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Lactobacillus HNC7-YLC92 Improves the Fermentation Quality of Cassava–Acerola Cherry Beverage

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Abstract: In order to promote the consumption and application of cassava in the food industry, the cassava–acerola cherry juice beverage was developed using lactic acid bacteria fermentation, which improved the flavor attraction and nutritional value, increased the added value of cassava and acerola cherry. The study investigated the effects of both pure and mixed fermentation using *Lactobacillus plantarum* (*L. plantarum*) HNC7 on the chemical compounds and antioxidant activities in cassava–acerola cherry juice. Following 72 h of fermentation, the fruit juice showed increased acidity, a decrease in pH, and higher consumption of soluble solids, and a significant rise in volatile substance content and antioxidant activity ($p < 0.05$). Notably, the HNC7-YLC92 binary combination demonstrated the most effective fermentation, resulting in a product with uniform color, moderate sourness and sweetness, and a delicate and smooth taste. Overall, the HNC7-YLC92 binary combination, due to its beneficial biological properties, shows great potential as the optimal strain for fermenting this juice. This provides a reference for selecting industrial fermentation conditions and strains, aiming to develop new value-added cassava products and increase their consumption.

Keywords: cassava–acerola cherry; *Lactobacillus plantarum*; organic acids; volatile compounds; antioxidant activities; mixed fermentation

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1. Introduction

Cassava is a tropical and subtropical root crop, which ranks among the world's three largest potato crops; originating from the Amazon Basin, it is now extensively cultivated in Chinese provinces such as Hainan, Guangdong, and Fujian [1]. Rich in nutrients like niacin, thiamine, calcium, potassium, and phosphorus [2], cassava is beneficial to human health as a food crop. Currently, cassava processing yields a relatively limited variety of products when compared to other food crops. The development and large-scale production of new processing technologies and products for cassava are still in their infancy, limiting the crop's potential economic benefits.

Currently, the beverage market is primarily composed of fruit, carbonated, and tea drinks, while tuber drinks are relatively less common. Utilizing abundant and inexpensive cassava resources for developing cassava beverages, not only can reduce production costs, enhance the added value of cassava but also can contribute to the beverage market's growth. Nowadays, with the focus on consumers' healthy diets, fermented products, especially non-dairy beverages, are gaining attention for their functionality [3]. Fermentation can enhance flavor [4], while the metabolites produced by lactic acid bacteria exhibit antibacterial and antioxidant properties [5]. Freire believes that integrating lactic acid bacteria (LAB) into

beverages [6], like bergamot juice [7], sea buckthorn [8], and pomegranate juice [9], is a growing trend following the launch of new fruit- and grain-based products. The traditional Brazilian beverage yakupa is a naturally fermented cassava drink, produced without controlled fermentation or environmental conditions [10]. However, cassava beverages often lack appeal due to their relatively unsweet taste and lack of a strong aroma.

The Acerola cherry is notable for its high sugar content, diverse phenolic compounds, and multiple vitamins, offering a pleasant flavor profile characterized by mild sweetness and sourness. However, they are prone to spoilage, leading to economic losses. Deep processing is an effective strategy to mitigate losses and enhance their added value. To promote the consumption and application of cassava in the food industry, acerola cherry juice was added to enrich the aroma, flavor, and ascorbic acid (AA) content, thereby creating a lactose-free functional beverage [11]. Compared with single fermentation, mixed fermentation can significantly reduce the fermentation time and yield a greater variety of acids, alcohols, aldehydes, and esters. So far, there are many studies on the application of mixed culture in fruit fermentation, information on its application in cassava–acerola cherry juice, either in pure or mixed forms, remains scarce. Building upon the previous study [12], *L. plantarum* HNC7 and two other *Lactic acid bacteria* were chosen for fermentation. The analysis included assessing viable bacteria count, organic acids, sugars, and volatile compounds in both pure and mixed fermentations. Then, the strains with favorable biological properties were chosen as fermenters of cassava–cherry juice. This not only provides the possibility of developing new products of cassava but also enriches the special bacterial stock of fruit fermentation.

2. Materials and Methods

2.1. Microorganisms and Culture Conditions

L. plantarum HNC7, *L. plantarum* J1, and *L. paracasei* YLC92 were isolated from cassava leaves, banana, and tofu fermentation respectively and supplied from the College of Food Science and Engineering of Hainan University, Haikou, China. These strains were retrieved from a −80 °C ultra-low temperature refrigerator (SANYO NDF-382E, Osaka, Japan) and reactivated in MRS broth (BD Company, Franklin Lakes, NJ, USA) at 37 °C for 48 h.

2.2. Preparation of Cassava-Acerola Cherry Juice

Cassava roots were sourced from local markets. Then, crushed in a juicer (Joyoung, Jinan, China) and thoroughly mixed with 85 °C water at a 1:6 ratio. Based on the mass ratio of cassava raw material, 100 U/g α-Amylase (Macklin, Shanghai, China) was added to cassava juice and enzymatic hydrolysis was conducted at 85 °C for 2 h. Subsequently, the juice was boiled for 10 min to halt the enzymatic reaction and filtered with a dense gauze. No viable cells were detected in the juice after heat-treatment.

Acerola cherries supplied from the acerola cherry base (Dingan, China). After pulping and pressing, the juice was filtered through a 140-mesh sieve. Then, the mixture of acerola cherry juice and cassava juice (in the ratio 1:4) was pasteurized for inoculation (85 °C water bath for 5 min). The total bacterial cells were counted after pasteurization, revealing no increase in either total bacterial cells or the count of LAB in the mixed juice (negative control).

2.3. Fermentation

Based on previous research, four fermentations were conducted, labeled as group A (*L. plantarum* HNC7), group B (*L. plantarum* HNC7 + *L. plantarum* J1), group C (*L. plantarum* HNC7 + *L. paracasei* YLC92), and group D (*L. plantarum* HNC7 + *L. plantarum* J1 + *L. paracasei* YLC92). Before fermentation, each bacterial culture was centrifuged, and the bacterial cells were washed twice, before being suspended in a 0.85% NaCl solution. Each suspension had a cell population of approximately 6.4 log CFU/mL and was inoculated separately into 500 mL conical flasks, each containing 200 mL of cassava–acerola cherry juice. Control samples consisted of naturally fermented bacteria in mixed fruit juices without inoculation.

Following a 3% inoculation dose, fermentations were carried out in a laboratory incubator (Yiheng; DHP-9012; Shanghai, China) at 37 °C for 72 h and fermentation juices were sampled every 24 h for analyses.

2.4. Viable Count and pH Measurement

The LAB were enumerated through spread-plating appropriate dilutions on an MRS agar (BD Company, Franklin Lakes, NJ, USA), while the pH of fermentation juice was measure using a pH meter ST2100 (Ohaus, Parsippany, NJ, USA).

2.5. Acidity and Soluble Solids

The fermentation sample was titrated with 0.1 N NaOH to determine acidity, which was expressed as the percentage of lactic acid. Soluble solids were measured with a portable refractometer (PX-B32T, Guangzhou Puxitong Instrument Co., Ltd., Guangzhou, China).

2.6. Determination of Organic Acids and Sugars

Organic acids (lactic acid, citric acid, malic acid, tartaric acid, and succinic acid) and sugars (glucose, fructose, and sucrose) were analyzed using high-pressure liquid chromatography (Agilent, Santa Clara, CA, USA), as described [13]. Organic acids were separated on an Athentgga C18-Wp column (4.6 × 250 mm, 5 μm) (ANPEL Labs Inc., Shanghai, China) and detected with an ultraviolet detector at 210 nm. For sugars, a refractive index detector was employed with a separation column of Athena NH2-RP (4.6 × 250 mm, 5 μm) (ANPEL Labs Inc., Shanghai, China).

2.7. Volatile Analysis

An analysis of the volatile compounds in fermentation juices was conducted using headspace–solid phase microextraction-gas chromatography-mass spectrometry (Agilent 7890 A-5975 C, Santa Clara, CA, USA) as described previously [14], with slight adjustments. The juices (2 mL) were mixed with sodium chloride (0.6 g) in a headspace flask, preheated at 50 °C for 20 min, and absorbed by the extraction head for 30 min. Then, the extraction head was inserted into the sample inlet of the gas chromatography and desorbed at 250 °C for 5 min. Volatile compounds were separated on a DB-5MS column using helium as the carrier gas, and the column temperature was increased from 50 °C to 250 °C. The scanning range of MS ranged from 30 to 350 amu, and the mass spectrum of volatiles was analyzed and compared with the NIST 14 library.

2.8. Ascorbic Acid Content

The AA content was determined using a 2,6-dichloroindophenol titration method. Initially, 10 mL fermentation juice was mixed with 10 mL of a 20 g/L oxalic acid solution, the resulting mixture was titrated with 2,6-dichloroindophenol until it turned reddish and remained so for 15 s. Meanwhile, 10 mL of oxalic acid solution was used to titrate the juices as a blank control, following the same procedure as described above. The result was calculated using the following formula: AA content (mg/100 g) = $[(V - V_0) \times T \times A]/m \times 100$, where V and V_0 are the volumes of 2,6-dichloroindophenol solution consumed by the samples and the control, respectively (mL); T is the titration of 2,6-dichloroindophenol solution (mL); A is the dilution ratio; m is the mass of the sample (g).

2.9. Antioxidant Activity

The antioxidant activity was measured according to the method of Hashemi [15] with slight adjustments. The fermentation juice was centrifuged at 4000× *g* for 5 min, and then 0.04 mL of obtained supernatant and 0.96 mL absolute ethyl alcohol were blended with 2 mL 0.05 mg/mL DPPH. The absorbance value was measured at 519 nm after the mixed solution was kept for 30 min in the dark. ABTS working mother liquor was obtained by mixing ABTS solution and oxidizer solution in equal volumes and storing at room temperature for 16 h away from light. The absorbance of ABTS working mother liquor was

0.65~0.75 at 734 nm when diluted with 80% anhydrous ethanol. After mixing the diluent with the sample for 6 min, the absorbance of the solution was measured.

The DPPH and ABTS radical scavenging activity (%) were calculated using the following formula: DPPH (%) = $[(A_0 - A)/A_0] \times 100$, ABTS (%) = $[(A_0 - A)/A_0] \times 100$, where A_0 and A are the absorbance value of the control and the samples, respectively.

2.10. Statistical Analysis

An analysis of variance (ANOVA) and Duncan's multiple range tests were performed using the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). $p < 0.05$ indicated a statistically significant difference. All experiments were repeated three times and the results were expressed as mean with standard deviations. Values with different lowercase letters at the same fermentation time point are considered significantly different ($p < 0.05$).

3. Results and Discussion

3.1. Bacterial Growth and Changes of pH during Fermentation

No significant difference was observed in the viable count before fermentation between the control sample and the experimental group. In the four fermentation trials, the viable counts in single and binary culture fermentations increased with the extension of fermentation time, which was significantly ($p < 0.05$) higher than that in ternary fermentation when reaching the peak at 48 h, and then it decreased. It was observed that the viable counts in HNC7-J1 binary combination and monoculture were not significant, but the viable counts in the HNC7-YLC92 binary combination were significantly ($p < 0.05$) greater than that of the remaining combinations at 72 h of fermentation. The results showed that the HNC7-YLC92 binary combination could maintain a higher biomass. Hashemi and Jafarpour reported that the interaction might stimulate or inhibit the growth of certain strains in mixed cultures, and the stimulation might be caused by the production of certain nutrients by other strains, which was interpreted as a compound produced by one microbe and then metabolized by another [16].

As shown in Figure 1b, the pH of cassava–acerola cherry juice in the four treatment groups and the control group significantly decreased within the initial 24 h, which was related to the rapid proliferation of LAB. In the late fermentation stage, the pH of all treatment groups reduced by around 0.27 units, and the pH of the binary culture treatments and the ternary mixed culture were the lowest. However, there was no statistically significant difference in pH between the single culture and the control group ($p > 0.05$).

3.2. Acidity and Soluble Solids

During the entire fermentation period, the acidity of fermented juices showed a significant increase ($p < 0.05$) compared with that of the control group (Figure 2a). The increase in acidity was mainly due to the rise in lactic acid (Table 1). It was observed that the acidity level of the HNC7-YLC92 binary combination was notably higher than that of other treatment groups after 72 h of fermentation. This was consistent with a downward trend in the pH values of the HNC7-YLC92 binary combination, where an increase in lactic acid correlates with a decrease in pH and an increase in acidity.

As one of the quality indicators [17], the soluble solid content of unfermented juices was 6.6° Bx (Figure 2b). In the fermentation process, the soluble solids content decreased in all treatment groups, and the maximum decrease of the HNC7-YLC92 binary combination was 1.4, followed by the HNC7-J1 binary combination. The degree of value decline was the least in single and ternary fermentations and there was no significant ($p > 0.05$) difference between the two treatments. Studies had shown that the change of soluble solids content had a strong correlation with malic acid and lactic acid and a low correlation with sugars [8]. The findings indicate that the HNC7-YLC92 binary group exhibits a higher utilization of soluble solids in cassava–acerola cherry juice.

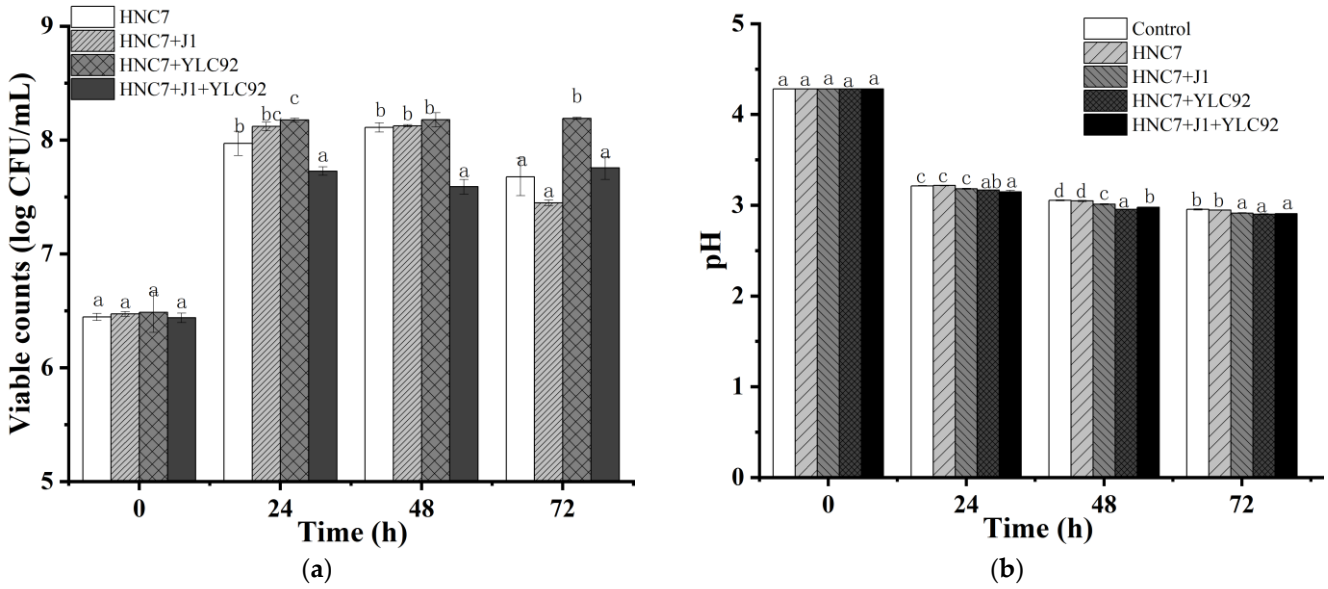

Figure 1. Changes in viable cell counts (**a**) and pH (**b**) during 72 h fermentation of cassava–acerola cherry juice with pure and mixed cultures of *L. plantarum* HNC 7. a–d indicate significant differences between data on the same day ($p < 0.05$).

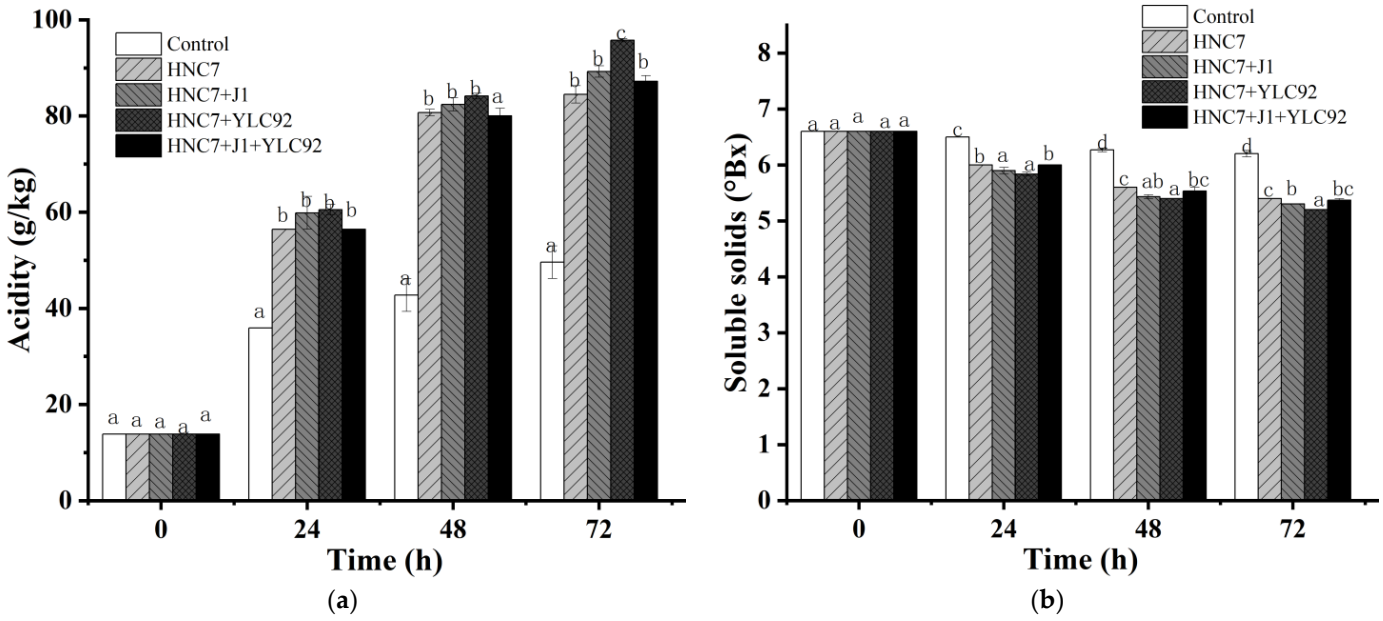

Figure 2. Changes of acidity (**a**) and soluble solids (**b**) during 72 h fermentation of cassava–acerola cherry juice with pure and mixed cultures of *L. plantarum* HNC 7. a–d indicate significant differences between data on the same day ($p < 0.05$).

3.3. Sugars and Organic Acids

The microbial activities will promote the metabolism of soluble carbohydrates. In unfermented cassava–acerola cherry juices, glucose was the predominant soluble carbohydrate, with the other three carbohydrates having concentrations below 1.0 g/L (Table 1). The same data were also observed in the untreated (sterilized without treatment, same after) cassava–acerola cherry juices. During the fermentation process, all treatment groups exhibited significant reductions ($p < 0.05$) in the concentrations of the four carbohydrates compared to unfermented juices. Notably, fructose showed the highest utilization rate (approximately 90.0%), followed by sucrose (about 39.0%), while glucose and maltose decreased by 27.0% and 13.0%, respectively. This observation indicated that LAB utilized

these carbohydrates as carbon sources, and the increase of viable bacteria and the decrease of sugar content identified with this trend [7,9]. After 72 h of incubation, the fructose content in all fermentation groups did not differ significantly ($p > 0.05$), though it decreased notably ($p < 0.05$) in the same groups with prolonged fermentation. At the end of fermentation, the residual glucose content in the single and binary culture of *L. plantarum* HNC7 was significantly ($p < 0.05$) lower than that in ternary mixed cultures, while the content of sucrose and maltose indicated no significant ($p > 0.05$) difference between four fermentation groups. Research has shown that LAB's carbohydrate metabolism is influenced by the substrate and fermentation duration, and varies among strains.

Table 1. The sugars and organic acids of cassava–acerola cherry juices fermented with single and mixed cultures of *L. plantarum* HNC7.

		Sugars (g/L)				Organic Acids (g/100 mL)			
		Fructose	**Glucose**	**Sucrose**	**Maltose**	**Malic Acid**	**Lactic Acid**	**Citric Acid**	**Succinic Acid**
Control		0.59 ± 0.01 a	1.79 ± 0.04 a	0.28 ± 0.03 a	0.96 ± 0.01 a	1.34 ± 0.12 e	Nd	0.55 ± 0.00 f	0.05 ± 0.01 b
group A	24 h	0.21 ± 0.02 b	1.38 ± 0.02 bcd	0.20 ± 0.01 b	0.91 ± 0.00 b	1.92 ± 0.08 f	4.66 ± 0.04 b	0.11 ± 0.03 d	0.01 ± 0.00 a
	48 h	0.11 ± 0.01 d	1.32 ± 0.03 def	0.19 ± 0.01 b	0.87 ± 0.01 bcde	0.88 ± 0.01 bcd	5.84 ± 0.02 c	0.09 ± 0.01 bcd	0.01 ± 0.00 a
	72 h	0.06 ± 0.01 e	1.30 ± 0.03 f	0.20 ± 0.01 b	0.86 ± 0.02 de	0.97 ± 0.07 bcd	6.89 ± 0.15 f	0.03 ± 0.02 ab	0.00 ± 0.00 a
group B	24 h	0.19 ± 0.01 b	1.35 ± 0.01 bcdef	0.19 ± 0.01 b	0.90 ± 0.00 bc	1.78 ± 0.27 f	4.52 ± 0.12 b	0.22 ± 0.04 e	0.01 ± 0.00 a
	48 h	0.10 ± 0.00 d	1.37 ± 0.03 bcde	0.20 ± 0.01 b	0.87 ± 0.00 bcde	0.92 ± 0.00 bcd	6.37 ± 0.00 de	0.04 ± 0.01 abc	0.01 ± 0.00 a
	72 h	0.08 ± 0.01 de	1.31 ± 0.01 ef	0.17 ± 0.01 b	0.85 ± 0.01 de	0.73 ± 0.01 bc	6.37 ± 0.00 de	0.02 ± 0.01 ab	0.00 ± 0.00 a
group C	24 h	0.19 ± 0.01 b	1.35 ± 0.01 bcdef	0.17 ± 0.01 b	0.88 ± 0.02 bcde	1.87 ± 0.02 f	4.52 ± 0.03 b	0.12 ± 0.06 d	0.01 ± 0.00 a
	48 h	0.10 ± 0.00 d	1.34 ± 0.01 cdef	0.18 ± 0.00 b	0.86 ± 0.00 cde	0.86 ± 0.01 bcd	6.05 ± 0.03 cd	0.11 ± 0.00 d	0.01 ± 0.00 a
	72 h	0.06 ± 0.00 e	1.31 ± 0.01 ef	0.17 ± 0.00 b	0.84 ± 0.00 e	1.17 ± 0.04 de	7.44 ± 0.01g	0.01 ± 0.00 a	0.00 ± 0.00 a
group D	24 h	0.20 ± 0.01 b	1.41 ± 0.00 b	0.18 ± 0.12 b	0.89 ± 0.04 bcd	0.65 ± 0.23 ab	3.85 ± 0. 19 a	0.11 ± 0.01 cd	0.01 ± 0.00 a
	48 h	0.14 ± 0.01 c	1.41 ± 0.00 b	0.19 ± 0.01 b	0.88 ± 0.00 bcde	1.04 ± 0.04 cde	6.66 ± 0.06 ef	0.09 ±0.01 bcd	0.00 ± 0.00 a
	72 h	0.08 ± 0.01 de	1.39 ± 0.01 bc	0.19 ± 0.01 b	0.86 ± 0.00 cde	0.38 ± 0.06 a	6.44 ± 0.28 e	0.01 ± 0.00 a	0.00 ± 0.00 a

Nd: not detected. The values are expressed as means ± standard deviation. Values with different lowercase letters in the same row are significantly different ($p < 0.05$).

The malic acid in the analyzed organic acids (malic acid, lactic acid, citric acid, and succinic acid) of unfermented juice was dominant, followed by citric acid, which also contained trace succinic acid (Table 1). The same data were also observed in the untreated cassava–acerola cherry juices. In the first 24 h, the malic acid levels in all treatment groups, except for the ternary combination, were significantly higher ($p < 0.05$) than in the control group, followed by a rapid decrease. This may be interpreted as the tricarboxylic acid cycle in the juices during lactic acid fermentation. As an intermediate in the tricarboxylic acid cycle, the content of malic acid dynamically changes with the associated biochemical reactions. The observation indicated that citric acid and succinic acid in the juices decreased notably ($p < 0.05$) in all treatment groups after 72 h of fermentation, and the citric acid content decreased by 96.0%. Studies revealed that the selected strains had a strong ability to metabolize citric acid, which was a high proportion of the available carbon source in the juices [18]. The increase in the total amount of analyzed organic acids from the initial value (1.94 g/100 mL) in the cassava–acerola cherry juice fermented with pure and mixed cultures of L. plantarum HNC7 was the result of lactic acid accumulation. During fermentation, lactic acid content significantly increased in all treatment groups. The highest increase was in the HNC7-YLC92 binary combination, reaching 6.89 g/100 mL at fermentation's end, followed by the single monoculture. The increases in the HNC7-J1 binary and the ternary combinations were not significant ($p > 0.05$). In contrast, a cereal fermentation study indicated higher lactic acid production in mixed cultures than in single monocultures [16], differing from our results. This discrepancy could be due to the strain-specific variance in lactic acid yield [8], or variations in the count of viable microorganisms throughout the fermentation process.

3.4. Volatile Analysis

The volatile components, including 8 alcohols, 6 esters, 2 acids, 2 ketones, and 3 phenols, were detected using GC-MS after 72 h of fermentation, and their relative contents were shown in Table 2. No significant difference was observed between the untreated and control cassava–acerola cherry juices. Compounds such as 3-methyl-3-buten-1-ol, prenol,

linalool, and acetic acid, detected in all treatment groups, showed no significant difference ($p > 0.05$) in relative content compared to the unfermented group. The relative contents of benzyl alcohol, 2-furanmethanol, and 1-octanol were highest in the juices fermented using the HNC7-YLC92 binary combination, and the relative contents in the remaining fermentation groups were similar to those in the unfermented group. Octaethylene glycol was detected in the unfermented group and all treatment groups except the ternary fermentation group, and the relative content was highest in HNC7-YLC92 binary fermentation group. Phenylethyl alcohol and 2,4-di-tert-butylphenol were detected in the fermented group and they notably ($p < 0.05$) decreased in all four fermentation groups. There was no difference ($p > 0.05$) in the relative content of isobornyl acrylate in all treatment groups and that in the unfermented group. The observation indicated that isobornyl acrylate was related to the substrate and was not metabolized by the cultures [6]. Isobornyl propionate, nonanoic acid, and 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester were detected only in the single and HNC7-J1 binary combination, while 2,6-Di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one was not detected only in the single fermentation group. The phenolics also included two esters that were detected in the unfermented juices, while chloroformic acid n-nonyl ester and 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester were only detected in the ternary and binary fermentation groups, respectively. The fermented juices exhibited a relatively higher concentration of volatile compounds, primarily alcohols, esters, and acids, compared to the unfermented group; these are also the flavor sources of fermented beverage [19].

Table 2. The volatile components and relative contents of the cassava–acerola cherry juice fermented with 72 h of single and co-culture fermentation.

Volatile Compounds	Control	Group A	Group B	Group C	Group D
Alcohols (mg/L)					
3-Methyl-3-buten-1-ol	Nd	1.75 ± 0.23 a	1.47 ± 0.28 a	1.85 ± 0.39 a	1.19 ± 0.15 a
Prenol	Nd	2.22 ± 0.00 a	1.64 ± 0.35 a	1.67 ± 0.10 a	1.64 ± 0.30 a
Linalool	Nd	1.79 ± 0.03 a	1.78 ± 0.35 a	3.04 ± 1.30 a	1.66 ± 0.31 a
1-Octanol	0.52 ± 0.00 a	1.21 ± 0.00 b	0.68 ± 0.18 a	0.87 ± 0.16 ab	0.51 ± 0.04 a
2-Furanmethanol	0.94 ± 0.07 a	0.97 ± 0.13 a	0.90 ± 0.20 a	1.94 ± 0.59 b	0.87 ± 0.17 a
Benzyl alcohol	0.83 ± 0.00 a	0.72 ± 0.14 a	0.57 ± 0.05 a	4.53 ± 2.12 b	0.76 ± 0.18 a
Phenylethyl alcohol	1.63 ± 0.22 b	0.82 ± 0.13 a	0.81 ± 0.13 a	0.69 ± 0.11 a	0.77 ± 0.12 a
Octaethylene glycol	1.34 ± 0.00 a	1.37 ± 0.13 a	1.02 ± 0.00 a	3.13 ± 0.25 b	Nd
Esters (mg/L)					
Isobornyl acrylate	5.09 ± 0.59 a	3.76 ± 1.20 a	4.23 ± 1.15 a	5.06 ± 0.61 a	4.05 ± 1.00 a
1,2-Benzenedicarboxylic acid,bis(2-methylpropyl) ester	Nd	2.12 ± 0.00 a	2.63 ± 0.00 a	Nd	Nd
Isobornyl propionate	Nd	4.65 ± 0.10 a	3.47 ± 0.12 a	Nd	Nd
Chloroformic acid n-nonyl ester	Nd	Nd	Nd	Nd	1.38 ± 0.2
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	Nd	Nd	0.03 ± 0.2	Nd	Nd
Dibutyl phthalate	0.85 ± 0.00 a	0.68 ± 0.12 a	Nd	Nd	Nd
Acids (mg/L)					
Acetic acid	Nd	5.06 ± 0.00 a	3.86 ± 0.95 a	2.59 ± 0.84 a	4.14 ± 0.66 a
Nonanoic acid	Nd	3.42 ± 0.12 a	3.41 ± 0.12 a	Nd	Nd
2,6-Di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one	1.18 ± 0.10 a	Nd	0.50 ± 0.00 a	3.13 ± 1.83 a	0.28 ± 0.07 a
Acetoin	Nd	Nd	1.13 ± 0.00 a	Nd	0.70 ± 0.09 a
Phenolics(mg/L)					
4-Vinylguaiacol	0.46 ± 0.00	Nd	Nd	Nd	Nd
2,4-Di-tert-butylphenol	52.09 ± 2.61 b	25.97 ± 2.63 a	29.34 ± 4.29 a	34.84 ± 10.14 ab	27.80 ± 9.34 a
Butylphen	0.26 ± 0.00 a	Nd	Nd	Nd	0.19 ± 0.00 a

Nd: not detected. The values are expressed as means ± standard deviation. Values with different lowercase letters in the same row are significantly different ($p < 0.05$).

In all treatment groups, the number of compounds in the HNC7-J1 binary fermentation group was higher than that in the remaining groups, while the relative contents of some compounds in the HNC7-YLC92 binary fermentation group were significantly higher than that in the other groups. The differences in volatile compounds between single and mixed cultures can be attributed to the microbial interaction [6].

3.5. Ascorbic Acid Content

The acerola cherries are rich in AA, which is easily damaged by adverse factors in processing due to its instability [20]. Figure 3 showed that the concentration of AA decreased with the extension of the fermentation time, with no significant difference ($p > 0.05$) in AA content between all treatment groups and the control group after 72 h of fermentation. Studies have shown that the AA content of bergamot juice fermented with lactobacillus decreased with the fermentation time [7], which was similar to our results. Lu [21] interpreted that this conclusion as a result of ascorbate oxidase in juices was not inactivated by pasteurization, leading to AA degradation. Another study also indicated that the changes of AA during fermentation were not significantly affected by the growth of LAB, with the oxidation process being the primary cause of AA degradation. The initial AA content in untreated cassava–acerola cherry juices was approximately 3768 mg/100 g, and the AA concentration in each treatment group notably decreased after fermentation, while the AA concentration of HNC7-YLC92 binary combination could still reach 1818.0 mg/100 g. Consequently, cassava–acerola cherry juices remain a good source of AA.

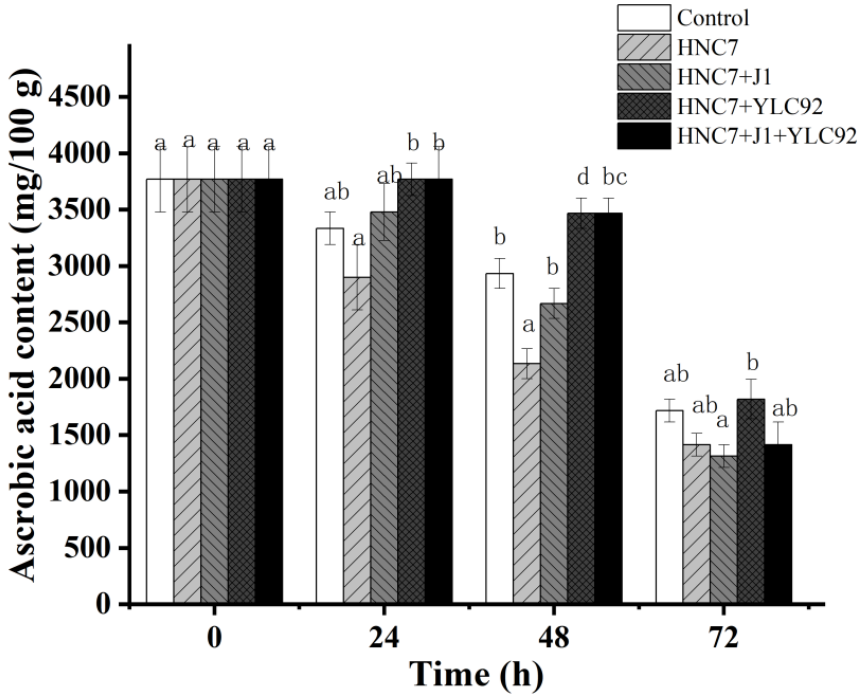

Figure 3. Changes of ascorbic acid content during 72 h fermentation of cassava–acerola cherry juice with pure and mixed cultures of L. plantarum HNC 7. a–d indicate significant differences between data on the same day ($p < 0.05$).

3.6. Antioxidant Activity

The antioxidant activity of cassava–acerola cherry juice was measured using the DPPH and ABTS radical scavenging activity (%), as shown in Figure 4. In all treatment groups, DPPH radical scavenging activity significantly improved ($p < 0.05$) within the first 24 h of fermentation and remained unaffected by further fermentation time, a trend also observed with ABTS radical scavenging activity.

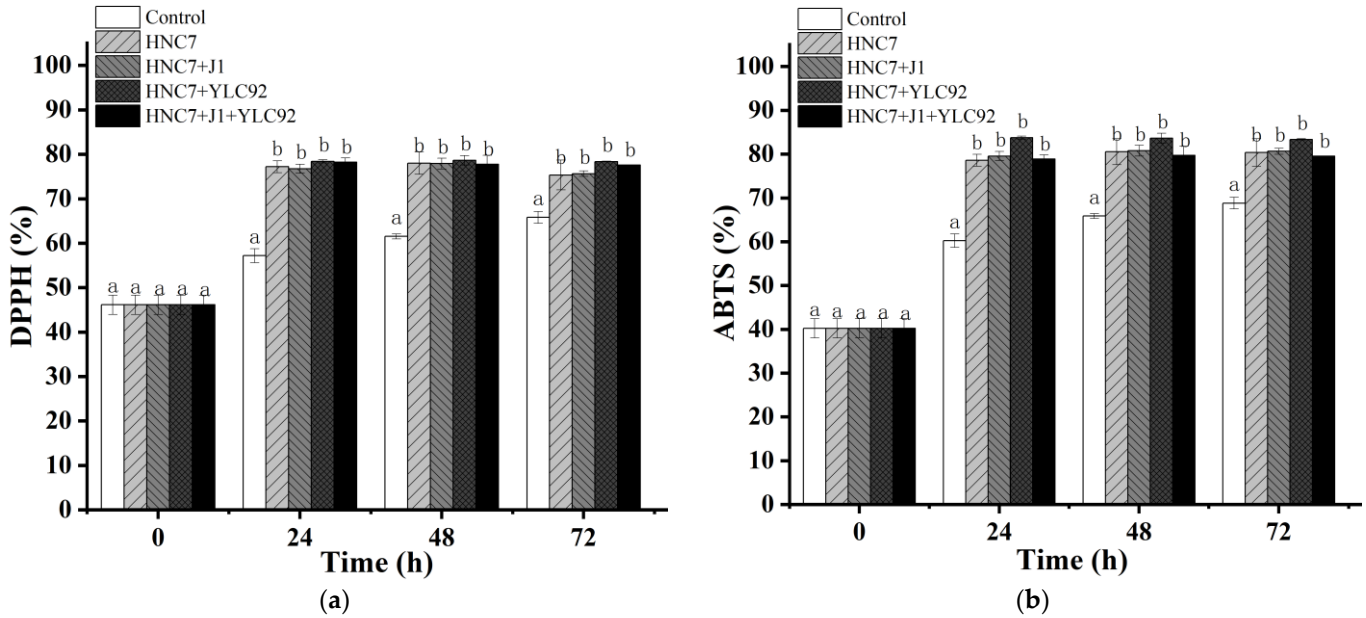

Figure 4. The DPPH (**a**) and ABTS (**b**) radical scavenging activity (%) during 72 h fermentation of cassava–acerola cherry juice with pure and mixed cultures of *L. plantarum* HNC 7. a,b indicate significant differences between data on the same day ($p < 0.05$).

The DPPH and ABTS antiradical ability of each treatment group did not significantly differ ($p > 0.05$) and were notably higher than those of the control sample. This observation indicated that the fermentation cassava–acerola cherry juice could notably improve its antioxidant activity, but the concentration of AA, as a strong antioxidant component in the juices, decreased with the fermentation time. The phenomenon may be explained as LAB produce enzymes such as β-galactosidase, which hydrolyze phenolic glycosides into aglycones with enhanced radical scavenging capacity during fermentation [22,23], and this explanation is consistent with the findings of Lee [24], who found that the β-galactosidase can liberate free phenolic compounds from bound forms, thereby augmenting antioxidant activity. Another study based on sea buckthorn fermentation found that *Lactobacillus plantarum* could promote the transformation or protection of active substances, thus effectively improving the antioxidant activity [8]. In principle, there are many factors affecting the antioxidant capacity, including the type and diversity of the strain, the metabolic level, the cell concentration, and the ability to inhibit the activity of polyphenol oxidase [22,25,26]. Therefore, the determination of antioxidant capacity in vitro is only a preliminary indicator that can provide a reference for in vivo studies [8].

4. Conclusions

This study found that various fermentation strain combinations were capable of growing in cassava–acerola cherry juice, metabolizing its sugars and organic acids. Among fermentation groups, the HNC7-YLC92 binary combination showed higher viable counts, increased acidity, greater pH decline, and more extensive consumption of soluble solids, sugars, and organic acids. All treatment groups showed similar trends compared with the control sample. There was no significant difference in the AA content between all treatments and the control group, but the fermented cassava–acerola cherry juice remained beneficial for AA absorption, meeting health requirements. Additionally, fermenting cassava–acerola cherry juice enhanced the juices' antioxidant activity and contained a higher number of volatile compounds compared to the unfermented group. In summary, fermenting cassava–acerola cherry juice with the HNC7-YLC92 binary combination was found to be optimal, offering valuable insights for the conditions and strains required for its industrial production. The addition of acerola cherry can improve the flavor and nutritional value, thus fostering the development of novel, value-added fermented cassava

products and increasing their consumption. Fermented cassava–acerola cherry juice is also considered a non-dairy functional beverage, making it particularly suitable for individuals with lactose intolerance.

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