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Kefir: a fermented plethora of symbiotic microbiome and health

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Abstract

In recent decades, a global shift in lifestyle and the ubiquitous consumption of junk foods have led to dysbiosis and other metabolic disorders significantly impacting human health. Recent studies performed on traditional foods have shown several health benefits and have gained the attention of the scientific community towards ethnic foods. In this regard, the consumption of ethnic foods with symbiotic properties is increasing gradually across the globe. Kefir is one such ethnic food with excellent functional properties. It is a unique traditional fermented drink comprised of kefir grains and probiotic microbes. Kefir grains are a gelatinous consortium of casein, milk solids coupled with yeasts, and lactobacilli-rich microbiota embedded in a poly-saccharide matrix. These components act as starters, initiating fermentation when introduced into fresh milk. This beverage bestows a myriad of symbiotic benefits, encompassing improved gut health and preventing several metabolic and other diseases through various biological mechanisms. Despite its millennia-long history, it has recently gained prominence due to emerging biotechnological and nutraceutical applications and researchers' burgeoning fascination. In this comprehensive review, we endeavour to provide a meticulous elucidation of the most recent advancements concerning kefir, encompassing its production and processing methodologies for both dairy and water kefir. Furthermore, we delve into the intricate mechanisms underlying its functional properties and the health benefits of kefir as a functional fermented beverage.

Keywords Dairy, Fermented food, Gut, Kefir, Metabolic disorders, Symbiotic

Introduction

In recent decades, a significant paradigm shift in global health has been observed, with the predominant use of several antibiotics and an increased consumption of junk foods directly affecting the composition of the intestinal microbiota, thereby leading to dysbiosis and several metabolic and non-metabolic-related disorders [1, 2]. According to the World Gastroenterology Organization, the gut of a healthy adult individual is believed to be composed of more than 10^{14} microorganisms [3, 4]. The development of the intestinal microbiota during childhood plays a pivotal role in the development of human health against a spectrum of diseases, including allergies, neurological disorders, and obesity [5]. This underscores the crucial role of the intestinal microbiota in shaping new approaches to maintaining human health [6, 7]. Functional foods have gained considerable attention because they offer supplementary advantages to human physiology and metabolic processes [8, 9].

In recent years, kefir, a fermented dairy product, has become a focal point of scientific investigation and public interest, owing to its diverse range of potential health benefits [10, 11]. It is a yellowish-white tart, viscous

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beverage that has gained worldwide popularity because of its health benefits and therapeutic effect, leading to its widespread consumption around the globe among people [12, 13]. With its origins rooted in the Caucasus Mountains, kefir holds both cultural and historical importance, celebrated for its distinctive flavour and reputed therapeutic qualities [14, 15]. Crafted through the fermented symbiotic plethora of milk and kefir grains—a complex amalgamation of yeast and bacteria—this tangy beverage has captured attention not only for its taste but also for its probiotic properties [16, 17]. Enriched with a plethora of beneficial microorganisms, kefir is believed to play a pivotal role in enhancing gut health and fortifying the immune system [18, 19]. Kefir exhibits an excellent protein content, manifesting in two distinct forms: intact protein and partially digested protein [20, 21]. These forms facilitate its utilization as a prebiotic by the organism and effectively function as a probiotic [22, 23]. The intricate interplay between its microbial composition and its impact on human physiology has spurred a surge in research exploring kefir's potential in alleviating various health conditions, including gastrointestinal disorders and metabolic diseases [24, 25].

This comprehensive review navigates the multifaceted realm of kefir, delving into its historical and cultural roots, the nuances of its fermentation process, and the scientific endeavours dedicated to unravelling its therapeutic potential. Through this exploration, we will try to illuminate kefir's promising role in the domain of functional foods and human health, shedding light on its intricate mechanisms and potential applications.

Production of kefir

Kefir can be produced through diverse methodologies, including: (1) traditional manufacturing, entailing the fermentation of milk with kefir grains; (2) a Russian or European approach; and (3) industrial-scale production, wherein kefir is fermented by the direct incorporation of commercial starter cultures into milk, as mentioned in Fig. 1 [26].

Types of kefir

Based on the fermenting substrate, kefir is categorized as dairy (milk) and non-dairy (water) [6, 27]. While the preponderance of scholarly research has traditionally focused on delineating the benefits associated with the consumption of kefir derived from milk substrates, currently the attention is shifting towards exploring non-dairy alternatives for the synthesis of kefir [28, 29]. Despite its natural synbiotic properties, many studies and medical evidence indicate the unsuitability of traditional dairy kefir for individuals with lactose intolerance, vegan dietary preferences, or dairy allergies [29]. It has led to a surge in adapting kefir fermentation to non-dairy substrates, offering an alternative approach to harnessing its health benefits [30]. This shift not only broadens the accessibility of kefir but also opens new avenues for researchers and innovations in the realm of functional foods and synbiotics.

However, milk and water kefir grains are traditionally blended with a plethora of vast symbiotic microbial consortiums and exhibit striking similarities in terms of characteristic structure, associated microbial composition, and metabolites [27, 31]. The diverse microbial constituents present within both kefir grains give rise to a spectrum of kefir products, each with distinct physicochemical, nutritional, microbiological, and sensory characteristics. Likewise, both kefirs exhibit distinctive functional attributes too [32]. While milk kefir yields substantial quantities of protein, probiotics, and prebiotics. In contrast, water kefir emerges as a crucial reservoir of probiotics, prebiotics, and antioxidants, particularly catering to the dietary needs of vegans and individuals with dairy allergies or intolerances [33, 34]. Understanding these intricate dynamics not only deepens our knowledge of kefir fermentation but also paves the way for tailored approaches to developing kefir-based products with diverse attributes and potential health benefits.

Both milk and water kefir are produced through the inoculation of the kefir grain as starter culture into substrates (milk or water-based solutions enriched with fruits, vegetables, and sugar sources) at variable proportions (ranging up to 20% w/v) and fermenting for 24 h at a varying temperature of 20–25 °C [35, 36]. The fermentation process commences as the yeasts and bacteria within the grains of kefir adapt to the specific culture conditions, leading to a 5–7% increase in grain biomass and the synthesis of diverse metabolites [37, 38]. Upon completion of the fermentation, kefir grains are then separated from the beverage through filtration and can be reused for subsequent inoculations [38].

Dairy kefir

Since millennia, milk has been the fundamental component of the human diet. In pursuit of augmenting its shelf life, surplus milk was subjected to fermentation and preserved to maintain its nutritional content [39]. Kefir, an artisanal dairy beverage, is produced by the fermentation of milk, which is facilitated by the diverse microbiota inherent in kefir grains. Typically, the fermentation process for kefir extends beyond 24 h at ambient room temperature and usually in containers such as goatskins, wooden vessels, or clay receptacles [40]. Milk sourced from various ruminants (cows, sheep, goats, buffalo, or camels) serves as a substrate

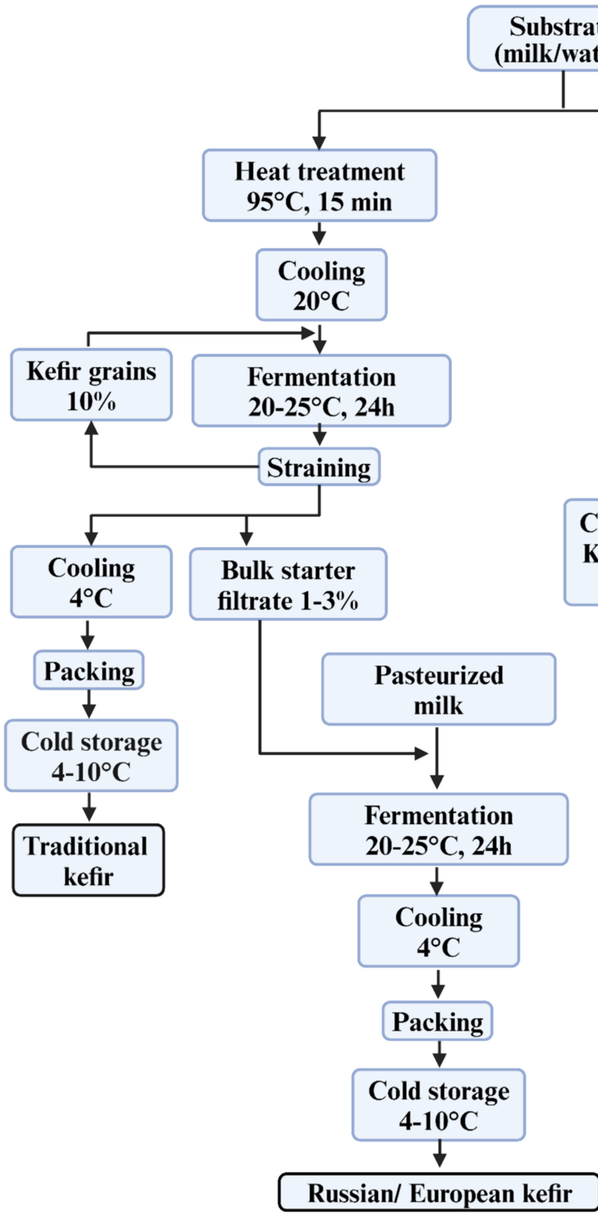

Fig. 1 Overview of various methods involved in production of kefir

for fermentation [6, 16]. The production of kefir can be executed through either conventional methodology or a commercial procedure that involves the inoculation of kefir grains into the milk substrate, ensuring precision and reproducibility of the product [41]. This intricate process contributes to the elucidation of kefir's microbiological dynamics and underscores its potential applications in promoting digestive health and overall wellness.

Milk kefir grains

Milk kefir is a cultured dairy product obtained from the symbiotic interaction between kefir grains and milk, resulting in a biologically enriched fermented beverage [41]. The granular composition of milk kefir is characterized by small, creamy, yellowish-to-white structures resembling cauliflower florets in an irregular and lobed shape, with a diameter ranging between 0.1 and 0.2 cm [6, 41]. On average, the grains exhibit a composition of 14% dry matter and 86% water, wherein the dry matter comprises 58% polysaccharide, 7% fat, 30% protein, and 5% ash. The microbiota, comprising lactic acid bacteria (LAB), acetic acid bacteria, and yeasts, is intricately entrenched within a bacterial polysaccharide matrix, coexisting in a symbiotic relationship [42]. However, it is imperative to acknowledge that these proportions may exhibit variations depending on the origin and source.

The gelatinous and slimy structure of the bacterial polysaccharide in milk kefir grains predominantly consists of an exopolysaccharide (EPS) named "*kefiran*" and a pentasaccharide known as "*kefirose*". Additionally, this structure includes a heteropolysaccharide comprising water-soluble glucogalactan with an equal distribution of galactose and glucose, incorporating 127 hexose units [43].

Microbiota profiling of milk kefir

Kefir consists of a plethora of symbiotic microbiomes and it was perceived that the microflora in kefir varies based on its culture condition, origin, and growth procedure. The characteristic microbiota in milk kefir comprises *Lactobacillus kefiranofaciens*, *Lb. kefiri*, *Lb. parakefiri*, and *Lb. kefirgranum*, as mentioned in Table 1 [42]. In a study where specific LAB and yeast from milk kefir were grown in various water-based substrates (fruit juices), there was a reduction in the colony forming units (CFU) of both LAB and yeast after fermentation. This implies that the microbiota from milk kefir requires a highly specific dairy-based growth medium (milk and whey) [44].

Further exploration unveiled, that milk kefir grains harbour an approximate composition of 50–55% *Lactobacillus* sp., 18–20% *Leuconostoc* sp., 10–12% *Streptococcus* sp., 8–10% *Pediococcus* sp., 7–9% *Lactococcus* sp., and 5–7% additional bacterial species [45]. *Lactococcus* varieties exhibit enhanced growth in milk as compared to yeast, catalysing the hydrolysis of lactose and the subsequent production of lactic acid, thereby fostering an ideal condition for yeast proliferation. Yeasts play a pivotal role by synthesizing diverse B-vitamin types and hydrolysing milk proteins, resulting in the generation of carbon dioxide (CO_2) and ethanol through aerobic metabolism [46].

In the context of kefir fermentation, LAB are introduced into milk to initiate the process. These microorganisms enzymatically convert lactose into lactic acid, leading to a reduction in pH. Thus, LAB influence the sensory attributes and extended shelf life of fermented milk [46]. Additionally, yeasts assume significance in kefir fermentation by producing ethanol and CO_2, fostering symbiotic interactions among microorganisms present in kefir. This phenomenon ultimately augments the olfactory and gustatory attributes of kefir. A study focused on Brazilian kefir revealed the abundance of LAB after post-fermentation with declining pH levels, citric acid, and lactose content, accompanied by an elevation in ethanol, acetic acid, glucose, propionic acid, galactose, and butyric acid content over the course of fermentation until the storage phase [46].

Table 1 Microbial diversity between dairy and non-dairy kefir

Type of kefir	Probiotic Species	References
Dairy based		
Lactobacilli	*Lactobacillus acidophilus*, *Lb. amylovorus*, *Lb. apis*, *Lb. crispatus*, *Lb. delbrueckii*, *Lb. fomicalis*, *Lb. gallinarum*, *Lb. gasseri*, *Lb. gigeriorum*, *Lb jensenii*, *Lb. kalixensis*, *Lb. kitasatonis*, *Lb. ultunensis*, *Lentilactobacillus otakiensis*, *Lenti Lb. parakefiri*, *Lentilactobacillus sunkii*, *Levilactobacillus brevis*, *Lentilactobacillus kefiri*, *Ligilactobacillus salivarius*, *Limosilactobacillus reuteri*, *Lactiplantibacillus pentosus*, *Lacticaseibacillus rhamnosus*	[6, 21]
Lactococci	*Lactococcus cremoris*, *Lc. garvieae*, *Lc. lactis* subsp. *cremoris*	[26, 27]
Streptococci	*Streptococcus durans*, *S. faecalis*, *S. thermophilus*	[33]
Acetic acid bacteria	*Acetobacter aceti*, *A. fabarum*, *A. genera*, *A. lovaniensis*, *A. orientalis*, *A. pasteurianus*, *A. syzygii*, *Gluconobacter japonicus*, *Gluconobacter morbifer*	[39–41]
Yeast	*Hanseniaspora uvarum*, *Kazachstania aquatica*, *K. aerobia*, *K. servazzii*, *K. solicola*, *K. turicensis*, *Saccharomyces cari-ocanus*, *S. servazzii*	[27, 40]
Other probiotics	*Pediococcus halophilus*, *P. lolii*, *P. pentosaceus*, *Lysinibacillus sphaericus*, *Enterococcus* species	[30, 31]
Non-dairy based		
Lactobacilli	*Lb. buchneri*, *Lb. casei*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. diolivoran*, *Lb. fermentum*, *Lb. helveticus*, *Lb. hilgardii*, *Lb. hordei*, *Lb. kefiranofaciens*, *Lb. kefiri*, *Lb. mali*, *Lb. nagelli*, *Lb. paracasei*, *Lb. paracasei* subsp. *paracasei*, *Lb. paracasei* subsp. *tolerans*, *Lb. parabuchneri*, *Lb. parafarraginis*, *Lb. perolens*, *Lb. plantarum*, *Lb. satsumensis*, *Lb. sunkii*, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Leuconostoc* species, *Oenococcus kitaharae*, *Oenococcus oeni*	[28, 46, 50]
Lactococci	*Lc. citreum*, *Lc. lactis*	[52, 53]
Yeast	*Candida boidinii*, *C. valdiviana*, *Dekkera anomala*, *D. bruxellensis*, *Hanseniaspora valbyensis*, *Issatchenkia orientalis*, *Kazachstania unispora*, *Kluyveromyces lactis*, *K. marxiamus*, *Lachancea fermentati*, *L. meyersii*, *Meyerozyma species*, *Pichia caribbica*, *P. cecembensis*, *P. fermentans*, *P. membranifaciens*, *Saccharomyces cerevisiae*, *Wickerhamomyces anom-alus*, *Yarrowia lipolytica*, *Zygosaccharomyces fermentati*, *Z. lipolytica*, *Zygotorulaspora florentina*	[54, 80]

Non-dairy kefir (water kefir)

Water kefir, sugary kefir, or tibico (known as tibico's tepache) has gained significant popularity in recent years. It emerges as a pivotal contributor to the dietary requirements of vegans and individuals with dairy allergies or intolerances, manifesting itself as an efficient reservoir of probiotics, prebiotics, and antioxidants [6, 29]. Water kefir grains are fermented with water substrate in a saccharine medium, with brown sugar serving as a primary substrate, whereas other auxiliary substrates include fruit juices (e.g., grape, pomegranate, apple, pineapple, and melon), vegetables (e.g., onion, ginger, soybean, and carrot), and molasses (e.g., honey, sugarcane) [26, 27]. This diversification augments an array of choices as an alternative to milk-derived kefir. These adaptations cater to the preferences of non-dairy consumers and vegans, allowing them to enjoy the benefits of kefir consumption [31].

The process of fermentation is based on the judicious selection of a substrate containing readily fermentable carbohydrates, such as glucose, fructose, and sucrose, among other compounds. These substrates are then homogenized with water kefir biomass (grains), maintaining controlled conditions at a temperature (~25 °C) for an approximate duration of 24 h [47]. Additionally, when carbonation is desired, a natural gasification process is implemented to generate an effervescent beverage [48].

Water kefir grains

Water kefir grains, are also known as "*Tibi* or *Tibico,*" "*Sugary kefir grains,*" "*Japanese water crystals,*" and "*Graines Vivantes,*" contain a dextran matrix [49]. The structure of dextran within water kefir grains is established by α-D-(1→6)-linked glucopyranosyl residues accompanied by (1→2) or (1→3) or (1→4)-linked side chains [36, 49]. The predominant microbiota accountable for the synthesis of the dextran structure in water kefir grains includes *Lactobacillus* sp. (*Lacticaseibacillus* (formally known as *Lactobacillus*) *casei, Lb. nagelii, Lb. hilgardii, Lb. hordei, and Leuconostoc mesenteroides*) [50–54].

Water kefir grains exhibit translucency and a grey-whitish color (but can be influenced by the colour of the substrate) are waxy and rubbery in consistency with a smooth texture and have rarely visible subunits, also resembling "rock salt." The diameter of water kefir grain ranges to a few millimetres [51].

Microbial composition of water kefir

The fermentation process of non-dairy (water) kefir is facilitated by kefir grains, which comprise a conglomerate of acetic acid bacteria, yeast, predominantly S*accharomyces, Candida*, and *Kluyveromyces*, as well as LAB, encompassing the species, *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Oenococcus*, *Pediococcus*, *Enterococcus*, and *Lactococcus,* as mentioned in Table 1. These micro-organisms are encapsulated within a naturally occurring matrix of exopolysaccharides (EPSs) recognized as kefiran [51, 52].

Generally, within the LAB category, the prevalence of the *Lactobacillus* genus is notably higher (like *Lacticaseibacillus paracasei*, *Lb. hilgardii*, and *Lb. nagelii*), followed by the *Lactococcus* genus. Concerning yeasts, there is greater variability in the profile; however, strains affiliated with the *Saccharomyces* (like *S. cerevisiae*) and *Kluyveromyces* genera are essential microbiota and exhibit increased growth. [53, 54]. Water kefir harbours an estimated composition of around 70–75% *Lactobacillus* sp., 10–12% *Leuconostoc* sp., 8–10% *Acetobacter* sp., 5–7% *Bifidobacterium* sp., and 3–5% other bacterial species, and as compared to dairy kefir, water kefir nurtures more genera of yeast (like species of *Guehomyces*, *Kloeckera*, and *Hanseniaspora*) [53, 55]. In a study, the microbiota originating from water kefir grains exhibited limited growth on a milk substrate. This phenomenon was attributed to the absence of a lactose-metabolizing mechanism within the microbiota derived from water kefir. Consequently, the essential polysaccharides necessary for augmenting biomass were not generated in the milk medium [54, 56].

Health aspects of kefir

Kefir has been highly relinquished by our ancestors for its positive health benefits and increased longevity. It exhibits preclusive, recuperative, and momentous physiological benefits for health due to the presence of its rich microbiota stemming from the impact of numerous bioactive components spawned during fermentation [57].

Anti-microbial properties of kefir

Kefir drinks made by fermenting the kefir grains potentially impede infections by inducing a bactericidal effect within the gastrointestinal tract. This is attributed to the existence of bioactive metabolites and peptides that confer anti-microbial properties during fermentation in accordance with low pH [58].

The antimicrobial efficacy of kefir is attributed to the presence of LAB. These micro-organisms actively engage in competition with pathogens for nutrients. Additionally, kefir fermentation triggers the endogenous synthesis of organic acids (specifically lactic and acetic acid), CO_2, acetaldehyde, bacteriocins, cathelicidin, and H_2O_2. These manifest an antimicrobial impact against a spectrum of pathogens [59]. Significantly, the proteolytic degradation of milk proteins in kefir is reported to exhibit antimicrobial efficacy against certain pathogenic bacterial strains [60]. This is attributed to bioactive molecules, peptides, and other constituents. Further research indicates that the microbes originated from kefir could potentially be used for the treatment of gastrointestinal disorders through the production of short-chain fatty acids (SCFAs). As SCFAs help in decreasing the pH and avoiding the growth of pH sensitive pathogenic bacteria in the intestine [61]. In a study to assess the antimicrobial efficacy of digested kefir (gastric and intestinal juices) against foodborne bacteria (*Escherichia (E.) coli*), it was revealed that the antimicrobial activity was attributed to its bacteriostatic nature rather than bactericidal. However, a vital factor of this study is the utilization of digested kefir in the analysis of antimicrobial activity, which stands in contrast to the prior studies on the antimicrobial properties of kefir that typically employ undigested counterparts of kefir [62].

An effort was made to comparatively study the antimicrobial properties of commercial and traditional kefir along with their microbial composition. The overarching evidence suggests that traditional kefir exhibits superior efficacy in terms of inhibiting pathogenic microbes [63]. Further exploration revealed that kefir exhibits potent anti-microbial activities against *E. coli*, *Enterobacter cloacae*, and *Enterococcus faecalis* strains, respectively. It was concluded that the anti-microbial property may be attributed to the antagonistic interactions among diverse microbiota within kefir, thereby producing antimicrobial peptide bacteriocins and cathelicidin, in synergist with low pH by organic acids [64].

An intriguing study was made on the antimicrobial properties of kefir from two different sources. It was reported that the microflora present in the kefir from source 1 were predominantly *Lactobacilaceae* (~55%), *Acetobacteraceae* (~30%), *Pseudomonadacea* (~12%), and *Streptococcaceae* (~3%), in contrast, the bacterial composition of the kefir from source 2 was overwhelmingly dominated by the *Lactobacillaceae* family (~95%). It was further reported that kefir from source 1 exhibited a bactericidal effect at pH 5 and bacteriostatic activity against pathogens at pH 7. Conversely, kefir from source 2 did not exhibit any discernible antimicrobial effect. Finally, it was concluded that the anti-microbial activity is intricately linked to the source and composition of the kefir grains, particularly with respect to the diversity of microbiota present [65].

So, it can be concluded that the anti-microbial efficacy of kefir is not solely attributable to its low pH by SCFAs but is intricately tied to the presence of specific inhibitory peptides, such as antimicrobial proteins (bacteriocins, cathelicidin) and polyalkenes which enhance or antagonize the antimicrobial effects of kefir, as mentioned in Fig. 2 and Table 2. Furthermore, the SCFAs such as propionate, butyrate, acetate, and formate that are generated through the process of sugar fermentation by LAB species offer several advantages. Short-chain fatty acids such as propionate, lactate, and formate inactivate pathogenic bacteria such as *E. coli* and *Salmonella* at pH 5.0. Butyrate has an inhibitory effect on *Clostridium perfringens*. Acetate exhibits both anti-inflammatory and anti-allergic activity [17, 18]. This exhibits the multifaceted nature of kefir's antimicrobial activity, which extends beyond acidity and encompasses a spectrum of bioactive compounds contributing to its inhibitory and biostatic effects on pathogens.

Anti-inflammatory properties of kefir

Globally, the consequences of neuro and chronic inflammatory disorders stand as the foremost contributors to morbidity and mortality. In recent years, mounting evidence derived from both ex vivo and in situ studies strongly affirms the unequivocal anti-inflammatory and immunomodulatory capabilities of kefir [66, 67]. This therapeutic approach elevates anti-inflammatory agents while concurrently suppressing pro-inflammatory cytokines. These findings suggest the potential of kefir in alleviating the adverse effects associated with neuro and chronic inflammatory conditions [68, 69].

Further studies revealed that fractions and isolated organisms from kefir demonstrated the promotion of the anti-inflammatory cytokines Th-2 response, while concurrently inhibiting the pro-inflammatory Th-1 response [70]. Supplementation of kefir has been reported to reduce the glycemia and enhance the equilibrium between pro- and anti-inflammatory cytokines, as indicated by the modulation of interleukin-1(IL-1), IL-6, and the tumor necrosis factor (TNF)/IL-10 ratio [71].

In a recent investigation, the anti-inflammatory properties of novel kefir exo-polysaccharides (KEPS) and kefiran (KE) were evaluated under in vitro conditions and demonstrated a remarkable capacity to mitigate the lipopolysaccharide-induced secretion of IL-6. The impact of KEPS or KE was studied by their oral administration for a week in a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)-luciferase+/+transgenic mouse (male and female) model and subjected to systemic injury. It was reported that both KE and KEPS markedly suppressed the expression of inflammatory signalling molecules (IL-6 and phosphorylated mitogen-activated protein kinase (p-MAPK)). These compelling conclusions underscore the potential therapeutic efficacy of KEPS in alleviating anti-inflammatory property by inhibiting

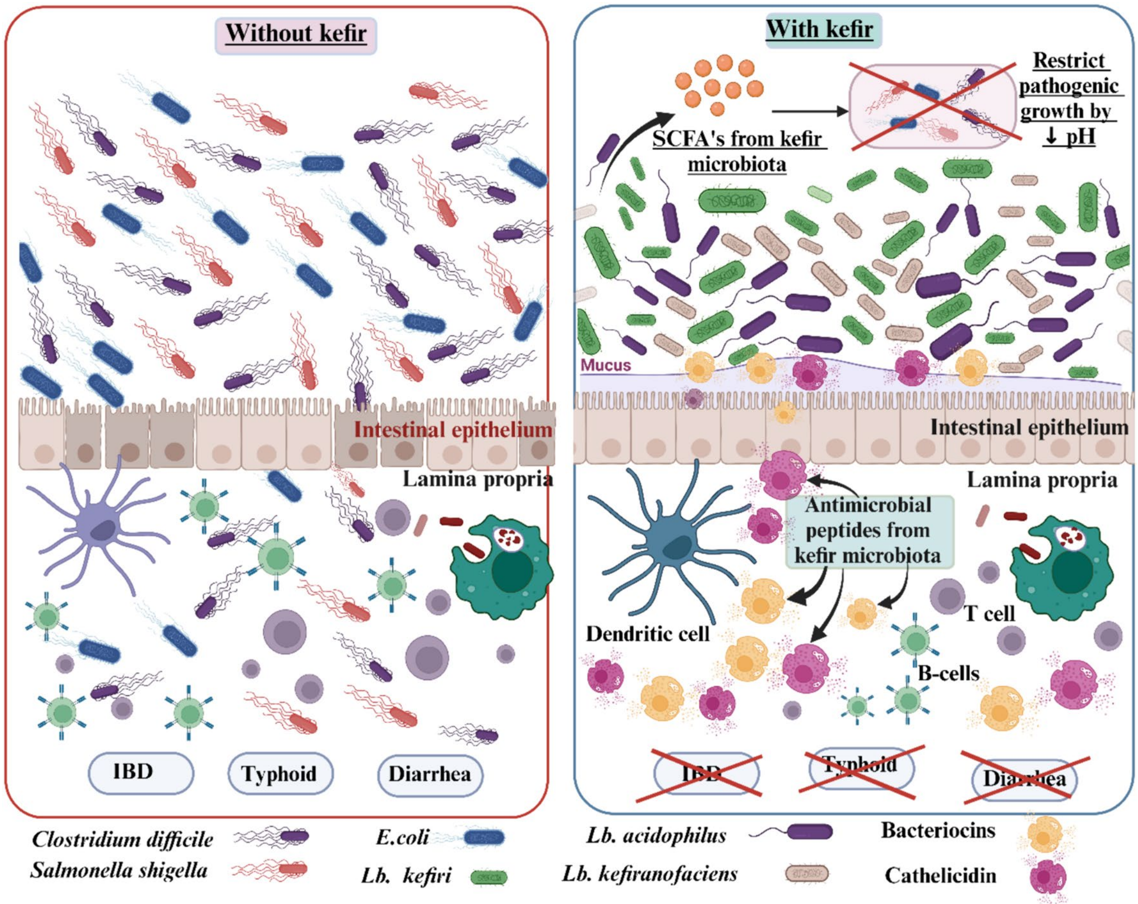

Fig. 2 Antimicrobial efficacy of kefir attributed to the synergistic effect of decrease in pH due to short-chain fatty acids production, and antimicrobial peptides. SCFAs, short-chain fatty acids; IBD, Inflammatory bowel disease

Table 2 Functional attributes of dairy and non-dairy kefir

Properties	Mechanism	References
Antimicrobial	Prevents the adhesion of pathogens to the gut epithelium through competitive binding. Produces anti-microbial peptides (bacteriocins, cathelicidin), along with acetaldehyde, CO_2, and H_2O_2, that manifest a bacteriolytic and bacteriostatic impact against a spectrum of pathogens Produce short chain fatty acids, which decrease the pH and avoid the growth of pH-sensitive pathogenic bacteria in the intestine	[58, 61, 63, 64]
Anti-inflammation	Kefir suppresses the pro-inflammatory cytokines (Th-1, IL-6, IL-1β, TNF-α, NF-kB, and p-MAPK), and elevates the anti-inflammatory cytokines (Th-2, and IL-10), and stimulates TLR-2 proteins	[69–73]
Anti-cancer	Kefir-induced apoptosis is associated with the upregulation of BAX, Cyto-c, Caspase 3, 8, and 9, TGF-β1 mRNA expression and by downregulation of TGF-α and Bcl-2 expression Furthermore, organic acids produced as secondary metabolites play a pivotal role in fostering cytotoxicity against HepG2 cells	[76–79]
Antioxidant	The donation of protons from the lactic acid by LAB during the fermentation of kefir scavenges free radicals, thereby augmenting the primary antioxidant property Lactate ions within kefir can prevent lipid peroxidation by scavenging free radicals	[83–86]
Anti-diabetic	Promotes the activation of phosphoinositide 3-kinase or other molecules within the insulin signalling pathway, leading to enhanced insulin secretion. It inhibits the activity of pancreatic α-amylase and α-glucosidases, resulting in a significant reduction in postprandial glucose levels in the blood	[92–97]

LAB lactic acid bacteria, *Th-1/2* T helper cells-1/2, *IL* interleukin, *TNF-α* tumor necrosis factor alpha, *TLR-2* toll-like receptor 2, *NF-kB* nuclear factor kappa B, *p-MAPK* Phospho-Mitogen-activated protein kinase, *TGF-α/β1* transforming growth factor alpha/beta1, *BAX* BCL2-associated X apoptosis regulator, *Bcl-2* B-cell lymphoma 2

NF-kB/ MAPK signaling pathways. Thereby suggesting a promising avenue for their utilization in the treatment of inflammatory disorders [72].

Similarly, a study was conducted to assess the anti-inflammatory properties of kefir-fermented in a dextran sodium sulfate (DSS)-induced colitis mouse model. It was reported that kefir supplementation manifested a mitigating effect on the inflammatory cascade, as evidenced by reductions in reticulum edema, neutrophil accumulation, and an elevation in autophagosomes. Furthermore, it enhanced the levels of acetate and propionate, thereby abating the intestinal damage of DSS-induced colitis. It was concluded that kefir may also have promoted epithelial barrier restoration, thus facilitating anti-inflammation [73].

Recently, the therapeutic potential of kefir peptides (KPs) in adjuvant-induced arthritis (AIA) was investigated using a mouse model. It was reported that TLR receptors present in epithelial cells are stimulated by pathogens and trigger the NF-kB and p-c-Jun N-terminal kinases (JNK) pathways, leading to the release of proinflammatory cytokines and chemokines. Thus, causing overexpression of immune cells and inflammation. Results demonstrated KPs' efficacy in mitigating AIA through suppression of phospho-IkappaB (p-IkB), NF-kB, phospho P38 (pp38), and p-JNK activation, along with reduced tumour necrotic factor (TNF-α) expression. KPs alleviated synovitis by downregulating rheumatoid arthritis-related inflammatory signalling molecules (TNF-α, Il-1β, and Il-6). Overall, KPs effectively attenuated AIA and minimized bone erosion by modulating NF-kB and MAPK pathways and reducing macrophage-related inflammatory signalling molecules expression [74].

It was investigated whether milk kefir's anti-inflammatory impact on mouse periodontitis. Wistar rats, divided into control (C), experimental periodontitis (EP), one-day fermented milk kefir (K1), one-day fermented milk kefir with EP (K1 + EP), four-day fermented milk kefir (K4), and four-day fermented milk kefir with EP (K4 + EP) groups, were fed for 28 days pre-EP induction. Results revealed the K4 + EP group exhibited significantly reduced alveolar bone loss, decreased expression of proinflammatory cytokines TNF-α, IL-6, and IL-Iβ, and elevated IL-10 expression (a potent anti-inflammatory cytokine) compared to EP groups [75]. Thus, it can be concluded that both isolated microbes and peptides derived from kefir elicited an upregulation of anti-inflammatory cytokines and a concurrent downregulation of pro-inflammatory cytokine responses, signifying their potential as anti-inflammatory agents, as mentioned in Table 2 and Fig. 3.

Anti-cancer properties of kefir

In accordance with the World Health Organization's report from 2018, cancer stands as the second leading cause of mortality worldwide, with its prevalence continuing to escalate further every year [76]. Exploration into the anti-carcinogenic attributes of kefir has unveiled an intricate link to its bioactive constituents, including polysaccharides and peptides. These biological activities in kefir induce macrophage activation, prompt nitric oxide synthesis, augment phagocytic capability, and play a pivotal role in the modulation of cellular processes such as apoptosis, proliferation, and transformation [77, 78].

An effort was made to explore the anti-cancer efficacy of the EPSs synthesized by *Lb. kefiri* derived from Chinese kefir grains in inhibiting the proliferation of colorectal cancer (HT-29) cell growth. It was evident that MSR101 EPS exhibited excellent antitumor activity against HT-29 colon cancer cells. Furthermore, the results elucidated that the apoptosis induction was associated with the upregulation of Bcl2-associated X protein (Bax), cytochrome c, caspase-3, -8, and -9, along with the suppression of B-cell lymphoma 2 (Bcl-2) expression [79]. Overall, it was concluded that the EPS synthesized by *Lb. kefiri* holds promising potential in the regulation of cancer and as an anti-tumor agent.

Recently, an effort was made to elucidate the anticancer attributes of fermented beetroot juice made from grains of water kefir on human hepatoma cell lines (HepG2). It was reported that betalains (a major bioactive compound) have the ability to inhibit HepG2 cell proliferation by inducing the arrest of the cell cycle at the G1 phase and instigating cell death by apoptotic means. Furthermore, betanin and betalain produced by beet root and organic acids produced as secondary metabolites by the probiotic bacteria present in kefir during the fermentation process played a pivotal role in fostering cytotoxicity against HepG2 cell lines, as mentioned in Table 2 and Fig. 4 [80]. It was reported that in the cell-free medium of kefir, an anti-proliferative impact was observed, accompanied by the induction of apoptosis. This effect was associated with the downregulation of transforming growth factor-α (TGF-α), and upregulation of TGF-β1 mRNA expression in *Human T lymphotropic virus type 1* (HTLV-1) negative malignant T-lymphocytes, as mentioned in [78]. In aggregate, it unveils the potential of kefir and its fractions as adjunctive components in cancer therapy.

Antioxidant properties of kefir

Antioxidants help in scavenging free radicals, mitigating damage caused by unstable molecules generated by the body in response to stress and environmental factors [81, 82]. Kefir exhibits robust antioxidant

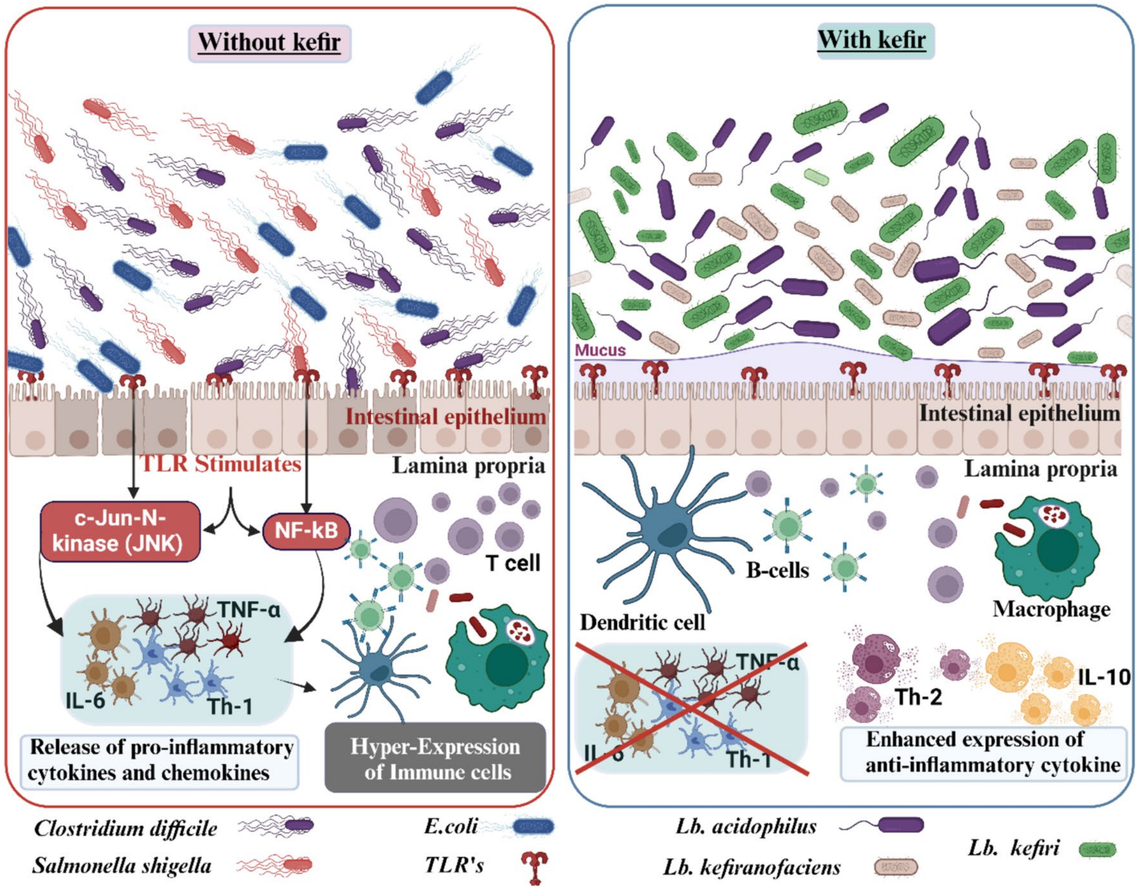

Fig. 3 Anti-inflammatory properties of kefir by elevating anti-inflammatory cytokines and suppressing pro-inflammatory cytokines by interacting with TLR present in gut epithelium. TLR, Toll-like receptor; IL-6, interleukin-6; TNF-α, Tumor necrosis factor alpha; Th-2, T helper cells-2; NF-kB, Nuclear factor kappa B; IL-10, interleukin-10

capabilities, substantiated through empirical validation in both ex vivo and in situ studies model [83].

In a recent investigation, the antioxidant activity of *Arthrospira platensis* and *Chlorella vulgaris* microalgae-enriched kefir was assessed using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity. Lower concentrations (0.5% w/v) of the above microalgae demonstrated a significant increase in antioxidant activity as compared to higher concentration (1.0% w/v). It indicates that a higher concentration of microalgae may inhibit the antioxidant potential of kefir. Furthermore, the intricate microbiota of milk kefir contains various beneficial bioactive compounds, including EPSs, bioactive peptides, and organic acids, particularly lactic acid and kefiran. The lactate ion within kefir can prevent lipid peroxidation by scavenging free radicals [84]. The enrichment of microalgae amplifies the presence of these compounds, potentially influencing DPPH results through factors beyond phenolic compounds.

In a similar study to assess the nutrient composition of kefir-spirulina (two different sample formulations) and its impact on antioxidant potential, DPPH activity displayed IC_{50} values of P1 (43.65 ppm) and P2 (42.00 ppm). Notably, an IC_{50} below 50 ppm signifies a highly potent activity. It was concluded that the antioxidative potential of kefir-spirulina experienced augmentation throughout the fermentation process [85]. This activity was attributed to the donation of protons from the acids produced by LAB during fermentation to scavenge free radicals, thereby augmenting the primary antioxidant capacity as mentioned in Table 2.

Further, an effort was made to ameliorate the effect of kefir and its impact on oxidative stress against γ-irradiation-induced liver damage in rats. It was reported that the administration of kefir resulted in the restoration of glutathione, total antioxidant capacity, and catalase activity. Moreover, kefir exhibited a mitigating effect on lipid peroxidation and nitric oxide production.

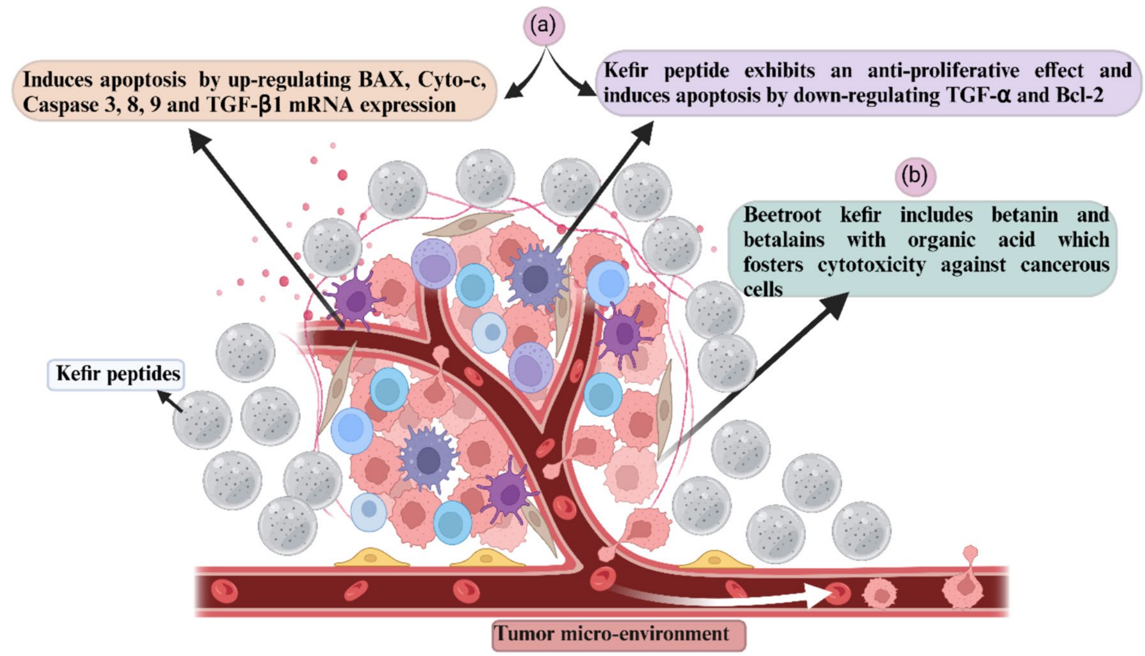

Fig. 4 **a** The kefir peptides induce apoptosis by upregulating BAX, Cyto-c, Caspases, TGF-β1 and down regulating TGF-α. **b** Cytotoxic effects of organic acids, betanin, and betalains produced during fermentation of beetroot kefir. TGF-α/β1, Transforming growth factor alpha/beta1; BAX, BCL2 associated X apoptosis regulator; Bcl-2, B-cell lymphoma 2

Additionally, it was elucidated that kefiran, a component of kefir, demonstrated various antioxidant activities, including superoxide, and nitric oxide radicals [86].

An effort was made to assess the impact of natural kefir grains on the intestinal microbiota and their antioxidant potential in BALB/c mice. Biochemical analyses revealed that the kefir exhibited an elevated total antioxidant status (TAS) value. The lower malondialdehyde values indicated an antioxidative effect, suggesting a potentially probiotic impact on the microbiota. The study concluded that the sera obtained from mice subjected to a diet incorporated with kefir enhanced the TAS value, emphasizing the notable antioxidant potential associated with this kefir formulation [87]. Probiotics fermented technology (PFT) kefir grain comprised of *Lb. kefiri* P-IF and small amount of various yeast has minimized the age associated oxidative stress in mice model studies [88]. In addition to the above supplementation of *Lb. kefiranofaciens* ZW3 from Tibetan kefir improved depression like behaviour in stressed mice by modulating gut microbiota [89]. Probiotic enriched kefir has improved the climbing ability, survival rate, and minimized the vacuolar lesions happens during neurodegeneration by modulating amyloidogenic pathways in *Drosophila melanogaster* model studies for Alzheimer's disease [90]. It was also reported that administration of soy milk kefir, and cow milk kefir displayed higher anti-depression, and anxiolytic effects on nicotine with drawl-induced depression and anxiety in rats [91].

Anti-diabetic properties of kefir

As per the statistics by the International Diabetes Federation, diabetes affects 1 in 11 adults with an age range of 20–79 years, accounting for a total of 463 million individuals, thus characterizing it as a worldwide pandemic. In the absence of appropriate therapeutic interventions, persistent hyperglycaemia can lead to glucose toxicity, progressively impairing insulin secretion [92]. Over the past decade, a mounting body of evidence has surfaced, substantiating the anti-diabetic attributes of kefir as a prospective and economically viable therapeutic agent [93].

One of the earliest antidiabetic properties of kefir was demonstrated by the water-soluble and methanolic fractions of kefram-kefir. It was reported that administration of kefir has significantly reduced the effect of type II diabetes through the uptake of an active agent in kefram-kefir, which was absorbed by the small intestine and transported to the liver. It leads to enhanced glucose uptake and upregulates glucose transporter4 (GLUT4), which activates phosphoinositide 3-kinase (PI3-K) and other molecules within the insulin signalling pathway [94]. Administration of kefir has inhibited the expression of hydrolytic enzymes, specifically pancreatic α-amylase

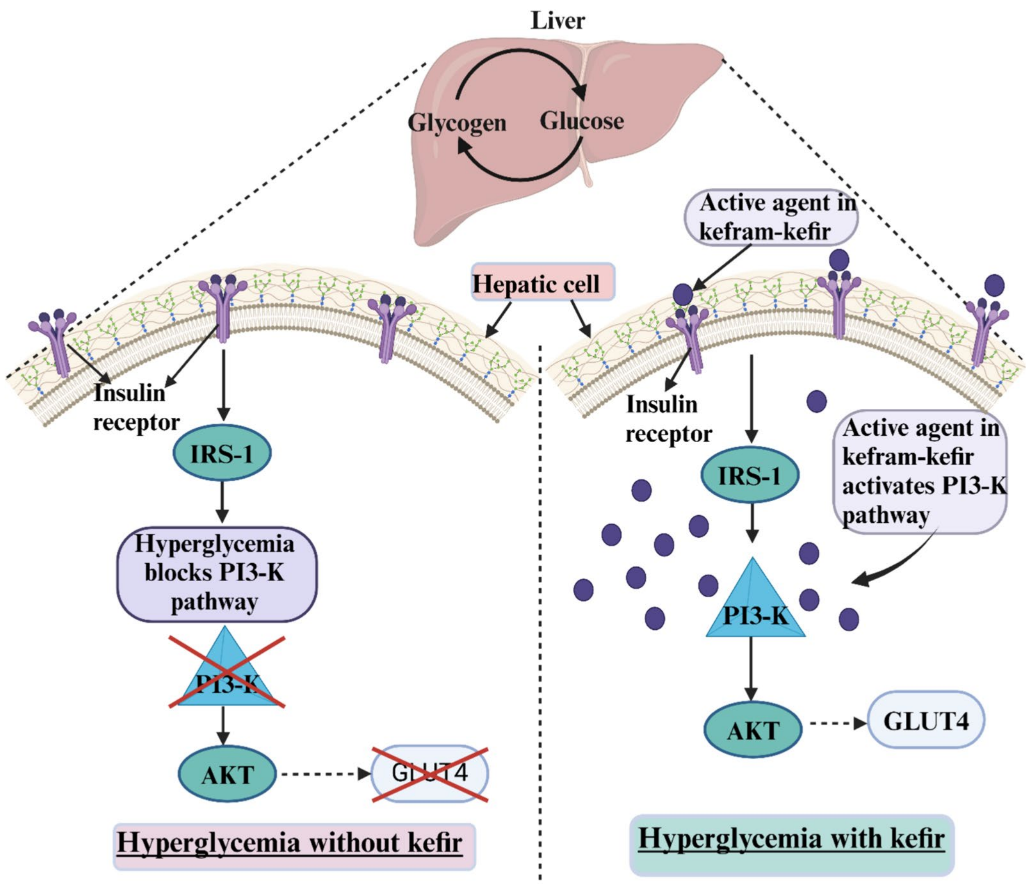

Fig. 5 Anti-diabetic potentials of kefram-kefir leads to the activation of PI 3-kinase augmenting the glucose uptake via the insulin signaling pathway. IRS-1, Insulin receptor substrate 1; PI3K, Phosphoinositide 3-kinase; GLUT4, Glucose transporter-4; PKB, Protein kinase B (also known as Akt)

and α-glucosidases, resulted in a significant reduction in postprandial glucose levels in the blood. An increase in α-glucosidase inhibitory activity was observed in soymilk kefir fermented with *Rhodiola* extracts, showcasing enhanced anti-diabetic functionality, as mentioned in Fig. 5 and Table 2 [95].

A recent study to evaluate the in vivo anti-diabetic potential of the isolated *Lb. paracasei* from Malaysian water kefir grains (MWKG) was carried out on a mouse model. The study revealed that the administration of *Lb. paracasei* from MWKG led to distinct alterations in the expression patterns of genes associated with glucose regulation and lipid homeostasis in the hepatic tissues of treated mice. It was concluded that the G protein-coupled receptor pathway emerged as the preeminent and significant regulatory pathway in the maintenance of glucose homeostasis. This pathway assumes a crucial role in diverse biological processes, such as insulin secretion, adipogenesis, metabolic functions, and endocrine regulation [96].

Probiotics present in kefir have the potential to stimulate the gut microbiota to generate insulinotropic polypeptides and glucagon-like peptide 1, thereby inducing glucose uptake by muscular tissues. The anti-diabetic potential of kefir depends on the substrate and fermentation technique employed [97]. Furthermore, the glucose-lowering effect of kefir may be attributed to its antioxidant activity, which involves several interacting pathways that eventually contribute to the regulation of blood sugar or the reduction of glucose absorption in the gastrointestinal tract [98].

Conclusion and future prospects

Due to changes in lifestyle and food behaviour, the global burden of diseases is increasing, significantly affecting human health leading to dysbiosis and several metabolic and non-metabolic-related disorders. In the past decade, functional foods have gained considerable attention from the scientific community. Recent studies on kefir have illuminated its multifaceted aspects, emphasizing its ethnic and scientific significance. The exploration of both dairy and water kefir, their production methodologies, and their microbial compositions has provided valuable insights into the diversity of this ethnic fermented beverage. Unlike traditional dairy kefir, non-dairy kefir is highly suitable for individuals with lactose intolerance, vegan dietary preferences, or dairy allergies. The intricate relationship between kefir's microbial constituents and their metabolic by products has been underscored, emphasising the potential health benefits of kefir consumption. Furthermore, compelling evidence supports kefir's remarkable antimicrobial, anti-inflammatory, anticancer, antioxidant, and anti-diabetic properties attributed to the synergistic effect of kefir peptides, immune and cytokine modulatory properties and free radical scavenging effects. However, numerous technical gaps still prevail, which need the attention of the scientific community. For instance, the symbiotic association of microbes with the kefir matrix and its composition are still unclear. Research needs to be performed on how the microbial diversity of kefir grains or kefir starters will affect the quality, flavour, and functional properties of beverage. Several animal and human trails need to be performed to signify the health-promoting attributes of kefir. In addition, research should be focused on optimizing production parameters (temperature, pH, starter, substrate) from different kefir grains (with varying microbial diversity), which are vital for enhancing the functional properties and development of kefir-based beverages (dairy and non-dairy) to cater to the needs of consumers across the world. Future research must be focused on developing kefir starter cultures responsible for the production of desired metabolites so that disease- specific functional kefir-based beverages can be developed. In essence, this review provides a foundation for further exploration of kefir and its applications in functional foods with diverse health-promoting attributes. The intricate interplay of kefir's metabolites and their potential therapeutic effects are yet to be explored to their full potential.

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