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Improvement in color expression and antioxidant activity of strawberry juice fermented with lactic acid bacteria: A phenolic-based research

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ABSTRACT

The aim of this study was to investigate the impact of lactic acid bacteria fermentation on color expression and antioxidant activity of strawberry juice from the perspective of phenolic components. The results showed that both *Lactobacillus plantarum* and *Lactobacillus acidophilus* were able to grow in strawberry juice, promote the consumption of rutin, (+)-catechin and pelargonidin-3-O-glucoside, and increase the content of gallic acid, protocatechuic acid, caffeic acid and p-coumaric acid compared to group control. Lower pH environment in fermented juice was likely to enhance the color performance of anthocyanins and increase its parameters *a** and *b**, making the juice appear orange color. In addition, the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant capacity (FRAP) were improved and closely related to polyphenolic substances and strain's metabolites in fermented juice.

Introduction

Strawberry, a common perennial herb of the Rosaceae berry family, has huge commercial and economic impact around the world, whether in the form of fresh fruit or processed products. It has been confirmed to have a remarkable effect on human health-promoting and disease prevention for its high content of phenolics, flavonoids, and micronutrients (such as minerals, folate, and vitamin C) (Afrin et al., 2016; Li et al., 2019). Polyphenols, especially the anthocyanins represented by pelargonidin and cyanidin along with its derivatives in strawberry, may act an important role in providing attractive red color in fresh strawberry fruit, as well as exert an influence on physiological regulation and disease resistance on human health. Previous studies have found that anthocyanins can prevent oxidative stress-related diseases through the approach of stimulating stress-related cell signaling, regulating enzyme expression pathways, and inducing cancer cell apoptosis (Wang et al., 2020). Moreover, some evidences based on cell and animal studies also suggest that polynpheols have strong impacts on antioxidant, anti-inflammatory, anti-atherosclerotic, and anti-cancer (Higbee, Solverson, Zhu, & Carbonero, 2022).

However, the quality of fresh strawberries with high moisture content is likely to decline in a short period of time after harvest, so it may cause great economic losses if they are not properly processed (Bhat & Stamminger, 2015; Xu et al., 2021). Currently, some common traditional products of low degree processing, such as quick-frozen strawberries, strawberry jam, strawberry powder and strawberry juice, still generate low economic benefits with huge loss of raw materials. For this reason, innovative processing methods and novel product development are needed to enhance the utilization and nutrition of strawberries for greater economic and health benefits.

Lactic acid fermentation is a long-established and potential biotechnological method to preserve food and transform its characteristics to enhance flavor and increase the content of functional ingredients. Over the decades, an increasing number of lactic acid bacteria (such as *Lactobacillus*, *Leuconostoc*, *Lactococus lactis*, *Bifidobacterium* and so on) have been used to produce various dairy, vegetable and fruit products, e.g., yogurt, kefir, cheese, sauerkraut and kimchi (Multari et al., 2020). Regular consumption of these fermented foods has been reported to antagonist pathogenic bacteria, reduce cholesterol and boost immunity by producing valuable compounds (organic acids, bacteriocins, hydrogen peroxide, and nitric oxide and so on) with lactic acid bacteria and stimulating immune responses (Castellone, Bancalari, Rubert, Gatti, Neviani, & Bottari, 2021). In addition, development of novel probiotic beverages based on fruit and vegetable as alternatives to

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consumers with intolerance to lactose or with vegetarian diets, could facilitate academic research and worldwide economic efficiency (Ruiz Rodriguez et al., 2021).

Although the potential of lactic acid bacteria fermentation to improve the efficacy of strawberries is theoretically promising, less information is available in current studies of lactic acid bacteria fermented strawberries juice, and the mechanism of change of its intrinsic properties is not clearly clarified. Therefore, it is necessary to conduct a comprehensive evaluation of the changes of phenolics, color expression and antioxidant activity in fermented strawberry juice, which not only helps deepen our knowledge of the properties of strawberry juice in fermentation process, but is also essential to develop product and manage quality control. Moreover, the improvement of color properties and health effects in fermented juice can help arouse consumers' purchase fervour, thereby increasing commercial benefits. Consequently, we eventually screened out two potential stains of *L. plantarum* and *L. acidophilus* from *L. plantarum*, *L. acidophilus*, *L. rhamnosus* and *L. paracasei* for further fermentation research. In this article, the purpose is to analyze and assess the change of color properties and antioxidant activity in vitro and their correlation with phenolic compounds in strawberry juice during fermentation, expecting to provide the theoretical basis for further investigation of other fermented fruit and vegetable products.

Materials and methods

Materials and reagents

Chemical (ferulic acid, sinapic acid, chlorogenic acid, vanillic acid, caffeic acid, gallic acid, syringic acid, p-coumaric acid, protocatechuic acid, quercetin, kaempferol, (+)-catechin and rutin) were purchased from DeSiTe Biological Technology Co., ltd (Chengdu, China). Pelargonidin-3-O-rutinoside were purchased from Wokai Biotechnology Co., ltd (Beijing, China). Pelargonium-3-O-glucoside and Cyanidin-3-O-glucoside were obtained in laboratory (HPLC $\geq$ 95 %).

Activation of starter culture

Lactobacillus plantarum (BNCC192567) and *Lactobacillus acidophilus* (BNCC185344) were purchased from BeNa Culture Collection (BNCC) (Henan, China). The strains in glycerol needed to be activated prior to being used. In a nutshell, 100 μL of bacterial suspension was mixed to 10 mL MRS broth to activate for 24 h at 37 ℃. Then repeated the above operation and obtained the second activated bacterial suspension. Strain cells were obtained through centrifugation at 5000 rpm for 10 min, and then washed with saline solution twice and collected after centrifuging. Afterwards, viable cells were diluted with saline solution to obtain the initial cell density of 3.0×10^{10} colony-forming units per milliliter (CFU/mL) determined by the standard plate count method.

Fermentation process

Fresh strawberries purchased from the market were sepals removed and washed, and then were crushed with a juicer (Joyoung products, Shandong, China). Then the juice was collected by filtration with gauze after 20 min of enzymatic hydrolysis by pectinase and cellulase. The juice was mixed with whey protein at 0.05 % (w/v) and adjusted to pH 4.20 with edible sodium bicarbonate, and then it was allowed to be kept at room temperature after pasteurization (85 ℃, 5 min). Finally, *Lactobacillus plantarum* (BNCC192567) and *Lactobacillus acidophilus* (BNCC185344) were added to strawberry juice at approximately 7 log CFU/mL, respectively, and the juice were allowed to stand at 37 ℃ for 48 h in a temersion oxcillator registration (Sapeen, Shanghai, China) at 100 rpm. Strawberry juice with same treatment but no strains was considered as the group control (CON).

pH measurement and total titratable acidity

pH is measured with a pH meter (Bante901, Shanghai, China). Total titrated acidity was obtained through potentiometric titration described by Ghosh with slight modifications (Ghosh et al., 2015). Briefly, 5 mL of sample and 50 mL of deionized water were mixed into a beaker and placed on a magnetic stirrer with stirring bar, calomel electrode and glass electrode; then titrated the sample using 0.1 M sodium hydroxide standard solution until the pH showed 8.2 $\pm$ 0.1, and recorded the volume (mL) of the sodium hydroxide solution consumed. The result was expressed in terms of lactic acid content (g/L) calculated by the formula $(c \times V_1 \times 1000 \times 0.09)/V_0$, in which c represented the concentration of standard sodium hydroxide solution, mol/L; V_1 represented the consumed sodium hydroxide Standard solution volume, mL; V_0 represented the volume of sample, mL; 0.09 represented the coefficient converted to grams of lactic acid.

Microbial counts

The number of lactic acid bacteria in fermented strawberry juice was counted by plate counting method. In short, the samples were serially diluted to $1/10^4$-$1/10^6$ with saline solution. Then, 50 μL of the treated sample was inoculated on MRS agar plates and cultivated anaerobically at 37 ℃ for 48 h. Eventually, the results were expressed as log CFU/mL.

Color assessment

According to the description of Suriano et al. (Suriano, Balconi, Valoti, & Redaelli, 2021) the color of the samples was detected with a WSF spectrophotometer (Shanghai INESA Physico optical instrument Co., ltd., Shanghai, China). Among them, the CIELAB color parameters L^*, a^* and b^*, reflect trend of light-darkness, red-greenness, and yellow-blueness, respectively. Besides, ΔE is used to describe the change in color presented visually by the juice during fermentation, calculated with formula $[(L_0–L^*)^2 + (a_0–a^*)^2 + (b_0–b^*)^2]^{1/2}$, in which L_0, a_0 and b_0 represented the light-darkness, red-greenness, and yellow-blueness of strawberry juice at 0 h, respectively; L^*, a^* and b^* represented the light-darkness, red-greenness, and yellow-blueness of the sample to be tested, respectively. It is described that the difference in juice color can be distinguished visually especially when ΔE is >3 (Wibowo et al., 2015).

Determination of phenolic substances content

Content of total phenolic in juice

The determination of the total phenolic content (TPC) of the samples referred to the method of Ghosh, with slight modifications (Ghosh, et al., 2015). Briefly, 0.1 mL of the diluted strawberry juice was mixed with 0.1 mL of distilled water and 1.0 mL of Folin-Ciocalteu to incubate for 5 min at 25 ℃, then immediately added 3.0 mL of 7.5 % Na_2CO_3 to stand for 30 min and finally recorded the absorbance at 760 nm. The result of TPC was shown as milligrams of gallic acid equivalents with a gallic acid standard curve ($y = 3.9016x-0.0397$, $R^2 = 0.9996$).

Content of total flavonoid in juice

The determination of the total flavonoid content (TFC) of the samples referred to the method of Ghosh, with slight modifications (Ghosh, et al., 2015). Briefly, 0.5 mL of the diluted strawberry juice was mixed with 0.5 mL of distilled water and 0.3 mL of 5.0 % $NaNO_3$ to incubate for 6 min, then added 0.3 mL of 7.5 % $AlCl_3$ to stand for 30 min. After that, added 2 mL of 4 % NaOH to stop the reaction and finally recorded the absorbance at 510 nm. The result of TFC was shown as milligrams of rutin equivalents with a rutin standard curve ($y = 1.7538x + 0.0003$, $R^2 = 0.9996$).

Content of total anthocyanin in juice

The determination of the total anthocyanin content (TAC) of the

samples referred to the method of Braga, with slight modifications (Braga et al., 2018). The samples were separately diluted with appropriate amount of hydrochloric-potassium chloride buffer (pH 1.0) and hydrochloric-sodium acetate buffer (pH 4.5), and incubated in the dark for 30 min. After that, the absorbance at 520 and 700 nm was recorded. The result of TAC was shown as milligrams of cyanidin-3-O-gucoside equivalents, calculated by $((A_{520}\text{-}A_{700})_{pH1.0}\text{-}(A_{520}\text{-}A_{700})_{pH4.5} \times MW \times DF \times 103)/(\varepsilon \times 1)$, in which "A" represented absorbance; "MW" represented Molecular mass (449.2); "DF" represented dilution factor; "ε" represented molar extinction coefficient, 26800; "1" represented cuvette pathlength, cm.

Anti-oxidation capacity assessment

DPPH radical scavenging activity

The determination of the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) of the samples referred to the method of Kedare (Kedare & Singh, 2011). 2.7 mL solution of DPPH (0.1 mmol/L) was mixed to 0.3 mL diluted fermented juice to stand in the dark for 30 min and then recorded the absorbance at 517 nm. The group blank was considered to replace the sample with distilled water. The result was shown as the percentage of scavenging capacity of DPPH (%), based on the formula $((A_{blank}\text{-}A_{sample})/A_{blank} \times 100$, in which "$A_{blank}$" represented group blank, and "A_{sample}" represented the absorbance of the sample solution.

ABTS radical scavenging activity

The determination of the scavenging capacity of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)($ABTS^{\cdot+}$) of the samples referred to the method of Miller (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993). 3.0 mL solution of ABTS was mixed to 30 μL diluted fermented juice to stand in the dark for 30 min and then recorded the absorbance at 734 nm. The group blank was considered to replace the sample with distilled water. It was worthy to mention that ABTS solution needed to be diluted to an absorbance of 0.70 ± 0.02 with ethanol before use. The result was shown as the percentage of scavenging capacity of ABTS (%), the same as DPPH.

FRAP assay

The determination of the ferric reducing antioxidant power (FRAP) of the samples referred to the method of Tang (Tang, Xing, Li, Wang, & Wang, 2017). Briefly, 3.0 mL of freshly prepared FRAP solution was added to 100 μL of fermented juice to stand for 30 min and then recorded the absorbance at 593 nm. The group blank was considered to replace the sample with distilled water. The result of FRAP was shown as moles of Fe^{2+} equivalents with a $FeSO_4$ standard curve ($y = 6.6086x\text{-}0.0034$, $R^2 = 0.9995$).

Analysis of phenolics by HPLC-PDA

The HPLC determination of samples was achieved by Shimadzu LC-20AT/SPD-M20 (Tokyo, Japan). The samples to be analyzed were processed by centrifugation (10,000 rpm, 10 min) and then filtered through a filter membrane into a brown liquid bottle. Material separation was performed using a Venusil ASB C18 column (4.6 mm × 250 mm × 5 μm, Tianjin, China). Before elution, mobile phases A and B were set to 2 % formic acid and 100 % methanol, respectively, and the gradient was set to following cases: 0.0–2.0 min, 0–6 % B; 2.0–30.0 min, 6–90 % B; 30.0–31.0 min, 90–95 % B; 31.0–34.0 min, 95–95 % B; 34.0–37.0 min, 95–6 % B; 37.0–40.0 min, 6–0 % B. At the same time, the column temperature, injection volume and flow rate were set to 30 ℃, 10 uL and 1 mL/L, respectively. The response values of phenolic acids, flavonoids and anthocyanins were detected at 280, 360 and 520 nm, respectively. In addition, chemical standards were eluted with methanol in a series of concentration gradients under the same conditions. The content of phenolics in strawberry juice was calibrated according to retention time and specific wavelength response.

Statistical analysis

All treatments and detection of samples in this experiment were performed in triplicate, and the data were shown as mean ± standard deviation for the further statistics and analysis. The acquisition of relevant graphs was achieved by Origin version 2020 (OriginLab, Northampton, USA.), while a significant difference analysis was performed by SPSS (SAS Institute Inc., USA)(ANOVA), where $P < 0.05$ indicated a significant difference between samples.

Results and discussion

Viable count, pH and total titratable acid assay

Microbial population is the simplest and most accurate indicator to reflect the adaptability of lactobacillus strains in fermented strawberry juice. The kinetics of cell growth of lactobacillus were studied under anaerobic culture conditions at 37 ℃ for 48 h. As shown in Fig. 1A, after a short lag phase both *L. acidophilus* and *L. plantarum* entered the logarithmic growth stage and almost increased to maximum levels of 9.257 ± 0.018 log CFU/mL and 9.167 ± 0.037 log CFU/mL at 12 h. However, no significant change in viable count (VC) of these two strains was observed in further fermentation process (12–48 h), which revealed that they may have entered a stationary phase (Mousavi et al., 2013). In comparison, no viable microorganism was detected in CON (0 log CFU/mL) during the entire fermentation process (0–48 h). The results indicated that the selected strains showed excellent growth fitness in strawberry juice with subtle differences. The viable count of LAFSJ (*L. acidophilus* fermented strawberry juice) was significantly higher than that of LPFSJ (*L. plantarum* fermented strawberry juice) at 6–12 h ($p < 0.05$), but the difference between the two gradually narrowed with the prolongation of fermentation time and they almost reached the same level at 48 h ($p > 0.05$). It indicated that *L. acidophilus* entered and crossed the log phase faster than *L. plantarum*, although the difference gradually disappeared during the stationary phase. Besides, a diversity of beneficial impacts on the human health could be exerted when the viable count of lactic acid bacteria in food is at a sufficient level of at least 6 log CFU/mL (Castellone, Bancalari, Rubert, Gatti, Neviani, & Bottari, 2021). Obviously, the strawberry juice fermented with *L. plantarum* and *L. acidophilus* could meet or even exceed this basic requirement.

Organic acids represented by lactic acid are produced by consuming sugar during lactic acid fermentation, resulting in a decrease in the pH of the juice. As illustrated in Fig. 1B, the pH of LPMBJ and LAMBJ declined to 2.96 ± 0.01 and 2.95 ± 0.01 (48 h) from 4.20 (0 h), respectively. Correspondingly, the total titratable acid (TTA) increased from 3.588 ± 0.145 to 13.416 ± 0.463 and 13.500 ± 0.483 g/L (Fig. 1C), respectively. In comparison, there was no obvious drop in pH and no significant increase in titratable acid content in CON with the same treatment. Interestingly, it was found that *L. acidophilus* started to produce and accumulate lactic acid faster than *L. plantarum*, although there was no difference in lactic acid content at the end of fermentation (48 h) ($p > 0.05$). As obligatory homo-fermentative lactic acid bacteria, both *L. acidophilus* and *L. plantarum* were able to convert hexoses such as glucose, fructose, mannose or galactose into pyruvate, which was then reduced to lactic acid under the action of lactate dehydrogenase; at the same time, citric acid and malic acid could also be metabolized to lactic acid through a similar pathway (Bintsis, 2018). This was the main reason for the pH drop after lactic acid fermentation. The concentration of lactic acid in strawberry juice was affected by the strains and displayed a certain difference relating to the growth status of strains and the ability to produce acid. But there was no doubt that the content of lactic acid in the system did not change significantly after it reached a peak value. Besides, other primary and secondary metabolism could generate

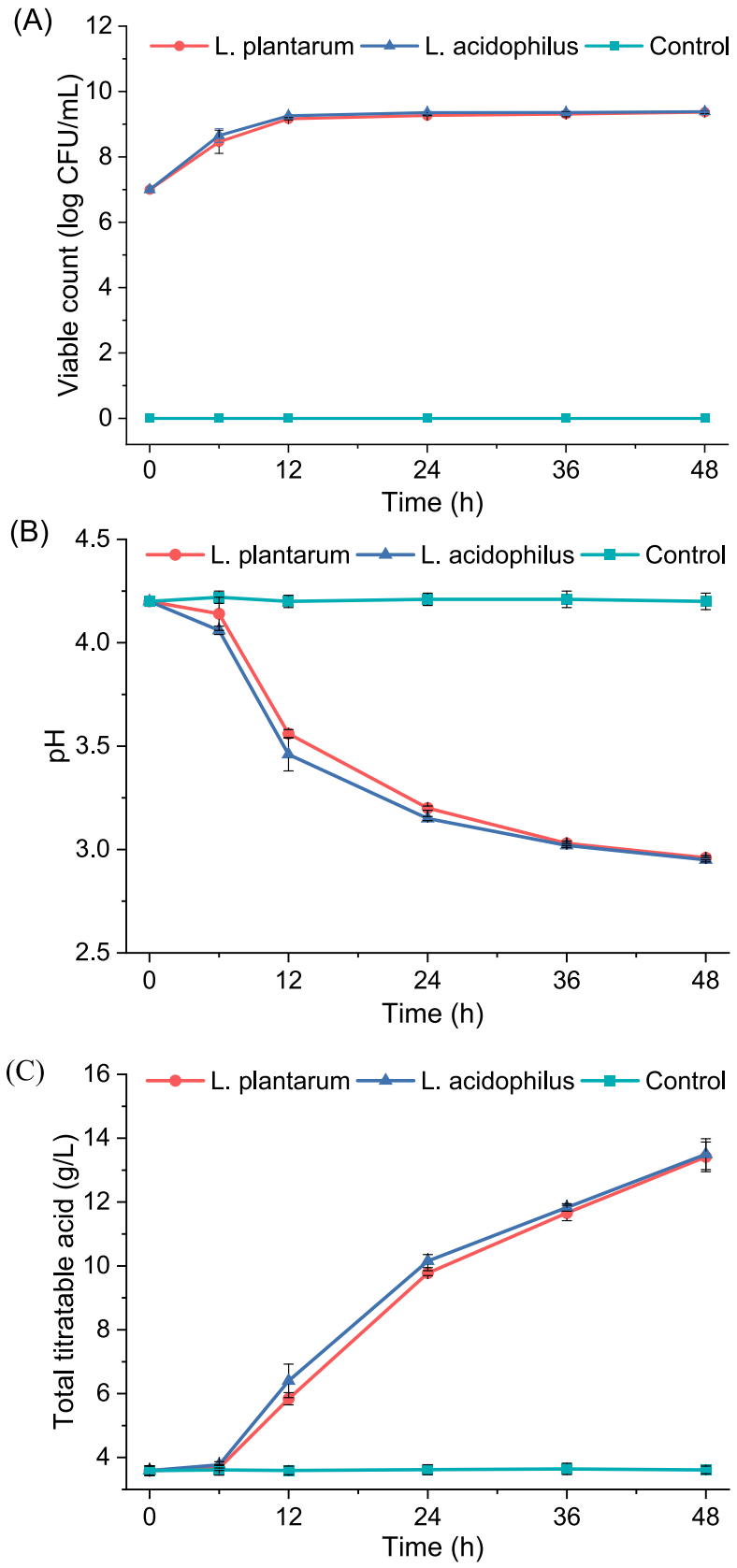

Fig. 1. Effects of fermentation on viable count (A), pH (B), and total titratable acid (C).

specific substances, such as fatty acids, diacetyls, volatile esters, terpenes and bacteriocins, which were determinants of antimicrobial activity, development of food flavor, and impacts on human health (Sousa, Rama, Volken de Souza, & Granada, 2020).

Evolution of phenolic compounds in lactic acid fermentation

The changes of content of total phenolic, total flavonoid and total anthocyanin in strawberry juice were differently affected by fermentation treatments. The total phenolic content was observed to be significantly higher in both LPFBJ and LAFBJ than that in CON ($p < 0.05$) after fermentation (12–48 h) (Fig. 2A), although it gradually decreased in fermentation process. A similar decreasing trend in concentration with fermentation time was found for total flavonoid and anthocyanin. However, in contrast to total phenolic content, the total flavonoid and anthocyanin content in both LPFBJ and LAFBJ were detected to be significantly lower than that in CON after fermentation ($p < 0.05$) (Fig. 2B, 2C).

The reduction in the content of these substances may be attributed to thermal processing (37 ℃), as heat treatment may induce complex physical and chemical reactions, including breaking the bonded form of phenolic compounds and degrading and transforming phenolics to others (Chen, Yu, & Rupasinghe, 2013). In this study, both LPFBJ and LAFBJ showed higher total phenolic content but lower total flavonoid and anthocyanin content than CON in the end of fermentation (48 h), which may be caused by the enzymes of lactic acid bacteria. Ávila et al. found that *Lactobacillus plantarum* and *Lactobacillus casei* strains exhibited high cell-envelope associated β-glucosidase activity against anthocyanin (delphinidin-3-glucoside) when investigating the enzymatic potential (Ávila et al., 2009). β-glucosidase is a hydrolase produced by lactic acid bacteria that can hydrolyze terminal β-D-glucose bonds to deliver glycosides and corresponding ligands. Anthocyanin monoglycosides and diglycosides can be deglycosylated by β-glucosidase and further degrade into phenolic acids corresponding to anthocyanin ring-B (Tian et al., 2019). Studies have shown that intestinal microorganisms including lactobacillus can convert anthocyanins to a wider range of phenolic acid types by deglycosylation, ring fission, reductive metabolism of phenyl acyl fragments, aromatic hydroxylation and dehydroxylation, reduction of carbon–carbon double bonds, as well as lengthening and shortening of aliphatic C chains (Gui et al., 2022). The degradation and oligomerization of flavonoids can improve absorption and bioavailability, and further exert physiological regulation functions. Besides, α-galactosidase and β-galactosidase are also found to be involved in the degradation and transformation of anthocyanins in lactobacillus fermentation of jussara pulp (Braga et al., 2018). Obviously, more than one enzyme is involved in the transformation and degradation of anthocyanins and flavonoids in lactic acid fermentation processing.

Similar findings have been reported when fermenting other plant-derived materials with lactic acid bacteria. Ghosh et al. (Ghosh, et al., 2015) fermented rice-based fermented beverage with Lactobacillus fermentum KKL1 and found that the total phenolic content increased to 63.42 mgGAEg^{-1} in the extracted part of the fermented rice, whereas that in unfermented rice was 11.80 mgGAEg^{-1}. Also, Wu et al. (Wu, Tsai, Hwang, & Chiu, 2012) found that an enhancement of total phenolic concentration was noted in Chingshey purple sweet potato (CPSP) when fermented with *L. acidophilus* (LA), *L. delbrueckii subsp lactis* (LDL), and *L. gasseri* (LGA), respectively, in which that of groups fermented were 107.89 to 108.83 mg gallic acid equivalent [GAE]/g dw) while that of group control was 29.45 mg GAE/g dw. It could be explicated that lactic acid fermentation facilitated the release of phenolics from their complex form in dietary fiber into freely soluble form through enzymes and acids produced by the strain. However, our findings on total flavonoid content in strawberry juice was not in agreement with previous literature (Ghosh, et al., 2015; Wu, Tsai, Hwang, & Chiu, 2012), which reported a general increase in total flavonoid content. In our study, a lower

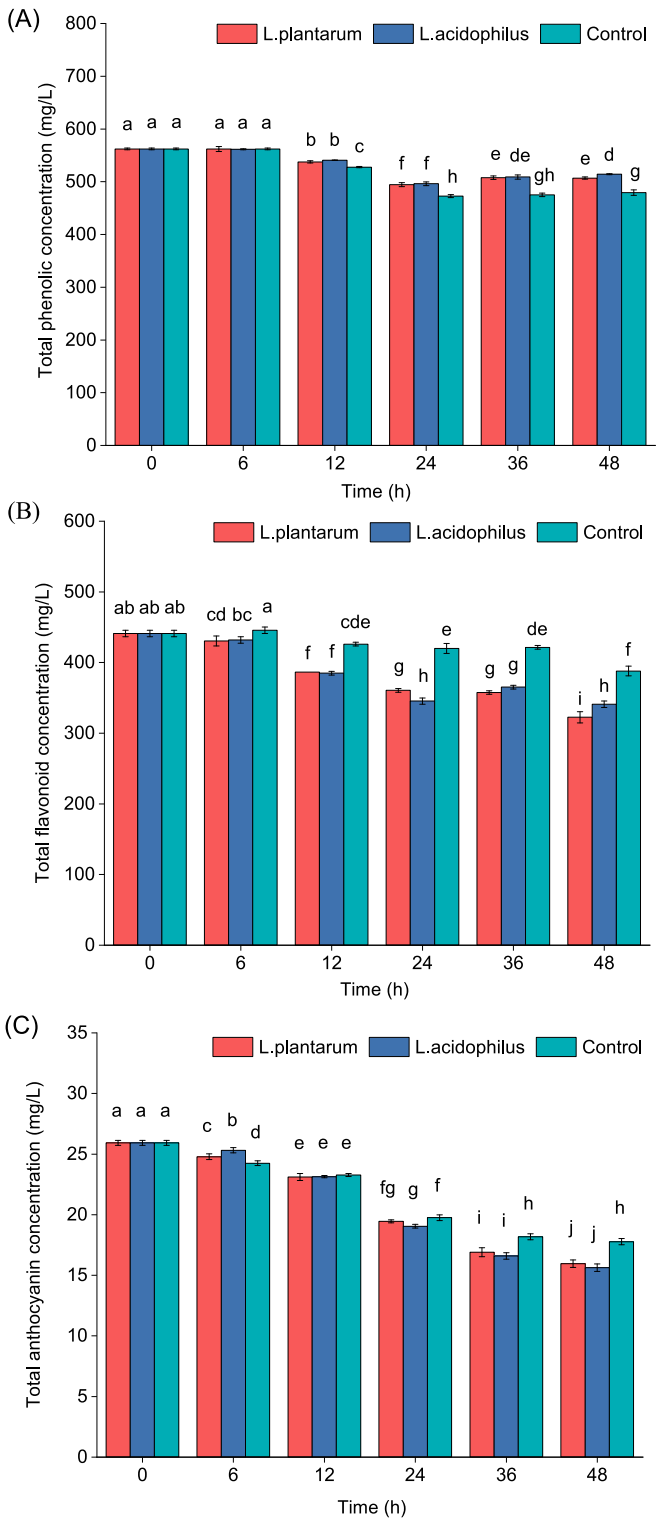

Fig. 2. Effects of fermentation on total phenolic concentrations (A), total flavonoid concentrations (B), and total anthocyanin concentrations (C). (Different letters indicate significant differences with $p < 0.05$).

concentration of total flavonoids was detected in fermented strawberry juice, suggesting that lactic acid bacteria may have the ability to utilize and metabolize flavonoids. In addition, it was found that the prolongation of fermentation time was accompanied by the reduction of phenolic components (Fig. 2A-C). It may illustrate an important point that the effect of fermentation conditions (such as temperature, fermentation time, etc.) on the phenolic components shouldn't be

ignored.

Effects of fermentation on color characteristics

The color characteristics of fermented juices are described using the classic CIELAB space model. Parameter L^* represents the brightness of the surface, with values ranging from 0 (minimum brightness) to 100 (maximum brightness). Positive values for parameter a^* indicate red tones, while negative values indicate green tones. Similarly, it is shown as a yellow tone for positive values of b* and a blue tone for the opposite. As shown in Fig. 3A, the changes of the color parameters of strawberry juice in process of fermentation were recorded. Parameters L^* of LPMBJ and LAMBJ showed a trend of first decreasing to the lowest level and then increasing gradually, while that of CON kept increasing. That pointed to a possible reduction in brightness caused by fermentation. In other respects, parameters a^* of LPMBJ and LAMBJ were observed to increase significantly and reach the peak level at around 24 h of fermentation ($p < 0.05$), while CON continued to decline on the contrary. It demonstrated that a redder transformation in the juice could be achieved by lactobacillus fermentation, which made it more attractive to consumers. Similarly, parameters b^* of LPMBJ and LAMBJ were found to increase gradually and reached the highest value at 24 h of fermentation as well. Unexpectedly, the parameter b^* of CON was also in a trend of increasing although it was significantly lower than that of LPMBJ and LAMBJ after 48 h fermentation ($p < 0.05$). Besides, a similar sustained upward trend occurred to ΔE as fermentation progressed. All evidence indicated that the color profile of strawberry juice changed significantly after fermentation, with the juice treated with lactobacillus becoming more orange but the untreated one turning to light yellow–brown (Fig. 3B).

A close relationship is considered to exist among color parameters and pigments. In particular, anthocyanins are regarded as the major pigment accountable for the color of fresh strawberry fruit, where pelargonidin-3-O-glucoside provides orange-red color and cyanidin-3-O-glucoside provides red color (Li et al., 2019). Suriano et al. reported that anthocyanin content was positively correlated with parameter a^* and b^*, as well as negatively correlated with parameter L^* (Suriano, Balconi, Valoti, & Redaelli, 2021). In other words, it can result in a decrease in parameters a^* and b^* and an increase in parameter L^* when the concentration of anthocyanins in the sample declines. However, the decrease of anthocyanin concentration did not cause the decrease in parameters a^* and b^* and an increase in parameter L^* in this experiment.

Besides anthocyanins, pH is considered to be another factor contributing to the color parameters of strawberry juice. When the pH is <4, the anthocyanins are enriched to the flavylium cation with the decrease of pH, which enhanced their red color characteristics. However, when the pH shifts to mildly acidic or basic conditions (pH 5–6), the anthocyanins will develop into other colorless forms such as carbinol pseudobase and chalcone (Lu et al., 2021). The decrease in pH during fermentation may cause the conversion of anthocyanins into more flavylium cation, thereby increasing a^* and b^* value of the juice and decreasing L^* value of the juice. Therefore, the parameter a^* and b^* of LPMBJ and LRMBJ rose rapidly and reached a peak level due to a sharp drop in pH at 0–24 h of fermentation, and then gradually declined with the decrease of total anthocyanin content because of less pH change (24–48 h). Similarly, it can also explain why their L^* value first decreased to the lowest value, and then gradually increased. As for CON, the decrease in total anthocyanin content led to a monotonic decrease in parameter a^* and a monotonic increase in parameter L^* as the pH hardly changed. However, it still remained a mystery that parameter b^* in CON continued to rise. Some studies have pointed out that the oxidation of phenols and the polymerization of quinones or semi-quinones could produce yellow–brown groups that can exacerbate the deterioration of the color of the juice (H. Li, Guo, & Wang, 2008). The potential factor that the oxygen unmoved in the bottle may participate in the oxidation of phenolic components and then result in the formation of yellow–brown color, may be primarily responsible for the rise of b^* values in unfermented strawberry juice. As illustrated in Fig. 2A, unfermented strawberry juice (CON) had a lower total phenolic content associated with the possibility that it underwent a deeper oxidation reaction. On the other hand, the anthocyanin-flavanol adduct formed by the polymerization of flavanols and anthocyanins can also emit a yellow color (Li & Duan, 2019). But the process is reported to take longer, so it seems to be less likely to be triggered in lactic acid bacteria fermented juice. Furthermore, carotenoids and their derivatives, such as violaxanthin, β-carotene, lutein, etc., may also be related to the color space value b^* (Lu, et al., 2021), which implies that the yellow property of the juice will be enhanced as these pigment are forced to release more under the influence of kinds of physical or chemical factors (Sánchez-Bravo, Zapata, Martínez-Esplá, Carbonell-Barrachina, & Sendra, 2018). In this way, the yellow color (b^* value) of the juice would be the result of a combination of multiple possible elements. In conclusion, the color of strawberry juice was mainly contributed by anthocyanins and was strongly influenced by pH, which appeared visually orange after fermentation with lactic acid

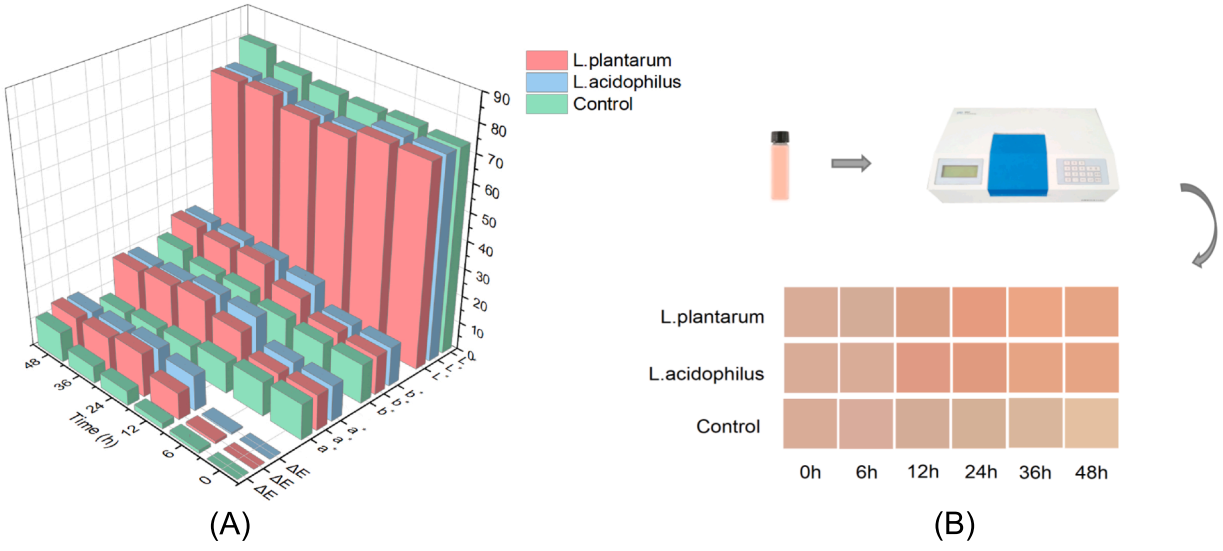

Fig. 3. Changes of color parameters of strawberry juice during fermentation (A), color images of strawberry juice constructed using CIELAB values (B). (L^* - Lightness, a^* - Redness, b^* - Yellowness, ΔE - Total color difference).

bacteria.

Antioxidant activity assay

Three different antioxidant evaluation methods were adopted to investigate the antioxidant capacity of fermented strawberry juice, including the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and the ferric reducing antioxidant power (FRAP) (Fig. 4).

DPPH radical scavenging activity was first proposed by Blois in 1958. According to the description, the single electron of DPPH· can be paired by a radical scavenger, which weakens its violet color and reduces its absorbance at 517 nm (Kedare & Singh, 2011). Fig. 4A exhibited that the percentage of scavenging capacity of DPPH (%) decreased significantly in fermentation process. The percentage of scavenging capacity of DPPH in LPMBJ and LAMBJ decreased from 68.74 % at the beginning (0 h) to 59.96 % and 59.75 % at the end of the fermentation (48 h), respectively,

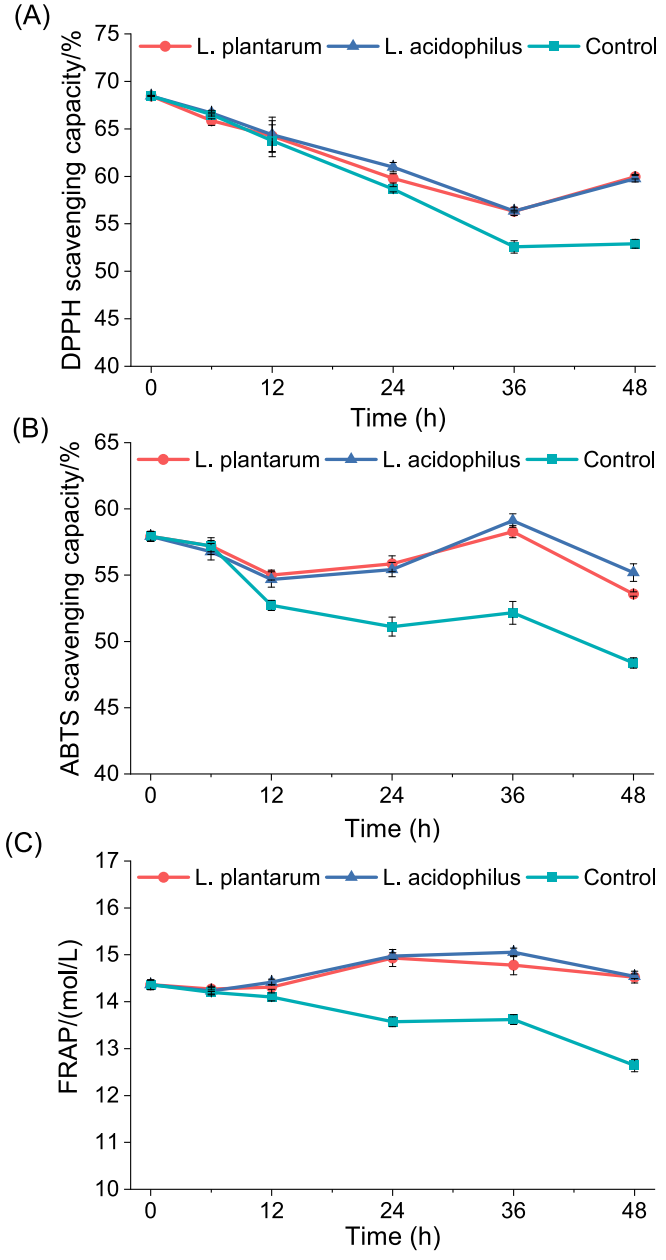

Fig. 4. Effects of fermentation on antioxidant activity of DPPH (A), ABTS (B) and FRAP (C).

while CON showed the lowest value of 52.90 % (48 h). The decrease in DPPH radical scavenging ability of all samples may be due to the loss of components with strong radical scavenging ability, such as phenolics and vitamin C (Panda, et al., 2017). The higher scavenging abilities of LPMBJ and LAMBJ could be explained by the release of more freely soluble phenolics, since more total phenolic content were detected in these two groups than CON in this study($p < 0.05$) (Fig. 2). This indicated that lactic acid bacteria fermentation contributed to the improvement of DPPH radical scavenging ability of strawberry juice.

Established by Miller in 1993, the determination of scavenging capacity of ABTS is based on the fact that the combination of antioxidants and $ABTS^{\cdot+}$ free radicals reduces its characteristic green color and causes a decrease in absorbance at 734 nm (Miller et al., 1993). As shown in Fig. 4B, the ABTS radical scavenging percentage of LPMBJ and LAMBJ appeared to be enhance in the fermentation process (12–36 h), while that of CON was decreased all the time. At the end of fermentation (48 h) LAMPJ was detected with the highest percentage of ABTS radicals scavenging (55.20 ± 0.66 %), followed by LPMBJ (53.58 ± 0.17 %) and CON (48.38 ± 0.39 %). This highlighted the positive contribution of lactobacillus fermentation to the composition of strawberry juice which promoted the transfer of electrons to enhance the scavenging effect of free radicals. There may be a close connection between ABTS radical scavenging ability and phenolic compounds, because some of them have higher electron delocalization due to free electron pair resonance to attract more radicals to bind for elimination (Granato, Katayama, & de Castro, 2011).

Similar results were found when the antioxidant activity of strawberry juice was evaluated by FRAP analysis, as shown in Fig. 4C. The FRAP antioxidant capacity of LPMBJ and LAMBJ was significantly increased in the lactobacillus fermentation process, while that of CON decreased. After fermentation (48 h), LPMBJ and LAMBJ showed higher FRAP antioxidant activities of 14.525 ± 0.127 and 14.541 ± 0.051 mol/L, respectively, while that of CON was 12.639 ± 0.134 mol/L. The FRAP antioxidant capacity of samples treated with lactic acid bacteria were significantly higher than that of CON ($p < 0.05$), while no statistical distinction was found between LPMBJ and LAMBJ. It demonstrated that lactic acid bacteria fermentation could enhance the ability of strawberry juice to donate electrons to promote the reduction of Fe^{3+} to Fe^{2+}, thus preventing the oxidation process of free radicals and other substances. Besides, *L. plantarum* fermentation broth with strong FRAP antioxidant capacity has also been described by other researchers (Tang, Xing, Li, Wang, & Wang, 2017).

Overall, the two selected strains of lactic acid bacteria exhibited strong antioxidant capacity including DPPH, ABTS and FRAP. Some scholars have found similar results in the fermentation of other juices. Kwaw et al. (Kwaw, et al., 2018) fermented mulberry juice with *L. plantarum*, *L. acidophilus*, and *L. parasite*, and observed an increase in DPPH, ABTS and reducing power capacity. Zhang et al. (Zhang et al., 2021) fermented blueberry juice with *L. plantarum* J26 and found the improvement of scavenging abilities of DPPH, superoxide anion radical and hydroxyl radical. The enhancement of antioxidant activity is considered to be inseparable from phenolics. Except for the release of phenolic components in dietary fiber from complexed to freely soluble forms, the metabolic process of lactic acid bacteria can also generate new phenolic compounds. Rupasinghe et al. (Rupasinghe, Parmar, & Neir, 2019) fermented cranberries with *Lactobacillus rhamnosus* and detected phenolic metabolites such as 4-hydroxyphenylacetic acid, 3-(4-hydroxyphenyl) propionic acid, hydrocinnamic acid, catechol and pyrogallol. Besides, vitamin C and carotenoids are mentioned to contribute to antioxidant activity as well (Oladeji, Akanbi, & Gbadamosi, 2017).

Determination of phenolic components in lactic-acid-fermented strawberry juice by HPLC

Phenolic components in strawberries were mainly phenolic acids (caffeic acid, gallic acid and p-coumaric acid), anthocyanins (cyanidin

and pelargonidin) and flavonols (catechin, quercetin and kaempferol) (Giampieri, Alvarez-Suarez, & Battino, 2014). In this study, 16 major phenolic compounds were identified in strawberry juice as shown in Table 1. Main phenolic acids in strawberry juice were protocatechuic acid (16.458 ± 0.394 mg/L), gallic acid (4.416 ± 0.024 mg/L), caffeic acid (3.849 ± 0.039 mg/L) and sinapic acid (2.258 ± 0.007 mg/L). The anthocyanins in strawberry juice were represented by pelargonidin-3-O-glucoside (26.756 ± 0.068 mg/L), followed by cyanidin-3-O-glucoside (11.458 ± 0.090 mg/L) and pelargonidin-3-O-rutinoside (1.447 ± 0.032 mg/L). In addition, (+)-catechin and rutin accounted for the majority of flavonoids at concentrations of 40.972 ± 0.559 and 6.879 ± 0.007 mg/L, respectively.

The presence of lactic acid bacteria would significantly influence the anthocyanin in the juice. An important factor was that the glycosides of anthocyanins could be utilized by lactic acid bacteria to obtain energy to sustain survival and reproduction. The total anthocyanin concentrations of LPMBJ and LAMBJ decreased from initial 39.661 ± 0.190 mg/L to 21.196 ± 0.239 and 21.074 ± 0.062 mg/L at the end of fermentation (48 h), respectively, while that of CON remained 27.320 ± 0.165 mg/L. Compared to CON, the total anthocyanin concentrations of LPMBJ and LAMBJ were detected to be significantly lower ($p < 0.05$), although no statistical distinction was observed between LPMBJ and LAMBJ. In more detail, pelargonidin-3-O-glucoside showed the largest decrease with 5.733–5.862 mg/L, followed by cyanidin-3-O-glucoside with 0.356–0.387 mg/L, and pelargonidin-3-O -rutinoside with 0.004–0.028 mg/L. Some researches on metabolism of anthocyanins by lactobacillus indicated that anthocyanins would first lose the glycosidic bond due to β-glucosidase and be further degraded to phenolic acids such as hydroxyphenylpropene, hydroxyphenylacetic acid and hydroxybenzoic acid through oxidative dehydroxylation, α-oxidation, β-oxidation, rearrangement reaction and methylation (Zhu et al., 2018). This process greatly enriched the types of phenolic acids and contributes to improving the bioavailability and health effects of anthocyanins (Tian, et al., 2019). As reported by Gui et al. (Gui, et al., 2022), gut microorganisms (including lactic acid bacteria) are able to cleavage anthocyanins into caffeic acid, syringic acid, gallic acid, protocatechuic acid and others, among which caffeic acid as an intermediate product can also further undergo chemical reactions such as methoxylation, dehydroxylation, and carbon chain extension or shortening and then convert into a wide variety of hydroxycinnamic acids (including vanillic acid, p-coumaric acid and its derivatives) or short-chain aromatic acids (including phenylacetic acid, hydroxy benzoic acid and its derivatives). Indeed, the concentration of degradation products related to anthocyanin was detected to be higher in strawberry juice treated with lactic acid bacteria in our study, like protocatechuic acid, caffeic acid, p-coumaric acid and vanillic acid. But it can't be confirmed that the degradation of anthocyanin is the only approach for the improvement of those substance concentration. Scalzini et al. (Scalzini, Giacosa, Río Segade, Paissoni, & Rolle, 2021) reported that some hydroxycinnamic acids generally exist in the form of tartaric acid esters and diesters, such as caffeoyltartaric acid, p-coumaroyltartaric acid and feruloytartaric acid, which can be hydrolyzed to generate the corresponding acid in the processing or storage, like caffeic acid, p-coumaric acid and ferulic acid. In addition, other phenolic acids and flavonols in LAMBJ and LPMBJ, like chlorogenic acid, syringic acid, ferulic acid, (+)-catechin and rutin, displayed lower concentrations than those of CON, which revealed that they might be also involved in the metabolic process of lactic acid bacteria, or converted into other substances. In conclusion, the transformation of phenols may have undergone complex chemical reactions in strawberry juice, which makes it difficult to identify the degradation and derivatization pathways of specific phenolics in detail. Therefore, further achievement of confirming on degradation and derivatization pathways of phenolics is needed through the establishment of univariate fermentation models and the analysis of strain-targeted metabolomic as well as the combination of multiple detection methods.

Table 1
Phenolic components concentrations of lactic-acid-fermented strawberry juice.

Components	MF	Concentrations (mg/L)			
		CON-0 h	LPMBJ-48 h	LAMBJ-48 h	CON-48 h
Phenolic acids					
Gallic acid	$C_7H_6O_5$	4.416 ± 0.024^{d}	15.326 ± 0.107^{a}	14.918 ± 0.093^{b}	13.271 ± 0.074^{c}
Protocatechuic acid	$C_7H_6O_4$	16.458 ± 0.394^{c}	23.738 ± 0.170^{a}	23.451 ± 0.137^{a}	20.950 ± 0.017^{b}
Chlorogenic acid	$C_{16}H_{18}O_9$	0^{c}	2.523 ± 0.126^{b}	2.623 ± 0.107^{b}	4.831 ± 0.122^{a}
Vanillic acid	$C_8H_8O_4$	0^{d}	7.020 ± 0.093^{a}	6.862 ± 0.026^{b}	6.488 ± 0.145^{c}
Caffeic acid	$C_9H_8O_4$	3.849 ± 0.039^{d}	5.651 ± 0.013^{a}	5.562 ± 0.031^{b}	4.301 ± 0.064^{c}
Syringic acid	$C_9H_{10}O_5$	0.428 ± 0.012^{c}	1.117 ± 0.030^{b}	1.168 ± 0.034^{b}	2.052 ± 0.046^{a}
p-Coumaric acid	$C_9H_8O_3$	0.294 ± 0.007^{d}	4.782 ± 0.032^{a}	4.490 ± 0.010^{b}	0.678 ± 0.016^{c}
Ferulic acid	$C_{10}H_{10}O_4$	0.267 ± 0.007^{d}	0.726 ± 0.010^{c}	0.762 ± 0.011^{b}	0.984 ± 0.028^{a}
Sinapic acid	$C_{11}H_{12}O_5$	2.258 ± 0.007^{d}	3.134 ± 0.022^{c}	3.320 ± 0.047^{b}	3.540 ± 0.011^{a}
Total		27.970 ± 0.490^{d}	64.017 ± 0.603^{a}	63.156 ± 0.496^{b}	57.095 ± 0.523^{c}
Anthocyanins					
Cyanidin-3-O-glucoside	$C_{21}H_{21}O^{+}_{11}$	11.458 ± 0.090^{a}	7.370 ± 0.078^{c}	7.401 ± 0.043^{c}	7.757 ± 0.095^{b}
Pelargonidin-3-O-glucoside	$C_{21}H_{21}O^{+}_{10}$	26.756 ± 0.068^{a}	13.044 ± 0.145^{c}	12.915 ± 0.009^{c}	18.777 ± 0.056^{b}
Pelargonidin-3-O-rutinoside	$C_{27}H_{31}O^{+}_{14}$	1.447 ± 0.032^{a}	0.782 ± 0.016^{b}	0.758 ± 0.010^{b}	0.786 ± 0.015^{b}
Total		39.661 ± 0.190^{a}	21.196 ± 0.239^{c}	21.074 ± 0.062^{c}	27.320 ± 0.165^{b}
Flavonols					
(+)-Catechin	$C_{15}H_{14}O_6$	40.972 ± 0.559^{c}	49.452 ± 0.329^{b}	49.694 ± 0.248^{b}	53.759 ± 0.128^{a}
Rutin	$C_{27}H_{30}O_{16}$	6.879 ± 0.007^{a}	2.448 ± 0.015^{d}	1.587 ± 0.023^{c}	3.403 ± 0.008^{b}
Quercetin	$C_{15}H_{10}O_7$	0.824 ± 0.003^{c}	1.021 ± 0.001^{b}	1.037 ± 0.006^{a}	0.800 ± 0.001^{d}
Kaempferol	$C_{15}H_{10}O_6$	1.157 ± 0.019^{a}	1.127 ± 0.005^{a}	1.101 ± 0.048^{a}	1.114 ± 0.018^{a}
Total		49.832 ± 0.587^{c}	54.048 ± 0.350^{b}	53.420 ± 0.325^{b}	59.076 ± 0.155^{a}
Total polyphenol concentration		117.463 ± 1.267^{d}	139.261 ± 1.192^{b}	137.65 ± 0.883^{c}	143.491 ± 0.843^{a}

*Different letters indicate significant differences with $p < 0.05$.

Pearson correlation analysis and principal component analysis

Pearson correlation coefficients were calculated to evaluate the relationship between the factors in fermented strawberry juice, such as pH, phenolic concentration, color parameters and antioxidant activity (Fig. 5A). There was a positive correlation between the viable count (VC) and TTA (0.660, $p < 0.05$), reflecting that lactic acid bacteria might produce lactic acid and cause an increase in TTA. In contrast, a strong negative correlation was found between pH and TTA (-0.973, $p < 0.05$), indicating that the decrease in pH of the juice might be caused by the accumulation of acid. Lactic acid bacteria could hydrolyze and utilize parts of flavonoids and anthocyanins to generate corresponding phenolic acids, which reduces the concentration of flavonoids and anthocyanins but increases that of phenolic acids on the contrary (Leonard, Zhang, Ying, Adhikari, & Fang, 2021). However, in this study, the VC of lactic acid bacteria had a moderate negative correlation with the total

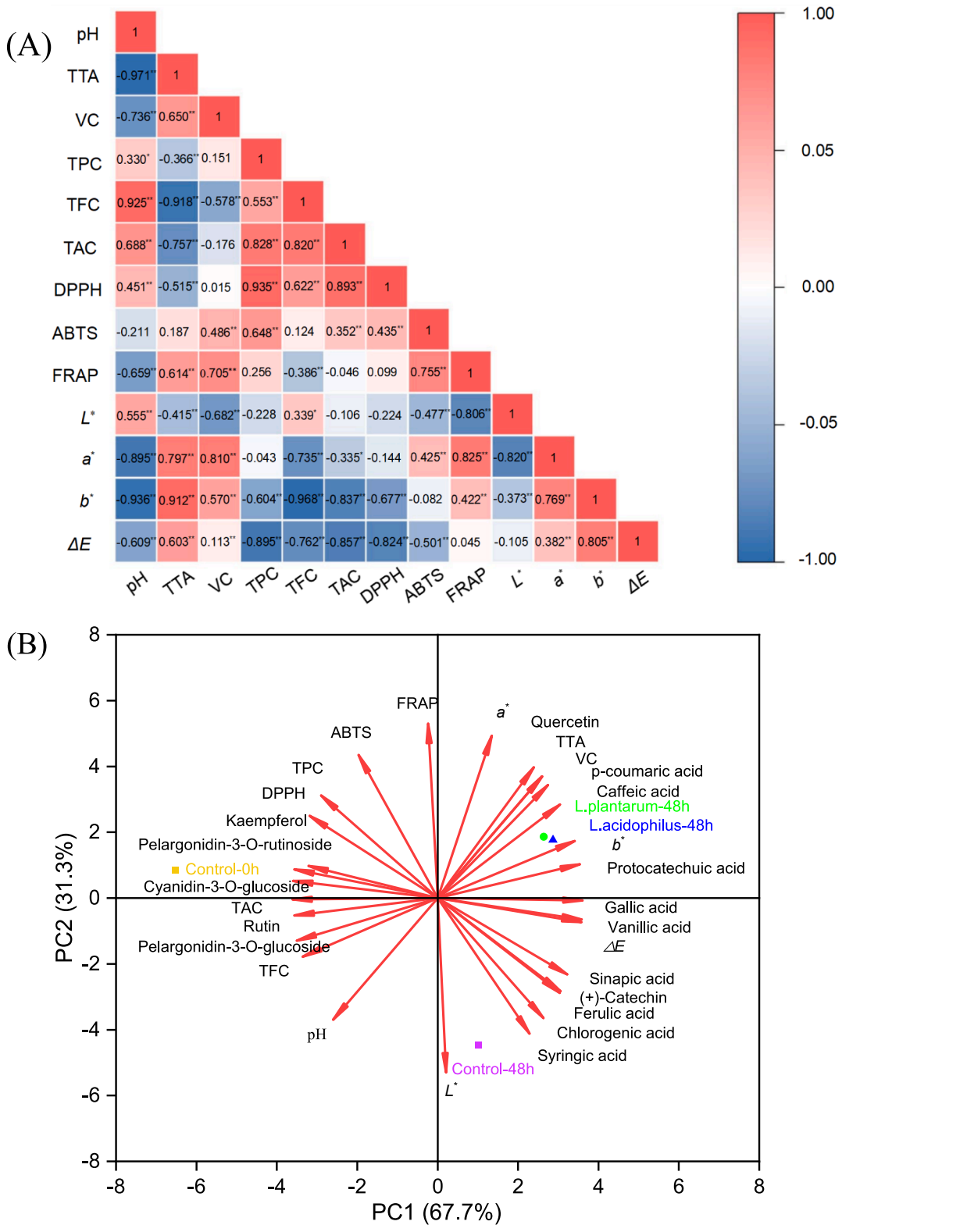

Fig. 5. Heatmap of Pearson's correlation coefficient (A) and principal component analysis plot (B) for phenolic composition, color characteristics and antioxidant capacity of fermented strawberry juice. (TTA- Total titratable acid; VC - Viable count of lactic acid bacteria; TPC - Total phenols concentration; TFC - Total flavonoid concentration; TAC - Total anthocyanin concentration; DPPH – 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; ABTS – 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation scavenging activity; FRAP - Ferric reducing antioxidant power; L^* - Lightness, a^* - Redness, b^* - Yellowness, ΔE - Total color difference).

flavonoid content (TFC) (-0.583, $p < 0.05$), and a low correlation with total phenolic content (TPC) (0.148, $p > 0.05$) and total anthocyanin content (TAC) (−0.180, $p > 0.05$). The connection between lactic acid bacteria and the concentration of phenolic components might be weakened to some extent due to the influence of the fermentation factors mentioned above (e.g. heat, oxidation). Besides, it might reveal that the utilization of phenolic compounds by strains is not limited to anthocyanins but include other types of flavonoids besides anthocyanins.

Significant positive correlation was observed between TPC and DPPH (0.935, $p < 0.05$) and ABTS (0.648, $p < 0.05$). This might reveal a strong relationship between TPC and antioxidant activity as phenolics are able to reduce $DPPH^{\bullet}$ to $DPPH_2$ by donating protons as well as convert $ABTS^{\bullet+}$ to ABTS by transferring electron (Cheng et al., 2020). Unexpectedly, TFC and TAC showed lower correlation with ABTS compared to TPC. Even, contrary to our speculation, FRAP did not seem to correlate well with phenolics. Phenolics including TPC, TFC and TAC contributed less to FRAP of the strawberry juice, which indicated that phenols had a weak ability to reduce Fe^{3+} to Fe^{2+} to prevent the oxidation process of free radicals and other substances. Moreover, it suggested that the antioxidants in strawberry juice might not only be phenols, but also other substances such as sterols, tocopherols, ascorbic acid and carotenoids (Nikolic, Mitic, Stankov Jovanovic, Dimitrijevic, & Stojanovic, 2019). Interestingly, the correlation coefficients between FRAP and VC and TTA were observed to reach 0.708 ($p < 0.05$) and 0.621 ($p < 0.05$), demonstrating that lactic acid bacteria and its metabolite-related substances might contribute to antioxidant capacity.

A low pH environment facilitates the enrichment of anthocyanins to the flavylium cation, which helps increase a^* and b^* values but decrease L^* values, which can also be observed from the strong correlation coefficients between pH and color parameters L^* (0.560, $p < 0.01$), a^* (-0.900, $p < 0.01$) and b^* (-0.938, $p < 0.01$) in Fig. 5A. Some studies have shown that TAC is positively correlated with a^* and b^* and negatively correlated with L^*, but our study is not in agreement with that. The conclusions of the literature are based on pH-invariant conditions, but in our study the pH was variable, and the correlation may be weakened by strong decrease in pH during fermentation. Therefore, a strong increase in parameters a^* and b^* and a slight decrease in parameter L^* were observed despite a decrease in anthocyanin content.

Principal component analysis (PCA) was carried out to highlight critical properties of strawberry juice before and after fermentation using pH, TTA and VC as well as phenolic components, color parameters and antioxidant capacity (Fig. 5B). Principal component analysis systematically summarized the data and clearly categorized each particular component, which provided approximately 99 % of the total variance, with PC1 and PC2 accounting for 67.7 % and 31.3 %, respectively. The figure showed a clear distinction between juices, where fresh juices (control-0 h) were in the second quadrant, unfermented juices (control-48 h) were in the fourth quadrant, and lactobacilli-fermented juices (L. plantarum-48 h and L. acidophilus-48 h) were in the first quadrant. All the different quadrants reflected the correlations between the juices and PC1 and PC2, in either positive or negative. Fresh strawberry juice (Control-0 h) was negatively correlated with PC1 but positively correlated with PC2, as was the characteristic parameters FRAP, ABTS, DPPH, TPC, kaempferol, cyanidin-3-O-glucoside and pelargonidin-3-O-rutinoside. Unfermented juice (Control-48 h) was positively correlated with PC1 but negatively correlated with PC2, maintaining the same characteristics as L^*, ΔE, gallic acid, vanillic acid, sinapic acid, (+)-catechin, ferulic acid, chlorogenic acid and syringic acid. Besides, lactic acid bacteria fermented juices (L. plantarum-48 h and L. acidophilus-48 h) which positively correlated with PC1 and PC2 were characterized by a^*, b^*, VC, TTA, quercetin, p-coumaric, caffeic and protocatechuic acids. PCA reflected that substances or indicators of homogeneous trends were grouped in the same region or adjacent regions, which facilitated further analysis of the target substances. Pelargonidin-3-O-glucoside was in a different region from cyanidin-3-O-glucoside and pelargonidin-3-O-rutinoside with a higher depletion rate, which suggested that pelargonidin-3-O-glucoside may be more easily consumed and used than the other two anthocyanins because of its simpler C-ring structure and glycoside and less spatially blocked. The content of p-coumaric acid, caffeic acid, protocatechuic acid and quercetin as well as viable count were found in the same region with an increasing trend, which reflected the possible production of phenolic metabolites by lactic acid bacteria grown in strawberry juice. Also, PCA results showed that lactic acid bacteria fermented juice and unfermented juice exhibited significant differences and were distributed in different regions. Furthermore, together with the aforementioned increase in color and antioxidant activity of the strawberry juice, it suggested that lactic acid bacteria fermentation was a worthwhile attempt to improve the properties of the strawberry juice.

Conclusion

Lactobacillus plantarum and *Lactobacillus acidophilus* had significant effects on strawberry juice characteristics. Both strains were able to grow in strawberry juice and change the content of different phenolic components. The total phenolic content of strawberry juice fermented with *Lactobacillus plantarum* and *Lactobacillus acidophilus* improved significantly compared to group control, while the content of total anthocyanin and total flavonoid in fermented juice decreased significantly compared to the control group. The lower pH value of fermented juice improved the color performance of anthocyanin, and increased its parameters a^* and b^*, making the juice appear orange color. In addition, antioxidant activities (DPPH, ABTS and FRAP) were detected to be significantly higher than those of group control after fermentation, which were highly related to phenolic components and metabolites of lactic acid bacteria. Nevertheless, further studies are needed to confirm the utilization and metabolism mechanism of phenolic components in strawberry by lactic acid bacteria.

CRediT authorship contribution statement

Wending Chen: Methodology, Investigation, Formal analysis, Writing – review & editing. **Caiyun Xie:** Writing – original draft, Investigation, Validation. **Qianqian He:** Visualization, Data curation. **Jianxia Sun:** Conceptualization, Project administration, Supervision. **Weibin Bai:** Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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