**Biochemical Insights into kefir: The Role of Yeasts and Bacteria in Health Benefits**

**Aida García García**

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**Academic tutor:** Dr. Francesc Puiggròs Llavinés, francesc.puiggros@urv.cat.

**In cooperation with:** U4IMPACT and Danone

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## Abstract

Kefir is a traditional fermented dairy beverage produced through the action of a complex microbiota, primarily composed of lactic acid bacteria and yeasts. This microbial diversity is responsible for its distinctive biochemical composition and a wide range of health-related properties, including improvements in gut health, immune function, antioxidant status, and metabolic regulation. This bibliographic review compiles and updates recent scientific evidence on the composition and functional effects of dairy kefir, highlighting the role of its microbial and bioactive components. Although kefir is defined as a fermented milk product, the increasing variety of products labelled as “kefir” does not always follow this definition, which may lead to differences in composition and potential health benefits. A better understanding and characterization of kefir is essential to support its role as a functional food.

**Key words:** Kefir, fermented foods, microbiota, probiotics, intestinal health, yeasts, dairy fermentation, kefiran, health benefits.

## Resumen

El kéfir es una bebida láctea fermentada tradicional producida mediante la acción de una microbiota compleja, compuesta principalmente por bacterias lácticas y levaduras. Esta diversidad microbiana es responsable de su distintiva composición bioquímica y de una amplia gama de propiedades beneficiosas para la salud, incluyendo mejoras en la salud intestinal, la función inmunitaria, el estado antioxidante y la regulación metabólica. Esta revisión bibliográfica recopila y actualiza la evidencia científica reciente sobre la composición y los efectos funcionales del kéfir lácteo, destacando el papel de sus componentes microbianos y bioactivos. A pesar de que el kéfir se define como un producto lácteo fermentado, la creciente variedad de productos etiquetados como "kéfir" no siempre se ajusta a esta definición, lo que puede generar diferencias en su composición y posibles beneficios para la salud. Una mejor comprensión y caracterización del kéfir es esencial para respaldar su papel como alimento funcional.

**Palabras clave**: Kéfir, bebidas fermentadas, microbiota, probióticos, salud intestinal, levaduras, fermentación de lácteos, kefiran, beneficios para la salud.

## 1. Introduction

Kefir is a type of dairy product obtained through the fermentation of milk by the action of microorganisms, resulting in a reduction in pH. The cultures of microorganisms used must be viable, active and abundant in the product until the date of minimum duration except if the product is thermally treated after fermentation (Organización de las Naciones Unidas para la Alimentación y la Agricultura, 2022). The starter cultures depend on the type of fermented milk that you want to produce so that the starter culture for kefir is prepared with kefir grains that generally contain *Lactobacillus kefiri*, species of the genus *Leuconostoc*, *Lactococcus* and *Acetobacter,* among other possible species used (FAO & OMS, 2022).

Kefir has its origin in the Caucasus region of Asia and is characterized by being a slightly alcoholic fermented milk with a slightly viscous texture and an acidic touch. Traditionally, milk fermentations were carried out by spontaneous fermentations or by back-slopping. However, during the great expansion that kefir has had during the last 10 years in global markets, its production has been standardized using a mixture of non-traditional and traditional starter cultures to try to maintain adequate organoleptic properties in all batches (Bintsis & Papademas, 2022). It is important to remark that, despite containing small amounts of ethanol due to fermentation, kefir is still considered a non-alcoholic beverage under most food regulations, as its alcohol content typically remains below 1.2% (v/v) (Konuspayeva et al., 2023).

The use of different methods of analysis including both traditional microbiology with plate culture techniques and other molecular biology techniques has demonstrated the complex microbiota of kefir granules that include a wide mix of bacteria and yeasts.

This fermented milk can be made with milk from different animal sources such as cow, buffalo, or goat, thus conferring different microbiota profiles. However, the analysis of kefir from different countries in Europe and America shows that although there are differences between the microbiota of kefir milk and grains, the populations present in fermented milk are more homogeneous than in the grains from which they were produced (Gentry et al., 2023).

Kefir grains have an irregular shape of globular structures with a diameter of 2-9mm.They are composed of the exopolysaccharide known as kefiran, proteins and different microbial cells. The microbial composition of these granules is complex and the presence of 4 different groups has been demonstrated: heterofermentative bacteria, homofermentative bacteria, lactose assimilating yeasts and lactose non-assimilating yeasts (Gentry et al., 2023). This structure and the distribution of the microbiota within it are important to understand the functions of the microbiota and the interactions between the different populations. All the species within the kefir grains interact in a way that allows direct and indirect interactions. In the direct interactions, physical connections facilitate cooperation, leading to formation of structures such as biofilms. On the other hand, indirect interactions occur through the action of extracellular metabolites impact, which impact on other components of the microbial consortium, the establishment of quorum sensing, an activity that continues to be a potential area of research (Nejati et al., 2020).

Traditional kefir production is carried out by adding kefir granules to pasteurized milk and letting it ferment at room temperature for 1-4 days. This way of producing only allows small quantities and the ability to scale production is limited. In industrial production, kefir is produced by isolated cultures of kefir grains that reproduce the flavor but exclude the effervescence of yeasts and heterofermentative cultures. It also increases production, improves reproducibility and avoids the need to filter the kefir grains in the final product (Gentry et al., 2023).

There are other methods of commercial kefir production such as the "Russian method", which stands out for producing kefir in a more traditional way on a large scale in a more traditional way. This involves using a mother culture or starter culture inoculated into milk, and in the following productions, a small amount of the previous fermentation is used to start the next fermentation, also known as back-slopping (Gentry et al., 2023).

In commercial production, food safety is a priority, and yeasts can have negative effects due to the excessive production of carbon dioxide and ethanol during secondary fermentation. This overproduction can modify the flavor of the kefir and lead to the swelling of the containers during storage (Lee et al., 2020).

The selection of strains is essential to produce initial cultures that maintain the desired characteristics of aroma, shelf life and functional benefits of the product.

Therefore, in the industry the production of initial cultures has also been studied by replacing the yeasts with probiotic strains of bacteria (Lee et al., 2020).

As a fermented food, kefir has a long tradition and has generated increasing interest in recent years due to its potential health benefits. Unlike other products, kefir contains a microbial diversity that includes bacteria and yeasts, giving it unique probiotic characteristics that favor digestion and intestinal health, among other positive effects. This microbial diversity is one of the key factors that distinguishes it from other fermented foods, such as yogurt, and gives it additional functional properties. However, the absence of specific legislation that determines what kefir is and the requirements it must meet at a functional and nutritional level generates uncertainty regarding its authenticity and market value. As a result, products labeled as "kefir" are being marketed that do not align with the traditional definition, including non-dairy alternatives such as water kefir or tea kefir, which creates confusion among consumers and production companies.

Therefore, it is essential to study and analyze the differences between traditional kefir, which includes both bacteria and yeast, and those versions without yeast. This analysis can not only clarify the specific properties of kefir, but also, can contribute to the understanding of its impact on the intestinal microbiota and human health in general. With the increasing consumption of fermented products, the scientific compilation on kefir both with yeasts and without them, is key to help establish clearer regulations, guarantee product quality and optimize its characterization and standardization in the food industry.

As previously mentioned, kefir has generated increasing attention in scientific research due to its wide variety of beneficial health effects. As a fermented food, it is recognized for its probiotic, antimicrobial, and immunomodulatory properties, which are its most widely documented benefits and are primarily associated with improved intestinal health, immune system modulation, and infection prevention. However, additional, more specific effects have also been identified that can have a significant impact on human health. These include antioxidant and anticancer properties, effects on wound healing, reduction in cholesterol levels (hypocholesterolemia), influence on the renin-angiotensin system (ACE system), and improvement in lactose digestion, among others, indicating kefir's potential to contribute to health in a complex way

(Bourrie et al., 2016; Dimidi et al., 2019; John & Deeseenthum, 2015; Lolou & Panayiotidis, 2019; Prado et al., 2015a; Rosa et al., 2017; Sharifi et al., 2017).

Despite the evidence presented in the various reviews, these studies highlight the need for further research to understand the mechanisms responsible for these effects. More specifically, they highlight the need to characterize the microbial strains involved, as well as the variability found in the designs of both *in vitro* and *in vivo* studies in animals and humans. Furthermore, while numerous benefits have been identified, associating each microorganism or metabolite remains a challenge.

While the understanding of kefir's health benefits has advanced significantly up until 2019, recent research may have expanded upon this foundation, allowing for a deeper insight into the molecular and biochemical mechanisms behind these effects.

Therefore, this review aims to update the current scientific knowledge of kefir by evaluating whether recent studies have advanced our understanding of the mechanisms underlying its health benefits, improved the characterization of the microorganisms involved, and refined experimental methodologies

From a biochemical perspective, this work aims to deepen the molecular and biochemical aspects of kefir, offering a comprehensive understanding of its composition and its effects on health.

## 2. Hypothesis

Recent scientific knowledge has consolidated and expanded the evidence supporting the health benefits of kefir consumption, particularly in relation to intestinal function, metabolic regulation, and immune response, with these effects being attributed to its bioactive compounds and microbiological composition.

## 3. Objective

Main objective:

This bibliographic review aims to compare and update current knowledge on the health benefits of consuming dairy kefir, focusing on its impact on multiple aspects of human health including improvements in intestinal, metabolic, and immune functions, as well as other physiological effects, and how its microbiological and bioactive composition influences these outcomes.

Secondary objectives:

1. Describe the nutritional and microbiological composition of milk kefir.

The biochemical characteristics of dairy kefir will be reviewed, with special attention to lactic acid bacteria, yeasts, and other microorganisms, as well as its nutritional profile and the role of these components in health benefits.

2. Review the effects of dairy kefir consumption on intestinal, metabolic, and immune health.

Review the mechanisms of action proposed in studies up until 2020 and comparting them with updated findings from 2020-2025.

3. Examine the labeling of commercially available dairy kefir products.

This objective will focus on analyzing the nutritional and microbiological information provided on the labels of commercial dairy kefir products. It will assess how these products are marketed, the health claims made, and how the composition is highlighted to appeal to consumer preferences, particularly with regard to probiotics, functionality, and health benefits.

## 4. Methods

The methodology used for this bibliographic review consisted of a systematic search for articles in databases such as PubMed and Scopus. The search was conducted in March 2025 using the advanced search tools available on these platforms and applying different keyword combinations, including: *"dairy kefir" and "health"*, “kefir and cholesterol”, *"kefir and health promotion"*, *"milk kefir"*, *"beneficial effects of kefir"*, “kefir and gut microbiota”, and *" kefir and health improvement"*.

Different inclusion and exclusion criteria were applied to ensure the relevance and quality of the selected studies. Specifically, since the objective of this review is to compile current scientific knowledge, the search was restricted to articles published between 2020 and 2025, ensuring that only studies from the last five years were considered. Given the large number of results obtained, the selection was further refined by prioritizing review articles to provide a more comprehensive understanding of the topic.

Additionally, only studies published in English or Spanish were included. To ensure accessibility, preference was given to free full-text articles and those available through the Digital Library Access Service (SABiDi), provided by the CRAI of Rovira i Virgili University. Studies that did not specifically address the relationship between kefir, its composition, and its health effects, as well as those outside the scope of fermented dairy products, were excluded.

A total of 20 review articles published between 2020 and 2025 were selected based on different keyword combinations. These articles were chosen to analyze the biochemical composition of kefir, evaluate its potential health benefits, and examine how its classification as a fermented dairy product influences these benefits, considering its microbial composition.

Additionally, other bibliographic sources that do not meet the inclusion and exclusion criteria for the comparative analysis have been consulted. These sources have been essential for introducing and understanding the research topic, providing a broader context on fermented foods and their impact on health

## 5. Results and Discussion

### 5.1. Nutritional and microbiological composition of kefir

To fully understand the health potential of kefir it is essential to examine its nutritional and microbiological composition. Although traditional kefir is made from cow's milk, kefir can also be produced using milk from other livestock, such as goats, sheep, buffalo, camels, or even donkeys, (Guzel-Seydim et al., 2021), resulting in slight variations in nutritional content.

The nutritional composition of kefir depends primarily on the type of milk used, the kefir grains or culture mixture, the additives used, and processing methods used in its production (Gul et al., 2015).. However, a typical composition includes at least 2.7% protein, 0.6% lactic acid, and less than 10% fat, depending on the type of milk (Prado et al., 2015b). Likewise, the presence of alcohol, protein, fat, and ash levels is influenced by the kefir grain levels and the fermentation pH (Farag et al., 2020).

The high nutritional value of kefir is due to its diverse biochemical composition, which includes proteins, prebiotic oligosaccharides, exopolysaccharides (EPS), minerals, vitamins, fats, and other bioactive metabolites such as bacteriocins (Hikmetoglu et al., 2020; Leite et al., 2013)

Kefir is a good source of amino acids such as valine, isoleucine, methionine, serine, threonine, phenylalanine, tryptophan, alanine, and lysine, which play an important role in the central nervous system. Furthermore, the proteins present in kefir can be partially digested, which enhances their absorption and bioavailability in the body (Simova et al., 2006). Moreover, milk fermentation leads to a decrease in lactose concentration and an increase in beta-galactosidase activity (Saleem et al., 2023), thereby improving digestibility in lactose-intolerant individuals.

On the other hand, the vitamin content of kefir is notable and depends not only on the quality of the milk used but also on the metabolic activity of the microorganisms involved in the fermentation process (Rosa et al., 2017). Kefir contains vitamins B1, B2, B5, C, A, K, and carotene. Similarly, the concentration of vitamin B12, folic acid, biotin, thiamine, riboflavin, and pyridoxine increase as fermentation progresses (Sarkar, 2008).

Among its minerals, kefir stands out as a great source of magnesium, calcium, and phosphorus. Other trace minerals such as zinc, copper, manganese, iron, cobalt, and molybdenum are also present (Rosa et al., 2017).

In addition, lactic acid, $CO_2$, and ethanol are the main end products of the fermentation. Other compounds also contribute to kefir’s aroma and flavour profile, including acetic acid, pyruvic acid, hippuric acid, propionic acid, butyric acid, diacetyl and acetaldehyde (Ahmed et al., 2013).

Beyond its nutritional value, the microbial composition of kefir plays an important role in shaping its functional properties and probiotic potential. This complex and diverse microbiota also directly influences the nutritional composition of kefir by producing bioactive compounds, synthesizing vitamins and modifying macronutrient profiles during fermentation, thereby enhancing its health benefits.

The microbiota present in kefir includes numerous species of bacteria, as well as yeasts and filamentous fungi that develop complex symbiotic associations. In this relationship, yeasts produce vitamins, amino acids, and other growth factors essential for bacterial growth. In turn, the metabolic products of the bacteria serve as an energy source for the yeasts, establishing a symbiotic relationship throughout the fermentation cycle (Rosa et al., 2017).

The estimated number of different microbial species in kefir exceeds 300, and this number varies depending on the origin of the kefir grains and the specific techniques used in its production. Table 1 lists some of the main species involved in kefir production.

## 5.2. Health-related benefits of kefir consumption

### 5.2.1 Digestive and Gatrointestinal Health

The impact of kefir and its microorganisms has been evaluated to identify their effects on the microbiota and gastrointestinal health. Kefir can modulate the microbiota by increasing and decreasing various bacterial taxa, contributing to the maintenance of a healthy gut microbiota (Dimidi et al., 2019). As mentioned by Yılmaz et al. (2018), studies in patients with inflammatory bowel disease showed that strains isolated from kefir, such as *Lactobacillus kefiri*, can establish themselves in the microbiota; after daily consumption of 800 ml/day for 4 weeks, the presence of *L. kefiri* in total stool increased. Also, Bekar et al. (2011), demonstrated how kefir consumption can contribute to the eradication of *Helicobacter pylori*. In this study, kefir was associated with a greater elimination of *H. pylori* compared to the control group, along with a reduction in diarrhea, abdominal pain, and nausea. However, other studies have failed to show significant improvements in symptoms (Dimidi et al., 2019; Merenstein et al., 2009), which may indicate that there are major limitations in the design of studies and differences in the microbial composition of the kefir used, which may explain these heterogeneous results.

Table 1. Microorganisms found in kefir.

| Group | Type | Species |
|---|---|---|
| Bacteria | Lactobacili | *Lactobacillus acidophilus, Lactobacillus amylovorus, Lactobacillus apis, Lactobacillus crispatus, Lactobacillus delbrueckii, Lactobacillus fomicalis, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus gigierioum, Lactobacillus jensenii, Lactobacillus kalixensis, Lactobacillus kitasatonis, Lactobacillus ultunensis, Lactobacillus otakiensis, Lactobacillus sunkiii, Levilactobacillus brevis, Lentilactobacillus kefiri, Ligilactobacillus salivarius, Limosilactobacillus reuteri, Lactiplantibacillus pentosus, Lacticaseibacillus rhamnosus* |
| | Lactococci | *Lactococcus cremoris*, Lactococcus garvieae, Lactococcusvlactis subsp. cremoris |
| | Streptococci | *Streptococcus durans*, Streptococcus faecalis, Streptococcus thermophilus |
| | Acetic acid bacteria | Acetobacter aceti, Acetobacter fabarum, Acetobacter genera, Acetobacter lovaniensis, Acetobacter orientalis, Acetobacter pasteurianus, Acetobacter syzygii, Gluconobacter japonicus, Gluconobacter morbifer |
| Yeast | | *Hanseniaspora uvarum, Kazachstania aquatica*, *Kazachstania* aerobia, *Kazachstania* servazzii, *Kazachstania* solicola, *Kazachstania* turicensis, Saccharomyces cariocanus, Saccharomyces servazzii |
| Other probiotics | | *Pediococcus halophilus,* Pedicoccus lolii, Pediococcus pentosaceus, Lysinibacillus sphaericus, Enterococcus species |

In recent years, the study of the effect of fermented foods on the microbiota has experienced a boom. Therefore, a wide range of literature has been obtained that ratifies the beneficial effects of kefir suggested before 2020. Efforts have been made to understand the different mechanisms by which kefir exerts beneficial health effects (Gates et al., 2022). Van de Wouw et al. (2020), confirm the effects suggested by Yılmaz et al. (2018), showing that the consumption of kefir increases bacterial diversity, promoting the prevalence of *L. Reuteri* and *Bifidobacterium pseudolongum*, which are related to the increase in the capacity of the microbiota to produce glutamate and gamma-aminobutyric (GABA), a neurotransmitter involved in the regulation of anxiety and stress responses. At the same time, it decreases the prevalence of others as *Bacillus Amyloliquefaciens* and *Candidatus Arthomitus*, both related with the increase in regulatory T cells.

Furthermore, although there are few data on the effect of specific probiotics on healthy humans, a randomized trial carried out by Toscano et al. (2017), showed that those fed with *L. kefiri* modulated the composition of their microbiota, leading to a reduction in bacteria directly associated with the beginning of the pro-inflammatory response and gastrointestinal diseases.

Regarding the effects of kefir in disease conditions, Dahiya & Nigam (2023), showed that the presence of probiotics in functional drinks, such as kefir, can restore the dysbiosis of the intestinal microbiota induced by antibiotic therapy. Likewise, a consumption of 140 mg/kg of kefir has demonstrated lower activities of biochemical markers of liver lesion, especially for non-alcoholic fatty liver (NAFLD), such as glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase in serum (H.-L. Chen et al., 2014).

Teruya et al. (2013), suggest that the supernatant of the kefir and dehydrated kefir powders can have radioprotective effects on the mucosa of the small intestine, suggesting that they can alleviate the effect of radiation therapy against cancer. Also, the oral administration of kefir could avoid diarrhea and enterocolitis caused by *Clostridium difficile* (Bolla et al., 2013).

In addition, the modulation of the microbiota demonstrated by kefir seems to affect metabolic parameters, characteristic of the metabolic syndrome, showing improvements in fasting insulin levels and the insulin resistance rate, as well as a decrease in proinflammatory cytokines such as TNF-α and IFN-γ, and reductions in both systolic and diastolic pressure. A relation has also been found between these parameters and the intestinal microbiota: a weight gain and the BMI is positively correlated with the relative abundance of *Firmicutes* and *Proteobacteria*, while it negatively correlates with the abundance of Clostridia. Correlations between microbiota and fat mass, waist circumference, LDL-cholesterol, homocysteine, insulin and blood pressure were also demonstrated (Bellikci-Koyu et al., 2019).

Evidence demonstrates that the potential effects of probiotics are strain- and host-dependent. Therefore, it is necessary to study specific strains with healthy subjects in human population (Lazda et al., 2020). For instance, (Toscano et al., 2017) conducted a randomized trial showing that the consumption of *Lactobacillus kefiri* isolated from kefir led to significant reduction in bacterial genera associated with gastrointestinal disorders. This highlights the importance of selecting and characterizing individual strains when evaluating probiotic effects.

Additionally, Wang et al., (2020) studied a well-characterized kefir and found a reduction in abdominal pain, distension, and appetite in male subjects. After stopping kefir consumption, *Bifidobacteria* levels increased, and in women, total anaerobic bacteria and total gastrointestinal bacteria also increased, suggesting that kefir consumption could improve gastrointestinal function.

Although the consumption of fermented milk is known to modulate the intestinal microbiota, this effect is generally attributed to the specific probiotics it contains. However, it is difficult to determine the individual influence of each microbial strain used. Nevertheless, the beneficial role of the modulation of the intestinal microbiota in human health becomes evident when consuming fermented dairy products with probiotics (Abd El-Salam et al., 2025).

It is also important to note that, since artisanal kefir is mainly selected for kefir studies, only a few studies report findings on industrial kefir, which remains a limiting factor when comparing types of kefirs (Vieira et al., 2021).

Therefore, kefir is a promising tool for the modulation of microbiota in health cases, but we can also highlight the potential positive effects by achieving eubiosis, which can help regulate different factors, such as weight gain, insulin resistance and blood pressure. These, in turn, are related to chronic diseases that involve a great expense for public health, such as cardiovascular disease, obesity and diabetes.

However, further studies with well-characterized kefir are essential to associate the effects of certain microorganisms and metabolites present, although this remains a challenge due to the wide variety of microorganisms present in this functional food.

### 5.2.2 Immune system

Immunomodulatory and anti-inflammatory effects

Multiple diseases, such as cancer, obesity, and diabetes have been associated with inflammation, which is the main reason of the study about the immunomodulatory effects of probiotics (Rosa et al., 2017).

In kefir's case, the immunomodulatory properties may arise from both the action of the microbiota and the various bioactive compounds produced during fermentation. These effects have been evaluated in several studies, such as J. Liu et al., (2006) and Rosa et al., (2017), who demonstrated that kefir consumption can reduce the inflammatory process in rats with edema and granuloma induction. Likewise, they suggested that kefir has potential in preventing food allergies because it may reduce intestinal permeability to food-borne antigens. Furthermore, it has been demonstrated that *Lactobacilli* isolated from kefir can suppress pro-inflammatory cytokines while promoting pro-inflammatory cytokines (Bourrie et al., 2016; Rosa et al., 2017). On the other hand, the cell-free fraction of kefir can also modulate the immune system, demonstrating *in vitro* its ability to reduce the activation of Caco-2-ccl20:luc cells, IL-1β or TNF-α (Iraporda et al., 2014)). A possible mechanism involves immune modulation similar to that induced by a 100 mM lactic acid solution, which reduces the activation of NF-κB and the expression of proinflammatory cytokines without affecting the normal function of enterocytes (Iraporda et al., 2014). Thus, kefir has immunomodulatory and anti-inflammatory properties, which are not only attributed to its microorganisms but also due to its metabolites present after fermentation.

Building upon these earlier findings, more recent studies have expanded our understanding of kefir's immunomodulatory effects, particularly focusing on the role of specific bioactive compounds.

Recent years' evidence confirms the anti-inflammatory and immunomodulatory properties of kefir. Thus, recent studies, such as that conducted by Liao et al. (2023), demonstrates how the EPS of kefir have anti-inflammatory properties, mitigating lipopolysaccharide-induced IL-6 secretion. Furthermore, oral administration of these compounds has shown suppression of the expression of inflammatory signalling molecules, demonstrating therapeutic potential through inhibition of the NF-kB/MAPK pathways, suggesting a promising tool for the treatment of inflammatory disorders (Liao et al., 2023).

Numerous inflammatory conditions have been studied to evaluate the effect of kefir supplementation. A notable example is the research conducted by Nascimento da Silva et al. (2023), evaluating kefir supplementation in a mouse model of dextran-induced colitis. In this study, kefir promoted epithelial barrier restoration through the presence of short-chain fatty acids and mitigated the effects of the inflammatory cascade, as evidenced by the reduction in neutrophil accumulation and reticular edema. Furthermore, the therapeutic potential of kefir peptides was also evaluated. They were shown to attenuate adjuvant-induced arthritis (AIA) in animal models and reduce bone erosion by modulating the NF-κB and MAPK pathways (Chuang et al., 2023).

The production of bioactive compounds such as kefiran and extracellular vesicles has been linked to various beneficial effects, making the genus *Lactobacillus* particularly relevant for their synthesis (Vieira et al., 2021). However, although several studies in animals have demonstrated the anti-inflammatory effect of the exopolysaccharides produced in kefir, including kefiran, can have an immunostimulatory effect in cases with no inflammatory insult (G. Vinderola et al., 2006). The dose of EPS used is very important; depending on the concentration, they can either stimulate or inhibit the secretion of TNF-alpha, IL-10 and IL-6 (You et al., 2020).

Therefore, the concentration and environmental context (presence or absence of inflammatory stress) are relevant factors to consider depending on the purpose of administering the bioactive compound.

Overall, kefir has been shown to have significant immunoregulatory capacity. However, considering that the production of different bioactive compounds depends on the strains present in kefir grains and that different results are obtained depending on the administered concentration of each compound, further research is needed to obtain optimal results in relation to the production of these compounds by specific microorganisms and their effect on the human body.

Antioxidant

The body has several antioxidant mechanisms to deal with the reactive oxygen species to which we are exposed. Several studies have sought to evaluate the antioxidant properties of kefir, such as J.-R. Liu et al., (2005), who demonstrated that kefir can neutralize 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and superoxide radicals after several weeks of consumption. Additionally, other studies have also shown that kefir can demonstrate an antioxidant effect superior to that of vitamin E against carbon tetrachloride toxicity (Güven et al., 2003). These findings suggest that kefir has potential for reducing cellular damage and could be used as a tool to prevent chronic diseases associated with increased free radicals (Rosa et al., 2017).

Recent studies have moved beyond general findings, aiming to identify the specific antioxidant compounds in kefir and understand how they act at molecular level.

Dairy kefir's antioxidant components include peptides, amino acids, vitamins A and E, carotenoids, phenolic compounds, enzyme systems, and EPS such as kefiran, highlighting its potential against oxidation (Chong et al., 2023; Vieira et al., 2021).

Several studies have identified microorganisms capable of producing antioxidant compounds. The antioxidant activity of EPS produced by kefir microorganisms contributes to the protection of biological molecules against oxidation. For instance, low concentrations of EPS have been shown to protect the bovine serum albumin protein from oxidation caused by APPH (2,20-azobis(2-methylpropionamidine) dihydrochloride), reducing protein oxidation by 31–96%, and offering greater protection than the negative control (protein without induced oxidation) (Z. Chen et al., 2016; Vieira et al., 2021).

Moreover, phenolic compounds resulting from the metabolic activity of kefir microorganisms can increase *in situ* antioxidant capacity by up to 120% during fermentation (Yilmaz-Ersan et al., 2018).

In addition, *in vitro* gastrointestinal digestion simulation analyses demonstrated that 50 g of kefir could release antioxidant activity equivalent to 300 mg of ascorbic acid (Carullo et al., 2022).

Furthermore, when evaluating the effect of kefir on oxidative stress in rats with gamma radiation-induced liver damage, it was observed that kefir consumption resulted in the restoration of glutathione, total antioxidant capacity, and catalase activity. In addition, kefir intake reduced lipid peroxidation and nitric oxide production, indicating a protective effect (Ali et al., 2020).

Although the precise mechanisms of action remain unclear, it is believed that EPS exert antioxidant effects through metal chelating activity and scavenging of hydroxyl and superoxide radicals. Additionally, specific peptide sequences with antioxidant potential have been found (Ebner et al., 2015), although their mechanisms of action appear to be independent of ion chelating ability (Gamba et al., 2020).

Thus, maintaining an adequate antioxidant intake may contribute to cardiovascular prevention by halting LDL oxidation, modulating cholesterol metabolism, and promoting muscle relaxation, leading to a decrease in blood pressure (Manach et al., 2004; Martínez Leal et al., 2018).

Therefore, kefir and its bioactive compounds exhibit promising antioxidant effects. However, it is essential to carefully determine optimal doses, identify the specific microorganisms responsible of producing these compounds, and elucidate their mechanisms of action. Likewise, to support its therapeutic potential, more standardized and well-organized *in vivo* studies are required to generate robust and reproducible scientific evidence on the effects of this fermented beverage in the human body.

#### 5.2.3 Antimicrobial and antiviral

Antimicrobial

Kefir, as a whole product, has demonstrated antimicrobial properties against various pathogenic bacteria of clinical and foodborne interest. Additionally, it has been shown to have this effect against fungi such as *Candida albicans* and *Aspergillus flavus*, among others, with a similar effect to certain antibiotics like ampicillin and amoxicillin (Bourrie et al., 2016). These effects may be attributed to the final product's composition, which is high in lactic acid, acetic acid, and hydrogen peroxide (Huseini et al., 2012; Lolou & Panayiotidis, 2019). Furthermore, some studies have demonstrated the production of bacteriocins by different species isolated from kefir. For example, *L. plantarum* ST8KF produces the bacteriocin ST8KF, while other *Lactobacillus* species such as *L. acidophilus* and *L. kefiranofaciens*, have shown activity against various pathogens such as *L. monocytogenes, S. aureus, S. typhimurium, S. enteritidis, P. aeruginosa, E. coli, and Y. enterocolitica* (Bourrie et al., 2016).

Thus, by 2020, the antimicrobial effects of kefir had been established primarily through *in vitro* studies. However, further *in vivo* studies are necessary to confirm these findings, determine effective dosages, and evaluate infection reduction ratios.

More recent research has continued to support kefir's potent antimicrobial activity, now including effects against microorganisms such as *Escherichia coli*, *Enterobacter cloacae*, and *Enterococcus faecalis* (Gut et al., 2022). This activity is attributed not only to the low pH and organic acids produced but also to the complex antagonistic interactions among kefir microorganisms and the production of bacteriocins.

Similarly, a comparative study of kefir from two different sources evaluated the antimicrobial activity of distinct kefir grains. It concluded that this activity depends not only on pH reduction but also on the microbial composition of the grains themselves, particularly the presence of strains capable of producing inhibitory peptides (Marques et al., 2020). This finding reinforces previous knowledge while emphasizing the need for precise strain identification.

Nonetheless, further *in vivo* investigations are essential to validate these antimicrobial effects and to identify the specific strains and compounds responsible for the most significant activity.

Antiviral

Although the antiviral function is closely related to anti-inflammatory and immunomodulatory properties, this aspect can be highlighted because in the years prior to the COVID-19 pandemic, only a few studies evaluated the potential antiviral effects of kefir. Following this event, several strategies to combat viral infections have been evaluated. Among them, natural products can strengthen the immune system to prevent viral infections (Hamida et al., 2021). Therefore, given that kefir contains both nutritional components and microorganisms capable of inhibiting proinflammatory cytokine activity, it may be valuable as a protective agent against viral infections.

The antiviral potential of some probiotics may involve virus entrapment, enhancement of immune responses, and the production of bacteriocins and hydrogen peroxide as antiviral agents (Al Kassaa et al., 2014). Likewise, the biomolecules present in fermented dairy beverages have been shown to promote the production of lymphocyte proliferation and immunoglobulins (Möller et al., 2008). C. G. Vinderola et al. (2005), demonstrated that kefir can regulate the immune response by increasing the number of intestinal and bronchial IgA+ cells, as well as enhancing phagocytic potential for peritoneal and pulmonary macrophages in mice.

The rising incidence of viral diseases has drawn attention to this topic, but there is still a lack of antiviral drugs to alleviate the development of resistance to antivirals. As a close relationship between human nutrition and the immune system has been well established, the search for dietary products that can enhance immune defences remains essential. Accordingly, several studies have reported that kefir has activity against the Zika virus, HCV, hepatitis B virus, influenza virus (H1N1), HSV, rhinoviruses, and retroviruses (Vieira et al., 2021).

Therefore, studying the effects of foods that support the immune system may represent a promising path forward, as it aligns with the growing trend towards healthy eating and the consumption of foods whit health-promoting properties.

### 5.2.4 Metabolism and chronic diseases

Hypocholesterolemic effects

The potential impact of probiotics on cholesterol metabolism has been a subject of investigation for several years, with various mechanisms proposed to explain the hypocholesterolemic effects observed in certain studies.

One proposed mechanism is the inhibition of endogenous cholesterol absorption by lactic acid bacteria (LAB). In this context, a high concentration of LAB in kefir may reduce the cholesterol content in the surrounding medium (Bourrie et al., 2016).

Another proposed mechanism involves the production of short-chain fatty acids (SCFAs), particularly propionate, which may contribute to cholesterol reduction by downregulating the expression of genes involved in its biosynthesis, such as those encoding 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase). Furthermore, SCFAs may promote the redistribution of plasma cholesterol and enhance both the synthesis and secretion of bile acids (Bourrie et al., 2016; Rosa et al., 2017).

Additionally, LAB have been shown to deconjugate bile acids, a process that facilitates their excretion and increases the utilization of cholesterol for the synthesis of new bile salts (Bourrie et al., 2016).

Despite numerous studies in animal models demonstrating that kefir consumption can reduce triglyceride levels and the atherogenic index (Bourrie et al., 2016; John & Deeseenthum, 2015; Prado et al., 2015a; Rosa et al., 2017), findings in human studies remain inconclusive. For instance, Ostadrahimi et al. (2015), conducted a nutrition intervention trial in diabetic patients in which kefir consumption did not result in significant changes in plasma lipid profiles.

Therefore, while the effects of kefir on lipid metabolism are promising, they remain controversial as of 2020. Further clinical research in humans, using standardized methodologies, is required to more clearly elucidate the true impact of this probiotic beverage on cholesterol regulation.

In recent years, studies on the effect of kefir consumption on cholesterol have not substantially increased in the scientific literature since 2020.

*In vitro* and *in vivo* studies have confirmed kefir's ability to reduce fat accumulation in adipocytes. EPS isolated from kefir strains such as *Lactobacillus kefiri* and *Leuconostoc mesenteroides* demonstrated up to 28% inhibition of lipid accumulation in adipocytes *in vitro*, generally in a dose-dependent manner (Seo et al., 2020). Therefore, the potential of EPS from kefir microorganisms shows promise as potential ingredients for the management of obesity. The mechanism of action in adipose tissue involves downregulation of proinflammatory genes such as Wdfc21 and

Hp, and genes related to fatty acid synthesis such as Fabp4 and Fsan. However, these changes in gene expression were not associated with changes in serum cholesterol or total lipid levels. A diet supplemented with heat-treated lactic acid bacteria with a polyphenol-rich wine grape seed flour was also evaluated, resulting in a significant reduction in serum triglycerides. This suggests that antioxidant activity may play a role in reducing fat accumulation (Seo et al., 2020; Vieira et al., 2021).

According to Ghizi et al. (2021), a clinical trial conducted in patients with metabolic syndrome who consumed kefir daily for 11 weeks showed no significant changes in anthropometrics or C-reactive protein levels. Nonetheless, kefir consumers experienced reductions in systolic and diastolic blood pressure, as well as triglyceride levels.

In summary, although the hypocholesterolemic effects of continue to be studied, notable discrepancies persist between *in vitro* and *in vivo* findings, both in animal models and in human clinical trials. Furthermore, as highlighted in the meta-analysis carried out by Kairey et al. (2023), many studies present methodological limitations, including the absence of dietary assessment, the lack of description of the products (kefir) used, the dosage and the duration of the intervention. Addressing these limitations is essential to obtain meaningful statistical comparisons and to draw more accurate conclusions regarding kefir's potential hypocholesterolemic effects.

<u>Antihypertensive</u>

According to this topic, previous findings up to 2020 have evaluated the antihypertensive effect of dairy kefir, with differing opinions about its mechanism.

Maeda et al., (2004), suggested that this property was due to the inhibitory effect of kefir against ACE activity. However, Hernández-Ledesma et al., (2011) described the mechanism as whether the bioactive peptides in kefir can inhibit the production of vasoconstrictor angiotensin I and the production of aldosterone, which causes the increase in blood pressure by increasing serum calcium concentration. Additionally, this bioactive peptide can also inhibit the cleavage of bradykinin, which has vasodilation action, contributing to the decrease in blood pressure (Rosa et al., 2017). Therefore, although results up to that point suggested a potential benefit of kefir consumption against hypertension, the underlying mechanisms still required further studies.

Quirós et al. (2005), also identified two peptides with strong ACE-inhibitory capacity, suggesting that caprine milk kefir may exhibit antihypertensive effects. In this sense, more recent studies conducted by Gamba et al. (2020), reported a 98.4% reduction in ACE activity in milk after fermentation with kefir grains for 24 hours. This effect was attributed to the bioactive peptides released by kefir microorganisms during fermentation. These findings reinforce the earlier hypotheses about kefir's potential role in hypertension management.

In conclusion, while the available data highlight kefir's potential as an antihypertensive agent, especially due to its bioactive peptides, further identification of the specific peptides involved is needed. In this regard, a peptidomic approach could be particularly useful to characterize and confirm the key peptides responsible for this activity. Moreover, standardized, large-scale studies are essential to confirm its clinical application.

Glucose homeostasis regulation

As previously demonstrated, probiotics consumed regularly can regulate the composition of the intestinal microbiota by reducing intestinal permeability, oxidative stress, and inflammation (Rosa et al., 2017). Similarly, given that kefir is also classified as a probiotic food, several studies have evaluated the effect of regular kefir consumption in animal models with induced diabetes, supporting this hypothesis (Hadisaputro et al., 2012). Clinical studies in diabetic patients reinforce these findings, reporting lower levels of fasting glucose and glycated hemoglobin compared to controls (Ostadrahimi et al., 2015).

Moreover, part of kefir's effect is believed to result from its probiotic nature, which helps maintain a healthy microbiota and prevents increased intestinal permeability. This, in turn, reduces the risk of metabolic endotoxemia, which initiates TLR4 receptor signaling cascades, leading to inflammation and increased insulin resistance (Rogero & Calder, 2018; Rosa et al., 2017). Therefore, these findings highlight kefir's potential as a promising tool in the management of diabetes. However, further positive results from well-designed and standardized clinical trials are still needed.

Over the last few years, the beneficial effects of kefir as a potential therapeutic agent against diabetes has gained attention (Salari et al., 2021). Recent literature by Talib et al. (2024), evaluated the antidiabetic potential of *Lb. paracasei* isolated from kefir grains, revealing this strain's ability to modulate the expression of genes involved in glucose regulation and lipid homeostasis in mouse liver tissue. As seen previously in 2020, the antidiabetic potential depends on the substrate and appropriate fermentation conditions. Nonetheless, the probiotics present in kefir may stimulate the gut microbiota to produce insulinotropic polypeptides and glucagon-like peptide-1, promoting glucose uptake in muscle tissue (Nurliyani et al., 2015; Ostadrahimi et al., 2015). Likewise, this hypoglycaemic effect of kefir may also be attributed to its antioxidant capacity, which involves various interconnected pathways that eventually contribute to regulating blood sugar levels or reducing glucose absorption in the gastrointestinal tract (Kahraman et al., 2021).

In short, although there have been no major discoveries in the past five years, different mechanisms have been linked. It is now understood that kefir probiotics can help regulate blood glucose by modulating the gut microbiota and reducing the generalized inflammation associated with hyperglycaemia (Chong et al., 2023; Peluzio et al., 2021; Tingirikari et al., 2024). Therefore, the antioxidant and anti-inflammatory potential of kefir may contribute to improving glucose levels in diabetic patients and those with insulin resistance. However, further scientific literature from human studies, as well as the characterization of the kefir grains used, is still needed. Furthermore, it is also important to highlight that these studies have been conducted in individuals with pre-existing conditions, so future research should explore potential effects in healthy individuals

Lactose digestion

The inability to digest lactose affects many adults worldwide, which is why several studies have been conducted to evaluate the effect of kefir on lactose digestion.

Up until 2020, several studies had been conducted to evaluate this effect. For instance, some studies demonstrated that kefir may contain different microorganisms with β-galactosidase activity, which can enhance lactose digestion (Rosa et al., 2017). Similarly, Hertzler & Clancy (2003), found that kefir consumption can improve lactose intolerance in human clinical studies, where milk-induced flatulence was reduced by 54–71%. Additionally, Alm, (1982) described that after fermentation, lactose concentration can be reduced by approximately 30%. However, further clinical trials are needed to understand the mechanisms and effects of kefir on lactose consumption, as well as the quantities and regularity of consumption needed to obtain the greatest benefit.

Although this beneficial effect has been observed in earlier studies, recent literature has not provided substantial updates. In recent years, a trend has emerged towards the production of kefir in different matrices, beyond milk. Water kefir, which uses a sugar-water base instead of milk, and kefir in plant-based beverages (such as soy, almond, or oat drinks) have gained popularity (Dahiya & Nigam, 2023). These alternatives have been introduced to meet the growing demand for vegan or lactose-free products. However, the lack of a clear definition of what constitutes kefir has caused some confusion in the market, as products that do not meet the traditional dairy base are being marketed under this name, highlighting the need for standardization and regulation in the food industry.

#### 5.2.5 Nervous system and mental health

<u>Neuroprotective effects:</u>

Kefir supplementation has shown promise in improving autism spectrum disorder (ASD) in animal models. *In vivo* studies in mice demonstrated reduced repetitive behavior and increased levels of anti-inflammatory Treg cells in mesenteric lymph nodes. On the other hand, *in vitro* studies using neuroblastoma cells, have demonstrated that kefir shows neuroprotective potential by modulating apoptotic genes such as Tp73, Bax, and Bcl-2 (Yegin & Sudagidan, 2024).

Additionally, kefir appears to protect neurons from degeneration through its anti-inflammatory properties. It may also activate receptors in the brain involved in learning and memory. From a cognitive perspective, kefir consumption has shown potential in alleviating depression and anxiety, particularly in nicotine-related models, likely through its high tryptophan content and subsequent serotonergic modulation (Peluzio et al., 2021).

One study linked supplementation with *Lactobacillus kefiranofaciens ZW3* to improved tryptophan metabolism, enhanced levels of anti-inflammatory cytokines, decreased pro-inflammatory cytokines, and favorable changes in gut microbiota composition (increased *Actinobacteria*, *Bacteroides*, *Lachnospiraceae*, *Coriobacteriaceae*, *Bifidobacteriaceae*, and *Akkermansia*, with decreased *Proteobacteria*). These alterations suggest that kefir may influence metabolic pathways involved in the development of depression (Peluzio et al., 2021).

Ton et al. (2020), demonstrated that kefir supplementation significantly improved global cognitive function in Alzheimer's disease (AD) patients, reduced ROS and plasma protein oxidation, and lowered pro-inflammatory cytokines and apoptosis levels.

Moreover, diseases such as encephalitis have been associated with gut microbiota dysbiosis, leading to elevated pro-inflammatory cytokine release and neuronal hyperactivity factors linked to epileptogenesis and neuroinflammation. Therefore, modulation of the gut microbiota through kefir consumption could be explored as a therapeutic strategy for epilepsy and other neuroinflammatory conditions (Pereira et al., 2021).

<u>Psychiatric disorders:</u>

Following the growing interest in the gut–brain axis, kefir is gaining attention as a potential psychobiotic, a type of probiotic that may offer mental health benefits. In early studies using murine models, kefir increased GABA production in the gut microbiota, likely due to a higher prevalence of *Lactobacillus reuteri*. It also reversed stress-induced reductions in colonic serotonergic signaling and improved behavioral deficits such as repetitive behavior (Yegin & Sudagidan, 2024).

Despite these promising findings, further research, particularly human clinical studies, is necessary to confirm the specific microbial strains responsible for the observed effects and to determine optimal dosing. Kefir has shown potential to modulate the gut–brain axis and may represent valuable non-pharmacological strategy for managing neurodegenerative diseases.

### 5.2.6 Anticancer potential

Cancer remains one of the leading causes of chronic diseases and one of the most common causes of mortality worldwide. In this context, there has been growing interest in complementary preventive strategies, including those related to diet and gut microbiota. Various studies have been conducted in this context, expanding our knowledge of kefir's effect on this disease.

According to some articles published before 2020, Sharifi et al., (2017), cancer results from the dysregulation of immune and inflammatory systems. Kefir may help restore balance in this dysregulation through various mechanisms, including decreased secretion of TGF-α, TGF-β, and Bcl2, while increasing the secretion of Bax to promote apoptosis; induction of ROS-mediated apoptosis and activation of endonucleases for DNA cleavage; and increased secretion of interferon-β, which exerts antiproliferative effects.

Moreover, as noted by Bourrie et al., (2016), kefir has already demonstrated antitumoral activity against various cell types. Different studies have shown that the cell-free part of kefir can exert a dose-dependent anti-proliferative effect on gastric cancer cells (SGC7901), and it can also induce apoptosis through upregulation of the gene Bax and downregulation of the oncogene bcl-2 (Sorenson, 2004). It has also been observed that prior consumption of kefir in a study with murine breast cancer models could reduce tumor size, increase apoptosis, and show higher levels of IL-10, IL-4, IgA+ cells, and CD4+ T cells, linking this effect to kefir’s immunoregulatory properties (de Moreno de LeBlanc et al., 2007).

Although studies prior to 2020 provided a solid foundation regarding the potential effects of kefir on cancer, in recent years, there has been a notable increase in research exploring its impact and underlying molecular mechanisms. These more recent studies have expanded our understanding of how kefir can influence cancer biology and refined some of the previously observed effects.

Recent research has also shown that kefir intake has additional effects on gut microbiota and colon health. In one study, kefir consumption led to an increase in cecal short-chain fatty acids, a reduction in the lactulose/mannitol ratio, and an increase in colonic levels of TNF-α, IL-1β, and catalase enzyme compared to control groups. These results suggest that kefir could exert protective effects against lesion development by modulating intestinal permeability, immune modulation, and enhancing colonic antioxidant activity (da Anunciação et al., 2024). Moreover, *in vitro* studies have revealed that bioactive compounds in kefir, particularly extracellular polysaccharides, have significant anticancer effects (Jenab et al., 2020; Vieira et al., 2021). It has been observed that kefiran, a type of EPS, reduces the viability of breast cancer cells (MCF7) by 45%, as well as in liver and cervical cells. The anticancer efficacy of these compounds appears to be concentration-dependent and mediated in part through the regulation of apoptotic genes such as Cyto-c, BAD, and BAX. However, it is crucial to note that high concentrations of these compounds can induce toxicity in normal tissues, limiting their clinical application without appropriate dosing (Vieira et al., 2021). On the other hand, bioactive peptides from kefir, particularly in estrogen-sensitive breast cancer models, have shown anticancer activity without affecting the proliferation of normal mammary epithelial cells, suggesting high specificity in their mechanism of action (C. Chen et al., 2007; Vieira et al., 2021).

In summary, current evidence suggests that kefir exhibits promising anticancer properties through multiple mechanisms. Both *in vitro* and *in vivo* studies support its potential to reduce tumor size, regulate oncogene expression, and enhance the production of anti-inflammatory and immunoregulatory cytokines. While further clinical research is needed to confirm its efficacy and safety in humans, kefir can become a valuable candidate for complementary cancer prevention and therapeutic strategies.

#### 5.2.7 Wound healing and oral health

<u>Wound Healing:</u>

As previously mentioned, kefir has anti-inflammatory properties that may play a role in the wound healing process. Moreover, several studies using *in vivo* models have shown that kefir administration may result in better wound healing outcomes than conventional treatments such as clostebol-neomycin emulsion and silver sulfadiazine, particularly in thermal injuries susceptible to infection by antibiotic-resistant pathogens like *P. aeruginosa* (Bourrie et al., 2016).

Additionally, some reports also mention that animal models treated with gels made from kefir or kefir grains were more effective in reducing the size of open wounds. This may be due to kefir's ability to inhibit bacterial growth, generating a cleaner surface that promotes faster healing (Bourrie et al., 2016).

On the other hand, beyond the antimicrobial effect, one study observed that microorganisms present in kefir, such as *S. cerevisiae*, can increase the expression of type I collagen and the transcription factor TGF-β1, thereby improving the biomechanical properties of the healing tissue (Lolou & Panayiotidis, 2019).

Building upon these experimental findings, which emphasize the anti-inflammatory and tissue-regenerating potential of kefir, recent studies have begun to associate specific microbial strains, and bioactive metabolites present in kefir with distinct wound healing mechanisms.

Recent studies have linked the wound healing effects of kefir to certain microorganisms present in the beverage. The effectiveness of kefir in wound healing may be explained by the presence of bioactive compounds such as acetic acid and lactic acid produced by bacteria. Additionally, microorganisms like *L. acidophilus* are believed to exert anti-inflammatory effects by accelerating tissue granulation and re-epithelialization. Kefir has also been shown to enhance human dermal fibroblast (HDF) cells proliferation and migration, reduce IL-1β, and modulate the expression of growth factor beta-1, along with stimulating basic fibroblast growth factor (bFGF)(Farag et al., 2020).

Furthermore, kefir appears to have a positive effect on diabetic foot ulcer (DFU) patients, significantly reducing ulcer size after 12 weeks of kefir supplementation (Farag et al., 2020).

Oral Health:

Dental caries is associated with changes in biofilm composition due to the proliferation of pathogenic bacteria. In the proposed mechanism, kefir, rich in probiotics, may help maintain oral microbiome eubiosis, which refers to a balanced and healthy microbial community in the oral cavity, where beneficial bacteria predominate and maintain homeostasis, preventing the overgrowth of harmful pathogens that can lead to diseases such as dental caries. The probiotics in kefir may outcompete harmful bacteria by adhering to tooth surfaces and producing bacteriocins and hydrogen peroxide, both of which can inhibit pathogenic microorganisms (González-Rascón et al., 2025).

Moreover, kefir has shown the ability to inhibit glucosyltransferase (GTF) activity, reducing the release of fructose, a by-product of GTF action on sucrose, which in turn decreases the cariogenic activity of species like *S. mutans* (González-Rascón et al., 2025).

Although the available literature on kefir and oral health is still limited, these findings suggest a growing interest in the potential of kefir as a preventive agent in this field. This could be considered emerging research, as few studies have explored its specific role in oral microbiota modulation and dental disease prevention.

In addition, studies such as the one conducted by Sefidgar et al. (2014), demonstrated that the reduction of *S. mutans* in saliva through kefir consumption is comparable to that achieved with sodium fluoride mouthwash. Altogether, kefir shows promise as a potential adjuvant in oral health management.

## **Summary of physiological effects of kefir**

After a comprehensive review of the various physiological effects of kefir, Table 2 provides a concise summary of the main health benefits attributed to its consumption. This table aims to facilitate a clear overview of kefir's multifaceted impact on human health. The levels of evidence indicated in the table reflect the strength and consistency of the scientific data supporting each effect. A high level of evidence (green) corresponds to well-established effects supported by multiple studies showing consistent results. A moderate level of evidence (yellow) indicates that there is reasonable support for the effect, although some studies may present limitations or variations. Effects classified as having preliminary evidence (orange) are those with early findings that are promising but require additional research for confirmation and better understanding. Finally, an inconclusive level of evidence (red) means that the current data are insufficient or conflicting, and no definite conclusions can be drawn at this stage.

Table 2. Summary of the main physiological effects attributed to kefir, their proposed mechanisms and the level of evidence supporting them.

| Physiological Effect | Proposed Mechanism | Evidence level |
|---|---|---|
| Digestive and gastrointestinal health | Gut microbiota modulation and pathogen reduction | 🟢High |
| Immunomodulatory and anti-inflammatory | Regulation of cytokines and immune cell activation | 🟡Moderate |
| Antioxidant | Reduction of oxidative stress markers and ROS | 🟡Moderate |
| Antimicrobial | Production of organic acids and bacteriocins | 🟡Moderate |
| Antiviral | Immune activation and viral inhibition | 🟠Preliminary |
| Hypocholesterolemic effects | Lipid metabolism regulation and cholesterol reduction | 🔴Inconclusive |
| Antihypertensive | Vasodilation and blood pressure regulation | 🟡Moderate |
| Glucose homeostasis regulation | Improved insulin sensitivity and glycemic control | 🟠Preliminary |
| Lactose digestion | Enhanced lactase activity | 🟠Preliminary |
| Neuroprotective effects | Anti-inflammatory and apoptotic gene modulation | 🟠Preliminary |
| Psychiatric disorders | Gut-brain axis and neurotransmitter regulation | 🟠Emerging |
| Anticancer potential | Apoptosis induction and immune regulation | 🟠Preliminary |
| Wound healing | Anti-inflammatory and antimicrobial tissue repair | 🟡Moderate |
| Oral health | Inhibition of oral pathogens and cariogenic activity | 🟡Moderate |

* Evidence levels are indicated by color and term: High (green), Moderate (yellow), Preliminary (orange), and Inconclusive (red) to reflect the strength of scientific support.

### 5.3. Kefir in the current functional food market

In recent years, the consumption of probiotic products has grown significantly worldwide, driven by new social media trends and a rising interest among consumers in maintaining optimal health through a balanced diet. Among these functional foods, kefir stands out not only for its ability to improve gut health but also for the various benefits it offers for overall well-being. According to recent estimates, the size of the kefir market has surpassed 1.11 billion euros, and it is expected to grow by at least 5% annually from 2025 to 2030 (Grand view research, 2024).

This growing demand has led companies to develop a wide variety of kefir products to meet consumer preferences. However, despite traditional kefir being made from milk fermented with a specific combination of bacteria and yeasts, the lack of clear legislation has enabled the commercialization of products that do not meet this classic definition. As a result, the ambiguity surrounding the composition of items labeled as ‘kefir’ has created uncertainty about what should be considered authentic kefir. This regulatory gap causes confusion both for consumers, who cannot always identify the real benefits of the products, and for regulators, who do not have a clear framework to monitor the quality of these items.

In the current market, many commercial kefir products do not contain yeasts, which raises the question of the authenticity of these products and whether they can offer the same benefits as traditional kefir. The absence of clear regulations allows companies to produce yeast-free products, as this extends shelf life and simplifies preservation by preventing oxidative changes that may occur during fermentation (Newbold & Koppel, 2018). Although this approach is understandable from an industrial perspective, it calls into question whether such products truly represent traditional kefir and its functional properties.

Traditional kefir is known for the synergy between the bacteria and yeasts present in kefir grains. This interaction enhances the bioavailability of key nutrients such as short-chain fatty acids and essential vitamins like folic acid, which are crucial for gut and metabolic health. By eliminating yeasts, many commercial products may lack these essential bioactive compounds, potentially reducing their health-promoting properties. Moreover, most scientific studies have been conducted on kefir that includes yeasts, suggesting that the observed benefits might be diminished in products without them.

Beyond the microbiological and compositional issues, it is also important to consider the regulatory limitations that influence how kefir products are labeled and perceived by consumers. Although kefir is commonly associated with a wide range of beneficial physiological effects, there is currently no specific European legislation defining what constitutes kefir or what health benefits can be attributed to it. While general regulations on health claims exist such as Regulation (EC) No 1924/2006, which permits only authorized claims based on scientific evidence evaluated by European Food Safety Authority (EFSA), no health claims related to the functional or probiotic properties of kefir have been approved to date. Consequently, the only permitted claims for kefir products relate to their nutritional composition, such as “source of calcium" or “high in protein”. Establishing clear definitions and specific health claims for kefir could help bridge the gap between scientific knowledge, consumers’ expectations, and regulatory frameworks.

Table 3 summarizes the characteristics of several commercial kefir products available on the market. It highlights a range of brands and products from different countries, showcasing the diversity of kefir offerings today. Notably, although many products do not specify the presence of yeasts, several list only lactic acid bacteria such as *Lactobacillus* or *Streptococcus thermophilus*. This suggest that some items may not meet the traditional definition of kefir, which requires a symbiosis between yeasts and bacteria.

Additionally, table 3 reveals that within the same brand or product line, the presence of yeasts may vary depending on the specific item. For instance, some items from brands like Carrefour or Olympus state that they do not contain yeasts, while others, such as Milbona or Kremly, mention the inclusion of yeasts. It is also important to note that some products, such as those from Nestlé or El Corte Inglés, do not clearly specify which yeasts or cultures are used, creating uncertainty about the probiotic properties of these products.

Table 3 also illustrates differences in nutritional profiles, including variations in protein, carbohydrate, and fat content. For example, some products contain higher carbohydrate levels, especially those with added fruit, such as Olympus Kefir Blueberry, which contains 14.5 g of carbohydrates per 100 ml due to the inclusion of blueberries. In contrast, products like Organic Goat Kefir have lower carbohydrate content but may include additives like calcium.

To complement the overview of current market offerings, Figure 1 presents four representative commercial kefir products available in the Spanish market. These examples illustrate the variety in packaging, composition, and ingredient labeling found today, including flavored options, traditional kefir, and products highlighting specific probiotics. The visual comparison of these packages helps to better understand the differences in labeling practices, which can significantly influence consumer perception and choice.

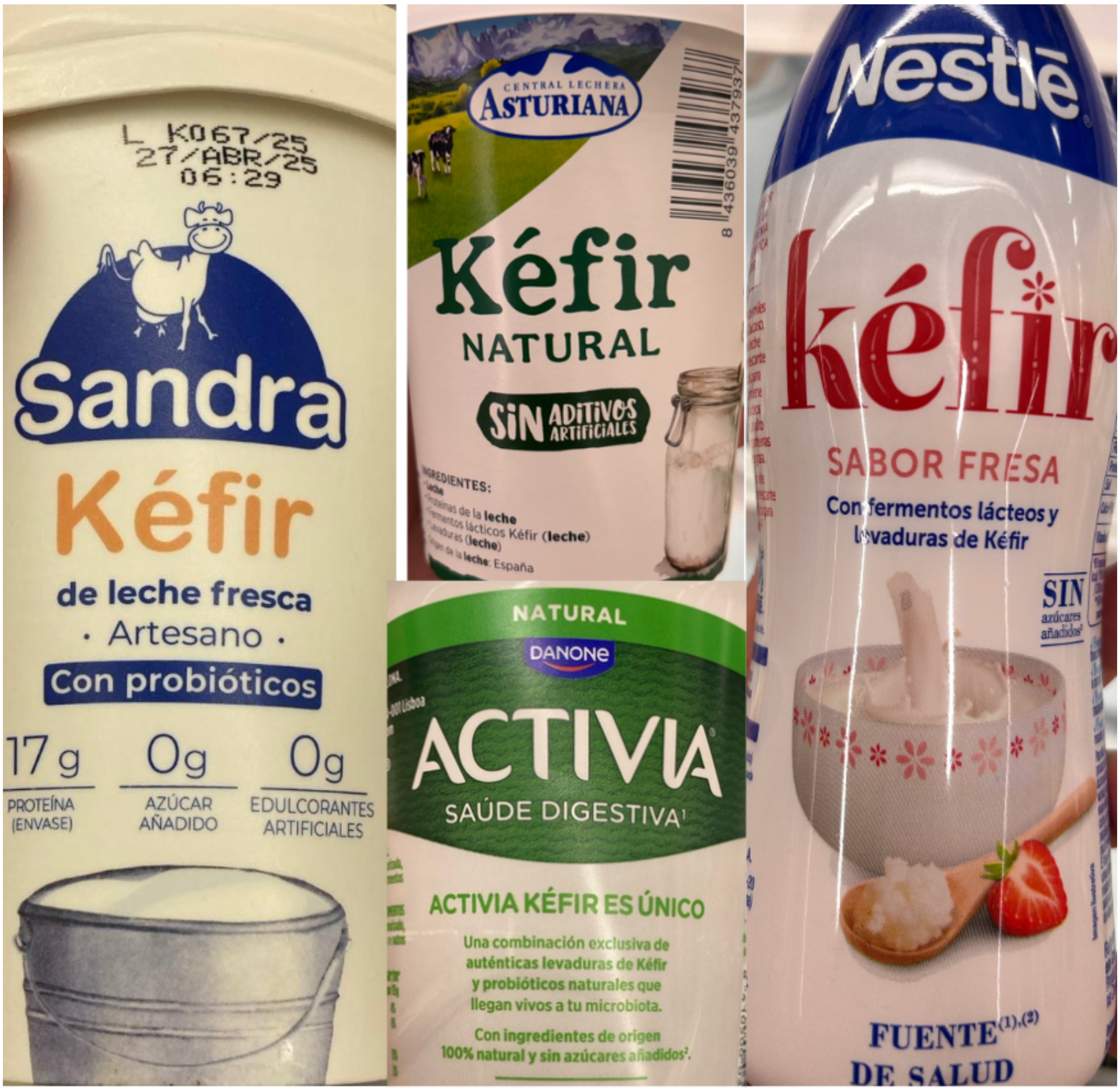


Figure 1. Front packaging of four commercial kefir products available in Spain: Sandra fresh milk kefir with probiotics, Central Lechera Asturiana Natural kefir, Activia Natural (Danone), and Nestlé Strawberry Flavour. Notable differences are observed in ingredient disclosure, probiotic claims, yeast content, and nutritional profiles.

The increasing popularity of kefir, driven by growing consumer demand for probiotic-rich foods, presents a major opportunity for the food industry. However, the lack of clear regulations surrounding the composition of kefir products has created inconsistencies in the market. While many commercial kefir products are marketed as probiotic, the absence of yeasts in some variations raises questions about whether they provide the same health benefits as traditional kefir. Furthermore, differences in nutritional profiles and microbial content further complicate the consumers' ability to make informed choices. To address this, it is essential for regulatory bodies to establish clear guidelines that promote transparency and consistency in kefir production. This would not only help consumers make better purchasing decisions but also support the growth of the kefir market by ensuring that items labeled as kefir meet the standards associated with its traditional health benefits.

In summary, the growing kefir market underscores the urgent need for clear regulations regarding its composition. Standardizing kefir products could help ensure that all commercial products meet a minimum list of ingredients and probiotic characteristics, thereby preserving the authenticity of kefir and its potential to improve health. Such regulation would eliminate confusion, ensuring that consumers can access products that truly deliver the expected benefits. Additionally, this would encourage further scientific research into the effects of kefir, facilitate comparisons between products based on their composition, and promote greater transparency for both consumers and producers.

Tabla 3. Nutritional and microbial characteristics of commercially available kefir productss (Innova Market In, 2021)

| Brand/Product | ¿presence of yeast? | Statement about MOs | Protein (g/100mL) | Carbohydrates (g/100mL) | Fat (g/100mL) | Other ingredients: |
|---|---|---|---|---|---|---|
| Milbona kefir | Yes | Not especified | 3,5 | 4 | 1,5 | |
| Carrefour-Sensation kefir Semi Skimmed fermented Milk | No | Lactic ferments (not especified) | 3,6 | 3,9 | 1,6 | Fiber |
| Carrefour-Sensation Natural kefir | Si | *Lactococus lactis, Lactococcus cremoris, Lactobacillus acidophilus, Streptococcus thermophilus, Bifidobacterium lactics, Lactobacillus helveticus, Debaryomyces hansenii* | 4,5 | 4,0 | 5,0 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Olympus kefir Blueberry | No | Not especified | 3,7 | 14,5 | 4,1 | blueberrys, fiber |
| Olympus kefir Naturale | ? | kefir ferments (not characterized) | 4,5 | 6,0 | 5,0 | |
| Miekovita Polski kefir | No | *Lactococcus lactis, Lactococcus cremoris, Lactococcus diacetylolactis, Leuconostoc, Streptococcus thermophilus, Bifidobacterium, Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. Bulgaricus* | 3,1 | 8,3 | 1,8 | |
| Activia Gut Health kefir | No | *Bifidobacterium lactis, Lactobacillus bulgaricus, Streptococcus thermophilus, Lactococcus lactics, Lactobacillus rhamnosus* | 3,6 | 8,7 | 3,1 | Calcium, fiber |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hacendado kéfir | ? | Kefir lactics ferments | 3,1 | 4,8 | 2,0 | |
| Livli kefir drink | ? | Kefir cultures | 2,9 | 11,8 | 0,9 | fiber |
| Kalekoi Quefir Natural | ? | Lactic ferments | 3,1 | 4,7 | 3,8 | |
| Krasnystaw kefir | ? | Kefir grains and bacteria cultures (not characterized) | 3,4 | 4,7 | 2,0 | |
| Oda Terra kefir Natural | ? | Lactic ferments (not especified) | 3,1 | 4,7 | 3,8 | |
| Lykas Health Choice kefir | ? | Kefir cultures | 3,3 | 4,0 | 1,5 | |
| Nestle kefir Natural | Si | Lactic ferments, kefir grains and grains (not characterized) | 5,5 | 3,9 | 2,9 | Vitamin D |
| Auchan kefir | Si | *Streptococcus thermophilus, Lactobacillus sp., Lactococcus* | 4,4 | 3,9 | 3,6 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *lactis, Leuconostoc sp., Debaryomyces sp., Saccharomyces sp.* | | | | |
| Milko kefir Naturalny | No | Kefir bacteria (not specified) | 3,6 | 4,6 | 1,5 | |
| Arla kefir Dryck Blabar: Blueberry flavour | Si | Lactic ferments and yeast (not specified) | 3,1 | 5,7 | 1,4 | Lactase, Riboflavin, B12 |
| Lowicz Kefir Gesty | Yes | Kefir yeast and bacterial cultures (not specified) | 3,2 | 4,5 | 1,5 | |
| Il Viaggiator Goloso kefir di Latte | Yes | *Lactobacillus sp., Lactococcus sp., Leuconostoc sp., Acetobacter sp., Saccharomyces sp.* | 3,3 | 3,5 | 1,5 | |
| El Corte Ingles Selection kefir | ? | Starter cultures (not specified) | 3,4 | 3,8 | 3,8 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Yayla Kefir | No | Lactic ferments | 5,0 | 7,9 | 3,5 | |
| El cabecico Cabra el kefir Natural | No | Kefir lactic ferments (not specified) | 3,5 | 4,3 | 5,0 | |
| Campina kefir Natuur | ? | Lactic ferments (not specified) | 3,7 | 3,8 | 7,0 | Calcium, B12, vitamin A |
| Kremly kefir | Yes | Yeast, kefir grains (not specified) | 3,7 | 5,4 | 1,5 | Vitamin D |
| Smarti Latte Di Kefir | Yes | Lactic ferments, yeast (not specified) | 3,3 | 3,5 | 1,5 | |
| kefir natural: central leche asturiana | No | Not specified | 3,9 | 2,6 | 3,5 | |
| kefir Sandra de leche fresca | No | *Lactobacillus rhamnosus, Lactobacillus paracasei, Bifidobacterium infantis,* | 3,4 | 4,3 | 3,6 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *Bifidobacterium lactis, Lactobacillus acidophillus* | | | | |
| kefir natural Activia | Yes | *Bifidobacterium sp.,* lactic ferments, yeast | 4,0 | 4,8 | 3,5 | Calcium |
| kefir ecológico de cabra desnatado: el canturo de letur | No | Lactic ferments | 4,5 | 3,7 | 0,3 | Calcium |
| kefir ecológico de vaca: el canturo de letur | No | Lactic ferments | 3,4 | 4,2 | 3,7 | Calcium |

## 6. Conclusions

This bibliographic review highlights the potential of dairy kefir as a functional food, due to its beneficial effects on multiple aspects of human health. These benefits are largely attributed to its complex microbiological composition and to bioactive compounds produced during fermentation.

Recent scientific studies published between 2020 and 2025 have reinforced previous findings and provided new insights into kefir's ability to modulate the gut microbiota and influence various health outcomes. While evidence supporting effects on intestinal and immune health is relatively consistent, some areas, such as lipid metabolism and mental health impacts, remain less well understood and require further research.

Most studies on kefir consumption have been conducted using traditional kefir that contains both lactic acid bacteria and yeasts, while kefir made solely with bacteria has been less explored. This gap in research limits our understanding of the comparative health benefits of yeast-free kefir.

An analysis of commercial kefir products reveals considerable variability in their microbiological and nutritional profiles, as well as differences in labelling and health claims. This variability, combined with the absence of clear regulatory definitions for kefir, creates uncertainty among consumers and producers alike.

To ensure product consistency and build consumer confidence, establishing clear regulatory standards is essential. Additionally, more well-designed clinical studies are needed to validate the health benefits attributed to kefir and to clarify the specific roles of its microbiological and bioactive components. Only through robust scientific evidence and regulatory clarity can kefir's potential contributions to public health be fully understood and responsibly promoted.

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