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Kefirin Biyoaktif Bileşenlerinin Antioksidan Etkileri

Antioxidant Effects of Bioactive Components of Kefir

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Biyoloji/ Biology **Derleme / Review**

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Öz

Kefir günümüzde popülaritesi dünya çapında yaygınlaşmış, besin değeri yüksek, sağlığı destekleyen kendine has tat ve aromalı bir fermente süt ürünüdür. Bu çalışmadaki amacımız: kefirin antioksidan etkileri hakkındaki mevcut bilgilerin biyokimyasal açıdan detaylı bir araştırılması yapılarak kefirdeki hangi etken maddelerin doğrudan veya dolaylı olarak serbest radikallerin etkilerini azaltma yönünde antioksidatif etkili olduğunu araştırmak oldu. Veritabanları kullanılarak, “kefir, probiyotik, oksidatif stres, antioksidan” anahtar kelimeleri kullanılarak zaman kısıtlaması yapılmaksızın ilgili makalelere ulaşıldı. Yapılan çalışmada kefirin antioksidan özelliğini kefirde bulunan peptid, polifenol, glutatyon, laktik asit bakterileri ve B_2, B_{12} vitaminleri sayesinde sağladığı söylenebilir.

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Abstract

Kefir is a fermented milk product with a high nutritional value and a unique taste and aroma that supports health, whose popularity has spread around the world today. Our aim in this study was to investigate which active ingredients in kefir have antioxidative effects in terms of directly or indirectly reducing the effects of free radicals by conducting a detailed biochemical investigation of the existing information about the antioxidant effects of kefir. By using the database, the keywords "kefir, probiotic, oxidative stress, antioxidant" were used to access the relevant articles without time restrictions. In the study, it can be said that kefir provides its antioxidant properties thanks to the peptide, polyphenol, glutathione, lactic acid bacteria and vitamins B_2 and B_{12} found in kefir.

1. INTRODUCTION

In recent years, kefir, as a natural beverage containing probiotic microorganisms and functional organic substances, has found a great response both as a food and as a supplementary treatment product for health thanks to its bioactivities. Kefir, which can be produced by commercial methods as well as by traditional methods, is a beverage with rich probiotic content, which is formed by the symbiotic fermentation of microorganisms in kefir grains and milk in symbiotic unity Guven et al., 2003, Guven et al., 2005: Abdel-mogheith & El-gendy; 2017). Kefir increases the absorption of minerals such as calcium, magnesium, etc.

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by multiplying beneficial bacteria in the intestinal flora, preventing pathogens from settling in the intestines and regulating the functioning of the intestines. Kefir, with its high nutritional content and probiotic richness, is associated with a wide range of nutraceutical benefits, including anti-inflammatory, anti-oxidative, anti-cancer, anti-microbial, anti-diabetic, anti-hypertensive and anti-hypercholesterolemic effects (Guven et al., 2003; Guven et al., 2005; Guven et al., 2018; Setyowati and Setyani 2016; Abdel-mogheith & El-gendy, 2017; Guven et al., 2021; Albuquerque Pereira et al., 2024). Kefir has been shown to be beneficial in the treatment of similar ailments such as joint inflammation, rheumatism, other inflammatory disorders and gout by reducing pro-inflammatory cytokines and increasing anti-inflammatory mediators (Lopitz-Otsoa et al., 2006; Rosa et al., 2017). Thanks to the acetic acid and hydrogen peroxide (H2O2) it contains, kefir prevents the development of all microorganisms, including harmful disease-causing microorganisms and viruses, and makes the body more resistant (Ghoneum et al., 2020). In this context, Kefir shows significant protection against viral infections such as Hepatitis A, B, C, and COVID19 by inhibiting the activation of the cytokinesis pre-inflammation it creates in the body (Reham et al., 2021; Albuquerque Pereira et al., 2024.). Another of the effects of kefir on health is that it enriches brain functions, provides internal balance and helps fight stress. This effect has been shown to increase focus, relaxation, and the brain's recall power (Peluzio et al., 2021; Eroglu FE, Şanlıer, 2022; Weber et al., 2023). However, kefir has been observed to block intestinal lipid uptake in obese mice by reducing hepatic and serum triglycerides, total cholesterol, and LDL-c, and by reducing the expression of genes linked to adipogenesis, lipogenesis, and proinflammatory cytokines in epididymal fat (Choi et al., 2017).

The effects of hyperactivity in the treatment of colds, flu, migraines, diarrhea, constipation, rickets, anemia, tissue stiffness, depression, stomach cramps, and sleep disturbance have also been supported by studies (Lin, Chen, & Liu, 1999; Daniells, 2006; Rosa, et al., 2017). Kefir also plays an important role in protecting the body against the harmful effects of radiation and other toxic impurities while treating similar gum conditions such as peridontitis and bad breath (Ali OSM et al., 2020; Fehmi, 2015). Kefir has also been shown to play an effective role in the treatment of allergies and liver disease, in the treatment of the gallbladder and in dissolving bile salts, in clearing chemical antibiotics from the body, in the treatment of kidney stones, and in eliminating vaginal odors (Daniells, 2006; Lopitz-Otsoa et al., 2006; Rosa et al., 2017; Weber et al., 2023). It has been suggested that people in the

Caucasus have a longer lifespan (Kim et al., 2015). All these health-promoting properties are linked to kefir microorganisms, their interactions and metabolic products during the fermentation process.

2. KEFIR

2.1. Kefir Grains and the Structure of Kefir

Kefir grains; It is called kefir in polysaccharide composition, which is formed by the activities of microorganisms, resembling small cauliflower flowers with irregular lobes, ranging in length from 1 to 6 cm, ranging in color from white to yellow, and is called kefiran (Güven et al., 2003).

Figure 1. Kefir grain and kefir drink

Microorganisms in the composition of kefir grain increase the nutritional value of kefir by breaking down some of the lactose and milk proteins in milk. Thus, kefir can be better absorbed by the body, further increasing its nutritional importance. Within this structure, lactic acid bacteria (LAB), yeasts and acetic acid bacteria (AAB) coexist in symbiotic connection, showing a gelatous and slimy natural matrix structure consisting of exopolysaccharides (EPS), kefiran and proteins (Bessa MK, Bessa 2023; Leite et al., 2015). In kefir grains, *Lactobacillus kefiranofaciens, Lacticaseibacillus paracasei (Lactobacillus paracasei), Lactiplantibacillus plantarum (Lactobacillus plantarum, Lactobacillus acidophilus and Lactobacillus delbrueckii subsp), Bulgaricus. as well as Saccharomyces cerevisiae, S.unisporus, Candidakefyr* and

Kluyveromycesmarxianus ssp. Marxianus yeasts are also found (Elias et al., 2019; Bourrie et al., 2016; Guven et al., 2003; Anonymous, 2009). As a result of the typical microbiological analysis of kefir, which was traditionally performed in Kars, the total number of mesophyll aerobic colonies, lactic acid bacteria, lactic streptococci, enterococci, total coliform and mold averages were found to be $1.04x10^9$ CFU/ml, $9.87x10^8$ CFU/ml, $4.38\ x10^8$ CFU/ml, $7.80x10^4$ CFU/ml, 0 CFU/ml, $1.26x10^5$ CFU/ml, while the chemical component was 86-89% water, 11-14% dry matter, 2.8-3.3% fat, .7-2.9% lactose and 0.7-0.9% mineral substance (Güven et al., 2003). This fermentation process has been supported by studies in which metabolic products such as acetic acids, carbon dioxide, acetaldehyde, acetoin and other volatile compounds, minerals, essential amino acids, vitamins, folic acid, bacteriocins, bioactive peptides and some nutraceutical components are also produced (Blasche et al., 2021; Dallas et al., 2016).

Studies have concluded that the composition and sensory differences in milk, the use of different milk in the kefir fermentation process may affect the population development of kefir microflora and kefir quality (Yaman et al. 2010; Gürel, et al., 2021).

2.2. Antioxidant Effect of Kefir

Free radicals and other reactive oxygen species play an important role in the formation of various diseases related to oxidative effects in humans. Unstable molecules or free radicals produced by the body under stress and other environmental pressures cause tissue, organ and cell damage. Enzymes found endogenously in the body and exogenously taken antioxidative substances act as free radical scavengers and reduce or destroy this damage of free radicals. In this context, one of the most important nutrients known for its antioxidative properties is kefir. The potent antioxidant potential of kefir has been proven in both in vitro and in vivo models (Ghoneum et al., 2020; Yılmaz-Ersan et al., 2023;). A study investigating the protective effects of kefir against oxidative stress in mice demonstrated significant antioxidant benefits. Kefir consumption notably increased the activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Additionally, it reduced oxidative stress biomarkers such as nitric oxide (NO) and malondialdehyde (MDA).

The study also found that kefir inhibited liver enzyme levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting a protective effect on liver function.

Furthermore, it enhanced total antioxidant capacity (TAC), glutathione (GSH) levels, and anti-hydroxyl radical content.

Moreover, kefir positively influenced lipid profiles by increasing high-density lipoprotein (HDL) levels while significantly reducing total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels (Ghoneum, 2020).

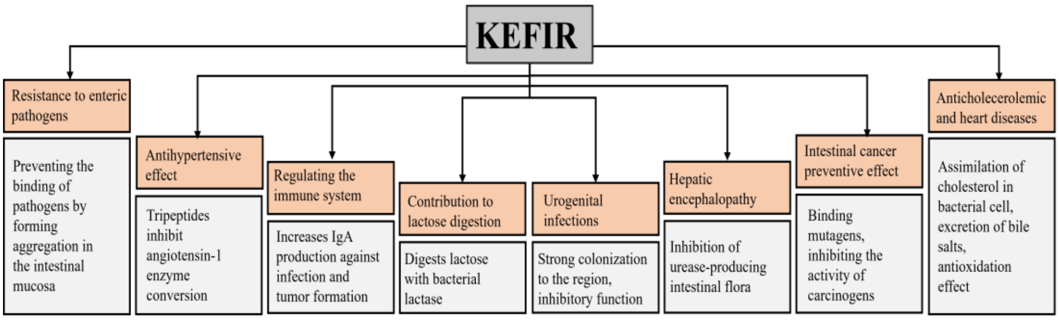

Figure 2. Probiotic effects of kefir on metabolism and health

2.3. Bacterial Antioxidative Effect

There are dozens of studies showing that kefir neutralizes free radicals and reduces their harmful effects on body cells and tissues. It has been determined that Lactobacillus plantarum, one of the bacteria found in kefir, regulates various enzymes involved in free radical synthesis. The presence of this bacterium in kefir contributes to its consistent antioxidative effect (Tang et al., 2017). Lactobacilli, in this context, have been associated with protection from pathogenic bacteria, modulation of the immune system against potentially reduced risk of allergies and cancer, reduction of free oxygen radicals (Ghoneum et al., 2014). It is stated that the ability to inhibit proliferation and induce apoptosis in cancer cells may be related to the modulation of the intestinal microbiota, reduction of tumor growth and DNA damage, as well as antioxidative processes and inhibition. Furthermore, strong evidence suggests that Lactobacillus plantarum and Lactobacillus casei, bacteria found in kefir, play a role in providing these effects (Yamane et al., 2018). In an animal model mimicking human depression, where mice were exposed to seven stressors for six weeks, a study investigating the effects of kefir-derived Lactobacillus kefiranofaciens ZW3 supplementation found several beneficial outcomes. Kefir supplementation improved tryptophan metabolism, increased anti-inflammatory cytokines, and decreased pro-inflammatory cytokines. Additionally, it significantly altered gut microbiota composition, leading to increased levels of *Actinobacteria,* *Bacteroides, Lachnospiraceae, Coriobacteriaceae, Bifidobacteriaceae,* and *Akkermansia*, while reducing Proteobacteria (Sun et al., 2019). An in vivo study using aged mice reveals that probiotic fermentation technology (PFT) containing specific microbes such as *Lactobacillus kefir P-IF, L. kefir P-B1, Kazachstania turicensis, Kazachstania unispora, and Kluyveromyces marxianus* reduces age-related oxidative stress. A six-week oral daily dosage of 2 mg/kg body weight SFT suppresses oxygen radical formation, increases GSH and total antioxidant capacity, and reduces NO and MDA levels, restoring age-related oxidative changes to levels seen in untreated youth (Ghoneum et al., 2020).

2.4. Nutritional Antioxidant Effect

Kefiran, a microbial polysaccharide derived from kefir grains, aids in the mental recovery of individuals with severe traumatic brain injury and prolongs the healthy life of the elderly (Bifari et al., 2017; Salari et al., 2022). Although its biological profile has been little studied, many biologically active peptides are produced through symbiotic metabolic interactions between different bacterial and yeast species in kefir, including ACE inhibitor peptides that block angiotensin-converting enzyme (ACE) and prevent angiotensin conversion (Ebner et al., 2015). Exopolysaccharide isolated from kefir grains show high antioxidant activity in vitro and concentration-dependent protection of protein from oxidative damage. This protection is also thought to be due to the action of exopolysaccharides found in kefir grains (Farnworth, 2005). The use of kefir peptides in various in vivo studies has also demonstrated its potent immunostimulating effects (Chen et al. 2019). Similarly, (Radhouani et al. 2018) showed that kefiran extract had the highest superoxide radical scavenging activities in the study evaluating the antioxidant properties of kefiran biopolymer in vitro, while kefiran extract showed great potential for nitric oxide radical scavenging. In their study, in which they investigated the protective abilities of kefir peptides against oxidative stress, inflammation, and their protective ability against renal dysfunction, they revealed a decrease in renal infiltration of inflammatory cells; reactive oxygen species (ROS) levels; histopathological lesions; and vascular cell adhesion molecule-1 (VCAM-1), monocyte chemoattractant protein-1 (MCP-1), endothelin-1 (ET-1), and cytokine nucleotide-binding oligomerization domain (NOD)-like receptor They stated that it provides strong protection in all organs by causing a significant increase in glomerular filtration rate and renal superoxide dismutase activity (Chen et al., 2018; Kesenkaş, 2011). On the other hand, he discovered that adding kefir to apple juice increased the total phenolic content and antioxidant activities, which were analyzed using diphenyl-2-picrylhydrazyl radical scavenging, reducing power, metal chelating effect, inhibition of linoleic acid autoxidation, and inhibition of ascorbate autoxidation assays (Sabokbar et al., 2015). A study reported that kefir fermented in peanut milk had a greater antioxidant effect than peanut milk alone. Similarly, kefir fermented with a mixture of cow's and soy milk showed improved antioxidant activity compared to kefir fermented with cow's milk alone, and the study found that the level of phenolic compounds increased after fermentation. The active peptides of kefir induce ROS-mediated apoptosis and stimulate CaMg dependent endonucleases for DNA cleavage (Pepe et al., 2013).

Kefir peptide therapy is also among the studies that significantly improve the development of atherosclerotic lesions by reducing oxidative stress, macrophage accumulation, endothelial dysfunction, aortic lipid accumulation and inflammatory immune response (Farag et al., 2020). In addition, it has been reported that fermented foods using *Lactobacillus paracasei* can positively affect gluten sensitivity by reducing the flow of gluten-related peptides. For individuals who cannot consume milk due to lactose intolerance, fermented milk products can be used as a tolerable alternative dairy product (Marco et al., 2016).

2.5. Antioxidant Effect According to the Characteristics of Milk

The antioxidant activity of kefir also varies depending on the characteristics of the milk used in kefir. It is known that in addition to cow's milk, sheep, goat, camel milk and even vegetable sources such as soy, rice and coconut milk are mostly used in kefir production. While the antioxidant capacity of kefir made from goat's milk is much higher than that made from cow's milk, the antioxidant capacity of kefir made with kefir grain in kefir production is higher than that made with kefir starter culture (Yilmaz-Ersan et al., 2018). A study conducted in Thailand investigated the antioxidant activity and bacterial inhibition of jasmine rice milk-kefir and cow's milk-kefir between 24 and 48 hours, and it was found that rice milk-kefir showed higher bioactivity than cow's milk-kefir. This is expressed by the high potential of rice milk-kefir as agents for bacterial inhibition and mitigating oxidative damage (Sirirat and Jelena., 2010). In the study conducted to examine phenolic antioxidant mobilization during the production of yogurt from soy milk, it was shown that the soluble phenolic content of kefir in soy milk increased by using active probiotic cultures of kefir, which increased its antioxidant activity (McCue et al., 2005). In another study, the antioxidant power of water kefir was examined, and it was stated that water kefir scavenged DPPH free radical from 9,88% to 63,17% and inhibited ascorbate oxidation by 6,08-25,57%, indicating the potential of kefir as an antioxidant agent (Alsayadi et al., 2014). Likewise, the properties of kefir fermented with a combination of black rice extract and goat milk and its effects on streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats were investigated, and it was revealed that the antioxidant activity of kefir prepared from the combination of black rice extract and goat milk was higher compared to goat milk kefir alone (Nurliyani et al., 2015.)

The study, which examined the antioxidant and antimutagenic properties of fermented kefir in milk and soy milk, showed that milk-kefir and soy milk-kefir led to significantly increased antimutagenic activity compared to milk and soy milk controls. Milk-kefir and soy milk-kefir also showed a greater DPPH scavenging activity, decreased glutathione peroxidase activity, and an inhibition effect on linoleic acid peroxidation. The study also showed that the fermentation of kefir grains in milk and soy milk did not alter the superoxide dismutase activity and iron ion chelating ability of the original materials (Liu et al. 1999; Gamba et al., 2020). In the study investigating the antioxidant activity of kefir fermented products in Bristle and Saanen goat milk, it was found that kefir grains fermented in goat milk showed higher total antioxidant activity and microbiota content (Satir and Güzel-Seydim, 2015). Also phenolic contents in goat milk-kefir samples ranged from 726.08 to 1359.32 mg gallic acid equivalent (GAE) L-1, while phenolic compounds in goat milk-kefir samples ranged from 0.36 to 5.09 mg 100 g -1 for catechin and 0.77 to 4.21 mg 100 g -1 for gallic acid, indicating that the bioactive substances in goat milk-kefir samples were significantly higher. In a study investigating the radioprotective effects of kefir and ascorbic acid against radiation-induced DNA damage and genotoxicity in the blood lymphocytes of mice, it was found that kefir and ascorbic acid when applied together reduced DNA damage in lymphocyte blood cells, resulted in high antioxidant activities in both DPPH radical scavenging and ferric-reducing antioxidant power analyses, and protected animal lymphocyte blood cells from radiation-induced DNA damage and genotoxicity. Koohian et al (2020) have been shown to prove antioxidant potential.

3. Discussion

With the awareness of the society, the demand for healthy and natural foods is increasing. In this context, kefir mediates antioxidative and protective effects through the intestinal microbiota and numerous molecular biomarkers and organic acids it produces and secretes in its microbiota, as well as its health-promoting effects attributed to improve overall health.

Conflict of Interest

There is no conflict of interest with any institution or person in the study.

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