Changes in the Physicochemical Properties and Flavour Compounds of Mulberry after Fermentation with *Lactobacillus Plantarum* NCU137

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Abstract

The physicochemical properties and volatile flavor compounds in fresh and fermented mulberry by *Lactobacillus plantarum* NCU137 were detected in this study. The physicochemical property in the initial stage of mulberry fermentation, significantly differed from that in the end of mulberry fermentation. After fermentation, the number of *Lactobacillus* increased from 107 CFU/mL to 109 CFU/mL in the mulberry fruits, while the sugar contents decreased during the mulberry fruits fermentation. During the fermentation, the organic acids contents increased from 9.51 g/L to 32.64 g/L, the pH value decreased from 3.92 to 3.25, the free amino acids reduced by 36.92%. A total of 54 components were detected in the initial stage of fermentation, among which 33 disintegrated. After fermentation, a total of 38 novel components were found in the mulberries. The aroma components of mulberries significantly changed after fermentation. The alcohols and alkanes content increased from 15.18%, 8.57% to 21.75% and 43.52%, respectively, whereas the percentages of aldehydes decreased from 36.32% to 2.07%.

Keywords: Physicochemical property; Mulberry fruits; *Lactobacillus plantarum*; Fermentation

Introduction

As the member of the Moraceae family, Mulberry (Murus alba L.) is wildly grown or cultivated in the tropical, subtropical, temperate, and sub-arctic regions of Asia, Africa, and Americas [1,2]. In general, the mulberry fruits are composed of the black (M. nigra), white (M. alba), and red (M. rubra) varieties [3]. Mulberry is also widely distributed in most areas of China. As the group of berries, Mulberry has the characteristics of thin skin, succulence and contains rich nutrition (e.g., vitamins, minerals, and phenolic acids) which have been related to the health benefits [4,5]. Besides, Mulberry fruits possesses active compounds (e.g., polyphenols, flavonols or anthocyanins), which could protect against liver and kidney damage, strengthen the joints, improve eyesight, and exert anti-aging effects [2,6-9]. However, the mulberry fruits are unfavorable for storage

and transportation because it is susceptible to spoilage at room temperature and easily be oxidized during storage, which affects the stability of the products. Moreover, during transportation of fresh fruits, soft skin on the surface of fruits is vulnerable to mechanical damage and is easily influenced by temperature change, thereby increasing the risk of bacterial pollution. In recent years, to prolong its shelf life and extend its use value, Mulberry can be consumed either fresh or used to make wine, fruit juice, jam, vinegar, and cosmetics, and even used as traditional Chinese medicine for the treatment of fever, hypertension, anemia, and sore throat due to their antioxidant activity, anti-inflammatory, and antagonism functions [10-12]. However, it is difficult to preserve fresh mulberry fruit due to its low acidity. For this reason, there is a real need to apply technologies to enhance the shelf-life, nutritional, organoleptic qualities

and health benefits of mulberry fruit.

Lactic acid fermentation has been applied to improve the nutritional, sensory, safety and shelf-life of fruits due to its economic value and biological activity [13,14]. Many LAB strains (e.g., Lactobacillus plantarum, Lactobacillus brevis and Leuconostoc mesenteroides) have been applied in fruit and vegetable fermentation [15,16]. Although there are some reports on the effect of fermentation on the phytochemical and antioxidant properties of lactic-acid-fermented mulberry juice [17]. However, there is a serious lack of detailed description on the non-volatile and volatile substances of mulberry before and after fermentation. The aim of this study was to investigate to the changes of non-volatile and volatile substances flavor compounds in mulberry after fermentation with Lactobacillus plantarum NCU137, being isolated and trained in our laboratory. This study will provide a guideline for future industrial production of mulberry fermented juice and even serve as a scientific foundation for developing probiotic foods and nutraceuticals.

Material and Methods
Preparation of mulberry samples

Mulberries were provided by Shangzhilvye Berry Co. Ltd. (Heilongjiang, China). Mulberries were washed by distilled water, mixed with high fructose corn syrup (HFSC, w/w, 10%), and beat to puree. The puree was packed into a triangle flask at a sterilization temperature of 90 °C for 20 min. Thereafter, the sterilized puree was cooled. Subsequently, the cooled blueberries was inoculated with the strains NCU137 for fermentation at 37 °C for 72h. The fermented mulberries were collected regularly to analyze the viable cell count of NCU137. Metabolites such as volatile flavor compounds, sugars, organic acids, free-amino acids, and anthocyanins were also detected.

Detection methods of the parameters

Microbiological analysis and determination of pH value: 0.5 g of fermentation samples was dissolved in 4.5 mL of sterile saline (0.85%NaCl, w/v) and diluted to three appropriate gradients. After shaking well, 100µL of the samples was coated on the flat plate of MRS medium. The pH values were determined by a pH meter FE30 (Mettler-Toledo Instruments Company, Shanghai, China). The process was repeated three times for each sample (n=3).

Determination of organic acids and sugars: The mulberry puree was treated following the method by Xiong et al. [18] Briefly, 1 g of puree samples was diluted by adding 2.5 mL of distilled water into the 5 mL centrifuge tubes. The mixture was centrifuged at 1200 rpm for 15 min

by using a high-speed micro-centrifuge (Hunan Xiangyi Labs, Hunan, China). Then, the supernatant was further filtered through the membrane filter (pore diameter, 0.22 µm). The standard curves of organic acids and sugars were established by Agilent 1260 HPLC (Agilent Technologies, Inc., Santa Clara, USA). Organic acids and sugars were separated by the Aminex® HPX-87H Ion Exclusion Column (300 mm.× 7.8 mm, 20 µm particle size, Catalog 125-0140) with sulfuric acid (0.6 mM) as the mobile phase at 45 °C. Refractive index detector and ultraviolet detector (210 nm) were used for the detection of sugars and organic acids, respectively. Thereafter, 20µL of each sample was injected into loading valve and run 25 min at 0.5 mL/min flow of the mobile phase.

Determination of free ammo acids: Free amino acids were analyzed according to the method by Wan et al. [19] with slight modification. Briefly, 8 g of sample was centrifuged at 3000 rpm for 5 min, and 1 mL of supernatant was added into 9 mL of sulfosalicylic acid (2%, w/v). After standing for 15 min, the mixtures were centrifuged at 3000 rpm for 20 min and filtered through the 0.22 µm membrane. Processed samples were loaded on a model S433D automatic amino acid analyzer (Sykam Corp, Munich, Germany) for amino acids analysis.

Determination of volatile constituents: Samples were treated for determination of volatile constituents following the method by Liu et al. [19] with slight modification. Briefly, 1g of NaCl was added to 5g of sample and stirred for 1 min, and then packed into a 15mL the solid-phase micro-extraction (SPME) bottle fitted with a politetrafluoroethylene/silicone septum/aluminum cap. After the bottle was heated at 45 °C for 30 min in water bath, SPME fiber was inserted to the bottle for another 30 min. The processed samples were analyzed by headspace solid-phase microextraction (HS-SPME) coupled with GC-MS (Agilent Technologies, Inc., Burwood, Australia). Parameters were set as follows: an SPME fiber, 50/30 mm DVB/Carboxen™/PDMS Stable Flex™, was mounted in the manual SPME holder. By insertion through the septum of the sample bottle, the fiber was exposed to the sample headspace prior to the desorption of the volatiles at 250 °C for 5 min into the splitless injection port of the GC-MS equipped with a 5973-mass selective detector and using an HP-5MS capillary column (30 m 0.25 mm I.D. and 0.25 mm film thickness). In addition, helium was used as the carrier gas. Programmed temperature elution was employed with an initial temperature of 40 °C for 5 min, which was then ramped to 240 °C at 10 °C/min and held at 240 °C for 2 min. Electron impact ionization was performed using an

electron energy of 70 eV and a mass range of 20–350 U. The components were identified by comparison of their relative retention times and mass spectra with the standards in the Wiley7n.1 library data of the GC-MS system.

Statistical analysis

The data such as the sugars, organic acids, pH value and microbiological growth curve were plotted using representative data from 12 fermentation stages. The error bars represent the standard deviation of three independent measurements (n=3). The graphs were drawn by SigmaPolt 12.5. The data were statistically analyzed using ANOVA (SPSS software, IBM Corporation, Armonk, New York, USA) for determination of statistically significant difference between the different values at 95% confidence interval.

Results and Discussion
The cell count and pH value changes

As shown in Figure 1, the initial number of the *Lactobacillus plantarum* NCU137 was $1.6×10^7$ CFU/mL, after inoculation, and remained at the inoculation level ($1.6×10^7$ CFU/mL) at the 4th hour fermentation, which could be related to the lactobacillus bacteria was adapted to the fermentative environment. During 4-32 h, and the NCU137 number increased from $1.6×10^7$ CFU/mL to $3.91×10^9$ CFU/mL and reached a peak at 32 h ($3.91×10^9$ CFU/mL). After 40h, the *Lactobacillus plantarum* NCU137 number sharply dropped to $1.31×10^9$ CFU/mL, and then maintained this level until the end of the fermentation. The pH value decreased from during 0-20h, from initial 3.92 to 3.47 at the 20th hour, and then dropped slowly to 3.25 at the end of the fermentation.
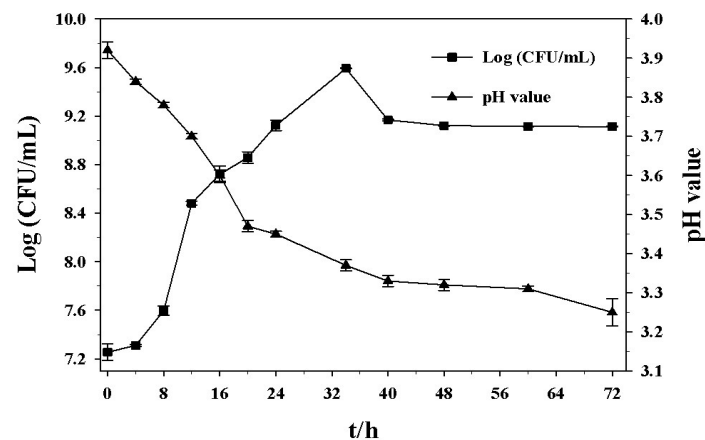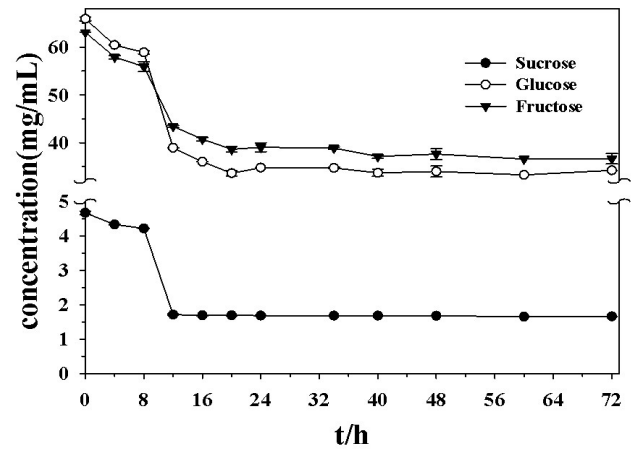

Figure 2: Change of sucrose, glucose, and fructose during the fermentation of mulberry by NCU137.

22]. As shown in Figure 2, the initial contents of sucrose, glucose, and fructose decreased from 4.67, 65.91, and 63.09 mg/mL to 3.02, 31.51, and 26.45 mg/mL at the end of fermentation, respectively, indicating the glucose and fructose were the major sugars utilized by NCU137 during fermentation. These findings were similar to the previous reports that glucose and fructose were the dominant sugar metabolized by Lactobacillus in fruits juice and an increase in glucose and fructose during the fermentation of juice by Lactobacillus [23]. Compared to the glucose, fructose was consumed more slowly during fermentation, which matched the result of cabbage fermentation by Xiong. During the 0-8h, the consumption of sugars (sucrose, glucose, and fructose) were relatively slow, which may be related to the lag period of lactic acid bacteria NCU137. After

Figure 1: Viable cell counts of NCU137 and change in pH value during mulberry fermentation.

Sugar and organic acid changes

Fructose, glucose, and sucrose are the major sugar components in fruits juice and could be utilized by LAB [20-
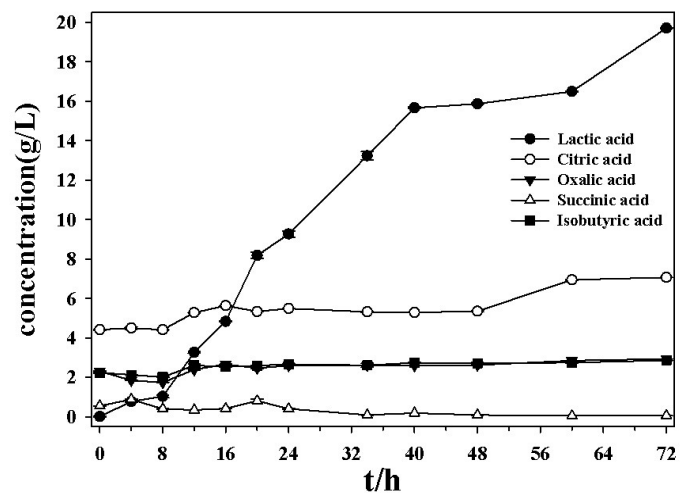

Figure 3: Change of organic acids during the fermentation of mulberry by NCU137.

8 h, the contents of (sucrose, glucose, and fructose) sharply decreased, and the NCU137 were in the logarithmic growth period as mentioned 3.1, leading to a large consumption of sugar.

As shown in Figure 3, the initial contents of citric acid, oxalic acid, succinic acid, and isobutyric acid were 4.41, 2.31, 0.55, and 2.24 g/L, respectively. In general, oxalic acid and citric acid are recognized as the principal organic acid in Mulberry [24,25]. After fermentation, the change of oxalic acid was relatively small, while the content of citric acid increased to 7.07 g/L, which was different that a decrease in citric acid is observed during the mulberry fermentation and other fruits juice. We speculate that a large amount of citric acid was released from the juice during fermentation. Although the partial catabolism of succinic acid by LAB remains unclear in fruits juice fermentation, the lactobacillus strains used in fermented juice and vegetables caused losses of succinic acid [26]. As the main organic acids of the end of fermentation, the lactic acid contents increased from 0 g/L to 19.71 g/L, which enhances the microbial stability and confers a pleasant taste to the fermented food products [26,27].These results indicated that fermentation not only changed the content of organic acids of mulberry but also enriched the kinds of organic acids of mulberry.

Changes of free amino acids after fermentation by the *Lactobacillus plantarum* NCU137

Sixteen amino acids were observed in this study. These amino acids included aspartic acid (Asp), serine (Ser), lysine (Lys), glycine (Gly), alanine (Ala), cysteine (Cys), valine (Val), methionine (Met), Isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), glutamate (Glu), arginine (Arg), and proline (Pro).As shown in Table 1, the total concentration of free ammo acids (FAA) in the initial stage of fermentation was approximately 1.0578 mg/mL and then decreased to about 0.667, which reduced by 0.3908 mg/mL after fermentation. It was noted that the serine is the most abundant amino acid and decreased from 53.54% to 34.00% of the original total free ammo acids, which could be due to the conversion of serine to pyruvate via serial reactions by deamination [28-30]. In total, a significant decrease ($P<0.05$) in the Asp, Ser, Ala, Val, Ile, Leu, Tyr, Phe, Arg, Pro was found after fermentation, while a significant increase ($P<0.05$) in the Gly, Cys, Met and Glu was found after fermentation. According to the taste characteristics, the amino acids are classed into bitter amino acids (histidine, arginine, leucine, lysine, valine, phenylalanine, and Isoleucine), sweet amino acids (glycine, alanine, proline, serine, threonine, and methionine), umami amino acids (aspartic acid and glutamate), and astringent

Table 1: Change of free ammo acids after fermentation of mulberry.

**correlation is significant at the 0.01 level, *correlation is significant at the 0.05 level.

Abbreviation	0 h	48 h	Taste
Asp[*]*	0.0570±0.0010	0.0117±0.0015	Umami
Ser**	0.5663±0.0031	0.3400±0.0010	Sweet
Lys	0.0110±0.0017	0.0097±0.0006	Bitter
Gly*	0.0039±0.0004	0.0057±0.0006	Sweet
Ala**	0.0567±0.0015	0.0097±0.0012	Sweet
Cys**	0.0032±0.0002	0.0143±0.0006	
Val**	0.0187±0.0006	0.0133±0.0006	Bitter
Met**	0.0043±0.0006	0.0157±0.0006	Sweet
Ile**	0.0120±0.0010	0.0053±0.0006	Bitter
Leu**	0.0260±0.0010	0.0117±0.0006	Bitter
Tyr**	0.0457±0.0006	0.0113±0.0006	Astringent
Phe**	0.0650±0.0050	0.0293±0.0006	Bitter
His	0.0327±0.0006	0.0310±0.0010	Bitter
Glu**	0.0723±0.0006	0.0843±0.0006	Umami
Arg**	0.0220±0.0010	0.0160±0.0010	Bitter
Pro*	0.0610±0.0010	0.0580±0.0010	Sweet

amino acid (tyrosine) [31,32]. It total, the contents of bitter amino acids, sweet amino acids, umami amino acids, and astringent amino acid decreased after fermentation. In particular, the content of sweet amino acids decreased the most. Therefore, after fermentation, the sweetness, bitterness and freshness of blueberry decreased, especially the sweetness.

Changes of volatile constituents after fermentation by the *Lactobacillus plantarum* NCU137

The volatile constituents of mulberry were detected by GC-MS (Figure 4). Compared with the volatile flavors of the initial stage of fermentation (Figure 4B), some new volatile flavors of the end of fermentation stage appeared at the acquisition time of 10-16 min (Figure 4A). Based on the variation of functional group, the volatile constituents were classified into nine parts (alcohols, acids, esters, aldehydes, ketones, phenols, alkanes, alkenes, and others) (Table 2). 54 and 59 kinds of volatile compounds were detected in the initial stage of mulberry fermentation and the end of fermentation stage, respectively. In total, a total of 92 kinds of volatile substances were detected in this study, and 21 kinds of volatile substances were both found At the beginning and end of mulberry fermentation, while 33 kinds of volatile substances were unique at the beginning of mulberry fermentation and 38 kinds of novel substances were unique at the end of mulberry fermentation, which indicated that fermentation process affects the flavor composition in mulberry. Alcohols (15.18%), aldehydes
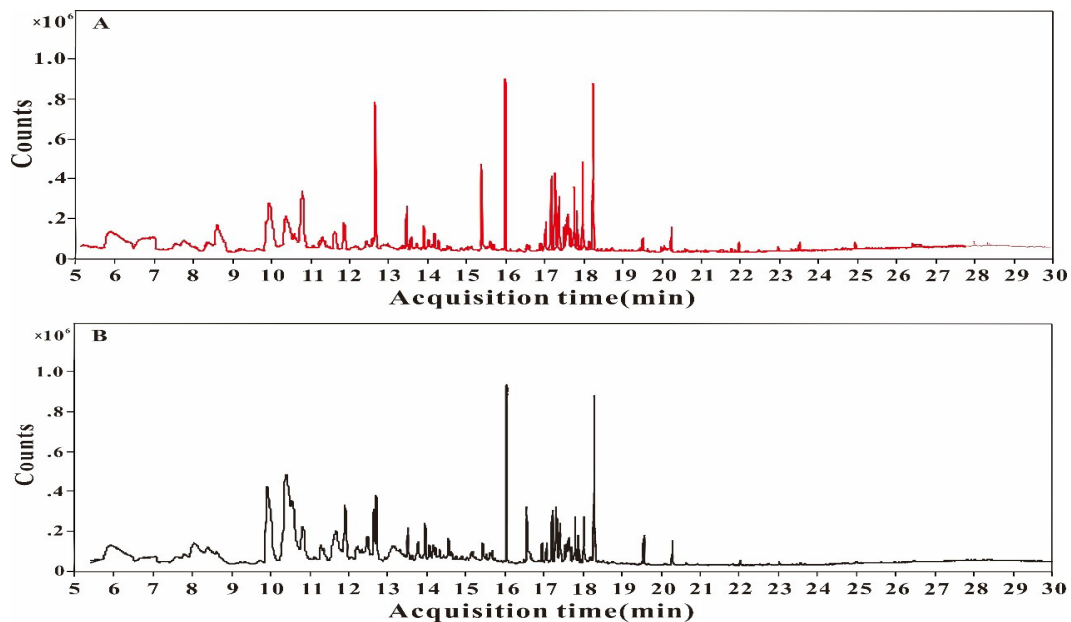
Figure 4: GC-MS total ion chromatogram of volatile compound profiles observed at 0 h and 48h.

Table 2: Change of volatile constituents after fermentation of mulberry.

Classify	Serialnumber	Name	Formula	CAS	Relative quantity %		
					0 h		48 h
alcohols	1	(S)-(+)-3-Methyl-1-pentanol	$C_6H_{14}O$	42072-39-9			4.913730298
	2	Cycloheptanol, 2-methylene	$C_8H_{14}O$	16240-38-3	1.4135075		
	3	Exo-fenchol,	$C_{10}H_{18}O$	22627-95-8	0.3107604		
	4	myrcenol	$C_{10}H_{18}O$	543-39-5	0.3816621		
	5	2-[(2R,5S)-5-Methyl-5-vinyltetrahydro-2-furanyl]-2-propanol	$C_{10}H_{18}O_2$	5989-33-3			0.263307889
	6	Trans-p-Menth-2-en-7-ol	$C_{10}H_{18}O$	19898-87-4			0.085068702
	7	Octahydro-4,7-methano-inden-1-ol	$C_{10}H_{16}O$	55255-97-5	0.1991281		
	8	(E,E)-2,4-Decadien-1-ol	$C_{10}H_{18}O$	18409-21-7	4.0263089		5.898096714
	9	3,7-dimethyl-1,6-Nonadien-3-ol	$C_{11}H_{20}O$	10339-55-6	0.5822987		
	10	(-)-Isolongifolol	$C_{15}H_{26}O$	1139-17-9			0.239002545
	11	2-phenylethanol	$C_8H_{10}O$	1960/12/8			0.907399494
	12	(-)-Trans-pinocarveol	$C_{10}H_{16}O$	547-61-5			0.311918576
	13	1-Heptatriacotanol	$C_{37}H_{76}O$	105794-58-9			0.162035624
	14	(-)-perillyl alcohol	$C_{10}H_{16}O$	18457-55-1			1.154503822
	15	Z-11-Pentadecenol	$C_{15}H_{30}O$	1000130-77-0			0.101272265

	No.	Compound	Formula	CAS			
alcohols	16	(-)-Terpinen-4-ol	$C_{10}H_{18}O$	20126-76-5	1.9505498		
	17	(-)-cis-Caran-trans-(5)-ol	$C_{10}H_{18}O$	6909-21-3	0.2111964		
	18	8-phenylmenthol	$C_{16}H_{24}O$	134256-18-1	5.723423		3.649852431
	19	2-methylene-, (3.beta.,5.alpha.)-Cholestan-3-ol	$C_{28}H_{48}O$	22599-96-8			0.016203562
	20	Estra-1,3,5(10)-trien-17.beta.-ol	$C_{18}H_{24}O$	2529-64-8	0.1599059		0.02618811
	21	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	1000131-11-4			0.028356235
	22	2,6,10,15,19,23-hexamethyltetracosa-2,6,14,18,22-pentaene-10,11-diol	$C_{30}H_{52}O_2$	153650-82-9	0.2247733		
	23	cis-Sabinol	$C_{10}H_{16}O$	3310/2/9			0.06076336
	24	strophantidol	$C_{23}H_{34}O_6$	560-54-3			0.02618811
	25	p-Mentha-1(7),8(10)-dien-9-ol	$C_{10}H_{16}O$	29548-13-8			0.020254454
	26	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-,(1.alpha.,3.alpha.,4.beta.,6.alpha.)-	$C_{10}H_{18}O$	38748-96-8			0.729160308
	27	3,7-dimethyl-1,6-Octadien-3-ol	$C_{10}H_{18}O$	78-70-6			3.155643778
					15.183514		21.74894628
acids	28	Benzyl oxy tridecanoic acid	$C_{20}H_{32}O_3$	1000289-36-6			0.06481425
	29	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	1000131-33-2			0.044559796
	30	(E)-5-hydroxy-3,4,4-trimethyl-2-Hexenoic acid	$C_9H_{16}O_3$	14919-59-6	1.2430418		
	31	cascarillic acid	$C_{11}H_{20}O_2$	35936-15-3	0.4978201		4.281791365
	32	10-12-Pentacosadiynoic acid	$C_{25}H_{42}O_2$	66990-32-7			0.016203562
	33	12-oxo-Tridecanoic acid	$C_{13}H_{24}O_3$	2345/12/2			0.247104326
					1.7408619	0	4.654473299
esters	34	6-tridecyloxan-2-one	$C_{18}H_{34}O_2$	1227-51-6			0.190391858
	35	Acetic acid,7-hydroxy-1,3,4,5,6,7-hexahydro-2H-naphthalen-4a-ylmethyl ester	$C_{13}H_{20}O_3$	1000188-89-1			0.587379137
	36	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	57156-91-9			0.765618324
	37	2-Phenyl-2-methylbutanoic acid methyl ester	$C_{12}H_{16}O_2$	62338-21-0	3.4862495		1.895816801
	38	10,12-Tricosadiynoic acid, methyl ester	$C_{24}H_{40}O_2$	1000333-59-4	0.0452564		0.295715014
	39	Geranyl isovalerate	$C_{15}H_{26}O_2$	109-20-6			0.032407125
	40	Butanoic acid, tridec-2-ynyl ester	$C_{17}H_{30}O_2$	1000299-12-6	0.9398241		1.523134866
	41	2,4,5,6,7,7a-Hexahydro-3,6-dimethyl-α-methylene-2-oxo-6-vinyl-5-benzofuranacetic acid methyl ester	$C_{16}H_{20}O_4$	19892-19-4			0.06481425
					4.4713299		5.355277375

Group	No.	Compound	Formula	CAS			
aldehydes	42	Hexanal	$C_6H_{12}O$	66-25-1	0.6185038		
	43	4-methylhexanal	$C_7H_{14}O$	41065-97-8	6.4942899		
	44	Phenylacetaldehyde	C_8H_8O	122-78-1	3.6536981		
	45	Nonanal	$C_9H_{18}O$	124-19-6	15.085459		
	46	2-ethylidene-6-methyl-3,5-Heptadienal	$C_{10}H_{14}O$	99172-18-6			0.044559796
	47	(Z)-7-Hexadecenal	$C_{16}H_{30}O$	56797-40-1	1.5900073		0.518513996
	48	(E,E)-2,4-Dodecadienal	$C_{12}H_{20}O$	21662-16-8	0.2323161		
	49	2,4-Dimethylbenzaldehyde	$C_9H_{10}O$	15764-16-6	0.3560168		
	50	cuminaldehyde	$C_{10}H_{12}O$	122-03-2	0.2971835		
	51	4-Ethylbenzaldehyde	$C_9H_{10}O$	4748-78-1			1.462371507
	52	perillyl aldehyde	$C_{10}H_{14}O$	2111-75-3	6.5998881		
	53	All-trans-retinal	$C_{20}H_{28}O$	116-31-4	0.2323161		0.040508906
	54	(Z)-14-methylhexadec-8-enal	$C_{17}H_{32}O$	60609-53-2	1.1585632		
					36.318241	0	2.065954206
ketones	55	5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione	$C_{11}H_{13}NOS$	86071-95-6	1.0559821		
	56	1-Hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one	$C_{14}H_{22}O_2$	1000189-14-9	0.5430765		0.980315525
	57	1,1,3a-Trimethyl-1a,3a,5,6-tetrahydro-1H-cyclopropa[c]pentalen-4-one	$C_{12}H_{16}O$	91531-54-3	4.786616		
	58	.γ.-Palmitolactone	$C_{16}H_{30}O_2$	730-46-1			0.085068702
	59	Tricyclo[5.4.3.0(1,8)]tetradecan-6-one, 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl	$C_{20}H_{32}O_2$	1000197-61-7	0.2428759		0.085068702
	60	1-(2,3-dihydro-7,8-dinitro-1,4-benzodioxin-6-yl)butan-1-one	$C_{12}H_{12}N_2O_7$	1000273-76-9	0.4073074		
					7.0358579		1.15045293
phenols	61	Toluene	C_7H_8	108-88-3	0.2443844		0.291664123
	62	o-Xylene	C_8H_{10}	95-47-6	1.4451869		1.454269725
	63	p-Xylene	C_8H_{10}	106-42-3	1.8524943		
	64	m-Xylene	C_8H_{10}	108-38-3	0.461615		
	65	Alpha,P-Dimethylstyrene	$C_{10}H_{12}$	1195-32-0			1.903918582
	66	4-Ethyl-1,2-dimethylbenzene	$C_{10}H_{14}$	934-80-5			0.717007636
	67	1,4-dimethyl-2,5-di(propan-2-yl)benzene	$C_{14}H_{22}$	10375-96-9	0.182534		
	68	1-(1,5-dimethylhexyl)-4-methylbenzene	$C_{15}H_{24}$	1461-02-5	0.2081793		
	69	3-hydroxy-4-methoxyallylbenzene	$C_{10}H_{12}O_2$	501-19-9			3.285272276
	70	Benzene, (1-methyl-1-propylpentyl)-	$C_{15}H_{24}$	54932-91-1	0.9232301		0.486106871
	71	2,6-Di-tert-butyl-4-sec-butylphenol	$C_{18}H_{30}O$	17540-75-9	0.3062348		
	72	2,4-ditert-butylbenzenethiol	$C_{14}H_{22}S$	19728-43-9	0.2308075		0.081017812
					5.8546665		8.219257026

Category	No.	Name	Formula	CAS			
alkanes	73	2-butyl-3-methyloxirane	$C_7H_{14}O$	14925-96-3			0.891195932
	74	2-hexyl-3-methyloxirane	$C_9H_{18}O$	56820-01-0			0.291664123
	75	2,6-diphenyl-3-methylheptane	$C_{20}H_{26}$	1000161-22-5		1.5070373	0.789923667
	76	2-cyclohexyl-2-phenylpropane,	$C_{15}H_{22}$	25683-97-0		3.7366681	2.02139441
	77	4-[[(2-Methoxy-4-octadecenyl)oxy]methyl]-2,2-dimethyl-1,3-dioxolane	$C_{25}H_{48}O_4$	16725-41-0		0.5822987	
						5.8260041	3.994178131
alkenes	78	7-methyloct-1-ene	C_9H_{18}	13151-06-9			1.227419852
	79	9-methyl-1-undecene	$C_{12}H_{24}$	74630-41-4		7.0735715	40.508906
	80	(3R)-(+)-Isosylvestren	$C_{10}H_{16}$	1461-27-4		0.411833	
	81	2-Formymethyl-4,6,6-trimethylbicyclo[3.1.1]hept-3-ene	$C_{12}H_{18}O$	135004-95-4			0.149882952
	82	1,6-Dimethylhepta-1,3,5-triene	C_9H_{14}	1000196-61-0			1.4218626
	83	1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	$C_{12}H_{18}$	130930-56-2		0.2413673	0.113424937
	84	α-longipinene	$C_{15}H_{24}$	5989/8/2		0.08297	0.097221374
	85	α--curcumene	$C_{15}H_{22}$	644-30-4		0.75729	
						8.5670319	43.51871772
others	86	2-hydroxy-2-phenylacetonitrile	C_8H_7NO	532-28-5		7.9500366	
	87	6,7-Dimethyl-3,5,8,8a-tetrahydro-1H-2-benzopyran	$C_{11}H_{16}O$	110028-10-9		0.6426405	
	88	2-Naphthol, 1,2,3,4,4a,5,6,7-octahydro-4a-methyl-	$C_{11}H_{18}O$	91253-94-0			0.06886514
	89	N-benzyl-3-phenyl-1,2,4-Thiadiazol-5-amine	$C_{15}H_{13}N_3S$	17467-59-3		0.5506192	
	90	(R)-camphor	$C_{10}H_{16}O$	464-49-3		0.1734828	
	91	N-benzyloxycarbonylglycine	$C_{10}H_{11}NO_4$	1138-80-3			7.008040739
	92	2,6-Di-tert-butyl-p-benzoquinone	$C_{14}H_{20}O_2$	719-22-2		5.6857093	2.215837159
						15.002489	9.292743038

(36.32%), and others (15.00%) were the major volatile at the beginning of mulberry fermentation. Other components, such as acids, esters, ketones, phenols, alkanes, and alkenes were approximately 1.74%, 4.47%, 7.04%, 5.85%, 5.83%, and 8.57%, respectively. After fermentation, the contents of alcohols, acids, esters, phenols and alkenes increased from 15.18%, 1.74%, 4.47% and 5.85%, 8.57% to 21.75%, 4.65%, 5.36%, 8.22% and 43.52%, respectively, while the contents of aldehydes, ketones and alkanes decreased from 36.32%,7.04% and5.83% to 2.07%,1.15% and 3.99%, respectively. Previous study reported that the number of alcohols and esters increased during fermentation, and improvement of alcohol content may potentially further enhance the fragrance of fermented juices [33], which indicated the aroma of mulberry could be improved after fermentation. Researchers have reported that the microorganisms can convert some aldehydes into alcohols [34]. Moreover, the increase of alcohol and decrease of aldehyde in fruit juice after fermentation are also reported in other studies [35]. In total, after fermentation, the variety

of volatile constituents in mulberry were greatly enriched and the content of total volatile constituents in mulberry also increased, indicating that fermentation plays an important role in improving the flavor of mulberry.

Conclusion

We analyzed the dynamic change of components for mulberry fermentation using the Lactobacillus plantarum NCU137 as the fermentative strain. In total, after fermentation, the Lactobacillus counts, organic acids contents, alcohols and alkanes content contents significantly increased, while the pH value and contents of sugar, free amino acids and aldehydes significantly decreased. In addition, the changes of non-volatile flavor and volatile flavor compounds in mulberry would improve the sensory properties and flavor of product significantly. Lactic acid fermentation can be used as a preservation technique for the processing of mulberry juice with enhanced phytochemical, volatile and sensory qualities. Furthermore, processing mulberry into fruit juice makes good use of mulberry, which could be used as a basis for studying the economic viability of producing fermented mulberry derivatives. Thus, fermented mulberry juice exhibits a huge development space and market potential. Further studies should be focused on studying the correlation between Lactobacillus plantarum NCU137 and the specific flavor using omics analysis techniques.

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Zhanggen Liu and Nengneng Su contributed equally to the article

Declaration of Interest

The authors declare that they have no conflict of interest.

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