THE FUNCTIONAL PROPERTIES OF SUGAR AND DAIRY BASED FERMENTED KEFIR BEVERAGE

Abstract

Probiotic microorganisms are found in great quantities in the fermented beverage kefir. While milk kefir grain has cauliflower appearance, water kefir grain has glassy and transparent appearance. Since the microbiota of both grains is different, the quality of the product obtained varies. Both kefir microbiotas include lactic acid bacteria (LAB), yeasts and acetic acid bacteria. *Lentilactobacillus kefir*, *Lentilactobacillus parakefiri*, *Kluyveromyces marxianus*, *Torula kefir*, *Acetobacter aceti* in milk kefir and *Lentilactobacillus hilgardii*, *Bifidobacterium aquikefiri*, *Zygosaccharomyces florentinus*, *Torulaspora pretoriensi*, *Acetobacter fabarum*, *Acetobacter orientalis* in water kefir constitute the dominant flora. The fat and protein content of the water kefir production medium is low and a probiotic drink is obtained as a result of sugar fermentation. Therefore, the composition, flavor composition, rheological properties and the amount of many biochemical metabolites such as antioxidant activity are different in both beverages. Therefore, the health effects of both beverages may also vary. In this study, the properties of the grains used in the production of milk and water kefir, product characteristics and their importance for health were investigated.

Keywords: Milk kefir, water kefir, human health, functional products, kefir grain

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I. INTRODUCTION

Various scientific studies conducted in recent years have shown that the relationship between the nutritional model and health has important effects on prolonging life span, leading a healthy life, and improving the quality of life. The direction of studies on healthy aging and disease prevention has also revealed the special physiological effects of nutrients. As a result of these developments, functional food, nutraceuticals, medical food, enriched food, diet food, and many similar products have started to take their place in the food market as health protectors and improvers [1]. Although urban life offers many opportunities to people, busy work lives and changes in lifestyles have led consumers to turn towards organic foods and functional foods, which they see as the key to body fitness and healthy nutrition. Functional foods are foods that bring a psychological or physiological benefit beyond their traditional nutritional value, some of whose compounds significantly and positively affect the functioning of the organism. This benefit may be the maintenance of health and well-being or the reduction of the risk of developing diseases, or both at the same time [2]. The concept of functional food emerged in Japan in the 1980s, and the concept called FOSHU (Foods For Specific Health Use) was accepted in other countries, especially in the USA, in the 1990s. Kefir consumption in the functional food market has increased day by day, showing a global market size of 1.73 million dollars in 2022. By 2026, it is estimated to reach 2.19 million dollars, with an increase of 5.99% [3, 4].

Healthy nutrition is an important starting point for producers in reaching consumers [5]. However, some factors such as the income status of consumers, the price of products, and consumers' lack of knowledge about functional foods and their scepticism towards the product limit the consumption of functional foods [6]. For example, it has been determined that the environmental interactions, news sources, and information acquisition methods of working women differ from those of non-working women and that having economic power is effective in their orientation towards functional foods [6]. In general, more than 90% of functional food sales are in the USA, Europe, and Japan. Among European countries, France, the Netherlands, and Germany are in the first place. The largest share of these food sales are confectionery, bakery products, cereal products, dairy products, and soft drinks. Among these, the most important group is dairy products [7]. Functional products are obtained by enriching, supplementing, modifying, or improving some components or living organisms added to milk. In addition to the probiotic microorganisms used, especially in dairy products, its functional properties can be increased by enriching it with some special food supplements that are not included in its composition. The most important feature of using probiotic bacteria in dairy products is that they improve intestinal health and the ambient flora. These beneficial microorganisms, which create an antagonistic effect, ensure that the intestinal flora is balanced. Thus, absorption of consumed nutrients is much better [8]. Hippocrates' statement, "All diseases begin in the intestine", proved the positive effects of intestinal microbiota on human health 2000 years ago [9]. For many years, milk kefir has been at the forefront of a probiotic dairy product. However, many consumers who prefer vegan diets have started to tend towards water kefir, which has high probiotic properties [10]. In addition to the important role of probiotic bacteria in water kefir fermentation, many effects on health have been identified. Mainly, it has properties such as preventing gastrointestinal infections, strengthening the immune system, preventing diarrhoea and some types of foodborne allergies [11].

II. GENERAL PROPERTIES AND PRODUCTION OF KEFIR

1. **Grain Properties and Microbiota of Milk Kefir and Water Kefir:** Although many definitions have been used for the word probiotic until today, one of the most widely accepted ones was made by Roy Fuller as “live microbial food additives that are beneficial to consumer health by maintaining or improving the intestinal microbial balance of individuals”. Guarner and Schaafsman defined it as “a certain number of live microorganisms that provide a certain health gain beyond ensuring healthy living” [12]. In the definition made by the World Health Organization (WHO), the word probiotic is stated as "live microorganisms that provide health benefits to the host if consumed in sufficient quantities" [13].

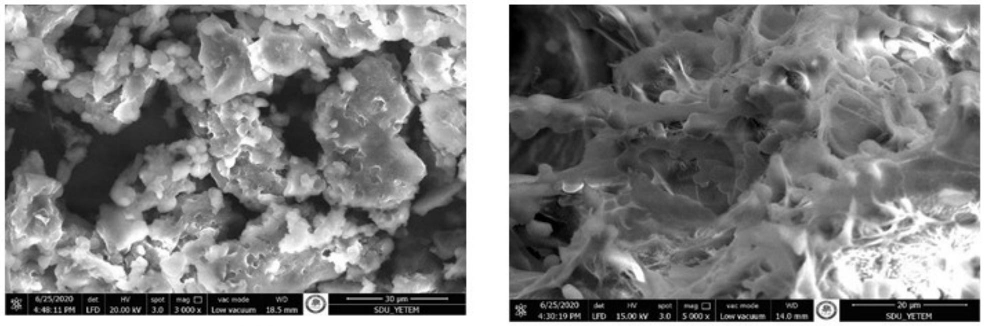
When kefir is mentioned, the first definition that comes to mind is a fermented drink with probiotic properties obtained from milk [14]. However, in recent years, water kefir drink, which is prepared in sugar-based solutions and has completely different properties than milk kefir, has also taken its place in the functional food market due to its probiotic properties [15]. While milk kefir grains need milk components such as protein, fat, and lactose, sucrose is an important carbon source for the development of water kefir grains, and grain development can be promoted with honey, molasses, and unrefined sugar [16]; fruits such as fig, grape, apple, carrot, apricot [17] and vegetables such as onion, ginger, soybean, carrot, or fennel [18]. However, besides the different origin of kefir grains, there may be changes in the grain microbiota according to the characteristics of the substrate used [19, 20]. The general and SEM appearances of milk and water kefir grains are shown in Figures 1 and 2.

(a) (b)

Figure 1: Milk (a) and water (b) kefir grain appearences

Depending on the type and fat content of the milk used, milk kefir is a bright pale color and resembles cauliflower in appearance. Depending on the country of origin, grain size ranges from 3 to 20 mm [21]. All around the World, water kefir grains are known also as “sugary kefir grains”, “Tibi” or “Tibico”, “Graines Vivantes” and “Japanese water crystals” [10, 22]. However, in many countries it is also known as “Kefir d'aqua”, “Japanese beer seeds”, “Beer plant”, “Ginger-beer plant”, “Australian bees”, “African bees”, “California bees”, “Ale nuts”, “Tepache de tibicos”, and “Balm of Gilead” [20, 23]. The diameter of the water kefir grain also varies according to the origin and characteristics of the grain. The diameter of the available water kefir grains varies from

0.2 mm to 6 mm [21, 24].



(a) (b)

Figure 2: Scan Electron Microscopy micrographs of milk kefir (a) and water kefir (b) grains

The most important factors in the production of milk kefir and water kefir are the diversity of grain microbiota, the amount of grain utilization, raw material composition, incubation temperature and time, and the use of flavorings agents (fruit, vegetables, etc.). When grain characteristics change, differences in product quality are also observed [25]. In order for milk kefir grains to remain alive, the growth medium (milk) must be renewed every day. In the exopolysaccharide matrix (kefiran) formed during the incubation period of about 20-24 hours, the grains grow, and their mass increases by 25% [26]. A modified version of the mechanism of kefir grain and kefiran formation [26] is shown in Figure 3. This situation is similar for water kefir grains. However, since the growth medium is a sugar-based solution, the basic structure of the kefir grain matrix is glucan. For this reason, in order for both kefir varieties to develop without deterioration of the grain characteristics, the environmental conditions must be at their optimum level. These polysaccharide structures are used in the production of packaging materials in the food industry as well as in the production of functional products such as food supplements [27].

Table 1: Basic differences of milk and water kefir grains

Parameter	Milk Kefir	Water Kefir	References
Appearance	Irregularly shaped, miniature cauliflower or popcorn	Translucent, waxy, rock salt, mucilaginous , jelly cristal	[21, 28, 29, 30]
Color	White -yellowish	Gray-white, slightly brown (according to sugar type, fruit and vegetable medium)	[21, 29, 31]
Dimention	3-20 mm	A few milimeters to centimeters	[21, 29]
Chemical composition	18.89% dry matter, 7.26% protein, 0.79% ash	17.5% dry matter, 0.20% protein, 0.06% ash	[14]
Exopolysaccharide structure	Kefiran, kefirose	α-Glucans, mannitol, mutan, fructan	[4, 32]

Studies show that the microorganisms in milk kefir grain are intensified differently in the inner and outer layers. It was found that the outer layer of the grain was concentrated with a denser group of microorganisms compared to the inner part, while the outer layer contained more lactobacilli, lactococci, and yeast, while the inner layer contained more yeast cells [33]. In another study, it was determined that the short rod-shaped ones, such as *Lactobacillus kefir* were found in the liquid part close to the grain surface, while the concentration of *Lactobacillus kefiranofaciens* in the form of long and thin rods increased as it descended towards the center [9]. The coke-shaped ones were localized close to the surface of the yeast cells. Yeast groups are located both in the center and on the surface of the grain. In general, the density of all groups is higher on the surface of the grain [33]. This is mainly due to their oxygen requirements and the change in pH value [34, 35].

While the composition of the raw material milk, incubation temperature and time, incubator mixing speed, vitamin and mineral supplementation are important in the diversity of milk kefir grain microflora, carbon (sugar) and nitrogen source (especially the use of dried figs), incubation temperature/time, addition of fruits or vegetables are seen to encourage grain growth in the development of water kefir grains [26, 36]. It is emphasized in many studies that the main reason for this is the nitrogen source from dried figs [29, 37]. In conclusion, although both kefir grain types mainly contain lactic acid bacteria and yeast (Table 2), the differences observed between the species can change the final product's quality characteristics. Unfavorable growing conditions negatively affect the reproductive capacity of milk kefir grains, leading to the deterioration of the grain structure, changes in their appearance, loss of resistance, and significant changes in microbial diversity [38]. However, it has been observed in many of our productions that water kefir grain structure deteriorates in low- or high-sugar-based solutions, and the exopolysaccharide structure dissolves, causing the kefir grains to deteriorate. When both kefir grains reach sufficient maturity, the grain mass increases, and division is observed, so that the newly formed small grains tend to grow again under favorable conditions [39]. The polysaccharide structure formed in milk and water kefir during both the growth of the grain and the fermentation of the product is called kefiran and glucan, respectively. Kefiran is a heteropolysaccharide containing mainly D-glucose and D-galactose [40]. Dextran, the main exopolysaccharide component of water kefir, is obtained by catalyzing α-glucan by dextransucrases [41].

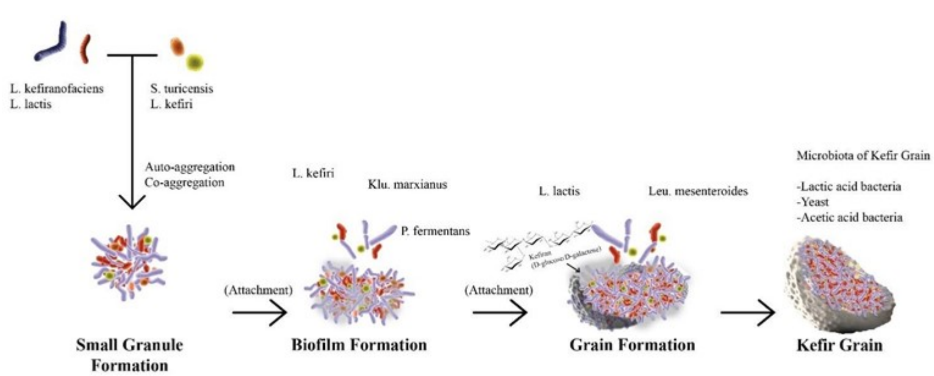


Figure 3: Kefir Grain Formation Mechanism

Table 2: Microbialdiversity of milkandwaterkefir grains

Microbial diversity	Milk Kefir	Water Kefir	References
Bacteria	*Lentilactobacillus kefiri Lentilactobacillus parakefiri Lentilactobacillus buchneri Lentilactobacillus parabuchneri Lactobacillus kefiranofaciens Lactobacillus delbrueckii subsp. lactis Lactobacillus acidophilus Lactobacillus acetotolerans Lactobacillus amylovorus Lactobacillus delbrueckii Lacticaseibacillus casei Lacticaseibacillus paracasei Lactiplantibacillus plantarum Levilactobacillus brevis Bifidobacterium psychraerophilum Lactobacillus helveticus Lactobacillus delbrueckii subsp. bulgaricus Liquorilactobacillus satsumensis Liquorilactobacillus uvarum Enterococcus durans Enterococcus faecium Leuconostoc lactis Leuconostoc mesenteroides Lactobacillus kefirgranium Lactobacillus amylovorus Pseudomonas putida*	*Lentilactobacillus hilgardii Gluconobacter cerinus Gluconobacter frateuri Bifidobacterium aquikefiri, Liquorilactobacillus hordei, Liquorilactobacillus nagelii Lactobacillus acidophilus, Levilactobacillus brevis, Liquorilactobacillu satsumensis, Lc. taiwanensis Lacticaseibacillus paracasei subsp. paracasei, Lactiplantibacillus plantarum Lentilactobacillus buchneri Lactobacillus fructiovorans Secundilactobacillus collinoides Lactococcus raffinolactis Schleiferilactobacillus harbinensis Leuconostoc mesenteroides subsp. mesenteroides, Leuconostoc mesenteroides subsp. dextranicum Enterobacter hormachei Bifidobacterium psychraerophilum Dekkera bruxellensis Kazachstania barnettii Kazachstania humilis Mortierella alpine Rhizopus arrhizus Saitozyma podzolica Pseudomonas fragi*	[14, 29, 36, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51]
Yeast	*Saccharomyces cerevisiae Kazachstania martiniae Kazachstania turicensis Kazachstania unispora Kazachstania exigua Candida inconspicua Candida humilis Geotrichum candidum Kazachstania aerobia Kazachstania exigua Kazachstania unispora Kluyveromyces lactis Kluyveromyces marxianus Lachancea meyersii Pichia fermentans Pichia guilliermondii Pichia kudriavzevii Torulaspora delbrueckii Torula kefir, Candida kefir*	*Saccharomyces bayanus Saccharomyces cerevisiae Zygosaccharomyces florentinus Torulaspora pretoriensis Zygotorulaspora florentina Hanseniaspora valbyensis Hanseniaspora vinae Hanseniaspora valbensis Hanseniaspora uvarum Pichia fermentans Pichia membranifaciens Teunomyces kruisii Candida ethanolica Candida tetrigidarum*	[42, 43, 45, 46, 52, 53, 54, 55, 56, 57, 58, 59, 60]
Acetic acid bacteria	*Acetobacter lovaniensis Acetobacter aceti, Acetobacter rasens, Acetobacter fabarum, Acetobacter syzygii Gluconobacter japonicus*	*Acetobacter fabarum Acetobacter orientalis Acetobacter tropicalis*	[60, 61, 62, 63]

2. **Milk Kefir Production and General Properties:** The word "kefir" is thought to be derived from the word "Keyf", which is used in Turkish in the sense of giving pleasure, and enrapturing. However, it is also known by various names such as kephir, knaphan, kiaphur, and kippi in different countries [64, 65, 66]. Kefir is a product rich in amino acids, potassium, calcium, phosphorus, folic acid, and B-vitamins in addition to protein, fat, lactose, mineral substances, and vitamins and its consumption has become widespread due to its positive effects on health [67]. In addition, the synthesis of some vitamins, during fermentation and the partial breakdown of proteins and lactose further increase the nutritional value of kefir [68]. Lactic, succinic, purivic, ketoglutaric, and oxalic acids formed during production give kefir a refreshing and acidic taste, while intermediate products consisting of carbonyl compounds, volatile fatty acids, and alcohols are responsible for the pleasant characteristic aroma [66]. Kefir is a product containing approximately 1% lactic acid and 0.5-2.0% ethyl alcohol as a result of lactic acid and yeast fermentation [69, 70], and its sensory properties are affected by microbial activity. Thus, some volatile fatty acids and some aromatic compounds such as CO2, alcohol, acetaldehyde, and acetone occur [39].

While kefir grains are added to pasteurized milk in traditional kefir production (Artisanal kefir), kefir starter culture is used in industrial production [71]. In the general kefir production method, quality controlled raw milk is pasteurized (93±2°C/10-15 min) and then cooled to 25°C. About 3-5% kefir grains or kefir starter culture is added to the milk taken into clean glass jars in home conditions or into fermentation tank or special fermented in factory conditions, and fermentation continues for 24-48 hours at a constant temperature (Figure 4). The rate of grain utilization may vary according to the characteristics of the kefir to be obtained. For example, while the amount of grain usage is 10 g /L milk for the production of a more viscous and low acidity kefir, 100 g /L milk can be preferred for the production of a less viscous and more acidic kefir [72]. During this process, as a result of the activities of microorganisms in the grain microbiota, acidity development occurs on the one hand and CO2 and flavor are formed on the other hand. At this stage, the consistency of kefir also changes depending on the dry matter of milk and especially the exopolysaccharide production of lactic acid bacteria. Low dry matter content of the milk used in production, unfavorable change in grain microflora, non-control of fermentation temperature and time cause some problems. Thus, there may be a risk of obtaining kefir beverages with weak structure, or excessive creep, excessive yeast growth, or weak flavour. In recent years, milk homogenization, whey powder or various additives have been used to improve the consistency of kefir or prevent serum separation [73]. Especially when making kefir with low fat content, fat replacements like inulin and structure-improving enzymes like transglutaminase are used to improve the structure and give the kefir a fatty feel [74]. In addition, nutritional fibers such as β-glucan [75], apple and lemon fiber [76] can also be added to improve nutritional value and functional properties. However, the addition of more than 2% of substances such as β-glucan may have the opposite effect and cause serum separation in the product [75] and fruit may change the acidity of kefir [76]. Fermentation is an important step in kefir production. Grain addition rate, incubation temperature and duration, and pH value at the end of incubation significantly affect product quality and microbiota diversity. For example, the use of grains and kefir starter cultures in production significantly affects the microbial diversity of the product. Because there is no yeast group in commercial kefir starter culture and the variety of lactic acid bacteria is limited [30]. Casein loses its stability with the acidity that develops in the fermentation stage that starts after the addition of kefir grains or starter culture to pasteurized milk. In addition, serum proteins, which are denatured at high temperatures, interact with casein through sulphydryl/disulfide bonds. Coagulation is completed with the conversion of lactose to lactic acid as a result of the activity of lactic acid bacteria, the separation of calcium phosphate from the micelle structure when the ambient pH reaches 5.1-5.2 and the formation of a three-dimensional gel structure at pH 4.6-4.7 as a result of casein aggregation [77].

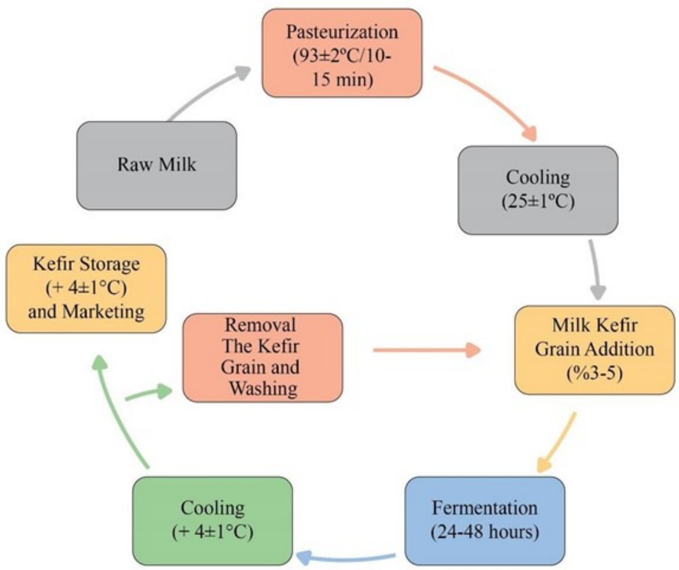


Figure 4: Milk kefir production stages

Kefir grain microbiome contains lactic acid bacteria that can produce exopolysaccharides (EPS), also known as extracellular polysaccharides, similar to starter cultures used in the preparation of yogurt. Kefir grain-specific kefiran is a water-soluble glucugalactone whose basic structural unit is composed of D-galactose and D-glucose. Although the ratios vary at different incubation temperatures, the kefiran composition contains 58.6-59.8% glucose and 40.2-41.3% galactose, but this ratio may vary depending on the microbiota of the kefir grain, the strain characteristics of lactic acid bacteria, and the incubation temperature and time [40]. Kefiran produced by lactic acid bacteria during fermentation shows Newtonian behavior in dilute solutions and pseoplastic behavior at higher concentrations. Kefiran croyojels are semi-rigid in appearance. In addition, due to its adhesive and water retention capacity, it causes an increase in viscosity and improves the structure [27]. The fermentation stage is usually terminated at pH 4.5-4.7 and the clot is allowed to rest and mature at +4°C in cold storage (Figure 4). If grain is used in production, it is removed with the help of a sterile strainer, and if commercial culture is used, it is directly packaged and shipped to the market.

In general, the composition of kefir varies according to the composition of the milk produced [78]. Depending on the alcohol and CO2 content of the product, it is classified as sweet, medium-hard, hard or very hard kefir (Table 2.2) [79]. Kefir obtained from cow's milk contains 88-90% moisture, 10-12% dry matter, 6% carbohydrate, 3-5%

fat, 3.1-3.6% protein, 0.7-0.8% ash, 1% lactic acid, 0.48% alcohol, and 1.98 g/L CO_2 [69, 80]. It is reported that the dry matter of kefir produced in Brazil is 9.62% and contains 3.91% protein and 2.34% fat [81].

The dry matter content of kefirs produced from milk of different animal species was determined as 11.39%, 11.03%, 17.33% and 11.06%; protein content as 3.17%, 2.75%, 6.20% and 3.14%; fat content as 3.01%, 3.61%, 7.11% and 4.02%; lactose content as 3.95%, 3.41%, 4.12% and 3.55% in cow, camel, sheep and goat milk, respectively [82]. The composition of kefir produced from buffalo milk was found to be 16.64% dry matter, 6.75% fat, 4.30% protein, 48.4% lactose, and 0.75% ash [83].

Table 2.2: Classification and characteristics of kefir [79]

Gross Composition	Sweet Kefir	Middle Sharp Kefir	Sharp Kefir	Very Sharp Kefir
Lactic acid (%)	0.8	0.6	0.7	0.9
Moisture (%)	88.2	88.9	89.4	89.0
Fat (%)	3.3	3.1	2.8	3.3
Casein (%)	2.9	2.7	2.9	2.5
Lactoalbumine (%)	0.3	0.2	0.1	0.1
Lactose (%)	2.7	2.9	2.3	1.7
Ash (%)	0.8	0.6	0.7	0.6
Ethyl Alcohol (%)	0.6	0.7	0.8	1.1

The type of animal fed as well as the animal breed change the composition of milk. Therefore, the quality values of the products produced also change. In a study conducted by Satır and Güzel-Seydim [84], the total dry matter content of kefir produced from milk of Saanen goat breeds raised with Intansive (SIGK) and Extansive (SEGK) diets was found to be 12. 09% and 13.34%, fat 3.79% and 4.5%, protein 4.0% and 3.69%, ash 0.77% and 0.77%, calcium 140.8 and 120.5 mg/100g, sodium 35.1 and 27.7 mg/100g. Biogenic amines such as putrescine, cadaverine, spermidine, and tyramine were also detected in kefir according to the activity of lactic acid bacteria [85]. Özdestan and Üren [86] found that the total biogenic amine content in kefir varied between 2.4 and 35.2 mg/L, and the highest amount among them was found in tyramine content, but the value found was within the legal limits.

3. **The Effect of Milk Kefir on Health:** The tendency of people who want to live a healthy life to eat functional foods is increasing day by day. Especially when the results of the studies on human health of functional dairy products such as kefir are examined, it is seen that it has started to be consumed more and more due to its positive effects on the human immune system, cholesterol, blood sugar, and blood pressure, as well as its antimicrobial, anticarcinogenic and antiallergic effects, reducing lactose intolerance, intestinal and digestive systems [87, 88]. In various studies, it has been reported that kefir has antagonistic effects against many microorganisms in the stomach and intestinal microbiota [85]. Due to the intestinal adhesion of probiotics to the intestinal epithelium, it is stated that they trigger the antagonism effect against pathogens, directly or indirectly.

According to another view, the co-aggregation of different types of cells that prevent the growth of pathogens and biofilm formation is the basis of the antimicrobial effect [89]. The antimicrobial effect against *Staphylococcus aureus* occurred after 48 hours, while *Cronobacter sakazakii* and *Salmonella enterite* are reported to be affected at 36 and 72 hours, respectively. The authors suggest that the metabolites of different strains formed as a result of fermentation contain different inhibitory compounds and interact with each other to enhance or antagonize their antibacterial effects during the incubation period [90]. In addition, regular consumption of kefir in the daily diet may provide inhibition by antagonistic effects on competitive pathogens in the intestinal mucosa due to acids and bacteriocins produced by the kefir microbiota [91]. In recent studies on yeasts in kefir, *S. cerevisiae* was found to be effective against *Klebsiella pneumoniae*, *Ps. aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. *Kluyveromyces lactis* was also found to be antagonistic against E. coli ATCC-25922, *Staphylococcus aureus* ATCC-BAA-42, and *Salmonella Typhimurium* BIOTEC019 [92]. In all these studies, the antimicrobial activity of kefir was ssociated with the production of organic acids, peptides, carbon dioxide, hydrogen peroxide, ethanol, and diacetyl. However, it is also reported that kefiran has antimicrobial activity against bacteria and *Candida albicans* [93].

Studies show that regular kefir consumption slows down and stops the formation of many types of cancer, such as breast cancer, chronic myelogenous leukemia, lung cancer, pancreatic cancer, prostate cancer, ovarian cancer, and colorectal cancer [68]. Kefir complex components are involved in the synthesis of anticancer bioactive components, including peptides, polysaccharides, and sphingolipids, in biological cell processes such as apoptosis, proliferation, and transformation, which play a vital role in different signaling pathways. In many studies, it has been determined that kefir reduces mutation and DNA damage, decreases the activities of some enzymes that affect cancer formation, such as ß-glucuronidase, nitroreductase, and azoreductase, inactivates substances that cause cancer formation, increases the production of short-chain fatty acids, and accelerates cancer cell apoptosis by increasing acidity, and thus shows an anticarcinogenic effect [94]. It is stated that metabolites formed in dairy products such as kefir and yogurt as a result of fermentation play a role in cancer prevention and suppression of tumor formation in the early stages act by delaying enzyme activities that convert carcinogenic compounds into carcinogens or activating the immune system [69]. This effect of kefir on cancer cells is thought to occur mainly as a result of four mechanisms (Figure 5) [95, 96, and 97].

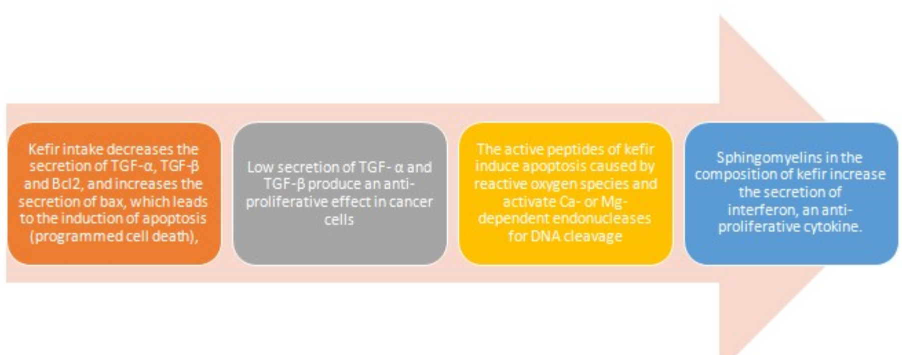


Figure 5: The effect mechanism of kefir on cancer cells

According to Liu et al. [98], they found that kefir may have anti-tumor characteristics and boost the mucosa's resilience to intestinal infections by inhibiting tumor growth, inducing apoptotic cell lysis in tumors, and significantly raising IgA levels in mice. Güzel-Seydim et al. [99] stated that this anticarcinogenic effect is due to the amino acid groups containing sulphur in the structure of milk proteins. In the study of Nagira et al. [100] on the effects of kefir on skin cancer cells, it was shown that kefir suppressed morphological changes in melanoma (skin cancer type) cell lines (HMV-1 and SK-MEL and TIG-1) caused by UV-C rays. The anti- tumour effects of soya milk kefir and milk kefir administered orally in mice bearing sarcoma (connective tissue) tumour cells showed that tumour growth was inhibited by 64.8% in the tumour bearing group, 64.8% in the tumour group containing milk kefir, and 70.9% in the soya milk kefir group compared to the control group [98]. In another study investigating the effect of kefir on leukemia, kefir consumption was found to increase apoptosis and decrease cell proliferation. It has been determined that kefir affects malignant T19 lymphocyte cells in leukaemia by acting on the substances in the body's immune system called Tran growing factor beta-1 (TGF-beta-1) and Tran growing factor-alpha, increasing GF-beta-1 and showing an anti-proliferative effect by reducing TGF-α [101]). In recent years, studies on the prevention of cancer formation by donkey milk and its products have gained increasing popularity. In a study about donkey milk kefir, it was determined that the tumour size of solid Ehrlich ascites carcinoma was reduced in mice fed kefir produced from donkey milk at a dose of 0.5 mL/day [102].

Obesity has become an important problem all over the world. For this reason, it has become a necessity for people to change their diet. Thus, the tendency towards probiotic products has increased in order to prevent many other health problems, and especially the demand for kefir consumption has increased. Probiotics in kefir prevent the accumulation of fat cells by further facilitating fat excretion and regulating insulin resistance. As a result, it is reported that regular kefir consumption supports the prevention of obesity by controlling weight gain [103].

Özkan [104] found that clinical, macroscopic, and histological colitis findings improved in rats given kefir at a 10% concentration in the Trinitrobenzene sulphanic acid (TNBS)-mediated experimental colitis model. However, in the group in which the kefir concentration was increased to 30%, it was determined that the incidence of diarrhea, weight loss, and histological colitis scores were similar to those in the colitis-control group. According to the tissue biochemical parameters performed on rats, it was observed that myeloperoxidase levels increased 3-4 times in colitis groups. In the study, while IL-1β levels did not cause a significant difference between the groups, IL-10 levels was suppressed, especially in the 30% kefir-colitis group. When evaluated in terms of TNF-α, TNF- α (tumour necrosis factor-alpha) was suppressed in the colitis groups while TNF-α was high in the control groups, contrary to expectations. In the TNBS-mediated experimental colitis model, although kefir given at 10% concentration had a protective effect against colitis, no protective effect was observed at 30% concentration.

In a study investigating the effect of kefir on the innate immune system, it was reported that metabolites in kefir strengthened the immune system by affecting the release of various cytokines such as TNF-α and IL-6 in peritoneal macrophages and adherent cells in Peyer's plaques [105]. In another study examining the effects of kefir produced from kefir grain and starter kefir culture on the immune system, 6-8-week-old, 20-25 gram, male Balb/c mice were fed 300 µl/day kefir by gavage for two weeks [106]. After sacrification, intestinal tissues of mice fed with kefir produced using natural kefir grain (KGI group), mice fed with kefir produced using starter culture (STI group) and control group mice fed with phosphate buffered saline (PBS) (CNI) were obtained, Immunoglobulin (Ig) A, Immunoglubulin G, Interleukin (IL)-4, Interleukin-10, Interleukin-12, Toll-like Receptor (TLR) -4 analyses were performed on intestinal fluid samples, and an immunological evaluation was performed. IgA values of CNI, KGI and STI groups were determined as 60.87, 72.78 and 55.31 ng/mL; IgG values as 26.59, 38.90 and 29.44 ng/mL; IL-4 values as 84, 40.28 and 53.28pg/mL; IL-10 values as 110.98, 175.91 and 134.77 pg/mL; IL-12 values as 53.90, 22.93 and 24.75 pg/mL; TLR-4 values as 0.53, 0.43 and 1.37 ng/mL, respectively. At the end of the study, it was stated that kefir has the capacity to modulate many immunological mechanisms. Although the evidence that kefir consumption reduces serum cholesterol has not been fully demonstrated, it is stated that bacteria and yeasts in kefir reduce cholesterol absorption by forming enzymes capable of bile acid degradation and reducing the activity of HMG-CoA reeducates, the most important enzyme in cholesterol synthesis [87]. Increasing the number of lactic acid bacteria in kefir provides up to 33% cholesterol binding due to the direct effect of bacteria on cholesterol through their metabolic products [107]. In kefir production, it has been determined that incubation of milk with kefir cultures for 24 hours leads to 28-65% assimilation of cholesterol [108]. In kefirs produced from sheep, cow and goat milk, it was found that kefirs obtained from sheep milk showed more hypocholesterolemic activity [109]. In another study investigating the hypocholesterolemic effect of milk and soya milk kefir, a cholesterol-free or cholesterol-enriched diet containing 10% skimmed milk, milk kefir, soy milk and soya milk kefir was tested on male hamsters for a period of 8 weeks. The soya milk, milk kefir, and soya milk kefir diets all resulted in reduced serum triacylglycerol and total cholesterol concentrations and reduced cholesterol accumulation in the liver, with the reduction in serum cholesterol concentration being mainly in the non-HDL fraction. In the study, it was determined that soya milk kefir caused a significant decrease in the serum ratio of HDL-cholesterol [110].

As a result of studies on blood sugar regulating effects, it is stated that water-soluble parts of kefiran and kefir increase glucose uptake in skeletal muscle cells. Therefore, it is thought to be used in the treatment of Type 2 diabetes. In a study investigating the protective effects of kefir in rats with experimental Type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD), a total of 30 Wistar albino female rats were examined. While no treatment was given to the control group animals, 80 mg/kg intraperitoneal streptozotocin was given to the rats at a single dose to induce T2DM, and rat feed with a high fat content was given to the rats to induce NAFLD. As a result of the experiment, it was determined that the blood glucose levels of rats with kefir in the ration decreased [111]. Regular consumption of kefir obtained as a result of fermentation suppresses the IgE and IgG1 responses and creates antiallergic effect. In addition, kefir use is recommended in the treatment of allergic bronchial asthma [112]. Another study has shown that kefir prevents ovalbumin-induced eosinophilia in lung tissue and mucus hypersecretion. Prevention of this suggests that kefir has great therapeutic potential for the treatment of allergic bronchial asthma [113].

In animal experiments, kefir was found to significantly reduce blood pressure and serum cholesterol levels, especially in animals consuming high amounts of dietary cholesterol [114]. The reduction in blood pressure is associated with suppressing the angiotens in-converting enzyme (ACE). ACE activity affects blood pressure in different ways. ACE converts AT I to AT II. AT II is a strong vasoconstrictor and stimulant. Two of the 16 peptides from kefir have been found to show ACE inhibitory activity ([115]. Today, ACE suppressors are used in the treatment of hypertension.

III. WATER KEFIR PRODUCTION AND GENERAL PROPERTIES

1. **Identification and Production of Water Kefir:** Water kefir is a white or yellowish, slightly foamy, carbonated probiotic drink containing trace amounts of alcohol depending on the use of white or brown sugar [4, 10]. Although there are two different theories about its origin, it is not known exactly how it emerged. One of these approaches is that soldiers returning from the Crimean War brought kefir grains from Western Europe. Another approach is that it spontaneously formed on the leaves of the Opuntia cactus in Mexico [116]. Water kefir, whose origin is known in Mexico, is also known as “Tibicus” or “Tibi”. However, in many countries it is also known as “Ginger-beer plants”, “Tibetan mushrooms”, “California bees”, “African bees”, “Ale nuts”, “Balm of Gilead”, “Bèbées”, “Japanese Beer Seeds”, “Sugary kefir”, “Acquakefir", etc. [20, 23, 45]. Unlike milk kefir, which is fermented with sheep, cow, or goat milk, water kefir is a natural beverage obtained by fermentation with water kefir grains in a boiled and cooled sugar and/or fruit-based solution. Especially water kefir, which has probiotic properties like fermented dairy products, is an indispensable probiotic drink for vegetarians and lactose intolerant people and is usually subjected to a secondary fermentation with fruit pieces or fruit juice [117]. Indeed, fruit juices contain water, sugar, proteins, amino acids, vitamins, and minerals, which are favorable and rich environments for microbial growth [118]. Moreover, fermentation of these substrates enables the appreciation of water kefir beverages with acidic flavor, refreshing, slightly carbonated, low alcohol, and acetic content [61]. Furthermore, it was determined that the addition of figs to the solution in water kefir production caused 10 times more lactic acid production than the fermentation product in the medium containing only sugar, as well as increasing TEAC and ORAC antioxidant activity [14].

   Although there is no standard method for the production of water kefir, the fermentation process is carried out at 20-37°C for 24-72 hours in a sugar-based solution containing between 3% and 10% sucrose (Figure 6). Depending on the grain characteristics, an inoculum rate between 3% and 8% are considered appropriate [15, 119, 120]. The most commonly used sugar source for water kefir fermentation is raw cane sugar, but beet sugar is also preferred as it is cheaper and easily available. Due to the presence of molasses in the composition of brown sugar, the mineral content is higher compared to white sugar. Therefore, it is thought that grain development and fermentation are encouraged. In addition, various fruits and vegetables, especially dried figs or raisins, are also preferred as a source of both carbon and nitrogen [14, 15]. The pH value of the water kefir beverage after fermentation may vary according to the fermentation temperature and duration, grain addition rate, carbohydrate source used, and fruit and vegetable variety. For example, Ph 4.71 in the sample containing only 5% sugar, pH 3.59 in the sample containing dried figs [121]; pH 3.71 in the control sample, 3.66 in the sample containing raisins, 3.56 in the sample containing grape juice in the beverage made with 2% dematerialized whey [15]; pH 4.1 for carrot juice, 4.40 for fennel juice, 3.6 for strawberry juice; and pH 4.2 for tomato juice [18]. In addition, the addition of fruits and vegetables to water kefir significantly affects total solids content, antioxidant activity, and flavor and microbiota diversity.

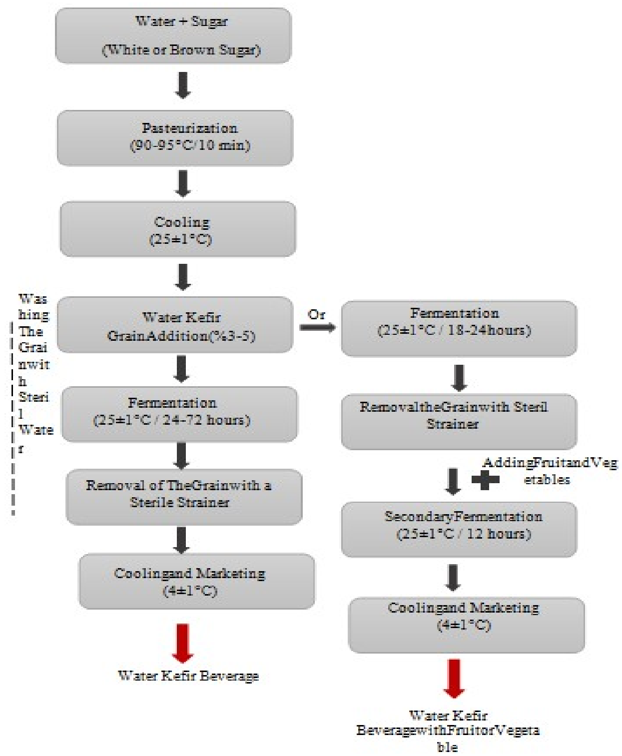


Figure 6: Water Kefir Production Stage

Table 4: Some chemical, antioxidant capacity and volatile aroma compounds of water kefir drinks made from different growth medium

Content of water kefir	Gross composition	Aroma compounds	References
Sugar solution (6 g unrefined cane sugar + 5 g dried fig)	pH 3.45 Sucrose 1.2 g/L	2-Methyl-1-propanol, Isoamyl alcohol, Ethyl acetate, Isoamyl acetate, Ethyl hexanoate, Ethyl octanoate, Ethyl decanoate, Ethyl butanoate, Ethyl 2- methyl-butanoate	[47]
Soy Whey	pH 3.47 Total Soluble Solid (Brix°) 4.43 Fructose 1.75 (g/L) Glucose 15.6 (g/L) Sucrose 1.42 (g/L)	Acetic acid, Hexanoic acid, Octanoic acid, n-Decanoic acid, 1-Octanol, 2,3-Butanediol, 2-Ethy-1-	[122]

	DPPH radical scavenging activity 40.55–72.34 (% ˙Inhibition) TEAC antioxidant capacity 38.65 – 87.06%	hexanol, Phenylethyl Alcohol, Nonanal, 1-Butanol 3-Methyl Hexanoic acid, ethyl ester, Octanoic acid ethyl ester	
Aronia pomage	pH 3.72 Total Soluble Solid (Brix°) 6.10 Sucrose 7.75 g/100 mL DPPH radical scavenging activity 58.7 mg TE/100 mL Total phenolic 7.33 mg GAE/L	1-Hexanol, 1-Hexanol, 2-Ethyl, 1- Octanol, Benzyl alcohol, Phenylethyl Alcohol, Ethanol, 1-Butanol, 2,3- Butanediol, 4-Nonanol, Hexanoic acid, 2-Ethyl, Octanoic acid, Nonanoic acid, Nonanal, Propanoic acid, n-Decanoic acid, Benzoic acid ethyl ester, Decanoic acid, ethyl ester, Hexanoic acid, ethyl ester, 7-Octenethiol, Benzaldehyde, Butylated Hydroxytoluene, 2,4-Di-butylphenol, CO_2	[123]
Shiraz Dried Grape	pH 3.62 Protein 0.3% Ash 0.51% Viscosity 12.40 cP Total Soluble Solid (Brix°) 5.08 DPPH radical scavenging activity 14.5 (% Inhibition) Total phenolic 30.6 mg GAE/L TEAC antioxidant capacity 21.2 µM TE/100 g	Ethanol, Oxalic acid, Acetic acid, 1- Pentene, 6-Methy l-Octanal, Formic acid, 4-Heptanone 2-methyl, 1-Hexanol, 2- Ethyl 1-Butanol 2-methyl, 1-Nonanol, 4-Octanone, Butylated Hydroxytoluen, Propene	[15]
Shiraz Fruit Juice	pH 3.43 Protein 0.41% Ash 0.53% Viscosity 13.20 cP Total Soluble Solid (Brix°) 5.80 DPPH radical scavenging activity 17.3 (% Inhibition) TEAC antioxidant capacity 23.5 µM TE/100 g Total phenolic 33.1 mg GAE/L	Ethanol, Oxalic acid, 3-Octanone, 1- Butanol 2-Methyl 2-Propanoic acid, Methoxyacetic acid, Octanoic acid, Acetic acid, 7-Octene 2-ol, 1-Hexanol 2-Ethyl 1-octanal, 1- Pentene, 1- Nonanol, Butylated Hydroxytoluen, Propene	[15]

N.A.: Not Analysed

In a study conducted with demineralized whey prepared to contain 2% and 5% lactose instead of sugar as a carbon source, the effect of raisin and grape juice obtained from different grape varieties on the properties of water kefir drink was investigated [15].

In the study, it was determined that the protein and total solid content of the water kefir beverage increased with the addition of fruit juice. Similarly, the increase in product viscosity was found to be directly proportional to the lactose content. The total phenolic and antioxidant activity of water kefir beverage produced with d whey containing 2% lactose was found to be significantly affected by the grape variety and higher in the samples with fruit juice addition. The total phenolic content of water kefir beverage produced with d whey containing 2% lactose was 24.1 mg GAE/L in the control sample, 28.3 mg GAE/L and 29.6 mg GAE/L in the samples containing fruit juice; 27.6 mg GAE/L, 31.2 mg GAE/L and 33.1 mg GAE/L in the samples containing 5% lactose, respectively. DPPH antioxidant activity (% inhibition) was determined to be 10.4 in the control sample, 15.62 and 16.3 in the samples made with Dimrit and Shiraz grapes, respectively. The TEAC values of the samples were determined as 12.44, 18.6 and 24.8 μM TE/100 g, respectively. In 5% lactose based water kefir, DPPH antioxidant activity (% inhibition) was 1.2, 14.9, 17.3; TEAC antioxidant activity were 13.2, 19.3 and 23.5 μM TE/100 g. According to the sensory evaluation results, it was observed that water kefir samples containing 5% lactose had a more acidic and yeasty flavour.

2. **The Effect of Water Kefir on Human Health:** The tendency of people who want to live a healthy life to eat functional foods is increasing day by day. Looking at the results of studies on human health, it is seen that functional dairy products such as kefir have started to be consumed more and more due to their positive effects on the human immune system, cholesterol, blood sugar, and blood pressure regulating effects, as well as antimicrobial, anticarcinogenic, and antiallergic effects, reducing lactose intolerance, and intestinal and digestive systems [87, 88, 110].

The fact that water kefir is seen as a food that has a positive effect on health is due to its lactobacilli, Bifidobacteria, and, to a lesser extent, Saccharomyces genus microorganisms, and its compatibility with fermentable sources, which enables the development of new probiotic products. According to the findings obtained from many studies on milk-based kefir products, it shows that it may cause health problems in people with problems such as lactose intolerance, lactose as a sugar source, and cholesterol levels. The presence of beneficial bacteria and yeasts that can easily adapt to various substrates, the low calorie content, zero cholesterol, and lactose-free properties of water kefir have enabled it to be considered an extremely attractive alternative to milk-based kefir products [20, 51], making water kefir an important probiotic source for vegetarian/vegan individuals. It has been determined that water kefir grains, which have a flora similar to milk kefir in terms of lactic acid bacteria and yeast flora, have the potential to be consumed by individuals with lactose intolerance problems, individuals with allergic constitution, individuals who prefer vegan diets. They can also be used in the production of innovative beverages containing different functional and sensory properties due to their ability to use different sugar sources [124].

Streptozotocin injections made adult Wistar rats diabetic, and they received water kefir at concentrations of 10% and 30% for five weeks. In the study, it was reported that water kefir had a beneficial effect not only on blood glucose but also on body weight and lipid profiles in streptozotocin-induced diabetic rats. It is stated that water kefir is an inexpensive supplement with hypoglycaemic and hypolipidemic effects even when consumed for a short period of time, and therefore, water kefir may be a potential health supplement for diabetic patients who need to control blood glucose levels and reduce cardiovascular risks [125].

In another study, the hepatoprotective (liver protective) effect of water kefir in rats was investigated based on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. A lethal dose of acetaminophen (640 mg/kg) was given to induce hepatocellular damage in experimental animals, and the effects of water kefir were measured. According to the results obtained in the study, it was determined that water kefir providing a hepatoprotective effect by significantly reducing the levels of AST and ALT enzymes. In addition, the effect of water kefir given at different dosages was found to be more significant. In the study, the amount of fermented water kefir providing hepatoprotective effect was determined to be 3 ml and 2 ml for AST and ALT, respectively. It was also observed that the increase in water kefir concentration caused a decrease in the AST level of rat blood samples [126]. Similar results were found in rats fed 2 and 3 ml doses of water kefir. It is thought that water kefir protects the mitochondria from oxidative stress caused by acetaminophen toxicity, thus protecting the integrity of the mitochondrial membrane [127].

In a study investigating the ability of water kefir to prevent and treat acute liver failure, it was concluded that the feeding dose of water kefir at 90 mL/kg BW, 180 mL/kg BW and 270 mL/kg BW for 14 days with silymarin could significantly improve aspartate aminotranferase (AST), alanine aminotransferase (ALT), and total protein levels, and there was an improvement in the level of liver necrosis [128]. In the other study, it was determined that water kefir given to rats had a protective effect against irritable bowel syndrome, TNF-a, NF-kB expressions, and histopathological effects were significant [129].

Intracellular and extracellular metabolites made by the microflora in water kefir during fermentation have a big effect on how well they get rid of DPPH radicals [125]. Similarly, the radical scavenging effects of dextran produced as exopolysaccharide are reported to be shown by reacting with free radicals derived from radical chain reactions or by converting radicals into more stable forms by giving them electrons. It is stated that this effect is caused by reducing hydroxyl and other functional groups such as -COOH, C=O, and -O- [32].

Additionally, the water kefir fermentation-induced microbial activity results in the hydrolysis of microbial enzymes, rupturing the substrate's cell wall, and releasing a variety of beneficial chemicals. Additionally, microbes metabolize these bioactive substances to create more straightforward substances, which increases their bioactivities [121].

IV. THE USE OF MILK AND WATER KEFIR EXOPOLYSACCHARIDES IN THE PACKAGING INDUSTRY

Exopolysaccharides formed by many lactic acid bacteria and yeasts are produced in two ways; intracellularly and extracellularly. Depending on the source, kefiran, dextran, mutan, alternan, levan are among the exopolysaccharides that are produced. Kefir grains are capable of producing partially branched α-1,6-linked dextrans. However, kefiran made from milk kefir fermentation contains repeated units of branched hexa- or hepta-saccharides, such as (1 6)-linked Glc, (1 3)-linked Gal, (1 4)-linked Gal, and (1 2, 6)-linked Gal [130, 131]. Expolisaccharides are employed in a variety of industries and applications (Table 5). As can be seen in the table, the usage areas of kefiran and dextran are quite different. Besides the effect of kefir on health and product quality, exopolysaccharides from milk and water kefir can also be utilized to make packaging materials, which is another useful property. Because polysaccharide-based films are relatively stiff, plasticizers are required to make handling easier [38]. Kefiran and dextran are combined with other compounds, particularly when creating edible composite films [132]. By using milk kefir grains, which are made up of proteins, polysaccharides, and a complex symbiotic microbial combination, *Lactobacillus kefiranofaciens* produces EPS kefiran [62]. Both homopolysaccharides and heteropolysaccharides can be produced by lactic acid bacteria in milk and water kefir [14]. The homopolysaccharides produced are glucans or fructans, which contain just one kind of monosaccharide (fructose or glucose) [133]. Ghasemlou et al. [132] investigated a novel edible film made from kefiran and plasticized with sorbitol and glycerol. Their findings showed that kefiran's film-forming agent played a fundamental role in these edible films. High concentrations of glycerol and sorbitol generated phase separation in the films, which was more successful than sorbitol as a plasticizer to improve the mechanical and physical qualities of kefiran films because their natural edible films had poor mechanical and physical properties. New biodegradable film materials were created by Rad et al. [134] using kefiran as the soft section and waterborne polyurethane (WPU) as the hard segment.

Table 5: Utilisation of exopolysaccharides from milk and water kefir in different industries

Exopolisaccahyride Type	Applications Field	Properties	References
Kefiran	Food	Decreases syneresis, increases viscosity, improves rheological properties, increases water-holding capacity	[29, 40]
Kefiran	Pharmaceutic	Thermal stability, good injectability and desirable viscoelastic properties, mechanical stability and elastic behavior	[135]
Kefiran	Clinical	Suppression of degranulation of mast cells, ability to produce cytokines, anti-inflammatory effect and regulation of the immune system	[136]
Kefiran	Clinical	The ability to change the balance of immune cells in the intestinal mucosa	[137]
Dextran	Biomedical	Emulsifying properties, film-forming ability, low photodegradation rate and good mucoadhesive properties	[138]
Dextran	Medicine, pharmaceutics and biotechnology	Blood-plasma expander and anticoagulant in medicine, drug carriers in pharmaceutics and matrix for chromatographic columns among others in biotechnology	[139]

According to what they found, when the amount of kefiran/WPU blends was less than 30 wt%, new properties like a uniform structure, higher tensile strength, the ability to mix, and thickness were seen. In another study, increasing the amount of chitosan in composite films by adding film-forming solutions with various kefiran-to-chitosan ratios. However, there was a considerable increase in tensile strength, elongation at break, puncture strength, and puncture deformation. There was a large decrease in moisture content, solubility in water, and water vapor permeability [140]. Coma et al. [141] produced films for use in sustainable packaging using only water kefir grain biomass. The authors assessed the effects of various glycerol plasticizer concentrations on the characteristics of films. Films with extremely low tensile strengths were produced depending on the concentration of this plasticizer, for instance, 1.9-0.3 MPa for films with 30% glycerol.

V. CONCLUSIONS

The number of probiotic beverages available is growing by the day. Although milk kefir has long held a place in the functional food industry, in recent years, consumers who are allergic to milk casein or follow a vegan diet have turned to water kefir, a sugar- based beverage. As a result, the focus of research has shifted to this product. Studies on water kefir, in particular, are rising by the day, and each year a new feature emerges. The basic characteristics and microbial diversity of both kefir beverages and health research were presented in this study. In general comparison, milk and water kefir show significant differences in grain structure and microbiota and are fermented in different production environments.

Water kefir is an important substitute for milk kefir for consumers who have a milk casein allergy or follow a vegan diet. Water kefir is also a good source of probiotics for people who don't want to consume dairy products. Both products fermented in solutions containing different components have varied nutritional value, chemical and microbiological qualities, flavor components, antioxidant capacity, and health impacts. However, both products hold a significant position in the probiotic market. The health benefits of water and milk kefir are the subject of several studies, and the functional aspects of the products are well investigated. Therefore, it is estimated that the consumption of both products will increase further.

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