

REVIEW ARTICLE

An alternative source of probiotics: Water kefir

Gizem Cufaoglu  | Ayse Nur Erdinc 

Faculty of Veterinary Medicine Department of
Food Hygiene and Technology, Kirikkale
University, Kirikkale, Turkey

Correspondence

Gizem Cufaoglu, Faculty of Veterinary
Medicine Department of Food Hygiene and
Technology, Kirikkale University, Kirikkale,
Turkey.
Email: gizemcufaoglu@kku.edu.tr

Abstract

The aim of this review was to compile the origin and distribution of water kefir, its production, microbial diversity, fermentation dynamics, health effects, and its place in national/international food legislation. Water kefir is a traditional, slightly carbonated fermented beverage with a lactic acid content of up to 2%. It is obtained by fermenting sugar by kefir grains, which are white or yellowish in color and translucent in appearance. The types of microorganisms in the water kefir differ depending on the sugar, carbon sources, and fermentation conditions. It has a complex microbiota mainly consisting of lactic acid bacteria, acetic acid bacteria, and yeasts. In addition, fermented water kefir contains various metabolites, including sugar, fruit-derived components, lactic acid, acetic acid, ethanol, carbon dioxide, mannitol, vitamins, and amino acids such as arginine. As a non-dairy fermented beverage, water kefir emerges as an alternative source of probiotics for individuals with lactose intolerance, people with dairy allergic reactions, and vegan consumers. However, although consumer demands have driven the food industry to increase the production of water kefir, the most common production still relies on the traditional method due to some difficulties in the industrial-scale production process. On the other hand, more *in vitro* and *in vivo* studies should be carried out to reveal the health benefits of water kefir.

KEYWORDS

fermentation, functional food, probiotic, vegan, water kefir

1 | INTRODUCTION

Strengthening immunity has gained importance in the prevention of diseases especially after COVID-19 pandemic. The immune system is affected by genetics, environmental factors, and dietary habits. As a result, adequate and balanced nutrition is receiving more attention than ever before in order to prevent diseases or overcome them with the least amount of harm. According to reports, consuming functional foods, which are not drugs or dietary supplements, can be increased for the continuity of health (Kamarli-Altun et al., 2021).

Kefir is one of the most widely consumed functional foods due to its health-promoting properties. Although milk kefir is the first thing that comes to mind when kefir is mentioned, the term kefir refers to a carbonated, acidic, and low-alcohol fermented beverage obtained by fermentation of kefir grains with milk or water (Azizi et al., 2021). Therefore, dairy and non-dairy features of kefir vary on the type of source used for fermentation. Most of the reported kefir studies are on the beneficial effects of milk kefir consumption (Koyu & Demirel, 2018; Nejati et al., 2020; Rosa et al., 2017). Water kefir, on the other hand, has probiotic properties like milk kefir, but besides milk kefir, it is a suitable

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2023 The Authors. *Food Frontiers* published by John Wiley & Sons Australia, Ltd and Nanchang University, Northwest University, Jiangsu University, Zhejiang University, Fujian Agriculture and Forestry University.

food for vegans and consumers with lactose intolerance or milk allergy (Fiorda et al., 2017).

Water kefir is a fruity, sour, and slightly carbonated traditional fermented beverage with a lactic acid content of up to 2% and usually less than 1% alcohol. It is obtained as a result of fermentation of sugar by water kefir grains in which dried fruits can be often included in the fermentation process (Moretti et al., 2022). In this review, we aimed to discuss the origin and distribution of water kefir, its traditional and industrial production, microbial diversity, fermentation dynamics, health effects, and its place in national and international food legislation.

2 | ORIGIN AND DISTRIBUTION OF WATER KEFIR

The origin of water kefir grains is not known exactly, but there are some theories. The grains called “ginger beer plant,” which were brought by the British soldiers while returning to their country from the Crimean War in 1855, were defined by Ward as “beverage containing a symbiotic mixture of yeast and bacteria, and containing sufficient amounts of nitrogenous organic matter and beet sugar or cane sugar in its aqueous solution” (Ward, 1892). This description is probably the first published account of the water kefir culture. As a different theory, Lutz (1899) reported “Tibi grains” which were plucked from the leaves of a Mexican cactus (*Optunia*). In the 19th century, it was called by various names such as “Japanese Beer Seeds” and “Gilead Balm” in different geographies (Kebler, 1912). In the late 1900s, Pidoux called them “sweetened kefir grains” to distinguish them from grains that ferment milk (Pidoux, 1989; Pidoux et al., 1990). These days, all such grains are referred to as “water kefir grains” or “sugary kefir grains” and are utilized for the fermentation of sugar–water solutions. Although its historical origin is uncertain, water kefir grains have been passed down through the generations, and they are quite preferred in various countries such as Japan, Thailand, Malaysia, the United Kingdom, Spain, the Netherlands, Brazil, Chile, Peru, and Argentina (Fiorda et al., 2017).

3 | WATER KEFIR PRODUCTION

Traditionally, water kefir fermentation is accomplished by incubating at 20–37°C (optimally 20–25°C) for 24–72 h in a dark environment using 6%–30% sugar and 6%–20% water kefir grains (Laureys et al., 2018; Pendon et al., 2021). After the fermentation is completed, the grains are filtered through a sterile sieve, separated from the medium, washed, and the same processes are repeated for the next fermentation. The filtered water kefir beverage is stored at 4°C before being prepared for consumption (Guzel-Seydim et al., 2021). The traditional production process of water kefir production is shown in Figure 1.

Historically, non-aseptic or barely aseptic conditions have been used to make water kefir in households (Horisberger, 1969; Pidoux et al., 1988). Starter culture should be used to standardize the final product, but the use of starter cultures in water kefir is not common and

there are few studies in this area. Laureys et al. (2017) conducted one of the studies reporting the industrial water kefir production process, including grain failure and/or slow growth and instability. In the study, the performance of the industrial water kefir production process was found to be insufficient. On the other hand, it was not found appropriate to store water kefir grains at −20°C. It has been reported that freezing and thawing irreparably harm the grain structure and/or related microbes because the grains contain 86%–90% water (Gulitz et al., 2013; Laureys et al., 2017). According to reports, using demineralized water diminishes the buffering capacity of the water because ions are removed, which has a negative impact on water grain growth (Laureys et al., 2017, 2019).

In addition to various sources of sucrose, fruits can also be used to make water kefir. Refined sugar and brown sugar are the most common sources of carbon, while dried fruits are added as a source of nitrogen. The type of fruit, the way it was processed (e.g., dried), the absence of preservatives, and the presence of microorganisms (e.g., *Enterobacteriaceae*, *Pseudomonas* spp.) that can contaminate water kefir from the fruit should be taken into account (Randazzo et al., 2016). In this context, it is noteworthy that the most used and studied fruit is the fig (Gulitz et al., 2013; Laureys & De Vuyst, 2014; Verce et al., 2019).

Other nutrients such as amino acids, vitamins, and minerals which are required for fermentation of water kefir are also provided from dried fruits. It is claimed that microbial species diversity, substrate consumption, and metabolite production during fermentation may be impacted by the quantity and/or kind of fruit used. Laureys et al. (2018) tried to show the effect of figs and sugar by adding 3% dried figs, 6% dried figs, and 6% sucrose, respectively. While slow fermentation, low metabolite concentration, and high pH are observed in the fig-free group, a rapid fermentation and high metabolite concentration were observed without a decrease in pH values in the group containing 6% dried figs (10 g). In normal nutrient concentration (3% dried figs [5 g]), high metabolite production has been reported, again without a decrease in pH. Furthermore, it is proposed that the slow fermentation progress is caused by the high sucrose concentration and the effect of osmotic pressure on microorganisms (Laureys et al., 2017). It is also reported that a wide range of sugar sources can be used to make water kefir since the microorganisms in water kefir grains are very adaptable to varied sources (Bueno et al., 2021).

Microorganisms in water kefir grains coexist symbiotically, and microorganisms can migrate from water kefir grains to water kefir liquid. After the fermented product is filtered through a sieve, the water kefir grains can be utilized for the following fermentation (Verce et al., 2019). Also, a process known as “black-slopping” can be used which is the practice of adding some of the previously fermented beverage along with the grains to the new fermentation (Garofalo et al., 2020). In water kefir production, after the water kefir grain is separated from the kefir liquid, the liquid part can be used for the second fermentation. It is reported that more flavored products can be obtained by adding fruit and fruit juice in the second fermentation stage and incubating at 4°C for 24 h (usually using apple, pineapple, lime, lemon, orange, mango, cherry, strawberry juices) (Bueno et al., 2021; Fiorda et al., 2017).



FIGURE 1 Water kefir production steps

4 | MICROBIAL DIVERSITY OF WATER KEFIR

Water kefir grains are small (1–10 mm in diameter) and translucent, have a fragile structure that deforms under pressure, and can vary in white or yellowish color depending on the type of sucrose and the fruit added to the culture medium (Figure 2). With the symbiotic association of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts, water kefir grains consist of a complex microbiological system within a matrix of polysaccharides (mainly dextran and to a lesser extent levan) produced by bacteria (Lynch et al., 2021; Pidoux et al., 1988). Each gram of water grain contains approximately 10^8 colony-forming units (CFU) of LAB, 10^6 – 10^8 CFU of AAB, and 10^6 – 10^7 CFU of yeast (Gulitz et al., 2011). Additionally, lactobacilli are dominant in the environment (10–100 times more than yeast), and the number of acetic acid bacteria varies according to the oxygen level in the system, but are mostly similar to lactobacilli (Gulitz et al., 2011). *Lactobacillus* spp. from lactic acid bacteria, *Acetobacter* spp. from acetic acid bacteria, and *Saccharomyces* spp. from yeast are the primary microbial members of water kefir grains (Laureys et al., 2018). Some species are known to be found frequently in

water kefir grains, but which species dominate varies depending on the geographical origin of the grains and the source of fermentation (Lynch et al., 2021).

The carbon and energy sources employed during fermentation have an impact on the variety and frequency of microbial species as well as the concentration of final bioproducts. These variations also affect grain granulation and microbial development (Hsieh et al., 2012). Significant steps have been taken to identify the variety of yeast and bacteria that participate in the fermentation of water kefir since the discovery of these organisms by Dr. Ward in London in 1892 and the subsequent development of molecular tools. Especially in the last 30 years, various studies on water kefir microbiology have been carried out in different countries such as Brazil, Belgium, Germany, Serbia, Taiwan, China, Ireland, Argentina, and Yemen (Alsayadi et al., 2013; Blaiotta et al., 2014; Davidovic et al., 2015; Diosma et al., 2014; Gulitz et al., 2011; Hsieh et al., 2012; Laureys & De Vuyst, 2014; Magalhães et al., 2010; Marsh et al., 2013). Studies examining the microbial composition of water kefir according to different fermentation times, temperatures, and grain origins are compiled in Table 1.

TABLE 1 Studies on the microbial composition of water kefir (Lynch et al., 2021; Pendon et al., 2021)

Country (source of grains)	Lactic acid bacterial species	Acetic acid bacterial species	Yeast species	Other bacterial species	Fermentation time	Fermentation temperature	Reference
Unspecified (said to have Tibi grains)	<i>Lb. brevis</i> , <i>St. lactis</i>	Undefined	<i>S. cerevisiae</i>	Undefined	-	-	Horisberger (1969)
France	<i>Lb. casei</i> , <i>Lb. hilgardii</i> , <i>Leuc. mesenteroides</i> ssp. <i>dextranicum</i> , <i>Lb. casei</i> ssp. <i>rhamnosus</i> , <i>Lb. plantarum</i> , <i>St. lactis</i> , <i>St. cremoris</i>	Undefined	<i>Zy. florentinus</i> , <i>Torulaspora pretoriensis</i> , <i>H. apiculata</i> , <i>C. lambica</i> , <i>C. valida</i>	Undefined	Unspecified	Room temperature	Pidoux (1989)
Italy	<i>Lb. casei</i> ssp. <i>casei</i> , <i>Lb. casei</i> ssp. <i>pseudopplantarum</i> , <i>Leuc. mesenteroides</i> ssp. <i>mesenteroides</i> , <i>Pediococcus</i> spp., <i>Lb. buchneri</i> , <i>Lb. fructivorans</i> , <i>Lb. collinoides</i>	Undefined	<i>S. cerevisiae</i> , <i>H. valbyensis</i> , <i>H. viniae</i> , <i>S. florentinus</i> , <i>S. pretoriensis</i>	Undefined	Not available	Not available	Galli et al. (1989)
Germany	Short and long rod-shaped lactobacilli, dominant bacterial cocci	Undefined	<i>Zy. florentinus</i> , <i>H. valbyensis</i>	Undefined	72 h	Approximately 22°C	Neve and Heller (2002)
Brazil	<i>Lb. paracasei</i> , <i>Lb. parabuchneri</i> , <i>Lb. kefir</i> , <i>Lb. bunchneri</i> , <i>Lactococcus lactis</i> , <i>Lb. casei</i> , <i>Lb. paracasei</i> ssp. <i>paracasei</i> , <i>Leuc. citreum</i> , <i>Lb. paracasei</i> ssp. <i>tolerans</i>	<i>A. loyensis</i>	<i>S. cerevisiae</i> , <i>Kluyveromyces lactis</i> , <i>La. meyersii</i> , <i>Kz. aerobia</i>	Undefined	24 h	25°C	Magalhaes et al. (2010)
Germany	<i>Lb. hordei</i> , <i>Lb. nagelii</i> , <i>Leuc. mesenteroides</i> , <i>Leuc. citreum</i> , <i>Lb. casei</i> , <i>Lb. hilgardii</i>	<i>A. fabarum</i> , <i>A. orientalis</i>	<i>S. cerevisiae</i> , <i>La. fermentati</i> , <i>H. valbyensis</i> , <i>Z. florentina</i>	Undefined	72 h	21°C	Gulitz et al. (2011)
Brazil	<i>Lb. casei</i> , <i>Lb. sunkii</i> , <i>Lb. kefir</i> , <i>Lb. satsumensis</i> , <i>Lb. paracasei</i> , <i>Lb. helveticus</i> , <i>Lb. buchneri</i> , <i>Lb. parakefir</i> , <i>Lb. kefiranoferiens</i>	<i>G. liquefaciens</i> , <i>A. loyensis</i>	<i>S. cerevisiae</i> , <i>P. cecembensis</i> , <i>Yarrowia lipolytica</i> , <i>P. membranifaciens</i> , <i>P. caribbica</i> , <i>P. fermentans</i> , <i>C. valdiviana</i> , <i>La. fermentati</i> , <i>Kz. aerobia</i> , <i>Kluyveromyces lactis</i> , <i>Kluyveromyces marxianus</i> , <i>La. meyersii</i>	<i>Bacillus cereus</i>	Not available	Not available	Miguel et al. (2011)

(Continues)

TABLE 1 (Continued)

Country (source of grains)	Lactic acid bacterial species	Acetic acid bacterial species	Yeast species	Other bacterial species	Fermentation time	Fermentation temperature	Reference
UK, Canada, USA	<i>Lactobacillus</i> , <i>Leuconostoc</i> , <i>Bifidobacterium</i>	<i>Gluconacetobacter</i> , <i>Acetobacter</i>	<i>D. anomala</i> , <i>D. bruxcellensis</i> , <i>S. cerevisiae</i> , <i>H. valbyensis</i> , <i>H. vineae</i> , <i>La. fermentati</i> , <i>Torulaspora</i> , <i>Zy. lentus</i> , <i>Meyerozyma caribbica</i>	<i>Zymomonas</i>	24 h	25°C	Marsh et al. (2013)
Germany	<i>Lb. hordei</i> , <i>Lb. nagelii</i> , <i>Lb. hilgardii</i> , <i>Lb. satsumensis</i> , <i>Leuc. citreum</i> , <i>Leuc. mesenteroides</i> , <i>B. psychraerophilum</i> , <i>Lb. casei</i>	<i>A. fabarum</i> , <i>A. orientalis</i>	Not studied	Undefined	72 h	21°C	Gulitz et al. (2013)
Belgium	<i>Lb. casei/paracasei</i> , <i>Lb. hilgardii</i> , <i>Lb. harbinensis</i> , <i>Lb. nagelii</i> , <i>Lb. hordei/mali</i> , <i>B. psychraerophilum/crudilactis</i>	<i>A. lovaniensis/fabarum</i>	<i>S. cerevisiae</i> , <i>D. bruxcellensis</i>	Undefined	192 h	21°C	Laureys and De Vuyst (2014)
Brazil	<i>Lb. casei</i> , <i>Lactococcus lactis</i> , <i>Lb. perolens</i> , <i>Lb. parafarraginis</i> , <i>Lb. diolivorans</i> , <i>O. oeni</i> , <i>Lb. kefirianofaciens</i> , <i>Lb. hilgardii</i> , <i>Lb. satsumensis</i> , <i>Lb. nagelii</i> , <i>O. kitaharae</i> , <i>Lb. mali</i>	Undefined	Not studied	<i>Klebsiella pneumoniae</i> , <i>Enterobacter ludwigii</i>	24 h	Room temperature	Zanirati et al. (2015)
Thailand	<i>Lb. perolens</i> , <i>Lb. rhamnosus</i> , <i>Lb. paracasei</i>	<i>Gluconobacter japonicus</i>	<i>S. cerevisiae</i> , <i>C. ethanolica</i> , <i>D. bruxcellensis</i>	<i>Bacillus cereus</i>	72 h	25–28°C	Sarikha et al. (2015)
Belgium	<i>Lb. hilgardii</i> , <i>Lb. nagelii</i> , <i>Lb. satsumensis</i> , <i>Lb. harbinensis</i> , <i>Lb. paracasei</i> , <i>Lb. mali/hordei</i> , <i>Lb. harbinensis</i> , <i>Oenococcus spp.</i> , <i>B. aquikefiri</i>	Undefined	<i>S. cerevisiae</i> , <i>Z. florentina</i> , <i>D. bruxcellensis</i>	Undefined	192 h	21°C	Laureys and De Vuyst (2017)
Mexican	<i>Lb. ghanensis</i> , <i>Lb. casei/paracasei</i> , <i>Lb. hilgardii</i>	<i>A. orientalis</i> , <i>A. tropicalis</i> , <i>A. okinawensis</i>	<i>S. cerevisiae</i> , <i>C. californica</i> , <i>P. membranifaciens</i>	<i>Pseudarthrobacter chlorophenolicus</i>	192 h	26°C	Martinez-Torres et al. (2017)
Belgium	<i>Lb. paracasei</i> , <i>Lb. hilgardii</i> , <i>Lb. nagelii</i> , <i>Lb. harbinensis</i> , <i>B. aquikefiri</i>	<i>G. roseus/oxydans</i> , <i>A. indonesiensis</i> , <i>A. fabarum</i>	<i>S. cerevisiae</i> , <i>D. Bruxellensis</i> , <i>C. smithsonii</i>	Undefined	72 h	21°C	Laureys et al. (2018)
Belgium	<i>Lb. harbinensis</i> , <i>Lb. hilgardii</i> , <i>Lb. nagelii</i> , <i>Lb. paracasei</i> , <i>Lb. hordei/mali</i> , <i>B. aquikefiri</i> , <i>Candidatus O. aquikefiri</i>	Undefined	<i>S. cerevisiae</i> , <i>D. bruxcellensis</i>	Undefined	192 h	21°C	Verce et al. (2019)

(Continues)

TABLE 1 (Continued)

Country (source of grains)	Lactic acid bacterial species	Acetic acid bacterial species	Yeast species	Other bacterial species	Fermentation time	Fermentation temperature	Reference
Belgium	<i>Lb. hilgardii</i> , <i>Lb. nagelii</i> , <i>Lb. paracasei</i> , <i>Leuc. pseudomesenteroides</i> , <i>Lb. harbinensis</i> , <i>Lb. mali/hordei</i> , <i>B. aquikefiri</i> , <i>Oenococcus</i> spp.	Undefined	<i>S. cerevisiae</i> , <i>D. bruxellensis</i>	Undefined	72 h	21°C	Laureys et al. (2019)
Malaysia	<i>Lb. hilgardii</i> , <i>Lb. harbinensis</i> , <i>Lb. satsumensis</i> , formerly <i>Lb. zeae</i> , <i>O. oeni</i> ,	<i>A. lovaniensis</i> , <i>A. tropicalis</i> , <i>G. oxydans</i> , <i>Komagataeibacter hansenii</i>	Undefined	Undefined	24 h	Room temperature	Kumar et al. (2021)

Note: *Lactobacillus* (Lb.), *Streptococcus* (St.), *Saccharomyces* (S.), *Leuconostoc* (Leuc.), *Candida* (C.), *Zygosaccharomyces* (Zy.), *Hanseniaspora* (H.), *Acetobacter* (A.), *Kazachstania* (Kz.), *Lachancea* (La.), *Zygorulasporea* (Z.), *Gluconobacter* (G.), *Pichia* (P.), *Dekkera* (D.), *Bifidobacterium* (B.), *Oenococcus* (O).

5 | FERMENTATION DYNAMICS OF WATER KEFIR

5.1 | Metabolites and metabolic interactions

Fermented water kefir contains various metabolites, including sugar and fruit-derived components, lactic acid, acetic acid, ethanol, carbon dioxide, mannitol, vitamins (especially B-complex vitamins), amino acids (such as arginine), mannitol, glycerol, esters, and other organic acids (Fiorda et al., 2017; Laureys & De Vuyst, 2014; Laureys et al., 2017). The fermented product also contains polysaccharides such as glucans (glucose polymers) and levans (fructose polymers) produced by microorganisms (Fels et al., 2018; Stadie et al., 2013). When microorganisms in water kefir reproduce under suitable conditions, glucan is synthesized, and the grain biomass increases. In addition, the microorganisms ferment sucrose; thus, the sucrose concentration decreases up to 98% in the first 24 h (Laureys & De Vuyst, 2014; Magalhães et al., 2010; Martínez-Torres et al., 2017). The main final products of fermentation are ethanol, lactic acid, and acetic acid.

The primary bio-product created during the fermentation process is ethanol. The ethanol concentration increases linearly until it exceeds 10% of the total volume. The ethanol level drops as ethanol is converted to acetic acid by acetic acid bacteria during the fermentation process (Martínez-Torres et al., 2017). During the first 24 h of fermentation, sucrose consumption is favorably correlated with yeasts' generation of ethanol (mainly *Saccharomyces cerevisiae*). Yeast contains the enzyme invertase, which hydrolyzes sucrose. Bacteria that produce lactic and acetic acids metabolize this enzyme, increasing fructose and glucose levels. The presence of yeasts in water kefir also contributes to increased sensory quality. The yeast flavor creates a refreshing and tangy taste (Magalhaes et al., 2010). Laureys and De Vuyst (2014) fermented 17% water kefir grains with 7.1% sugar for 72 h. They reported 2% ethanol, 0.5% lactate, 0.1% acetate, 0.2% glycerol, and 0.08% mannitol concentrations in water kefir, and these concentrations remained constant even after 192 h. Similar results were reported in the presence of 5% panela (sugar cane product) and 1% water kefir grains (Martínez-Torres et al., 2017).

5.2 | Factors affecting biomass increase

The biomass increase of water kefir grain is greatly affected by factors such as the frequency of inoculations, microbial properties of the grain, available nutrients, temperature, and fermentation time (Laureys et al., 2017; Laureys & De Vuyst, 2019). Laureys et al. (2017) performed sequential fermentations using three water kefir grains from different houses using a sterile and standard environment at 21°C. They noticed that one of the grains did not gain weight after each fermentation or gained weight very slowly, another gained weight up until the fifth passage before losing weight, and the third grain's growth capacity was reduced by subsequent fermentation procedures. In grains obtained from a household in Argentina, 5% sucrose and 1% figs were inoculated with 3% tap water, and their initial weights were reported to increase by 55% and 155%, respectively, after 24 and 72 h of incubation at



FIGURE 2 Water kefir grains

20°C. The weights of grains grown under identical conditions but for a different household did not increase at all (Pendon et al., 2021).

The availability of nutrients also has an impact on the biomass growth of water kefir grains. Low nutrient concentrations have been linked to a gradual loss of biomass, while high nutrient concentrations have been shown to promote grain growth up to a certain point but not significantly alter grain growth rate (Laureys et al., 2018). The fermentation medium's sugar composition has an impact on grain growth as well. According to some reports, substituting glucose or fructose for sucrose reduces the growth of the water kefir grains because glucose is fermented more quickly than fructose (Laureys et al., 2021). To better understand the environmental elements influencing grain growth, Laureys et al. (2019) assessed the impact of the buffer capacity and calcium concentration of the water used in the eight-sequenced pass fermentation process. They discovered that when the water buffer capacity and/or calcium content dropped below a critical level, the growth of the water kefir grain gradually slowed down. A glucan-type homopolysaccharide is the primary component of the water kefir grain matrix (Fels et al., 2018). This polysaccharide can be synthesized by *Lactobacillus hilgardii* and is thought to contribute to the grain growth of this microorganism (Pidoux, 1989). It has been reported that microorganisms found in water kefir grains such as *Lactobacillus casei*, *Leuconostoc mesenteroides*, *Lactobacillus nagelii*, *Lactobacillus hordei*, and *Lactobacillus satsumensis* contribute to grain growth by producing dextran from sucrose (Côté et al., 2013; Davidovic et al., 2015). It is clear that more than one bacterium contributes to the production of the water kefir grain matrix when distinct glucan sucrases and polymers produced by various microbes in water kefir grains are taken into account.

Freezing of water kefir grains (contain ~86% water) can damage the polysaccharide structure and the cell membrane of microorganisms, so freeze-thaw process can also reduce the biomass increase of the grains (Laureys et al., 2017). No increase or little increase in water kefir grain biomass is considered common in water kefir fermentation; however, it limits a successful production of water kefir beverages on a homemade or industrial scale. Sufficient grain biomass is crucial for the

biochemical and microbial characterization of the starter in order to understand the microbial diversity and fermentation dynamics of the grain (Laureys & De Vuyst, 2017).

5.3 | Effect of different fermentation sources

In addition to sugar, different carbon and nitrogen sources can also be included in the fermentation process of water kefir. Most commonly, fresh or dried figs are used. Among all fruits, figs offer the best conditions for fermentation according to Reiß (1990). It has been found that leaving out the fig greatly slows down the intake of glucose and, consequently, the pace of fermentation, while substituting with other dried fruits (raisins, dates, and plums) affects the rate of lactic acid and acetic acid generation. In the study, an increase in water kefir grain mass was observed for 18 days, and a significant decrease was observed in all but fig-containing media. Moreover, biomass increase was noted lower in fruits and vegetables such as bananas, raisins, plums, apricots, potatoes, and carrots, while no increase was observed in apple, pasteurized grape juice, and milk (Reiß, 1990). However, it has also been noted by Bueno et al. (2021) that water kefir grains are highly adaptable to various food sources and can be utilized to create a wide range of fermented beverages.

Dried figs have high calcium content. A total of 100 g of fresh figs contain 35 mg of calcium, while 100 grams of dried figs contain 162 mg of calcium (USDA, 2019). Since calcium is another important factor promoting grain growth, the effect of fig on water kefir fermentation and biomass increase can be understood more clearly. Additionally, it is anticipated that hard waters with higher levels of calcium and magnesium ions will be better suited for water kefir fermentation given their capacity as buffers and the beneficial effects of calcium concentration on fermentation and grain growth (Lynch et al., 2021). The increasing role of calcium content in water kefir grain biomass is attributed to glucan biosynthesis. Calcium in water kefir fermentation affects glucan synthesis by changing glucan sucrose activity (Pendon et al., 2021).

Glucan sucrases have a calcium-binding site near their active center and their activity is regulated by calcium ions (Vujičić-Žagar et al., 2010).

As sources of fermentation, in addition to fruits and vegetables including carrots, ginger, fennel, and onions (Fiorda et al., 2017), dairy products such as cow's milk and goat's milk (Hsieh et al., 2012), as well as milk substitutes like soy, have also been used. In a study in which water kefir grains were inoculated into brown sugar, cow's milk and goat's milk, it was observed that the grains contain many different microorganisms that can adapt to different environments. Therefore, the distribution of strains during fermentation may vary depending on the available carbon and energy sources (Hsieh et al., 2012). In South America, a drink called "Tepache" that contains brown sugar, pineapple, and cinnamon fermented with water kefir grains is quite popular (Fiorda et al., 2017; Fuente-Salcido et al., 2015). The origin of the grains, their diversity, and the dominant strains are all greatly influenced by the carbon and nitrogen sources (Hsieh et al., 2012; Marsh et al., 2013; Miguel et al., 2011). Alves et al. (2021) analyzed the microbial diversity of water kefir grains produced with water-soluble coconut extract fortified with inulin, and reported species were never previously reported in water kefir before such as *Lactobacillus uvarum*, *Gluconobacter albidus*, *Lasiodiplodia brasiliensis*. *Oenococcus* species, which are claimed to come from the surface of fresh or dried fruit added during water kefir fermentation, are also suggested to contribute to its microbial diversity (Verge et al., 2020).

6 | POTENTIAL EFFECTS OF WATER KEFIR ON HEALTH

The potential health-promoting properties of water kefir are influenced by a number of variables, including the kind of substrate, fermentation temperature, and time. Most of the beneficial effects in milk kefir are related to the fermentative action of LAB and yeasts. Despite the difference in the substrate, water kefir also consists of a similar type of microbiota, suggesting that water kefir has the potential for beneficial effects like milk kefir (Lynch et al., 2021). Many studies have reported the nutritional and health benefits of regular milk kefir consumption (Koyu & Demirel, 2018; Nejati et al., 2020; Rosa et al., 2017). However, few studies have examined the beneficial health effects of water kefir.

Studies examining various properties of microorganisms isolated from water kefir grains (such as non-pathogenicity, tolerance to gastrointestinal conditions, adhesion to the gastrointestinal mucosa, ability to colonize, and competitive exclusion of pathogens) have reported that these microorganisms have different probiotic properties (Golowczyc et al., 2011; Rodrigues et al., 2005; Schneedorf, 2012; Soccol et al., 2010). Therefore, the positive properties of water kefir on health are associated with the presence of probiotic microorganisms (Marsh et al., 2013). In this context, the presence of *Bifidobacteria* in water kefir samples could be considered important. There are studies that report *Bifidobacterium psychraerophilum/crudilactis* presence in water kefir samples (Gulitz et al., 2013; Hsieh et al., 2012; Marsh et al.,

2013). *Bifidobacteria* are anaerobic, obligatory bacteria that produce more acetate than lactate. For this reason, metabolic body activities may increase due to the high acetate concentration that may occur in water kefir fermentation (Laureys & De Vuyst, 2014). Also, *Bifidobacteria* is one of the most beneficial probiotic microorganisms that supports the immune system (Laureys et al., 2016).

The functional qualities of the probiotic microorganisms in water kefir are directly related to the quantity consumed. In this context, immunomodulatory and anti-inflammatory (Diniz et al., 2003; Rodrigues et al., 2016), anti-carcinogenic (Zamberi et al., 2016), anti-hyperglycemic and anti-hyperlipidemic (Alsayadi et al., 2014; Rocha-Gomes et al., 2018; Rosa et al., 2017), antioxidant (Alsayadi et al., 2013; Kumar et al., 2021; Ozcelik et al., 2021), antimicrobial (Gonda et al., 2019; Romero-Luna et al., 2020; Silva et al., 2009) effects of water kefir have been demonstrated in various studies.

Considering the studies and the data obtained, it should not be concluded that a particular water kefir can provide all these benefits, as each water kefir is produced from different grains and as a result of different fermentation conditions that affect the microbial/chemical composition. The FAO/WHO definition of probiotic is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). As highlighted in the definition, the health benefits of any probiotic product are dependent on the administration of an adequate number of living microbes in the ingested formulation (typically defined as CFU per dose). Probiotic bacteria have a positive impact on the immunological and digestive benefits, but robust and systematic clinical investigations are required to substantiate their alleged health advantages. In addition, it should not be ignored that the probiotic effects are strain specific (Wright, 2019). Identification of strains in microbiota is extremely important for the safety, growth conditions, and metabolic characteristics of a particular strain (FAO/WHO, 2002).

7 | WATER KEFIR IN FOOD LEGISLATION

Water kefir is a popular beverage that promotes good health (Hsieh et al., 2012). Although a lot of semi-commercial, commercial water kefir can be obtained in different markets across the world, home-made water kefir accounts for the majority of this consumption. Water kefir is not included in the food legislation of most countries and commonly sold as a "traditional beverage." However, in recent years, regulations regarding probiotic strains in the world have reached water kefir (Moretti et al., 2022). As a result, it has become necessary to include it in food legislation in the countries where it is consumed. Water kefir is included in the legislation of some international organizations. In Australia, water kefir is classified in the fermented soft drink category of "Soft Drinks and Fermented Soft Drinks" (Food Standards Australia, 2016). The product is detailed as a fermentation process made from sugar, water, and one or more fruit or vegetable extracts or infusions, and is declared to contain no more than 1.1% alcohol by volume. The FDA considers water kefir as one of the items that might provide a plant-based replacement to milk, along with other

plant-based milk substitutes (FDA, 2021). Argentina has reported that water kefir should be included in the codex (Comisión Nacional de Alimentos, 2021). Water kefir is manufactured industrially and sold on the market in countries including the United States, Belgium, Italy, and Argentina (Moretti et al., 2022). In Turkey, only a few companies have commercially offered water kefir grains and water kefir beverages, but there is no mention of water kefir in the Turkish Food Codex.

8 | CONCLUSION AND RECOMMENDATIONS

Water kefir, which is a functional, fermented, and probiotic beverage, continues its importance from the past to present due to its beneficial effects on health. Water kefir emerges as an alternative source of probiotics for individuals with lactose intolerance, people with dairy allergic reactions, or those who prefer not to consume milk and dairy products. In addition to the symbiotic relationship between the lactic acid bacteria, acetic acid bacteria, and yeast, the microbiota of the water kefir grain can be influenced by the time of fermentation, temperature, the presence of oxygen, and the type and concentration of sucrose. Although consumer demands have driven the food industry to increase the production of water kefir, the most common production still relies on the traditional method due to some difficulties in the industrial-scale production process. Considering the potential health benefits of water kefir due to the probiotic microorganisms it contains, it is noteworthy that *in vitro* and *in vivo* studies on water kefir are insufficient. It should be commercialized and included in the category of "Non-Alcoholic Drinks" in the food legislations.

ACKNOWLEDGEMENT

This review includes sections from the master's thesis of Ayşe Nur Erdinc, one of the authors of the article.

ETHICS STATEMENT

None declared.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

ORCID

Gizem Cufaoglu  <https://orcid.org/0000-0001-8639-532X>

Ayşe Nur Erdinc  <https://orcid.org/0000-0002-6597-0548>

REFERENCES

- Alsayadi, M., Al-Jawfi, Y., Belarbi, M., & Sabri, F. Z. (2013). Antioxidant potency of water kefir. *The Journal of Microbiology, Biotechnology and Food Sciences*, 2(6), 2444–2447.
- Alsayadi, M., Al-Jawfi, Y., Belarbi, M., Soualem-Mami, Z., Merzouk, H., Sari, D. C., Sabri, F., & Ghalim, M. (2014). Evaluation of anti-hyperglycemic and anti-hyperlipidemic activities of water kefir as probiotic on streptozotocin-induced diabetic Wistar rats. *Journal of Diabetes Mellitus*, 4(2), 85–95. <https://doi.org/10.4236/jdm.2014.42015>
- Alves, V., Scapini, T., Camargo, A. F., Bonatto, C., Stefanski, F. S., Jesus, E. P., & Treichel, H. (2021). Development of fermented beverage with water kefir in water-soluble coconut extract (*Cocos nucifera* L.) with inulin addition. *LWT—Food Science and Technology*, 145, 111364. <https://doi.org/10.1016/j.lwt.2021.111364>
- Azizi, N. F., Kumar, M. R., Yeap, S. K., Abdullah, J. O., Khalid, M., Omar, A. R., Osman, M. A., Mortadza, S. A. S., & Alitheen, N. B. (2021). Kefir and its biological activities. *Foods*, 10(6), 1210. <https://doi.org/10.3390/foods10061210>
- Blaiotta, G., Di-Capua, M., Romano, A., Coppola, R., & Aponte, M. (2014). Optimization of water curing for the preservation of chestnuts (*Castanea sativa* Mill.) and evaluation of microbial dynamics during process. *Food Microbiology*, 42, 47–55. <https://doi.org/10.1016/j.fm.2014.02.009>
- Bueno, R. S., Ressutite, J. B., Hata, N. N., Henrique-Bana, F. C., Guergoletto, K. B., Oliveira, A. G., & Spinosa, W. A. (2021). Quality and shelf-life assessment of a new beverage produced from water kefir grains and red pitaya. *LWT—Food Science and Technology*, 140, 110770. <https://doi.org/10.1016/j.lwt.2020.110770>
- Comisión Nacional de Alimentos. (2021). Expedientes CONAL. www.conal.gob.ar/expedientes_conal.php
- Côté, G. L., Skory, C. D., Unser, S. M., & Rich, J. O. (2013). The production of glucans via glucansucrases from *Lactobacillus satsumensis* isolated from a fermented beverage starter culture. *Applied Microbiology and Biotechnology*, 97(16), 7265–7273. <https://doi.org/10.1007/s00253-012-4606-Y>
- Davidović, S. Z., Miljković, M. G., Antonović, D. G., Rajilić-Stojanović, M. D., & Dimitrijević-Branković, S. I. (2015). Water kefir grain as a source of potent dextran producing lactic acid bacteria. *Hemijška Industrija*, 69(6), 595–604. <https://doi.org/10.2298/HEMIND140925083D>
- Diniz, R. O., Garla, L. K., Schneedorf, J. M., & Carvalho, J. C. T. (2003). Study of anti-inflammatory activity of Tibetan mushroom, a symbiotic culture of bacteria and fungi encapsulated into a polysaccharide matrix. *Pharmacological Research*, 47(1), 49–52. [https://doi.org/10.1016/S1043-6618\(02\)00240-2](https://doi.org/10.1016/S1043-6618(02)00240-2)
- Diosma, G., Romanin, D. E., Rey-Burusco, M. F., Londero, A., & Garrote, G. L. (2014). Yeasts from kefir grains: Isolation, identification, and probiotic characterization. *World Journal of Microbiology and Biotechnology*, 30(1), 43–53. <https://doi.org/10.1007/s11274-013-1419-9>
- Fels, L., Jakob, F., Vogel, R. F., & Wefers, D. (2018). Structural characterization of the exopolysaccharides from water kefir. *Carbohydrate Polymers*, 189, 296–303. <https://doi.org/10.1016/j.carbpol.2018.02.037>
- Fiorda, F. A., de Melo-Pereira, G. V., Thomaz-Soccol, V., Rakshit, S. K., Pagnoncelli, M. G. B., de Souza-Vandenbergh, L. P., & Soccol, C. R. (2017). Microbiological, biochemical and functional aspects of sugary kefir fermentation. *Food Microbiology*, 66, 86–95. <https://doi.org/10.1016/j.fm.2017.04.004>
- Food and Agriculture Organization/World Health Organization. (2002). *Guidelines for the evaluation of probiotics in food*. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food.
- Food and Drug Administration. (2021) Enhancing Retailer Standards in the Supplemental Nutrition Assistance Program (SNAP). <https://www.fns.usda.gov/snap/fr-121516>
- Food Standards Australia New Zealand. (2021). Standard 2.6.2 Non-alcoholic beverages and brewed soft drinks. <https://www.foodstandards.gov.au/code/Documents/2.6.2%20Non-alco%20drinks%20v157>
- Fuente-Salcido, N. M., Castañeda-Ramírez, J. C., García-Almendárez, B. E., Bideshi, D. K., Salcedo-Hernández, R., & Barboza-Corona, J. E. (2015). Isolation and characterization of bacteriocinogenic lactic bacteria from M-Tuba and tepache, two traditional fermented beverages in México. *Food Science and Nutrition*, 3(5), 434–442. <https://doi.org/10.1002/fsn3.236>
- Galli, A., Fiori, E., Franzetti, L., Pagani, M. A., & Ottogalli, G. (1995). Microbiological and chemical composition of sugar kefir grains. *Annali Di Microbiologia Ed Enzimologia*, 45(1), 85–95.

- Garofalo, C., Ferrocino, I., Reale, A., Sabbatini, R., Milanović, V., Alkić-Subašić, M., Boscaino, F., Aquilanti, L., Pasquini, M., Trombetta, M. F., Tavoletti, S., Coppola, R., Coccolin, L., Blesić, M., Sarić, Z., Clementi, F., & Osimani, A. (2020). Study of kefir drinks produced by back-sloping method using kefir grains from Bosnia and Herzegovina: microbial dynamics and volatile profile. *Food Research International*, 137, 109369. <https://doi.org/10.1016/j.foodres.2020.109369>
- Golowczyc, M. A., Silva, J., Teixeira, P., De Antoni, G. L., & Abraham, A. G. (2011). Cellular injuries of spray-dried *Lactobacillus* spp. isolated from kefir and their impact on probiotic properties. *International Journal of Food Microbiology*, 144(3), 556–560. <https://doi.org/10.1016/j.ijfoodmicro.2010.11.005>
- Gonda, M., Garmendia, G., Rufo, C., León-Peláez, Á., Wisniewski, M., Droby, S., & Vero, S. (2019). Biocontrol of *Aspergillus flavus* in ensiled sorghum by water kefir microorganisms. *Microorganisms*, 7(8), 253. <https://doi.org/10.3390/microorganisms7080253>
- Gulitz, A., Stadie, J., Ehrmann, M. A., Ludwig, W., & Vogel, R. F. (2013). Comparative phylobiomic analysis of the bacterial community of water kefir by 16S rRNA Gene amplicon sequencing and ARDRA analysis. *Journal of Applied Microbiology*, 114(4), 1082–1091. <https://doi.org/10.1111/jam.12124>
- Gulitz, A., Stadie, J., Wenning, M., Ehrmann, M. A., & Vogel, R. F. (2011). The microbial diversity of water kefir. *International Journal of Food Microbiology*, 151(3), 284–288. <https://doi.org/10.1016/j.ijfoodmicro.2011.09.016>
- Guzel-Seydim, Z. B., Gökirmaklı, Ç., & Greene, A. K. (2021). A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. *Trends in Food Science and Technology*, 113, 42–53. <https://doi.org/10.1016/j.tifs.2021.04.041>
- Horisberger, M. (1969). Structure of the dextran of the Tibi grain. *Carbohydrate Research*, 10(3), 379–385. [https://doi.org/10.1016/S0008-6215\(00\)80897-6](https://doi.org/10.1016/S0008-6215(00)80897-6)
- Hsieh, H. H., Wang, S. Y., Chen, T. L., Huang, Y. L., & Chen, M. J. (2012). Effects of cow's and goat's milk as fermentation media on the microbial ecology of sugary kefir grains. *International Journal of Food Microbiology*, 157(1), 73–81. <https://doi.org/10.1016/j.ijfoodmicro.2012.04.014>
- Kamarli-Altun, H., Karacil-Ermumcu, M. S., & Seremet-Kurklu, N. (2021). Evaluation of dietary supplement, functional food and herbal medicine use by dietitians during the COVID-19 pandemic. *Public Health Nutrition*, 24(5), 861–869. <https://doi.org/10.1017/S1368980020005297>
- Kebler, L. F. (1912). California bees. *The Journal of the American Pharmaceutical Association*, 10(12), 939–943. <https://doi.org/10.1002/jps.3080101206>
- Koyu, E. B., & Demirel, Z. B. (2018). Fonksiyonel bir besin: kefir. *Beslenme ve Diyet Dergisi*, 46(2), 166–175. <https://doi.org/10.33076/2018.BDD.301>
- Kumar, M. R., Yeap, S. K., Mohamad, N. E., Abdullah, J. O., Masarudin, M. J., Khalid, M., Leow, A. T. C., & Alitheen, N. B. (2021). Metagenomic and phytochemical analyses of kefir water and its subchronic toxicity study in BALB/c mice. *BMC Complementary Medicine and Therapies*, 21(1), 1–15. <https://doi.org/10.1186/s12906-021-03358-3>
- Laureys, D., Aerts, M., Vandamme, P., & De Vuyst, L. (2018). Oxygen and diverse nutrients influence the water kefir fermentation process. *Food Microbiology*, 73, 351–361. <https://doi.org/10.1016/j.fm.2018.02.007>
- Laureys, D., Aerts, M., Vandamme, P., & De Vuyst, L. (2019). The buffer capacity and calcium concentration of water influence the microbial species diversity, grain growth, and metabolite production during water kefir fermentation. *Frontiers in Microbiology*, 10, 2876. <https://doi.org/10.3389/fmicb.2019.02876>
- Laureys, D., Cnockaert, M., De Vuyst, L., & Vandamme, P. (2016). *Bifidobacterium aquikefiri* sp. nov., isolated from water kefir. *International Journal of Systematic and Evolutionary Microbiology*, 66(3), 1281–1286. <https://doi.org/10.1099/ijsem.0.000877>
- Laureys, D., & De Vuyst, L. (2014). Microbial species diversity, community dynamics, and metabolite kinetics of water kefir fermentation. *Applied and Environmental Microbiology*, 80(8), 2564–2572. <https://doi.org/10.1128/AEM.03978-13>
- Laureys, D., & De Vuyst, L. (2017). The water kefir grain inoculum determines the characteristics of the resulting water kefir fermentation process. *Journal of Applied Microbiology*, 122, 719–732.
- Laureys, D., Leroy, F., Hauffman, T., Raes, M., Aerts, M., Vandamme, P., & De Vuyst, L. (2021). The type and concentration of inoculum and substrate as well as the presence of oxygen impact the water kefir fermentation process. *Frontiers in Microbiology*, 12, 628599. <https://doi.org/10.3389/fmicb.2021.628599>
- Laureys, D., Van-Jean, A., Dumont, J., & De Vuyst, L. (2017). Investigation of the instability and low water kefir grain growth during an industrial water kefir fermentation process. *Applied Microbiology and Biotechnology*, 101(7), 2811–2819. <https://doi.org/10.1007/s00253-016-8084-5>
- Lutz, L. (1899). Recherches Biologiques Sur la Constitution du Tibi. *Bulletin de la Société Mycologique de France*, 15, 68–72.
- Lynch, K. M., Wilkinson, S., Daenen, L., & Arendt, E. K. (2021). An update on water kefir: Microbiology, composition and production. *International Journal of Food Microbiology*, 345, 109128. <https://doi.org/10.1016/j.ijfoodmicro.2021.109128>
- Magalhães, K. T., de Pereira, G. V. M., Dias, D. R., & Schwan, R. F. (2010). Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. *World Journal of Microbiology and Biotechnology*, 26(7), 1241–1250. <https://doi.org/10.1007/s11274-009-0294-x>
- Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2013). Sequence-based analysis of the microbial composition of water kefir from multiple sources. *FEMS Microbiology Letters*, 348(1), 79–85. <https://doi.org/10.1111/1574-6968.12248>
- Martínez-Torres, A., Gutiérrez-Ambrocio, S., Heredia-del-Orbe, P., Villatanaca, L., & Hernández-Rodríguez, C. (2017). Inferring the role of microorganisms in water kefir fermentations. *International Journal of Food Science and Technology*, 52(2), 559–571. <https://doi.org/10.1111/ijfs.13312>
- Miguel, C. P. M. G., Cardoso, P. G., Magalhães, K. T., & Schwan, R. F. (2011). Profile of microbial communities present in Tibico (sugary kefir) grains from different Brazilian States. *World Journal of Microbiology and Biotechnology*, 27(8), 1875–1884. <https://doi.org/10.1007/s11274-010-0646-6>
- Moretti, A. F., Moure, M. C., Quiñoy, F., Esposito, F., Simonelli, N., Medrano, M., & León-Peláez, Á. (2022). Water kefir, a fermented beverage containing probiotic microorganisms: From ancient and artisanal manufacture to industrialized and regulated commercialization. *Future Foods*, 5, 100123. <https://doi.org/10.1016/j.fufo.2022.100123>
- Nejati, F., Junne, S., & Neubauer, P. A. (2020). Big world in small grain: A review of natural milk kefir starters. *Microorganisms*, 8(2), 192. <https://doi.org/10.3390/microorganisms8020192>
- Neve, H., & Heller, K. J. (2002). The microflora of water kefir: A glance by scanning electron microscopy. *Kieler Milchwirtschaftliche Forschungsberichte*, 54(4), 337–349.
- Ozcelik, F., Akan, E., & Kinik, O. (2021). Use of Cornelian cherry, hawthorn, red plum, rosehip and pomegranate juices in the production of water kefir beverages. *Food Bioscience*, 42, 101219. <https://doi.org/10.1016/j.fbio.2021.101219>
- Pendón, M. D., Bengoa, A. A., Iraporda, C., Medrano, M., Garrote, G. L., & Abraham, A. G. (2021). Water kefir: Factors affecting grain growth and health-promoting properties of the fermented beverage. *Journal of Applied Microbiology*, 133, 162–180. <https://doi.org/10.1111/jam.15385>
- Pidoux, M., Brillouet, J. M., & Quemener, B. (1988). Characterization of the polysaccharides from a *Lactobacillus brevis* and from sugary kefir grains. *Biotechnology Letters*, 10(6), 415–420.
- Pidoux, M., Marshall, V. M., Zannoni, P., & Brooker, B. (1990). *Lactobacilli* isolated from sugary kefir grains capable of polysaccharide production and minicell formation. *The Journal of Applied Bacteriology*, 69(3), 311–320. <https://doi.org/10.1111/j.1365-2672.1990.tb01521.x>

- Pidoux, M. (1989). The microbial flora of sugary kefir grain (the gingerbeer plant): Biosynthesis of the grain from *Lactobacillus hilgardii* producing a polysaccharide gel. *MIRCEN Journal of Applied Microbiology and Biotechnology*, 5(2), 223–238.
- Randazzo, W., Corona, O., Guarcello, R., Francesca, N., Germanà, M. A., Erten, H., Moschetti, G., & Settanni, L. (2016). Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. *Food Microbiology*, 54, 40–51. <https://doi.org/10.1016/j.fm.2015.10.018>
- Reiß, J. (1990). Metabolic activity of Tibi grains. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 191(6), 462–465. <https://doi.org/10.1007/BF01193095>
- Rocha-Gomes, A., Escobar, A., Soares, J. S., Silva, A. A., Dessimoni-Pinto, N. A. V., & Riul, T. R. (2018). Chemical composition and hypocholesterolemic effect of milk kefir and water kefir in Wistar rats. *Revista de Nutrição*, 31(2), 137–145.
- Rodrigues, K. L., Araújo, T. H., Schneedorf, J. M., Souza-Ferreira, C., Oliveira-Isac-Moraes, G., Coimbra, R. S., & Rodrigues, M. R. (2016). A novel beer fermented by kefir enhances anti-inflammatory and anti-ulcerogenic activities found isolated in its constituents. *Journal of Functional Foods*, 21, 58–69. <https://doi.org/10.1016/j.jff.2015.11.035>
- Rodrigues, K. L., Gaudino-Caputo, L. R., Tavares-Carvalho, J. C., Evangelista, J., & Schneedorf, J. M. (2005). Antimicrobial and healing activity of kefir and kefir extract. *International Journal of Antimicrobial Agents*, 25(5), 404–408. <https://doi.org/10.1016/j.ijantimicag.2004.09.020>
- Romero-Luna, H. E., Peredo-Lovillo, A., Hernández-Mendoza, A., Hernández-Sánchez, H., Cauich-Sánchez, P. I., Ribas-Aparicio, R. M., & Dávila-Ortiz, G. (2020). Probiotic potential of *Lactobacillus paracasei* CT12 isolated from water kefir grains (Tibicos). *Current Microbiology*, 77(10), 2584–2592. <https://doi.org/10.1007/s00284-020-02016-0>
- Rosa, D., Dias, M., Grześkowiak, Ł., Reis, S., Conceição, L., & Peluzio, M. (2017). Milk kefir: Nutritional, microbiological and health benefits. *Nutrition Research Reviews*, 30(1), 82–96. <https://doi.org/10.1017/S0954422416000275>
- Sarikka, P., Nitisoravut, R., Poljungreed, I., & Boonyarattanakalin, S. (2015). Identification of bacteria and yeast communities in a Thai sugary kefir by Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) analyses. *The Journal of Industrial Technology*, 11(2), 25–39.
- Schneedorf, J. M. (2012). Kefir d'aqua and its probiotic properties. E. Rigobelo (Ed.), *Probiotic in animals* (pp. 53–76). London: IntechOpen.
- Silva, K. R., Rodrigues, S. A., Filho, L. X., & Lima, Á. S. (2009). Antimicrobial activity of broth fermented with kefir grains. *Applied Biochemistry and Biotechnology*, 152(2), 316–325. <https://doi.org/10.1007/s12010-008-8303-3>
- Soccol, C. R., Souza-Vandenberghe, L. P., Spier, M. R., Medeiros, A. B. P., Yamaguchi, C. T., De Dea-Lindner, J., Pandey, A., & Thomaz-Soccol, V. (2010). The potential of probiotics. *Food Technology and Biotechnology*, 48(4), 413–434.
- Stadie, J., Gulitz, A., Ehrmann, M. A., & Vogel, R. F. (2013). Metabolic activity and symbiotic interactions of lactic acid bacteria and yeasts isolated from water kefir. *Food Microbiology*, 35(2), 92–98. <https://doi.org/10.1016/j.fm.2013.03.009>
- United States Department of Agriculture, Food Data Central. (2019). <https://fdc.nal.usda.gov/fdc-app.html#/query%20=%20fig>
- Verge, M., De Vuyst, L., & Weckx, S. (2019). Shotgun metagenomics of a water kefir fermentation ecosystem reveals a novel *Oenococcus* species. *Frontiers in Microbiology*, 10, 479. <https://doi.org/10.3389/fmicb.2019.00479>
- Verge, M., De Vuyst, L., & Weckx, S. (2020). The metagenome-assembled genome of *Candidatus Oenococcus aquikefiri* from water kefir represents the species *Oenococcus siceræ*. *Food Microbiology*, 88, 103402. <https://doi.org/10.1016/j.fm.2019.103402>
- Vujičić-Žagar, A., Pijning, T., Kralj, S., López, C. A., Eeuwema, W., Dijkhuizen, L., & Dijkstra, B. W. (2010). Crystal structure of a 117 kDa glucanase fragment provides insight into evolution and product specificity of GH70 enzymes. *Proceedings of the National Academy of Sciences*, 107(50), 21406–21411.
- Ward, H. M. V. (1892). The ginger-beer plant, and the organisms composing it: A contribution to the study of fermentation-yeasts and bacteria. *Philosophical Transactions of the Royal Society of London (B.)*, 183, 125–197. <https://doi.org/10.1098/rstb.1892.0006>
- Wright, A. V. (2019). Safety assessment of probiotics in the European Union. G. Vinderola, A. Ouwehand, & S. Salminen (Eds.), *Lactic acid bacteria: Microbiological and functional aspects* (pp. 723–734). CRC Press. <https://doi.org/10.1201/9780429057465>
- Zamberi, N. R., Abu, N., Mohamed, N. E., Nordin, N., Keong, Y. S., Beh, B. K., Zakaria, Z. A. B., Nik-Abdul-Rahman, N. M. A., & Alitheen, N. B. (2016). The antimetastatic and antiangiogenesis effects of kefir water on murine breast cancer cells. *Integrative Cancer Therapies*, 15(4), NP53–NP66. <https://doi.org/10.1177/1534735416642862>
- Zanirati, D. F., Abatemarco-Jr, M., Cicco-Sandes, S. H., Nicoli, J. R., Nunes, Á. C., & Neumann, E. (2015). Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starter or probiotic cultures. *Anaerobe*, 32, 70–76. <https://doi.org/10.1016/j.anaerobe.2014.12.007>

How to cite this article: Cufaoğlu, G., & Erdinc, A. N. (2023).

An alternative source of probiotics: Water kefir. *Food Frontiers*, 4, 21–31. <https://doi.org/10.1002/fft2.200>