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REVIEW ARTICLE

Analyzing the Effects of Kefir on the Gut Microbiota Strains in Alzheimer's Patients

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A B S T R A C T

Alzheimer's disease (AD) is a neurological disorder that deteriorates over time, gradually depriving sufferers of their memory, cognitive capacities, and, finally, the capacity to do even the most basic tasks, as well as affecting the gut microbiome. According to research, alterations in gut microbial diversity and gut-brain axis anomalies are linked to Alzheimer's disease. Probiotics, which have multiple health advantages, are well-acknowledged as one of the most effective preventative therapies against cognitive decline in AD. The gut microbiota need resources to develop, including Lactobacilli and Bifidobacteria, both of which are usually associated with kefir's medicinal potential. It has been shown to influence the immune system and preserve gut health by altering the gut microbiota as it decreases inflammation and oxidative stress, alleviating the course of Alzheimer's disease. Thus, this research discusses the potential advantages of probiotics, particularly kefir, on gut microbiota strains in Alzheimer's patients. A literature search was undertaken on scientific sites such as Google Scholar and PubMed. Given the inclusion criteria and the constraint on the publication year, 53 scientific papers were chosen for further examination. Keyword searches were restricted to the following. (1) Alzheimer's illness; (2) gut microbiota; (3) probiotics; and (4) kefir. The findings of this study clarify the association between probiotics and AD in altering the gut microbiota by improving the proliferation of *Lactobacillus* and *Bifidobacterium* and subsequently slowing the progression of AD.

K E Y W O R D S

Alzheimer's disease (AD), Gut microbiota, Probiotics, Nutrients, Bioactive compounds, Kefir

H I G H L I G H T S

- Alzheimer's disease (AD) is a progressive neurological disorder with few treatment options, necessitating the search for new therapeutic techniques that target the gut-brain axis.
- Kefir, a fermented dairy beverage high in probiotics, has the potential to benefit the gut microbiota, which has been linked to Alzheimer's disease development.
- Kefir's bioactive ingredients and nutritional profile are being assessed for their potential to raise digestive health, inflammatory processes, and immune function—all of which may contribute to attenuating AD course.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurological condition that affects people over 60 years old and accounts for 80% of dementia cases worldwide (Naomi et al., 2022). According to the Global Burden of Disease study, approximately 43.8 million people worldwide were diagnosed with Alzheimer's disease in 2016. In Indonesia alone, statistics projected that there were 1.2 million people with dementia in 2016, which is expected to increase to 2 million in 2030 and 4 million in 2050 (Brasti et al., 2021). Alzheimer's disease is characterized by losses in memory, cognition, and motor function caused by the accumulation of amyloid-beta (Aβ) plaques outside neurons. As a result, vascular degeneration, neuroinflammation, and abnormalities in calcium homeostasis ensue, all contributing to neuronal death (Daulatzai, 2015). In severe stages, considerable loss of brain function might result in dehydration, infection, malnutrition, or deadly consequences (Mayo Clinic, 2023).

To date, there is no treatment to cure AD. Although some drugs can prevent cognitive and memory loss, long-term solutions remain elusive. Recent research has focused on the human microbiome's function in regulating several neurochemical pathways via the gut-brain axis and the quest for novel treatment strategies for microbiota modulation. Numerous dietary strategies have shown promise for improving particular cognitive abilities or for preventing or slowing neurodegeneration. Specifically, research suggests that the beneficial bacteria found in kefir could support brain health and protect against neurodegenerative conditions by stimulating neurotransmitters and their precursors (Romanenko et al., 2021; Wiatrak et al., 2022). Probiotics, which are live microorganisms that have health benefits when consumed adequately, may emerge as a novel approach to help prevent and slow the progression of neurodegenerative diseases such as AD by regulating the body's pH levels, maintaining the health of the gut lining, modulating the immune system, and promoting brain-derived neurotrophic factors, all of which are crucial for proper neurodevelopment (Naomi et al., 2022).

Kefir, an example of a probiotic, appears to be related to three main aspects of Alzheimer's disease—systemic inflammation, blood cell damage, and oxidative stress and may be a viable adjuvant therapy against the disease's progression (Ton et al., 2020). Kefir has a high concentration of bioactive chemicals, macromolecules, and nutrients. The compounds include purified exopolysaccharides, peptides, and organic acids comprising acetic acid, hippuric acid, propionic acid, and pyruvic acid, which all showed a prominent effect on the amyloid protein-induced plaques that develop in AD (Gangalla et al., 2021). If these elements are eliminated from the brain, memory loss and a severe decline in cognitive ability may result.

Though accumulating evidence from previous clinical trials supports the potential role of probiotics in alleviating neurodegenerative disease, results were still inconsistent, and probiotics are not yet recommended as a standalone treatment for Alzheimer's disease (Xiang et al., 2022). More extensive and robust studies are required to establish probiotics' effectiveness and appropriate use. Hence, this review paper aims to unveil the possible beneficial effects of kefir as a probiotic-containing drink on the gut microbiome strains in Alzheimer's patients based on current evidence.

MATERIAL AND METHODS

The proximate effects of probiotics on health underlie broad elements, starting with the digestive system, immunity, and cognitive function. As it has been stated as an alternative to reduce the risk of certain diseases, the literature review method was narrowed down to digestive and cognitive mechanisms. These identifying determinant words, such as diseases, risk factors, Alzheimer, and gut microbiota, are explained in several subchapters. The paper discussion started by reviewing general information about AD and its related factors. It continues by explaining the gut microbiota in the human body and comparing the

differences between the functions of healthy individuals and Alzheimer's patients. To give nourishment to the gut microbiota it requires the function of probiotics, nutrients, and bioactive compounds. Probiotics as an alternative were reviewed for their activity in altering gut microbiota, along with the roles of nutrients and bioactive compounds in this process. Eventually, the paper examined how kefir serves as a probiotic and alters the gut microbiota strains in Alzheimer patients.

To identify the problem, keyword searches were conducted on scientific platforms using Google Scholar and PubMed. Keywords limited to the literature search were determined, namely “Alzheimer's disease,” “gut microbiota,” “probiotic,” “nutrient,” "bioactive compound,” and “kefir.” During the research, some words were combined to make the topic more specific, for instance, combining “gut microbiota” and “Alzheimer's disease.” This paper used materials and contextual elements based on worldwide data not specified in certain regions. The newest and most trusted publications of papers and articles might be utilized as additional references to support the data. These papers investigated the samples from various ranges of ages and were not limited to certain populations, using references published in the last eleven years (2012–2023).

ALZHEIMER’S DISEASE (AD)

In the 21st century, Alzheimer’s Disease (AD) has become one of the challenges faced in the medical field as it is costly and mortal and is the most prevalent etiology of dementia characterized by a progressive decline in memory, behaviour, thinking, and social skills; “early indications of Alzheimer's disease development include changes in cognition or unconscious conduct, memory difficulty for new knowledge, and dysfunctional changes in language and speech.” (Scheltens et al., 2021; Mayo Clinic, 2023; Guo et al., 2020). These alterations have an impact on a person's ability to operate on a daily basis.

AD may be differentiated into three main phases of disease progression: early (mild), mid (moderate), and late (severe). In the early stages of AD, one can still perform daily activities independently, though memory lapses may occur. As the disease progresses, dementia symptoms become more apparent, and one may start requiring assistance in performing routine tasks due to further damage to the nerve cells in the brain (National Institute on Aging, 2021a). This is known as middle-stage AD. A person may become fully bedridden or chair-bound in later stages, requiring full-time care and assistance with daily living and personal care. Severe dementia, inability to swallow, incontinence, depression, and apathy are particularly common in this stage (National Institute on Aging, 2021b; Alzheimer’s Society, 2021). Though the rate of disease progression varies, an early diagnosis is crucial for prognosis and advanced-care planning to increase the quality of life (Galvin et al., 2021).

Etiology

AD is distinguished by two detrimental brain abnormalities: neurofibrillary tangles and amyloid plaque. Amyloid plaques are the slow buildup of protein fragments between neurons produced by AD, which disrupts the brain's natural disposal mechanism and ultimately impairs cognitive function. Neurofibrillary tangles are formed when twisted filament bundles known as tau proteins accumulate within healthy neurons. This kind of protein binds to the internal structures of neurons in a healthy brain, aiding in the distribution of nutrients and chemicals throughout the cell (DeTure & Dickson, 2019). However, in AD, there are mutations of presenilin 2 (PSEN2), amyloid precursor protein (APP), or presenilin 1 (PSEN1) genes, which stimulate the misfolding of tau and Aβ proteins in neurofibrillary tangles and amyloid plaques, respectively (Scheltens et al., 2016).

A study mentioned by Tokuraku and Ikezu (2014) stated that the Aβ protein is responsible for the pathogenesis of AD. The failure of this protein clearance in the brain is also enhanced by the expression of the APOE4 gene, increasing the risk factor for AD, which is correlated with the neurodegenerative state in

the brain of the patients (Scheltens et al., 2016). Additionally, the tau protein abnormal folding can also be a biomarker of AD as it indicates neurodegeneration in patients (Scheltens et al., 2016). Furthermore, according to Jiang et al. (2017), the alteration of gut microbiota due to ageing can be one of the causative agents in AD neuroinflammation development and vice versa due to the bidirectional relationship where AD progression modifies the gut microbiota composition and amounts. Changes in the gut microbiota have an impact on gut microbiome-derived metabolites and peripheral immunity by influencing the expression of peripheral immune cell genes and cytokine release. Modifications in peripheral immunity, potentially gut-derived metabolites, and vagus nerve-trafficked gut-derived hormones might then modify the phenotype of the blood-brain barrier (BBB) and central nervous system cell types (microglia, astrocytes, neurons), modulating amyloidosis, neurodegeneration, and tauopathy, and contributing to disease pathogenesis (Chandra et al., 2023).

Risk factors

Risk factors associated with AD include age, family history and genetics, traumatic brain injury, and trisomy 21 (down syndrome). Alzheimer's is not caused by ageing, yet it is the most important established risk factor for the condition. Beyond the age of 65, the number of people with AD roughly doubles every 5 years (National Institute on Aging, 2019). APP and PSEN1/2 gene mutations are the most closely related causal gene mutations for rare forms of early-onset familial AD (Armstrong, 2019). Additionally, having a first-degree relative with AD raises the overall chances of having the condition by 10% to 30% (Kumar et al., 2022). Other risk factors that may increase the risk for AD include excessive alcohol consumption, lifestyle and heart health, mental disorders, and other comorbidities such as heart disease, diabetes, stroke, high blood pressure, and high cholesterol (National Health Services, 2021; National Institute of Aging, 2022). This is because alcohol consumption and cardiovascular disease may affect the brain and neuropsychological functioning, and the absence of these factors may inhibit inflammation and oxidative stress in the brain (Dhana et al., 2020). However, the specific pathways are still limited.

Pathophysiology

There are some hypotheses about the pathophysiology of Alzheimer’s disease, including hyperphosphorylated tau protein and Aβ, oxidative stress or mitochondrial dysfunctions, metal ion dyshomeostasis, and cholinergic dysfunctions. According to the hyperphosphorylated tau protein and Aβ hypothesis, there is a formation of senile plaques (SP), which are spherical microscopic lesions with an extracellular Aβ-peptide as the core. This happens because of the deposition of Aβ, a small soluble peptide produced by the APP by the action of proteases such as α-, β-, and γ-secretase. This will cause an imbalance of Aβ between production and clearance, leading to some types of toxic oligomers, including protofibrils, fibrils, and plaques, during the extent of oligomerization (Thakur et al., 2018). It may also cause the formation of tau aggregates, forming twisted paired helical filaments, namely neurofibrillary tangles, leading to AD (Kumar et al., 2022).

The second hypothesis is oxidative stress; reactive nitrogen species (RNS) and reactive oxygen species (ROS) can damage the cellular structures more in the brain since the brain consumes oxygen in higher amounts compared to other tissues. The neuron's polyunsaturated fatty acids can then react with the ROS, causing a lipid peroxidation reaction and molecular apoptosis. Moreover, the decreased number of glutathione in the neurons may also lead to oxidative stress injury. These can then cause damage to the brain, leading to AD. Another hypothesis is metal ion dyshomeostasis, which may also cause neurodegenerative diseases like AD. The imbalanced levels of redox transition metals, such as copper (Cu), manganese (Mg), iron (Fe), aluminium (Al), and zinc (Zn), may lead to AD, where their levels in the brain are higher in AD patients. Lastly, the cholinergic hypothesis proves that the binding of the cholinergic receptors is diminished in specific brain regions of early to moderate AD. It is then related to the slower processing

speed of the patient as well as the neuropsychiatric symptoms, such as apathy, depression, and sleep disorders (Thakur et al., 2018).

GUT MICROBIOTA

The term “microbiota” refers to the entire population of microorganisms that colonize a certain location, including fungi, viruses, archaea, and protozoans (Jandhyala et al., 2015). Gut microbes collaborate with hosts via the "gut-brain axis" to modulate the immune system's development and function. The gut-brain axis connects the intestinal and central neural systems, forming a bidirectional communication network. The network comprises not only anatomical communication pathways but also endocrine, humoral, metabolic, and immunological communication channels. The hypothalamic-pituitary-adrenal (HPA) axis, nerves, and the autonomic nervous system inside the GI tract all connect the gut and the brain, allowing the brain to change intestinal functions (Carabotti et al., 2015).

As a result, this process may have an impact on human health, which is related to the type of bacteria in the gut, and damage to microbial communities can result in a number of neurological diseases (Morais et al., 2021). To examine the gut microbiota, feces samples must be collected and separated from participants. The great majority of gastrointestinal microbes, however, are difficult to separate, identify, and count using traditional culture-based methods. Because of the availability of high throughput gene sequencing technology, two major steps—16S rRNA-based gene sequencing and bioinformatics analysis—can be done rapidly and with strong findings (Jandhyala et al., 2015). With the assistance of the Human Microbiome Project and MetaHit data, researchers have acquired the most detailed picture of human-associated microbial diversity. The data recorded 2172 human-isolated species, which were grouped into 12 separate phyla; 93.5% of these species belong to *Proteobacteria, Bacteroidetes, Actinobacteria,* and *Firmicutes* (Thursby & Juge, 2017).

Gut microbiota and its function in healthy individuals

Studies discovered that Alzheimer’s patients might have different viable strains of microorganisms in their digestive system. King et al. in 2019 recognized that healthy humans would have gut microbiomes from 109 species, 59 genera, 38 orders, 23 classes, 18 families, and 8 phyla. Furthermore, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* are known to be the predominant inhibitors of healthy individuals. From the *Clostridia* class, *Firmicutes* were deemed to be the most profuse, subsequently followed by *Bacteroidia*, *Bifidobacteriales*, *Enterobacterales*, and lastly *Lactobacillales*. Apart from the aforementioned microorganisms, *Akkermansia miciniphilai* from *Verrucomicrobia* was also observed in the digestive tract of those with healthy conditions.

Based on the study conducted by Siddiqui et al. in 2013, these microbes are advantageous due to their ability to digest complex carbohydrates (e.g., dietary fiber), which the human itself cannot digest through enzyme production. Hence, the dietary fibers and other complex substances could be uptaken by the body. Another crucial role played by the beneficial bacteria is the fermentation of fibers and other prebiotics to form short-chain fatty acids (SCFA), namely acetate, propionate, and butyrate, crucial for appetite regulation; and inducing leptin and ghrelin (appetite regulators) to maintain energy balance through hunger and satiety (David et al., 2014). Additionally, SCFA regulates immune function and glucose metabolism while providing energy for colonocytes, supporting intestinal barrier integrity. With the existence of these microbiotas, the immune system could be regulated by T-cell formation, which can also lower the risk of inflammation. Pathogenic bacteria count is also suppressed as beneficial bacteria can create antimicrobial compounds or be competitors for nutrients (Hsu & Nanan, 2015). There are, however, several distinct functions from certain bacteria which are not found in other strains. *Actinobacteria* and *Bacteroidia* are instances of microorganisms able to produce vitamin K and vitamin B12, which are integral parts of ensuring ultimate, long-term overall health. Lactic acid, which curtails pH, is synthesized by

Bifidobacteriales and *Lactobacillales*, inhibiting the growth of pathogenic bacteria. The gut mucin formation is also enhanced in terms of production by *Akkermansia muciniphila* to protect the intestinal walls (Kamada et al., 2013).

Gut microbiota alteration in Alzheimer's patients

Numerous literature studies have found that the balance of gut microbiota present in Alzheimer's patients is distinct from that of healthy individuals. Beneficial bacteria such as *Bifidobacterium* for the immune system, *Faecalibacterium* for SCFA synthesis, *Lactobacillus* to induce neuroprotective characteristics, and *Akkermansia muciniphila* to maintain gut barrier integrity were found to be reduced. Conversely, *Escherichia coli* from the *Proteobacteria,* which is a potentially pathogenic bacteria that is capable of stimulating adverse effects, was elevated, thus increasing the risk for inflammation due to its proinflammatory characteristics (Cattaneo et al., 2017).

Varesi et al. (2002) emphasized a number of possible mechanisms for the alterations in gut microbiota in AD patients, although the specific pathway is yet to be determined. As the gut-brain axis is bidirectional, the gut microbiota may promote or halt the progression of AD. A proposed factor is ageing, which is the life process where most people are diagnosed with AD. In the ageing process, the gut microbiota composition may be modified and imbalanced, known as dysbiosis (Belizário et al., 2015). This, in turn, supports the proliferation of pro-inflammatory bacteria, namely *Eubacterium* and *Faecalibacterium,* while impeding the development of anti-inflammatory bacteria. Consequently, local systemic inflammation is stimulated, causing the gastrointestinal tract permeability to escalate and disrupting the BBB, endorsing neuroinflammation and AD progression (Clemente et al., 2012). As stated by Bhattacharjee and Lukiw (2013), in AD patients, tau tangles and Aβ plaques from the brain are found in the gut, caused by broadening through the vagus nerves and leading to inflammation, which modifies the gut microbiota composition, specifically the lessening of *Bifidobacteria*, *Akkermansia municiphila*, and *Lactobacillus* although there has not been a clear consensus on the mechanism.

Besides the intrinsic factors, such as physiological processes, extrinsic factors, such as diet and medication, also bring about gut microbiome alterations (Mohajeri et al., 2018). Diets with excessive amounts of fat and sugar could possibly promote the growth of pro-inflammatory and oxidative stress microbiomes, which play a role in AD progression. Conversely, those with AD could have altered gut microbiomes due to appetite decline and challenges in eating, affecting the types of food consumed. As a result, gut microbiota composition would not be similar to those with balanced and varied diets since some bacteria could either be reduced or survive based on the provided nutrients (Caracciolo et al., 2014). Medications administered by AD patients could subsequently modify the gut microbiota. Several commonly prescribed medications include aducanumab, donepezil, galantamine, and rivastigmine (National Health Services, 2020; National Institute on Aging, 2021c). Even though the exact mechanism of these medications has not been established, rat studies and literature have alluded to the fact that gut microbiota, which is attributed to anti-inflammatory properties, increased while inflammation-inducing bacteria decreased.

BENEFICIAL CONSTITUENTS FOR GUT MICROBIOTA

Probiotics

Probiotics are described as living organisms that, when consumed in sufficient amounts, provide health benefits, such as regulating body pH levels, supporting the development of brain-derived neurotrophic factors, protecting intestinal lining integrity, and serving as antibiotics (Naomi et al., 2022). Since then, probiotics, mostly *Bifidobacterium* and *Lactobacillus* species, have been widely marketed and consumed, primarily as functional foods or nutritional supplements. Neuroinflammation, a critical factor in the development of AD and amyloid buildup, is suppressed by *Bifidobacterium* and lactic acid bacteria.

Three strains of *Lactobacillus (L. fermentum, L. casei,* and *L. acidophilus)* and *Bifidobacterium bifidum* have been demonstrated to enhance cognitive abilities in AD patients (D'Argenio & Sarnataro., 2021; John et al., 2021). Probiotics positively impact the human gut microbiota, which regulates the structure and composition of the intestinal flora and greatly improves the immune system. Its mode of action includes intestinal microbial community manipulation, pathogen eradication, stimulating epithelial cell differentiation and proliferation, immunomodulation, and reinforcing the intestinal barrier (Hemarajata & Versalovic, 2013; Wang et al., 2021).

Probiotics generate antimicrobial substances or metabolic compounds that inhibit other microorganisms' growth or compete with them for receptors and binding sites in the intestinal mucosa (Hemarajata & Versalovic, 2013). Some probiotic bacteria, notably *Bifidobacterium bifidum* and *Lactobacillus acidophilus*, create antimicrobial compounds, such as bacteriocins and organic acids. These chemicals can suppress the growth of pathogenic bacteria in the stomach, including *Escherichia coli* and *Clostridium difficile*, promoting a healthy microbiome balance (Rabetafika et al., 2023). Lactic acid bacteria generate antimicrobial substances, including hydrogen peroxide and diacetyl, in which those inhibitory substances generate hostile conditions for foodborne pathogens. In addition, *Bacillus clausii* produces bacteriocins to inhibit pathogen growth (Nagpal., 2012). Mechanisms-wise, probiotics can directly impact the host independently on the gut microbiota, such as by immunological regulation or the synthesis of bioactive substances (Valdes, 2023). For example, several probiotic strains have been demonstrated to influence the synthesis of bioactive compounds in the gut, such as short-chain fatty acids (SCFAs). These SCFAs help to maintain gut health, regulate inflammation, and promote general well-being. Probiotics can boost the formation of SCFAs in the colon by fermenting dietary fibers, which have various health benefits for the host (Zhou et al., 2024). Additionally, other than SCFA, bioactive compounds, including bacteriocins, amino acids, vitamins, enzymes, and oligosaccharides are also produced by *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus casei,* and *Saccharomyces boulardii* (Indira et al., 2019). To further elaborate, by stimulating the host to create IgA and β-defensin, probiotics in the gastrointestinal tract of humans can modify gut microbial communities and inhibit the growth of infections. Probiotics strengthen the intestinal barrier by promoting tight junctions and mucin production. Bacteria use food substrates to create secreted soluble components and metabolites, including SCFAs and vitamins. These bioactive molecules alter the function of the intestinal epithelium and mucosal immune cells, prompting them to produce cytokines and other associated substances consisting of B-cell activation factors and proliferation-inducing ligands. Furthermore, the release of cytokines via probiotic-mediated immunological regulation influences the proliferation and development of immune cells (T cells) and epithelial cells (Hemarajata & Versalovic, 2013).

Nutrients and bioactive compounds

According to the National Institutes of Health (2022), in order to sustain life, all microorganisms, including humans and gut microbiota, require a substance called nutrients, which are usually acquired through diet but could also be supplemented in supplement form. Nutrients are generally classified into two major categories: macronutrients and micronutrients. Macronutrients encompass carbohydrates, proteins, and fats, all of which contribute to energy production. Meanwhile, micronutrients include vitamins and minerals with their own further groupings. Unlike macronutrients, micronutrients do not contain any energy, although they are equally paramount for health. Similarly, the gut microbiota requires nutrients to thrive and proliferate. Nutrients serve as substrates and energy sources to support gut bacteria activity, especially *Lactobacilli* and *Bifidobacteria* (Sonnenburg & Sonnenburg, 2014).

Complex carbohydrates such as human-indigestible fiber are broken down into short-chain fatty acids (SCFA), which serve as a source of energy for the gut microbiota while preserving the epithelium and modulating the immune system. Those carbohydrates, especially dietary fiber, are the potential of

prebiotics. However, prebiotics can also be dietary fiber, although dietary fiber is not necessarily prebiotic. The dietary fibers that are classified as prebiotics are oligosaccharides, such as fructooligosaccharides (FOS), transgalactooligosaccharides (TOS), galactooligosaccharides (GOS), isomaltooligosaccharides (IMO), and xylooligosaccharides (XOS). Additionally, polysaccharides, including inulin, hemicellulose, cellulose, pectin, or reflux starch, are also considered prebiotics. Prebiotics are not digested or only partially digested in the upper parts of the digestive tract. Thus, they are preferentially fermented in the colon by potentially advantageous bacteria. Prebiotic substances specifically stimulate the intestinal ecosystem of the host. It stimulates the fermentation activity of the microbiota in the intestinal tract, which supports the development of anaerobic bacteria (*Bacteroides*) to generate SCFA, subsequently lowering the gut pH to be more acidic and inhibiting pathogenic bacteria. During the fermentation process of fiber by the bacteria, oxygen is uptaken, making the environment anaerobic (David et al., 2014; Markowiak & Slizewska, 2017).

The components, particularly functional foods that offer advantageous health effects, are known as bioactive compounds. It has the ability to treat, control, and prevent chronic persistent diseases, including Alzheimer's and cancer (Martirosyan & Miller, 2018). Organic acids and bioactive peptides are the bioactive substances frequently associated with kefir's therapeutic potential. Microorganisms create these beneficial compounds in kefir during fermentation and preservation (Vieira et al., 2021). They change the makeup of the gut's microbiota by either having specific prebiotic effects or acting as an antimicrobial on pathogenic bacteria (Sharma et al., 2022)

KEFIR

Kefir is a beverage that originally comes from the Caucasus Mountains of Russia and contains probiotics as a result of introducing kefir grains to milk or water, producing fermented beverages with many health benefits such as anti-inflammation, antioxidant, anti-cancer, antimicrobial, and other effects (John & Deeseenthum, 2015; Azizi et al., 2021). Kefir is often made up of a natural matrix of exopolysaccharides (EPS) called kefiran and proteins in which lactic acid bacteria (LAB), yeasts, and acetic acid bacteria (AAB) live symbiotically. Because of its nutraceutical advantages and inexpensive cost, this beverage is extensively consumed, including but not limited to persons with health issues (Azizi et al., 2021). Moreover, research performed by Vasquez et al. (2020) revealed that kefir, with its probiotic properties, can help cure gut microbial dysbiosis for Alzheimer's patients.

Kefir content

According to Marco et al. (2017), kefir was commonly recognized as a probiotic-rich beverage with microbial diversity and in amounts higher than average supplements. In general, probiotic supplements comprise *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Streptococcus thermophilus,* and *Saccharomyces boulardii* (Hill et al., 2014). Meanwhile, kefir contains a more diverse microbial community compared to supplements (Jeong et al., 2013). When compared to kimchi and tempeh, with only 10 strains of microorganisms, kefir is still considered superior with its 20 microbial strains such as *Lactobacillus kefiri*, *Lactobacillus kefiranofaciens*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* sub. *bulgaricus*, *Streptococcus thermophilus*, *Lactococcus lactis* sub. *cremoris*, *Acetobacter aceti*, *Acetobacter raisins*, *Saccharomyces cerevisisiae*, *Leuconostoc mesenteroides* sub. *cremoris*, and many others (Prado et al., 2015; Pangastuti et al., 2019).

When the kefir beverages are fermenting and being stored, the bacteria in the kefir grain have the ability to produce bioactive compounds. Peptides, or bioactive compounds, which are the particular protein fragments that have beneficial impacts on the metabolic functions of the body and may influence health (e.g., antimicrobial, antioxidative, and antihypertensive), were detected in kefir, not naturally occurring in

raw milk, since microbiota proteases released them from milk caseins (Vieira et al., 2021; Sanchez & Vazquez, 2017). Based on a study by Ebner et al. (2019), 257 peptides were discovered, mostly from β-casein, and the majority, which was 236 of the peptides, were only found in kefir. This proves that fermentation or applying microflora to the milk boosts proteolytic activity compared to unfermented milk and changes the peptide fraction's composition. The proteolytic degradation of milk proteins results in several peptides with various biological properties, including antioxidant, antibacterial, antihypertensive, and immunomodulatory actions (de Lima et al., 2017).

Along with peptides, the other bioactive compound in kefir is an organic acid. In kefir, it acts as a flavour contributor as well as an antimicrobial and antimutagenic agent (Turker et al., 2014). The highest amount of organic acid present in kefir is acetic acid, hippuric acid, propionic acid, and pyruvic acid, with the first-mentioned being the highest amount. The presence of this acid could be introduced by the conversion of organic compounds in dairy by acetic acid or lactic acid microorganisms in kefir grains through heterofermentative mechanisms (Sanli et al., 2018). Furthermore, the metabolites (i.e., organic acids) produced by the aforementioned fermentative microorganisms decrease the pH of kefir, providing the optimal environment for lactic acid bacteria to grow (Putri et al., 2020).

Besides its bioactive and microbial content, kefir provides an extensive range and variety of nutrients. A study by the USDA in 2019 revealed that for every 100 mL of kefir consumption, 3.3 grams of protein could be acquired, making kefir considered high in protein. With milk as the raw ingredient, kefir is deemed calcium-rich, reaching 120 mg for every 100 mL of kefir. Phosphorus, another major mineral, is also present in kefir in a 90 mg/100 mL concentration. In addition, 11 mg of magnesium would be consumed for every 100 mL of kefir, along with small amounts of zinc and potassium. Not just macrominerals; vitamins are also abundant in kefir. For instance, vitamin B12 is sourced from kefir, with a content of 0.4 µg/100 mL. Another vitamin present in significant numbers within kefir is vitamin K2 (6 µg/100 mL kefir), together with trace concentrations of vitamin D, B1, and B6. Furthermore, kefir for every 100 mL consists of only 41 kcal of energy, 3.5 grams of carbohydrates, and 1 gram of fat. It is suitable for a wider variety of consumers and inclusive for patients with diverse non-communicable diseases such as diabetes and hyperlipidemia (Peluzio et al., 2021). When compared with other probiotic beverages such as yoghurt, kefir comprises a greater variety of nutrients and higher amounts of vitamin K2, calcium, and phosphorus, although yoghurt contains a greater value of vitamin B12 and protein. Despite the nutrient composition, yoghurt is higher in energy, fat, and sugars, making it less inclusive than kefir (Gaware et al., 2011; Zhu et al., 2020).

Table 1. Nutrition information of kefir per 100 g (USDA, 2019)

Nutrient	**Type**	**Kefir**
Energy (kcal)	-	35
Macronutrients	Carbohydrate (g)	3.5
	Fat (g)	1
	Protein (g)	3.3
Micronutrients (Vitamin)	Vitamin B12 (µg)	0.4
	Vitamin B1 (µg)	trace amounts

	Vitamin B6 (μg)	trace amounts
	Vitamin D (μg)	trace amounts
	Vitamin K2 (μg)	6
Micronutrients (Mineral)	Calcium (mg)	120
	Phosphorus (mg)	90
	Magnesium (mg)	11
	Zinc (mg)	trace amounts
	Potassium (mg)	trace amounts

Effects of kefir consumption on AD patients' gut microbiota

From the literature acquired, kefir was found to improve and amplify the proliferation of *Lactobacillus* and *Bifidobacterium* in AD patients, which leads to an increment of SCFA with abilities to lower inflammation and improve the brain's function (Batista et al., 2021). Chen et al. (2019) stated that 300 mL of kefir intake daily in a 12-week period significantly elevated the *Lactobacillus* and *Bifidobacterium* population. In an animal experiment by Peluzio et al. (2021) using mice, kefir administration for 12 weeks with the kefir peptides dissolved in a saline solution with a concentration of 500 mg/kg successfully elevated antioxidant enzymes whilst suppressing pro-inflammatory cytokines, thus lowering the prevalence of inflammation as well as oxidative stress. Furthermore, this study noticed that kefir enhanced gut microbiota diversity significantly and was associated with overall digestive system health and immunity. Another study discovered that kefir intake improved the gut barrier function, a complex system between the blood circulation and the gut comprising proteins and cells, ensuring substance flow regulation. As justification, a study by Bellikci-Koyu et al. (2019) supplemented people with metabolic syndrome with kefir for 12 weeks. The treatment resulted in a notable rise in Actinobacteria and alterations in the genera of Bacteroidetes and Firmicutes phyla, which enhanced the gut microbiota diversity and the gut barrier function. With the probiotics in kefir, the tight junction protein expression escalated, impeding the toxic substances from being transported (Chen et al., 2020). Moreover, peptides as bioactive compounds contained in kefir can inhibit *Escherichia coli*. In addition to being able to attach to the genetic material of *Escherichia coli*, peptides have the ability to raise the permeability of both the outer and inner bacterial membranes (Miao et al., 2016). The organic acid improves gut microbiota by providing an acidic environment, inducing the lactic acid bacteria (LAB) growth while inhibiting the pathogenic bacteria (Putri et al., 2020). The nutrients found in kefir, namely vitamin K2 and calcium, were found to be anti-inflammatory, which are prevalent in AD patients. With the reduction of gut inflammation, more beneficial bacteria can proliferate whilst reducing the progression of AD (Popescu & German, 2021). Additionally, vitamin B12 acts as a cofactor for enzymes that synthesize SCFA that reduces neuroinflammation and aids nutrient absorption for the gut microbiota, which induces microglia immune cells in the brain with a major role of lowering Aβ plaques, AD's hallmark (Yuan et al., 2022; Roth & Mohamadzadeh, 2021)

CONCLUSION

Alzheimer's disease is a progressive neurological disorder affecting the memory, cognition, and motor of patients. This can be caused by the alteration in the gut microbiota. Probiotics, on the other hand, are living organisms that can provide health benefits, and one example is kefir. Thus, the effects of kefir as a probiotic-containing drink on the gut microbiota in AD patients are examined throughout this paper. According to the findings, kefir can improve the proliferation of *Lactobacillus* and *Bifidobacterium*, some beneficial bacteria that are reduced in AD patients. It is proven that kefir can affect the digestive system health and immunity of AD patients through the bioactive compounds contained in kefir, such as peptides, organic acids, and exopolysaccharides, as well as its nutrients, including vitamins and minerals. In addition, further research may focus more on the mechanism of action for how the gut microbiota in AD patients is modified and the mechanism of action for how kefir can affect the AD patient's gut microbiota.

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