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Effects of Lactic Acid Bacteria Fermentation on Organic Acids, Volatile Aroma Components, and Sensory Quality of Hawthorn Pulp

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Abstract. The purpose of this paper is to investigate the effects of lactic acid bacteria (LAB) fermentation on the volatile aroma components and sensory qualities of hawthorn pulp (HP). High performance liquid chromatography (HPLC) and electrons Nasal combined with HS-SPME-GC-MS were used to analyze the effects of lactic acid bacteria fermentation on the organic acid content and volatile compounds of hawthorn pulp (HP). The results showed that the content and type of organic acids and volatile compounds in HP were significantly improved after fermentation of lactic acid bacteria, and the sensory score was also significantly increased.

1. Introduction

Hawthorn (*Crataegus pinnatifida*) distributed in North America, East Asia, Central Asia and Europe, belonging to the Rosaceae family. Hawthorn fruit contains organic acids such as citric acid, malic acid and oxalic acid. These organic acids can increase the secretion of gastric protein and contribute to food digestion and waste excretion. Due to the unique sour taste of hawthorn, the intake of hawthorn fruit is greatly limited. In order to overcome this challenge, Hawthorn has been processed into beverages [1], canned hawthorn, hawthorn sauce, hawthorn preserves and other products.

It has been reported that phenolic compounds such as proanthocyanidins, hyperoside, vitexin, chlorogenic acid, erythroic acid, isoquercetin and isoquercitrin are highly contained in hawthorn [2]. However, due to the sharp acidic taste and high sugar content of the hawthorn, the consumption of consumers is severely restricted. Therefore, in recent years, probiotics such as LAB have been added to juice to increase the probiotic effect of the juice and improve the taste of the juice [3]. Processing fruits and vegetables into fermented beverages can increase probiotic and sensory qualities.

Little research has been done on the effects of LAB fermentation on the volatile aroma components, organic acids and sensory qualities of hawthorn pulp [4]. Therefore, in order to improve the utilization rate and product diversity of hawthorn, this paper aims to study the effects of LAB fermentation on the volatile aroma components, organic acids and sensory qualities of HP.

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2. Materials and methods

2.1. Chemicals and plant material
Oxalic acid, malic acid, lactic acid, acetic acid, citric acid and succinic acid were purchased from Shanghai Yuanye Biological Technology Co., Ltd (Shanghai, China).

2.2. Preparation and fermentation of hawthorn pulp
The cleaned hawthorn was beaten in a food grade juicer for 10 min (material to water ratio 1: 15). 100 mL of hawthorn pulp was placed in a 250 mL Erlenmeyer flask and autoclaved at 121 ℃ for 15 min. The HP was prepared. Activated lactic acid bacteria (CICC 20265: CICC 20248: CICC 20241 is 1:1:1, 10 % inoculum) were inoculated into sterile haw pulp and incubated for 12 h in the 37 ℃ incubator.

2.3 Determination of organic acid content by HPLC
The column was a ZORBAX SB-C18 column (4.6 * 150 mm, 5 μ m, Agilent Technologies, Ltd, US); the mobile phase was 0.1 % phosphoric acid solution and pure methanol; the flow rate was 1 mL/min; the injection volume was 20 μL; the column temperature was 40 ℃, and the detection wavelength was 210 nm. The elution procedure: First, the elution procedure was carried out for 10 min with a flow equal ratio of 0.1 % phosphoric acid solution-methanol (95: 5), and then the mobile phase was adjusted to a ratio of 0.1 % phosphoric acid solution-methanol (98: 2) and equilibrated for 8 min. Finally, the methanol phase was brought to 100 % with a short time gradient and equilibrated for 5 min [5].

2.4 Determination of volatile aroma components

2.4.1 Electronic nose for determination of volatile aroma components. The electronic nose analysis of volatile compounds was carried out base on the method of Li et al [6] and Chen et al [7]. For the electronic nose detection, 10 g samples were weighed, sealed in a 500 mL beaker with plastic wrap, and allowed to stand for 10 min at 25 ℃. The sensor cleaning time was 60 s and the detection time was 120 s.

2.4.2 Determination of volatile aroma components by HS-SPME-GC-MS. HS-SPME was performed with a slight modification as described by Kwaw et al [8]. 6 mL of samples and 1.2 g of NaCl were placed in a 15 mL vial, capped and sealed, and the aged extraction head (PDMS-DVB-CAR, SUPELCO, US) was inserted into the top of the vial. At 250 rpm and 50 ℃, the mixture was equilibrated for 10 min and adsorbed for 30 min. The adsorbed extraction head was removed and inserted into the GC injector and the desorption was performed for 3 min.

The measurement of the volatile components of the samples was determined by GC-MS (GC-MS-TQ 8040, Shimadzu Enterprise Management Co., Ltd, Shanghai, China). The volatile components were separated on a VF-WAX ms column, 60 m × 0.25 mm × 0.5 μm film thickness. GC conditions: the inlet temperature was 230 ℃; temperature increase program: the initial temperature was 35 ℃ for 3 min, the temperature was raised to 130 ℃ at 6 ℃/min for 10 min, the temperature was raised to 210 ℃ at 10 ℃/min for 7 min, and the temperature was raised to 230 ℃ at 20 ℃/min for 3 min; the carrier gas was helium; the flow rate was 1 mL/min; a splitless injection was used.MS conditions: electron impact (EI) ion source; the electron energy was 70 e V; the ion source temperature was 200 ℃; the interface temperature was 230 ℃ and the mass scan range was m / z 35 - 400.

2.5 Sensory analysis
Sensory assessment of the samples was done using the method reported by Kwaw et al [9]. Two samples (HP, FHP) were randomly presented to the panellist in clear plastic cups (15 mL) with a 3-digit code number. Each panellist evaluated samples (10 mL) for taste, mouth feel, aroma, color,

organizational form and overall acceptability using a 9-point hedonic scale (1 = extremely dislike; 2 = very much dislike; 3 = dislike; 4 = slightly dislike; 5 =neither like nor dislike, 6 = slightly like; 7 = like; 8 =like very much; 9 = like extremely) .

3. Results and discussion

3.1. Effect of Lactic Acid Bacteria Fermentation on Organic Acid Content of Hawthorn Pulp

The changes of organic acid content before and after fermentation of lactic acid bacteria fermented hawthorn pulp were shown in Table 1. It could be seen from Table 1 that there was no significant change in the oxalic acid content in the hawthorn pulp after lactic acid bacteria fermentation ($P > 0.05$). Compared with the hawthorn pulp before fermentation, the content of malic acid and citric acid in the fermented hawthorn pulp decreased significantly ($P < 0.05$), which decreased by 0.938 g/L and 0.651 g/L, respectively. After the fermentation of the hawthorn pulp, a large amount of lactic acid was produced, and the acetic acid content was also significantly increased ($P < 0.05$), which was increased by 0.396 g/L. Compared with unfermented hawthorn pulp, the total acid content of fermented hawthorn pulp increased by 2.417 g/L ($P < 0.05$). These changes might be due to the decomposition of lactic acid bacteria to malic acid and citric acid to produce lactic acid and acetic acid, which was consistent with Herrero et al [10].

Table 1. Changes of organic acids in Hawthorn pulp before and after lactic acid bacteria fermentation.

Organic acid （g/L）	Oxalic acid	Malic acid	Lactic acid	Acetic acid	Citric acid	Acid value
HP	0.408±0.033^{a}	2.073±0.014^{a}	ND	0.437±0.023^{a}	8.316±0.006^{a}	11.234±0.07^{a}
FHP	0.319±0.004^{a}	1.135±0.002^{b}	3.699±0.005^{d}	0.833±0.012^{b}	7.665±0.030^{b}	13.651±0.053^{b}

ND: Not detected.

3.2. Electronic nose analysis of volatile compounds

Figure 1 is a radar chart for sensor response for analyzing the volatile aroma of hawthorn pulp before and after fermentation by electronic nose. As could be seen from Figure 1, there was a significant difference in the radar profile of the fermented hawthorn pulp compared to before fermentation. The Sensors W5S, W1W and W2W had the highest response values to the volatile odor of the fermented hawthorn sample. Lactic acid bacteria fermentation had a significant effect on the volatile odor of hawthorn pulp. Compared to the hawthorn pulp before fermentation, the fermented hawthorn pulp had higher aroma intensity. Therefore, the content of nitrogen oxides, terpenes and aromatic compounds increased after fermentation of the hawthorn pulp.

Figure 2 was a PCA analysis before and after fermentation of hawthorn pulp. As could be seen from Figure 3, the contribution rate of the first principal component was 99.93 %, the contribution rate of the second principal component was 0.06 %, and the sum of the contribution rates of the two principal components was 99.99 %, of which greater than 95 %. Therefore, the two principal components could better reflect the information of the original high-dimensional matrix, which could reflect different aroma substances of different samples [11]. It could be seen from Figure 3 that the positive change of the fermented hawthorn pulp along the PC 1 and PC 2 axes was more obvious than that of the hawthorn pulp before fermentation. It could be seen from PCA that the hawthorn pulp before and after fermentation had obvious differences, and the difference effect was remarkable.

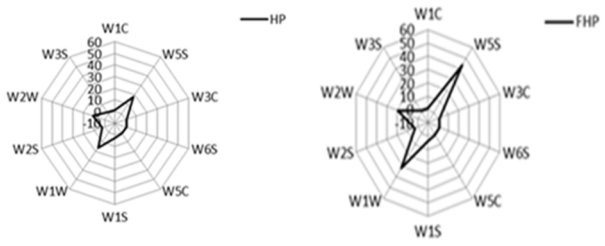

Figure 1. The electronic nasal response of volatile components in the samples.

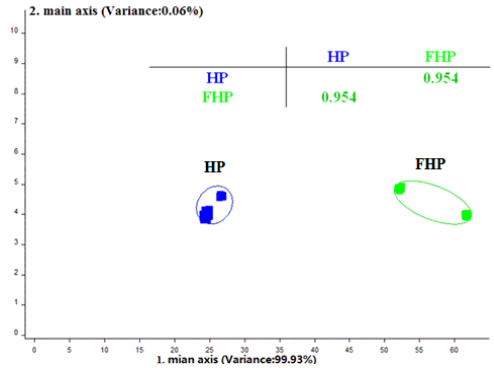

Figure 2. PCA analysis of volatile components in the samples.

3.3. HS-SPME-GC-MS analysis

As could be seen from Table 2, 54 volatile components were detected in the hawthorn pulp before fermentation and the hawthorn pulp after fermentation. A total of 41 volatile components were detected in the hawthorn pulp before fermentation, including 3 ketones, 4 decenes, 15 alcohols, 8 aldehydes, 2 acids, and 5 esters. There were one type of furan, ether, phenol and amide. The ketones accounted for 4.01 % of the total peak area, the oxime olefins accounted for 3.43 %, the alcohols accounted for 33.44 %, the aldehydes accounted for 29.93 %, the acids accounted for 4.88 %, the esters accounted for 8.62 %, the furans accounted for 0.65 %, and the ethers 0.8 %, phenols accounted for 13.62 %, and amides accounted for 0.62 %. The relatively high aroma substances are 3-furfural (20.36 %), eugenol (13.62 %), α-terpineol (12.62 %), α, α, 4-trimethyl-3-cyclohexene. 1-Methanol (6.91), p-Methylphenylisopropanol (4.72 %), acetic acid (3.99 %), dimethylbenzyl acetate (3.89 %).

A total of 41 volatile components were detected in the fermented hawthorn pulp. There were 4 kinds of ketones, 2 kinds of terpene olefins, 17 kinds of alcohols, 3 kinds of aldehydes, 7 kinds of acids, and 4 kinds of esters. There were one type of furan, ether, phenol and amide. Among them, ketones accounted for 31.58 % of the total peak area, decane olefins accounted for 0.84 %, alcohols accounted for 16.41 %, aldehydes accounted for 10.75 %, acids accounted for 21.99 %, esters accounted for 3.29 %, furans accounted for 0.25 %, ethers accounted for 0.49 %, phenols accounted for 13.63 %, and amides accounted for 0.77 %. The relatively high content of aroma substances were 2,3-butanedione (25.6 %), acetoin (4.62 %), acetic acid (17.71 %), eugenol (13.63 %), and α-terpineol (5.17 %) , 3-furfural (8.51 %), α,α,4-trimethyl-3-cyclohexene-1-methanol (2.97 %).

After the fermentation of hawthorn pulp by LAB, the carbonyl compounds and acid substances increase, the types of alcohols increase, and the ester substances and oxime olefins decrease. New substances were formed in the pulp after fermentation, such as: 2, 3-butanedione, acetoin, n-hexanol, etc. After the lactic acid bacteria, the volatile aroma of the hawthorn pulp changed significantly.

Table 2. Changes of volatile aroma components in the samples.

Category	RT (min)	Compound name	Percentage of compound in total area (%)	
			HP	FHP
Ketones	12.46	2,3-butanedione	ND	25.6
	16.88	3-penten-2-one	0.72	ND
	21.719	Acetoin	ND	4.62
	23.017	Methylheptenone	1.82	0.68
	38.43	P-methylacetophenone	1.47	0.68
Olefins	18.855	D-Limonene	0.43	ND
	21.355	(+)-4-Carene	0.89	ND
	25.651	2-methyl-6-methylene-2-octene	0.62	0.23
	36.37	Terpinene	1.49	0.61
Alcohols	18.694	2-methyl-1-butanol	ND	0.36
	22.820	alpha,alpha-dimethyl-phenethyl alcoho acetatel	6.91	2.97

	23.139	Hexanol	ND	0.23
	24.555	Cis-4-hexen-1-ol	0.69	0.31
	27.143	Trans-2-octene-1-ol	0.87	ND
	27.144	1,7-octadien-3-ol	ND	0.36
	29.001	(E)-furanyl sterol oxide	1.22	ND
	29.3	2-ethyl-1-hexanol	0.80	0.41
	32.114	1-octanol	0.51	0.54
	33.025	1-Terpineol	1.0	0.35
	34.017	4-nonenol	0.65	0.31
	34.645	4-Isopropenyl-1-methylcyclohexanol	0.75	0.27
	35.091	1-decanol	0.47	ND
	35.438	2,2,4-trimethyl-3-cyclopentene-1-ethanol	ND	1.08
	35.551	2,6-Dimethyl-5,7-octadien-2-ol	1.24	ND
	36.111	(Z)- 2-(3,3-dimethylcyclohexylidene)-Ethanol	0.50	0.49
	36.306	α- terpineol	12.62	5.17
	39.544	P-methylisopropanol	4.72	2.2
	40.393	Benzyl alcohol	ND	0.44
	41.24	Phenylethanol	ND	0.67
	45.436	4-(1-methylethyl)-1,3-cyclohexadiene-1-methanol	0.49	0.25
Aldehydes	25.185	Nonanal	1.41	ND
	29.13	3-furfural	20.36	8.51
	30.219	Decanal	1.55	ND
	30.482	(E,E)-2,4-heptadienal	0.58	ND
	31.972	Benzaldehyde	3.11	1.66
	33.395	5-methylfuraldehyde	1.44	0.58
	35.441	Phenylacetaldehyde	0.85	ND
	38.907	Crocusaldehyde	0.63	ND
Acids	28.39	Acetic acid	3.99	17.71
	35.743	2-methylbutyric acid	0.89	1.09
	38.525	4-methylvaleric acid	ND	1.13
	39.399	Caproic acid	ND	0.85
	41.734	Heptanoic acid	ND	0.48
	44.385	Heptanoic acid	ND	0.47
	46.698	Tannic acid	ND	0.26
Esters	27.514	Dimethyl benzyl acetate	3.89	1.65
	28.995	Ethyl -2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-carboxylate	1.22	0.58
	38.517	Methyl salicylate	1.02	ND
	39.854	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	0.87	0.33 0.33
	40.118	Methyl 1-methyl-2-oxocyclohex-3-enecarboxylate	1.62	0.73
Furans	31.019	2-Acetyl furan	0.65	0.25
Ether	45.025	2-tert-butyl-4-hydroxyanisole	0.8	0.49
Phenolics	47.339	Eugenol	13.62	13.63
Amide	38.11	N,N-dibutylformamide	0.62	0.77

ND: Not detected.

3.4. Sensory evaluation

It could be seen from Figure 3 that there was a significant difference in the taste, mouth feel, aroma and color of the hawthorn pulp before and after the fermentation of the lactic acid bacteria ($P < 0.05$). After the lactic acid bacteria are fermented, the taste, mouth fell, aroma and color of the hawthorn pulp are better than those before the fermentation. There was no significant change in the organizational form of the hawthorn pulp before and after fermentation ($P > 0.05$). After the fermentation of lactic acid bacteria, the overall acceptability of the hawthorn pulp was significantly improved, indicating that the fermentation can improve the sensory of the hawthorn pulp.

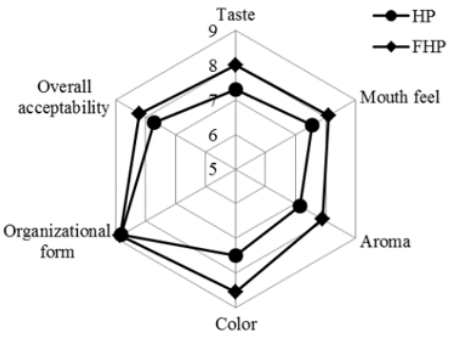

Figure 3. Sensory evaluation.

4. Conclusions

Lactic acid bacteria fermentation has a great influence on the organic acid, volatile aroma components and sensory quality of hawthorn pulp. After fermentation by lactic acid bacteria, lactic acid appeared. The volatile aroma components of the fermented hawthorn pulp are more prominent and coordinated. The sensory score of the fermented hawthorn pulp is also higher than that of the hawthorn pulp before fermentation. Lactic acid bacteria fermentation can significantly improve the quality of hawthorn pulp.

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