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Nutritional value and health benefits of an ethnic dairy product Kefir

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Abstract

Kefir is a fermented milk drink with an acidic taste and creamy consistency produced by bacterial fermentation of kefir grains. Kefir is a popular ethnic dairy product that is constantly subjected to development, different additives of flavors, milk types, fibers, grains, and many more have been examined for the market. Kefir exhibits many health benefits owing to its antimicrobial, anticancer, gastrointestinal tract effects, gut microbiota modulation, anti-diabetic effects, wound healing, anti-stress properties, immune-modulation, cholesterol-lowering, anti-allergenic, anti-asthmatic, anti-microbial, chemoprevention against colon cancer, aside from its gastrointestinal beneficial effects. Such health benefits are attributed to Kefir's protein, vitamin, lipid, mineral, amino acid, and microelement composition. Moreover, the fermentation process enriches the content of vitamins B1, B12, K, folic acid, calcium and amino acids, adding to Kefir's health benefits. As such, kefir contains various bacteria and yeasts which influence its chemical and sensory characteristics. A mixture of two kinds of milk promotes Kefir sensory and rheological properties aside from improving its nutritional value. However, kefir is well recognized for its potential health value as an excellent source of probiotics, some limitations in kefir consumption need to be recognized.

Keywords: Bacteria; Probiotic; Dairy Product; Kefir; Milk Kefir; Water Kefir; Fermentation; Yeast

1. Introduction

Kefir is a dairy product that can be prepared from different milk types, such as goat, buffalo, sheep, camel, or cow, donkey milk via microbial fermentation (inoculating milk with kefir grains) [1-35-139]. Kefir is a fermented milk drink with an acidic taste and creamy consistency produced by bacterial fermentation of Kefir grains [1-52-139]. Kefir is a viscous, slightly carbonated dairy beverage, which has its origins in the Caucasian, Tibetan and Mongolian mountains. It comprises a complex microbial consortium of mainly lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeasts, and is considered a functional dairy product as it has been associated with a wide range of health benefits [1-139]. Three *Lactobacillus* species have been identified in the microbiota of the traditional dairy product Kefir, namely *Lentilactobacillus kefiri* (basonym: *Lactobacillus kefir*), *Lactobacillus kefiranofaciens* and *Lentilactobacillus parakefiri* (basonym: *Lactobacillus parakefir*) [1-138]. Kefir exhibits many health benefits owing to its antimicrobial, anticancer, gastrointestinal tract effects, gut microbiota modulation, anti-diabetic effects, wound healing, anti-stress properties, immune-modulation, cholesterol-lowering, anti-allergenic, anti-asthmatic, anti-microbial, chemoprevention against colon cancer, aside from its gastrointestinal beneficial effects [1-139]. Such health benefits are attributed to Kefir's protein, vitamin, lipid, mineral, amino acid, and microelement composition [1-148]. Moreover, the fermentation process

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enriches the content of vitamins B1, B12, K, folic acid, calcium and amino acids, adding to Kefir's health benefits [1-139]. As such, Kefir contains various bacteria and yeasts which influence its chemical and sensory characteristics [1-35]. Kefir, which is made up of complex microbiota mainly *Lactobacillus* spp., has been suggested as a potential reservoir for probiotics [1-23-148]. Benefits of probiotics to human health includes aiding in balancing gut microflora by increasing number of microbes in the diet, triggering the immune system to combat pathogens, and antimicrobial properties [1-25,26-139-148].

Kefir is a traditional fermented dairy beverage, first consumed in Caucasus Mountains of Russia centuries ago[1-140]. Nowadays, its popularity has been expanded worldwide and it is considered as a healthy product with high nutritional value in Europe, Asia, and South and North America [1-140]. It is a stimulating and self-carbonated beverage produced through fermentation of different types of milk such as sheep, cow, goat, etc[1-136]. However, currently, alternative Kefir drinks have been proposed, substituting milk and applying other carbohydrate sources, such as fruit juices, molasses, etc. [1-136]. The study of probiotic products is one of the fastest growing ventures among other functional foods due to an increase in consumer awareness towards its multitude of beneficial effects on health [1-100, 136]. Probiotics are defined as living microorganisms that offer health benefits to the host when consumed in an adequate quantity [1-69-140]. Probiotics are functional foods that have been demonstrated to be effective treatments to control several diseases such as inflammatory bowel disease, allergy, diarrhea, etc. [1-7-136]. The gut is the key target for probiotics foods because it acts as the main interface between diet and metabolic pathways in human health as it modulates the intestinal microflora [1-8-136]. Many genera and species of microorganisms can be considered as potential probiotics, but the genera that are commercially used in probiotic foods are the *Lactobacillus* and *Bifidobacterium* [1-140]. *Lactobacillus* acts as an important probiotic because of its strain-specific properties, which are beneficial to health, mainly towards the microflora of the gastrointestinal tract [1-136]. It also has been declared to have generally regarded as safe (GRAS) status and is regulated by the FDA (food and drug administration) for human and animal consumption[1-136].

A mixture of two kinds of milk promotes Kefir sensory and rheological properties aside from improving its nutritional value [1-135-148]. Additives such as inulin can also enrich Kefir's health qualities and organoleptic characters[1-15]. Several metabolic products are generated during Kefir production and account for its distinct flavor and aroma: Lactic acid, ethanol, carbon dioxide, and aroma compounds such as acetoin and acetaldehyde [1-135]. During the storage process, microbiological, physicochemical, and sensory characteristics of kefir can further undergo changes, some of which improve its shelf life[1-125]. The term Kefir is derived from the word kef, which means 'pleasant taste' in Turkish [1-135]. Kefir grains are the natural starter for Kefir and are recovered after the fermentation process [1-135]. The grains contain a mixture of microorganisms (bacteria and yeast), which coexist and interact to produce a unique fermented dairy product [1-140]. The physicochemical properties of Kefir include an acidic pH of 4.6, alcohol of 0.5%–2%, acidic taste, and yeasty flavour [1-140]. Additionally, carbon dioxide produced by the yeast flora contributes to its sharp acid and yeasty flavour[1-140]. In the following section the preparation, nutritional and health benefits of Kefir has been updated and discussed.

2. Preparation of Kefir

The traditional method of preparing dairy-based Kefir used in private households is to incubate milk with Kefir grains [1-140]. Kefir is produced through the fermentation of milk using Kefir grain as a starter culture [1-142]. The Kefir grains are inoculated into sterilized milk and fermented at 25° C until a pH of 4.4 is reached[1-105-142]. The grain and milk are then separated using a sterilized plastic filter at the end of the fermentation process [1-17-90]. In contrast, a water kefir is a homemade fermented beverage based on a sucrose solution with different dried and fresh fruits [1-140]. In the traditional process of sugary Kefir preparation, Kefir grains are placed into a solution containing 8% sucrose, dried fruits (typically figs) and some slices of lemon [1-140]. Fermentation for one or two days at room temperature results in a cloudy, carbonated and straw-colored drink, poor in sugar, slightly alcoholic, and acidic [1-18-98]. The milk is first pasteurized at 90 ◦ C for 15 min and is then cooled to 25 ° C to improve its microbiological quality. The cooled milk is mixed with 5% Kefir grains and incubated at 18–34 ° C for 18 h, and the Kefir grains are later separated via a sieve under aseptic conditions [1-149]. The Kefir is then stored at 4 ° C[1-18-142]. The fermentation step is used to speed up the microorganisms action and the metabolic changes occurring in milk composition [1-20]. A backslopping strategy is employed to increase Kefir beverage generation, with a 50-fold production yield increase while maintaining the same Kefir characteristics (physicochemical, microbiological and nutritional value) as traditional Kefir except for the differences in the lactobacillus Kefir population (7.94 vs. 8.89 log CFU/mL) and decreased yeast count (7.1 vs. 5.22 log CFU/mL) [1-17-142]. This method is considered cheap and reliable, especially in less developed countries, with only a few shortcomings observed in product consistency and microbiological diversity[1-135-142].

Kefir grains include heterogeneous microorganisms embedded in a polysaccharide matrix called Kefiran which is considered a biofilm; it also has many uses due to its therapeutic values [1-144]. Kefiran is a microbial exopolysaccharide (EPS) obtained from the flora (acid-lactic bacteria and yeasts) of Kefir grains and glucose units, in almost the same proportion [1-144]. Kefiran has prebiotic nature agitating the growth of probiotics in the gastrointestinal tract of the human entity. It extends certain therapeutic benefits by balancing the microbiota in the intestine [1-144]. Kefiran is a functional exopolysaccharide produced by *Lactobacillus kefiranofaciens* originated from Kefir, traditional fermented milk in the Caucasian Mountains, Russia. Kefiran is attractive as thickeners, stabilizers, emulsifiers, gelling agents and also has antimicrobial and antitumor activity[1-144]. However, the production costs of Kefiran are still high mainly due to high cost of carbon and nitrogen sources[1-144].

Water Kefir has a hazy and sticky consistency, a blond to yellowish color, an even, organized, bubbling texture, an acidic (about 2 % lactic acid content) and yeasty flavor (less than 1 % alcohol), with a tinge of sweetness. It is a locally or homemade fermented beverage produced from sucrose solution incorporated with fresh and dried fruits along with water Kefir grains. Water Kefir is a convenient dairy-free alternative to dairy-based fermented beverages. It is prepared by fermenting a sucrose solution with fresh and dried fruits using water Kefir grains, and demineralized whey can be used in water Kefir production [1-143]. Water Kefir grains and substrates, including brown sugar, dried and fresh fruits, vegetables, and molasses, used in the production influence the fermentation characteristics and composition of water Kefir[1-143]. Generally, the microorganisms used for the production of water Kefir grains belong to lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts, and fermentation brought about by water kefir grains is spontaneous. However, a few reports have identified the strains of *Lentilactobacillus hilgardii* that produce dextran (α-(1 → 6)-linked glucose polymer) forming granules. *Glycosyltransferase* is mainly responsible for dextran production. Lactic acid bacteria, acetic acid bacteria, and yeasts are the microorganisms involved in the fermentation process. *Lactobacillus* species are the most common microorganisms found in water Kefir [1-143]. Water Kefir contains various bioactive compounds that have potential health benefits. Water Kefir may inhibit the growth of certain pathogenic microorganisms and food spoilage bacteria, resulting in various health-promoting properties, including immunomodulatory, antihypertensive, anti-inflammatory, anti-ulcerogenic, antiobesity, hypolipidemic, and hepatoprotective activities [1-143]. However, there are some challenges in water Kefir production, such as unstable fermentation, low grain growth, and the sensitivity of probiotic microorganisms to physicochemical stresses. Contaminants and improper handling can also pose health risks[1-143].

3. Microbial composition of Kefir

Lactobacillus kefiranofaciens is a key indicator of Kefir because it is a dominant and stable bacterial species found in kefir grains [1-140]. It is responsible for producing a unique exopolysaccharide called Kefiran, which is a basic component of the Kefir grain structure and is crucial for the formation and maintenance of the grains themselves[1-140]. The presence of this specific bacteria is considered a marker of genuine Kefir fermentation, making it a reliable indicator of the product [1-140]. The starter culture employed in Kefir production exhibits a significant effect on its viscosity and on chemical composition [1-22-98-140]. The Kefir microbial community encompasses a complex mixture of lactic acid bacteria (LAB) (*Leuconostocs, Lactobacilli, Streptococci, lactococci, Enterobacter, Acinetobacter*, *Enterococcus*, and *Pseudomonas* spp.), acetic acid bacteria and yeasts (*Kluyveromyces, Candida, Torulopsis, Saccharomyces, Rhodotorula* and *Zygosaccharomyces* [1-140]. More than 23 different yeast species have been isolated from kefir grains and from fermented beverages of different origins[1-69-108-135]. However, the predominant species are *Saccharomyces cerevisiae, S. unisporus, Candida kefyr*, and *Kluyveromyces marxianus* ssp. *marxianus* [1-73-108-140]. There is a symbiotic relation between the microorganisms present in kefir grains, wherein the bacteria and yeast survive and share their bioproducts as power sources and microbial growth factors [1-69-103]. This microorganism association is responsible for lactic and alcoholic fermentation [1-73-108]. The microbial composition may vary according to Kefir origin, the substrate used in the fermentation process and the culture maintenance methods [1-69-108]. Tibetan Kefir, which is used in China, is composed of *Lactobacillus, Lactococcus*, and yeast. Additionally, acetic acid bacteria have been identified in Tibetan kefir, depending on the region in China from where it was obtained. Aadditionally, Tibetan kefir composition differs from that of Russian kefir, Irish kefir, Taiwan kefir, Turkey fermented beverage with kefir [1-63-106-135]. However, it is known that this microbial diversity is responsible for the physicochemical features and biological activities of each Kefir [1-69-108-140]. The vast majority of the Kefir-derived lactobacilli belong to the *L. kefiranofaciens* species, which are Gram-positive, non motile, capsulated, non-spore-forming rods and includes two subspecies, namely *kefiranofaciens* and *kefirgranum.* Among them *L. kefiranofaciens* is outstanding and has been revealed to exhibit various probiotic properties, such as antimicrobial activity, immunomodulation, reduction in cholesterol levels, risk of allergies and cancer[1-69-108-140].

One of the main lactic acid bacterial species found in the Kefir grain ecosystem worldwide is *Lactobacillus kefiranofaciens,* exhibiting strong auto-aggregation capacity and, therefore, being involved in the mechanism of grain

formation [1-69-108-140]. Its occurrence and dominance in Kefir grains of various types of milk and geographical origins have been verified by culture-dependent and independent approaches using multiple growth media and regions of the 16S rRNA gene, respectively, highlighting the importance of their combination for its taxonomic identification [1-69-108-140]. L. kefiranofaciens comprises two subspecies, namely *kefiranofaciens* and *kefirgranum*, but only the first one is responsible for the production of kefiran, the water-soluble polysaccharide, which is a basic component of the kefir grain and famous for its technological as well as health-promoting properties [1-69-108-140]. *L. kefiranofaciens*, can be involved in mechanisms affecting intestinal health, immunomodulation, control of blood lipid levels, hypertension, antimicrobial action, and protection against diabetes and tumors[1-69-108-140]. These valuable bio-functional properties place it among the most exquisite candidates for probiotic use as a starter culture in the production of health-beneficial dairy foods, such as the Kefir beverage[1-69-108-140].

4. Aroma and Flavour of Kefir

Kefir should exhibit acceptable aroma, flavour, and good mouth [1-63-106-135]. When flavour attributes in kefir, such as sour, sweet, salty, bitter, creamy, cheesy, sharp, gas, alcohol and metallic flavour tastes were compared, camel and cow milk kefirs scored differently[1-63-106-135]. Camel milk Kefir sample was found to be more sour, cheesy and have a sharper aroma than kefir prepared from cow milk, though its consistency and appearance had a lower score than that of cow milk Kefir [1-63-106-135]. Camel milk Kefir received an overall better preference score from the panelists, mostly on account of its higher sourness [1-63-106-135]. These characteristics are influenced first by the milk type used and its effect on kefir properties (textural, rheological and organoleptic properties) [1-63-106-135]. Kefirs produced from camel, cow, goat or ewe milk were found to have similar microbiological properties [1-63-106-135]. Kefir prepared using non-animal milk, i.e., soymilk and 2% sucrose, exhibited acceptable flavour and aroma compounds[1-63-106-135]. After two weeks of the cold storage, decreased levels of ethanol, acetaldehyde, diacetyl, and acetoin were noted [1-63-106-135]. The addition of less than 5% pomegranate juice reduced pH value concurrent with an increase in viscosity, while the addition of more than 5% pomegranate juice substantially reduced protein and Kefran concentration [1-63-106-135]. The addition of thickening-agent additives, such as inulin, during Kefir production did not significantly affect its chemical, microbial composition, odour or flavour, though it exhibited a higher viscosity value [1-63-106-105]. The aroma, flavour and good mouth feel properties appear to be influenced by additives such as Kefir grain, inulin, and sucrose, whereas milk type influenced Kefir's textural and rheological properties [1-63-106-140].

5. Nutritional value and Health Benifits of Kefir

Kefir possesses a myriad of bioactive properties to include anticancer, antimicrobial, anti-inflammatory, hypocholesterolemic, wound healing, antioxidant and gastrointestinal aiding properties [1-63-106-140]. Most of these health effects are based on biochemical or laboratory assays and need to be substantiated by studies in animals and humans to be more conclusive[1-63-106-140]. Additionally, monitoring changes in the human gut microbiome after ingesting the different probiotics available in Kefir products can provide a better understanding of its many health benefits [1-63-106-140]. The nutritional values of Kefir are due to its rich chemical composition, including minerals, sugars, carbohydrates, proteins, peptides, vitamins and fats [1-106-140]. Macro-elements enriched in kefir include calcium, magnesium, potassium, and sodium that aid in the utilization of carbohydrates, fats and proteins for cell growth, maintenance, and energy. Kefir also contains micro-elements, including iron, zinc, and copper, which are of special value in cellular metabolism and blood production [1-106-140]. Aside from such chemical makeup, it is the fermentation process that further enhances kefir's nutritional value owing to secondary bioactive ingredients such as catechin, vanillin, ferulic acid, and salicylic acid [1-106-135]. The latter has been identified in kefir produced from peanut milk [1-106-135]. Kefir is enriched in vitamins B1, B2, B5 and C, minerals and essential amino acids that are of value for improving fitness, the healing process and homeostasis[1-106-135]. The vitamin composition in kefir is affected by the type of milk and microbiological flora used in its production [1-106-135]. *Propionibacterium peterssoni* and *Propionibacterium pituitosum* produced vitamin B12, whereas *Freudenreichii* subsp. *Propionibacterium Shermani*i supported more vitamin B6 production [1-67]. Kefir is rich in the amino acids serine, threonine, alanine, lysine, valine, isoleucine, methionine, phenylalanine and tryptophan, which play a major role in the central nervous system [1-106-135]. Kefir also contains partially digested proteins (e.g., caseins) that aid in its digestion and absorption by the body [1-68-103]. The essential amino acids found in abundance in Kefir also regulate protein, glucose and lipid metabolism and exhibit a positive effect on the regulation of body weight, maintenance of immune response and energy balance [1-106-135]. Amino acids prevent disability and prolong the healthy life expectancy of elderly subjects [1-69,70], and the branched-chain amino acids that are also found in Kefir improve the cognitive recovery of patients with severe traumatic brain injury [1-70]. Peptides are regarded as a unique and important class of compounds generated during milk fermentation and account for much of fermented milk products' health benefits [1-106-135]. In bovine Kefir

derived from β-casein proteolysis, 236 peptides were detected and found to display antimicrobial, antioxidant, angiotensin-converting enzyme (ACE)-inhibitory, immunomodulation and antithrombotic effects [1-72-140].

Kefir is a popular ethnic dairy product that is constantly subjected to development; different additives of flavors, milk types, fibres, grains, and many more have been examined for the market [1-106-135]. Kefir is made from various types of milk (cow, goat, camel, buffalo, or mare), and is usually produced by mixing two types of milk to enhance its benefits, flavor, and texture, and subjected to secondary fermentation or the addition of additives such as inulin to improve the final product properties [1-106-135]. The fermentation of Kefir grains in a raw sugar solution or sugar from fruits or vegetables without using milk is another way of producing kefir[1-106-140]. This product is called sugary Kefir. These additives and different production methods, aside from affecting palatability, also affect kefir's physicochemical properties and health benefits[1-106-135]. The type of milk used, the Kefir grain and the fermentation conditions of production (time and temperature) should be monitored during manufacture as any changes in these variables can impact kefir's chemical and microbiological composition[1-106-135]. A myriad of macro- and micronutrients are encompassed within kefir including proteins, lipids, amino acids, and vitamins[1-106-135]. These components account for kefir's antibacterial, immunological, chemopreventive, and hypocholesterolemic effects and for why Kefir can be consumed by people who have lactose intolerance [1-106-135].

6. Limitations of Kefir

Faraget et al., (2020) [8] reported that kefir is well recognized for its potential health value as an excellent source of probiotics, some limitations in Kefir consumption need to be recognized [1-106-135]. These limitations are mostly due to its cholesterol-rich content and potential to trigger allergic reactions [1-106-135]. A brown sugar solution is the main alternative substrate used nowadays in the fermentation of kefir and produces a beverage known as sugar Kefir [8]. Sugary Kefir possesses a similar microbial association as traditional milk kefir fermentation, especially lactic acid bacteria such as *Lactobacillus, Leuconostoc, Kluyveromyces pichia* and yeasts, S*accharomyces*[1-106-135]. Sugary Kefir was found to be more effective at improving the lipid profile in mice than milk Kefir [1-140]. As far as structure, associated microorganisms and products formed during the fermentation process are concerned, sugar kefir grains are quite similar to the milk Kefir grains [1-106-140]. Melon, carrot, onion, tomato, fennel and strawberry juices have been used as fermentable substrates to produce Kefir on which LAB and yeasts could grow [1-16-140]. Among these substrates, microorganisms showed the best growth on melon juice. Esters were the major volatile compounds in onion, melon, and strawberry juices, and terpenoids were abundant in fennel and carrot[1-106-140]. Aside from improving Kefir's aroma, onion, tomato and strawberry juices were found to contribute to its strong antioxidant effect [1-126]. *Lactobacilli* are microorganisms that are useful in dairy technology (cheese, yoghurt and fermented milk) and have a long documented history of food use. *L. kefiri* strains did not cause α-or *β*-hemolysis and were found susceptible to tetracycline, clindamycin, streptomycin, ampicillin, erythromycin, kanamycin and gentamicin. L. kefiri strains could inhibit pathogens from both gram-positive and negative bacteria [1-106-140].

7. Conclusion

Kefir is a dairy product that can be prepared from different milk types, such as goat, buffalo, sheep, camel, or cow via microbial fermentation (inoculating milk with kefir grains). Many studies have demonstrated the biological activities of kefir highlighting its potential as an antioxidant, antimicrobial, anti-inflammatory, wound healing and hypocholesterolemic agent. Macro-elements enriched in Kefir include calcium, magnesium, potassium, and sodium that aid in the utilization of carbohydrates, fats and proteins for cell growth, maintenance, and energy. Kefir also contains micro-elements, including iron, zinc, and copper, which are of special value in cellular metabolism and blood production. Moreover, the fermentation process enriches the content of vitamins B1, B12, K, folic acid, calcium and amino acids, adding to Kefir's health benefits. As such, kefir contains various bacteria and yeasts which influence its chemical and sensory characteristics. A mixture of two kinds of milk promotes kefir sensory and rheological properties aside from improving its nutritional value. However, kefir is well recognized for its potential health value as an excellent source of probiotics, some limitations in kefir consumption need to be recognized. The natural starter for kefir production is Kefir grains, which house many yeast and bacterial species in a polysaccharide matrix, called Kefiran. Kefiran has many beneficial properties, including therapeutic and rheological, that make it a valuable biopolymer with widespread industrial applications, including food packaging, food stabilizing, tissue engineering, and drug or probiotic delivery.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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