, including fermented milk products and beverages containing probiotic cultures. Several probiotic products are available to cater to the increasing demand for safe and quality health food products, minimizing the demand for expensive dietary supplements. De Simone reported on the unregulated probiotic market [2]. Along these lines, fermented dairy products are a suitable and economical option for functional dietary sources combined with recognized health benefits.

Although yogurt is a more popular probiotic food in the Western diet, it is normally prepared using inoculum of specific strains of lactic acid bacteria; hence, the final product contains only bacterial strains. However, another fermented milk product, kefir, is actually a much more effective source of a symbiotic mixture of probiotic microorganisms. However, kefir is consumed as a beverage comparable to yogurt, which has a set consistency. Kefir is considered as a health-promoting fermented milk comprising complex microbiota, containing both bacteria and yeast bound together within a polysaccharide matrix known as kefiran. During the fermentation of milk, kefir grains are synthesized by kefir strains. Kefiran polysaccharide molecules grow in their biomass, holding both types of probiotic microorganisms within it.

Traditional kefir is a milk product prepared under the condition of natural fermentation. This is made using only dairy-sourced milk and bioprocessed by inoculum of live mixed bacteria and yeast cultures present in kefir grains. Frag et al. reported kefir as a probiotic beverage with a potential for health support in several ways. They studied kefir fermented dairy products for quality characteristics, flavour chemistry, nutritional value, health benefits, and safety [3]. Current research studies associate the regular consumption of kefir with a wide range of health-promoting features and support the relationship between kefir's probiotic strains and its bio-functional components in the enhancement of the immune system as well as providing support for gastrointestinal health [4].

#### *Process for Kefir Preparation*

Kefir-making first began in regions of Eastern Europe and Southwest Asia. Its name has been derived from *keyif*, a Turkish word which refers to "feeling good after eating". Kefir is traditionally prepared using dairy milk sourced from cows or goats in a stationary state fermentation process. The process is started by inoculating dairy milk with specific preculture in the form of kefir grains. The starter culture's grain-like clusters are made of a mixed population of yeast and lactic acid bacteria. The clusters or kefir grain are very similar in appearance to the florets of a cauliflower. In the incubation process of 24–36 h, the microorganisms present in the kefir grains multiply and ferment the sugar content of the milk (or dairy-free alternative substrates) in the fermentation medium, bio-converting milk into a probiotic beverage. After the fermentation is accomplished, the microbial clusters of kefir grains are separated from the fermented milk and stored as a stock starter culture to perform more batches of fermentations at a later stage when needed [5]. There are two products obtained in such fermentation: the first is the main product, a fermented beverage named "kefir", and the second by-product is harvested microbial cells developed during the fermentation, named "kefir grains" [6].

The lactic acid bacteria, the major component of kefir grains, metabolize lactose sugar content as the main carbon source available in dairy milk, synthesizing lactic acid as their metabolite. The taste of kefir is developed due to this lactic acid, similar to the process of fermentative production of natural yogurt. However, the difference between the two milk products is that the kefir is not a set product like yogurt. Kefir has a liquid consistency after the removal of microbial cells (kefir grains); hence, it is used as a beverage [7]. Kefir has established itself as a nutritional probiotic dairy-based beverage, since its consumption has been linked to a wide range of health benefits. Nutritional, microbiological, and health benefits of milk kefir were reported by Rosa et al. [8]. It constitutes a distinctive symbiotic association of microorganisms, comprising mainly lactic acid bacteria, probiotic strains of yeasts, and sometimes acetic acid bacteria. Though the quality of kefir varies, its physical, chemical, microbiological, and functional properties are strongly stimulated by three main factors: (1) the geographical origin of its starter culture, i.e., the kefir grains; (2) the source of dairy milk used as raw material, i.e., the cow, goat, other local cattle, or water (for dairy-free); and (3) the process of fermentation used, i.e., at traditional domestic level or in an industrial setting [9].

Furthermore, the preparation of kefir can be modified using different substrates other than dairy milk as raw material for fermentation [10], which allows the production of new functional beverages to provide choices for consumers. There is a growing demand for kefir as a functional food; therefore, several studies have been conducted on its probiotic preparations using milk-free kefir, such as using a water medium for water-based kefir with health-promoting properties [11]. This review presents selected information in the following sections based on some of the relevant reports published in the last few years, summarizing therapeutic and dietary support for the gastrointestinal tract using kefir. This nutraceutical beverage can be prepared with two options, either milk-based for lactose-tolerant consumers or plant-sourced dairy-free kefir for the lactose-intolerant and vegan populations.

## 2. Therapeutic Function of Kefir and Its Probiotic Strains

Actively growing recommended dietary microorganisms constituting a functional food or beverage product, or used in the formulation of synbiotic supplements, have been suggested as nutraceuticals to contribute to human health [12]. Compared with the traditional domestic preparations and consumption of naturally fermented foods in many Eastern societies, there was a lesser intake of fermented foods comprising live beneficial microbes in Western culture. This difference in the dietary practice of functional foods corresponded with a rise in modern auto-immune diseases. Sonnenburgs analysed the ancestral and industrialized gut microbiota and their implications for human health [13].

The consumption of kefir beverages is associated with a wide range of nutraceutical benefits, as is discussed in a recent article by Ganatsios et al. [4]. The beneficial properties associated with the regular consumption of natural probiotic beverage kefir include anti-inflammatory, anti-oxidative, anti-cancer, anti-microbial, anti-diabetic, anti-hypertensive, and anti-hypercholesterolemic effects as discussed by de Oliveira Leite [14]. In addition, other therapeutic functions of kefir have been reported in the modulation of the gut microbiome, improved digestive system, mitigation of the effects of obesity, reduced cardiac hypertrophy, tolerance to lactose, control of plasma glucose, anti-allergenic activity, reduced kidney hypertrophy, and anti-mutagenic effect. Yilmaz-Ersan et al. also studied a comparison of the antioxidant capacity of kefirs prepared from milk sourced from cows and ewes [15].

### 2.1. Protection against Infections Caused by Bacteria and Virus

Kefir has been studied for its antimicrobial activities, including antibacterial and antiviral properties as discussed in the following sections.

#### 2.1.1. Antibacterial Properties

Antibacterial properties of some probiotic strains present in the composition of kefir provide protection against gut infections. This includes the probiotic *Lactobacillus kefiri*, which is a unique strain of lactic acid bacteria isolated from kefir. Carasi et al. reported on antimicrobial properties and safety characterization [16]. Studies demonstrated that probiotic strains can inhibit the growth of various harmful bacteria, including *Escherichia coli*, *Salmonella*, and *Helicobacter pylori* [17].

Kefiran, a type of carbohydrate present in kefir, has also shown antibacterial properties; Rodrigues et al. reported the antimicrobial and healing activity of kefir and kefiran extract [18]. Kefir microorganisms used in the fermentation of cheese whey were studied by the research team of Lontero for their antagonism effect against *Salmonella* and its immunomodulatory capacity [19]. Kefir sample was used to isolate a probiotic strain *Lactobacillus kefiranofaciens* DN1, which produced a metabolite referred to as a novel exopolysaccharide by Jeong et al., with the characteristic of possessing antibacterial activity [20]. Kefir fermented milk was also studied for its antifungal effect and shelf-life improvement of corn arepas by Gamba et al. [21]. For the production of functional fermented milk, Terpou et al. evaluated an antimicrobial agent sourced from a natural matrix-forming material, chios mastic gum, for the encapsulation of probiotic bacterial cells, which were then used in milk fermentation [22].

#### 2.1.2. Antiviral Properties

Recently, Hamida et al. studied the effectiveness of kefir as a protective dietary supplement against viral infection [23]. The consumption of probiotics was reported by Baud et al. to flatten the curve of the coronavirus disease COVID-19 pandemic [24]. A study published by Stavropoulou et al. stated that probiotics could be used as a weapon in the fight against COVID-19 [25]. A food-grade antimicrobial peptide nisin is produced by lactic acid bacteria in milk fermentation, which was examined by the team of Bhattacharya et al. for virus infection control. Their work was published in the Virology journal for the probable interaction of nisin with the human ACE2 (hACE2) receptor at the site where

spike protein of SARS-CoV-2 binds [26]. A potential peptide from soya bean cheese produced using *Lactobacillus delbrueckii* WS4 was studied by Chourasia et al. for its effective inhibition of SARS-CoV-2 main protease and s1 glycoprotein [27]. The activity of probiotics to prevent viral infections, with an emphasis on COVID-19, has been reported by Sundararaman et al. [28].

## 2.2. Protection against Cancer

Cancer is one of the world's leading causes of death. It occurs when abnormal cells in our body grow uncontrollably, such as in a tumour. The activity of kefir has been studied on glioblastoma cancer cells, and it was reported recently as a new treatment by Fatahi et al. [29]. Kefir's anticancer properties were explored by Hatmal et al. under the influence of different culture conditions [30].

The probiotics in fermented dairy products are believed to reduce tumour growth by stimulating the immune system. There are reports on the potential role of probiotics in cancer prevention and its treatment; therefore, it is feasible that kefir consumption can be used as a biotherapeutic agent alongside other medical therapies for cancer [31]. Fiorda et al. experimented with the development of kefir-based probiotic beverages with DNA protection and antioxidant activities using soybean hydrolyzed extract, colostrum, and honey [32].

The protective role of kefir has been demonstrated in in vitro studies, and researchers reported that kefir exhibited anti-proliferative and pro-apoptotic effects on colon adenocarcinoma cells with no significant effects on cell migration and invasion [33]. There are reports on kefir's anticancer activity, where kefir was found to induce cell-cycle arrest and apoptosis in HTLV-1-negative malignant T-lymphocytes [34].

In a study performed by Chen et al., it was found that kefir extract reduced the number of human breast cancer cells by 56% compared with only 14% with yogurt extract. Kefir extracts suppressed in vitro proliferation of estrogen-dependent human breast cancer cells; however, no effect was observed on normal mammary epithelial cells [35]. Anti-tumor activity was detected in exopolysaccharides produced by the *Lactobacillus kefir* strain, which was isolated from Chinese kefir grains and was characterized for its therapeutic properties by Riaz Rajoka et al. [36]. The gut microbiota influenced by the intake of probiotics and functional foods has been reviewed for the sustainability of wellness through the alleviation of gut inflammation and colon cancer [37]. Kefir beverage can be used in dietary biotherapy, as the probiotic strain constituents of fermented food and beverage products have been studied as therapeutic agents for the restoration of gut microbiota to relieve gastrointestinal tract inflammation, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and to aid in the prevention of cancer [38].

## 2.3. Protection against Allergy and Inflammatory Responses

Allergic reactions are caused by inflammatory responses to certain foods or substances. The anti-allergic effect has been shown by kefir's lactobacilli [39]. People with an oversensitive immune systems are more prone to allergies, which can provoke conditions such as asthma. The anti-inflammatory and anti-allergic effects of kefir have been demonstrated in a mouse asthma model [40]. The inhibitory effect of kefir was reported on ovalbumin-induced lung inflammation in a murine model of asthma [41]. The roles of gut microbiota, probiotics, and prebiotics have been reported in cardiovascular diseases [42].

The therapeutic effects of kefir grain *Lactobacillus*-derived extracellular vesicles were studied in mice with inflammatory bowel disease induced with 2,4,6-trinitrobenzene sulfonic acid [43]. Kefir peptides have been studied for anti-inflammatory, anti-fibrotic, and anti-oxidant effects on renal vascular damage and dysfunction induced by salt in aged stroke-prone spontaneously hypertensive rats by Chen et al. [44]. A probiotic strain, *Lactobacillus plantarum*, isolated from kefir was investigated for its activity to protect cultured Hep-2 cells against Shigella invasion [45].



#### 2.4. Restoration of Gut Health

The consumption of kefir as a functional beverage constituting active probiotic strains of bacteria and yeasts has been useful in the restoration of gut microbiota balance. The gastroprotective effect of oral kefir and its impact on oxidative stress was studied on acute gastric lesions, which were induced by indomethacin in mice [46]. Bourrie et al. reported the effectiveness of the traditional preparation of kefir in the reduction of weight gain and improvement in plasma and liver lipid profiles in a mouse model of obesity [47]. The presence of probiotic cultures in functional beverages, like in kefir, supports the restoration of gastrointestinal microbiota in cases of antibiotic-therapy-induced gut dysbiosis, which disturbed the balance in gut microbiota [48]. Fermented beverages consumed for probiotic strains act as potential biotics for improving gut health and cognition via the microbiome–gut–brain axis [49]. The clinical potential of probiotic strains used in fermentation for functional beverages has been suggested for their effectiveness as psychobiotics for cognitive treatment through gut–brain signaling [50].

### 3. Dietary Function of Kefir and Its Probiotic Strains

Kefir was initially developed as a staple preserved stock food in many societies; however, it has become an extremely popular fermented food among Western communities and is preferred as a natural food health product. Kefir is categorized as a nutraceutical for its dual role—firstly, as a dietetic supplement due to being rich in nutrients, and secondly, as very beneficial for the regulation of the digestive system and sustaining gut health due to being a good source of probiotics. In fact, kefir can be considered part of functional diets due to its mixed cultural profile of bacteria together with yeast species [4].

#### 3.1. Source of Bioactive Compounds and Aroma

Kefir contains a wide variety of nutrients, including organic acids and peptides that contribute to its health benefits. The microbial diversity of traditional kefir grains contributes to the specific aroma in kefir. Dertli and Con studied the microbial diversity of traditional kefir grains and their role in the aroma profile of kefir produced using the inoculum of different types of kefir grains. Analysis by gas chromatography–mass spectrometry showed a high level of diversity in the volatile components of kefir samples, which were produced with selected kefir grains. The volatile compounds present in kefir products were acids, alcohols, aldehydes, carboxylic acids, esters, and ketones. Acetic acid was detected as the main carboxylic acid in kefir, with 2-butanone being the important volatile component contributing aroma in kefir similar to that of yogurt. *L. kefiranofaciens* has been associated with the production of carboxylic acids and ketones, contributing to cheesy flavours, and with the production of esters, contributing to fruity flavours in kefir [51].

Therefore, kefir grains have been purposely cultivated using different types of carbohydrate substrates and have been used as a novel baking starter in sourdough wheat bread-making [52]. Kefir-sourced *Lactobacillus casei* have been immobilized on brewery-spent grains for use in sourdough wheat bread-making as a source of functional dietary source [53]. Kefir grains were studied to contribute a better aroma and texture, with their application in bread-making as a replacement for baker's yeast [54]. Researchers have evaluated the performance of freeze-dried kefir co-culture as the starter in the production of feta-type cheese to enhance its organoleptic properties [55].

#### 3.2. Probiotics in Kefir Support Digestive System

Probiotic strain constituents of kefir can help the development of friendly bacteria in our gut. That is why the intake of kefir beverage is highly effective at treating gastric problems, such as *Clostridium difficile*-associated diarrhoea [56]. Studies have suggested that probiotic diets can alleviate many digestive concerns, including irritable bowel syndrome [57]. Ruggiero studied the use of probiotics as a dietary treatment for gastric ulcers caused by *Helicobacter pylori* infection [58]. Ayala et al. explored alternative treatments for *H. pylori* infection using natural resources, such as probiotics and nutraceuticals [59]. For this reason,

kefir can be recommended as a functional beverage for the therapeutic effect of its probiotic bacteria to help with issues with digestion and as a relief in gastrointestinal diseases [60].

Microflora of kefir include probiotic cultures, including bacterial and yeast strains. Several food items are prepared using mixed strains of yeasts and bacteria. For example, sourdough bread has an improved texture for better digestibility and contains aromatic compounds due to the evolution of aroma volatiles during the storage of sourdough bread made by mixed cultures of yeast and bacteria. Such bread is preferred by consumers compared with other types of bread prepared from a dough without fermenting it with probiotic cultures [61]. Plessas et al. studied the application of yeast *Kluyveromyces marxianus* and bacteria *Lactobacillus delbrueckii* Ssp. *bulgaricus* and *L. helveticus* for sourdough bread-making [62].

### 3.3. Milk-Based Kefir Supports Bone Density

Consumption of milk-based kefir is a good source of calcium, and its consumption as a nutraceutical beverage helps in avoiding osteopenia and osteoporosis. The condition of osteopenia shows a lower bone density than the average level for a certain age group, but still not as low as to be graded as osteoporosis. Hence, it is a preliminary stage of osteoporosis. It is characterized by the deterioration of bone tissue and is a major concern in Western countries. Osteoporosis is especially common among older women and dramatically raises the risk of fractures. The intake of fermented-milk-based kefir can ensure calcium intake, which is one of the dietary ways to improve bone health by slowing down the progression of osteoporosis [63].

Kefir prepared from the fermentation of full-fat milk is a good source of not only calcium but also vitamin K2, which is required for the metabolism of calcium. The supplementation of calcium with K2 leads to improved bone density, which helps in preventing bone fractures. Chen et al. reported the use of kefir in animal studies for increased calcium absorption in bone cells, improving bone mass and microarchitecture in an ovariectomized rat model of postmenopausal osteoporosis [64].

The stability of probiotic strains is required in fermented products, and the cultures should be in viable numbers at the time of consumption of products. Therefore, Bosnea et al. experimented with preserving probiotic strains in biopolymer-based coacervate structures to enhance their cell viability when used in milk-fermented products [65]. A calcium-containing probiotic product was prepared using a probiotic strain by Vassiliki et al. The researchers studied the stability of fermenting bacteria *Lactobacillus casei* by the process of entrapment of bacterial cells in the viscous matrix of a natural resin *Pistacia terebinthus* [66]. Another milk product as a source of calcium was prepared fermenting milk with probiotic bacteria immobilized on wheat bran for their enhanced probiotic viability and aromatic profile in the fermented product [67].

## 4. Fermentative Production of Traditional Kefir products from Dairy Sources

Kefir is traditionally prepared by fermentation of dairy milk, and it is recognized as a functional beverage with an established population of probiotic bacteria that coexist in symbiotic association with other microorganism yeasts. Kefir prepared from dairy sources is an acceptable probiotic beverage for lactose-tolerant and non-vegan consumers. The microflora of kefir grains were found unusually stable; they retained viability and fermentation activity for years after they were preserved and incubated under appropriate cultural and physiological conditions. There are certain factors which control the health-promoting quality of kefir, and these should be taken into consideration in the process of kefir preparation [4]. Important factors include the microbial composition of kefir grains used for inoculum, and kefir-grain-to-milk ratio for optimum inoculum size duration of fermentation and incubation temperature. Then, the downstream processing steps are also equally important, such as maintaining hygiene and a clean environment during the separation of kefir grains from fermented milk, washing of kefir grains, and the safe cold storage for their repeated use in the fermentation of further batches of milk. These

parameters affect the kefir quality and the microflora of the kefir grains. The bacterial populations in international artisanal kefir were discussed by Sindi et al. [68].

The common microorganisms isolated from kefir grains have variations in different regions. The bacteria contained in kefir grains are usually various homo- and heterofermentative species of lactic acid bacteria, such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus*, and of acetic acid bacteria of *Acetobacter* species. These microorganisms, characterized as probiotics [69], will have beneficial effects on health when ingested in numerous ways, aiding digestion, weight management, and mental health. Kefir grains contain more than 60 strains of bacteria and yeasts, making them a very rich and diverse probiotic source, although this diversity may vary [70]. The mixture of microorganisms constituting the kefir microflora contributes to symbiotic relationships. Kefir preparations depend on the quality of kefir grains used for inoculum and the source of milk. Kefir could contain yeasts, such as *Kluyveromyces*, *Candida*, *Saccharomyces*, and *Pichia*, and lactic acid bacteria (as described above). Other fermented dairy products, yogurt, and sour milk, are made from far fewer strains, mainly bacteria, and do not contain any yeast. Plessas et al. reported the microbiological assessment of different types of kefir grains [71]. The bacteria of the kefir grains and their fermented dairy products from different regions are listed in Table 1.

**Table 1.** Kefir prepared from dairy sources as a probiotic beverage for lactose-tolerant and non-vegan consumers.

Dairy-Based Kefir Product	Probiotic Cultures	Reference
Commercial preparation from cow’s milk	<i>Bifidobacterium</i> , <i>Lactobacillus acidophilus</i> , <i>L. casei</i> , <i>L. rhamnosus</i> , and <i>L. plantarum</i>	[72]
Dairy-milk based + 1 billion active cells	Easy kefir freeze-dried grains sachets with more than 3 billion live bacteria and more than 50,000 lactic yeast cells per gram	[73]
Commercial kefir-yogurt from cow’s milk	<i>Bifidobacterium</i> , <i>Streptococcus thermophilus</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i> , <i>L. casei</i>	[73]
Normal kefir	<i>Lactococcus lactis</i> (34%), <i>Lactobacillus kefir</i> (34%)	[74]
Kefir with 1% anthocyanin from black carrots	<i>Lactobacillus kefir</i> (17%), <i>Leuconostoc mesenteroides</i> (9%), <i>Lactococcus lactis</i> (5%)	[74]
Kefir with 5% anthocyanin from black carrots	<i>Lactobacillus kefir</i> (72%), <i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> (3%)	[74]
Non-commercial domestic preparation kefir	Bacterial strains: <i>Lentilactobacillus kefir</i> , <i>Leuconostoc mesenteroides</i> , and <i>Lactococcus lactis</i> Yeast strains: <i>Kluyveromyces marxianus</i> and <i>Saccharomyces cerevisiae</i>	[75]
Industrial preparation	Bacterial strains: <i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> , and <i>Lactocaseibacillus rhamnosus</i> Yeast strains: <i>Debaryomyces hansenii</i> and <i>Kluyveromyces marxianus</i>	[75]
Artisanal Turkish kefir grains A	Dominant bacterial strain: <i>Bifidobacterium longum</i> Dominant yeast strain: <i>Naumovozyma dairensis</i>	[76]
Artisanal Turkish kefir grains G	Dominant bacteria: <i>Lactobacillus kefiranofaciens</i> Dominant yeast: <i>Zygosaccharomyces rouxii</i>	[76]
Traditional Turkish kefir grains.	Bacteria: <i>Lactobacillus kefiranofaciens</i> <i>Enterobacter</i> , <i>Acinetobacter</i> , <i>Enterococcus</i> , and <i>Pseudomonas</i> spp. Yeast: <i>S. cerevisiae</i> , <i>Kazachstania</i> , <i>Candida</i> , <i>Issatchenkia</i> , <i>Rhodotorula</i> species	[51]

### 5. Kefir from Plant Sources as Dairy-Free Option for Vegan and Lactose-Intolerant Consumers

Alternative plant-based milk has been fermented with kefir culture to produce kefir for lactose-intolerant and vegan consumers [77]. For the reason, regular dairy milk contains a



natural sugar called lactose, and many people are unable to break down and digest lactose properly, suffering from a condition called lactose intolerance [78]. The lactic acid bacteria in the fermentation of milk to produce dairy foods, such as kefir and kefir yogurt, bioconvert the lactose sugar into lactic acid, so these foods are much lower in lactose than dairy milk. The fermented products also contain enzymes that can help break down the lactose even further. That is why kefir is generally better tolerated by people with lactose intolerance compared with normal unfermented milk. Hertzler and Clancy reported that kefir improves the digestion of lactose and its tolerance in adults with problems of lactose maldigestion [79].

Plant-based milk substitutes were studied by Aydar et al. for the presence of bioactive compounds and their health effects. Dairy-free kefir can be prepared with coconut water, coconut milk, or other liquids, including sugar-water and fruit juices, although these will not have the same texture and organoleptic profile as the dairy-based kefir [80]. Characterized probiotic strains can also be used for the fermentation of dairy-free liquids to produce kefir for vegans. Such cultures have been explored for their significance and applicability in the fermentation of non-dairy-based substrates sourced from plants, including vegetables, grains, fruits, and nuts, to produce nutraceutical food products that are beneficial for gut health and nutrition [81,82].

Some options of kefir beverages as 100% lactose-free options have been prepared using cereals, nuts, fruits, or other non-dairy substrates [83]. Randazzo et al. used Mediterranean fruit juices to develop non-dairy beverages from the fermentation of fruit juices by probiotic strains, which were obtained from water-based kefir [84]. Kefir-like beverages have also been produced from vegetable juices, and their properties were studied by Corona et al. [85]. Water-based kefir preparations have a base of water and sugar with or without the addition of vegetables or fruits, and spices may be added for flavour supplementation. Water-based kefir grains instead of milk-based kefir grains are used as inoculum in the fermentation process for probiotic kefir beverage preparation for vegan consumers. Yépez et al. studied the formulation of different kefir-like cereal-based beverages with riboflavin fortification using selected LAB strains [86]. Table 2 presents substrates and strains used in some of the dairy-free kefir beverages.

**Table 2.** Dairy-free probiotic kefir prepared from plant-sources for lactose-intolerant and vegans populations.

Plant-Based Substrates Used	Cultures Used	Reference
<u>Protein-rich beverages:</u> Almond kefir; Cashew kefir; Walnut kefir; Peanut kefir; Yogurt–nut kefir (Cashews, almonds, and pea protein);	Easy kefir freeze-dried grains sachets with more than 3 billion live bacteria and more than 50,000 lactic yeasts per gram	[83]
<u>Carbohydrate-rich beverages:</u> Oat kefir; Flaxseed kefir; Rice kefir; Coconut kefir	Easy kefir freeze-dried grains sachets with more than 3 billion live bacteria and more than 50,000 lactic yeast cells per gram	[87]
<u>Vitamin/mineral/oil-rich beverages:</u> Pine nut kefir; Hazelnut kefir; Pumpkin seed kefir; Macadamia nut kefir; Pistachio kefir; Pecan kefir; Sunflower seed kefir	Easy kefir freeze-dried grains sachets with more than 3 billion live bacteria and more than 50,000 lactic yeasts per gram	[88]

Table 2. Cont.

Plant-Based Substrates Used	Cultures Used	Reference
Pomegranate juice novel beverage	Kefir grains isolated from a domestic preparation	[89]
Oat, coconut cream, rice flour, (tapioca starch and pectin added as stabilisers), a commercial non-dairy-based probiotic beverage kefir	Live vegan kefir cultures <i>Bifidobacterium</i> , <i>Lactobacillus acidophilus</i> , <i>L. bulgaricus</i> , <i>L. rhamnosus</i>	[72]
Mediterranean fruit juices from quince, grape, kiwifruit, prickly pear, and pomegranate juices	Water kefir microorganisms	[84]
Soya milk	Kefir grains	[90]
Sugary kefir	Various lactic acid bacteria and yeast	[91]
Water kefir	<i>Bifidobacterium tibiigranuli</i> sp. nov.	[92]
Sugary kefir (apple juice, cherry juice, and a solution of sugar-water, enriched with plums)	Inoculum from fruit kefir grains Dominating strains in products: <i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces marxianus</i> , <i>Bacillus amyloliquefacien</i> , <i>Lactobacillus rhamnosus</i>	[93]
Rice cereal-based kefir	Sugary kefir grains <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Acetobacter</i> , <i>Saccharomyces</i> , <i>Kluyveromyces</i> , <i>Lachancea</i> , <i>Kazachstania</i> .	[10]
Water kefir	Water kefir grains	[94]
Cereal-based kefir-like beverages in situ riboflavin-fortified	Andean lactic acid bacteria strains	[86]
Pomegranate and orange juices for low-alcohol fruit beverages	Kefir grains	[95]
<i>Cornus mas</i> L. juice for low-alcohol fruit beverages	Lactic acid bacteria from kefir grains	[96]

## 6. Conclusions and Future Perspective

Consumers have selective product preferences for low-fat, fat-free, or full-fat milk products; hence, to cater to their requirements, kefir beverages can be produced from natural full-fat, semi-skimmed, or even skimmed dairy-sourced milk. The microbial strains used in fermentation produce health-promoting bioactive molecules, such as  $\gamma$ -aminobutyric acid (GABA), useful for mental well-being [97]. Hence, kefir is a nutraceutical beverage, contributing therapeutic, dietary, and mental health benefits to consumers. The traditional kefir was produced mainly via microbial fermentation of cow's milk, but reports indicate that kefir can also be prepared using milk from local cattle available in different geographical regions, for example, sheep, goats, buffalo, camels, and donkey [98]. A dairy-free kefir beverage is prepared from materials sourced from plants, which offers a suitable option for lactose-intolerant and vegan populations. A wider range of dairy-free kefir can be prepared from a variety of vegetable sources, such as soya beans, rice, coconut, oats, etc., although these preparations will not present a similar organoleptic profile as the dairy-milk-based kefir. However, these products can be consumed as a source of probiotic bacteria and yeast cultures useful for the supply of gut microbiota, providing a recommended daily intake of beneficial microbes [12]. On the basis of several studies and reports published in the last few years, as discussed in this review, the conclusion can be drawn that kefir as a nutraceutical beverage can be consumed by lactose-tolerant/intolerant and vegan consumers as a natural live functional food enriched with therapeutic properties and dietary benefits.

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