Mini-Review: The potential of raffinose as a prebiotic

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Abstract. Prebiotics are dietary fiber components that cannot be digested by the human gastrointestinal tract but can be selectively fermented by bacteria in the gastrointestinal tract. Therefore, prebiotics provide health effects for humans. The specificity of prebiotics is determined by the bacteria that are specifically capable of fermenting the prebiotic substrate. The characteristics of prebiotic substrate need to be investigated in vitro and in vivo to determine the function and effectiveness of the substrate as a prebiotic. The prebiotic production process must be able to keep the prebiotic components stable. Raffinose is an oligosaccharide that has potential as a prebiotic. This article will discuss raffinose chemical structure, degrading enzymes, and health benefits as a prebiotic. Raffinose consists of 3 monomers, namely \( \alpha \)-D-galactose, \( \alpha \)-D-glucose, and \( \alpha \)-D-fructose. Substrates containing raffinose-family oligosaccharides (RFOs) are the source of raffinose. Those substrates can be degraded to raffinose by the \( \alpha \)-1,6-galactosidase. Raffinose can increase the growth of lactic acid bacteria, suppress the growth of pathogenic bacteria, increase short-chain fatty acids (SCFA), reduce constipation, inhibit the formation of putrefactive compounds from protein, and reduce the risk of cardiovascular diseases.

1. Introduction
Food processing, whether in the food processing industry or at home, often takes a long time. Thus, it reduces the content of functional compounds. This phenomenon then causes unhealthy eating habits. This unhealthy eating habit increases the diseases related to diet. However, lately, consumer awareness is increasing on the importance of healthy food. Several studies have shown a relationship between the health of the microbiota and several diseases. One component of functional food that maintains the health of the gut microbiota is prebiotics.

Prebiotics cannot be digested in the upper human gastrointestinal tract. However, according to ISAPP (International Scientific Association for Probiotics and Prebiotics), prebiotics act as substrates that can be used selectively by microorganisms in the colon and benefit the host of these microorganisms. The host is a human or an animal [1].

*Lactobacillus* and *Bifidobacterium* can selectively use prebiotics. Based on the ISAPP definition, the term selective is not limited to these two types of bacteria but can be extended to several other bacteria. However, the types of microbes whose growth is increased due to prebiotics must be limited. The latest technological development in the form of 16S rDNA sequencing has proven the existence of other microbes whose growth is increased due to the effect of prebiotics, namely *Pediococcus*, *Lactococcus*, *Streptococcus* [2], *Anaerostipes* [3], and *Bacteroidetes* [4].

Some prebiotic components are indigestible oligosaccharides such as fructooligosaccharides and galactooligosaccharides. Those are found in breast milk or cow’s milk. Inulin is a commercial prebiotic extracted from the tubers of Jerusalem artichoke, chicory, dahlia, and yacon. Other prebiotic
components that have been widely studied include soluble fiber and dietary fiber. Polyphenols also have criteria as prebiotics, but further studies are still needed to support this hypothesis. Substrates that affect the composition of the microbiota through mechanisms that do not involve selective use of the substrate by microorganisms cannot be called prebiotics. These substrates include antibiotics, minerals, vitamins, and bacteriophages [1].

The development of new prebiotic sources continues today, one of which is raffinose [5]. Reports show that raffinose can increase the composition of *Lactobacillus* and *Bifidobacterium* [6,7], decrease the composition of *Clostridium perfringens* [7] and ammonia-producing bacteria [6], and increase the production of SCFA [6]. Raffinose has health benefits for modulating the gut microbiota composition [6], treating constipation [7], lowering blood cholesterol levels [8], reducing the formation of putrefactive compounds from protein [9], and others.

Initially, raffinose was considered an anti-nutritional compound because it caused flatulence due to excessive gas production. The production of CO$_2$ and H$_2$ gases is carried out by the gut microbiota, which indicates a fermentation process by the gut microbiota. This article will discuss the latest research related to the chemical structure, degrading enzyme, and benefits of raffinose as a prebiotic.

### 2. Chemical structure of raffinose

Raffinose is an oligosaccharide found in legumes, peas, lentils, soybeans [10], seeds, roots[11], and leaves, such as the leaves of the herbal plant *Eupatorium* [12]. The raffinose content in lupine nuts depends on the type of cultivar. Some cultivars are high sources of raffinose. The raffinose content in lupine nuts can be increased by dehulling, which is a process to remove the outer shell of lupine nuts [13].

![Figure 1. Chemical structure of raffinose](image)

D-(+)-raffinose is a pentahydrate crystalline oligosaccharide. It consists of three monosaccharide units, namely: α-D-galactose, α-D-glucose, and β-D-fructose with 1→6 and 1→2 glycosidic bonds.
The full name of raffinose is O-\(\alpha\)-D-galactopyranosyl-[1-6]-\(\alpha\)-D-glucopyranosyl-[1-2]-\(\beta\)-D-fructofuranoside. The five water molecules in raffinose hydrate are linked in a chain of sugar molecules gula [11]. Raffinose contains a disaccharide, which is either melibiose or sucrose (Figure 2). Raffinose is the smallest compound in the raffinose-family oligosaccharides (RFOs). While members of RFOs are raffinoses, stachyose, verbascose, and others. RFOs consist of several \(\alpha\)-D-galactose bound to \(\alpha\)-1,6 galactoside, \(\alpha\)-D-glucose, and \(\beta\)-D-fructose (Figure 2).

3. Raffinose degrading enzyme
The degradation of raffinose (and RFOs in general) requires the enzyme \(\alpha\)-1,6-galactosidase (EC 3.2.1.22) to hydrolyze the \(\alpha\)-1,6-galactosidic linkage (Figure 3). The human digestive system cannot produce the \(\alpha\)-1,6-galactosidase enzyme so that humans cannot digest raffinose. However, gut microbiota can metabolize raffinose [14]. The metabolism of raffinose by these microbes produces \(\text{CO}_2\) and \(\text{H}_2\) gases, causing flatulence in the stomach [6]. Thus, raffinose was formerly referred to as an anti-nutritional compound. However, recent research shows that raffinose has the potential as a prebiotic by specifically stimulating the growth of microbes in the gastrointestinal tract of humans and animals.

Figure 3. RFOs' degrading enzymes (http://bit.ly/38hY1Br).
4. Benefits of raffinose as a prebiotic

4.1. Modulate the composition of the gut microbiota

There are 300-500 species of bacteria in the human gut. The most dominant bacterial phyla are Bacteroidetes and Firmicutes, constituting >90% of the microbiota population. Bacteroidetes include *Bacteroides* and *Prevotella*, while Firmicutes contain *Clostridium*, *Eubacterium*, and *Ruminococcus*. The phyla Actinobacteria, Proteobacteria, Fusobacteria, Spirochaetes, Verrucomicrobia, and Lentisphaerae are also present in the human gut, although in lower composition (Figure 4). Every human being has a different composition of gut bacteria [14].

An in vitro study of raffinose fermentation using human feces from 2 healthy adult donors was carried out. Fermentation was carried out for 48 hours under anaerobic conditions. It showed changes in the composition of probiotics, namely *Lactobacillus* and *Bifidobacterium*. There was an increase of *Lactobacillus* in donor 1 (Figure 5A and 5B) and *Bifidobacterium* in donor 2 (Figure 5C and 5D) [6]. In addition, Proteobacteria pathogenic bacteria decreased during the fermentation process. Proteobacteria are the cause of various gastrointestinal tract diseases, for example, *Escherichia coli* [6].

Besides, ammonia gas produced during the raffinose fermentation decreased significantly compared to the control (Figure 5). It indicated that the population of ammonia-producing bacteria, namely *Escherichia coli* and *Clostridium sp*, decreased after consuming raffinose [6]. Therefore, it could be concluded that raffinose could change the microbiota composition in the human gut.

![Figure 5](image-url) The gut microbiota composition after 48 hours of in vitro fermentation from healthy adult donor 1: A, in control media without prebiotics and B, in media with raffinose. The gut microbiota composition after 48 hours of in vitro fermentation from healthy adult donor 2: C, in control media without prebiotics and D, in media with raffinose (D) [6].
The composition of microbes whose growth is influenced by raffinose also has differences in each person. An in vivo study reported the effect of raffinose on the composition of the pool of fecal microbiota in normal-weight children, over-weight children, and healthy adults (Figure 6). It showed that raffinose consumption increased the Firmicutes bacteria composition and decreased the Actinobacteria in over-weight children. Enriched growth of Firmicutes in over-weight children was related to the growth of *Streptococcus* and *Solobacterium*. The raffinose fermentation increased the Actinobacteria in normal-weight children. The expanded population of *Bifidobacterium*, *Collinsella*, and *Senegalimassilia* could be responsible for the high Actinobacteria growth. Raffinose also stimulated the growth of Actinobacteria and Proteobacteria in healthy adults [10].

![Figure 6. Gut microbiota population in the fermentation of levans (LevR, LevS, and LevT), raffinose (Raf), melibiose (Mel), and raffinose-mix (Mix) from the fecal pools of healthy adults (AD), over-weight children (OW), and normal-weight children (NW). The darkest the blue color, the more population of the microbiota [10].](image)

**4.2. Increase SCFA production**

Prebiotic fermentation by gut microbiota will produce SCFA. Production of lactic acid and other SCFA increased during the in vitro fermentation of raffinose for 48 hours under anaerobic conditions, using normal adult human feces. The maximum production of lactic acid and other SCFA was reached when the fermentation lasted between 36–48 hours. Most of the raffinose was consumed rapidly during the 24-hour fermentation duration. Figure 7 showed that acetic acid was the most abundant SCFA produced in the raffinose fermentation process. Acetic acid was reported to have an essential role in the prevention of colorectal cancer. Lactic acid was the second dominant metabolite produced in raffinose fermentation. However, butyric acid was not produced in the raffinose fermentation process [6].

Besides, normal-weight children and over-weight children who consumed raffinose also showed differences in the composition of SCFA. Metabolites in normal-weight children were primarily acetic acid and butyric acid. In contrast, the dominant metabolites in over-weight children were lactic acid and acetic acid. The difference in SCFA produced by normal-weight children and over-weight children indicated a difference in the microbiota composition in their gastrointestinal tract [10].
Figure 7. SCFA production during fermentation on raffinose for 48 hours, using feces from two donors. A negative control did not contain any prebiotics, while a positive control contained 10 g/L lactulose. Raf sample contained 10 g/L raffinose. Analyzed SCFA included lactic acid ( ), acetic acid ( ), propionic acid ( ), and butyric acid ( ) [6].

Lactic acid is a SCFA precursor that is reported to have bioactive components for human health, namely regulating the critical functions of macrophages and dendritic cells in the immune system and regulating inflammatory activity in epithelial cells. Lactic acid and lactic acid bacteria can affect parts outside the intestine, such as the vagina. It also defends the system against pathogenic microbes in the vagina, such as bacterial vaginosis, aerobic vaginitis bacteria, viruses, fungi, and protozoa [15].

4.3. Reduce constipation
The characteristics of constipation are the frequency of defecation less than 2-3 times a week, small, dry or hard stools, and the process of defecation is difficult. The microbiota composition in the intestines of constipated patients shows a decrease in the population of obligate bacteria and an increase in pathogenic bacteria. Therefore, one way to reduce constipation is to regulate the composition of the gut microbiota.

A total of 103 patients with constipation were treated to consume 5 g of DSG (Deshipu stachyose granules) for 30 days. DSG contained RFOs such as stachyose (55.3%), raffinose (25.8%), verbascose (9.7%), and sucrose (6.9%). Consumption of DSG rich in RFOs could improve bowel function in constipated patients by increasing bowel movement frequency, softer stools, and easier defecation [7].

Cecum weight in RD rats fed a high-fat, high-calorie diet decreased compared to RD rats fed a regular diet. However, raffinose supplementation on a high-calorie, high-fat diet increased cecum weight in RD rats [16].

4.4. Inhibit the putrefactive compounds from protein
Intestinal microbes can be beneficial for humans as well as harmful effects. Gut microbes can convert protein residues in the intestine into putrefactive compounds, such as ammonia, amines, phenols, and indole. These putrefactive compounds are thought to contribute to tumor formation. Wistar rats fed with a soy protein diet experienced an increase in indole in the intestine. Tryptophan-rich soy protein can be converted to indole by the gut microbiota. In vitro study was performed using media inoculated with human feces using soy protein substrate. This study reported the effect of raffinose on the formation of putrefactive compounds. Gut microbiota had grown on media containing raffinose. It resulted in lower concentrations of ammonia, indole, and phenol. It might be caused by the high lactic acid production leading to a pH decrease [9]. Raffinose could prevent the formation of putrefactive compounds resulting from protein metabolism by the gut microbiota.

4.5. Reduce the risk of cardiovascular disease
Cardiovascular disease is a threat to human health. This disease is caused by dietary habits, especially the consumption of saturated fatty acids and animal cholesterol. However, recent data suggest that L-carnitine had a positive correlation with the accumulation of endothelial dysfunction, which was an early phase of cardiovascular disease. L-carnitine was abundant in red meat and contained a trimethylamine structure. The gut microbiota then metabolized trimethylamine to trimethylamine-N-
oxide (TMAO), which caused cardiovascular disease. Consumption of L-carnitine in high concentrations also caused metabolic disturbances in cholesterol, sterol, and lipid levels in the blood and reduces nitric oxide (NO) synthesis. NO was a major relaxing factor derived from vascular endothelial cells and essential in maintaining vascular homeostasis and endothelial function [8].

Mice were allowed to consume 3% L-carnitine for three weeks. Then the mice consumed RFOs as much as 200, 400, and 800 mg/kg bw. The results of this study showed that RFOs at concentrations of 400 and 800 mg/kg bw were significantly (p<0.01) able to reduce cholesterol, triglyceride, and low-density lipoprotein concentrations, as well as increase high-density lipoproteins. In addition, these concentrations of RFOs were also able to prevent a decrease in NO and an increase in C-reactive protein markers, which was a marker of cardiovascular disease [8].

High blood cholesterol levels are also one of the causes of cardiovascular disease. There are two types of cholesterol sources in the human body: 1) derived from biosynthesis in the liver, and 2) derived from the diet, especially from animal fat. Currently, drugs to lower cholesterol are available, such as statins. However, there are some reports of side effects from using these drugs. Prebiotics and probiotics are safe alternatives to lower blood cholesterol [17].

The mechanism of reducing blood cholesterol levels caused by prebiotics and probiotics is still being debated. This cholesterol-lowering mechanism requires the synergistic performance of prebiotics and probiotics. The proposed mechanism was as follows [17]:

4.5.1. **Bile salt deconjugation by BSH.** The bile salt deconjugation process is carried out by probiotic bacteria’s bile salt hydrolase (BSH) enzyme. The deconjugation process will reduce cholesterol. Generally, conjugated bile salts are circulated through the enterohepatic tract. In contrast, deconjugated bile salts are more soluble in water and can be excreted in the feces. Bile salts that are excreted in the feces must be replaced by new bile salts synthesized from blood cholesterol. Therefore, the more bile salts that come out through the feces, the more cholesterol is taken from the blood, lowering cholesterol levels in the blood.

4.5.2. **Coprecipitation of cholesterol with deconjugated bile.** Prebiotic fermentation by probiotic bacteria produces SCFA, which results in a decrease in pH. Cholesterol will coprecipitate with deconjugated bile salts at a pH lower than 5.5.

4.5.3. **Cholesterol use by cellular membranes.** Cholesterol is absorbed by the cellular membrane of probiotic bacteria and used for the growth of these bacteria. *Lactobacillus* carries out this mechanism. This mechanism causes a decrease in cholesterol absorption by the blood in the intestines, thereby reducing cholesterol levels in the blood.

4.5.4. **Cholesterol assimilation by probiotics.** Probiotic bacteria such as lactic acid bacteria carry out cholesterol assimilation. Cholesterol is bound to the cellular membrane of probiotics and is used in the phospholipid layer of the bacterial membrane. This assimilation process occurs under anaerobic conditions.

5. **Conclusion**
Prebiotics benefit from maintaining the composition of the gut microbiota by becoming substrates for the fermentation of probiotic bacteria. The fermentation can produce metabolites such as SCFA and offer health benefits to both humans and animals. Prebiotics are functional food components that can prevent cardiovascular disease and other degenerative diseases. The search for new sources of prebiotics continues. Raffinose is one of the novel prebiotics. The potential of raffinose as a prebiotic has been studied recently. Raffinose has many benefits for human health. Furthermore, it is necessary to conduct further research on the use of raffinose in various food products to maintain the benefits of prebiotics and maintain the sensory properties of these food products.

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7. References

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