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Fermented Apple Juice by Two Commercial *Lactobacillus species*: Changes of Physicochemical Composition and Antioxidant Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was aimed to compare the changes of physiochemical composition, antioxidant activities of fermented apple juice with non-fermented apple juice by two commercial lactic acid bacteria (LAB), *Lactobacillus casei* CICC 20975 and *Lactobacillus bulgaricus* CICC 21101. The antioxidant activity was evaluated by three systems including DPPH, ABTS free radical scavenging methods and Fe^{3+} reducing power. The results showed that fermentation significantly increased the content of total phenols in apple juice (*P<0.05*). After fermentation, all malic acid was converted into lactic acid during fermentation with the lactic acid content up to 381.78 mg/kg. Free proline 21.55 mg/kg and lysine 21.99 mg/kg were also significantly increased. Similarly, fermented apple juice showed significantly higher antioxidant activities when compared to non-fermented apple juice. The scavenging activity of DPPH, ABTS free radical and the reducing power of Fe^{3+} in fermented apple juice increased by 22.4%, 35.0%, 9.7%, respectively. In conclusion, fermented apple juice by two commercial lactic acid bacteria (*L. casei* CICC 20975 and *L. bulgaricus* CICC 21101) exhibited a more satisfied property and possessed great application potentials.

Keywords: Fermented apple juice; lactic acid bacteria; physicochemical composition; antioxidant activity.

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1. INTRODUCTION

Lactic acid bacteria (LAB) was a group of probiotics that can utilize carbohydrate of matrix to produce a large amount of lactic acid, including *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Oenococcus* species [1]. With an extremely wide carbohydrate utilizing profiles ranging from common monosaccharides to complex plant polysaccharides, LABs were especially suitable to ferment various vegetable and fruits juice. Fermented vegetable and fruit juice combined the beneficial role of probiotics with bioactive phytochemical compounds in plants such as flavonoids and polyphenols, becoming a kind of unique functional food. Nowadays, fermentation by different LAB was an effective technology that had been used not only to improve the flavor and stability of food but also to improve the economic and nutritional value of the original product [2,3].

Apple was one of the special local fruit products in China. According to the latest statistics, China had become the world's largest producer of apples, apple planting area and output accounted for more than 50% of the world. However, most of the apple products sold were fresh food with low processing output, and mainly concentrated apple juice, apple vinegar, which had a single processing type, single taste and low additional value. Among various choices, fermented apple juice was a good choice to add economical value for apples.

In many literature, Lactic acid bacteria such as *L. acidophilus, L. rhamnosus*, *L. casei*, *L. plantarum* in fermented apple juice had been reported [4,5]. Previous studies in our laboratory optimized various LAB to ferment apple juice and compared their consumer preferences and the number of viable bacteria (data not published). The result showed that apple juice fermented with *L. casei* CICC 20975 and *L. bulgaricus* CICC 21101 had typical apple flavor, soft taste, delicious with slight sweetness and sour. On the basis of the above test, in the present study, we compared the physicochemical compounds, antioxidative abilities as well as aroma profile of the fermented apple juice with non-fermented apple juice to indicate the nutritional and functional changes before and after fermentation.

2. MATERIALS AND METHODS

2.1 Microorganisms and Culture

Both *L. casei* CICC 20975 and *L. delbrueckii* subsp. *bulgaricus* (termed L. *bulgaricus* hereafter) CICC 21101 were purchased from China's Industrial Microbial Preservation Management Center (CICC). All bacterial cultures were stored frozen at -20°C in MRS medium (Aoboxing Biotech Co. Ltd, Beijing, China) containing 20% glycerol. The strains were reactivated by means of double passage on MRS when needed.

2.2 Raw Materials and Reagents

In this work, fuji apples were purchased from orchard worker in Jixian, Shanxi, China. The fuji apples were cleaned with filtered water and chopped into small pieces. Then they were soaked in color protector liquid (mixed solution of ascorbic acid concentration of 3.5 mg/L and citric acid concentration of 7.5 mg/L) for 30 minutes. The apple juice was obtained through mechanical process by pressing the pulp in juice extractor, and then apple juice was heated by microwave heating for 2 min. The heated fuji apple juice was stored frozen (-20°C) prior to use. No additive was added to the juice.

Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Merck (Germany); MRS broth medium, AGAR and nutrient broth medium (NB) were purchased from Beijing Aoboxing Biotech Co. Ltd. (Beijing, China); Fructose, maltose, sucrose and free amino acid were purchased from Sigma Chemical Co. Sigma (USA); amino acid standards were purchased from Shanghai Anpel laboratory Technology Co., Ltd. All other chemicals and reagents used in the experiments were of analytical grade.

2.3 Preparation of Fermented Apple Juice

L. casei CICC 20975 and *L. bulgaricus* CICC 21101 were cultivated on MRS broth at 37°C for 24 h. Cells at the late exponential phase was obtained, harvested by centrifugation (10,000×g, 10 min, 4°C), washed twice with 50 mM sterile phosphate buffer solution (PBS, pH 7.0), and then re-suspended in sterile distilled water to the final optical density. After that, *L. casei* CICC 20975 (3.0×10^6 CFU/mL) and *L. bulgaricus* CICC 21101 (3×10^6 CFU/mL) were inoculated into 200 mL freshly prepared apple juice. Mixtures were statically cultured at 38°C for 60 h. And the final live bacterial counting was performed in MRS and the results showed that the live LAB numbers in fermented apple juice were up to 1.5-3.0×10^9 CFU/mL. In the present study, an uninoculated apple juice was used as an experimental control.

2.4 Determination of Free Sugars

The concentration of free sugars was determined by HPLC (1200 Series, Agilent, USA) equipped with a refractive index detector (RID) according to a modified version of the method described by Mousavi with some modifications [6]. The mobile phase was acetonitrile and water at a volume ratio of 7:3. Chromatographic analysis was achieved using a column (Agilent amino column 4.6 mm×250mm, 5 μm) maintained at 35°C. The injection volumes of 10 μL were chromatographically separated at a flow rate of 1.0 mL/min for both samples and standards. Sugar content was calculated using external standards. The following chromatographic grade free sugars were used as standards.

2.5 Analysis of Organic Acids

The chromatographic system was used to quantify the organic acids consisted of a Diode array detector (DAD) (1200 Series, Agilent, USA) according to a modified version of the method described by Belguesmia et al. with some modifications [7]. The mobile phase was K_2HPO_4 (10 mM, pH 2.55). One milliliter of sample was added 5 mL of mobile phase, ultrasonic extracted for 30 min and left for 1 h at 60 °C water bath. After being centrifuged for 10 min at 12000×g at 4 °C, the supernatant was kept for 1 h at 4 °C before filter-sterilization (0.45 μm pore size). The samples were then kept at –80 °C until analysis. Chromatographic analysis was achieved using a column (Agilent AQ 4.6 mm×250 mm, 5 μm) maintained at 30 °C. The injection volumes of 10 μL were chromatographically separated at a flow rate of 0.5 $mL \cdot min^{-1}$ for both samples and standards. The wavelength was set at 210 nm to detect the organic acids. The following chromatographic grade organic acids were used as standards. To ensure accuracy, the working standards were prepared daily. The HPLC results were qualitatively analyzed by peak retention time and quantified by peak area using the external standard method. Extractions and injections were conducted in triplicate for each fermentation replicate.

2.6 Determination of Free Amino Acids

FAAs analysis was carried out using acid hydrolysed (0.2 mM HCl) samples by reverse-phase high-performance liquid chromatography (HPLC) after precolumn derivatization by phenylisothiocyanate (PITC), by a modified method adapted from Zhao [8].

Extraction of FAAs from apple juice: One gram of sample was added with 8 mL of 0.2 mM hydrochloric acid, shook in a vortex for 5 min, then extracted by ultrasound for 10 min. After standing in the dark for 2 h, it was centrifuged at 2057×g for 10 min, taking the supernatant for later use.

Instrument method: The FAAs contained in the samples were separated using an amino acid SHISEIDO C18 column, 5 μm (250×4.6 mm) attached to Agilent 1260 Chromatography system equipped with UV-Vis detector monitoring at 254 nm. The injection volume was set at 10 μL and the column was kept at 40°C. The gradient mobile phase, consisting of eluent A (prepared by mixing the 0.1 mol/L sodium acetate with acetonitrile at a ratio of 97: 3) mix well and adjust pH to 6.5 (31.815 g sodium acetate plus 3880 mL water plus 120 mL acetonitrile) and eluent B (80% acetonitrile, 20% Milli-Q water) was injected at a flow rate of 1 mL / min throughout the experiment. The gradient program was defined as follows: 100 % A at start, 85 % A and 15 % B at 14 min, 66 % A and 34 % B at 29 min, 0 % A and 100 % B at 30 min and for 7 min, 100% A at 38 min and for 9 min, 100 % A at 45 min, allowing the column to equilibrate for 15 min until the 60th min.

2.7 Determination of the Total Phenolic Content

Total phenolic content was determined based on the *Folin-Ciocalteu* colorimetric method as described by Xu et al. [9]. Gallic acid was used as a reference standard, and the values of total phenols were expressed as milligram of gallic acid equivalent (GAE) per milliliter of fruit juice. The juice was diluted with a mixture of methanol (80%)-water and centrifuged at 6650×g for 10 min. The supernatant was used as a test sample for determining the total phenolic content. Briefly, an aliquot (0.5 mL) of appropriately diluted apple juice, 2.5 mL of deionized water and 0.5 mL of 1.0 M *Folin-Ciocalteu* reagent were mixed within 10 mL volumetric flasks and vortexed. After 8min, 1.5mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured using a spectrophotometer at 765 nm wavelength after incubation for 2 h at room temperature. Methanol was used as the blank, and gallic acid (GA) was used for calibration of the standard curve (0-500 $mg \cdot L^{-1}$). Phenolic content was expressed as gallic acid equivalents (milligrams of GAE per gram juice).

2.8 Determination of the Content of Total Flavonoid

The total flavonoid was measured by the method of by Feng and Xu [10]. Rutin was used as a reference standard, and the total flavonoid content was calculated as the equivalent of rutin content per mL of sample.

2.8.1 Determination of the content of ascorbic acid (Vc), total titratable acidity (TTA), pH, and total soluble solids (TSS)

The content of Vc was determined by 2, 6-dichlorophenol titration method according to the publication of AOAC [11]. TAA content was measured by using the method of AOAC [11]. Briefly, samples were 10-fold diluted, and then titrated with 0.1 N NaOH with phenolphthalein as indicator. The pH of the juice was measured using a pH meter at 25□. TSS in the juice sample was determined by a benchtop digital refractometer (PAL-1, Atago, Japan).

2.9 Determination of Antioxidant Activity

2.9.1 DPPH free radical scavenging activity

The DPPH free radical scavenging activity method according to the method as previously described by Guo et al. [12]. Briefly, each of sample solutions was serially diluted to various concentrations in methanol respectively, and then a 0.5 mL of samples was mixed with 2.5 mL of 60 μM DPPH dissolved in methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was measured at 517 nm against a solvent blank. The scavenging rate on DPPH free radical was calculated according to the formula: Scavenging rate (%) = [1- (Asample – Ablank) /Acontrol] ×100. Where, A was the absorbance of the sample, blank, or control, as indicated. The DPPH free radical scavenging activity of apple juice was expressed in mM of Vc.

2.9.2 ABTS free radical scavenging activity

The ABTS free radical scavenging activity method as described by Xu et al. [13]. ABTS free radical cation was generated by a reaction of 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate. The reaction mixture was allowed to stand in the dark at room temperature for 16−24 h before use and was used within 2 days. The ABTS solution was diluted with methanol to an absorbance of 0.700 ± 0.050 at 734 nm. One hundred microliters of the diluted samples were mixed with 2.0 mL of diluted ABTS solution. The mixture was allowed to stand for 6 min at room temperature, and the absorbance was immediately recorded at 734 nm. The scavenging rate were calculated using the equation described for DPPH assay. The ABTS free radical scavenging activity of apple juice was expressed in mM of Vc.

2.9.3 Determination of Fe^{3+} reducing power

The Fe^{3+} reducing power method as described by Kwaw et al. [14]. An aliquot of the apple juice (1 mL, diluted at 1:100) was mixed with 0.05 mL of HCl (0.01 M), 0.4 mL of potassium ferricyanide (0.02 M), 0.4 mL of 0.02 M $FeCl_3$ and 0.7 mL of distilled H_2O. The mixture was consequently incubated at 37 ^oC in the dark for 30 min and the absorbance read at 720 nm. The Fe^{3+} reducing power of apple juice was expressed in mM of Vc.

2.10 Electronic Nose Measurements

The aroma profiles of the juice samples were analyzed using an electronic nose system (PEN3, Airsence, Germany) [15]. Briefly, each sample (15 mL) was put into a 100 mL glass jar. Then the glass jar was sealed with three layers of plastic wrap and the headspace inside it was equilibrated for 30 min at 45°C under agitation at a speed of 500 rpm. The headspace volatiles were put into the electronic nose for 9 sec at a rate of 7.7 $mL \cdot min^{-1}$. To acquire stable signals, the acquisition duration for the sensors was 90s. The analysis system was purged with processed dry and pure air before each analysis. Each sample was tested 6 times to ensure the accuracy of the data, and the last measured three data were used in the subsequent analysis. The serial number, main applications, and references of the ten sensors were listed in Table 1 [16].

3. RESULTS AND DISCUSSION

3.1 Changes of Sugars Content in Apple Juice

The content of the free sugars and total soluble solids (TSS) in fermented and non-fermented apple juice were shown in Table. 2. As we can see from Table. 2, the levels of TSS, fructose and glucose decreased significantly (*P<0.05*). The soluble solid (TSS) of apple juice decreased by 3.4% during fermentation. The soluble solids utilization was high in fermented apple juice

compared to non-fermented apple juice due to the higher microbial load [17]. There was literature reported that the decrease in sugar concentrations during fermentation was largely due to not only bioconversion into lactic acid, but also the utilization for growth and metabolism of lactic acid bacteria [18]. Besides, 5.3% of fructose and 1.3% glucose were utilized during fermentation, respectively. Similar results were reported for Sohiong juice [19] and Pomegranate Juice [7]. It had been reported that fructose and glucose were efficient carbon and energy source for most of *Lactobacillus* strains [20]. Among the free sugars, the concentrations of sucrose and maltose showed no significant difference when compared fermented juice with non-fermented juice (*P>0.05*). This may be the result that sucrose and maltose were not utilized by lactic acid bacteria during fermentation generally. In addition, there were no detectable lactose in fermented and non-fermented juices.

3.2 Changes of Acid Substances of Apple Juice

The total acid (TA), pH and the content of nine free sugars in fermented and non-fermented apple juice were shown in Tab. 3. As shown in Table. 3, the total acid content of fermented apple juice increased significantly (*P<0.05*), and the pH content decreased significantly (*P<0.05*). The total titratable acid concentration decreased by 28.1% from 0.69 $g \cdot kg^{-1}$ to 0.96 $g \cdot kg^{-1}$. At the meantime, the pH decreased by 29.4% from 5.21 to 3.68. The low pH and high acidity were in agreement with the findings reported by Ibanoglu et al. [21].

3.3 Changes of Free Amino Acid Content of Apple Juice

It had been reported that soluble substances, some fat and some vitamins in fruit juice can be metabolized by probiotics into amino acids, fatty acids and so on [22]. The varieties and contents of free amino acids in fermented and non-fermented apple juice were shown in Table. 4. According to Table. 4, a total of 17 amino acids were detected in fermented and non-fermented apple juice, among which 7 were essential amino acids for human body. Changes in the content of nonessential amino acids (NEAA) were similar to total free amino acids (TFAA), there were no significant differences in fermented and non-fermented apple juice (*P>0.05*). Among the 17 detected amino acids, aspartic acid was the most abundant, and there was no significant change after fermentation, followed by glutamic acid and serine, which decreased significantly from 49.13 $mg \cdot L^{-1}$ to 14.58 $mg \cdot L^{-1}$ and decreased from 293.68 $mg \cdot L^{-1}$ to 255.86 $mg \cdot L^{-1}$, respectively. Similar results were reported by Xu et al. [23]. A total of seven total essential amino acids were detected and represented 4.6% and 3.6% of the total free amino acids in fermented and non-fermented apple juice, respectively. Meanwhile, cysteine, phenylalanine and methionine were not detected. Moreover, the content of proline was up to 21.55 $mg \cdot kg^{-1}$ and 3.7 times higher than the juice without fermentation. The similar trend was also found in the content of lysine with a concentration of 21.99 $mg \cdot kg^{-1}$ in fermented juice.

3.4 Changes of Phytochemical Contents in the Apple Juice

The contents of phytochemical compounds, including total polyphenols, flavonoids and Vc, fermented and non-fermented apple juice were shown in Fig. 1. As indicated in Fig. 1, except for the content of total polyphenols, the contents of the other two active compounds in the juice with and without fermentation showed no significant differences. Interestingly, total polyphenols significantly increased by 14.4% and the value was up to 0.97 milliequivalent of gallic acid. Previous studies showed that vegetable or fruits juice fermented by *Lactobacillus* showed higher contents of polyphenols and flavonoids, suggesting a bio-conversion role of LAB in this process [24]. As for the change of Vc content, Kaprasob et al. also reported that cashew apple juice fermented with *L. plantarum* retained a matchable level. This may be due to the protective effect of LAB fermentation and they may prevent the degradation of Vc. All of these results indicated that LAB fermentation can biotransform some natural botanical compounds into bioactive compounds or prevent the loss of bioactive phytochemicals, and finally enhance the quality of products.

3.5 Changes of Antioxidant Activity of Apple Juice

The changes of antioxidant activity of in fermented and non-fermented apple juice were shown in Fig. 2. It can be seen from Fig. 2 that the antioxidant activity of fermented apple juice was significantly increased (*P<0.05*). After fermentation, the DPPH free radical scavenging rate reached up to 56.9% equivalent to 7.32 $mmol \cdot L^{-1}$ Vc and it was 1.3 times of that non-

Table 1. Sensors used and their main applications in PEN 3

Number in Array	Sensor name	General description	Reference (ppm)
S1	W1C	Aromatic compounds	Toluene,10
S2	W5S	Very sensitive, broad range sensitivity, react on nitrogen oxides, very sensitive with negative signa	NO_2, 1
S3	W3C	Ammonia, used as sensor for aromatic compounds	Benzene, 10
S4	W6S	Mainly hydrogen, selectively (breath gases)	H_2, 0.1
S5	W5C	Alkenes, aromatic compounds, less polar compounds	Propane, 1
S6	W1S	Sensitive to methane broad range	CH_3, 100
S7	W1W	Reacts on sulfur compounds, sensitive to many terpenes and sulfur organic compounds, which are important for smell, limonene, pyridine	H_2S, 1
S8	W2S	Detects alcohols, partially aromatic compounds, broad range	CO, 100
S9	W2W	Aromatics compounds, sulfur organic compounds	H_2S, 1
S10	W3S	Reacts on high concentrations, sometime very selective (methane)	CH_3, 100

Table 2. Changes of sugar content in fermented and non-fermented apple juice

Sample	Soluble solids (^oBrix)	Free sugar				
		Fructose ($g·100g^{-1}$)	Glucose ($mg·kg^{-1}$)	Sucrose ($g·100g^{-1}$)	Maltose ($ug·g^{-1}$)	Lactose ($g·100g^{-1}$)
AJ	12.8±0.5a	6.24±0.20a	4.46±0.23a	0.77±0.09a	0.12±0.04a	NF
FAJ	9.4±0.3b	5.91±0.19b	3.80±0.16b	0.79±0.21a	0.15±0.03a	NF

Different letters within a column indicate statistically significant differences between the means (P<0.05); NF, not found; AJ, apple juice; FAJ, fermented apple juice.

Table 3. Changes of acid substances of fermented and non-fermented apple juice

Sample	TTA ($g·kg^{-1}$)	pH	Organic acid ($ug·g^{-1}$)								
			oxalic acid	tartaric acid	malic acid	lactic acid	acetic acid	citric acid	oxalic acid	maleic acid	fumaric acid
AJ	0.69±0.12b	5.21±0.64a	NF	0.59±0.18a	204.30±9.21	NF	NF	NF	NF	NF	NF
FAJ	0.96±0.06a	3.68±0.43b	NF	0.45±0.09b	NF	381.78±7.89	NF	NF	NF	NF	NF

Numbers represent mean values of three independent replicates ± SD; TTA, Abbreviations of total titratable acid; NF, not found; AJ, apple juice; FAJ, fermented apple juice; Different letters within a column indicate statistically significant differences between the means (p < 0.05).

Table 4. Changes of free amino acid content (mg·kg^{-1}) of fermented and non-fermented apple juice

Amino acids	AJ	FAJ	Amino acids	AJ	FAJ
aspartic acid	415.61±10.91a	418.13±9.88a	proline	5.84±0.38b	21.55±0.52a
glutamate	49.13±5.14a	14.58±2.12b	tyrosine	NF	NF
cystine	NF	NF	valine	7.02±0.23	NF
serine	293.68±9.35a	255.86±8.89b	methionine	NF	NF
glycine	4.48±0.23	NF	isoleucine	6.11±0.41a	2.42±0.34b
histidine	NF	NF	leucine	1.53±0.08a	1.43±0.19a
arginine	NF	NF	phenylalanine (Phe)△	NF	NF
threonine	2.52±0.21a	1.66±0.17a	lysine	20.3±3.18b	21.99±2.91a
alanine	12.04±0.75a	10.61±2.21a			
TEAA	37.48±3.34a	28.92±2.26b			
NEAA	780.78±18.23a	782.31±26.39a			
TFAA	818.26±10.45a	811.23±12.18a			

Numbers represent mean values of three independent replicates ± SD; NF, not found; AJ, apple juice; FAJ, fermented apple juice; Abbreviations of amino acids (TEAA, total essential amino acids; NEAA, nonessential amino acids; TFAA, total free amino acids); Different letters within a column indicate statistically significant differences between the means (P<0.05).

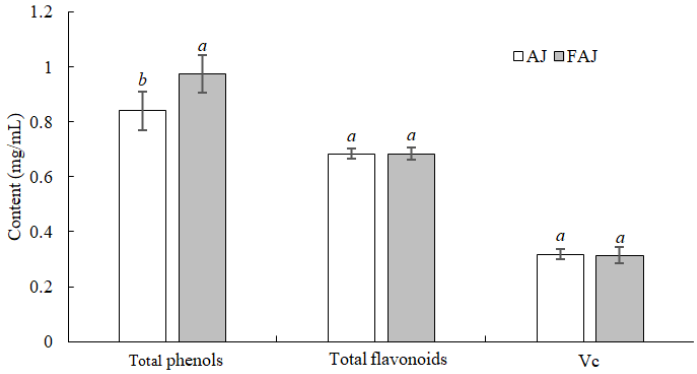


Fig. 1. Changes of antioxidant substances of fermented and non-fermented apple juice
Different letters within a column indicate statistically significant differences between the means (P<0.05); AJ, apple juice; FAJ, fermented apple juice.

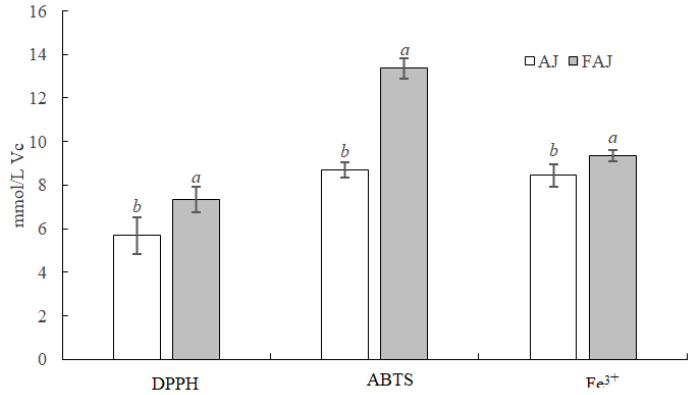


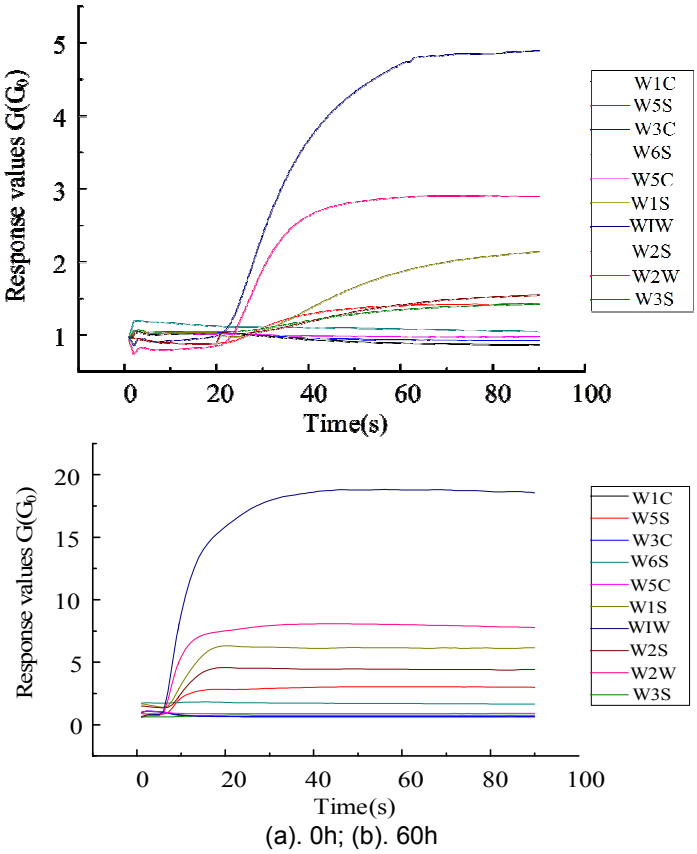
Fig. 2. Changes of antioxidant activity of fermented and non-fermented apple juice
Different letters within a column indicate statistically significant differences between the means (p < 0.05); AJ, apple juice; FAJ, fermented apple juice

fermented apple juice. As for ABTS free radical scavenging rate, the activity reached up to 58.9% equivalent to 13.45 mmol·L^{-1} Vc and had a 50% increase. The Fe^{3+} reducing power of fermented apple juice was 82.3% equivalent to 9.35 mmol·L^{-1} Vc and it was 1.1 times of that non-fermented apple juice. Similar results reported that Noni juice fermented with *Bifidobacterium longum* had greater antioxidant activity than non-fermented noni juice.

3.6 Analysis of Aroma Composition of Apple Juice

The aroma profiles of apple juice during fermentation were examined using an electronic nose. Fig. 3 displayed a typical response of ten sensors during measurement of apple juice (0h) and fermented apple juice (60h) in which each curve represented a different sensor response with time. The ordinate represented the changing ratio between G and G_0. It was apparent that, the responsive values of the sensors, after an initial period of low responsive values, increased sharply and then stabilized after 60 s. In this research, the responsive values of each sensor at 60 s point were used in analysis.

As shown in Fig. 3, compared with Fig. (a), the (b) of response values of W5S, W1S, W1W, W2S, W6S and W2W were enhanced to varying degrees. With the increase of fermentation time, the response values of W1W and W2W decreased at first (0h~12h) and then increased (12h~60h), while the response values of W5S, W1S and W2S gradually increased to stable with the increase of fermentation time, but the response values of other sensors did not change obviously. The differences in flavor of apple juice during different fermentation stages may be due to the changes in the substances represented by the sensors. Therefore, the characteristic flavor of fermented apple juice may be derived from esters, nitrogen, methane, sulfur compounds, alcohols, hydrogen, alcohols, and the dynamic changes of these substances affected the overall flavor.



(a). 0h; (b). 60h

Fig. 3. Response curves of electronic nose sensors to fermented and non-fermented apple juice

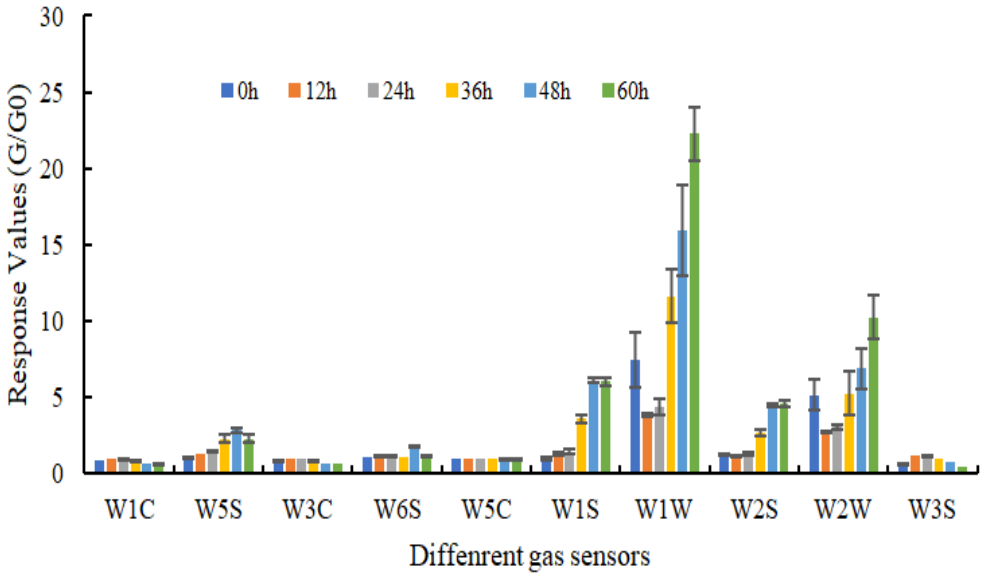


Fig. 4. Changes in response curves of electronic nose sensors to apple juice at different fermentation stages

4. CONCLUSION

The results showed that the LAB might had utilized carbohydrates and produced large amounts of organic acid, thus lowering the pH of the samples during fermentation. Lactic acid bacteria fermentation can release the content of organic acids, free amino acids and convert phenolic compounds to enhance the antioxidant activity. In addition, the characteristic flavor of fermented apple juice may be derived from nitrogen, methane, sulfur compounds, alcohols, hydrogen, alcohols.

These findings highlighted the beneficial effect of probiotic fermentation on the quality of apple juice. Probiotic fermentation changed the phytochemical composition of fruits juice, which thus enhanced their antioxidant activity. All of these contribute to a more satisfied quality of the final product from both stability and nutrition perspectives. Based on the findings in this study, apple juice fermented by *L. casei* CICC 20975 and *L. bulgaria* CICC 21101 was satisfied leavening agents for a health beverage. However, additional research was needed on the control of the fermentation process and the identification of the key active compounds and flavor substances produced during fermentation. All these works assist in obtaining more desirable organoleptic qualities in fermented food products.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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