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Effects of Lactic Acid Bacteria Fermentation on Physicochemical Properties, Functional Compounds and Antioxidant Activity of Edible Grass

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Abstract: Fermented foods are known worldwide for their functional health properties. In order to promote the relative product development of edible grass, *Lactobacillus plantarum* (Lp) and *Lactobacillus rhamnosus* (Lr) were used to ferment edible grass in this study. Effects of fermentation using Lp and Lr in monoculture and binary mixture on physicochemical properties, the contents of functional compounds and the antioxidant activity of edible grass at different fermentation times were investigated by colorimetric method and high-performance liquid chromatography (HPLC). Results show that the pH value and total sugar content of the three fermented edible grasses at the 4th day were lower than those of unfermented water extract (defined as the control sample) and kept almost unchanged at the 7th day. The total polyphenol content and total flavonoid content of the three fermented edible grasses were lower than those of the control sample by the oxidation of phenolic compounds caused by polyphenol oxidases. The highest soluble protein content and superoxide dismutase (SOD) activity were found in the binary mixture of Lp and Lr fermentation at the 7th day, which were respectively 11 and 1.78 times higher than those of control sample. The oxalic acid content of all fermented edible grasses shows a significant decrease with increasing fermentation time, especially for the binary mixture at the 7th day, reaching only 24% of the control sample. However, the contents of lactic acid and succinic acid of the three fermented edible grasses were higher than those of the control sample because of the metabolism of the microorganism. Functional compounds including soluble protein, SOD, lactic acid and succinic acid played the main positive roles in antioxidation, while oxalic acid had a negative correlation with antioxidation. Therefore, the antioxidant activity of edible grass was dramatically enhanced by *Lactobacillus* strain fermentation.

Keywords: edible grass; lactic acid bacteria; fermentation; functional compounds; antioxidant activity

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1. Introduction

Edible grass (*Rumex patientia* L. × *Rumex tianschanicus* A. LOS) is a member of genus *Rumex*, and it is bred from female parent Rumex K-1 (*Rumex patientia* L. × *Rumex.tianschanicuscv*) and male parent *Rumex patientia* L [1]. It is worth noting that the protein content of edible grass is over 30%, which is almost equivalent to the content of soybean protein [2]. Edible grass is also rich in superoxide dismutase (SOD) activity which is beneficial to human health [3]. In prior work, the water extract of edible grass exhibited better free radical scavenging activity than some other *Rumex* plants, indicating its good antioxidant activity [4]. Although the beneficial effects presented by edible grass are noticeable, there is a scarcity of processed products made from edible grass. In addition, oxalic acid (regarded as an antinutrient component) in edible grass presents with high content [5], which is known to chelate

minerals affecting their bioavailability [6]. Therefore, it is necessary to develop advanced processing technologies for edible grass to enrich the product variety, reduce the oxalic acid content and enhance the health benefits.

Vegetables and fruits are considered as good vehicles of lactic acid bacteria (LAB) owing to their abundant nutrients [7]. Because of the high nutritional quality and health benefits of probiotic functional products, the demand for them has been dramatically increased [8]. LAB fermented products generally exhibit antimicrobial activity due to the production of organic acids, hydroperoxide and bacteriocins [9]. Moreover, LAB fermentation could improve the nutritional quality of vegetable and fruit products and convert some macromolecular compounds to small molecular compounds with higher bioactivity [7,10]. For example, prior studies confirm that the fermentation of pear juice and vegetable-fruit beverage significantly improved the total polyphenol content and antioxidant activity [7,11]. Furthermore, LAB could effectively degrade oxalate, achieved through a mechanism involving the transportation of oxalate by permease into the cells where it is converted into oxalyl-CoA by formyl-CoA transferase and further into formate and carbon dioxide by oxalyl-CoA decarboxylase [12]. However, to the best of our knowledge, no research on changes in characteristic physicochemical properties, functional compounds contents and antioxidant activity in edible grass after LAB fermentation has been reported.

Therefore, LAB fermentation is expected to be used in edible grass processing to reduce the content of oxalic acid and improve the functional properties of edible grass. This work aims to investigate the effects of two LAB strains (*Lactobacillus plantarum* and *Lactobacillus rhamnosus*) fermentation on the physicochemical properties, functional compounds and antioxidant activity of edible grass. Furthermore, the relationship between antioxidant activity and metabolizing functional compounds was evaluated. This work will provide a theoretical direction for the production and characterization of processed products from edible grass in the future.

2. Materials and Methods

2.1. Materials

Edible grass was obtained from Shaoxing, Zhejiang, China. Two commercial LAB Direct Vat Sets, *Lactobacillus plantarum* (Lp, 1×10^{11} CFU/g) and *Lactobacillus rhamnosus* (Lr, 1×10^{11} CFU/g), were purchased from Shandong Junle Biotechnology Co., Ltd. (Weifang, China). Folin & Clocalteu's phenol reagent, gallic acid, and rutin were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). The BCA protein concentration assay kit and SOD assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Organic acid standards were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

2.2. Edible Grass Fermentation

Edible grasses were cleaned with deionized water and dried at room temperature. The mixture of dried raw material, sucrose and sterile water at a ratio of 1:1:2 (*w*/*w*/*v*) was treated by ultrasound (ultrasonic power = 400 W) at 50 °C for 2 h and then pasteurized at 65 °C for 30 min. After cooling to room temperature, the pasteurized mixture was inoculated with single strain or binary mixture strains at the ratio of 1:1 (*w*/*w*). The initial lactic acid bacterial concentration was 1.5 × 109 CFU/mL. Fermentation was conducted for 7 days at 35 °C. All fermentation processes were performed in triplicate, and each fermentation sample was collected at the 4th and 7th days, respectively. The dried edible grass was subjected to the same treatment steps, except for LAB inoculation which was used as control.

2.3. Determination of pH and Total Sugar Content

The pH meter supplied by Hangzhou Qiwei Instrument Co., Ltd. (Hangzhou, China) was used to measure the pH of the samples.

Total sugar content was determined by the anthrone-sulphuric acid method [13] with slight modifications. At first, 200 µL of diluted sample was mixed with 800 µL of 2 mg/mL anthrone-sulphuric acid solution at 0 °C. The mixture was incubated in a boiling water bath for 10 min and then cooled down to room temperature. The absorbance was measured at 620 nm. Using glucose as a standard, total sugar content was calculated from the calibration curve ($y = 0.0046x + 0.0689$, $R^2 = 0.9940$) and expressed in terms of mg/mL of glucose in the sample.

2.4. Determination of Total Polyphenol Content and Total Flavonoid Content

Total polyphenol content (TPC) was determined by the Folin-Ciocalteu method [14]. In brief, 100 µL of the diluted sample solution was added to 500 µL of 10% (*w*/*v*) Folin-Ciocalteu reagent. Then, 400 µL of 7.5% (*w*/*v*) Na_2CO_3 solution was added after 3 min. Subsequently, the mixture was incubated in the dark for 1 h, and the absorbance was measured at 765 nm. Using gallic acid as a standard, TPC was calculated from the calibration curve ($y = 0.0049x + 0.0552$, $R^2 = 0.9996$) and expressed as the amount of gallic acid equivalent (µg GAE/mL).

The $NaNO_2$-$Al(NO_3)_3$-NaOH method was used for measuring total flavonoid content (TFC) with minor modifications [7]. 30 µL of 5% (*w*/*v*) $NaNO_2$ was added to 500 µL of diluted sample solution and incubated for 6 min. Thereafter, 30 µL of 10% (*w*/*v*) $Al(NO_3)_3$, 400 µL of 1 mol/L NaOH and 40 µL of deionized water were the added to the mixture for reaction for 15 min, and the absorbance was read at 510 nm. Rutin was used as a standard to prepare the calibration curve ($y = 0.0051\ x + 0.086$, $R^2 = 0.9995$). TFC was defined as the rutin equivalent (µg RE/mL).

2.5. Determination of Soluble Protein Content and SOD Activity

Soluble protein content and SOD activity were determined following the procedures provided by BCA protein concentration assay kit and SOD assay kit supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China), respectively.

2.6. Determination of Organic Acids

The measurements of four organic acids including lactic acid, malic acid, citric acid and succinic acid were conducted on high-performance liquid chromatography (HPLC) (Waters e2695, Waters, Massachusetts, USA) by installing an AtlantisRR T3 column (250 mm × 4.6 mm, 5 µm) and UV detector according to the previously reported method [15] with slight modifications. Column oven temperature was 20 °C. The mobile phase was a mixture of solvent A (methanol) and solvent B (0.01 mol/L KH_2PO_4, pH 2.7) with the ratio of 2:98 (*v*/*v*), and the flow rate was 1.0 mL/min. The wavelength of the UV detector was 210 nm, and the injection volume of the sample filtered through 0.22 µm microporous membrane was 10 µL. Separation was obtained with isocratic elution. The concentrations of the organic acids of the samples were quantified by the calibration curves (Table 1) of the corresponding pure standards.

Table 1. Calibration curves of organic acids.

Organic Acids	Calibration Curve	R^2
Lactic acid	$y = 4.49 \times 10^6 x - 3.83 \times 10^3$	0.9965
Malic acid	$y = 5.7 \times 10^6 x + 1.11 \times 10^4$	0.9951
Citric acid	$y = 4.72 \times 10^6 x - 2.53 \times 10^4$	0.9968
Succinic acid	$y = 3.28 \times 10^6 x - 9.18 \times 10^3$	0.9968

Interference peaks appeared easily when the oxalic acid content was measured by the above HPLC method. Therefore, the content of oxalic acid was determined by the coloration method [16]. 72 μL of diluted sample solution was added to the mixture of 80 μL of 0.5 mg/mL $FeCl_3$, 800 μL of 2 mol/L KCl (pH 2.0), 48 μL of 0.5% (*w/v*) sulfosalicylic acid and incubated for 30 min. The absorbance was read at 510 nm. Using sodium oxalate as a standard, oxalic acid content was calculated from the calibration curve ($y = 0.3351\ x + 0.0047$, $R^2 = 0.9939$) and expressed as mg oxalate equivalent/mL of sample.

2.7. Antioxidant Activities Analysis

2.7.1. Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated using the method described by Li et al. [17] with slight modifications. 600 μL of diluted sample was added to the solution made up with 1.2 mL of 0.1 mmol/L DPPH-methanol and 135 μL of 50 mmol/L Tris-HCl (pH 7.4). The mixture was incubated in the dark at 25 °C for 30 min, and the absorbance (A) was measured at 517 nm by micro-plate reader (SpectraMax iD5, Molecular Devices, Shanghai, China). Results were calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - (A_1 - A_2))/A_0 \times 100$$

where A_0 is the absorbance of the blank control group, A_1 is the absorbance of the sample group, and A_2 is the absorbance of the sample background group.

2.7.2. Determination of ABTS Radical Scavenging Activity

ABTS radical scavenging activity was evaluated according to the previously reported method [18] with slight modifications. Equivalent-volume of 7 mmol/L ABTS solution and 2.45 mmol/L $K_2S_2O_8$ was incubated in the dark at room temperature for 16 h to ABTS cation radicals. Then the solution was diluted with 80% ethanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. 100 μL of diluted sample was mixed with 1 mL of ABTS solution and then incubated at 30 °C for 1 h in the dark. The absorbance was measured at 734 nm by micro-plate reader. Results were calculated using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = (A_0 - (A_1 - A_2))/A_0 \times 100$$

where A_0 is the absorbance of the blank control group, A_1 is the absorbance of the sample group, and A_2 is the absorbance of the sample background group.

2.8. Statistical Analysis

Data were analyzed using Origin (Version 2022, OriginLab, Northampton, Massachusetts, USA) and IBM SPSS Statistics (Version 26, IBM, Armonk, NY, USA). Significant differences among means of samples were tested by one-way analysis of variance (ANOVA) at $p < 0.05$. Results were expressed as the mean ± standard deviation of three independent data in the diagram. The Pearson method was used for correlation analysis.

3. Results and Discussion

3.1. pH and Total Sugar Content

Changes in pH of edible grasses fermented with Lp, Lr and mixed strains (Lp:Lr = 1:1 (*w/w*)) at 35 °C at different fermentation times are shown in Figure 1. pH value is one of the important indicators of LAB fermentation, which can reflect the acid production capacity of LAB to a certain extent. The pH values of the three fermented edible grasses were significantly ($p < 0.05$) lower than that of the control sample and decreased from 4.34 to between 2.79 and 3.10 during the fermentation period, which was consistent with the phenomena observed in kiwifruit fermented with Lp [19]. At the 4th day, the pH value of edible grass fermented with Lr was lower than those fermented with Lp and mixed strains. With increasing fermentation time, the pH values of the three fermented edible grasses kept almost unchanged, which was also observed in the vegetable-fruit beverage fermented

with two Lp strains [11]. The decrease of pH was considered due to the production of organic acids by LAB.

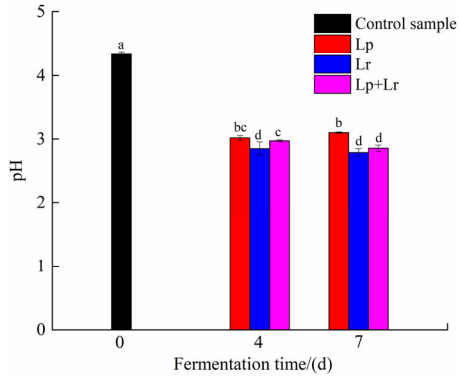

Figure 1. The value of pH in edible grass during fermentation. Different superscript letters in the columns indicate significant difference ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp + Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

As shown in Figure 2, the total sugar content of the three kinds of fermented edible grasses had no significant difference ($p > 0.05$) at the 4th day, decreasing by 36% compared to the control sample. This result agreed with the phenomena observed in cashew apple juice fermented with Lp, whose total sugar content decreased from 4.74 to 3.61 g/L during fermentation [20]. The total sugar content of edible grass fermented with Lp at the 7th day was higher than that at the 4th day, only reaching 82% of the control sample. The consumption of total sugar was mainly attributed to the utilization by microorganisms for cellular growth and bioconversion into organic acids such as lactic acid [21,22].

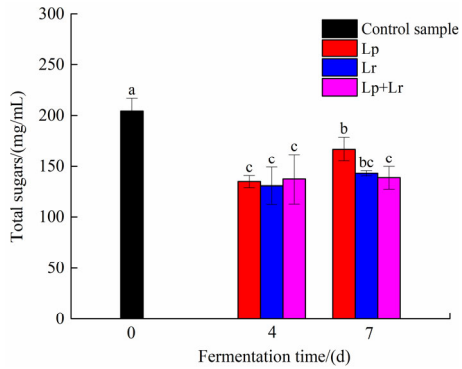

Figure 2. Total sugar content of edible grass during fermentation. Different superscript letters in the columns indicate significant difference ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp+Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

3.2. Total Polyphenol Content and Total Flavonoid Content

The total polyphenol content (TPC) of edible grass during fermentation is shown in Figure 3. The TPC of the fermented edible grass at the 4th day was lower than that of control sample, which was probably due to the diffusion and oxidation of phenolics caused by polyphenol oxidase [23]. With the hydrolysis of large polymeric phenolics into simple new phenolic compounds conducted by the microorganisms [24], the TPC increased significantly ($p < 0.05$) at the 7th day, especially for the mixture of Lp and Lr fermentation, increasing by 42%. Although the TPC of the fermented edible grass was lower than that of the control sample, it was still higher than that of some fermented fruits and vegetables reported in other research. For example, the TPC of edible grass fermented with mixed strains at the 7th day (~414 μg GAE/mL) was higher than the highest TPC

of fermented pear juice (~361 μg GAE/mL) [7] and fermented vegetable-fruit beverage (~121 μg GAE/mL) [11].

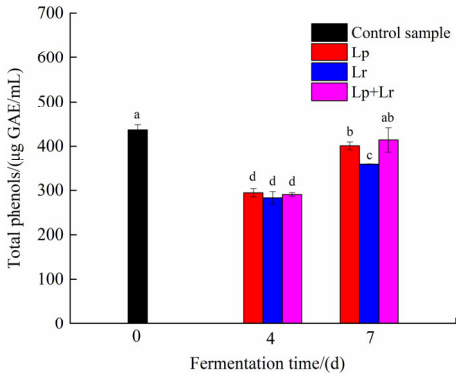

Figure 3. Total polyphenol content of edible grass during fermentation. Different superscript letters in the columns indicate significant difference ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp+Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

As shown in Figure 4, the total flavonoid content (TFC) of the edible grasses fermented with Lp and Lr presented the dramatic decrease during fermentation, and a significant difference was not found ($p > 0.05$) between the edible grass fermented with mixed strains at the 7th day and that at the 4th day. Briefly, the TFC of all fermented edible grasses was lower than that of the control sample, which was consistent with the findings observed in apple juice fermentation that decreasing TFC by 33.2% during LAB fermentation [17]. This result was closely associated with the oxidation of phenolic compounds including flavonoids caused by polyphenol oxidases [25].

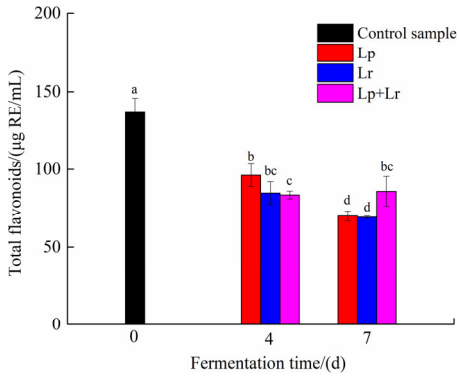

Figure 4. Total flavonoid content of edible grass during fermentation. Different superscript letters in the columns indicate significant difference ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp+Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

3.3. Soluble Protein Content and SOD Activity

As shown in Figure 5, significant ($p < 0.05$) interactions were found between the LAB-fermented edible grasses and time of fermentation with respect to the soluble protein content. The soluble protein content of the three fermented edible grasses increased with increasing fermentation time (Figure 5). At the 7th day, the protein content of the mixture of Lp and Lr fermentation was higher than those of other fermented edible grasses, which was over 11 times higher than that of control sample. According to the increase in soluble protein content during edible grass fermentation, a similar result from the fermentation of goji berry (*Lycium barbarum* L.) juice was also reported by Liu et al., where they found that fermentation increased the protein content in fermented goji juice by at least 31.18% with a short fermentation time of 20 h [26]. Proteases produced by bacterial strains can

break down macromolecular proteins and convert them into small protein molecules [26]. Therefore, the increase of soluble protein content was possibly attributed to the action of proteases.

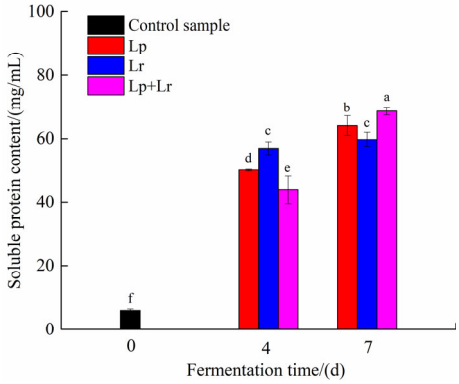

Figure 5. Soluble protein content of edible grass during fermentation. Different superscript letters in the columns indicate significant difference ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp+Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

As shown in Figure 6, significant ($p < 0.05$) interactions were found between the LAB-fermented edible grasses and time of fermentation with respect to SOD activity. The SOD activity of edible grass fermented by Lp at the 4th day was similar to other fermented edible grasses and was about 1.2 times higher than that of the control sample. At the 7th day, edible grasses fermented with mixed strains exhibited the highest SOD activity, which was 1.78 times higher than the control sample. Similarly, a significant increase in the value of SOD activity was observed in red cabbage sprouts as a result of LAB fermentation treatment compared to unfermented [27]. Some LAB can produce SOD in the fermentation period [19], so the increasing SOD activity during edible grass fermentation may be due to the growth of LAB.

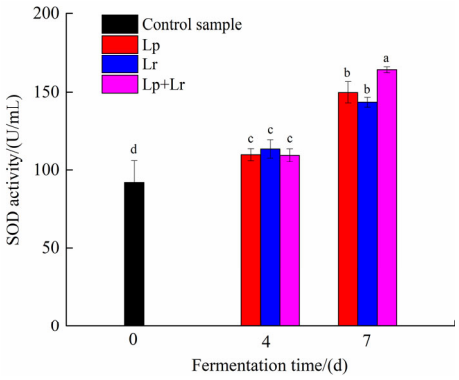

Figure 6. SOD activity of edible grass during fermentation. Different superscript letters in the columns indicate significant difference ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp+Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

3.4. Organic Acids

The variety and content of organic acids are important contributors to the bioactivity of fermented edible grass. Table 2 shows the changes in organic acids contents during the fermentation of edible grass. The total contents of organic acids of edible grasses fermented with Lp and Lr at the 4th day were significantly ($p < 0.05$) higher than the control sample, while the decreased trends were found in edible grasses fermented with Lr and mixed strains at the 7th day. The increase in organic acid content was associated with the extremely elevated amounts of lactic acid and succinic acid, while the total content of

organic acids decreased as oxalic acid was consumed at the 7th day. The lactic acid content increased up to 3.877 mg/mL after fermentation, which was more than 31 times that of the control sample of edible grass. Generally, lactic acid can be generated by the glycolytic pathway and the decarboxylation of malic acid [28,29]. Therefore, the content of malic acid significantly decreased after fermentation, consistent with the studies of fermented black chokeberry and sea buckthorn juices [30]. With the increase of fermentation time, the content of malic acid in three kinds of fermentations had no significant change ($p > 0.05$). Oxalic acid was the major organic acid in unfermented edible grass, while it decreased with the increase of fermentation time. The lowest oxalic acid content was found in the edible grass fermented by the mixed strains at the 7th day, which was only about 24% compared to that of the control sample. Reduction in oxalic acid content was possibly due to the oxalate-degrading activity of lactic acid bacteria achieved by the transportation of oxalate by permease into the cells [12]. Citric acid is an intermediate metabolite of the tricarboxylic acid cycle and can be used as the second carbon source by lactic acid bacteria [31]. Therefore, the citric acid contents of the three fermented edible grasses were lower than that of the control sample. The content of succinic acid increased significantly ($p < 0.05$) after fermentation, and the succinic acid content of edible grass fermented with mixed strains was about 0.479 mg/mL at the 7th day, which was more than 4 times higher than that of the control sample. Another study has shown similar results, wherein up to 1.14 mg/mL of succinic acid was found in matured coconut water fermentation [32]. The increase of succinic acid may be caused by the metabolic pathways of the reductive branch of the TCA cycle and the nitrogen metabolism [33].

Table 2. The profile of organic acids content of edible grass during fermentation (mg/mL).

Organic Acids		Fermentation Time/(d)		
		0	4	7
Lactic acid	Lp	0.115 ± 0.016^{d}	2.149 ± 0.341^{b}	2.364 ± 0.294^{b}
	Lr		3.877 ± 0.280^{a}	3.633 ± 0.369^{a}
	Lp + Lr		2.452 ± 0.536^{b}	1.286 ± 0.323^{c}
Oxalic acid	Lp	2.423 ± 0.015^{a}	2.061 ± 0.109^{b}	0.757 ± 0.003^{d}
	Lr		2.029 ± 0.012^{b}	0.738 ± 0.016^{d}
	Lp + Lr		1.633 ± 0.055^{c}	0.582 ± 0.006^{e}
Malic acid	Lp	0.926 ± 0.182^{a}	0.315 ± 0.086^{b}	0.363 ± 0.108^{b}
	Lr		0.151 ± 0.027^{c}	0.150 ± 0.019^{c}
	Lp + Lr		0.148 ± 0.078^{b}	0.371 ± 0.159^{b}
Citric acid	Lp	0.182 ± 0.079^{a}	0.117 ± 0.011^{b}	0.129 ± 0.011^{b}
	Lr		0.092 ± 0.048^{b}	0.129 ± 0.011^{b}
	Lp + Lr		0.115 ± 0.001^{c}	0.157 ± 0.036^{b}
Succinic acid	Lp	0.107 ± 0.026^{b}	0.343 ± 0.085^{a}	0.343 ± 0.085^{a}
	Lr		0.253 ± 0.120^{b}	0.435 ± 0.071^{a}
	Lp + Lr		0.147 ± 0.051^{b}	0.479 ± 0.183^{a}
Total organic acids	Lp	3.753 ± 0.318^{de}	4.985 ± 0.632^{bc}	3.956 ± 0.501^{cd}
	Lr		6.402 ± 0.487^{a}	5.085 ± 0.486^{b}
	Lp + Lr		4.495 ± 0.721^{bcd}	2.875 ± 0.707^{e}

Values in the same row with different letters are significantly different ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp+Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

3.5. Antioxidant Activity

Generally, DPPH radical scavenging activity and ABTS radical scavenging activity were used to measure antioxidant capacities of biological samples. Therefore, the free radical scavenging activities of the three fermented edible grass samples at the 7th day were evaluated because of the higher contents of metabolites obtained on that day. As shown in Figure 7, significant differences ($p < 0.05$) were identified between fermented edible

grasses and the control sample. DPPH radical scavenging activities in all fermentations were increased to 25 to31% as compared with the control sample. Moreover, edible grasses fermented with Lp, Lr and mixed strains exhibited higher ABTS radical scavenging activity, increasing by 25%, 20% and 35%, respectively, as compared with the control sample. This agreed with reports on fermented apricot juice, where fermentation significantly increased the DPPH and ABTS radical scavenging activities compared to the free radical scavenging activities in unfermented apricot juice [34]. The increases in DPPH radical scavenging activity and ABTS radical scavenging activity suggested that LAB fermentation could enhance the antioxidant activity of edible grass, achieved by affecting the transformation or protection of bioactive compounds like other plant-based fermentations [35].

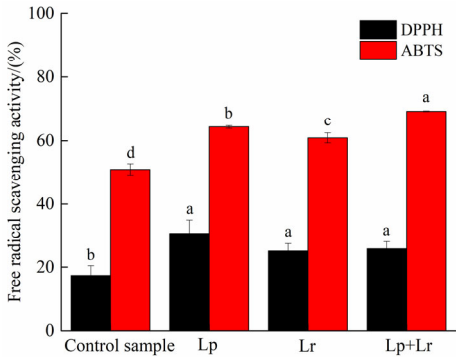

Figure 7. DPPH radical scavenging activity and ABTS radical scavenging activity of edible grass during fermentation. Different superscript letters in the columns indicate significant difference ($p < 0.05$). Abbreviations: Lp, *Lactobacillus plantarum*; Lr, *Lactobacillus rhamnosus*; Lp+Lr, mixture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus*.

Pearson correlations between metabolites and antioxidant indexes were studied to elucidate the contributions of metabolites to the antioxidant activity of edible grass (Figure 8). Highly significant positive correlations ($p < 0.001$) were observed between DPPH and soluble protein ($R^2 = 0.84$), DPPH and SOD ($R^2 = 0.92$). Additionally, succinic acid ($p < 0.01$, $R^2 = 0.71$) and lactic acid ($p < 0.05$, $R^2 = 0.62$) also had positive correlations with DPPH. Similarly, soluble protein ($p < 0.001$, $R^2 = 0.85$), SOD ($p < 0.01$, $R^2 = 0.81$) and succinic acid ($p < 0.01$, $R^2 = 0.79$) had positive correlations with ABTS. However, there were significant negative correlations between oxalic acid and DPPH ($p < 0.01$, $R^2 = -0.82$) as well as oxalic acid and ABTS ($p < 0.001$, $R^2 = -0.88$). The above results indicate that the improvement of antioxidant activity of fermented edible grass was mainly attributed to the comprehensive effects of the increased contents of soluble protein, succinic acid, lactic acid and SOD activity, and the decreased content of oxalic acid.

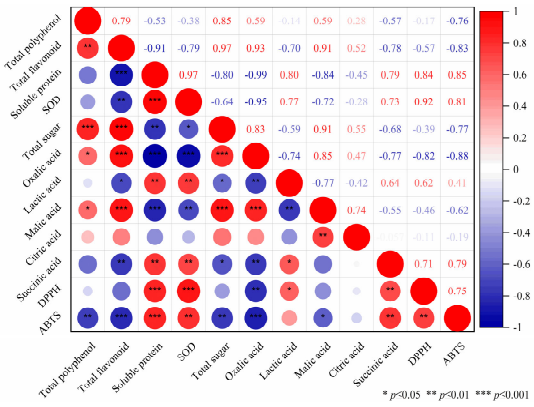

Figure 8. Pearson's correlation coefficients of antioxidant activities and phytochemical content.

4. Conclusions

In this study, edible grass was fermented by Lp and Lr to develop a possible functional product. Compared with the control sample of edible grass, fermentation of edible grass with LAB had positive impacts, increasing soluble protein content and SOD activity and improving antioxidant activity. It also reduced the oxalic acid content, which is usually considered to be an antinutritional factor and the cause of tartness of edible grass. Antioxidant activity (evaluated by DPPH radical scavenging activity and ABTS radical scavenging activity) of edible grass dramatically improved by LAB fermentation, especially for Lp fermentation and a binary mixture of Lp and Lr fermentation, as observed in the results of the improvement in soluble protein and organic acid (succinic acid and lactic acid) content and SOD activity and the reduction in oxalic acid content. The results of this study prove that LAB fermentation could reduce the oxalic acid content and improve the health benefits of edible grass and provided a guideline for processed product development made from edible grass. Further study should focus on the investigation of fermentation effects on other active parameters and the evaluation of other biological activities of fermented edible grass, to provide more scientific evidence of fermented edible grass for human health benefits.

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