Agronomy Science and Biotechnology

Review article

Characterization of milk and water-based kefir microbiota

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Citation: Destro, T. M., Souza Junior, H., Spinosa, W. A., & Scherer, A. (2024). Characterization of water and milk-based kefir microbiota. Agronomy Science and Biotechnology, *10*, 1-19 https://doi.org/10.33158/ASB.r202.v10.2024

Received: February 7, 2024.
Accepted: March 16, 2024.
Published: September 19, 2024.

English by: Helio de Souza Junior and Tainá Miranda Destro

Abstract

The production of functional foods is expanding and requiring research results in the areas of food, health, chemistry, pharmaceuticals, etc. Within this context, the inclusion of kefir in the diet has been gaining ground, as it meets the requirements of a functional food. Kefir can be defined as a fermented, acidic milk with a low alcohol content and produced from grains that contain microorganisms that carry out the fermentation process. Kefir can be produced using various means, such as milk or water-based substrates. In both process, the production is very similar. Kefir is composed of microorganisms such as bacteria and yeasts that are often welcomed by the population as a probiotic source and are regularly used in the production of food and/or medicine by the industry. To produce water kefir, sucrose is used, and the process is carried out at temperatures ranging from 20°C to 25°C, with an incubation time between 12 and 72 hours in this context. The objective of this work was to present a literature review on the characterization of milk and water-based kefir.

Keywords: Fermented drink; tibicos; functional foods; *Lactobacillus;* acetic acid; bacteria.

Introduction

Kefir is defined by Brazilian legislation as fermented milk, acidic flavor, with a low alcohol content and produced from grains that contain microorganisms that carry out the fermentation process. There are two main types of kefir, what differentiates one from the other is the substrate, one being water-based and the other milk-based. Milk has an estimated origin in 2000 BCE (Before the Common Era), with shepherds from the Caucasus Mountains, and its use spread to other regions after arriving in Russia in 1908. Commercially, it is found in countries such as Russia, Turkey, United States, Canada and France (Abraham & Antoni, 1999; Food and Agriculture Organization / World Health Organization [FAO/WHO], 2002).

The drink has been a success, gaining popularity in several locations around the world, with the annual market estimated at 78.7 million euros in the USA alone (Marsh, Hill, Ross, & Cotter, 2014a; Marsh, Sullivan, Hill, Ross, & Cotter, 2014b). Kefir is widely explored, especially in Eastern Europe. For example, in Russia, produced from bovine milk, it is manufactured by such dairy industries as Danone ("Activia Kefir" and "Danone Kefir"), Samaralakto ("BioBalance", "Prostokvashino" and "Dr. Brandt"), Ufamolagroprom (Veselyimolochnik) and Lianozovo ("Domik v derevne") (Enikeev, 2012).

The word Kefir is derived from keif or kef, which originated in Turkey and means "well-being". It is the product of a starter culture in a synergistic system called mutualism, where the efficiency of the microorganisms that carry out the fermentation process is optimized. The efficiency of cultivation can be proven when the microorganisms present in kefir are isolated and purified and these, in isolation, reduce its growth and metabolic activity.

Yeasts develop after acidification of the medium by bacteria, which, in turn, have their growth stimulated by the production of growth factors (vitamins) and soluble nitrogenous compounds, produced by yeasts (Monar, Dávalos, Zapata, Caviedes, & Ramírez-Cárdenas, 2014). This symbiosis can also be observed, between lactic acid bacteria (LAB) and yeast, in the fermentation process of other traditional drinks, such as Shubat, made from camel milk, and gari and cauim, made from cassava (Magalhães, Pereira, Dias, & Schwan, 2010). This work focuses on the literature review about kefir, its microbiota, probiotic properties and production in the water substrate.

Kefir produced with water is similar to kefir produced with milk. They are both fermented by a symbiosis between bacteria and yeasts contained in the "grains". Also known as tibi, tibicos, sugar kefir, California bees, African bees, balm of Gilead, beer brew, Japanese beer seeds, Tibetan crystals, sweetened kefir grains, prophet grains, and others. The origin of kefir from water is uncertain. The first scientific description was of "ginger beer grains", published by Ward in 1892, which were used by British soldiers in the Crimean War in 1855. In 1899, Lutz described grains called "tibi", originating in Mexico, as granules fermented from the sap of the Opuntia cactus and were collected from the leaves of the plant (Laureys & Vuyst, 2017).

Fermentation occurs by adding water, cactus grains, sugar, figs and lemon to improve the flavor and provide nutrients for the fermentation process (Miguel, Cardoso, Magalhães, & Schwan, 2011; Stadie, Gullitz, Ehrmann, & Vogel, 2013; Marsh et al., 2014a,b; Pidoux, 1989; Waldherr, Doll, Meibner, & Vogel, 2010; Horisberger, 1969).

Beneficial properties of kefir

There are reports in the literature about the benefits of fermented functional foods, such as kefir, due to the interaction of the ingested microbiota (probiotic effect) and the bioactive metabolites formed during fermentation (biogenic effect), such as vitamins, peptides, organic and fatty acids (Stanton, Ross, Fitzgerald, & Van, 2005).

In Brazil, ANVISA (Agência Nacional de Vigilância Sanitária [ANVISA], 2021), defines functional food as any food or ingredient that, in addition to basic nutritional functions, when consumed as part of the usual diet, produces metabolic and/or physiological effects beneficial to health (reduction of the risks of chronic non-communicable diseases), and must be safe for consumption without medical supervision (Zanirati et al., 2015).

ANVISA Resolutions No. 18 and 19, dated April 30, 1999, establish the basic guidelines for analyzing and proving functional properties in the food labeling procedure (ANVISA, 2021). Functional property is defined as: "metabolic or physiological role that the nutrient or non-nutrient has in the growth, development, maintenance and other normal functions of the human organism". Among the recognized functional foods and active compounds, we can mention omega-3 fatty acids, vegetable fibers, lycopene, lutein, zeaxanthin, resveratrol, carotenoids, flavonoids, phospholipids, organosulfurs, polyphenols, phytosterols, chitosan, psyllium, prebiotics and probiotics (ANVISA, 2021).

The results are obtained from functional foods through interaction with research bodies and industry. As it is a recent and expanding subject, the area of functional food production is being increasingly studied by professionals both in the food sector and in other related areas, such as health, chemistry and pharmaceuticals.

When evaluating foods with probiotic action, it is worth considering that half of the wet weight of colonic material is due to the presence of bacterial cells, which number exceeds 10 times the number of cells in the tissues that make up the human body. This is a consequence of the presence of almost 500 species of microorganisms in an individual's digestive tract, which continually compete for survival and maintain a balance that provides protection to the host's organism (FAO/WHO, 2002). This balance, however, can be disrupted, for example, through specific diets, intestinal infections, antibiotic treatment, chemotherapy or stressful situations (Fonseca & Costa, 2010).

The term probiotic derives from the Greek and means "pro-life". Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). To be effective, they must meet certain criteria, including non-pathogenic properties, resistance to technological processes, adhesion to epithelial tissues, stability in the presence of acid and bile, ability to persist in the gastrointestinal environment, influence metabolic activities and improve the immune system (Fonseca & Costa, 2010).

Data in the literature on the properties and functionality of live microorganisms in food have suggested that probiotics play an important role in immunological, digestive and respiratory functions. The dissemination of this information in the scientific community resulted in increased interest on the part of health professionals, merchants and consumers (FAO/WHO, 2002).

The increase in personal hygiene and environmental care contributed to the reduction of child mortality and diseases such as asthma and allergies and the increase in life expectancy. Within this context, it is important to highlight that our immune system needs to be constantly challenged by exposure to non-pathogenic microorganisms to increase our immunity (Forsythe, 2013). Consuming water kefir is a food option that meets this functionality.

Milk and water kefir microbiota

The composition of the kefir microbial population can vary depending on factors such as the origin of the grains, substrate and cultivation medium used for preparation (Marsh et al., 2014a,b; Pidoux, 1989). There is a smaller amount of acetic acid bacteria (AAB), and a predominance of lactic acid bacteria (LAB), in the proportion of 2 to 10 times the number of yeast cells (Laureys & Vuyst, 2017). Species of bacteria isolated from water and milk kefir are presented and can be found in the Table 1.

Table 1. Genera and species of bacteria isolated from water and milk kefir.

Genera of bacteria	Bacteria isolated from water kefir	Bacteria isolated from milk kefir	References
Acetobacter	*A. fabarium,* *A. lovaniensis,* *A. orientali*	*A. lovaniensis,* *A. orientalis,* *A. sicerae*	Laureys, Cnockaert, Vuyst, & Vandamme, 2016; Bourrie, Willing, & Cotter, 2016; Garofalo et al., 2015; Korsak et al., 2015; LI et al., 2014; Gulitz, Stadie, Ehrmann, Ludwig, & Vogel, 2013; Marsh et al., 2013; Gao et al., 2013; Gulitz, Stadie, Wenning, Ehrmann, & Vogel, 2011; Magalhães et al., 2010
Bifidobacterium		*B, brief,* *B. choerinum,* *B. longum,* *B. pseudolongum*	Marsh, O'Sullivan, Hill, Ross, & Cotter 2013; Dobson, Sullivan, Cotter, Ross, & Hill, 2011
Lactobacillus	*L. brevis, L. buchneri,* *L. married* *L. casei subsp. got married,* *L. casei subsp. rhamnosus,* *L. diolivorans,* *L. fermentum,* *L. harbinensis,* *L. collinoides* *L. fructiovorans,* *L. hilgardii,* *L. hordeii,* *L. kefiranofaciens,* *L. kefiri,* *L. lactis,* *L. mali,* *L. nagelli,* *L. parabuchneri* *L. paracasei,* *L. parafarraginis,* *L. pearlens,*	*L. acidophilus,* *L. brevis,* *L. buchneri,* *L. caseisubsp. pseudoplantarum,* *L. crispatus,* *L. curvatis* *L. delbrueckii,* *L. fermentum,* *L. helveticus,* *L gasseri,* *L. garvieae,* *L. instestinalis,* *L. kefiranofaciens,* *L. kefiri,* *L. kefirgranum,* *L. otakiensis, L.paracasei,* *L. parakefir,* *L. parabuchneri,* *L. plantarum,*	Fiorda, Pereira, Soccol, Rakshit, & Soccol, 2016; Bourrie et al., 2016; Laureys et al., 2016; Zanirati et al., 2015; Gulitz et al. 2013; Gulitz et al. 2011; Kesmen & Kacmaz, 2011); Magalhães et al. 2010; Sabir, Beyatli, Cokmus, & Onal-Darilmaz, 2010; Waldherr et al., 2010; Chen, Wang, & Chen, 2008; Witthuhn, Schoeman, & Britz, 2004; Simova et al., 2002; Garrote et al., 2001; Galli, Fiori, Franzetti, Pagani, & Ottogalli, 1995; Pidoux, 1989; Moinas,

	L. plantarum, *L. satsumensis.*	*L. rhamnosus, L. sake,* *L satsumensis* *L. sunkii,* *L. viridescens*	Horisberger, & Bauer, 1980
Leuconostoc	*L. citreum,* *L. mesenteroides*	*L. mesenteroides,* *L. pseudomesenteroides*	Fiorda et al., 2016; Bourrie et al., 2016; Gao et al., 2013; Gulitz et al., 2013; Kacmaz 2011; Gulitz et al., 2011; Sabir et al., 2010; Magalhaes et al., 2010; Waldherr et al., 2010; Garrote et al., 2001
Lactococcus		*L. cremoris,* *L. garvieae,* *L. lactis, L. raffinolactis*	Bourrie et al., 2016, Gao et al, 2013, Magalhaes et al., 2011, Kesmen & Kacmaz, 2011; Sabir et al., 2010; Yuksekdag, Beyatli, & Aslim, 2004
Pediococcus		*P. acidilactici,* *P. claussenii,* *P. damnosus,* *P. dextrinicus,* *P. halophilus, P. lolii,* *P. pentosaceus*	Nalbantoglu et al, 2014; Sabir et al., 2010.
Streptococcus		*S. durans,* *S. thermophilus*	Gao et al, 2013; Kacmaz, 2011; Chen et al., 2008; Yuksekdag et al., 2004; Simova et al., 2002
Other species	*Acinetobacter, Bifidobacterium psychraerophilumDekkeraanomala, Dekkerabruxellensis, Dysgonomonas, Enterobacter hormacheiGluconacetobacter, GluconobacterfrateuriLysinibacillussphaericus, Oenococcuskitaharae, OenococcusoeniPelomonas, Pseudomonas, Shewanella, Weissella, Zymomonas*		Fiorda et al., 2016; Zanirati et al., 2015; Nalbantoglu et al., 2014; Gulitz et al., 2013; Marsh et al., 2013; Gao et al., 2013; Waldherr et al., 2010

LAB bacteria are Gram-positive, non-sporulating, do not produce catalase and can carry out the process of fermenting carbohydrates to compounds such as lactic acid, acetic acid, alcohol and carbon dioxide. The formation of peptides with the bioactive action of bacteriocins also occurs. They prefer microaerophilic conditions, however, they are aero tolerant. Some health benefits have been attributed to LAB bacteria, such as: inhibition of pathogens and improvement of the immune system (Monar et al., 2014; Silva, Dutra, Silveira, Loures, & Bastos, 2018).

AAB belong to the Acetobacteraceae family, which includes several genera and species. These bacteria are strictly aerobic, Gram-negative, produce catalase and are rod-shaped, and can occur singly, in pairs or in chains. They are mesophilic microorganisms, and their optimal growth is situated between 25 and 30°C. They oxidize ethanol or sugar (glucose) to corresponding organic acids, such as acetic acid and gluconic acid, in the presence of oxygen (Gao et al., 2013).

The AAB play an important role in the production of foods and drinks, such as vinegar and Kombucha, but can be harmful to other foods such as wine, beer and fruit. They are also used in the production of other compounds such as gluconic acid, sorbose and bacterial cellulose (Raspor & Goranovič,2008). Species belonging to the genera Acetobacter, Gluconobacter, Gluconacetobacter (currently called Komagataeibacter) are mainly responsible for the production of vinegar due to their high capacity to oxidize ethanol to acetic acid (Yetiman, & Kesmen, 2015).

Yeasts are unicellular fungi that reproduce asexually by budding or gemmuletion and have a high physiological diversity, which makes them suitable for use in many products. Yeasts are chemoheterotrophic organisms and belong to a distinct group of organisms, including ascomycetes, basidiomycetes, and deuteromycetes (imperfect fungi). They obtain their energy from organic compounds and can grow on a wide range of carbon sources, including carbohydrates (most important in biotechnological use), alcohols, organic acids, amino acids, n-alkanes and lipids (Querol, & Fleet, 2006). They are 10 times larger than bacteria and can prevent the colonization of pathogenic bacteria, however they comprise less than 0.1% of the intestinal microbiota.

The yeast *Saccharomyces cerevisiae*, common in kefir grains, is associated with the production of B vitamins and some amino acids and the species *S. boulardii* is recognized as a probiotic species (Monar et al., 2014; Marsh et al., 2014a,b).

The metabolism of sugars occurs differently for monosaccharides, the metabolism of hexoses (glucose, fructose and mannose) is carried out mainly via the glycolytic pathway. The utilization of galactose requires the action of the Leloir pathway. The use of pentose is taxonomically restricted and requires activities of xylose reductase, xylitol dehydrogenase, xylulokinase and the pentose-phosphate pathway. The utilization of oligosaccharides and polysaccharides is initiated by hydrolysis to make their monosaccharide components available, and expression of the appropriate hydrolases may also be taxonomically restricted (Querol, & Fleet, 2006). The yeasts species isolated from water and milk kefir are presented in the Table 2. Comparing water and milk-based kefir, there is a stimulation of Saccharomyces and AAB species metabolism in the first one (Fiorda et al., 2016), and both present a strong dominance of *Lactobacillus* group.

Pyruvate formed by glycolysis can be fermented to ethanol or oxidized to CO_2 by the citric acid cycle. In both cases, the NADH produced by glycolysis is reoxidized to NAD^+. In fermentation, pyruvate is cleaved into acetaldehyde and CO_2 by pyruvate decarboxylase. The acetaldehyde then formed is reduced to ethanol by dehydrogenase. Acetaldehyde is also used in the production of acetyl-coenzyme A (CoA), which is used for the biosynthesis of lipids and amino acids (Querol, & Fleet, 2006).

Yeasts and their products have been used by the food industry and their consumption has been highlighted for its health benefits and nutritional aspects. Their products include: vitamins, sterols, carotenoids, lipids, enzymes, nucleic acids, polysaccharides, as well as modified compounds. As derivatives of yeast cell components, there are proteins modified chemically (acylated, phosphorylated enzymes, encapsulated and immobilized), physically (partially denatured or textured), enzymatically, partially digested by acid or enzymatic treatment (with amino acids, nucleotides and nucleosides covalently linked). In addition to flavoring substances that can replace salt or flavorings (Laureys et al., 2016).

Table 2. Genera and species of yeasts isolated from water and milk kefir.

Yeasts	Yeast isolates from water kefir	Yeast isolates from milk kefir	References
Candida	*C. kefir* *C. lipolytica* *C. valid*	*C. iconspicua, C. kefir, C. krusei, C. maris, C. lambica, C. valida*	Witthuhn et al., 2004; Witthuhn, Schoeman, & Britz, 2005; Simova et al., 2002; Pidoux, 1989
Sacharomyces	*S. bayanus, S. cerevisiae, S. florentinus, S. pretoriensis*	*S. cerevisiae, S. turicensis*	National Center for Biotechnology Information [NCBI], 2017; Fiorda et al., 2016; Laureys et al., 2016; Gulitz et al., 2013; Puerari, Magalhães, & Schwan, 2012; Magalhaes et al., 2010;Waldherr et al, 2010; Wang, Chen, Liu, Lin, & Chen, 2008; Simova et al., 2002; Galli et al., 1995
Pichia	*P. kudriavzevii, P. membranifaciens*	*P. fermentans*	Fiorda et al. 2016, Wang et al., 2008
Lanchancea	*L. fermentati, L. meyercii.*	*L. meyercii*	NCBI, 2017; Fiorda et al., 2016; Gulitz et al 2013; Magalhaes et al., 2011; Magalhaes et al., 2010
Kluyveromyces	*K. lactis, K. marxianus*	*K. lactis*	Puerari et al., 2012; Magalhães, Pereira, Campos, Dragone, & Schwan, 2011; Magalhães et al., 2010; Wang et al., 2008; Garrote et al., 2001
Kazachstania	*K. aerobia, K. unispora.*		Puerari et al., 2012; Magalhaes et al., 2010

leprosy	*H. uvarum,* *H. yalbyensis*		Fiorda et al., 2016; Gulitz et al., 2013; Franzetti, Galli, Pagani, & Noni, 1998
Other species	*Dekkera bruxellensis,* *Issatchenki aorientalis,* *Torulaspora,* *Zygosaccharomyces fermentati,* *Zygotorulaspora florentine*	*Cryptococcus humicolus,* *Geotrichum candidium,* *Zygosaccharomyces fermentati*	NCBI, 2017; Fiorda et al., 2016; Laureys et al., 2016; Gulitz et al 2013; Witthuhn et al., 2005; Neve et al., 2002

In the food industry that carries out fermentation processes, it is interesting that there is immobilization of the microorganisms involved, resulting in greater optimization of production. Examples of the procedures performed are increased separation rates of microbial cells at the end of the fermentation stage and accumulation of high concentrations of cells (Querol, & Fleet, 2006).

Prior to information on the nutritional and growth requirements of microorganisms, it was believed that less than 1% of microorganisms present in different environments could be cultivated under artificial conditions (Hugenholt et al., 2013; Lewis, Epstein, D'onofrio, & Ling, 2010). Currently, information obtained in literature and research using molecular techniques, 16S rRNA gene sequences and others, contributes to this advancement in the area of microbiology and food production using microorganisms. Several methods can be used together to increase discriminatory power, such as DNA-DNA hybridization, sequencing of protein-coding genes and some specific taxonomic parameters. Currently, more specific methods that use DNA-fingerprinting techniques, based on the polymerase chain reaction (PCR), such as the amplification of repetitive elements of bacterial DNA (rep-PCR), highlighting the (GTG)5-PCR, generate a highly complex banding pattern, distinguishes species, subspecies and even lineages (Felis & Dellaglio, 2007; Gevers, Huys, & Swings, 2001).

Kefir samples collected from a project enrolled in the National Center for Biotechnology Information (2017) from the United Kingdom, Canada and the USA were subjected to genetic sequencing and revealed that the bacterial portion of each water kefir and its respective grains was dominated by the genus Zymomonas. This bacterium produces ethanol and had not previously been detected in other analyzes (Catchmark et al., 2012). The other genera detected were ALB, AAB, Lactobacillus, Leuconostoc, Acetobacter and Gluconacetobacter. In the analysis of the fungal component, the genera Dekkera, Hanseniaspora, Saccharomyces, Zygosaccharomyces, Torulasporae and Lachancea were found (NCBI, 2017).

Water kefir grains

Kefir grains are not generally sold in Brazil. They are composed of an insoluble matrix of exopolysaccharides (EPS), a homogeneous polymer of D-glucose with α 1-6 glycosidic bonds, called dextran, analogous to the so-called kefiran produced by kefir cultured in milk. Kefiran is produced by LAB, especially Lactobacillus hilgardii. This bacterium is also present in the fermentation of wines and cocoa and contributes to the formation of grains, through the production of extracellular enzymes called glycosyltransferases (Monar et al., 2014; Laureys & Vuyst, 2017).

The genera Komagataeibacter and Gluconacetobacter can also produce EPS and this process can be detected by the formation of a gelatinous film produced on the surface of liquid media, when cultivated in static conditions, or by the appearance of spherical and/or irregular masses, up to 10 mm in size, cultivated under agitated conditions (Catchmark et al., 2012). Gulitz et al. (2013) observed that *Lactobacillus casei*, *Lb. hordei*, *Lb. nagelii*, *Lb. hilgardii* and *Lc. Mesenteroides* could produce EPS using sucrose as the source.

The grains are translucent, gelatinous, irregular in size, have an average diameter of 3 to 35 mm and tend to settle at the bottom of the container (Figure 1). Some grains rise to the surface due to the accumulation of CO_2 and descend again when the gas is lost. They increase in size and divide in the culture medium, resulting in a greater number of cells to be immobilized. Fission is determined by internal CO_2 pressure and the gas can leave the grains hollow. Its chemical composition (% weight/weight) is 89-90% water, 0.2% lipids, 3.0% proteins, 6.0% carbohydrates and 0.7% ash. They can be preserved by freezing, freeze-drying and refrigeration (Pidoux, 1989; Monar et al., 2014; Silva et al., 2018).

Figure 1. Water-based kefir grains.
Source: Photo of the authors.

Pidoux (1989) suggests that the polysaccharide can be used as a gelification agent and Waldherr et al (2010) state that the high production of glucans offers many industrial applications that have not yet been explored in food and biotechnology area. It is possible to distinguish in the grains a more compact outer layer with many microorganisms surrounded by dextran and a spongy internal structure (Moinas et al., 1980) (Figure 2 and 3).

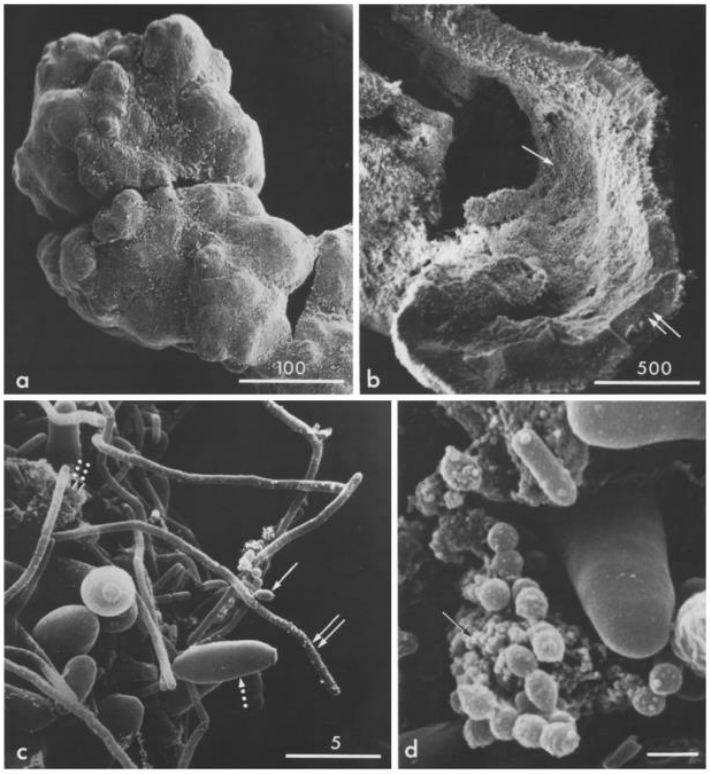

Figure 2. Scanning electron micrographs of water-based kefir grains.(**a**) Grain in the fission process; (**b**) Fractured grain with its internal spongy structure (arrow) and an external compact layer (double arrow); (**c**) Streptococci (arrow), lactobacilli (double arrow), yeast (dotted arrow) and dextran (double dotted arrow); (**d**) Streptococci incorporated into dextran (arrow). The bar represents 1µm or a multiple of it.
Source: Moinas et al., 1980.

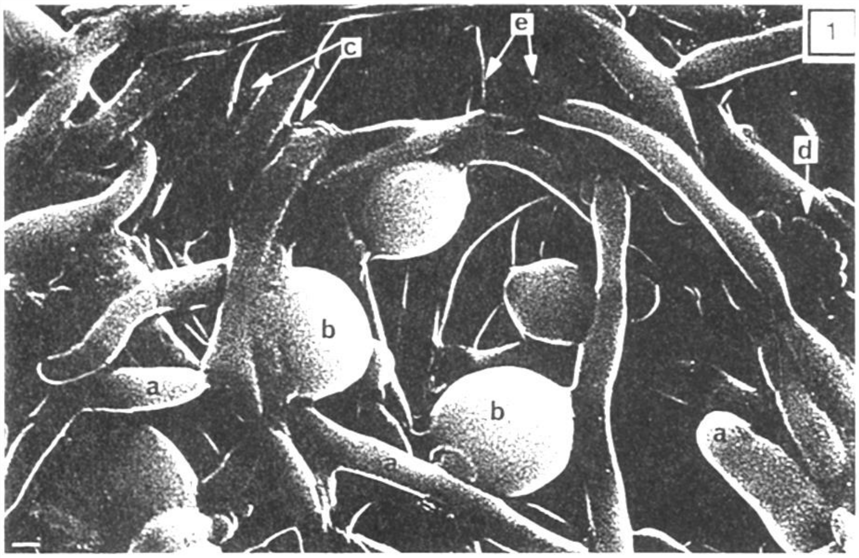

Figure 3. Scanning electron microscopy of the outside of a sweetened kefir grain. Network of pseudomycete (a), blastospores (b), cocci (c) and bacilli (d) held together by an adhesive substance. The bar represents 1µm.
Source: Pidoux, 1989.

Fermentation by water kefir grains

The fermentation process can be defined as a set of reactions that involve oxidation of glucose into pyruvic acid (glycolysis) and its reduction. Depending on the organic molecule that is produced from pyruvic acid, it can be classified as lactic (lactic acid) or alcoholic (ethanol), which can be oxidized and form acetic acid, by the bacterium *Acetobacter aceti* under aerobic conditions (Nelson & Cox, 2014). Microorganisms, when fermenting a food, add properties that make it more nutritious, tastier, flavored, in addition to increasing its shelf life (He et al., 2017).

The main metabolites produced during water kefir fermentation are ethanol, lactic acid, glycerol, acetic acid and mannitol; and the aroma-forming compounds are 2-methyl-1-propanol, isoamyl alcohol, ethyl acetate, isoamyl acetate, ethyl hexanoate and ethyl octanoate (Laureys & Vuyst, 2017).

Fermentation of beverages with health-promoting properties is native to many regions of Asia, Africa, Europe, the Middle East, and South America. Evidence from ceramic vessels shows that fermented rice, honey, and fruit beverages date back to 7000 a.C. in China (Steensels & Verstrepen, 2014; Marsh et al., 2014a,b).

Some fermented drinks provide beneficial effects through direct and indirect microbial probiotic action through the production of metabolites and breakdown of complex proteins. For example, metabolites such as organic acids can lower the pH of beverages (Marsh et al., 2014a,b; Alsayadi et al., (2014), carried out studies with rats with streptozotocin-induced diabetes and concluded that water kefir can be used as a food for diabetics, aiming to control glucose and lipid levels.

Antioxidant activity is increased thanks to the addition of microorganisms to foods, when they favor the fermentation process, due to the metabolites formed (Alsayadi, Jawfi, Belarbi, & Sabri, 2013).

Water kefir production

To prepare water kefir, sucrose is used as the main substrate for microorganisms. Between 3 and 10% (m/v) of sugar is used, with around 8% being more common. It is recommended to use unrefined sugars, such as brown sugar, which contributes to greater grain growth. Due to this, Silva et al., (2018) tested the antimicrobial activity of kefir using demerara sugar, cane molasses and brown sugar, and concluded that brown sugar was more effective against the pathogenic microorganisms *Candida albicans*, *Salmonella typhi*, *Shigella sonnei*, *Staphylococcus aureus* and *Escherichia coli*.

The drink resulting at the end of the sugar fermentation process is cloudy, straw-colored, with a slightly acidic flavor (formation mainly of lactic acid and acetic acid), fermented, refreshing and with a low alcohol content, with low effervescence and sweetness. The incubation time generally varies between 12 and 72 hours, with the grains entering the stationary phase after 48 hours of fermentation. The most used fermentation temperature is ambient, most commonly between 20 and 25^0C (Alsayadi et al., 2014; Silva et al., 2018). You can see the components for preparing water kefir in the Table 3.

Table 3. Composition of water kefir formulations.

Water	Sugar	Kefir grains	others	Source
85%	10%	2%	-	Januário et al., 2016
1000 mL	-	40 g	50 mL carrot, melon, onion, tomato, fennel and strawberry juice	Corona et al., 2016
1000 mL	-	40 g	50 mL apple, quince, pomegranate, grape, kiwi and prickly fig juice	Randazzo et al., 2016
300 mL	22.5 g (7%)	18 g (5.6%)	-	Monar et al., 2014
500 ml	8.5 g (1.7%)	Three tablespoons	-	Federhen & Ruschel, 2014
65 mL	6 g (8.5%)	15 g (21%)	20 mL fig extract	Laureys et al., 2016,
88.5%	6.5%	5%	5 g/L fresh apple pieces	Alsayadi et al., 2014
93%	6.5%	5%	5 g/L apple pieces	Alsayadi et al., 2013
1000 ml	80 g (7.4%)	Not specified	100 mL fig extract	Gulitz et al., 2013
1000 mL	100 g	Not specified	2 dehydrated figs and 1 organic lemon slice	Gulitz et al., 2011
2138 mL	112ml (5%)	250 g (11%)	-	Magalhães et al., 2010
95%	5%	5 g	-	Bergmann et al., 2010
1000 ml	80 g (7.4%)	60 g (5.6%)	half a fresh lemon and a dehydrated fig	Waldherr et al., 2010
2000 ml	60g (0.34%)	Not specified	2 dehydrated figs and 1 sliced lemon	Moinas et al., 1980

Final comments

The consumption of fermented functional foods has presented many health benefits. Among these foods, kefir stands out, a fermented drink produced from grains that contain microorganisms that carry out the fermentation process.

Kefir can be produced using a water or milk-based substrate. Water-based can be prepared using substrates such as fruit and vegetable juices and peels. The use of fruit and vegetable peels and even the production of juices can be an option to add value to rural producers' agribusinesses and products that are often discarded can be used to produce kefir on a large scale. Both water and milk-based kefir contain health-beneficial microorganisms such as lactic acid bacteria and yeasts.

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