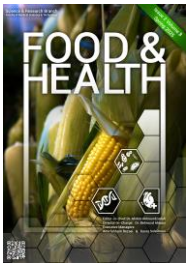

Food & Health Journal

Journal homepage: sanad.iau.ir/journal/fh

An Overview of the Biological Effects of Kefir: A Scientific Perspective

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ARTICLE INFO

Original Article

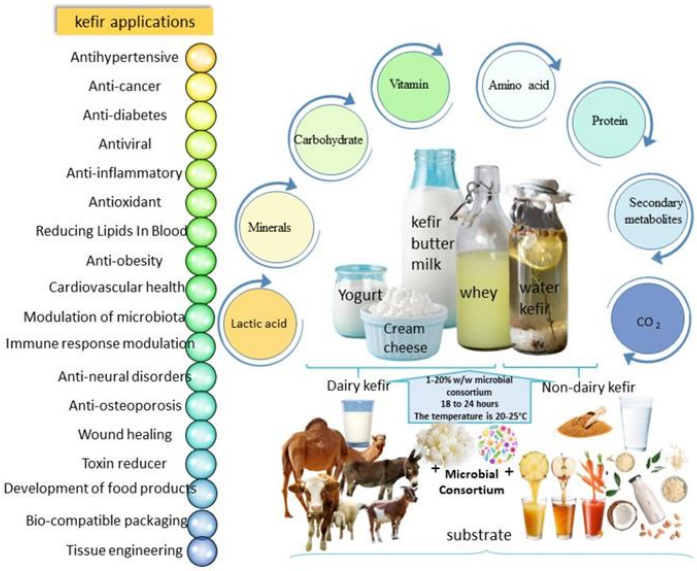
Article history:
Received 26 November 2024
Revised 1 January 2025
Accepted 17 January 2025
Available online 20 May 2025

Keywords:
Kefir,
Probiotics,
Postbiotics,
Prebiotics,
Medicinal food supplement

ABSTRACT

The occurrence of global epidemiological changes, such as cardiovascular diseases, cancer, diabetes, etc., has led researchers to examine the significant causal relationship between the disturbance of microbiota balance and the prevalence of these diseases. It has been determined that there is a direct relationship between them. Fermented foods have held a significant place in the human diet for a long time. The development of chemical drugs and their side effects has led people to consume more beneficial foods. Kefir is a natural beverage containing helpful microorganisms and several critical bioactive compounds. The presence of mineral compounds, vitamins, and proteins makes this beverage a particularly unique combination. Kefir is produced in two forms, dairy, and non-dairy, and its sensory and physicochemical characteristics depend on temperature, substrate, microbial consortium, etc. In this article, in addition to examining the general characteristics of kefir and its production method, the valuable properties of kefir, including its antimicrobial, anti-cancer, anti-fat accumulation and obesity, and antiviral effects, as well as its impact on reducing the symptoms of neurological and skin diseases, will be discussed. Additionally, the effects of kefir's probiotic, postbiotic, and prebiotic aspects will be investigated. Finally, kefir can be considered a medicinal food supplement.

Graphical Abstract



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1. Introduction

The intestinal environment is a bioreactor with thousands of different species of microorganisms (1, 2). The symbiotic microorganisms in the gut play a central role as a key elements in the body's health (3). An unbalanced microbiota and the predominance of harmful microorganisms cause dysbiosis and various diseases (2-4). Therefore, the ISAPP proposed the concept of "normobiosis" to define a healthy microbiota (3). For several decades, research has focused on using probiotics to modify microbiota (3, 4). Probiotics are live microorganisms that, when consumed in sufficient amounts, provide health benefits to the host (3-5). Today, probiotics are divided into food and medicinal food supplements (5). In addition to probiotics, new aspects such as prebiotics, postbiotics, and synbiotics have been proposed (2, 6, 7).

The uncontrolled population growth has created fundamental challenges in social systems, the economy, and the health of societies. Changing lifestyle factors, including diet, physical activity, and hygiene, significantly impact these challenges. One of the critical approaches to changing and regulating the microbiome is choosing the proper diet (7, 8). Worldwide, public awareness of the importance of healthy nutrition in improving lifestyle and promoting health has been increasing, and existing evidence confirms the critical role of food in the body's health (3, 9). Therefore, attention to fermented food products has increased dramatically (10, 11). For this purpose, consuming fermented foods with probiotic properties as microbiota modifiers and health promoters has gained more emphasis (7, 12). Among these products, functional beverages with health-promoting properties are the most active category of functional foods and exert various beneficial biological effects on the body (9, 11). Among the popular drinks with probiotic properties, the traditional kefir beverage has garnered attention due to its numerous benefits and has been the focus of scientific studies (1). The nutritional value of this beverage is attributed to various compounds such as minerals, sugars, proteins, peptides, vitamins, and fats, as well as secondary compounds from fermentation such as catechin, and vanillin. (10). Kefir is an inexpensive, accessible foodstuff with easy storage conditions, high functional potential, and a long history of providing health benefits, including modulation of gut microbiota (3, 12).

Kefir is an acidic, carbonated, fermented beverage with low alcohol content, obtained by fermenting kefir grains in milk or water (8, 9). Kefir grains are irregularly shaped, interconnected lobes resembling small cauliflowers. They are slimy, and hard, and range in color from white to light yellow, with sizes varying from 1 to 4 cm (8, 13, 14). The grains consist of a symbiosis of beneficial bacteria and yeast species within an inert matrix of polysaccharides and proteins, resulting in a slimy, gelatinous structure. Matrix and substrate fermentation is the synergistic effect of a microbial consortium of seeds and their metabolic activities (8). Kefir originates from the North Caucasus Mountains and has been produced and consumed in countries such as the Balkans for centuries (8, 10, 15). Kefir is one of the contributing factors to longevity among Caucasians. Due to its health-promoting effects, there is significant interest in its consumption in other parts of the world.

Most previous research has concentrated on a specific type of this beverage and its specific properties. Therefore, the present study aims to offer a comprehensive review of the similarities and differences among the various types of this drink, as well as their associated health benefits.

2. Types of kefir

Kefir is traditionally made by fermenting kefir grains in milk, and it is a popular fermented dairy product. Kefir is recognized as a source of probiotics with health benefits, but this functional beverage faces challenges and limitations in consumption. Among the most important of these restrictions is the use of milk as the traditional base of this beverage (8) For example, the limitations of drinking milk include its high cholesterol content, the presence of lactose, and the potential to cause allergic reactions (9, 16). Additionally, the consumption of dairy products is sometimes limited by individuals with religious beliefs, lactose intolerance, or special diets which has led to the scientific discovery and popularization of water kefir. Non-dairy or water kefir is produced on various substrates such as vegetable juice, herbal spirits, fruit juice, vegetable milk, and molasses (8, 10, 13, 17). Therefore, an alternative method for everyone to enjoy the benefits of this drink is to change the dairy substrate (9, 17).

2.1 Dairy kefir

Since ancient times, milk has held a special place in the human diet. People discovered that sour fermented milk could be stored for an extended period while retaining its beneficial properties and nutrients. Now, milk-based fermented foods, such as yogurt, cheese, and dairy beverages, are produced and consumed. These products are known as functional dairy products containing probiotics and prebiotics. Dairy kefir is highly popular because of its health benefits (5, 17).

Milk fermented with kefir grains or industrial starters produces a carbonated beverage with high viscosity and consistency, containing approximately 0.08% to 2.0% alcohol. The flavor of kefir results from the metabolic activities of the kefir microbial consortium, including the proteolytic and lipolytic degradation of milk compounds (9).

Dairy kefir is prepared from various types of milk (cow, camel, goat, buffalo, etc.) or a mixture of two types of milk, which enhances its nutritional value, rheological properties, and sensory qualities. Additionally, incorporating compounds such as inulin and vitamins improves the product's characteristics. Reports indicate that consuming dairy kefir offers more benefits than its non-dairy counterparts (10).

2.2 Non-dairy kefir

Water kefir, sugar kefir, or tibico became very popular during the 20th century (10, 18). The earliest documentation of water kefir usage dates back to the 1890s. In some countries in Latin America and Italy, water kefir is considered a traditional drink. Non-dairy or water kefir is prepared through microbial fermentation with a sugar solution, various juices (pineapple, grape, apple, coconut, etc.), vegetables (ginger, soybean, carrot, etc.), molasses, honey, and more (10, 13, 19).

3. Preparation of kefir

Different kefir grains are similar in their structural relationships, microbial consortia, and metabolites produced. However, the structure, microbial diversity, and biological products may vary depending on the available substrates used in the fermentation process (20).

Types of kefir are obtained from the inoculation of a microbial starter in the respective substrate with variable ratios (from 1 to 20% w/w), and fermentation begins under anaerobic conditions for 18 to 24 hours at 20 to 25°C. Consumption of the substrate leads to an increase of 5-7% in the biomass of the grains, the formation of various metabolites, the production of gas, and a decrease in acidity. Kefir grains are divided into smaller grains and free-living cells during the process. It is important to adhere to the conditions and protocols of the fermentation process (20). In the traditional method, kefir fermentation occurs over 24 hours at room temperature and typically employs goat skin, wooden buckets, or clay pots, which can influence the quality and characteristics of the final product (6). In industrial kefir production, standardized containers and methods are used.

3.1 Traditional and commercial production of kefir

Kefir is prepared and produced using both traditional and industrial methods (21). Traditional kefir is produced using natural kefir grains, while industrial kefir is made using a primary culture of selected bacteria and yeasts. Some studies attribute greater therapeutic potential and health effects to industrial kefir (14). Due to the limitations on the shelf life of the product and the complexities of using natural grains, the industrial production of kefir has garnered attention for utilizing special microbiota or properties similar to those of traditional kefir, but with a higher quality guarantee (8, 17). For this reason, the commercialization of kefir has a promising outlook (9).

Conducting studies on large-scale industrial kefir production to standardize its physiological benefits and health-giving properties is necessary for the development of production (5). Also, due to the short shelf life of kefir and the high costs of packaging and storage, dry powder kefir has been developed. In this regard, there are two methods: spray drying and freeze-drying, each with its advantages and disadvantages (10).

4. Effective factors in the properties of kefir

According to the geographical, climatic, and cultural origins and diversity of microorganisms, different types of kefir with varying tastes and physical and chemical properties have been identified (5, 8, 14). Other factors, such as the ratio of kefir grains to substrate, fermentation period, type of substrate, temperature, and stirring speed, may affect the population and activity of the microbial consortium, as well as the sensory and physicochemical properties (5, 8, 17).

5. Kefir microorganisms

The microorganisms in industrial starters or kefir grains belong to diverse genera of bacteria and fungi, particularly yeasts (6, 8, 10, 22). Yeasts are essential for initiating the fermentation process; they create suitable environmental conditions for bacterial growth by producing metabolites. Bacteria, mainly Lactobacillus species, produce and secrete exopolysaccharides (EPS) such as dextran and kefiran, which lead to the creation of a suitable substrate for the attachment of microorganisms and the formation of kefir grains. The most common bacterial and fungal species found in kefir grains are shown in Figure 1 (23-25).

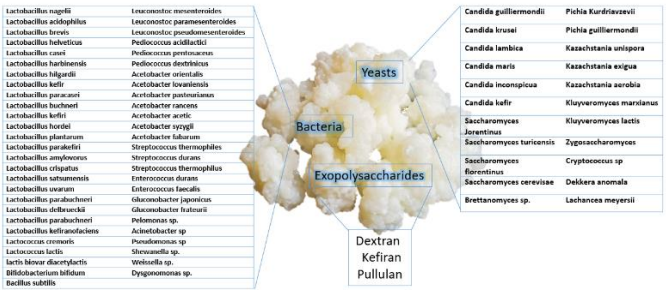

Figure 1- Composition of kefir grains

6. Kefir compounds

Kefir contains 6% sugar, 3% protein, 1% lactic acid, 0.2% fat, 0.7% ash, 0.48% alcohol, and 201.7-277 ml/liter of CO2 (10). The international and national standards for kefir compounds are outlined in Table 1. In general, kefir contains compounds such as various biomolecules (carbohydrates, fats, minerals, peptides, proteins, vitamins), secondary metabolites (such as salicylic acid, ferulic acid, vanillin, catechin, etc.), vitamins (various B vitamins, C, etc.), various microelements (such as sodium, potassium, magnesium, calcium, copper, zinc, iron, etc.), and amino acids (tryptophan, threonine,

serine, lysine, alanine, valine, methionine, isoleucine, phenylalanine) (14, 15, 24).

Table 1. Comparison of international and national standards

Quantity	International food standard CXS 2003-243	National Standard No.1177 of Iran
Initiating microorganisms	*Lactobacillus kefiri, Lactococcus* spp., *Leuconostoc* spp., *Acetobacter* spp., *Saccharomyces unisporus, Saccharomyces cerevisiae, Saccharomyces exiguus, Kluyveromyces marxianus*	*Lactobacillus kefiri, Lactococcus* spp., *Leuconostoc* spp., *Acetobacter* spp., *Saccharomyces unisporus, Saccharomyces exiguus, , Kluyveromyces marxianus*
Allowed ingredients	Drinking water Raw milk Starter cultures of the mentioned microorganisms Salt Non-dairy ingredients (flavorings)	Drinking water Raw milk Salt Cultivation of starter Natural flavorings Natural ingredients for thickening
Protein	At least 2.7% (m/m)	At least 5% (w/w)
Lipid	Less than 10% (m/m)	Maximum 50% (w/w)
Acid titer	At least 0.6% (m/m)	At least 0.6% (w/w)
Total starter microorganisms	At least 10^7 cfu/g	Maximum 10^6 cfu/ml
Yeasts	At least 10^4 cfu/g	Maximum 10^2 cfu/ml
Ethanol	-	Maximum 0.5% (w/w)

* m/m: mass by mass, w/w: weight by weight and cfu/ml: colony-forming units per milliliter

7. The role of kefir in health

Figure 2- The uses of kefir in health

The famous phrase "Let food be thy medicine and medicine be thy food" was coined by Hippocrates (400 years BC), and this phrase has been used to illustrate the importance of diet in maintaining health throughout history. Kefir has been recommended for the treatment of several diseases, including cancer, tuberculosis, and digestive disorders (5, 6, 9). In recent years, following studies on its nutritional value, organic substances, bioactive compounds, and health benefits, kefir has been reported as a functional food product (8, 11, 12). It can also be considered a medicinal food supplement. Recent studies have reported various health effects and uses for kefir, which are shown in Figure 2 and discussed in the following paragraphs (5, 17, 21).

7.1 Antihypertensive

Hypertension is one of the critical factors in the development of cardiovascular diseases, and if left unchecked, it can lead to severe consequences such as heart attacks and strokes. Unfortunately, half of the people with this condition are aware of their disease, and few of them are under the supervision of a specialist doctor and taking medication. The antihypertensive effect is one of the benefits of kefir beverage in maintaining health. The effective substances resulting from the coexistence of microorganisms in this beverage, with various functions such as lipolytic and proteolytic decomposition, produce many biologically active peptides, such as ACE inhibitors, which then reduce the production of aldosterone. Additionally, these factors prevent the breakdown of bradykinin. As a result, they prevent the increase in blood pressure. Therefore, kefir beverage can act as a modulator of blood pressure (9, 14).

Various studies have linked the antihypertensive effect of kefir to both bacterial and non-bacterial components, attributing this effect to changes and modulation of gut

microbiota as well as the effects of microorganisms in kefir. These effects depend on the specific strains involved. Bioactive compounds produced by microbial strains vary; for instance, two tripeptides derived from milk fermented with Lactobacillus helveticus can suppress the activity of angiotensin I-converting enzyme and, consequently, inhibit the rise in blood pressure (9, 14).

7.2 Anti-cancer

The increase in the prevalence of cancer, expensive diagnostic and treatment methods, and some cases being untreatable have led to this disease becoming the second leading cause of death worldwide. Various factors, including genetics and environmental conditions, are effective in causing cancer. Studies show that changing lifestyle can prevent up to 50% of some cancers; one of the critical factors in changing lifestyle is altering diet and consuming practical and functional foods. The nutritional aspects of kefir, as a synbiotic compound, are effective in preventing and helping to treat cancer. This drink exerts its anti-cancer effects by delaying the growth of tumor cells, inducing apoptosis, reducing tumor growth, inducing an immune response, interfering with the antioxidant process, and modulating the gut microbiota (9, 10, 14).

7.3 Anti-diabetes

Hyperglycemia, or high glucose levels in the blood, is caused by a problem in the secretion or function of insulin, or both. According to the IDF, one in every 11 adults (aged 20 to 79 years) has diabetes. Without proper treatment, this condition contributes to diabetes becoming a global epidemic. To address this problem, prescribing and taking insulin is used as an expensive treatment. Investigating alternatives and improving methods for managing these conditions is essential. One of the key aspects in this field is the use of functional foods that possess both medicinal and nutritional properties (9, 19). For this purpose, studies conducted in previous decades have provided substantial evidence of kefir's anti-diabetic properties, and this drink has emerged as a promising low-cost therapeutic option (10, 12, 14).

7.4 Antimicrobial

One of the critical aspects of kefir is the presence of various probiotic microorganisms. This microbial community can produce various antimicrobial compounds such as hydrogen peroxide, ethanol, carbon dioxide, diacetyl, peptides (bacteriocins), and organic acids, which help prevent the growth of pathogens (9). In various studies, evidence has been presented regarding the bacteriostatic effects of kefir on gram-negative and gram-positive pathogenic bacteria (10, 14).

7.5 Antiviral

Several studies have investigated the antiviral potential of kefir, with one study demonstrating its protective ability against the SARS-CoV-2 virus. The bioactive compounds and probiotic content of kefir stimulate immune responses and suppress pro-inflammatory cytokines, helping the immune system confront and overcome viruses (10, 15).

7.6 Anti-inflammatory

All over the world, chronic inflammatory diseases can lead to death. Extensive studies in laboratory conditions and animal models have investigated the effects of kefir on the immune system, reporting its ability to modulate the immune response and reduce pro-inflammatory cytokines (9).

7.7 Antioxidant

Antioxidant molecules are crucial because they neutralize free radicals, stop chemical chain reactions, and protect proteins and DNA, thereby preventing cellular damage (9, 10, 14). Subsequent studies have proven that kefir drink has strong antioxidant potential. In these studies, the evidence indicates that the level of phenolic compounds in the final product resulting from the mixture of the two substrates has increased (9).

7.8 Hypocolesterolemic

Among the properties of kefir confirmed in studies, we can refer to its effect on reducing blood cholesterol. In a study, two strains of Lactobacillus rhamnosus and Lentilactobacillus kefiri, isolated from Indonesian kefir grains, reduced cholesterol in the environment from 68.75% to 22.08% (9). Another study investigated the effect of Lactobacillus plantarum in an animal model of rats on a diet rich in cholesterol. The results showed a decrease in serum cholesterol, liver cholesterol, and triglycerides, along with an increase in fecal cholesterol (9, 10).

7.9 Cardiovascular health

By choosing a modern lifestyle and reducing physical activity, the chances of people suffering from cardiovascular diseases have significantly increased. The term "heart disease" is commonly used to refer to a heart attack; however, heart diseases include heart failure, coronary artery disease, cardiac arrhythmia, heart attacks, and cardiomyopathy (8).

One of the known risk factors for cardiovascular diseases is dyslipidemia, which is associated with dysbiosis in the gut. It leads to changes in intestinal permeability, increased inflammation, and negative health effects (8, 12).

7.10 Neurological disorders and Alzheimer's disease

Neurodegenerative diseases affect various societies and are often related to aging. Notable examples include Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, which cause damage to motor or cognitive functions through the accumulation of abnormal proteins and the loss of nerve cells (14).

Today, many studies have been conducted on the definition of the gut-brain axis and the relationship between microbiota balance and nerve-related diseases such as Alzheimer's disease. The connection between them has been investigated and proven. Consumption of kefir as a functional food with a synbiotic composition can adjust microbiota, support nerve health, and improve the treatment of diseases such as Alzheimer's (8).

7.11 Modulation of gut microbiota

It has been proven in many studies that the community of symbiotic intestinal microorganisms has both a direct and indirect effect on the health of the host. In recent decades, extensive research has been conducted on the impact of microbiota balance on the health of the digestive system, immune system, nervous system, and more. Accordingly, maintaining the balance of microbiota and preventing dysbiosis is one of the critical factors in preserving health. Food and pharmaceutical products based on probiotics, such as kefir, are among those that modulate gut microbiota (12, 14, 15).

7.12 Anti-fat accumulation and obesity

Obesity is one of the problems that people in different societies face and has a significant relationship with various diseases, especially cardiovascular diseases. Lifestyle and diet are important factors in causing obesity (8, 12, 14). Bioactive compounds in kefir, a functional beverage, are effective in reducing fat accumulation, cholesterol levels, and obesity (12, 14).

7.13 Effect on Osteoporosis

According to the National Health Association, osteoporosis is a skeletal disorder that primarily occurs in old age. Its characteristic feature is a decrease in bone strength, which puts individuals at risk of bone fractures and curvature of the back, often caused by osteoporosis. Menopause and the subsequent natural decrease in female reproductive hormones between the ages of 40 and 50 make women more susceptible to this disease than men (14).

Studies have shown that the removal of the ovaries in female mice leads to osteoporosis along with a decrease in the hormone estrogen. The ovaries are the main organs of estrogen production, which balance osteoblastic and osteoclastic activities in bones. The absence of this organ and the hormones it produces can lead to many bone problems for the individual. Research has proven that the consumption of peptides in kefir, a functional drink, has an inhibitory effect on osteoporosis in the model of mice whose ovaries have been removed. These peptides can improve bone density, trabecular number, trabecular bone volume, mechanical properties, and the hardness of bones. Therefore, the use of peptide supplements can eventually reverse the condition of osteoporosis (14).

7.14 Wound healing

Various factors cause wounds, and numerous studies have explored effective compounds for their repair and treatment. Research has shown that the topical use of 70% kefir gel is effective for treating wounds in the Wistar rat model with skin wounds due to the presence of substances such as lactic acid and acetic acid. The effect of increasing the dosage and frequency of probiotic gel on its effectiveness is still under investigation (10).

7.15 Reduce toxins

Aflatoxin G1 is one of the toxic contaminants with potential health risks in nuts, grains, animal feed, and other products, making AFG1 depletion a significant food safety concern. According to the results of a study, the use of kefir seeds is effective in reducing AFG1 contamination in pistachio nuts. The optimization method of biological detoxification using kefir grains treated at 70°C can be suitable for removing AFG1 from pistachios (10).

7.16 Application of kefir in skin and skin diseases

Atopic dermatitis is a chronic inflammatory skin disease associated with gut dysbiosis and immune system dysfunction. Intestinal disorders such as dysbiosis have been shown to negatively affect skin function by increasing intestinal permeability. As a result, the gut-skin axis may be modified and modulated through dietary changes or probiotic consumption; Kefir is an ancient probiotic that positively impacts the overall state of the digestive system, including the gut microbiota. Despite the relationship between the improvement of skin parameters and the digestive system after kefir consumption, the role of kefir as a potential moderator of the gut-skin axis in healthy individuals and those with atopic dermatitis is emphasized (26). In addition to its indirect effects through the intestinal axis and the skin, kefir can be used topically to treat wounds and skin problems (27).

8. Other uses of kefir

8.1 The use of kefir as a symbiotic

Kefir grains can be defined as a synbiotic composition because kefir consists of a community of diverse bacterial and fungal microorganisms in a polysaccharide substrate. Types of lactic acid bacteria, such as Lactobacillus spp., types of yeasts, Streptococcus spp., and Lactococcus spp., are known as probiotics, while the exopolysaccharides of kefir grains are recognized as prebiotics. For example, it has been shown that polysaccharides produced by Liquorilactobacillus satsumensis obtained from non-dairy kefir in sucrose-containing media are selectively utilized only by probiotics; they are not usable by pathogens or digestive enzymes. The presence of synbiotic compounds in kefir enhances the growth of kefir probiotic bacteria and increases short-chain fatty acids. On the other hand, it has been proven that polydextrose and galactofructose affect the survival of probiotic bacteria and possess synbiotic properties (16, 18).

8.2 Application of kefir in cheese making process

The growing interest in consuming kefir and the greater recognition of its many benefits have led to the use of kefir to start the cheese-making process. Additionally, kefir grains are used in whey-based fermented beverages, which is very innovative. Using whey as a substrate preserves the properties of kefir. Today, the general approach to grain-free products (freeze-dried or heat-dried kefir) at the commercial level has changed. In research, the application of frozen kefir as a starter culture in the cheese-making process accelerated the ripening of the cheese and improved the sensory characteristics of the product. In other studies that used heat-dried kefir starter culture, the resulting product showed better aroma, taste, and texture. Finally, it should be noted that there are microorganisms and other bioactive compounds in kefir with unique biological properties. For instance, the antimicrobial properties increase the stability of this product (9).

8.3 The use of kefir in the development of food products

Despite the health benefits of kefir for humans, the consumption of milk-based kefir can sometimes be limited for individuals with milk sensitivity due to lactose and other factors. However, because kefir can be compatible with non-dairy substrates such as fruits and vegetables, non-dairy fermented drinks based on kefir can be prepared and recommended for these individuals (8). The presence of kefir and the fermentation process in beverages shows significant effects, such as reducing sugar content and increasing acidity and total phenol. It is also known that the fermentation process can change characteristics, such as the color composition of the product (8, 9, 19).

8.4 Using kefir in Making Biocompatible Packaging Compositions

Today, the use of natural and biocompatible polymers has become an essential goal for related companies. Recently, studies have shown that incorporating kefir in the production of edible films increases the density of the resulting composition while maintaining the thickness of the layers and improving hydrophilic properties. Adding kefir to the mix enhances the color and transparency of the layers, indicating that kefir can be utilized to improve packaging materials. Another study found that adding kefir can lead to the destruction of harmful microorganisms within 10 days. These potential benefits distinguish kefir in terms of environmental sustainability and human health (9).

8.5 Application of kefir in tissue engineering and medical applications

In addition to the development of food products, several biopolymers have been identified in kefir, such as kefiran, which can have significant applications in tissue engineering and medicine. For example, in research, kefir biopolymers were used to produce biological scaffolds with approved molecular structures. The resulting composition has been found to have acceptable characteristics. It should be noted that kefiran extracts and scaffolds exhibited no cytotoxic effects. All of the mentioned cases position kefiran scaffolds as a significant option in tissue engineering and medicine (8). Some uses of kefir in health and medicine, such as its antimicrobial activity, ability to help heal wounds, induction of apoptosis, and potential in cancer treatment, are listed in Table 2.

Table 2. Examples of Medical studies conducted on kefir

Number	Type of Beverage	Microorganism (Fungus or bacteria)	Growth medium	Geographical origin	The aspect under consideration	Effective substance	Biological effects	Mechanism of action	Study conditions	Reference
1	Dairy kefir	*Lactobacillus plantarum* ST8KF	Pasteurized milk	South Africa	Postbiotic	Bacteriocin 3.5 kDa (bacST8KF)	Antimicrobial	Bacteriostatic effect against *Enterococcus mundtii* *Lactobacillus curvatus*	In vitro	(30)

Table 2 (continued): Examples of Medical studies conducted on kefir

Number	Type of Beverage	Microorganism (Fungus or bacteria)	Growth medium	Geographical origin	The aspect under consideration	Effective substance	Biological effects	Mechanism of action	Study conditions	Reference
2	Dairy kefir	*Lactococcus* bacteria and yeasts	Pasteurized milk and Sodium carbonate	Egypt	Probiotic and Postbiotic and Prebiotic	Kefir beverage	Antimicrobial	Inhibitory effect *on* Aspergillus flavus AH3 Streptococcus faecalis KR6 Fusarium graminearum CZ	In vitro	(31)
3	Dairy kefir	Various lactic acid bacteria and yeasts	Pasteurized milk and soy milk	Taiwan	Probiotic and Postbiotic and Prebiotic	Kefir beverage	Induction of apoptosis and lysis of cancer cells	Modulating the activity of T cells, Natural killer cells, and macrophages	In vivo	(32)
4	Dairy kefir	*Various lactic acid bacteria and yeasts*	Pasteurized milk and soy milk	Taiwan	Probiotic and Postbiotic and Prebiotic	Kefir beverage	Reduction of serum total cholesterol	Cholesterol clearance through increased hepatic LDL receptor, Decreased intestinal absorption of cholesterol	In vivo	(33)
5	Dairy kefir	*Various lactic acid bacteria and yeasts*	Soy milk and goat milk	Indonesia	Probiotic and Postbiotic and Prebiotic	Kefir beverage	Immune system modulator	Increasing the proliferation of lymphocytes, increasing IL-10 production, and decreasing TNF-a production	In vivo	(34)
6	Non-dairy kefir	*Various lactic acid bacteria and yeasts*	MRS Broth	Iran	Probiotic	Kefir gel, produced from the extract of kefir grains in MRS broth	Antimicrobial and wound healing	Antimicrobial effects of kefir grains Wound treatment, anti-inflammatory effect, increased angiogenesis And improving the performance of silver sulfadiazine on skin wounds infected with *Pseudomonas aeruginosa*	In vitro	(35)
7	Dairy kefir	*Lactococcus spp.*	Pasteurized cow's milk	Germany	Postbiotic	αS1-casein, αS2-casein, β-casein, and κ-casein	Hypotension	ACE[2] inhibitor	In vitro	(36)
8	Dairy kefir	*Various lactic acid bacteria and yeasts*	Pasteurized milk	Brazil	Probiotic and Postbiotic and Prebiotic	Kefir beverage	Lower blood pressure and tachycardia	Vascular endothelial repair and reduction of oxidative stress	In vivo	(37)
9	Dairy kefir	*Various lactic acid bacteria and yeasts*	Pasteurized milk	Brazil	Probiotic and Postbiotic and Prebiotic	Kefir beverage	Amelioration of acute renal failure (AKI)	Reducing oxidative stress and reducing cell apoptosis	In vivo	(38)
10	Dairy kefir	-	Pasteurized milk	Korea	Postbiotic and Prebiotic	Kefir without microorganisms	Anti-Cancer effect	In colon cancer by increasing the efficacy of the drug doxorubicin (DOX) on HT-29 cells	In vitro	(39)
11	-	*Lentilactobacillus kefiri*	Kefir grains	United States of America	Postbiotic and Parabiotic	Probiotic bacteria killed by heat	Anti-Cancer effect	Induction of apoptosis by activation of caspase-3 enzyme in human myeloid leukemia cells (HL60/AR)	In vitro	(40)
12	Dairy kefir	*Lentilactobacillus kefiri* MSR101	Pasteurized milk	Syria	Probiotic and Postbiotic	Exopolysaccharide treated with sodium bicarbonate (alkaline kefir)	Anti-Cancer effect	Induction of apoptosis in human sarcoma cells	In vitro	(41)
13	-	*Lentilactobacillus kefiri*	Kefir grains	United States of America	Postbiotic and Parabiotic	Probiotic bacteria killed by heat	Anti-Cancer effect	Induce apoptosis in gastric cancer cells of human	In vitro	(42)
14	Non-dairy kefir	*Various lactic acid bacteria* and yeasts	Water	Japan	Postbiotic	Aqueous extract of kefir	Antioxidant effect	Antioxidant effect by preventing the production and induced apoptosis in human melanoma cell line	In vitro	(43)
15	Dairy kefir	*Lactobacillus acidophilus and Bifidobacterium spp.*	Pasteurized milk	Iran	Probiotic and Prebiotic	Kefir beverage	Lower HbA_1C levels	The effects of probiotics on the production of insulinotropic polypeptides and glucagon-like peptides, and as a result, glucose absorption increases the hepatic absorption of glucose.	In vivo	(44)
16	Dairy kefir	*Lactobacillus kefiri* CIDCA 8321	MRS Broth	Argentina	Probiotic	Lactobacillus kefiri CIDCA 8321	Antimicrobial	The production of antimicrobial molecules by *Lactobacillus* against *Pseudomonas aeruginosa* and the absence of pathogen transmission in Blood, spleen, or liver	In vivo, In vitro	(45)

[2] Angiotensin-converting enzyme

Table 2 (continued): Examples of Medical studies conducted on kefir

Number	Type of Beverage	Microorganism (Fungus or bacteria)	Growth medium	Geographical origin	The aspect under consideration	Effective substance	Biological effects	Mechanism of action	Study conditions	Reference
17	Dairy kefir	*Lactobacillus kefiranofaciens* DN1	MRS Broth	Korea	Postbiotic	Exopolysaccharide	Antimicrobial	Production of antimicrobial molecules by different strains of *Lactobacillus* against *Listeria monocytogenes* and *Salmonella enteritidis*	In vitro	(46)
18	Dairy kefir	*Lactococcus, Streptococcus, Lactococcus lactis subspecies lactis, Lactobacillus delbrueckii subspecies bulgaricus, Lactobacillus helveticus, Lactobacillus casei*	MRS Broth	Arabia	Probiotic	Kefir beverage	Anti-inflammatory effect	Decreased expression of IL-1, interferon γ, IL-6 and TNF-α	In vivo	(14)
19	Non-dairy kefir	*Lactobacillus perolens, Lactobacillus parafarraginis, Lactobacillus diolivorans,* and *Oenococcus oeni*	Brown sugar solution	Brazil	Probiotic	Kefir beverage	Having probiotic potential based on the relevant standard	-	In vitro	(47)
20	Non-dairy kefir	*L. Mesenteroides* BD1710 *Leuconostoc pseudomesenteroides* YB-*Sporidiobolus pararoseus* JD-2	Mineral water and brown sugar	Brazil	Postbiotic	Exopolysaccharide	Antibacterial activity	*Escherichia coli (*MIC[3] = 2.0 mg mL^{-1}) Staphylococcus aureus (MIC = 3.0 mg mL^{-1})	In vitro	(48)
21	Non-dairy kefir	*Lacticaseibacillus paracasei*	MRS Broth	Malaysia	Probiotic	Lacticase ibacillus paracasei	Antidiabetic effect	Regulation of glucose homeostasis and lipid metabolism in diabetic rats	In vivo	(49)
22	Dairy kefir	standard starter culture *Streptococcus thermophiles Lactobacillus delbrueckii subsp. bulgaric Lactobacillus paracasei* KC 39	Pasteurized cow's milk + Taro leaf extract	Spain	Kefir beverage	Synbiotic postbiotic	Antimicrobial activity	E. Cole BA 12296 Salmonella Senftenberg ATCC8400 (inhibition 19.3 ± 1.02 mm and 18.53 ± 0.75 mm) *Fusarium oxysporum ITEM 12591 (*inhibition area 12.93 ± 1.17 mm) S. aureus NCTC107888 (11.33 ± 0.84 mm)	In vitro	(50)
23	Non-dairy kefir	Kefir grains	Soy milk	Northern Taiwan	Kefir beverage	Synbiotic	Improving effect on obesity, type 2 diabetes, high blood fat, and liver-kidney toxicities	Ameliorative effect in obesity increased motor function. And reduced intestinal and pancreatic lipase activity by 34% and 35%. This led to a reduction in TC[4] and LDL-c and 66%. and an increase of HDL-c[5] in HFFD rats by 32%, which helped reduce body weight by 20%. It inhibited intestinal and pancreatic alpha-amylase activity by 26% and 31%, thus reducing blood sugar by 36%.	In vivo	(51)
24	Non-dairy kefir	Kefir grains and commercial starter.	Pasteurized water kefir	Switzerland	Kefir beverage	Synbiotic	The modulator of gut microbiota	It increased beneficial short-chain fatty acids, decreased harmful proteolytic compounds, increased the Bifidobacterium genus, Improved inflammation, and increased IL-1β, and IL-10.	In vitro	(52)
25	Dairy kefir	Kefir grains	donkey milk	Türkiye	Kefir beverage	Synbiotic	Anti-Cancer effects	The tumor volume decreased. Proliferated cell nuclear antigen levels increased. The number of apoptotic cells increased. In the donkey milk kefir group, iNOS[6] levels were decreased, and eNOS[7] levels were within normal range. It had anti-proliferative and anti-mutagenic effects on breast cancer.	In vivo	(53)
26	Dairy kefir + Non-dairy kefir	Kefir grains	Cow's milk and almond milk	Malaysia	Kefir beverage	Synbiotic	Antimicrobial effects	Growth inhibition Escherichia coli, Staphylococcus aureus, Salmonella typhi	In vitro	(54)

[3] Minimum inhibitory concentration
[4] Total Cholesterol
[5] High-Density Lipoprotein Cholesterol
[6] Inducible nitric oxide synthase
[7] Endothelial nitric oxide synthase

Table 2 (continued): Examples of Medical studies conducted on kefir

Number	Type of Beverage	Microorganism (Fungus or bacteria)	Growth medium	Geographical origin	The aspect under consideration	Effective substance	Biological effects	Mechanism of action	Study conditions	Reference
27	Dairy kefir	*Acetobacter pasteurianus, Lactococcus lactis, Leuconostoc mesenteroides, L. kefiri, L. kefiranofaciens, P. fermentans, S. cerevisiae, Kazachstania unispora and Kluyveromyces marxianus*	animal milk	Canada	Kefir beverage	Synbiotic	Anti-obesity physiological effects	Cholesterol decreased by about 35%, and liver TAG decreased by about 55%. The expression of cholesterol and lipid metabolism genes (3-hydroxy-3-methylglutaryl-coenzyme A reductase, PPARγ,[8] and CD36[9] decreased in the liver.	In vivo	(54)
28	Dairy kefir	Kefir grains	Cow's milk	Brazil	Postbiotic	Bioactive peptides	Anti-Alzheimer	It investigated motor function, brain morphology, and oxidative stress due to molecular binding between peptides and main proteins related to pathology in flies.	In vivo	(55)
29	Dairy kefir	Commercial kefir grains	goat milk	Taiwan	Postbiotic	Bioactive peptides	Prevent osteoporosis	Modulation of microbiota, lower trabecular separation, increased bone density, bone volume, and trabecular number.	In vivo	(56)
30	Dairy kefir	Two types of kefir grains	Cow's milk	Ireland	Synbiotic	Kefir beverage	Gut-brain axis modulator	Two types of kefirs increased gut microbiota to produce GABA and rise in *Lactobacillus reuteri*.	In vivo	(57)

9. Discussion and Conclusion

The intestine is related to the function of the immune system, chronic diseases, and various infections, and maintaining the balance of this system is also the responsibility of the gut microbiota. The reason for the change in the intestinal microbiome is often related to the use of antibiotics and an improper diet; in this case, the use of probiotics is effective in stimulating gut microbiome homeostasis and helps control gut microbiome-related diseases, such as antibiotic-associated diarrhea (AAD), inflammatory bowel disease (IBD), Crohn's disease (CD), and colorectal cancer (CRC). For example, regular consumption of kefir is beneficial for reducing the risk of the aforementioned diseases, gut dysbiosis, and for changing or improving gut microbiota (9).

Adjusting the gut microbiota, as one of the most important ways to prevent and treat many diseases, has led societies to consume probiotics and fermented foods as a nutritional alternative to drug treatments. Kefir is a complex probiotic containing a combination of bacteria and yeast; it can be considered a beneficial probiotic beverage due to its safety for animals and humans, affordability, ease of preparation, and microbiological composition which contains bioactive substances, metabolites, and peptides. In addition, it has other beneficial effects such as modulating the immune system, lowering blood cholesterol, reducing blood pressure and improving glycemic control, aiding in the treatment of neurological disorders, reducing aflatoxin, promoting wound healing, and influencing the treatment of osteoporosis, among others. Most of these effects are related to the presence of kefiran exopolysaccharide, which is used as a new strategy to improve deficiencies without adverse side effects or opportunistic infections, especially in infants and children. It is expected that in the not-so-distant future, kefir will become one of the most promising and health-giving food compounds with potential probiotic, postbiotic, and prebiotic properties (12, 28).

Despite all the mentioned properties, and the fact that kefir is a product of natural origin, with the absence of possible side effects, there are limiting factors in using this composition. These limitations may include an individual's resistance to consuming kefir or other personal preferences. Additionally, the acidic taste of kefir may not appeal to everyone; however, its sensory characteristics can be enhanced by incorporating bioactive compounds such as honey (29).

Glossary

Probiotics: Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.

Dysbiosis: An imbalance within a community of microorganisms coexisting in a microbiome.

Postbiotic: A postbiotic is a preparation of inanimate microorganisms and/or their components that confer health benefits to the host.

Prebiotics: Prebiotics are high-fiber foods that serve as nourishment for microbiota.

Acknowledgments

Not applicable.

[8] Peroxisome proliferator-activated receptor gamma
[9] TheCluster of Differentiation 36

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Shahrzad Sadeghi Amjad: Writing – original draft, Resources, Methodology, Investigation, Conceptualization. Hoora Dadgostar: Writing – original draft, Resources, Methodology, Investigation, Conceptualization. Ali Akhavan Sepahi: Writing – review & editing, Resources. Mohaddeseh Larypoor: Project administration, Validation.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Ethics approval and consent to participate

Not applicable.

Data availability

No data was used for the research described in the article.

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