



A review of the mechanism of prebiotic polyphenols in apples to enhance the activity of probiotics in fermented foods

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Abstract

Apple is one of the most popular and sought-after fruits worldwide because it contains large amounts of nutrients, antioxidants, and bioactive compounds that play a significant role in human nutrition and health. The polyphenols in apples are known as natural antioxidants and play a significant role in improving general health. The main objective of this study is to investigate the function of polyphenols as probiotics and their effects on the activity of probiotics in fermented foods. For this purpose, Google scholar, sciencedirect and PubMed/Medlin databases were used to collect relevant studies from ۲۰۱۲ to ۲۰۲۴. And articles related to the properties and compositions of apple polyphenols, their effective mechanisms on the intestinal microbiota, their synergistic effects with probiotics and their application in the development of fermented foods were reviewed. Polyphenols, due to their specific chemical structure, have the ability to influence the composition and function of the intestinal microbiota. These compounds act as prebiotics and substrates to nourish and stimulate the growth of beneficial intestinal bacteria, thereby improving gastrointestinal health. Furthermore, apple polyphenols can improve the efficacy and viability of probiotics by interacting with probiotics in fermented foods, increasing the effectiveness of these products in improving consumer health. Future research should focus on the clinical validation of these benefits and innovative processing techniques to maximize the bioavailability and efficacy of these valuable compounds in everyday diets.

Keywords: Polyphenols, apples, prebiotics, probiotics, fermentation



۱-Introduction

The apple is one of the most widely consumed temperate fruits in many parts of the world. Due to its availability throughout the year, it is considered as a fresh fruit or a processed food (drinks such as juices, concentrates and purees). The apple is rich in dietary fiber, vitamins (vitamins E-C) and minerals such as potassium, hence promoting general human health. The rich fiber content helps maintain a healthy digestive tract. These fruits contain a wide range of phytochemicals with high levels of polyphenols, which have protective effects in preventing degenerative diseases and cardiovascular diseases and cancer (۱). Polyphenols are a class of natural compounds widely present in fruits, vegetables and beverages such as tea and wine. They can be divided into several categories including flavonoids and phenolic acids. The latter compounds are well known for their potent antioxidant properties, which play an important role in combating oxidative stress. In the human body, polyphenols modulate various biological pathways, enhance cellular defense mechanisms, and reduce inflammation. Thus, they help prevent the development of diseases such as cancer and cardiovascular disorders. Apples are a rich source of polyphenols, including quercetin and catechin. Both polyphenols found in apples play an important role in promoting health by protecting cells from oxidative damage and supporting metabolic processes (۲, ۳). The gut microbiota plays an important role in gut health, and prebiotics or probiotics play an important role in this ecosystem. By definition, prebiotics are non-digestible food substances that selectively stimulate the growth of beneficial bacteria. While the term probiotics is used for live microorganisms that, when consumed in sufficient amounts, are beneficial to the host. The interaction of prebiotics with probiotics is crucial in maintaining gut health by improving nutrient absorption, modulating immune responses, and inhibiting the growth of pathogenic bacteria. In



fact, some studies have confirmed that the dietary fiber in apple can act as a prebiotic, thereby stimulating the proliferation of beneficial bacteria such as bifidobacteria and lactobacilli. This symbiotic relationship is important for gut integrity (٤, ٥). The aim of this study was to investigate the functional role of apple polyphenols as prebiotics and their potential to enhance probiotic activity in fermented foods. This article provides a comprehensive review of how these bioactive compounds can be used to promote gut health by combining current research findings on the biological significance of apple polyphenols and their impact on the interaction mechanisms of prebiotics and probiotics in the gut microbiome.

١-١ Characteristics and Composition of Apple Polyphenols

Apples are a rich source of bioactive polyphenolic compounds, the amount of which is higher in the skin of the apple than in its pulp. This is likely to protect the fruit from the effects of ultraviolet radiation and pathogens. On the one hand, this highlights the importance of consuming whole apples to maximize their health benefits (١). In general, the polyphenols found in apple skin and pulp include:

a- Flavonoids: This group consists of flavanols such as catechin, epicatechin, procyanidin, dihydrochalcones (phloridzin and its derivatives), chloridzin, and quercetin glycoside. These compounds are known for their antioxidant properties and potential health benefits (٦).

B- Phenolic acids: These include caffeic acid, picomeric acid, and hydroxycinnamic acids (including chlorogenic acid), with chlorogenic acid being the most common phenolic acid in apple and contributing significantly to its antioxidant capacity (٧). Furthermore, the composition of these polyphenols can vary significantly based on the apple variety, with certain cultivars exhibiting higher concentrations of certain compounds. For example, Lee et al. (٢٠٢١) measured phenolic compounds from five different apple varieties grown in Australia (Royal Gala, Pink Lady,

Red Delicious, Fuji, and Smitten) using LC-MS/MS and HPLC. According to their observations, Red Delicious had the highest total phenolic content (121.78 ± 3.40 mg/g b.w.) and total flavonoid content (101.23 ± 3.70 mg/g b.w.) among the five apple samples (⁸). In another study of three apple varieties grown in Chile, the total polyphenol content in whole apple and pulp was shown to be Grani Smith > Royal Gala > Fuji (⁹).

Several factors influence the concentration of polyphenols in apple, including: a- Cultivation methods: Growing conditions such as soil quality, climate and agricultural practices can significantly affect polyphenol levels. For example, a study analyzed and compared three apple varieties grown in organic and conventional orchards in Poland in terms of the concentration of antioxidants such as phenolic acids, flavonols and vitamin C and showed that the organic production system has the potential to provide apple fruits rich in phenolic antioxidants (¹⁰). b- Storage conditions: Storage conditions (temperature and storage time) after harvest also play a decisive role. Its polyphenol level and antioxidant capacity can decrease during long-term storage due to enzymatic activity and oxidative processes (¹¹). C. Processing methods: Different processing techniques can alter the polyphenol profile. For example, juicing or drying apples may concentrate certain polyphenols while reducing others due to heat exposure or oxidation. Research has shown that mild treatments and high-pressure processing increase dihydrochalcones, hydroxycinnamates, and proanthocyanidins and decrease flavonols. In apples, standard heat treatment increased the concentration of flavonols and dihydrochalcones (¹²). Understanding these factors is essential to optimize the nutritional quality of apples and enhance their health benefits through agricultural and processing innovations.

۲.۱ Mechanisms of action of polyphenols as prebiotics



Polyphenols play an important role in modulating the gut microbiota, acting as prebiotic substrates that promote the growth of beneficial bacteria while inhibiting pathogenic species. Research suggests that polyphenols can increase the number of probiotics, such as *Bifidobacterium* and *Lactobacillus*, which are crucial for maintaining gut health. By altering the composition of the gut microbiota, polyphenols contribute to a balanced microbial ecosystem, which is essential for optimal digestive function and overall health (۱۳, ۱۴). Despite extensive research on various metabolites, little is known about the chemical metabolic pathways used by different bacterial species. However, studies support the idea that polyphenols promote the growth of beneficial gut bacteria (۱۴). For example, polyphenolic compounds have been shown to stimulate the proliferation of key bacterial species such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and various strains of *Lactobacillus*. These beneficial bacteria not only enhance the integrity of the intestinal barrier, but also have anti-inflammatory effects, thereby helping to improve metabolic health. The antimicrobial properties of polyphenols facilitate this process by reducing the population of harmful bacteria, creating ecological niches for the growth of beneficial species (۱۳). Polyphenols provide ideal conditions for the growth of beneficial bacteria by stimulating mucin production. Thus, polyphenols appear to exert effects comparable to those of probiotics on a small scale by inducing a host response that alters the bacterial niche (۱۲). Since prebiotics are usually carbohydrates with stable chemical structures that are resistant to hydrolysis by intestinal enzymes over a wide range of temperatures, pH, and temperatures, they are fermented by the lower intestinal microbiota and act as a stimulus for microbial growth. Therefore, the mechanisms through which polyphenols can exert their prebiotic effects include fermentation by intestinal bacteria and the production of metabolites such as short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate. These SCFAs play multiple physiological roles in the



human body. These metabolites are produced and absorbed in the large intestine and cause a concomitant decrease in the pH of urine and feces, which inhibits the growth of pathogenic microorganisms. In addition, they provide energy for colonocytes, regulate intestinal motility, modulate glucose and lipid metabolism, and inflammatory responses, and enhance the immune system (^۱).

۳.۱ Synergism of polyphenols and probiotic activity

The interaction between apple polyphenols and probiotics in fermented foods is a vital area of research, highlighting the potential of polyphenols to enhance the health benefits of these products. Polyphenols can act as prebiotic substrates, promoting the growth of beneficial bacteria (such as Bifidobacteriaceae and Lactobacillaceae) while simultaneously inhibiting pathogenic strains (such as *Escherichia coli* and *Clostridium perfringens*). This dual action, termed “bipolybiotic,” suggests that polyphenols can modulate the microbial community during fermentation, thereby improving the overall quality and bioactivity of fermented foods. During fermentation of polyphenol-rich foods, microorganisms can metabolize complex polyphenolic compounds into smaller, more bioavailable forms, increasing their nutritional value and health benefits (^{۱۴}). For example, two specific strains of lactic acid bacteria (LAB), *Lactobacillus paracasei* and *Lactobacillus plantarum*, have been shown to modify the phenolic compounds of apple juice during fermentation, increasing the antioxidant capacity of fermented apple juice by producing metabolites such as alcohol, acid, and ketone (^{۱۵}). Research has shown that the incorporation of apple polyphenols into fermented foods can significantly increase the survival rate of probiotics. For example, one study showed that apple peel polyphenol extracts (APPE) improved the survival and adherence of *Lactobacillus acidophilus* in frozen yogurt (^{۱۶}). The presence of these polyphenols not only increases the



survival of probiotics, but also improves their metabolic function, leading to increased production of beneficial metabolites such as lactic acid (۱۷). Furthermore, apple juice fermented by lactic acid bacteria strains showed how polyphenols can enhance the activity of probiotics during fermentation, leading to higher viable cell counts and higher lactic acid levels (۱۸). Several studies have investigated the polyphenolic composition of apple in various fermented food products:

A- Yogurt: A study investigated the effect of apple peel extract on the survival of probiotics in frozen yogurt stored at -20°C for 90 days. The results showed that the addition of 5% apple peel polyphenolic extract significantly increased the survival of probiotics compared to the control samples. Increased acidity and improved sensory properties were also noted (۱۶).

B- Kombucha: Research on the fermentation of kombucha with 3 sources of black tea, green tea and oolong tea has shown that the addition of polyphenol-enriched apple juice can increase the growth of beneficial bacteria while improving antioxidant properties. The fermentation process resulted in an increase in bioactive compounds and improved sensory properties of the product due to the synergistic effects between apple-derived polyphenols and probiotic cultures (۱۸).

C- Apple juice fermentation: In a study involving the use of *Lactobacillus* strains (*acidophilus*, *casei* and *plantarum*) in the fermentation of apple juice for ۷۲ hours, a significant increase in the number of viable cells (from $۷.۵ \log \text{CFU/mL}$ to $۸.۳ \log \text{CFU/mL}$) was observed, along with an increase in antioxidant activity and improved antibacterial properties after fermentation. This suggests that apple polyphenols not only support the growth of probiotics, but also contribute to the functional quality of the fermented product (۱۹).



D. Apple cider vinegar fermentation: Fermentation of apple cider vinegar using specific strains of *Acetobacter* increases the concentration of bioactive compounds, including SCFAs, which are beneficial for gut health. The presence of apple polyphenols during this process helps maintain the stability and efficacy of probiotics (۲۰).

۲- Material Method

In this review, a search was conducted to identify studies conducted by referring to the Google scholar, sciencedirect and PubMed/Medlin databases to collect relevant studies from ۲۰۱۲ to ۲۰۲۴.

۳- Discussion and Conclusion

In summary, apple polyphenolic compounds play an important role in the development of functional foods and act as key bioactive compounds that help promote health and prevent diseases. On the other hand, new trends are being directed towards the integration of prebiotics and probiotics into daily food with an emphasis on gut health. This trend has arisen in response to the growing consumer demand for products that have health-promoting benefits in addition to their primary nutritional benefits. In recent years, the incorporation of apple-derived phenolic compounds into functional foods has been in line with this trend, as these components have a high potential to promote probiotic activity and gut microbial homeostasis, which is why there is innovation in the formulation of products such as probiotic-containing snacks and beverages enriched with polyphenols (۱۶, ۱۷). Future research should focus on the clinical validation of these



benefits and innovative processing techniques to maximize the bioavailability and efficacy of these valuable compounds in everyday diets.

4-Reference

۱. Ribeiro JA, dos Santos Pereira E, de Oliveira Raphaelli C, Radünz M, Camargo TM, da Rocha Concenço FIG, et al. Application of prebiotics in apple products and potential health benefits. *Journal of Food Science and Technology*. ۲۰۲۲;۱-۱۴.
۲. Williamson G. The role of polyphenols in modern nutrition. *Nutrition bulletin*. ۲۰۱۷;۴۲(۳):۲۲۶-۳۵.
۳. Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, et al. Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in pharmacology*. ۲۰۲۲;۱۳:۸۰۶۴۷۰.
۴. Yoo S, Jung S-C, Kwak K, Kim J-S. The Role of Prebiotics in Modulating Gut Microbiota: Implications for Human Health. *International Journal of Molecular Sciences*. ۲۰۲۴;۲۵(۹):۴۸۳۴.
۵. Shirbhate U, Bajaj P, Chandak M, Jaiswal P, Sarangi S, Suchak D, et al. Clinical implications of probiotics in oral and periodontal health: a comprehensive review. *Cureus*. ۲۰۲۳;۱۵.(۱۲)
۶. Starowicz M, Achrem-Achremowicz B, Piskula MK, Zieliński H. Phenolic compounds from apples: reviewing their occurrence, absorption, bioavailability, processing, and antioxidant activity—a review. *Polish Journal of Food and Nutrition Sciences*. ۲۰۲۰;۷۰(۴):۳۲۱-۳۶.
۷. Benvenuti L, Bortolini DG, Fischer TE, Zardo DM, Nogueira A, Zielinski AAF, et al. Bioactive compounds recovered from apple pomace as ingredient in cider processing: monitoring of compounds during fermentation. *Journal of Food Science and Technology*. ۲۰۲۲;۱-۱۰.
۸. Li H, Subbiah V, Barrow CJ, Dunshea FR, Suleria HA. Phenolic profiling of five different Australian grown apples. *Applied Sciences*. ۲۰۲۱;۱۱(۵):۲۴۲۱.
۹. Quitral V, Sepúlveda M, Schwartz M. Antioxidant capacity and total polyphenol content in different apple varieties cultivated in Chile. *Revista Iberoamericana de Tecnología Postcosecha*. ۲۰۱۳;۱۴(۱):۳۱-۹.
۱۰. Średnicka-Tober D, Barański M, Kazimierczak R, Ponder A, Kopczyńska K, Hallmann E. Selected antioxidants in organic vs. conventionally grown apple fruits. *Applied Sciences*. ۲۰۲۰;۱۰(۹):۲۹۹۷.



۱۱. Matthes A, Schmitz-Eiberger M. Polyphenol content and antioxidant capacity of apple fruit: effect of cultivar and storage conditions. *Journal of Applied Botany and Food Quality*. ۲۰۱۲;۸۲(۲):۱۵۲-۷.
۱۲. Salazar-Orbea GL, García-Villalba R, Bernal MJ, Hernández A, Tomás-Barberán FA, Sánchez-Siles LM. Stability of phenolic compounds in apple and strawberry: Effect of different processing techniques in industrial set up. *Food Chemistry*. ۲۰۲۳;۴۰۱:۱۳۴۰۹۹.
۱۳. Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D, Desjardins Y, Roy D. Polyphenol-mediated gut microbiota modulation: toward prebiotics and further. *Frontiers in Nutrition*. ۲۰۲۱;۸:۶۸۹۴۵۶.
۱۴. Plamada D, Vodnar DC. Polyphenols—Gut microbiota interrelationship: A transition to a new generation of prebiotics. *Nutrients*. ۲۰۲۱;۱۴(۱):۱۳۷.
۱۵. Liang JR, Deng H, Hu CY, Zhao PT, Meng YH. Vitality, fermentation, aroma profile, and digestive tolerance of the newly selected *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* in fermented apple juice. *Frontiers in Nutrition*. ۲۰۲۲;۹:۱۰۴۵۳۴۷.
۱۶. Ahmad I, Khalique A, Junaid M, Shahid MQ, Imran M, Rashid AA. Effect of polyphenol from apple peel extract on the survival of probiotics in yoghurt ice cream. *International Journal of Food Science & Technology*. ۲۰۲۰;۵۵(۶):۲۵۸۰-۸.
۱۷. Zhao R, Ran J, Ruan X, Du H, Li G, Zhao L, et al. Apple polyphenol biotransformation using probiotics in vitro and dynamic simulated digestion by bionic rats. *Journal of the Science of Food and Agriculture*. ۲۰۲۳;۱۰۳(۱۱):۵۴۹۰-۹.
۱۸. Osiripun V, Apisittiwong T. Polyphenol and antioxidant activities of Kombucha fermented from different teas and fruit juices. *Journal of Current Science and Technology*. ۲۰۲۱;۱۱(۲):۱۸۸-۹۶.
۱۹. Yang J, Sun Y, Gao T, Wu Y, Sun H, Zhu Q, et al. Fermentation and storage characteristics of “Fuji” apple juice using *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum*: Microbial growth, metabolism of bioactives and in vitro bioactivities. *Frontiers in nutrition*. ۲۰۲۲;۹:۸۳۳۹۰۶.
۲۰. Mathew B, Agrawal S, Nashikkar N, Bundale S, Upadhyay A. Isolation of acetic acid bacteria and preparation of starter culture for apple cider vinegar fermentation. *Advances in Microbiology*. ۲۰۱۹;۹(۶):۵۵۶-۶۹.