Effects of Fermentation with Different Lactic Acid Bacteria on Nutritional Components and Antioxidant Activity of Aloe Juice

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Abstract [**Objectives**] This study was conducted to elucidate the fermentation characteristics of different bacterial strains and identify the optimal fermentation strain, so as to provide a theoretical basis for the development of functional aloe beverages. [**Methods**] Using fresh juice from *Aloe vera* L. as the raw material, the proliferation characteristics of *Lactobacillus plantarum* (LP), *Lactobacillus reuteri* (LR), *Lactobacillus rhamnosus* (LGG) and *Pediococcus pentosaceus* (PP) during fermentation were investigated. Changes in pH, total viable bacterial count, total sugars, acemannan (AC), total flavonoid content (TFC) and total phenol content (TPC), and their effects on antioxidant activity (DPPH, ABTS, FRAP) were measured before and after fermentation. [**Results**] Aloe juice served as an excellent growth substrate for all four lactic acid bacterial strains, with viable bacterial counts significantly increasing after fermentation. The LGG group achieved the highest viable count (12.82 lg CFU/ml), followed by the LP group (12.77 lg CFU/ml). All four strains significantly enhanced the total phenol content and antioxidant capacity of the aloe juice. The LP group demonstrated the most outstanding performance, achieving the highest increase in TPC, the smallest reduction in TFC (from 0.054 mg/ml to 0.019 mg/ml), and the strongest comprehensive antioxidant activity (DPPH scavenging rate: 80.33%, ABTS scavenging rate: 93.15%, FRAP value: 0.167 mmol/L). Additionally, it also better preserved the functional component AC. [**Conclusions**] *L. plantarum* demonstrated optimal performance in enhancing the antioxidant activity and preserving nutritional components of aloe juice, making it an ideal strain for aloe juice fermentation. This study provides a foundation for developing functional aloe beverages.

Key words Aloe juice; Probiotics; Fermentation; Bioactive compound; Antioxidant capacity

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Aloe (*Aloe vera* L.), as an important functional economic crop, is widely used in the food, pharmaceutical, and cosmetics industries. Its juice is rich in polysaccharides (such as acemannan), phenols, flavonoids, amino acids, and active enzymes, and possesses multiple functions including antibacterial, anti-inflammatory, and antioxidant properties[1], making it highly favored by consumers. According to market research data, the global market size for fresh aloe juice has reached billions of dollars, with a compound annual growth rate exceeding 5%, and the Asia-Pacific region is the primary consumer market. *Lactobacillus* fermentation is a crucial method for regulating the functional properties of fruit and vegetable juices. Fermentation with lactic acid bacteria serves as a significant technique for modulating the functional properties of fruit and vegetable juices. Previous studies have shown that lactic acid bacteria can alter the composition of organic acids, sugars, and phenolic compounds in juices to regulate their antioxidant activity, while also generating new volatile substances to improve flavor and stability[2]. For instance, different lactic acid bacterial strains can influence the color and antioxidant capacity of orange juice by regulating its phenolic content, thereby enhancing the nutritional value and bioavailability of the juice[3-4]. The polysaccharides, proteins and phenolic compounds in aloe juice provide ample nutrients for the growth of lactic acid bacteria[5]. Moreover, lactic acid bacteria can enhance the antioxidant function of juices through pathways such as scavenging free radicals, chelating metal ions, and improving antioxidant enzyme activity[6].

In this study, four common lactic acid bacterial strains were selected to ferment aloe juice, and the fermentation characteristics of different bacterial strains were elucidated by monitoring dynamic changes in nutritional components (total sugar content, acemannan, toal flavonoid content, toal phenol content) and antioxidant activity, so as to identify the optimal fermentation strain. This study provides a theoretical basis for the development of functional aloe beverages.

Materials and Methods

Materials and reagents

Raw materials *A. vera* was collected from Jiangdong, Yuanjiang County, Yuxi City, Yunnan Province. The plants were fresh and undamaged, and were processed immediately after peeling.

Bacterial strains *Lactobacillus plantarum* (LP), *Lactobacillus reuteri* (LR), *Lactobacillus rhamnosus* (LGG) and *Pediococcus pentosaceus* (PP) were all purchased from Shanghai Yangkong Industrial Co., Ltd.

Reagents Total antioxidant capacity (T-AOC) assay kit (Nanjing Jiancheng Bioengineering Institute); DPPH (analytically pure, Nanjing Jiujia Technology Co., Ltd.); ABTS (analytically pure, Woruida Technology Co., Ltd.); Folin-Ciocalteu reagent

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(analytically pure, Woruida Technology Co., Ltd.); plant total sugar assay kit (DNS micro plate method, Leagene Biotechnology Co., Ltd.); gallic acid, rutin and aluminum chloride (analytically pure, Shanghai Macklin Biochemical Co.,Ltd.).

Experimental methods

Preparation of aloe juice Fresh, undamaged aloe leaves were selected and rinsed with drinking water, peeled, and homogenized into pulp. The pulp was then filled into 100 ml fermentation bottles. The bottled aloe juice was sterilized in a water bath at 90 ℃ for 10 min[7].

Preparation of lactic acid bacteria starter cultures[8] The four lactic acid bacterial strains were individually inoculated into MRS medium and cultured at 37 ℃ for 12 h. Streaking was performed, followed by continued culture for 24 h. Single colonies were selected for purification culture. The four purified lactic acid bacterial strains were then separately inoculated into MRS broth medium and cultured at 37 ℃ for 24 h. Each bacterial suspension was transferred into MRS broth and cultured again at 37 ℃ for 12 h. The resulting bacterial cultures were centrifuged at 8 000 r/min and 4 ℃ for 10 min to collect the bacterial cells. After washing twice with sterile physiological saline, the bacterial suspensions were prepared for subsequent inoculation and fermentation.

Fermentation of aloe juice with lactic acid bacteria The four different lactic acid bacterial strains were inoculated into the aliquoted aloe juice with a final concentration of 5×10^6 CFU/ml. The inoculated samples were then sealed and subjected to static fermentation at 37 ℃ for 48 h.

Measurement methods Determination of total viable bacterial count in fermentation broth: A 1 ml sample of the fermentation broth was diluted with physiological saline to concentrations ranging from 10^7 to 10^{11} times (with dilution factors adjusted appropriately based on fermentation time). After thorough mixing, the samples were incubated at 37 ℃ for 48 h. Colony counts were recorded as lg (CFU/ml) using MRS medium, with measurements taken every 12 h[3]. MRS culture medium was used for counting, and the colony counts were recorded as lg (CFU/ml), which was determined every 12 h[3].

pH measurement: The pH values of the aloe juice before and after fermentation with the four lactic acid bacterial strains were measured using a pH meter.

Total sugar content determination: Changes in total sugar content during fermentation were measured using plant total sugar and reducing sugar assay kits (DNS microplate method). Following the kit instructions, the samples were hydrolyzed and extracted to obtain the extract, and the absorbance was measured at 540 nm. The results, calibrated against a glucose standard curve (0 – 1 mg/ml, $R^2 = 0.99$), were expressed as glucose equivalents (mg/ml) of the sample.

O-acetyl group (acemannan) content determination[9]: A 1 ml aliquot of sample supernatant was mixed with 2 ml of alkaline hydroxylamine hydrochloride (2.5 mol/L), and the mixture was allowed to stand for 5 min. Then, 1 ml of HCl (4 mol/L) was added to adjust the pH to 1.0 – 1.2, followed by the addition of $FeCl_3$-HCl (0.37 mol/L) and thorough mixing. The absorbance was measured at 540 nm using an ultraviolet-visible spectrophotometer. The results, calibrated against an O-acetyl group standard curve (0 – 45.4 mg/100 ml, $R^2 = 0.99$), were expressed as O-acetyl equivalents (mg/ml) of samples.

Determination of total phenolic content using the folin-ciocalteu colorimetry[10]: The total phenol concentration was determined using the Folin-Ciocalteu method. To a 1 ml aliquot of sample supernatant, 1.5 ml of freshly prepared Folin-Ciocalteu reagent (diluted at a 1∶9 volume ratio) was added, followed by dilution to 10 ml with water. Then, 6 ml of a 75 g/L sodium carbonate solution was added, and the mixture was allowed to stand for 5 min. Subsequently, it was incubated at 37 ℃ in the dark for 2 h. After incubation, the absorbance was measured at a wavelength of 760 nm. The experimental results were calibrated against a gallic acid standard curve (concentration range: 0 – 12 mg/ml) and expressed as gallic acid equivalents (GAE) of samples.

Determination of total flavonoid content by aluminum chloride method: The total flavonoid concentration was determined using the aluminum chloride colorimetric method[11], with some modifications.

Determination of DPPH free radical scavenging capacity: The DPPH free radical scavenging activity was measured based on a previously reported method[10] with some modifications. A 2 ml sample was added to 2 ml of a 40 mg/L DPPH ethanol solution. After mixing, the solution was incubated in the dark for 30 min, and its absorbance was measured at 517 nm.

Determination of ferric ion-reducing power by FRAP assay: The ferric ion-reducing power before and after fermentation was measured using the total antioxidant capacity (T-AOC) assay kit via the FRAP method. The supernatant of above fermentation broth was appropriately diluted, and the ferric ion-reducing power was determined according to the kit instructions. The results, calibrated against a $FeSO_4 \cdot 7H_2O$ solution standard curve (0 – 1.5 mmol/L, $R^2 = 0.99$), were expressed as ferric ion reducing equivalents (mmol/L).

Results and Analysis

Characterization of aloe juice before and after fermentation

The parenchyma cells of aloe contain a transparent mucilaginous jelly, known as aloe gel, which becomes an olive-gray liquid with high fluidity after juicing. After fermentation with the four different lactic acid bacterial strains, the aloe juice became more turbid and significantly darker in color than the pre-fermentation state. Notably, the juice fermented with LGG turned light pink, and the LGG fermentation group exhibited a light pink color (Fig. 2). The color difference was closely related to the transformation of anthraquinone phenolic compounds in aloe. During the metabolic process, lactic acid bacteria may secrete enzymes that catalyze the hydrolysis of anthraquinones, or atmospheric oxygen may trigger their oxidation, ultimately generating colored quinone

compounds[12]. Moreover, LGG exhibited stronger specificity in the transformation of phenols, resulting in a unique pink color, while other strains (such as LP) showed lower conversion efficiency for anthraquinones, manifesting only as darkening of the color.

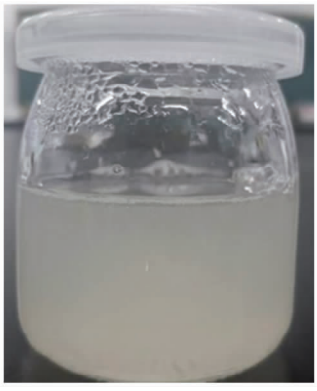
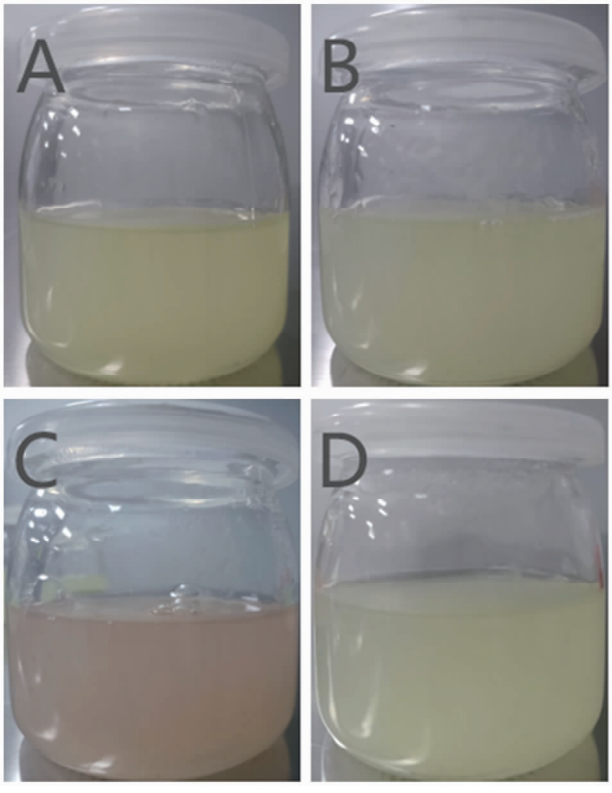


Fig. 1 Aloe juice before fermentation



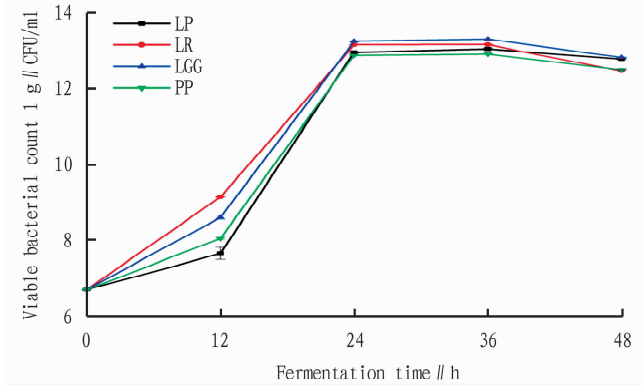
A: *L. plantarum*; B: *L. reuteri*; C: *L. rhamnosus*; D: *P. pentosaceus*.

Fig. 2 Aloe juice after fermentation

Changes in total viable bacterial count

Vegetables and fruits serve as excellent carriers for the growth of lactic acid bacteria due to their rich nutritional composition, maintaining significant cellular viability over time. The growth and viability of probiotics are crucial for the quality and stability of fermented products. Aloe contains polysaccharides, proteins, minerals, phenols, anthraquinones, enzymes, and amino acids, all of which provide optimal nutrients for microbial growth[5]. The four lactic acid bacterial strains exhibited similar proliferation characteristics in aloe juice: a lag phase (slow growth) from 0 to 12 h, followed by a logarithmic phase (rapid proliferation) from 12 to 24 h, a stationary phase (peak viable count) from 24 to 36 h, and finally a decline phase (slight reduction in viable count) after 36 h due to nutrient depletion and lactic acid accumulation (pH decline), as shown in Fig. 3. The order of viable bacterial counts during the stationary phase was as follows: LGG (12.82 lg CFU/ml) > LP (12.77 lg CFU/ml) > PP (12.51 lg CFU/ml) > LR (12.13 lg CFU/ml). It indicated that components such as polysaccharides and amino acids in aloe juice effectively supported the proliferation of lactic acid bacteria[5], and LGG demonstrated the strongest proliferation capacity.

The viable bacterial counts during the stationary phase ranked as LGG (12.82 lg CFU/ml) > LP (12.77 lg CFU/ml) > PP (12.51 lg CFU/ml) > LR (12.13 lg CFU/ml). The differences stemmed from the capacities of the strains to utilize nutrients in the aloe juice. LGG exhibited the strongest proliferation capacity, likely due to its secretion of specific glycosidases that efficiently degraded acemannan (AC) to obtain carbon sources (subsequent Fig. 5 shows that the AC reduction in the LGG group reached 32.6%, the highest among the four groups). Although LP showed slightly lower proliferation capacity than LGG, it demonstrated superior performance in subsequent functional indicators (such as antioxidant activity and AC retention). It indicates that the "proliferation capacity" of lactic acid bacteria is not directly positively correlated with their "nutrient-activity regulation capacity", and strain selection should be based on targeted functional requirements.



A: *L. plantarum*; B: *L. reuteri*; C: *L. rhamnosus*; D: *P. pentosaceus*. Error bars represent the standard deviation of two independent samples.

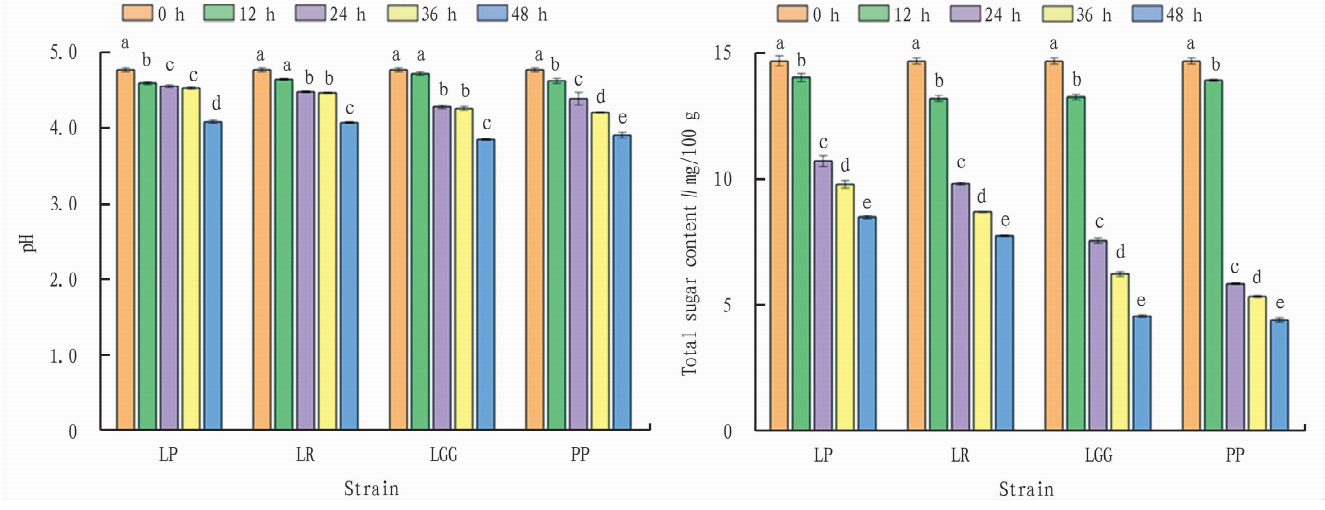
Fig. 3 Changes in viable bacterial count during fermentation

Effects of fermentation with lactic acid bacteria on the physicochemical properties of aloe juice

pH and total sugar content The initial pH of the aloe juice before fermentation was 4.76. From 0 to 12 h, due to the weak metabolic activity of lactic acid bacteria (in the lag phase), lactic acid production was limited, and the pH decreased slowly. After 12 h, as the lactic acid bacteria entered the logarithmic phase, significant accumulation of lactic acid occurred, leading to a notable pH drop to 3.84 – 4.08. The LGG and PP groups exhibited the lowest pH values (3.84 – 3.87), which was correlated negatively with viable bacterial counts (higher viable counts resulted in

more acid production), as shown in Fig. 4 (left).

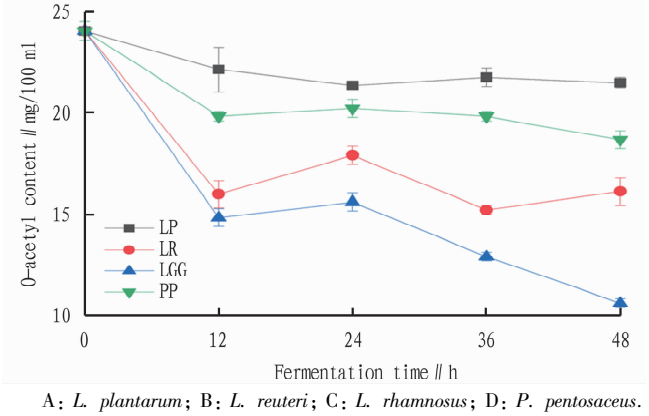
Changes in total sugar content showed an inverse correlation with viable bacterial counts. From 0 to 12 h, as lactic acid bacteria proliferated slowly, total sugar consumption was minimal. From 12 to 24 h, with the rapid increase in viable bacterial counts, total sugars were extensively metabolized into lactic acid and energy, leading to a sharp decline in content. After 24 h, the growth of lactic acid bacteria entered the stationary phase, the consumption of total sugars exhibited a trend of getting stable, as shown in Fig. 4 (right). The consumption of total sugars varied significantly among different bacterial strains during aloe juice fermentation. The LGG and PP groups showed the highest consumption (10.12 – 10.26 mg/100 g), while the LP group had the lowest consumption (8.74 mg/100 g). The characteristic helps retain some sweetness in the aloe juice while reducing flavor deterioration caused by excessive sugar metabolism, offering the potential for developing low-GI aloe beverages.



A: *L. plantarum*; B: *L. reuteri*; C: *L. rhamnosus*; D: *P. pentosaceus*.

* Error bars represent the standard deviation of two independent samples. For columns in the same treatment, different superscript letters indicate significant differences ($P < 0.05$).

Fig. 4 Changes in total sugar content and pH



A: *L. plantarum*; B: *L. reuteri*; C: *L. rhamnosus*; D: *P. pentosaceus*.

Error bars represent the standard deviation of two independent samples.

Fig. 5 Change in O-acetyl content

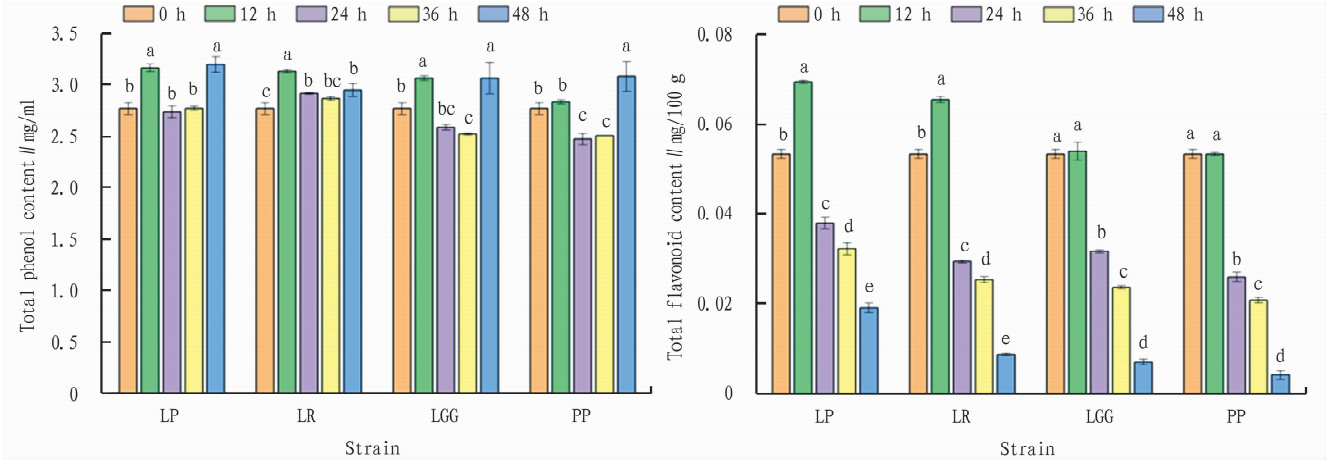
Changes in total flavonoid content and total phenol content

Total phenol content (TPC) and total flavonoid content (TFC) in fruits and vegetables exist in both bound and free forms. During fermentation, the metabolic activities of lactic acid bacteria can lead to an increase in TPC. The mechanism lies in that lactic acid bacteria hydrolyze compounds through the activities of glycosidase or decarboxylase and release phenolic substances bound to plant cell walls, converting phenolic compounds into free states, and then, they hydrolyze complex phenolic molecules into simpler molecular forms, thereby enhancing the bioavailability of phenolic compounds[17]. Different lactic acid bacteria secrete distinct enzymes during fermentation, leading to varying effects on phenolic and flavonoid compounds. As shown in Fig. 6, fermentation with all four lactic acid bacterial strains significantly increased the TPC of aloe juice, with consistent trends. In specific, from 0 to 12 h, lactic acid bacteria secreted hydrolases that hydrolyzed bound phenols into free monomers, causing a rapid rise in TPC. From 12 to 24 h, TPC sharply declined due to the binding of free phenols with proteins, oxidation, or precipitation. From 24 to 48 h, during the

Acemannan content Acemannan (AC) is the core functional polysaccharide in aloe, known for its immunomodulatory and tissue repair properties[15]. During fermentation, the AC content in the LP and PP groups initially decreased and then became stable (with reductions of 12.3% and 15.7%, respectively), while the LGG and LR groups showed a continuous decline (with reductions of 32.6% and 28.9%, respectively) (Fig. 5). The differences stemmed from the varying degradation capabilities of the bacterial strains to AC. LGG likely secreted highly active mannanase, which efficiently cleaved the main chain of AC to obtain carbon sources, resulting in the greatest reduction in AC content. In contrast, LP secreted mannanase with lower activity, leading to weaker degradation of AC and better preservation of this functional component. This characteristic is particularly crucial for the development of aloe products aimed at "immune activity", as the retention of AC helps maintain the original biomedical application potential of aloe juice[16].

stationary phase, TPC showed a slight recovery or remained stable (Fig. 6, left). The TPC increases after fermentation ranked as LP (3.19 mg/ml) > PP (3.02 mg/ml) > LGG (2.98 mg/ml) > LR (2.75 mg/ml), indicating that LP had the strongest capacity to release phenol compounds.

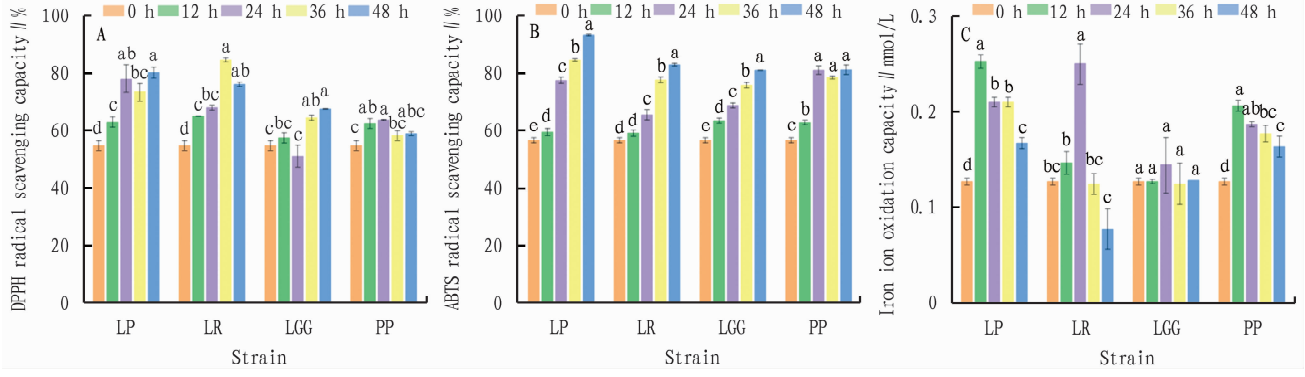
The trend of TFC change showed a trend of "increasing first and then decreasing". From 0 to 12 h, bound flavonoids were hydrolyzed into free forms, leading to a peak in TFC. After 12 h, TFC gradually decreased due to the metabolism of free flavonoids by lactic acid bacteria (e.g., LR exhibited high activities of degradation enzymes toward flavonoids) or oxidation (Fig. 6, right). Among them, the LP group showed the smallest decrease in TFC (from 0.054 mg/ml to 0.019 mg/ml), suggesting that it had lower activity of flavonoid-degrading enzymes (such as flavonoid glycosidases) while its acidic production environment inhibited flavonoid oxidation. In contrast, the LR group exhibited the largest decrease (dropping to 0.012 mg/ml), indicating a stronger metabolic preference for flavonoids, which is unfavorable for retaining flavonoid-related antioxidant functions[18].



A: *L. plantarum*; B: *L. reuteri*; C: *L. rhamnosus*; D: *P. pentosaceus*.

* Error bars represent the standard deviation of two independent samples. For columns in the same treatment, different superscript letters indicate significant differences ($P<0.05$).

Fig. 6 Changes in total phenol and flavonoid contents



A: *L. plantarum*; B: *L. reuteri*; C: *L. rhamnosus*; D: *P. pentosaceus*.

* Error bars represent the standard deviation of two independent samples. For columns in the same treatment, different superscript letters indicate significant differences ($P<0.05$).

Fig. 7 Changes in DPPH (A), ABTS (B) and iron ion oxidation capacity (C)

Changes in antioxidant capacity

Aloe juice is rich in various active substances, such as phenols, which can effectively scavenge free radicals and interrupt free radical chain reactions, thereby exerting antioxidant effects[19]. This study evaluated changes in the antioxidant activity of aloe leaf juice fermented with four lactic acid bacterial strains using three methods: DPPH, ABTS, and FRAP. The experimental results (Fig. 7) showed that fermentation with all four lactic acid bacterial strains enhanced the antioxidant activity of aloe juice, with the LP group performing the best. Specifically, the DPPH scavenging rate was highest in the LP group (80.33%, a 46% increase compared with unfermented juice), and its trend aligned with TPC fluctuations ("increasing-decreasing-increasing"), suggesting that TPC fluctuations were the main reason for DPPH activity variations. The ABTS scavenging rate exhibited a continuous upward trend in all four groups, with the LP group achieving the highest value (93.15%, a 65% increase). This trend did not fully synchronize with changes in TPC content. The increase in free phenolic compounds and the variety of phenolic transformation products were the reasons for the enhanced antioxidant

capacity[19-20]. The lowest ABTS activity in the LR group was associated with the smaller increase in TPC caused by its poor capacity to release free phenols (Fig. 7B).

FRAP values: All four groups showed a trend of "increasing first and then decreasing", peaking in the period of 12 – 24 h, consistent with TFC changes (Fig. 7B). It confirms that flavonoids exert antioxidant effects by chelating iron ions and scavenging peroxyl radicals[21]. The LP group achieved the highest peak (0.167 mmol/L, a 31% increase) due to its optimal TFC retention, while the LGG group, with greater flavonoid degradation, reached only 0.132 mmol/L (Fig. 7C). It is noteworthy that while both TPC and TFC contents increased in the LGG group, there were no significant changes in DPPH and FRAP activities. It indicates that the antioxidant activity of phenols is not solely determined by their total content, but is also critically influenced by factors such as composition, molecular structure and interactions with other components[20, 22].

Conclusions and Discussion

This study investigated the effects of four lactic acid bacterial strains on the physicochemical properties and antioxidant activity of aloe juice. Aloe juice served as an excellent growth substrate for all four lactic acid bacterial strains, manifested in the significantly-increasing viable bacterial counts after fermentation. The LGG group achieved the highest count (12.82 lg CFU/ml), followed by the LP group (12.77 lg CFU/ml). After fermentation with the four lactic acid bacterial strains, the aloe juice showed a significant decrease in total sugar and pH, a notable increase in TPC, and varying degrees of reduction in AC and TFC. Among them, the LP group demonstrated the best retention of AC (12.3% reduction), the highest increase in TPC (3.19 mg/ml), and the smallest decrease in TFC (64.8%). Antioxidant assays indicated that the LP group exhibited the strongest overall antioxidant activity (DPPH scavenging rate: 80.33%, ABTS scavenging rate: 93.15%, FRAP value: 0.167 mmol/L). In summary, among the four lactic acid bacterial strains, *L. plantarum* (LP) was the optimal strain for fermenting aloe juice. It can be used to develop functional aloe beverages or combined with other lactic acid bacteria to optimize product flavor and nutritional value.

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