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# New frontiers in pharmaceuticals and cosmetics for kefiran, a unique and versatile biopolymer

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In recent years, there has been a growing demand for food-derived polysaccharides in materials research, due to increasing concerns with synthetic biopolymers. Kefiran, is a unique polysaccharide produced exogenously by a member of the *Lactobacill*us sp., found ubiquitously in kefir grains. Recently, a lot of interest has been focused on this edible biopolymer, which is a water-soluble branching glucogalactan with nearly equal quantities of D-glucose and D-galactose in a chain sequence. Kefiran has been established as safe for human use, in addition to having many other advantageous characteristics such as, anti-microbial, anti-tumor and wound healing properties. Apart from this, it also has moisturizing and antioxidant effects, which makes it ideal for pharmaceutical and cosmeceutical applications. This review aims to focus on the kefiran properties while providing a broad overview of its potential applications in the growing pharmaceutical and cosmeceutical industries.

**Keywords: Kefiran, polysaccharide, applications, pharmaceutical industry, cosmeceutical industry**

## INTRODUCTION

Polysaccharides can be described as long chains of mono-saccharides such as glucose, fructose and galactose, which are bound together by glycosidic bonds to form polymeric carbohydrate molecules (Guo et al. 2017). Polysaccharides are in continuous demand since they are extensively used in many industries such as food, pharmaceuticals and cosmeceuticals. However, very often, supplies are inconsistent and cannot sufficiently meet demands and there is a great need for more variety in polysaccharides with similar characteristics (Chen et al. 2018). Polysaccharides may be obtained from various sources such as plants, animals and microbes. Production of polysaccharides from plants or animals is time consuming and exploits huge tracts of land in addition to requiring extensive labor. Polysaccharide production by microorganisms such as bacteria and it is free from these constraints (Wu et al. 2018) and has the added advantage of a much shorter production period with a more well-defined and controlled production process.

Microbial polysaccharides have garnered great interest since they are known to have a wide range of applications due to their pharmaceutical and therapeutic properties (Dailin et al. 2019; Nordin et al. 2020; Dailin et al. 2021). Microbial polysaccharides represent only a small portion of the current biopolymer market **(**Piermaría et al. 2016). This could be due to inherent problems related to the bioprocess involving the mass production of microorganism. In recent years though, a growing interest in isolating and identifying new microbial polysaccharides with better characteristics, have emerged (Sutherland, 2001), although microbial polysaccharides have long been known and are widely used in industry. Dextran was the first biopolymer discovered in 1880 (Sudiman et al. 2018) and also, the first microbial polysaccharide approved for food application and commercialization (Sun et al. 2019). Today, there are even more commercially produced polysaccharides with industrial applications such as kefiran (Dailin et al. 2020), xanthan (Ramezani et al. 2014), pullulan (Ma et al. 2014), levan (Sarilmiser et al.

2014), alginate (Díaz-Barrera et al. 2014) and gellan (Zhang et al. 2015). In nature, these microbial bioactive polysaccharides often function as protection against adverse conditions (Park et al. 2016). They hide the bacterial surface and substratum as protection against harsh environments and they help with the formation of cell aggregates in rhizosphere communities besides supporting biofilm structures as a stabilizer. They have also been reported to act as signaling molecules relaying information (Badel et al. 2011). In extreme conditions when nutrients are scarce, polysaccharides can be catabolized to serve as a potential energy reserve (Patel and Patel, 2011). Anionic exopolysaccharides may bind and affect the penetration of both beneficial and toxic ions through the cell surface (Cesco et al. 2012) thus controlling movement of substances into or out of the cell. Vast numbers of microorganisms also possess the ability to synthesize extracellular polysaccharides in the form of soluble or insoluble polymers (Massalha et al. 2017). Lactic acid bacteria (LAB) producing polysaccharides exogenously, have received considerable interest from both consumer groups and manufacturers, especially since, products from LABs are Generally Regarded As Safe (GRAS) (Patel and Prajapat, 2013).

The growth of bacteria is always followed with the production of exo-polysaccharides which have been shown to have ecological and physiological roles (Dertli et al. 2016). Polysaccharides produced by different LAB show large variations in composition, charge, spatial arrangement, rigidity, and ability to interact with proteins (Ishola and Adebayo., 2012). Besides the type of LAB strains, culture conditions and medium compositions may also determine the characteristics of polysaccharides produced and it is difficult to define a universal benchmark for the polysaccharides titers desired (Hassan et al. 2012).

Studies have shown that in LAB metabolism, large proportions of carbon are converted into lactate, which in turn, inhibits polysaccharides production. However, it has also been demonstrated that lactate formation could be reduced if the production of polysaccharides were coupled to cell growth, indicating that the culture should be maintained at its log phase (Caggianiello et al. 2016).

Currently, the area of study involving microbial polysaccharides as an important source of natural biopolymers, is growing exponentially (Dailin et al. 2015; Dailin et al. 2016). Most of these polysaccharides, such as kefiran, have great economic value (Dailin et al. 2022). Kefiran is a water-soluble polysaccharide, produced exogenously and predominantly by *Lactobacillus kefir* sp. (Kandler and Kunath, 1983). It is composed of equal repeats of galactose and glucose (Zamberi., 2016). Since this polymer was first isolated from kefir grains, it was aptly named kefiran by La Riviere et al. (1967) who discovered it.

Kefiran is produced in both the extracellular soluble form (broth kefiran) and the capsular form (capsular kefiran) (Korsak., 2015) although the molecular weight ($1\times10^7$ Da) (Magalhães., 2011) and the composition between these forms are the same, indicating that this polysaccharide has different physical structures but are chemically similar (Yokoi et al. 1990). Kefiran has great promise in both the pharmaceutical and cosmeceutical industries as there are many potential applications for it, based on both its characteristics and GRAS (Generally Regarded As Safe) status (Ahmed et al. 2013). It has also been used in regenerative medicine since it has shown exceptional performance in tissue biocompatibility with excellent biodegradability properties (Ahmed et al. 2013a). This review aims to present details on kefiran properties and provide an overview of its potential applications in the pharmaceutical and cosmeceutical industries.

## Kefiran characteristics

Kefir is a fermented drink prepared by the inoculation of cow, sheep or goat's milk with kefir grains. (May consider the inoculation from non-dairy products such as soy bean milk or almond milk). The hydrolysis of lactose occurs during the fermentation process. Kefiran, is a consortium of various exo-polysaccharides and microorganisms, of which, the kefir grains are a constituent (Marquinaet al. 2002). The polysaccharides present in kefir grains was first discovered and named kefiran by La Riviere ´re et al. (1967). Kefiran constitutes of water-soluble polysaccharides that are produced by the lactic acid bacteria known as *L. kefiran ofaciens,* which actually constitutes about 24 wt.% of the dry weight in a kefir grains (Durango et al. 2006)). It is usually produced either as a soluble extracellular polysaccharide or an encapsulated polysaccharide without any apparent difference in either the molecular weight or the sugar compositions between these forms (Yokoi et al. 1990).

Mukai et al. (1990) have simplified the chemical structure of kefiran extracted from kefir grains isolated from *Lactobacillus* sp. As shown in Figure 1, the structure proposed is a water soluble branched glucogalactan (Durango et al. 2006). It contains approximately equal amounts of D-glucose and D-galactose residues (Ghasemlou et al. 2012). It was also noted that no significant change in specific rotating power was observed when different carbon sources (glucose, lactose, maltose, sucrose, fructose, galactose) we reused in the fermentation medium during the production of kefiran, (Wang and Bi, 2008; Kanmani and Lim., 2013), indicating that the molecular structure was stable.

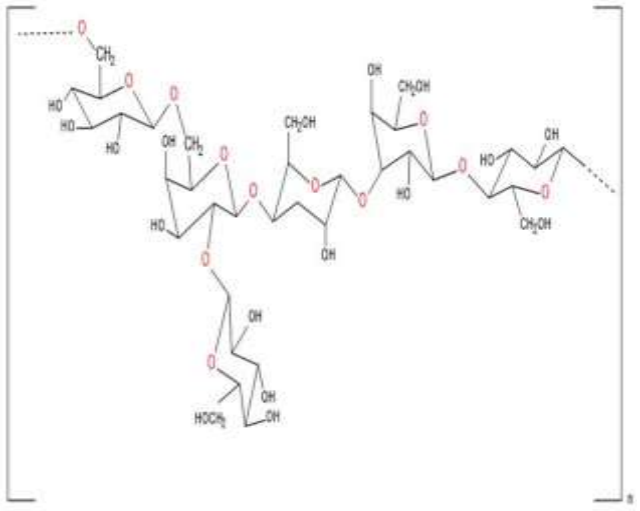

**Figure 1: Proposed chemical structure of kefiran (Prado et al. 2015)**

Different values for kefiran molecular weight have been reported by many authors. According to Mukai et al. (1990) the average molecular weight of kefiran was1.0×$10^6$ Da when *L. kefiran ofaciens* K, was grown for 3 days at 30°Cin IKPL medium under anaerobic conditions. However, when either maltose or lactose were employed as the sole carbon source in the fermentation broth of *L. kefiran ofaciens* JCM6985, a nearly 7-fold (1.5 × $10^5$Da) and 4-fold (2.4 × $10^5$Da) decrease respectively were observed (Wang and Bi, 2008). Nevertheless, it must also be pointed out that apart from different strains used in the two studies, the culture conditions were also different. In the study with *L. kefiran ofaciens* JCM6985, the fermentation broth was aerated at 100 rpm while in the other, it was anaerobic. In another report, Maeda et al. (2004) published a 1.3-fold decrease in molecular weight value for kefiran (7.6×$10^5$ Da) compared to that reported by Mukai et al. (1990). In this study, *L. kefiranofaciens* WT-2B$^T$was cultured in rice hydrolysate medium under anaerobic conditions. These findings strongly indicate that different strains and substrates may play a very significant role in affecting the molecular size of the product.

Apart from substrates and strains, the observed average yields of kefiran varied with different culture medium and probably also, was affected by the period of the fermentation process. The maximum yield of kefiran using rice hydrolysate as the culture medium, was 2500 mg/L (Maeda et al. 2004) while the reported values for kefiran yield with IKPL (improved kefir grain polysaccharide-producing lactobacillus) medium, was 63mg/L (Mukai etal., 1990). Maltose or lactose as single carbon sources also showed better yields than IKPL at 2270 and 1760 mg/L (Wang and Bi, 2008), despite having the same fermentation time as reported by Mukai et al. (1990).

Both Mukai et al. (1990) and Wang and Bi (2008) conducted 3-day long fermentations while Maeda et al. (2004) extended their culture period to 7 days. Although it is highly unlikely that prolonged cultivation can determine variations in molecular size, it is more probable that yields could be determined by duration of the fermentation process. It is clearly apparent that culture media composition and length of the fermentation process is important for good kefiran yields. Nevertheless, it is still important to consider the molecular size of the product yield as this feature may be critical to the intended function of the polysaccharide. Therefore, the relationship between molecular size and functional impact warrants further investigations since the applications for kefiran widely range from tissue restructuring (Radhouani et al.2019) to regulating hyperlipidemia (Tung et al. 2018).

**Potential Application of kefiran in Pharmaceutical and Cosmeceutical Industry**

kefiran is ubiquitously present in kefir drinks, it would undoubtedly be interesting to examine the systemic effect of this microbial polysaccharide, with respect to the immune system. An enhancing effect on the production of interferon ß cortisol and noradrenaline in human cell lines have been reported and thus, a possible use for this polysaccharide as a stress reducing food component has been hypothesized (Davras et al. 2018).

Apart from this, kefiran has been reported to also have antibacterial (Rodrigues et al. 2005), anti-tumour (Cevikbas et al. 1994), anti-fungal (Cevikbas et al. 1994), anti-inflammatory (Kwon et al. 2008; Furuno and Nakanishi, 2012) and cicatrizing (to become healed by the formation of scar tissue (Rodrigues et al. 2005). As such, the therapeutic potential of this polysaccharide is enormous, ranging from infectious diseases to cancer treatment and pain management. Moreover, kefiran is also reported to protect Caco-2 cells from cytopathic effects induced by *Bacillus cereus* infection (Medrano et al. 2009) which suggests that it may have viral protective properties

**Hyperlipidemia**

In 2005, another study showed that kefiran could lower serum cholesterol levels in spontaneous stroke-prone rats (Maeda et al. 2005). While kefiran does not inhibit the absorption of cholesterol from foods, it was shown that it could trap enter hepatic-circulating cholesterol within the intestine. This was evident in the observed treatment of rats fed with cholesterol and kefiran (Maeda et al. 2005). Amounts of cholesterol, bile acid and sterol excreted were proportional to the quantity of kefiran consumed, indicating a dose-dependent effect. Such an effect suggests that kefiran may help in averting cardio-vascular diseases (CVD), commonly caused by cholesterol build-up.

Several reports have also shown that there was a decrease in total serum cholesterol and phospholipids, when rats were fed with a high cholesterol diet

supplemented with kefir (Barbosa et al. 2011) although biomarkers such as high-density lipoprotein (HDLL) and serum triglycerides remained unaffected. This strongly suggests that kefir may play a role in reducing low density lipoproteins (LDLL), which are also commonly known as "bad cholesterol", and therefore, kefir consumption may prove beneficial in controlling hyperlipidaemia.

The findings of Liu et al. (2006) further substantiates this as it demonstrates that both the milk kefir and soy milk kefir lowered total cholesterol concentrations in hamsters. The authors also further cited other studies which had shown that kefir could indeed assimilate cholesterol in milk, and, inhibit exogenous cholesterol absorption from the small intestine. This proposed inhibition mechanism involved a host of activities such as cholesterol and bile salts binding to bacterial cells, deconjugation of biles salts to prevent systemic reabsorption and cholesterol assimilation (Brashears et al.1998).

Deconjugation of bile acid was shown to be directly linked to hydrolase activity of *Lactobacilus* (Begley etal., 2006). Wang etal. (2019), in fact, demonstrated that the expression of cholesterol liver X receptor (LXR) and low-density lipoprotein receptor (LDLR) genes were upregulated when mice were fed with a high cholesterol diet supplemented with either *L. plantarum* AR113 or *L. casei* pWQH01. These strains of lactobacilli are noted for their high bile salt hydrolase (BSH) activity while LXR regulates genes which control systemic cholesterol overload (Dahlman-Wright et al. 2010) and LDLR, is critical for the uptake of LDLL and the maintenance of plasma levels of cholesterol (Wang and Tontonoz, 2018).

The cholesterol lowering effects of kefir was also demonstrated in a dose dependent effect by Cenesiz et al. **(**2008**).** Serum cholesterol in chicken decreased over 42 days, in proportion to the amount of kefir added in the drinking water of the birds. These workers proposed that the decrease in cholesterol levels was primarily due to the reduction in both the biosynthesis of cholesterol in the liver and the increase of bile acid degradation by *Lactobacillus* species present within the kefir in the drinking water. The study also noted that there was no significant change in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels between control and treatment chicken, indicating that kefir was not detrimental to liver function.

It is evident, based on all the above findings regarding kefir and its cholesterol lowering effects, that the value of this fermented drink cannot be overemphasized. In addition to this, it would seem that soy-milk kefir compared to milk kefir may have a further advantage. Liu et al. (2006) postulated that the cholesterol lowering properties of soy-milk kefir, could also be attributed to other hypocholesterolaemic compounds, apart from genistein, present in soy-milk kefir. This was based on the increased faecal clearance of cholesterol and bile acid observed in soy-milk kefir compared to milk kefir, which indicated better anti-hyperlipidaemia effects.

**Tissue Engineering**

Besides a therapeutic role, kefiran based polysaccharides have also been increasingly used in tissue engineering. Tissue engineering can be defined as the production of tissue cells to replace, for the purpose of repairing, to replace for the purpose of restoring, or even, to regenerate any diseased or damaged tissues by artificial means (Edgar et al. 2016). The main consideration in tissue engineering is the effects exhibited by the scaffolds which function as the matrix for the cells to grow and achieve its desired shape (Gimble and Nuttal, 2011). Kefiran's capability of 3D morphological control and alterable mechanical properties (Radhouani, 2018) makes the kefiran based scaffold one of the most promising materials for tissue engineering (Wartenberg., 2017). The reported tensile strength and elongation of kefiran is noteworthy, recording between 5 to 18 MPa for tensile strength, and, 40% to 160% for elongation. These values indicate that kefiran has properties of a truly desirable polymeric matrix in the applications of tissue engineering (Han., 2016).

**Drug Delivery**

In engaging these attributes, Radhouani et al. (2019) demonstrated that kefiran cryogel was also ideal for drug delivery. A Kefiran-based scaffold developed by freeze gelation technique, showed high porosity (82.3 %) combined with a delayed degradation profile over 28 days, allowed a slow and sustained release of diclofenac over two weeks. In order to validate conserved tissue metabolism, human Adipose-derived Stem Cells were cultured onto the kefiran scaffold and metabolic activity was monitored. It was found that the cells remained metabolically active even after 72 hours, firmly establishing the biocompatibility of kefiran in sustaining metabolic activity. Thus, this strongly indicates that kefiran cryogel could be a promising and safe candidate for drug delivery of controlled bioactive molecules, especially in a tissue engineered scaffolding.

**Anti-tumor**

Tumors can be classified as carcinomas or sarcomas (Gimble and Nuttall , 2011). Sarcoma tumors are believed to be derived from the connective tissues of fat, bone and cartilage (Silva et al. 2014), while carcinomas are believed to have developed from the epithelial cells. Anti-carcinogen can be defined as the ability to inhibit the carcinogenic activity or the development and the growth of any type of carcinoma. According to studies conducted by Liu et al.(2002), freeze dried kefiran which was produced from cow (64.8% kefiran)or soy milk (70.9% kefiran)and tested in mice diagnosed with tumors, significantly inhibited the growth of tumors when compared with the control group. In another study, with breast cancer induced mice, the mice received kefir and cell-free fraction of kefir for 27 days in cyclic feeding of 2 and 7 days (De LeBlanc et al. 2007). The results showed that both kefir

and the cell-free fraction had significant effects on reducing tumour growth and increasing the number of apoptotic cells. These findings clearly indicate that it was the non-microbial components in kefir which was the source of the antitumorigenic activity observed.

The anti-tumorigenic mechanism of action in kefiran however seems to be indirect. According to Rattray and Connell (2011), kefiran inhibited Ehrlich carcinoma and Sarcome 180, a murine sarcoma cell-line, by stimulating the immune system of the host through T-cell activity, instead of directly attacking the cancer cells. Other studies have also shown that kefiran and kefiran bacterial isolates cannot directly inhibit the development of tumors in animal models (Rattray and Connell, 2011). Additionally, kefiran produced by microorganisms exhibited anticancer properties in two types of human cancer cells that were tested, including cervical and hepatocellular cancer cells, while its safer profiles in animals (zebrafish embryos) suggest that it may be a potential anticancer agent that does not interfere with normal tissue growth (Elsayed et al. 2017). A list of potential pharmaceutical applications for kefiran is presented in Table 1.

**Table1: Applications of kefiran in pharmaceuticals industries**

| Pharmaceutical Applications | References |
|---|---|
| Anti-bacterial<br>Anti-oxidant | Cevikbas et al. 1994;<br>Rodrigues et al. 2005<br>Dailin et al. 2020 |
| Anti-tumor/Anti-Cancer<br>Anti-fungal | Cevikbas et al. 1994<br>Cevikbas et al. 1994;<br>Ghazvini et al. 2018 |
| Anti-inflammatory | Kwon et al. 2008<br>; Furuno and<br>Nakanishi, 2012) |
| Cicatrizing agent | Rodrigues et al. 2005 |
| Stress reducing agent | Kabayama et al. 1997;<br>Cenesiz et al. 2008 |
| Lowered serum<br>cholesterol level | Maeda et al. 2004;<br>Cenesiz et al. 2008 |
| Reduces atherosclerosis | Uchida et al. 2010 |
| Immuno-modulator<br>Preventive for liver<br>disorder and<br>decreased of intestinal | Medrano et al. 2011<br>Maeda et al. 2005 |

## Cosmeceuticals

Within the cosmeceutical field, physical characteristics of an excipient become more important as the product must also gratify sensory demands. Piermaria et al. (2015) compared shear and extensional properties of kefiran with other neutral polysaccharides which were normally used in cosmetics. Interestingly, the results obtained for kefiran showed similar physical characteristics to methylcellulose, locust bean gum and guar gum, all of which are common binding agents used in the pharmaceutical and cosmeceutical industry. Kefiran, however is also gaining more interest because of its excellent dispersion stability enhancement, which can improve product texture and sensory performance. Its biodegradability and biocompatibility with human skin is also an additional advantage their degradation products are classified as GRAS(Tan e et al. 2020).

The production of kefiran for cosmeceuticals will require large-scale manufacture in order to ensure sufficient supply of the bioactive ingredient. Lactic acid bacteria (LAB) have commonly been used globally as the source bacteria in producing various kinds of cosmetic ingredients through fermentation technology (Chiba 2007). However, the extraction of kefiran from kefir grains traditionally cultured in milk historically produced meagre yields, while the process itself was quite complicated. Fortunately, nearly two decades ago Taniguchi et al. (2001) successfully established an enhanced yield process for producing kefiran from *Lactobacillus*. The study focused on optimizing the processing parameters such as media and culture conditions, ethanol addition, culture time, effects of aeration and yeast cell addition, in order to achieve higher kefiran yields. Such advancements in LAB fermentation technology can project high yields of cosmetic ingredients for improved skincare. Since kefiran, has exhibited good antimicrobial properties, this slimy polysaccharide matrix, may potentially replace the use of antibiotics in acne treatment or many other similar ailments.

Chen et al. (2006) studied the potential of kefir whey (kefir whey, peptides, lactic acid) in relation to skincare properties which included acne treatment and skin whitening effect. The results obtained proved that all the components in kefir whey had the ability to inhibit melanin synthesis, and its degree of inhibition was much greater than milk whey. The increased concentration of lactic acid produced (>60mg/mL) also played a role in inhibiting the growth of *Propionibacterium acne,* the primary cause of acne(Chen et al. 2006). Currently, the antibiotic Erythromycin, is generally used to treat acne inflammation caused by bacterial infection, but *P. acnes* is reportedly becoming resistant to this drug Dreno et al. (2001). Fortunately kefir components which have added antioxidant properties provided by copper chelates, may provide a suitable alternative for acne treatment(Liu et al. 2006).

Jaya et al. (2019) studied the microbiological properties of goat milk kefir in relation to facial mask cream. Kefir is believed to be a potential assembly of probiotics, polysaccharides, peptide and organic acids. These are bioactive compounds which have properties for skincare product development. The optimum incubation period for goat kefir in relation to facial mask cream production, has also been determined by Jaya et al. (2019). Kefir whey peptides and lactic acid have the ability to lighten the skin (whitening effect) while the chelation of copper inhibited tyrosinase activity. In another study, Vinderola et al. (2006) reported that kefiran had antimicrobial and wound healing properties which could be

incorporated into skincare products to function as antiseptics.

**Kefiran as a Prophylaxis**

Irritable Bowel Syndrome (IBS) is a large intestine disorder which predominantly causes stomach cramps and diarrhea, but can also include bloating, gas, and even constipation. It affects nearly 11% of the global population (Canavan et al. 2014) and is more common in the elderly, who also have greater prevalence of rectal urgency and fecal incontinence. Up-regulation of histamine has been widely implicated in the pathophysiology of IBS (Fabisiak et al. 2017) and one of the strategies proposed for managing this disorder, is to suppress histamine.

Maeda et al. (2005) noted that there was a negative correlation with kefiran uptake and histamine concentration in the solid waste of rats fed with cholesterol. They suggested that this was due to kefiran inhibition of histamine synthesis in the cecum. These findings present encouraging prospects for people afflicted with IBS as the nutritious kefir may help in controlling the severity of their condition. Also, since IBS is a known familial disorder, it can act as a prophylaxis for those who have genetic links to this disease.

Kefiran has also been shown to elevate protective immune responses. Medrano et al. (2011) noticed that goblet cells in the villus of mice treated with kefiran, increased in number. They suggested that this was caused by kefiran inducing changes in the vilus tissue panorama. They also noted that together with these changes, the concentration of immunoglobulin A (IgA) also increased, forming a protective layer in the epithelium of the vilus. IgA accounts for more than two-thirds of the body's total immunoglobulin production. IgA serves as an important first-line barrier limiting the access of intestinal antigens to the gut mucosa. It also controls the intestinal micro biota and dampens pro-inflammatory immune responses (Pabst, 2012). Thus, it is apparent that consumption of Kefir can support a healthy immune system which is no doubt essential for the prevention of undesirable infections.

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) are liver enzymes which are also used as markers to monitor liver function. Increases in these markers suggest poor liver conditions and are usually the result of insults caused by alcohol abuse, cholesterol overload and viral assault. Maeda etal. (2005) observed that GOT and GPT were down regulated in mice fed with high cholesterol or orotic acid diets supplemented with kefiran compared to the controls without kefiran. Based on these findings, they concluded that kefiran could support the prevention of hepatic disorders.

## CONCLUSION

The variety and depth of studies conducted on the kefiran macromolecule in recent years have been astounding, and they are progressing swiftly. This overview aimed to present a comprehensive picture of the scientific developments and intriguing studies in several domains up to the present. The potential of kefiran in both the pharmaceutical and cosmeceutical sectors, is huge, especially since it is a food-derived biopolymer and recognized as GRAS. Due to its unique physical properties and therapeutic effects, it is fast emerging as a very promising formulation component which can be safely used in these industries.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

DJD, VMN, FR, and AZB were involved in data collection and writing the manuscript. SNZ, DNAZ and HAE reviewed the manuscript. All authors read and approved the final version.



## REFERENCES

Ahmed Z, Wang Y, Ahmad A, Khan ST, Nisa M, Ahmad H, Afreen A. 2013. Kefir and health: a contemporary perspective. Critical reviews in food science and nutrition, 53(5), 422-434.

Ahmed Z, Wang,Y, Anjum N, Ahmad A, Khan ST. 2013. Characterization of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir–Part II. Food Hydrocolloids, 30(1),343-350.

Babaei-Ghazvini A, Shahabi-Ghahfarrokhi I, Goudarzi V. 2018. Preparation of UV-protective starch/kefiran/ZnO nanocomposite as a packaging film: Characterization. Food packaging and shelf life, 16, 103-111.

Badel S, Bernardi T, Michaud P. 2011. New perspectives for *Lactobacilli exopolysaccharides*. Biotechnology Advances, 29(1), 54-66.

Barbosa AF, Santos PG, Lucho AMS, Schneedorf JM. 2011. Kefiran can disrupt the cell membrane through induced pore formation. Journal of Electroanalytical Chemistry, 653(1-2), 61-66.

Begley M, Hill C, Gahan CG. 2006. Bile salt hydrolase activity in probiotics. Applied and environmental microbiology, 72(3), 1729-1738.

Brashears MM, Gilliland SE, Buck LM. 1998. Bile salt deconjugation and cholesterol removal from media by *Lactobacillus* casei. Journal of Dairy Science, 81(8), 2103-2110.

Caggianiello G, Kleerebezem M, Spano G. 2016. Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms. Applied microbiology and biotechnology, 100(9), 3877-3886.

Canavan C, West J, Card T. 2014. The epidemiology of irritable bowel syndrome. Clinical epidemiology, 6, 71-80.

CenesizS, Yaman H, Ozcan A, Kart A, Karademir G. 2008. Effects of kefir as a probiotic on serum cholesterol, total lipid, aspartate amino transferase and alanine amino transferase activities in broiler chicks-in English. Medycyna Weterynaryjna, 64(2), 168.

Cesco S, Mimmo T, Tonon G, Tomasi N, Pinton R, Terzano R, Neumann G, Weisskopf L, Renella G, Landi L, Nannipieri P. 2012. Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. A review. Biology and Fertility of Soils, 48(2), 123-149.

Cevikbas A, Yemni E, Ezzedenn FW, Yardimici T, Cevikbas U, Stohs SJ. 1994. Antitumoural antibacterial and antifungal activities of kefir and kefir grain. Phytotherapy Research, 8(2), 78-82.

Chen F, Ran L, Mi J, Yan Y, Lu L, Jin B, Li X, Cao Y. 2018. Isolation, characterization and antitumor effect on DU145 cells of a main polysaccharide in pollen of Chinese wolfberry. Molecules, 23(10), 2430.

ChenMJ, Liul JR, Sheu JF, Lin CW and Chuang CL. 2006.Study on skin care properties of milk kefir whey. Asian-australasian journal of animal sciences, 19(6), 905-908.

Chiba K. 2007. Development of functional cosmetic ingredients using lactic acid bacteria in Japan. Japanese Journal of Lactic Acid Bacteria 18(3), 105-11

da Silva LP, Cerqueira MT, Sousa RA, Reis RL, Correlo VM, Marques AP. 2014. Engineering cell-adhesive gellan gum spongy-like hydrogels for regenerative medicine purposes. Acta biomaterialia, 10(11), 4787-4797.

Dahlman-Wright K, Zhao C, 2010. LXR in cholesterol metabolism. Journal of Endocrinology, 204, 233-244.

Dailin D. J., Malek R.A., Ting T.L., Wehbe, R., Ramli S., Elsayed A.A., Leng, O.M., Ho T., and El-Enshasy H. (2021). Biosynthesis, Production and Application of Kefiran In Food Industry: A Review. Bioscience Research,18(1): 102-119.

Dailin DJ, Elsayed EA, Malek RA, Hanapi SZ, Selvamani S, Ramli S, Sukmawati D, Sayyed RZ, El-Enshasy HA,. 2020. Efficient kefiran production by Lactobacillus kefiranofaciens ATCC 43761 in submerged cultivation: Influence of osmotic stress and nonionic surfactants, and potential bioactivities. Arabian Journal of Chemistry, 13(12), 8513-8523.

Dailin DJ, Elsayed EA, Othman NZ, Malek R, Phin HS, Aziz R, Wadaan M, El-Enshasy HA. 2016. Bioprocess development for kefiran production by *Lactobacillus kefiranofaciens* in semi industrial scale bioreactor. Saudi journal of biological sciences, 23(4), 495-502.

Dailin DJ, Elsayed EA, Othman NZ, Malek RA, Ramli S, Sarmidi MR., Aziz R, Wadaan MA, El-Enshasy HA. 2015. Development of cultivation medium for high yield kefiran production by *Lactobacillus kefiranofaciens*. Int. J. Pharm. Pharm. Sci, 7, 159-163.

Dailin, D. J., Selvamani, S., Michelle, K., Jusoh, Y. M. M., Chuah, L. F., Bokhari, A., ... & Show, P. L. (2022). Production of high-value added exopolysaccharide by biotherapeutic potential Lactobacillus reuteri strain. *Biochemical Engineering Journal*, *188*, 108691.

Dailin, D.J., Low, L.Z.M.I., Malek, R.A., Wan Azelee, NI; Abdul Manas, NH, Keat, H.C., Sukmawati, D., Enshasy, H.A. (2019). Pullulan, a biopolymer with potential applications in pharmaceutical and cosmeceutical: A review. *Bioscience Research*, 16(3): 2604-2616.

Davras F, Guzel-Seydim ZB, Tas TK. 2018. Immunological effects of Kefir produced from Kefir grains versus starter cultures when fed to mice. Functional Foods in Health and Disease, 8(8), 412-423.

De LeBlanc ADM, Matar C, Farnworth E, Perdigón G. 2007. Study of immune cells involved in the antitumor effect of kefir in a murine breast cancer model. Journal of Dairy Science, 90(4), 1920-1928.

Dertli E, Mayer MJ, Colquhoun IJ, Narbad A. 2016. EpsA is an essential gene in exopolysaccharide production in *Lactobacillus johnsonii* FI9785. Microbial biotechnology, 9(4), 496-501.

Díaz-BarreraA, Gutierrez J, Martínez F, Altamirano C. 2014. Production of alginate by *Azotobacter vinelandii* grown at two bioreactor scales under oxygen-limited conditions. Bioprocess and biosystems engineering, 37(6), 1133-1140.

Dreno B, Reynaud A, Moyse D, Habert H, Richet H. 2001. Erythromycin-resistance of cutaneous bacterial flora in acne. European Journal of Dermatology, 11(6), 549-53.

Durango AM, Soares NFF, Andrade NJ. 2006. Microbiological evaluation of an edible antimicrobial

coating on minimally processed carrots. Food control, 17(5), 336-341.

Edgar L, McNamara K, Wong T, Tamburrini R, Katari R, Orlando G. 2016. Heterogeneity of scaffold biomaterials in tissue engineering. Materials, 9(5), 332.

Elsayed AE, Farooq M, Dailin DJ, El-Enshasy HA, Othman NZ, Malek R, Danial E, Wadaan M (2017). In vitro and in vivo biological screening of kefiran polysaccharide produced by *Lactobacillus kefiranofaciens*. Biomedical Research, 28 (2).

Fabisiak A, Włodarczyk J, Fabisiak N, Storr M, Fichna J. 2017. Targeting histamine receptors in irritable bowel syndrome: a critical appraisal. Journal of neurogastroenterology and motility, 23(3), 341-348.

FurunoT, Nakanishi M. 2012. Kefiran suppresses antigen-induced mast cell activation. Biological and Pharmaceutical Bulletin, 35(2),178-183.

Ghasemlou M, Khodaiyan F, Jahanbin K, Gharibzahedi SMT, and Taheri S. 2012. Structural investigation and response surface optimisation for improvement of kefiran production yield from a low-cost culture medium. Food chemistry, 133(2), 383-389.

Gimble ME, Nuttall A. 2011. Adipose-derived stromal/stem cells (ASC) in regenerative medicine: pharmaceutical applications. Current pharmaceutical design, 17(4), 332-339.

GuoMQ, Hu X, Wang C,Ai L. 2017. Polysaccharides: structure and solubility. Solubility of polysaccharides, 2, 8-21.

Han ME, Kim SH, Kim HD, Yim HG, Bencherif SA, Kim TI, Hwang NS. 2016. Extracellular matrix-based cryogels for cartilage tissue engineering. International journal of biological macromolecules, 93,1410-1419.

Hassan AN, Frank JF, Qvist KB. 2002. Direct observation of bacterial exopolysaccharides in dairy products using confocal scanning laser microscopy. Journal of Dairy Science, 85(7), 1705-1708.

Ishola RO, Adebayo-Tayo BC. 2012. Screening of lactic acid bacteria isolated from fermented food for bio-molecules production. AU Journal of Technology, 15(4), 205-217.

Jaya F, Thohari I, Susilorini TE, Asmara DR. 2019. Microbiological properties of preparing facial mask cream from goat milk kefir. In IOP Conference Series: Earth and Environmental Science 230(1), 12-105. IOP Publishing.

Kandler O, Kunath P. 1983. *Lactobacillus kefr* sp. nov., a component of the microflora of kefir. Systematic and *Applied Microbiology.* 4(2), 286-294.

Kanmani, P, Lim ST. 2013. Development and characterization of novel probiotic-residing pullulan/starch edible films. Food chemistry, 141(2), 1041-1049.

Korsak N, Taminiau B, Leclercq M, Nezer C, Crevecoeur S, Ferauche C, Detry E, Delcenserie V, Daube G. 2015. Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments. Journal of dairy science, 98(6), 3684-3689.

KwonOK, AhnKS, Lee MY, Kim SY, Park BY, Kim MK, Lee IY, Oh SR, Lee HK. 2008. Inhibitory effect of kefiran on ovalbumin-induced lung inflammation in a murine model of asthma. Archives of Pharmacal *Research.* 31(12) 1590-1596.

La Riviere JWM, Kooiman P, Schmidt K. 1967. Kefiran, a novel polysaccharide produced in the kefir grain by *Lactobacillus brevis*. Archiv für Mikrobiologie. 59(1) 269-278.

LiuJR, Wang SY, Chen MJ, Chen HL, Yueh PY, Lin CW. 2006. Hypocholesterolaemic effects of milkkefir and soyamilk-kefir in cholesterol-fed hamsters. British Journal of Nutrition, 95(5), 939-946.

LiuJR, Wang SY, Lin YY, Lin CW. 2002. Antitumor activity of milk kefir and soy milk kefir in tumor-bearing mice. Nutrition and Cancer, 44(2), 182-187.

Maeda H, Mizumoto H, Suzuki M, Tsuji K. 2005. Effects of kefiran-feeding on fecal cholesterol excretion, hepatic injury and intestinal histamine concentration in rats. Bioscience and microflora, 24(2), 35-40.

MaedaH, Zhu X, Suzuki S, Suzuki K, Kitamura S. 2004. Structural characterization and biological activities of an exopolysaccharide kefiran produced by *Lactobacillus kefiranofaciens* WT-2B$^{T}$. Journal of Agricultural and Food Chemistry, 52(17), 5533-5538.

Magalhães KT, Pereira GVDM, Campos CR, Dragone G, Schwan RF. 2011. Brazilian kefir: structure, microbial communities and chemical composition. Brazilian Journal of Microbiology, 42(2), 693-702.

MarquinaD, Santos A, Corpos A, Munoz J, Zazo J, Peinado JM. 2002. Dietary influence of kefir on microbial activities in the mouse bowel. Letters in Applied Microbiology, 35(2), 136-140.

Massalha, H., Korenblum, E., Tholl, D. and Aharoni, A. 2017. Small molecules below-ground: the role of specialized metabolites in the rhizosphere. The Plant Journal, 90(4), 788-807.

MaZC, Fu WJ, Liu GL, Wang ZP, Chi ZM. 2014. High-level pullulan production by *Aureobasidium pullulans* var. *melanogenium* P16 isolated from mangrove system. *Applied Microbiology and Biotechnology,* 98(11) 4865-4873.

Medrano M, Racedo SM, Rolny IS, Abraham AG, Pérez PF. 2011. Oral administration of kefiran induces changes in the balance of immune cells in a murine model. Journal of Agricultural and Food Chemistry. 59(10), 5299-5304.

Medrano M, Hamet MF, Abraham AG, Perez PF. 2009. Kefiran protects Caco-2 cells from cytopathic effects induced by *Bacillus cereus* infection. Antonie Van Leeuwenhoek,96(4), 505-513.

MukaiT, Toba T, Itoh T, Adachi S. 1990. Structural

investigation of the capsular polysaccharide from *Lactobacillus kefiranofaciens*K1. *Carbohydrate* Research, 204, 227-0232.

Nordin NZ, Rashidi AR, Dailin DJ, Malek R, Azelee NIW, Manas NH., ... & El Enshasy, H. (2020). Xanthan biopolymer in pharmaceutical and cosmeceutical applications: critical review. *Bioscience Research, 17 (1)*, 205-220.

Pabst O, 2012. New concepts in the generation and functions of IgA. Nature Reviews Immunology, 12(12), 821-832.

Park JY, Shin MS, Kim SN, Kim HY, Kim KH, Shin KS, Kang KS. 2016. Polysaccharides from Korean *Citrus hallabong* peels inhibit angiogenesis and breast cancer cell migration. International Journal of Biological Macromolecules, 85: 522-529.

PatelA, Prajapat, JB. 2013. Food and health applications of exopolysaccharides produced by lactic acid bacteria. Advance in Dairy Research, 106, 1-12.

PatelRM, Patel VP. 2011. Microbial polysaccharides: current innovations and future trends in medical science. Journal of Current Pharma Research. 1(2), 204-209.

Piermaría J, Bengoechea C, Abraham AG, Guerrero A. 2016. Shear and extensional properties of kefiran. Carbohydrate polymers, 152, 97-104.

Prado MR, Blandón LM, Vandenberghe LP, Rodrigues C, Castro GR, Thomaz-Soccol V, Soccol CR. 2015. Milk kefir: composition, microbial cultures, biological activities, and related products. Frontiers in microbiology, 6, 1177.

Radhouani H, Bicho D, Gonçalves C, Maia FR, Reis RL, Oliveira JM. 2019. Kefiran cryogels as potential scaffolds for drug delivery and tissue engineering applications. Materials Today Communications, 20, 100554.

RamezaniA, Jafari M, Goodarzi T, Alavi SM, Salmanian AH, Azin M. 2014. Lactose consuming strains of *Xanthomonas citri* subsp. *citri* (*Xcc*) insight into the emergence of natural field resources for xanthan gum production. World Journal of Microbiology and Biotechnology, 30(5), 1511-1517.

Rattray FP, Connell MJ. 2011. Fermented Milks Kefir. In: Fukay JW, editor. 392 Encyclopedia of Dairy Sciences. 2th ed. Academic Press; San Diego, USA. 393 518-524.

RodriguesKL, Carvalho JCT, and Schneedorf JM. 2005. Anti-inflammatory properties of kefir and its polysaccharide extract. Inflammopharmacology, 13(5) 485-492.

SarilmiserHK, Ates O, Ozdemir G, Arga KY, OnerET 2014. Effective stimulating factors for microbial levan production by *Halomonas smyrnensis* AAD6 $^{T}$. Journal of Bioscience and Bioengineering, 119(4), 455-463.

Sudiman S, Hsu YH, He JL, Kong ZL. 2018. Dietary polysaccharide-rich extract from Eucheuma cottonii modulates the inflammatory response and suppresses colonic injury on dextran sulfate sodium-induced colitis in mice. PloS ONE, 13(10), 1-15.

Sun Y, Shi X, Zheng X, Nie S, Xu X. 2019. Inhibition of dextran sodium sulfate-induced colitis in mice by baker's yeast polysaccharides. Carbohydrate polymers, 207, 371-381.

SutherlandIW. 2001. Microbial polysaccharides from Gram-negative bacteria.International Dairy Journal, 11(9), 663-674.

Tan KX, Chamundeswari VN, & Loo SCJ (2020). Prospects of kefiran as a food-derived biopolymer for agri-food and biomedical applications. *RSC advances*, *10*(42), 25339-25351.

Tung YT, Chen HL, Wu HS, Ho MH, Chong KY, Chen CM. 2018. Kefir Peptides Prevent Hyperlipidemia and Obesity in High-Fat-Diet-Induced Obese Rats via Lipid Metabolism Modulation. Molecular nutrition & food research, 62(3), 1700505.

UchidaM, Ishii I, Inoue C, Akisato Y, Watanabe K, Hosoyama S, Toshihiko T, Ariyoshi N, Kitada M. 2010. Kefiran reduces atherosclerosis in rabbits fed a high cholesterol diet Journal of Atherosclerosis and Thrombosis, 17, 980-988.

VinderolaG, Perdiogon G, Duarte J, Farnworth E, Matar C. 2006. Effects of theoral administration of the exopolysaccharide produced by *Lactobacilluskefiranofaciens* on the gut mucosal immunity. Cytokine 36(5-6), 254-260.

Wang B, Tontonoz P. 2018. Liver X receptors in lipid signalling and membrane homeostasis. Nature Reviews Endocrinology, 14(8), 452-463.

Wang G, Huang W, Xia Y, Xiong Z. Ai L. 2019. Cholesterol-lowering potentials of Lactobacillus strain overexpression of bile salt hydrolase on high cholesterol diet-induced hypercholesterolemic mice. Food & function, 10(3), 1684-1695.

WangM, Bi J. 2008. Modification of characteristics of kefiran by changing the carbon source of *Lactobacillus kefiranofaciens*. Journal of the Science of Food and Agriculture, 88(5) 763-769.

Wartenberg A, Weisser J, Thein S, Suso HP, Schmidt R, Matthias S. 2017. Cryogels as potential scaffolds for wound healing applications. J. Med. Mater. Technol., 1(2), 26-29.

Wu J, Gao W, Song Z, Xiong Q, Han Y, Yuan J, Zhang R, Cheng Y, Fang J, Li W. 2018. Anticancer activity of polysaccharide from Glehnia littoralis on human lung cancer cell line A549. International Journal of Biological Macromolecules, 106, 464-472.

YokoiH, Watanabe T, Fuji Y, Toba T, Adachi S. 1990. Isolation and characterization of polysaccharide-producing bacteria from kefir grains. *Journal of Dairy Science.*73(7), 1684-1689.

Zamberi NR, Mohamad NE, Yeap SK, Ky H, Beh BK, Liew WC, Tan SW, Ho WY, Boo SY, Chua YH, Alitheen NB. 2016. 16S metagenomic microbial composition

analysis of kefir grain using MEGAN and BaseSpace. Food Biotechnology, 30(3), 219-230.

ZhangJ, Dong YC, FanLL, JiaoZH, Chen QH. 2015. Optimization of culture medium compositions for gellan gum production by a halobacterium *Sphingomonas paucimobilis*. Carbohydrate Polymers, 115, 694-700