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Microorganisms in probiotic beverage fermentation: Health benefits and mechanisms in dairy and non-dairy products

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ABSTRACT

Background: Probiotics are microorganisms that actively improve health by improving the balance of intestinal microflora when consumed alive in adequate amounts. Probiotics are directly able to help the microflora in the digestive tract to inhibit pathogenic bacteria that can interfere with the digestive tract. This review aims to find out some examples of probiotic fermented beverage products, types of microorganisms that play a role and their benefits for human health. **Methods**: This article is a descriptive explanation using the literature review method. The stages of the literature review include collecting literature sources that are relevant to the research topic. Furthermore, analysis and evaluation of important findings from previous studies are carried out. Finally, a synthesis is carried out to formulate conclusions. **Finding:** Fermented dairy products such as yogurt, soy sauce, kefir, and yakult, as well as non-dairy products such as kombucha tea, are good sources of probiotics for health. The fermentation process in dairy products converts lactose into lactic acid, while in kombucha tea, fermentation occurs through a solution of tea and sugar with a kombucha microbial starter (a symbiosis of bacteria and yeast). **Conclusion**: Both fermented dairy products and non-dairy products contain probiotics that are beneficial for health. Fermentation of dairy products occurs by converting milk lactose into lactic acid. **Novelty/Originality of This Study:** This review uniquely compares dairy and non-dairy probiotic beverages, highlighting the symbiotic fermentation in kombucha tea as an alternative to traditional dairy products. It offers a fresh perspective on the diverse microbial mechanisms and health benefits of probiotics from both sources.

KEYWORDS: fermentation mechanism; fermentation products; probiotics.

1. Introduction

Fermented beverages are a type of functional beverage processed using fermentation techniques. Currently, the process of making beverages Some fermented beverages use standard cultures or are added with probiotic bacteria to provide a healthy effect. Standard cultures are common cultures used in the process of making fermented beverages, such as in the process of making yogurt using starter cultures of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus (Fazilah et al., 2018) and in making kombucha

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using Acetobacter and yeast starters. Meanwhile, probiotic bacteria such as Bifidobacterium and Lactobacillus acidophilus are usually supplemented with food ingredients and then fermented to produce a product called probiotic fermented beverage.

Probiotic bacteria are a group of living microorganisms consumed by humans and animals that can provide healthy effects and increase the body's resistance to intestinal pathogens (Sopandi & Wardah, 2014). Various types of probiotics that have been identified are Lactobacillus sp., Bifidobacteria, Streptococcus sp., Enterococcus, and Leuconostoc. Probiotic bacteria can produce various compounds such as lactic acid, acetic acid, bacteriocins, and reuterin that can inhibit the growth of pathogenic bacteria in the gut (Fazilah et al., 2018). The organic acids produced are not only to lower pH but also as toxins for pathogenic microbes.

The many health benefits of probiotic bacteria are certainly very beneficial for consumers if found in functional beverage products such as probiotic fermented beverages. Some studies mentioned that probiotic drinks are very useful as a good source of macronutrients, especially as a source of high protein nutrition (Rahmawati & Suntornsuk 2016), control weight and obesity (Barengolts et al., 2019), overcome diarrhea and digestive tract infections (Suzuki et al. 2017), prevent cancer, and reduce the risk of cardiovascular disease (Bayar et al., 2018). Therefore, this review was conducted to find out some examples of probiotic fermented beverage products, the types of microorganisms that play a role, and their benefits for human health.

2. Methods

The research method used in this article is descriptive with a literature review approach. This approach was chosen to provide a detailed and comprehensive explanation of the research topic based on existing literature. Instead of collecting primary data from the field, the researcher relies on various relevant and credible academic sources as the foundation for analysis. The first stage of the literature review involves collecting literature relevant to the research topic. The selected materials include books, journal articles, previous research findings, and other trusted academic references. This selection process is carried out systematically to ensure that each source contributes meaningfully and reliably to the discussion. After the literature has been gathered, the next steps are analysis and evaluation of significant findings from previous studies. The researcher compares arguments, methodologies, and results across sources to develop a broad and in-depth understanding of the topic's development. The final stage is synthesis, where the analyzed information is combined to formulate logical conclusions that support the research objectives.

3. Results and Discussion

3.1 Yoghurt

Yogurt is a fermented milk drink that has a semi-solid structure due to the coagulation of milk. Its sour taste comes from the effects of acetaldehyde, lactate, diacyl, and acetate (Sopandi & Wardah, 2014). Basically, yogurt is classified into 2 groups, namely yogurt with standard culture and probiotic yogurt. Standard yogurt usually uses starter cultures of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, while probiotic cultures are supplemented with probiotic bacterial strains such as Bifidobacterium and *Lactobacillus acidophilus*, which are claimed to have a healthy effect on the body (Fazilah et al., 2018). Some studies related to the types of microorganisms used as starters in probiotic yogurt fermentation come from groups of lactic acid bacteria such as *Lactobacillus rhamnosus* (Kamal et al., 2018), *Lactobacillus fermentum* KU200060 (Lim et al., 2020), *Lactobacillus paracasei* subsp. paracasei F19, *Lactobacillus acidophilus* (Gu et al., 2020), and some Bifidobacteria groups such as *Bifidobacterium longum* and *Bifidobacterium lactis* (Bayar et al., 2018).

3.1.1 Mechanism of yogurt fermentation process

The bacteria used in yogurt fermentation are *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Aryana & Olson, 2017). The optimum temperature for *Lactobacillus bulgaricus* is 41-43 oC and *Streptococcus thermophilus* grows at 40-45 oC. Temperatures higher than 43.3°C cause the growth of *Lactobacillus bulgaricus* to be higher and dominant, resulting in lower acid production and reduced flavor. Temperatures lower than 43.3°C cause *Streptococcus thermophilus* growth to be high and dominant, resulting in reduced flavor production and increased flavor. Therefore, the right temperature to produce the right aroma and flavor in the yogurt fermentation process is 43.3°C. At first, *Streptococcus thermophilus* grows rapidly in the presence of dissolved oxygen and produces formic acid and CO_2. Then *Lactobacillus bulgaricus* will grow in the presence of formic acid, CO2, and anaerobic conditions. *Lactobacillus bulgaricus* will produce peptides and amino acids from milk protein because it has exoproteinase and peptidase enzymes. Some amino acids, such as glycine, valine, histidine, leucine, and methionine, are good for the growth of *Streptococcus thermophilus*. The two bacteria also have a synergistic effect in producing acetaldehyde and have a galactosidase system that can hydrolyze lactose (Sopandi & Wardah, 2014).

During the fermentation process, several volatile compounds are formed. Based on research (Moineau-Jean et al., 2020), there are 32 volatile compounds potentially involved in the aroma of yogurt. The most important aromatic components are acetaldehyde, acetone, acetoin, diacetyl, acetic acid, formic acid, butanoic acid, and propanoic acid. Acetaldehyde is produced in two ways, namely from glucose via pyruvate by *Streptococcus* spp. and from threonine available through milk proteolysis by *Lactobacillus* sp. Low concentrations of acetaldehyde can give stale and tart flavors, but concentrations that are too high will give yogurt a green color (Sopandi & Wardah, 2014).

The addition of probiotic bacteria Bifidobacteria and Lactobacillus to milk by combining with yoghurt starter culture results in probiotic bacteria growing slowly in milk because probiotic bacteria have lower essential proteolytic activity when compared to yoghurt starter culture (Fazilah et al., 2018). However, the addition of a probiotic starter will increase the exopolysaccharide content so that yoghurt fermentation products with better texture will be produced. Microbial exopolysaccharides can function as emulsifiers and gelling agents, thickening agents, and stabilisers (Liu et al., 2017).

3.1.2 Yogurt prebiotics

Prebiotics are nutrients needed by probiotic microorganisms for growth. The combination of probiotics and prebiotics is called a synbiotic. In yoghurt, the use of *Lactobacillus delbrueckii* ssp. bulgaricus starter culture cooperates with *Streptococcus thermophilus* and lactulose during the fermentation and storage process (Delgado-Fernández et al., 2020). Both species have a galactosidase system that can hydrolyse lactose into glucose and galactose (Sopandi & Wardah, 2014).

Lactic acid bacteria rapidly metabolize glucose released from lactose to produce lactic acid and acetic acid. In addition, free fructose released from lactulose at high concentrations is also metabolized into carbon sources and CO2 by different pathways (Delgado-Fernández et al., 2020). Another study is the addition of glucose oxidase to probiotic yogurt of *Lactobacillus bulgaricus*, *Lactobacillus acidophillus*, *Bifidobacterium* and *Streptococcus thermophilus* bacteria stored at refrigeration temperature for 30 days showed the results that glucose oxidase is effective in the production of lactulose minimizes oxidative stress and enhances the defense of probiotic bacteria. Furthermore, yogurt containing glucose oxidase produces low pH values and high proteolysis values, higher fat content and lower sodium calcium, has higher levels of diacetyl, acetaldehyde, conjugated linoleic acid and polyunsaturated fatty acids and similar lactic acid and acetic acid values (Batista et al., 2015).

3.2 Soyghurt

Soy milk has advantages including not containing lactose, the protein does not cause allergies, low fat, cholesterol-free, highly nutritious, relatively easy manufacturing technology and relatively low production costs. However, soy milk has a disadvantage on the organoleptic side, namely that it has a strong odor. One way to reduce the odor of soy milk is to process it further into soy yoghurt, also known as soyghurt. Soy yoghurt has several advantages compared to yoghurt, namely being lactose-free, cholesterol-free, and containing lower amounts of fat (Winarno, 2017). The acidity level of soygurt is generally lower than the growth of probiotic bacteria and the fermentation time tends to be longer due to the low concentration of soluble carbohydrates in soy milk (Fazilah et al., 2018). Soyghurt is a fermented soy milk product using *Streptococcus thermophiles* and *Lactobacillus bulgaricus* bacteria, which are essential microbial species that are active in symbiotic relationships and have been commonly used in the process of making yoghurt (Layadi et al., 2009). Soygurt is a plant-based alternative to animal milk-based yoghurt. Besides being a source of probiotics, soy yoghurt is rich in isoflavones, antioxidant compounds that help prevent degenerative diseases.

3.2.1 Bakteri that play role

Some types of probiotic bacteria used in making soygurt are *Lactobacillus acidophilus* NCDC14 (Mridula & Sharma, 2015), a combination of *Bifidobacterium animalis* subsp. *lactis* BB-12, *Lactobacillus acidophilus* La-5 and *L.rhamnosus* (Cui et al., 2021), 5 probiotic strains such as *St. thermophilus* TH-4, *Lactobacillus acidophilus* LA-5, *L. rhamnosus* LGG, *L. fermentum* PCC, and L. reuteri RC-14 (Albuquerque et al., 2017), gabungan bakteri *Bifidobacterium bifidum*, *Lactobacillus casei*, and *L. plantarum* (Zhang et al., 2017). The variety and quantity of live microorganisms are important characteristics of soyghurt product quality. In the study (Horáčková et al., 2015) showed results that yoghurt culture YC-381 (*Streptoccocus thermophilus* dan *Lactobacillus bulgaricus*) and probiotic cultures (*Bifidobacterium animalis, Bifidobacterium bifidum*) were able to grow in both test media, namely soy milk and cow's milk. *Bifidobacterium animalis* is suitable for the production of fermented soy milk and cow's milk products due to its lower acetic acid content. In soy milk, *Bifidobacterium bifidum* showed extensive isoflavone hydrolysis and higher acetaldehyde production. Acetaldehyde, acetic acid and lactic acid significantly contribute to the organoleptic quality of soyghurt fermented products (Horáčková et al., 2015).

3.2.2 Mechanism of soyghurt manufacturing process

Soygurt, as a plant-based alternative to animal milk-based yoghurt, is produced through a series of steps involving the fermentation of soy milk by lactic acid bacteria. The process begins with the preparation of the soy milk, where the soybeans are soaked for 8–12 hours to soften their texture; the soy milk filtrate is then crushed with water in a ratio of 1:4. The soy milk filtrate obtained was heated to 90-100°C for 15-30 minutes to inactivate the lipoxygenase enzyme that causes the odour, as well as to kill pathogenic microbes. After that, the soy milk is cooled to 42°C and inoculated with bacterial starter cultures such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, in concentrations of 5–7%, for further fermentation (Fifendy & Syukryani, 2019).

During incubation at 37-45°C for 10-20 hours, lactic acid bacteria ferment simple sugars such as glucose into lactic acid through glycolysis and homolactic fermentation. This process begins with the hydrolysis of sucrose into glucose and fructose using the enzyme invertase, followed by glycolysis which converts glucose into pyruvate. Pyruvate is then reduced to lactic acid with the help of the enzyme lactate dehydrogenase. This lactic acid production causes a decrease in the pH of soygurt to 4.0-4.5, giving it a distinctive sour taste while increasing viscosity and creating a thick texture. In addition, this fermentation produces volatile compounds such as diacetyls that give a distinctive aroma, and increases the bioavailability of isoflavones, such as genistein and daidzein, which act as antioxidants (Fifendy & Syukryani, 2019).

After fermentation is complete, soygurt is refrigerated at 4°C to stop microbial activity and extend shelf life. In some formulations, additives such as red dragon fruit peel extract are often used to increase antioxidant content, provide bright color, and add aesthetic value to the product. Red dragon fruit peel is known to be rich in anthocyanins that not only provide a bright red color but also improve the functional properties of soygurt. With this fermentation process, soygurt not only becomes a more delicious and digestible product, but also offers significant health benefits, including improved digestive health, reduced risk of degenerative diseases, and enhanced immune function. The combination of a standardized process and the utilization of innovative additives makes soygurt a functional food alternative with high nutritional value.

3.2.3 Benefits of soyghurt

In terms of nutritional value, soy yoghurt has almost the same protein content as cow's milk, but without the cholesterol. The fat content also varies depending on the formulation and additives. Research shows that starter concentration and fermentation duration greatly affect product quality, including acidity, viscosity, and flavour. Soygurt with a starter concentration of 5-7% and a fermentation time of 10-20 hours produced a product that met national standards for protein content, pH, and total lactic acid bacteria (LAB), with good viscosity and a taste that panellists liked.

In addition to its nutritional value, soygurt also has tremendous health potential. Regular consumption of yoghurt can support gastrointestinal health by increasing the population of good bacteria in the gut, helping prevent diarrhoea, boosting immunity, and reducing the risk of degenerative diseases such as cancer. With these benefits, soy yoghurt is an ideal choice for people who want to maintain their health without sacrificing taste and nutritional value.

As a sustainable food product, soygurt also has great economic potential, especially in countries with high soy milk consumption. With diversification through the addition of local ingredients such as red dragon fruit skin, soygurt can be a superior product that supports local food-based innovation. Further research and development can pave the way for optimization of the production process, resulting in a product that is not only highly nutritious but also economical and environmentally friendly.

3.3 Yakult

Yakult is a food product of milk fermentation by lactic acid bacteria *Lactobacillus casei* Shirota (LcS) which has a distinctive flavor and fresh sour taste containing 10^8 live LcS hidup per milliliter. LcS has an important role in the human digestive tract. One of them can increase the frequency and consistency of stool and reduce constipation when consuming LcS for 3 days regularly as much as 80 mL (Baskaran et al., 2019).

3.3.1 Mechanism of the fermentation process

Lactobacillus is a homofermentative group (able to convert 85% of carbohydrates or simple sugars into lactic acid through the fermentation process, producing two molecules of ATP from one molecule of monosaccharide), has no spores, and is thermobacterium because it can grow at 45ºC, and these bacteria are often found in the human digestive tract. Lactobacillus functions as a flavor maker and can play a role in balancing the human gut microflora. LcS can break down more than just glucose. It can also do this with lactose, fructose, sucrose, ribose, mannose, galactose, sellobiose, trehalose, melezitos, mannitol, esculin, salicin, and amyglidin (Surono, 2016).

LcS that are alive and ingested into the body will reach the intestinal tract and some will settle, multiply and produce metabolite components such as lactic acid that can repel pathogenic bacteria and control fecal secretion by stimulating the intestinal wall (Baskaran et al., 2019). The gut microbiota differs from individual to individual depending on nutrition, health (healthy, sick or stressed), medications eaten, and environmental conditions.

3.3.2 The role of LcS in preventing diarrhea

Probiotics are effective for the primary and secondary prevention and treatment of diarrhea. The mechanism works by increasing the number of probiotic bacteria in the digestive tract. This makes sure that all of the epithelium of the intestinal mucosa is occupied by probiotic bacteria through receptors in intestinal epithelial cells. This makes it impossible for pathogenic bacteria to attach to intestinal epithelial cells and eventually colonize (Lai et al., 2019).

3.3.3 The role of LcS in reducing constipation and improving stool consistency

As shown in the study, both Yakult and yoghurt have constipation-relieving effects. Both Yakult and yoghurt have demonstrated an increase in the frequency of evacuation and an improvement in the consistency of faeces in the Bristol table. They also felt fewer constipation symptoms, such as straining and sensation of incomplete evacuation, after consuming Yakult and yoghurt (Baskaran et al., 2019).

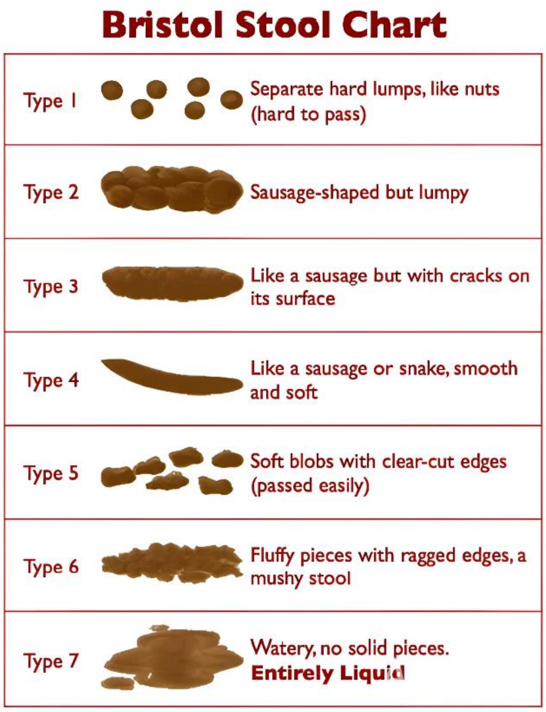

Fig. 1. Bristo stool chart
(Baskaran et al., 2019)

3.3.4 The role of LcS in maintaining gut health

Lactic acid, a weak acid, is an organic acid that results from the fermentation of sugar. Weak acids inhibit pathogenic bacterial growth by accumulating anions in cells, which in turn slows down the speed of macromolecular synthesis. Undissociated weak acids are more toxic than in dissociated form so they can inhibit microbial growth (Chen et al., 2019).

The inhibitory process is carried out by LcS bacteria against pathogenic bacteria by competing for substrates or nutrient sources and altering pH. Lactobacillus casei can reduce constipation and diarrhea, help increase defense against pathogenic bacteria, and prevent congenital *traveler's diarrhea.*

3.4 Kefir

Kefir is a fermented milk product using kefir seeds. Like milk fermentation, kefir seeds contain multiple microorganisms. Kefir seeds have a generally small and irregular shape with a diameter ranging from 1 to 6 cm (Bengoa et al., 2019). The quality of milk fermentation using kefir seeds is influenced by the type of milk, dosage of kefir seeds, incubation time, and microorganisms contained in kefir seeds (Sulmiyati et al., 2019b).The type of milk will affect the final composition of the fermented product; kefir fermentation usually uses animal milk products that are commonly consumed by people ranging from goat milk, cow milk, and vegetable origin, namely soy milk (Dertli & Çon, 2017; Gamba et al., 2020).

Dosing kefir seeds for fermentation will affect the increasing ability of microbes to break down glucose to produce the primary metabolites of kefir seeds, namely lactic acid and alcohol (Triwibowo et al., 2020). Incubation time is one of the most important factors in terms of fermentation; fermentation that is too short will produce low lactic acid and vice versa. If the incubation time is long, it will produce low lactic acid and high lactic acid (Sulmiyati et al., 2019b).

Table 1. Composition of kefir from Indonesia and Turkey

Indonesia Kefir Grain[a]		Turkey Kefir Grain[b]	
Bacterial Composition	Fungal Composition	Bacterial Composition	Fungal Composition
Lactobacillus fermentum 1	*Candida krusei*	*Lactobacillus kefiranofaciens*	*Alternaria spp.*
Lactobacillus paracasei ssp. Paracasei	*Candida Parapsilosis*	*Enterobacter amnigenus*	*Aspergillus amstelodami*
Lactobacillus brevis 3	*Saccharomyces cerevisiae*	*Enterobacter hormaechei*	*Dipodascus geotrichum*
Lactobacillus plantarum 1	*Candida glabrata*	*Lactobacillus kefiri*	*Dipodascaceae spp.*
Lactococcus lactis ssp lactis 1	*Kloeckera spp.*	*Lactobacillus apis*	*Candida parapsilosis*
Lactobacillus delbrueckii ssp delbrueckii	*Saccharomyces cerevisiae 2*	*Lactobacillus ultunensis*	*Candida zeylanoides*
		Acinetobacter rhizosphaerae	*Issatchenkia orientalis*
		Enterococcus lactis	*Saccharomyces cerevisiae*
		Acinetobacter calcoaceticus	*Malassezia spp.*
		Pseudomonas azotoformans	*Rhodotorula dairenensis*
		Pseudomonas aeruginosa	*Rhodotorula mucilaginosa*
		Pseudomonas otitidis	*Trichosporon spp.*
		Propionibacterium acnes	*Mucor circinelloides*
		Enterobacter soli	*Yarrowia lipolutica*
			Aspergillus spp.
			Kazachstania unispora
			Cryptococcus victoriae

(Dertli dan Çon 2017[b]; Yusuf *et al.* 2020[a])

Microorganisms in kefir seeds will affect the fermentation that occurs in dairy products. Microorganisms contained in kefir seeds obtained in four different regions of Turkey

(Istanbul, Kayseri, Balıkesir, and Izmir) and also in four regions in Indonesia (namely Bogor, Bandung, Jakarta, and Yogyakarta) have been found using next-generation sequencing (NGS) techniques for Turkey Grain, and the analytical profile index (API) is as follows Table 1. The microorganisms contained will be responsible for the flavour that will be produced in the final product, along with the volatile compounds formed using GC-MS analysis.

Table 2. Volatile compounds in kefir grains originating from Türkiye

Acidic compounds	Odor relevance(s) Sweaty	Source of metabolism
	Asam	
iso-valeric acid	*Sweaty, cheesy*	Lipid metabolism
Alkohol		
Etanol	*Dry, dust*	Carbohydrate metabolism
2-Heptanol	*Green, floral, fruity*	Carbohydrate metabolism
3-Metil-1-butanol	*Fresh chees, alcoholic, fruity*	Amino acid metabolism
Benzen etanol	*Fresh, wine*	Carbohydrate metabolism
	Aldehid	
3-Methylbutanal	*Cheesy, dark chocolate, cocoa*	Amino acid metabolism
Asetaldehida	*Fruity, alcoholic, wine*	Carbohydrate metabolism
	Asam karboksilat	
Asam benzoat	*Fruity, alcoholic*	Lipid metabolism
Carbamic acid	*Sour*	Amino acid metabolism
Asam asetat	*Vinegar, green, fruity, sour*	Carbohydrate metabolism
Asam propanoat	*Sour*	Lipid metabolism
Asam butanoat	*Acrid, acidic, cheesy, bad*	Carbohydrate metabolism
Asam heksanoat	*Sweaty, cheesy, sarp, acidic*	Lipid metabolism
Asam oktanoat	*Cheesy, rancid, pungent*	Lipid metabolism
Asam dekanoat	*Soapy, waxy, stale, buttery*	Lipid metabolism
Asam dodekanoat	*Soapy, waxy, fatty*	Lipid metabolism
	Ester	
1,2-Benzenedicarboxylic acid, diethyl ester	*Plastic*	Carbohydrate metabolism
2-hydroxy-isocaproic acid methyl ester	*Pleasent fresh planty*	Amino acid methabolism
Acetic acid ethyl ester	*Solvent, pineapple, fruity, apples*	Carbohydrate metabolism
	Keton	
2-Heptanon	*Blue cheese, spicy, Roquefort*	Lipid metabolism
2-Butanon	*Buttery, sour milk, etheric*	Amino acid methabolism
2-Nonanon	*Malty, fruity, hot milk*	Lipid metabolism
2,6-Dimetil-4-heptanon	*Fruity, sweet*	Lipid metabolism
	Sulfur	
Dimetil sulfone	*Sulfurous, hot milk, burned*	Amino acid methabolism

(Dertli & Çon, 2017)

3.4.1 Fermentation mechanism

Fermentation mechanism according to Sulmiyati et al. (2019a) Milk is preprocessed such as heating at 105°C for 5 minutes after which cooling is carried out until it reaches a temperature of 37°C, then kefir seeds are added and incubated for 12 hours at 37°C. Next, the kefir seeds are separated and the kefir can be cooled and ready to drink. Visually, it can be seen in the following Fig. 1.

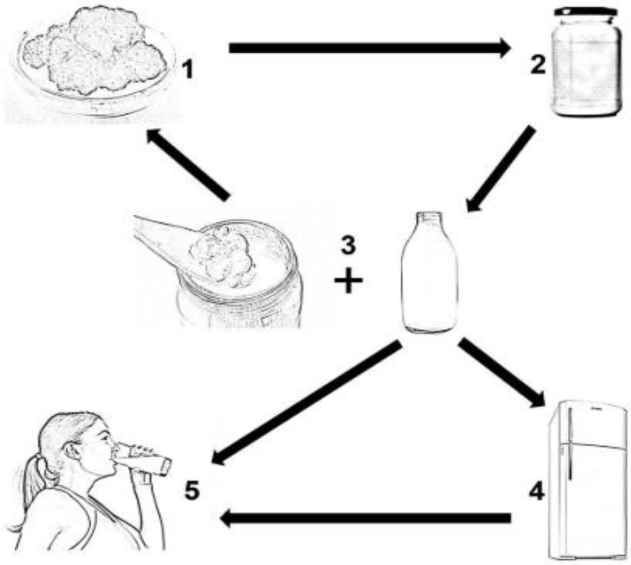

Fig2. Kefir seed (1), addition of kefir seed to milk (2), filtration and sparation of kefir seed (3), kefir can be stored at refrigration temperature (4), kefir can be consumed (4)
(Rosa et al., 2017)

3.4.2 Fermentation result

Fermentation mechanism according to Sulmiyati et al. (2019b). Milk is preprocessed, such as heating at 105°C for 5 minutes, after which cooling is carried out until it reaches a temperature of 37°C, then kefir seeds are added and incubated for 12 hours at 37°C. Next, the kefir seeds are separated and the kefir can be cooled and ready to drink. Visually, it can be seen in the following picture.

Table 3 Average of physicochemical, microbiological properties of different concentrations of kefir seeds

Variabel	Kefir Seed Concentration		
	2%	4%	6%
pH	5.40±0.100[a]	4.96±0.114[b]	4.76±0.540[c]
Lactic acid bacteria (%)	0.14±0.007[a]	0.26±0.620[b]	0.41±0.410[c]
Etanol (%)	0.69±0.005[a]	1.41±0.262[b]	1.78±0.130[c]
Changes Kefir seed weight (%)	7.21±0.647[a]	7.10±0.587[a]	5.08±0.181[b]
Total Lactic Acid Bacteria (10^7 FU/mL)	1.64±0.008[a]	1.71±0.130[b]	1.76±0.015[c]

(Sulmiyati et al., 2019b)

Incubation time is one of the determining factors of the results of fermentation products; below are data from the research of Sulmiyati et al. (2019a) on kefir fermentation products made from goat milk with varying incubation times.

Table 4 Average of physicochemical, microbiological properties of various incubation time at 2% kefir seed concentration

Variabel	Incubation Time (hour)		
	12	18	24
pH	5.40±0.100[a]	5.10±0.100[b]	4.16±0.089[c]
Lactic Acid Bacteria (%)	0.14±0.007[a]	0.17±0.011[a]	0.24±0.039[b]
Etanol (%)	0.69±0.005[a]	0.70±0.037[a]	0.75±0.044[a]
Total Lactic Acid Bacteria (10^7 CFU/mL)	1.64±0.008[a]	1.74±0.008[b]	1.24±0.008[c]

(Sulmiyati et al., 2019a)

Table 4 illustrates a significant impact of the variation in kefir seeds. A greater kefir concentration correlates with an increased overall bacterial count in the product (Sulmiyati, et al., 2019b). The duration of fermentation affects lactic acid production; extended fermentation results in increased lactic acid formation, however concurrently diminishes the population of lactic acid bacteria, as evidenced by research conducted by Sulmiyati et al. (2019a).

3.5 Kombucha

Kombucha is a non-alcoholic fermented beverage that originated in Asia and spread to Europe. Kombucha is a sweet tea-based fermented beverage that has been known since the time of the Tsin Dynasty in China around 220 BC. The microbes in kombucha are divided into two parts. The first part is the cellulose biofilm layer and the second is in the base solution. Kombucha tea is a traditional beverage product resulting from the fermentation of tea and sugar solution using a kombucha microbial starter (symbiosis of bacteria with yeast). The substances produced from kombucha fermentation become a bulwark against the attack of pathogenic bacteria. Some of them are various vitamins (vitamin B complex, vitamin C), organic acids (acetic acid, folic acid, glucuronic acid, lactic acid), and some compounds that function as antibiotics. The properties of kombucha tea can affect the body as a whole by stabilizing the body's metabolism and offering toxins (Naland, 2008). Kombucha is produced through a fermentation process by a symbiotic culture of bacteria and yeast known as SCOBY (Symbiotic Culture of Bacteria and Yeasts). Kombucha has gained widespread attention for its diverse health benefits, such as antioxidant, antimicrobial, and anticarcinogenic activities. The kombucha fermentation process involves brewing black or green tea and adding sugar, followed by inoculation of SCOBYs and starters from previous fermentations. Fermentation is carried out at 20–30°C for 7–14 hari, producing bioactive compounds such as polyphenols, organic acids, vitamins, and minerals.

3.5.1 Microorganisms that play a role

The microorganisms that play a role in the process of fermenting tea into Kombucha cannot be known with certainty (Jayabalan et al., 2014). This depends on the source of the inoculum used to ferment the tea. Based on some research results, the most abundant group of bacteria is the acetic acid bacteria, especially those from the genera Acetobacter, Gluconacetobacter, and Komagataeibacter (Chakravorty et al., 2016). These bacteria form a network layer on the surface of the fermented tea Gluconacetobacter sp. has the ability to produce D-saccharic acid-1,4-lactone (DSL) (Jayabalan et al., 2014). In addition to acetic acid bacteria, there are many yeast species in kombucha. *Saccharomyces, Saccharomycodes, Schizosaccharomyces, Zygosaccharomyces, and Mycoderma* species. The yeast of the Saccharomyces species is identified as Saccharomyces sp. In addition to acetic acid bacteria and yeast, lactic acid bacteria are also found in Kombucha. The lactic acid bacteria that were successfully identified were *Lactobacillus* sp., *Lactococcus* sp., *Leuconostoc* sp., and *Pediococcus pentosaceus* (Bogdan et al., 2018; Chakravorty et al., 2016; Marsh et al., 2014).

3.5.2 Mechanism of fermentation process

The best substrates for the kombucha fermentation process are black tea and sugar. Although green tea can also be used as a substrate. The tea leaves are put into boiled water for 10 minutes. A total of 50 g/L sucrose is added to the tea solution and then cooled. The tea is then poured into a larger container and vinegar or a kombucha starter is added. The container was wrapped with cloth and tied well. Incubation was carried out at room temperature (20°C-30°C) for 1±8 weeks. During the fermentation process, mold begins to form on the surface of the tea. The mold was removed from the surface and fermentation continued. The tea solution was filtered with a cloth and stored in sealed bottles at 4°C. The flavor of kombucha changed during fermentation to a mild vinegar-like flavor with prolonged incubation.

Acetic acid bacteria such as Acetobacter xylinum have the ability to synthesize the cellulose tissue layer in the Kombucha fermentation process. Acetic acid bacteria convert glucose into gluconic acid and fructose into acetic acid. Acetic acid stimulates yeast to break down sucrose into fructose and glucose and produce ethanol and then ethanol can help acetic acid bacteria to grow and produce acetic acid again (Liu et al., 1996). Both synergize in this fermentation process. The major components produced during kombucha fermentation are acetic acid, ethanol, and glucoronic acid, while the minor components produced are lactic acid, phenolic acid, B vitamins, and enzymes Kombucha also contains organic compounds that are beneficial to the body, namely vitamin B complex, organic acids, and other compounds that function as antibiotics (Naland, 2008).

3.5.3 Benefits of kombucha

Kombucha has various health benefits thanks to its bioactive content. Antioxidant activity mainly comes from green tea polyphenols, which can reduce the risk of cardiovascular and neurodegenerative diseases. In addition, Kombucha exhibits effective antimicrobial activity against pathogens such as Escherichia coli, Salmonella typhi, and Candida spp. Fermentation also increases the content of bioactive compounds such as gluconic acid, acetic acid, and glucuronic acid, which are known to have detoxifying, anti-inflammatory effects, and support liver function. Kombucha also has the potential to aid in the management of diabetes through reduced blood glucose levels and improved insulin sensitivity, as evidenced in several preclinical studies. Other benefits include anti-inflammatory and anticarcinogenic properties that have the potential to reduce inflammation and prevent the spread of cancer cells. Kombucha's chemical composition and biological activity are greatly influenced by the type of tea used, sugar concentration, and fermentation parameters such as duration and temperature. Green tea, for example, has higher polyphenol content than black tea, providing greater health benefits. Optimal fermentation time results in an ideal balance between flavor and bioactive content, while prolonged fermentation may result in an overly acidic flavor.

Factors that affect the quality and composition of kombucha include the type of tea used, sugar concentration, fermentation duration, and environmental conditions such as temperature and pH. Green tea tends to produce kombucha with higher polyphenol content than black tea, thus providing greater health benefits. The optimal fermentation duration is important to achieve a balance between flavor and bioactive content. Prolonged fermentation may cause the beverage to become too acidic due to the accumulation of organic acids, which may reduce its flavor quality and potential health benefits.

Kombucha safety is also an important concern. Although Kombucha is generally considered safe for consumption, reports of side effects such as poisoning due to microbial contamination or excessive consumption of overly acidic products require special attention. Therefore, the fermentation process should be carried out under hygienic and controlled conditions. Kombucha formulated with a pH below 4.5 is considered safe, as it can prevent the growth of pathogenic bacteria. However, this drink is not recommended for certain groups, such as children under 4 years old, pregnant women, patients with impaired kidney function, or those with weakened immune systems.

In addition to the health benefits, Kombucha also has great economic potential. The global kombucha market has grown rapidly in the past decade, with the estimated market value reaching billions of dollars. Kombucha is not only available in traditional flavors but also in various flavor variants that use additional ingredients such as fruits, herbs, and juices to attract more consumers. The use of alternative substrates, such as salak juice, grape juice, or herbal infusions such as rosemary and thyme, has been reported to increase antioxidant activity and provide added value to this beverage.

From a research perspective, there are still many opportunities for further exploration regarding the benefits of Kombucha. Human clinical studies are needed to confirm claims of health benefits, such as anticancer, antidiabetic, and liver function improvement activities. In addition, the specific identification of bacterial and yeast strains in SCOBY that contribute to the biological activity of Kombucha can help develop a superior product. Research also needs to focus on improving production technology to ensure the quality, safety, and efficiency of the fermentation process.

Overall, Kombucha is a functional beverage with promising health benefits and great market potential. The combination of the bioactive content of tea and the metabolic activity of microorganisms makes Kombucha an attractive option for consumers looking for healthy products. With the right research approach and innovation in production, Kombucha has the prospect of becoming an important part of the growing global health beverage industry (ref Appendix 1).

4. Conclusions

Fermented dairy products such as yogurt, soy yogurt, kefir, and yakult, and non-dairy products such as kombucha tea, are good sources of probiotics for health. The mechanism of the fermentation process in dairy products (yogurt, soy yogurt, kefir, and yogurt) is to convert milk lactose into lactic acid, while for kombucha tea, it is the fermentation of tea and sugar solution using a kombucha microbial starter (symbiosis of bacteria with yeast).

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Appendix 1. Health benefits of probiotic drinks

No	Function	Dosage	Mechanisms	Source
		Yakult		
1.	Relieve symptoms stress-related symptoms by modulating interactions gut-brain	100 mL of LcS fermented milk once daily for 8 weeks	Modulates HPA axis activation at appropriate levels and suppresses corticosterone secretion in situations	(Takada et al., 2016)
2.	Relieve symptoms constipation of stool consistency	100 mL of yakult containin 10^{10} LcS colony-forming units daily for 28 days.	There is an accumulation of anions in the cells that will inhibit microbial growth because the rate of macromolecular synthesis decreases..	(Chen et al., 2019)
3.	Determines the effect of (Yakult) in increasing stool frequency, improving stool consistency and reducing constipation.	yakult (80ml) for 3 days regularly after lunch. After 3 days, any changes in bowel habits according to the Bristol stool table were recorded.	-	(Baskaran et al., 2019)
4.	Find out the effect of *Lactobacillus casei* variety rhamnosus (Lcr35®) on clinical symptoms, gut microbiota and inflammatory markers in diarrhea in children (6 months - 6 years)	Twice daily for seven days in capsule form containing 2 × 108 colony forming units (CFU)/ 250mg	Activating local macrophages to enhance antigen presentation to T cells, then T cells release cytokines to activate B lymphocytes, and finally B lymphocytes synthesize immunoglobulins, namely IgA. So probiotics do not directly improve IgA	(Lai et al., 2019)

Yoghurt

No	Fuction	BAL Types and Mechanisms	Source
5	Inhibits the growth of pathogenic bacteria *Escherichia coli* O157:H7, S. *aureus, Yersinia enterocolitica* and *Salmonella* Typhimurium	Usage strains probiotics *L. rhamnosus* as a biocontrol agent	(Kamal et al., 2018)
6	Broad-spectrum antimicrobial activity (Gram- positive and Gram-negative bacteria and some fungal species)	*Lactobacillus plantarum* isolated from cow's milk	(Liu et al., 2017)
7	Prevention and therapy of infectious diseases, treatment of intestinal inflammation and for eradication of neoplastic host cells and higher absorption of lactose and essential minerals	Reuterin, a bacteriocin produced in fermented milk inoculated by *Lactobacillus acidophillus, Lactobacillus fermentum, Lactobacillus paracasei, Enterococcus faecium* and probiotic bacteria	(Bayar et al., 2018)

		Kefir	
8.	Aantimicrobial activity • Kefir with label A will inhibit *B. cereus, E. coli, S. Enteritidis, P. aeruginosa,* and *C. sakazakii.* • Kefir labeled L, M, and S will inhibit *S. aureus, P. aeruginosa,* and *C. sakazakii*	Antimicrobial compounds in kefir drinks can be formed during the fermentation process. During the fermentation process there will be a decrease in pH which will have an antimicrobial effect..	Kim *et al.* (2016)
9.	Activates the innate immune system that can inhibit damage caused by viruses and bacteria	Using kefir supernatant showed an increase in IL-10 (Interleukin-10) cytokine levels which is produced by Th2 (T-helper 2) cells. IL-10	(Wisudanti 2017)
		Kombucha	
10.	Helps the growth of useful microbes in the digestive tract. The collection of microbes in kombucha can be utilized as synbiotics	Kombucha provides short-chain fatty acids and other metabolites that boost immunity	(Kozyrovska et al., 2012)
		Soyghurt	
11.	Reduced HFD-induced body weight, liver and abdominal fat index and reduced hyperlipidemia in test animals	Bacteria Bifidobacteria bifidum, L.casei and L.plantarum cause inhibition of dietary fat, reduction of hyperlipidemia and liver damage due to decreased production of liver LPS, TNF-a and oxidative stress and promote adipose leptin production.	(Zhang et al., 2017)
12	Meet your daily intake of folate	The combination of two starter cultures of *Streptococcus thermophilus* ST-M6 and TA-40 and five probiotic strains of *S. thermophilus* TH- 4, *Lactobacillus acidophilus* LA-5, *L. rhamnosus* LGG, *L. fermentum* PCC, and L. *reuteri* RC-14 used to ferment soy milk and the addition of fructooligosaccharides had a symbiotic effect.	(Albuquerque et al., 2017)