



Microbial Communities in Home-Made and Commercial *Kefir* **and Their Hypoglycemic Properties**

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Abstract: Kefir is a popular traditional fermented dairy product in many countries. It has a complex and symbiotic culture made up of species of the genera Leuconostoc, Lactococcus, and Acetobacter, as well as Lactobacillus kefiranofaciens and Lentilactobacillus kefiri. Though kefir has been commercialized in some countries, people are still traditionally preparing kefir at the household level. Kefir is known to have many nutritious values, where its consistent microbiota has been identified as the main valuable components of the product. Type 2 diabetes mellitus (T2DM) is a common diet-related disease and has been one of the main concerns in the world's growing population. Kefir has been shown to have promising activities in T2DM, mostly via hypoglycemic properties. This review aims to explain the microbial composition of commercial and home-made kefir and its possible effects on T2DM. Some studies on animal models and human clinical trials have been reviewed to validate the hypoglycemic properties of kefir. Based on animal and human studies, it has been shown that consumption of kefir reduces blood glucose, improves insulin signaling, controls oxidative stress, and decreases progression of diabetic nephropathy. Moreover, probiotic bacteria such as lactic-acid bacteria and Bifidobacterium spp. and their end-metabolites in turn directly or indirectly help in controlling many gut disorders, which are also the main biomarkers in the T2DM condition and its possible treatment.

Keywords: *Kefir*; T2DM; hypoglycemic; probiotics; *Lactobacillus*; animal studies; human studies; metagenomics

1. Introduction

Kefir is a viscous and slightly fizzy beverage obtained by the fermentation of milk and *kefir* grains comprising lactic acid, acetic-acid bacteria, and yeasts [1] and is defined as a "natural complex probiotic" due to its rich microbial community structures [2]. *Kefir* might have originated in the Balkan–Caucasian regions and is probably the most widely studied fermented milk product in the world [3]. *Kefir* is consumed in many countries, including Russia, Turkey, Bosnia and Herzegovina, Belgium, Czech Republic, Estonia, Greece, Hungary, Ireland, Italy, Latvia, Norway, Poland, Romania, Slovakia, Ukraine, China, Taiwan, Malaysia, South Africa, Mexico, Argentina, and Brazil [4]. Though it is still commonly prepared at home, in some countries commercial *kefir* is also available. Besides the milk, the microbial and nutritional composition of *kefir* are influenced by starter cultures/*kefir* grains, the *kefir* grain to milk ratio, the source of inoculum, additives such



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). as fruits and inulin, the duration and temperature of its fermentation, and its storage conditions (Figure 1) [5,6].

The factors affect chemical composition of kefir





Kefir microbiota and its metabolites have been known to confer health benefits and gut immunomodulatory effects by stimulating, regulating, and enhancing the gut intestinal microbiota and functions [7,8]. Many microorganisms isolated from *kefir* have desirable probiotic properties such as high resistance to the low pH and bile salts in the gastrointestinal tract; bile salt hydrolase activity; non-cytotoxicity; and high adhesion to Caco-2 cells [9]. One of the commonest diet-orientated diseases is diabetes, which has affected around 10% of the world's population [10]. Traditionally, the ethnic people of the *Balkans* have believed that the consumption of home-made *kefir* has several therapeutic values [11], including its hypoglycemic properties [12,13]. Although in recent years some clinical studies have shown the hypoglycemic effects of *kefir*, according to our best knowledge, there is no such comprehensive review on the hypoglycemic effects of *kefir*. Therefore, this review aims to highlight the microbial communities in both homemade and commercial kefir and validate their hypoglycemic properties through animal studies and human clinical trials.

2. Microbial Composition of Home-Made and Commercial Kefir

The microbial diversity of kefir has been thoroughly studied using culture-dependent techniques; however, in recent times, due to the application of sequence-based taxonomical tools, its microbial diversity has revealed more interesting findings among the homemade and the commercial varieties of kefir [14]. The widely reported microbiota present in

kefir includes bacteria (lactic-acid bacteria, acetic-acid bacteria) and eukaryota (yeast and fungi) [7] make it a probiotic food product with a good source of probiotic microorganisms. Overall, the predominance of Bacillota (basonym: Firmicutes) [15] has been reported through various studies in both home-made and commercial *kefir* products [16], whereas the predominance of phylum Actinomycetota (basonym: Actinobacteria) [15] in the traditionally prepared *kefir* of Mexico has also been reported [17]. Metagenomics-based studies of *kefir* microbiota have been mostly studied using targeted amplicon sequencing and shotgun metagenomics [18–22]. So far, about 23 species (bacteria and eukaryota) have been reported from commercial kefir samples, among which Streptococcus thermophilus, Lactococcus lactis, and Leuconostoc mesenteroides are the main predominant species [21]. Among eukaryota, Debaryomyces hansenii and Kluyveromyces marxianus are the only two yeast species to have been reported [22]. Similarly, among the home-made kefir samples, the main predominant species belong to the lactic-acid bacterial group, where Lactobacillus kefiranofaciens and *Lentilactobacillus kefiri* have been reported as the most abundant species through metagenomics (both amplicon and shotgun) studies [23]. Interestingly, in some varieties of kefir of Turkey, the predominance of Bifidobacterium longum is also reported [24]. To date, about 66 species (bacteria and eukaryota) have been reported from metagenomics studies from home-made kefir samples (Figure 2), among which lactic-acid bacteria and acetic-acid bacteria are predominant [17–19,21–31]. Eukaryota (yeast and fungi) is also present to a significant degree in the home-made *kefir*, as compared to that of commercial *kefir* (Figure 2). However, homemade *kefir* shows the presence of many unwanted contaminants that would hamper the quality of the samples. From the above research studies, only the species viz., Lactococcus lactis, Leuconostoc mesenteroides, Streptococcus thermophilus, and Acinetobacter sp. are found to be shared among the homemade and commercial kefir, and only one yeast *Kluyveromyces marxianus* has been reported from both the *kefir* varieties (Figure 2).

2.1. Hypoglycemic Properties of Kefir

Type 2 diabetes mellitus (T2DM) is a serious long-term disorder [32] that is primarily caused due to defective insulin secretion by pancreatic β -cells and the inability of insulinsensitive tissues to respond to insulin [33], preventing the proper circulation of glucose for cell uptake [34]. It has been reported that some fermented milk products have hypoglycemic properties [35,36]. Biochemically, T2DM is mainly associated with glucose and lipid absorption in the human gut and is one of the main therapeutic measures to control the enzymes associated with these phenomena, such as lipid and carbohydrate hydrolyzing enzymes *viz.*, lipases, and α -amylases [37]. Subsequently, it has also been shown that upon kefir administration, the reduction of α -amylase and lipases was significant [38]. Kefir exhibits a negative effect on α -amylase, one of the important enzymes that is regulated in the type two diabetes pathway [38], and reduces insulin resistance [39]. The intake of kefir has been demonstrated to have a great impact in the treatment of type 2 diabetes and increases the life span in treated rats [12,13] via the mechanisms that will be mentioned in following sections. Many probiotic bacteria have been shown to help ameliorate type 2 diabetes disorder through various means such as reducing insulin resistance and improving glucose homeostasis [40]. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate have been shown to be important targets in the treatment of type 2 diabetes [41], which has also been reported in kefir [31], showing positive colonic and immune modulation [42]. The MK-9 variant of menaquinone (Vitamin K_2) has been detected in *kefir* [43], which plays a role in improving insulin resistance and reducing type 2 diabetes by means of its anti-inflammatory and lipid-lowering effects [44]. Some studies on animal models and human clinical trials have been conducted to validate the hypoglycemic properties of both homemade and commercial *kefir*, which are reviewed below.



Figure 2. A Sankey diagram represented the total microbiota reported from both commercial and home-made *kefir* as reported through metagenomics studies. A total of 23 genus/species have been reported from commercial varieties, whereas about 66 have been reported form home-made *kefir*, which consists of lactic-acid bacteria (LAB), acetic-acid bacteria (AAB), non-AAB/LAB, yeasts, and fungi. *Bacillota*-associated lactic-acid bacterial species *viz.*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus*; *Pseudomonadota*-associated species, *Acinetobacter* sp., and yeast-*Kluyveromyces marxianus* are the five most commonly shared microbes reported from the two varieties of *kefir* [16–31].

2.2. Animal Studies

Few animal models were investigated to validate the hypoglycemic properties of *kefir*. The blood glucose level of group 4 of rats was found to be lower than that of group 3 (p < 0.001) after feeding with *kefir*, indicating better control of oxidative stress and a decrease in the progression of diabetic nephropathy [45]. In another study, rats were divided into three different groups (the control group, fructose group, and fructose and *kefir* group), and fructose was given for 15 weeks, while *kefir* (1 mL/100 g/day) was given only for the last 6 weeks of the duration [46]. *Kefir* treatment improved insulin signaling in the liver via insulin receptor substrate 1 (IRS-1) and phospho-endothelial nitric oxide synthase (peNOS) [46]. The effect of *kefir* on metabolic syndrome was investigated [47]. Rats were divided into three different groups (negative control, without metabolic syndrome), positive control (with metabolic syndrome), and kefir group (with metabolic syndrome) in a study. The fasting insulin, glucose, and HOMA- β values of the *kefir* group were found to be lower than in the positive control group. When compared before and after the study, the highest decrease in the AUC value was observed in the group fed with kefir [47]. The effects of *kefir* on oxidative stress in diabetic animals were studied, and it was observed that kefir may contribute to the better control of glycemia and oxidative stress, indicating its consumption to delay the progression of diabetic complications [48].

Based on an animal study, the effect of the combination of goat and soy milk on lipid profile, plasma glucose, glutathione peroxidase (GPx) activity, and the improvement of pancreatic β -cell in diabetic rats was noted [49]. It was found that *kefir* combination can maintain the serum triglyceride, decrease plasma glucose, increase GPx activity, and improve pancreatic β -cells [49]. The effects of the consumption of a prebiotic (catechin-rich wine grapeseed flour, GSF) and a probiotic (*Lactobacillus kefiri* DH5) by obese mice on their hepatic steatosis were studied, and it was observed that the amelioration of high fat-induced hepatic steatosis after the consumption of GSF and *Lentilactobacillus kefiri* was partially mediated via the alteration of cecum propionate and intestinal permeability [50]. Nurliyani et al. (2022) conducted another experiment to determine the effect of goat milk *kefir* supplemented with synbiotic *kefir* and goat milk *kefir* without probiotic *kefir* on blood glucose, haemoglobin A1c (HbA1c), and insulin-producing cells in rats fed a high-fat and high-fructose (HFHF) diet. There was a decrease in the HbA1c level of the group receiving symbiotic *kefir*, which improved the health of rats fed an HFHF diet [51].

2.3. Human Studies

Recently, few human studies on the effect of *kefir* consumption have been reported. The glycaemic index, known as the effect of raising blood sugar, of foods containing carbohydrates is quite significant for patients with diabetes [52]. The study was conducted on 30 adult individuals aged 18 and 45 to determine the glycemic, insulinemic, and satiety indices of 3 types of *kefir* in three phases [53]. In phase I, low-fat strawberry *kefir* or orange kefir (containing 50 g carbohydrate) were given. In phase II, low-fat plain kefir (containing 25 g CHO) was given, and the glycaemic index was checked with equivalent glucose. In phase III, the insulinemic index and satiety index of 3 *kefir* samples were determined by comparing 1000 kJ portions of each 3 kefir samples with white bread with the same energy content. It was determined that while low-fat strawberry kefir, orange kefir, and low-fat plain kefir values of the area under the curve (AUC) were lower than the AUC value of the glucose solution given in equal amounts, they had similar values with the AUC value of the bread [53]. Judiono et al. (2014) conducted human studies on the effects of clear kefir on the biomolecular nature of the glycemic status of T2DM patients. One hundred and sixty individuals with diabetes were divided into 3 different groups: group 1 (T2DM, HbA1c <7) was given a standard diet and 200 mL of *kefir*, group 2 (T2DM, HbA1c >7) was given a standard diet and 200 mL of kefir, and group 3 was given a standard diet for 30 days. It was concluded that clear kefir reduced blood glucose levels (HbA1c, FBG, and PBG) in the individuals and also increased c-peptide [54]. In a study of 42 adult men with T2DM, the participants were divided into 2 groups. The men in the first group received only

metformin, while the second group consumed metformin and 1 cup of *kefir* for 10 weeks. At the end of the study, it was determined that *kefir* intervention decreased the fasting blood glucose level and the HbA1c value (p < 0.05) [55].

In addition to medical treatment for diabetes, the effect of probiotic *kefir* on glucose and lipid profile control has also been investigated in 60 diabetic patients aged between 30 and 65 [12]. For 8 weeks, the probiotic group (n:30) consumed 600 mL/day of probiotic fermented milk (kefir) containing Lacticaseibacillus casei, Lactobacillus acidophilus, and Bifidobacteria, while the control group (n:30) consumed 600 mL/day of conventional fermented milk. At the end of 8 weeks, the blood glucose level of the kefir group decreased from 161.63 \pm 57.71 mg/dL to 139.22 \pm 46.66 mg/dL, suggesting that probiotic *kefir* can be useful as adjuvant therapy in the treatment of diabetes [12]. In another similar study, 60 mL/day of kefir (Streptococcus thermophilus) or 600 mL/g of conventional fermented milk (S. thermophilus and Lb. bulgaricus) was given to 60 diabetic patients for 8 weeks to investigate the effect of probiotic *kefir* on the serum level of insulin and homocysteine in the type 2 diabetes patients [40]. The level of homocysteine decreased significantly in patients with probiotic fermented milk and conventional fermented milk consumption [40]. Similarly, a study on the effects of regular kefir consumption on gut microbiota composition, and their relation to the components of metabolic syndrome, was conducted [56]. In a randomized controlled trial, 180 mL/day of kefir (Lactococcus lactis subsp. lactis, Lc. lactis subsp. cremoris, Lc. lactis subsp. diacetylactis, Leuconostoc mesenteroides subsp. cremoris, *Lentilactobacillus kefiri, Kluyveromyces marxianus, and Saccharomyces unisporus)* were given to 11 metabolic syndrome patients and 180 mL/day of unfermented milk to 11 metabolic syndrome patients. At the end of 12 weeks, the fasting HOMA-IR and insulin level decreased in the kefir group (p < 0.05) [56]. In another study conducted on patients with metabolic syndrome, 24 patients (group 1) were given kefir and 24 patients (group 2) curd for 12 weeks. The HbA1c value was found to be similar between the two groups, whereas the fasting blood glucose level decreased from $95 \pm 9 \text{ mg/dL}$ to $83 \pm 8 \text{ mg/dL}$ in the kefir group (p < 0.05) [57]. In a case study, a 58-year-old individual whose diabetes could not be controlled for 15 years was given probiotic kefir for 90 days. At the end of the study, the body weight of the patient decreased from 88 kg to 84 kg and HbA1c decreased from 7.9% to 7.1% [58].

Bellikci-Koyu et al. (2022) divided 62 individuals into two different groups. The first group (n:31) was given 180 mL/day of probiotic *kefir* (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lentilactobacillus kefiri*, *Kluyveromyces marxianus*, and *Saccharomyces unisporus*), while the second group (n:31) consumed 180 mL/day of unfermented milk for 12 weeks. At the end, insulin, Homa-IR, HbA1c, and blood glucose were found to be similar in both groups because milk itself contains many bioactive components. It can be concluded that the regular consumption of probiotic *kefir* can provide favourable effects in the management of metabolic syndrome [59].

When the effect of *kefir* fermented by lactic acid, acetic-acid bacteria, and yeasts on diabetes was examined, it was observed that it regulated glucose hemostasis and insulin in human and animal models (Farag et al. 2020), but some studies did not find the same effects. Moreover, some studies have shown that synbiotic foods may be more effective on diabetes [60].

3. Future Directions and Prospective

Kefir is widely produced and consumed in many countries, mainly because of its biological activities such as antidiabetic properties. Many human and animal studies have shown that *kefir* may be beneficial for the management of T2DM, even though there are still some research gaps. Firstly, many human clinical trials use dairy products as control groups to understand the effects of fermentation. However, the studies that use non-dairy products as control groups also face problems regarding fermentation factors. Secondly, while planning the controlled and blinded studies, it is important to match populations in

terms of age, gender, education, health status, socioeconomic level, and other factors that may affect nutritional behaviors. With the current data, it is possible to recommend further studies, especially randomized controlled trials, to clarify the hypoglycemic property activities of both commercial and homemade *kefir*.

Though metagenomics has its own flaws, metatranscriptomics may be carried out to determine the actual expressed gene profile that could provide a clearer picture of *kefir* fermentation, its microbial role in health-beneficial functions, and its mechanism of action in many diseases/disorders such as type 2 diabetes mellitus. From many metagenomics analyses, the presence of uncultured and unknown microbes is being reported, and recently new novel microbial candidates have been reported from *kefir*. However, little knowledge is present of their culturability, which is an important aspect to be focused on as this could enrich the value-added property of *kefir* and its product improvement. Integrative multiomics studies, such as metagenomics, proteomics, and metabolomics, may be initiated by researchers working on *kefir*.

4. Conclusions

Kefir is a nutritious fermented milk product that is rich in health-positive properties and probiotic bacteria. Unlike many other fermented milk products, kefir is a well-studied fermented product that has been also commercialized in many countries. Though bacteria are found in more abundance in kefir, yeasts have also been detected in both varieties. Furthermore, even though there is a difference in the microbial composition between the home-made and the commercial varieties, kefir generally still possesses consistent healthpromoting bacteria. The predominance of naturally occurring potential health-beneficial microorganisms, as reported by metagenomics studies, in this food product has paved the ways for many studies investigating alternative means to control many health disorders, including T2DM. As many other fermented foods have been focused on as a potential measure to control T2DM, kefir intake has demonstrated, through much experimental evidence (animal and human studies), the positive effects on the T2DM. The presence of much predominant microbiota specific to kefir has also been correlated to gut function, in which they may directly or indirectly help alleviate the disease, thereby making this product an important dietary food. Though the fact that the presence of unwanted contaminants has been detected in many home-made varieties is a concern, there is a need to improve this product by using *kefir*-specific strains/species with health benefits. The regulation of commercial kefir is still not clear in many countries; it needs to be standardized as per the food safety and standards policy of each country.

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