

# Changes in antioxidant activities and volatile compounds of mixed berry juice through fermentation by lactic acid bacteria

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**Abstract** The objectives of this study were to analyze antioxidant activities and identify volatile compounds in mixed berry juice after fermentation by lactic acid bacteria (LAB). Antioxidant activity of the mixed berry juice increased significantly from 209.57±2.93 to 268.30±1.75 μmol TE/g after 24 h of fermentation. After LAB fermentation, 34 volatile compounds were identified. Among them, three compounds—benzoic acid, benzaldehyde, and vitispirane—showed significant changes in their concentrations. Peak areas of benzoic acid and benzaldehyde, which are known to possess antioxidant activities, increased by 64 and 188%, respectively, after fermentation. However, the peak area of vitispirane, which is the most abundant terpene compound in berry juices, decreased by 92% after fermentation.

**Keywords:** berry, fermentation, antioxidant activity, volatile compound, GC-MS

## Introduction

Various foods or beverages were recently developed using different berries such as the acai berry, blue berry, black berry, raspberry, aronia, and cranberry (1). Berries show positive influences on various lifestyle-related diseases, including obesity, hypertension, cerebrovascular diseases, arteriosclerosis, myocardial infarction, cancers, or rheumatoid arthritis (2-4). Therefore, there is high demand for berry-based products from consumers. Berries are also known as natural antioxidants that are capable of eliminating reactive oxygen species (ROS) like hydroxyl, peroxide radicals, and radicals of other reactive forms of oxygen (5). The U.S. Department of Agriculture selected 12 foods containing high amounts of antioxidants, and six among these include berries (6).

There have been many reports regarding the antioxidant activities of blackberry, raspberry, cranberry, and blueberry, and recently, several studies were performed using the acai berry, which shows high antioxidant activity (1,6-12). Berries contain high contents of vitamin C and polyphenols, mainly anthocyanins, phenolic acids, flavanols, and tannins, which are known natural antioxidants (13,14). Vitamin C and polyphenols are known to exist as glycosides in berries (15,16). When glucose was removed from glycoside in berries by enzyme glycoside hydrolase, antioxidant activities of berries improved

(9,17). Antioxidants activities from natural compounds increased after fermentation by fungi, yeasts, or lactic acid bacteria (LAB) (10,18). A highly active β-1,4-xylosidase, one of the glycoside hydrolase enzymes, was reported from *Leuconostoc* spp. (7,19).

Several studies investigated volatile compounds of various berries such as the blackberry, raspberry, cranberry, blueberry, acai berry, and aronia (20-25). Kraujalyte *et al.* (20) reported that ester compounds such as ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, and ethyl decanoate play an important role in the aroma of aronia. It is known that 1-(*p*-hydroxyphenyl)-3-butanone, or raspberry ketone, primarily imparts the characteristic aroma to raspberries (21). Recently, β-damascenone, *trans*-linalool oxide, (Z)-3-hexenol, and 2-phenylethanol were reported as aroma-active compounds in acai berries (22).

Recently, we found that the antioxidant activities of acai berry or blackberry juices were enhanced by 31 or 15%, respectively, after fermentation by LAB (26). In this study, we investigated the following properties of mixed berry juice: solid content, pH, sugar content, and antioxidant activity. After fermentation of the mixed berry juice by *Lactobacillus plantarum* LP-115, changes in the antioxidant activities were analyzed by oxygen radical absorbance capacity (ORAC). In addition, volatile compounds were analyzed by solid phase micro-extraction (SPME) and gas chromatography-mass spectrometry (GC-MS).

## Materials and Methods

**Materials and strain** The mixed berry juice was supplied by Dongwon F&B Co., Ltd. (Seongnam, Korea). It comprised 73% of acai berry, 17% of aronia, and 10% of cranberry. All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the fermentation of the mixed berry juice, *L. plantarum* LP-115 (Danisco USA Inc., Madison, WI, USA) was used.

### Analysis of physicochemical properties of the mixed berry juice

The solid content of the mixed berry juice was analyzed by the freeze-drying method (27). The sugar content was analyzed using a Brix meter (Atago, Tokyo, Japan). Concentrations of glucose and xylose were determined by high-performance liquid chromatography (HPLC 1200 Series; Agilent Technologies, Santa Clara, CA, USA) using a Rezex ROA-Organic Acid column (Phenomenex Inc., Torrance, CA, USA) equipped with a refractive index detector. The column was eluted with 0.005 N H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.6 mL/min at 50°C.

### Analysis of antioxidant activity of the mixed berry juice

Antioxidant activity was analyzed using ORAC assay kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's instructions (28). Briefly, the sample (25 µL) and 150 µL of fluorescein solution were added in a 96-well immune plate and incubated at 37°C for 10 min. After 10 min, 25 µL free radical initiator was added. Fluorescence was detected every minute for 60 min using a Microplate fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) at 480 nm excitation and 520 nm emission. Trolox (Cell Biolabs) was used as reference, and the standard calibration curve was used for quantitative analysis. Antioxidant capacity measured by ORAC assay was expressed as µmol TE/g of the sample.

### Fermentation of the mixed berry juice by *L. plantarum* LP-115

pH of the mixed berry juice was adjusted from pH 3.2 to pH 7.0, using 5 M NaOH solution, for optimum growth of LAB, and 1 g/L of freeze-dried *L. plantarum* LP-115 was inoculated in the juice. Fermentations were performed using a 500-mL flask with 100 mL of working volume at 37°C and shaking at 200 rpm for 36 h.

### Extraction of volatile compounds from the mixed berry juice

Volatile compounds were extracted from the mixed berry juice by the SPME method (29–31). The mixed berry juice (10 mL) was placed in a 20-mL teflon-capped headspace vial and allowed to equilibrate at 40°C for 30 min. Volatile compounds were extracted at 40°C for 30 min using an SPME fiber (50/30 µm divinylbenzene/carboxene/polydimethylsiloxane). The fiber was then retracted and immediately injected into the injection port of gas chromatography (200°C) for 1 min to desorb volatile compounds.

**Analysis of volatile compounds by GC-MS** GC-MS was performed to analyze the volatile compounds from mixed berry juice. Agilent

6890N GC/Agilent 5973 mass selective detector (Agilent Technologies) was used with a DB-wax column (60 mm length×0.25 mm i.d.×0.25 µm film thickness; J & W Scientific, Folsom, CA, USA). Operation conditions were as follows: injection port temperature, 200°C; detector temperature, 250°C; capillary direct interface temperature, 280°C; ionization voltage, 70 eV; mass range, 33–350 amu; flow rate of helium, 1.0 mL/min. Oven temperature was maintained at 40°C for 5 min, increased to 200°C with increments of 5°C/min, and maintained at 200°C for 20 min.

### Identification of volatile compounds from the mixed berry juice

Positive identification was based on the comparison of retention indices and mass spectra of unknowns with those of authentic standard compounds. Tentative identification was based on matching the mass spectra of unknowns with those in the Wiley/7n mass spectral database (Agilent Technologies) and previous reports (25,26).

### Statistical analysis

All experiments were performed in triplicate and error bars indicate standard deviation. Statistical analyses were performed using the statistical package for the social sciences (SPSS) (21.0 version, IBM, Armonk, NY, USA), and Scheffe's multiple range test was performed for testing the significance of the differences (32).

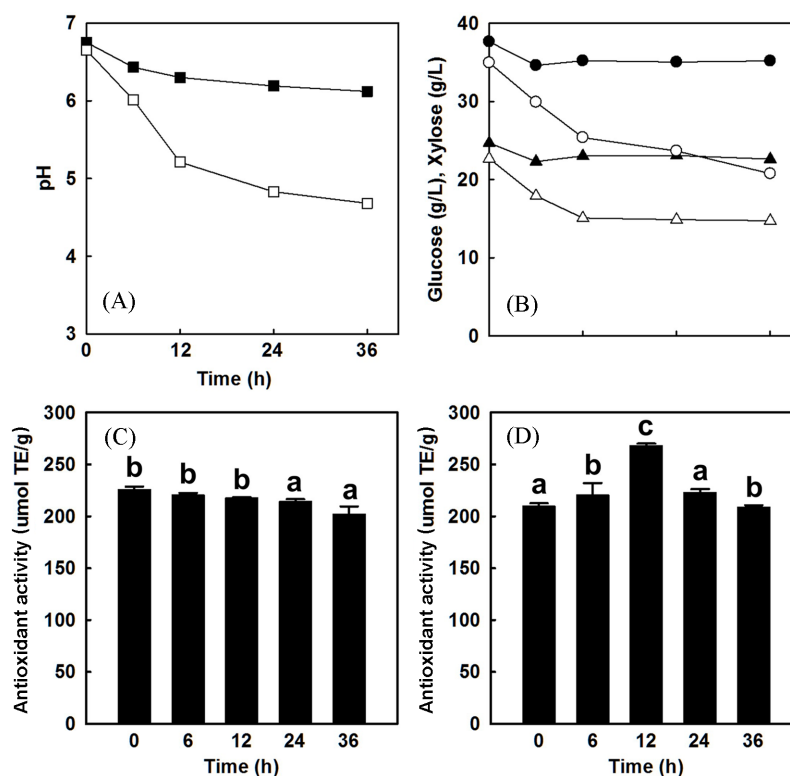
## Results and Discussion

### Physicochemical properties of the mixed berry juice

The mixed berry juice comprised the acai berry, aronia, and cranberry. After freeze drying, the solid content, pH, and sugar content of the mixed berry juice were 0.10±0.00 g/mL, 3.22±0.01, and 8.67±0.06 Brix, respectively. HPLC analysis showed that the mixed berry juice contained 34.96±0.01 g/L of glucose and 22.69±0.01 g/L of xylose. In addition, the mixed berry juice showed 251.43±0.53 µmol TE/g of antioxidant activity by ORAC assay. In general, acai berries showed much higher antioxidant activity than other berries such as raspberries, blackberries, cranberries, and blueberries (26). As the mixed berry juice contained 73% acai berry, the antioxidant activity of the mixed berry juice was much higher than those of raspberry (159.40±3.72 µmol TE/g), blackberry (125.85±10.23 µmol TE/g), cranberry (103.27±2.68 µmol TE/g), or blueberry (101.40±2.06 µmol TE/g) (26).

### Improved antioxidant activities of the mixed berry juice after fermentation by *L. plantarum* LP-115

In this study, *L. plantarum* LP-115 was selected to improve the antioxidant activity of the mixed berry juice containing 73% of acai berry, based on previous reports that *L. plantarum* LP-115 showed 20–30% increase in the antioxidant activity of acai berry juice after fermentation (26). To ferment the mixed berry juice by *L. plantarum* LP-115, the pH of the mixed berry



**Fig. 1.** Fermentation results of the mixed berry juice by *L. plantarum* LP-115. (A) Time profile of pH without *L. plantarum* LP-115 inoculation (closed square) or with *L. plantarum* LP-115 inoculation (open square). (B) Time profiles of glucose (circles) and xylose (triangles) consumptions without (closed symbols) or with (open symbols) *L. plantarum* LP-115 inoculation. (C) Changes in antioxidant activities without *L. plantarum* LP-115 inoculation. (D) Changes in antioxidant activities with *L. plantarum* LP-115 inoculation.

juice was adjusted to 7.0, using NaOH solution, prior to bacterial inoculation. During the fermentation, 14.21 g/L of glucose and 7.97 g/L of xylose were consumed, 28 g/L of lactic acid was produced, and the pH decreased drastically from 6.65 to 4.68 after 36 h of fermentation. The negative control without *L. plantarum* LP-115 inoculation showed no sugar consumption and a slight change in pH (Fig. 1A and 1B). Without *L. plantarum* LP-115 inoculation, the antioxidant activity of the mixed berry juice showed a 10% decrease from  $225.58 \pm 2.98$  to  $202.00 \pm 7.51$   $\mu\text{mol TE/g}$  (Fig. 1C). However, the antioxidant activity of the mixed berry juice increased significantly from  $209.57 \pm 2.93$  to  $268.30 \pm 1.75$   $\mu\text{mol TE/g}$  after 24 h of fermentation (Fig. 1D). The antioxidant activity of the mixed berry juice improved by about 28% after fermentation by *L. plantarum* LP-115. These results suggested that glycoside hydrolase or other metabolites from *L. plantarum* LP-115 may improve the antioxidant activity of the mixed berry juice during fermentation. Similar phenomena were reported using fungi, yeasts, or LAB (10,18). After 24 h of fermentation, the antioxidant activity decreased.

**Changes in volatile compounds in the mixed berry juice during fermentation by *L. plantarum* LP-115** During fermentation of the mixed berry juice by *L. plantarum* LP-115, volatile compounds were extracted by the SPME method and identified by GC-MS. As shown in Table 1, 34 compounds were identified, apart from three unknown

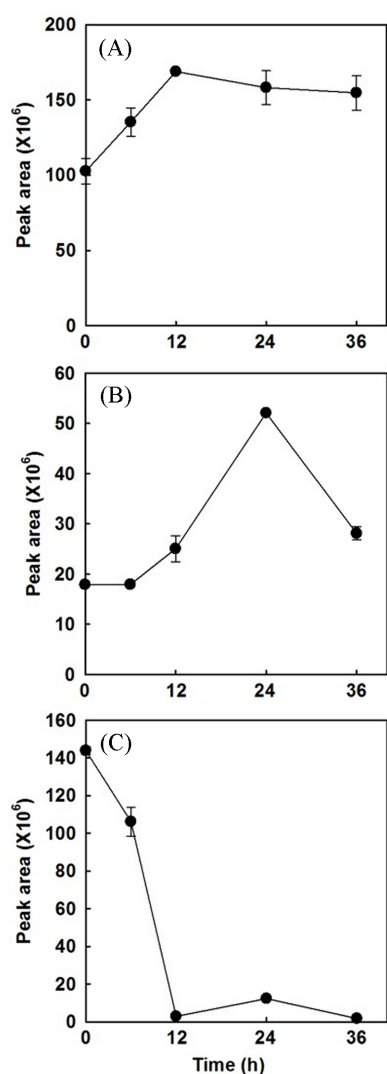
compounds, from the mixed berry juice during fermentation: seven alcohols, eleven terpene compounds, three ketones, four acids, three aldehydes, two esters, one lactone, one phenol, and one miscellaneous. The 34 identified compounds were classified into 8 groups: acid, aldehyde, alcohol, terpene, ketone, ester, furan, and phenol. The sum of peak areas of acid and aldehyde groups increased (47 and 122%, respectively), and the peak area of terpene decreased (64%) significantly during fermentation by *L. plantarum* LP-115.

Among acidic compounds, the peak area of benzoic acid increased significantly from  $102.65 \times 10^6$  to  $168.84 \times 10^6$  after 12 h of fermentation (Fig. 2A) and remained unchanged thereafter. Benzoic acid is generally used as a preservative in the food industry because of its antimicrobial activity (33). In addition, it was reported that benzoic acid and its derivatives showed antioxidant properties against different types of free radicals (34). Among aldehydes, the peak area of benzaldehyde increased sharply (191%) from  $17.89 \times 10^6$  to  $52.06 \times 10^6$  after 24 h of fermentation (Fig. 2B). Benzaldehyde produced from *Photorhabdus temperata* M1021 exhibited high antioxidant activity, determined by the DPPH method, as well as antimicrobial and insecticidal activity (35). The peak area of 2,6,6-trimethyl-10-methylidene-1-oxaspiro[4.5]dec-8-ene (vitispirane), which is the most abundant terpene compound in the mixed berry juice, decreased drastically (92%) from  $143.87 \times 10^6$  to  $12.30 \times 10^6$  after 12 h of

**Table 1.** Changes in the volatile compounds of the mixed berry juice during fermentation by *Lactobacillus plantarum* LP-115

No	RI <sup>1)</sup>	Compound name	Peak area ( $\times 10^6$ ) <sup>2)</sup>				
			0 h	6 h	12 h	24 h	36 h
<b>Alcohol</b>							
1	1384	1-Hexanol	2.77±0.04	2.60±0.06	1.89±0.01	2.04±0.05	1.95±0.02
2	1387	(Z)-3-Hexenol	36.22±1.00	34.56±0.62	24.20±0.02	26.50±0.61	24.82±0.08
3	1492	2-Ethyl-1-hexanol	2.42±0.31	1.85±0.00	1.71±0.01	1.86±0.04	1.64±0.08
4	1560	1-Octanol	2.14±0.26	1.43±0.11	1.49±0.08	1.49±0.10	1.29±0.03
5	1898	Benzyl alcohol	2.68±0.09	2.75±0.05	3.05±0.06	2.72±0.01	2.90±0.07
6	1934	2-Phenyl ethanol	3.81±0.23	3.69±0.06	4.57±0.38	3.64±0.13	3.90±0.10
7	1970	1-Dodecanol	11.99±1.49	3.57±0.31	8.59±1.33	5.61±0.23	4.03±0.07
		Total	62.03±1.86	50.45±0.71	45.50±1.39	43.86±0.68	40.53±0.18
<b>Terpene</b>							
8	1169	Isocineole	7.77±0.78	5.62±0.46	tr <sup>3)</sup>	3.25±0.25	tr
9	1213	1,8-Cineole	3.39±0.41	4.40±0.31	1.36±0.16	3.66±0.24	tr
10	1458	Trans-linalool oxide	21.73±1.17	21.74±0.57	20.31±0.08	20.60±0.10	22.11±0.42
11	1485	Cis-linalool oxide	7.69±0.26	7.87±0.44	8.18±0.04	7.56±0.08	9.43±0.30
12	1548	2,6,6-Trimethyl-10-methylidene-1-oxaspiro[4.5]dec-8-ene	143.87±2.20	106.17±7.63	2.86±0.42	12.30±0.75	1.73±0.13
13	1550	Linalool	13.48±1.48	12.88±0.24	11.02±0.22	12.22±0.21	11.60±0.10
14	1584	1-Terpineol	2.49±0.10	2.46±0.06	2.23±0.24	2.23±0.00	2.16±0.03
15	1615	4-Terpineol	4.66±0.37	4.08±0.08	3.69±0.18	3.96±0.09	3.87±0.16
16	1641	$\beta$ -Terpinol	6.70±0.29	5.71±0.09	4.53±0.04	4.50±0.11	4.77±0.15
17	1711	$\alpha$ -Terpineol	36.46±1.33	36.91±0.91	32.85±0.43	32.67±0.30	35.34±0.65
18	1713	$\gamma$ -Terpineol	7.02±0.26	7.09±0.19	6.12±0.02	5.89±0.05	6.49±0.07
		Total	255.26±5.13	214.93±7.74	93.15±0.74	108.84±0.92	97.50±0.88
<b>Ketone</b>							
19	1139	3-Penten-2-one	321.23±7.71	323.65±3.69	292.24±5.14	332.41±4.90	321.88±0.43
20	1478	4-Hydroxy-2-pentanone	1.24±0.17	1.16±0.09	1.43±0.05	1.38±0.07	1.43±0.09
21	1845	$\beta$ -Damascenone	4.01±0.33	3.51±0.15	3.16±0.15	3.54±0.14	2.52±0.28
22	2179	$\gamma$ -Decalactone	1.82±0.09	2.64±0.32	1.50±0.01	ND <sup>4)</sup>	ND
		Total	329.82±7.72	332.26±3.71	299.76±5.14	338.67±4.90	327.13±0.52
<b>Acid</b>							
23	1468	Acetic acid	21.43±2.07	19.49±1.14	20.80±1.14	19.30±0.23	20.05±0.07
24	1677	2-Methylbutanoic acid	17.76±0.51	18.73±1.12	18.58±0.36	18.26±0.48	20.41±0.55
25	2171	Nonanoic acid	1.40±0.55	0.50±0.02	1.97±0.30	tr	tr
26	>2200	Benzoic acid	102.65±8.60	135.32±9.40	168.84±0.28	158.18±11.31	154.69±11.53
		Total	143.24±8.87	174.04±9.53	210.19±1.26	195.74±11.32	195.15±11.54
<b>Aldehyde</b>							
27	1489	Furfural	4.77±0.23	4.16±0.15	3.47±0.02	4.26±0.14	3.83±0.06
28	1545	Benzaldehyde	17.89±0.41	17.93±0.15	25.03±2.64	52.06±0.14	28.09±1.31
29	1703	$\alpha$ -4-Dimethyl-3-cyclohexene-1-acetaldehyde	4.55±0.11	4.63±0.04	4.04±0.06	4.22±0.00	4.34±0.08
		Total	27.21±0.48	26.72±0.21	32.54±2.64	60.54±0.19	36.26±1.31
<b>Ester</b>							
30	1594	Methyl-2-furoate	6.06±0.26	5.60±0.17	6.01±0.51	5.57±0.14	5.66±0.10
31	1748	Benzyl acetate	11.14±3.34	4.07±0.50	6.19±0.53	5.35±0.31	3.59±0.13
		Total	17.2±3.35	3.67±0.52	12.20±0.73	10.92±0.34	9.25±0.16
<b>Furan</b>							
32	1529	3,9-Epoxy-1-p-menthene	1.83±0.06	1.77±0.07	1.36±0.07	1.71±0.02	1.56±0.03
		Total	1.83±0.06	1.77±0.07	1.36±0.07	1.71±0.02	1.56±0.03
<b>Phenol</b>							
33	1881	Guaiacol	0.69±0.01	0.46±0.00	0.43±0.01	0.65±0.00	0.40±0.02
		Total	0.69±0.01	0.46±0.00	0.43±0.01	0.65±0.00	0.40±0.02
<b>Miscellaneous</b>							
34	1112	2-Ethenyltetrahydro-2,6,6-trimethyl-2H-pyran	22.01±0.61	6.84±0.58	ND	ND	ND
		Total	22.01±0.61	6.84±0.58	ND	ND	ND
<b>Unknown</b>							
35	1203	Unknown	29.16±0.15	16.44±1.33	ND	7.54±0.57	tr
36	1211	Unknown	33.30±0.81	19.25±0.76	tr	5.98±0.18	tr
37	1540	Unknown	16.94±0.83	16.14±0.33	9.05±0.07	12.78±0.12	8.16±0.23
		Total	79.40±1.17	51.83±1.56	9.05±0.07	26.30±0.61	8.16±0.48

<sup>1)</sup>Retention indices were determined on DB-wax using C8-C22 as the external reference.<sup>2)</sup>Mean±standard deviation.<sup>3)</sup>tr, trace.<sup>4)</sup>ND, not detected.



**Fig. 2.** Peak area changes in benzoic acid (A), benzaldehyde (B), and vitispirane (C) from the mixed berry juice during fermentation by *L. plantarum* LP-115.

fermentation (Fig. 2C). Vitispirane was one of the major nor-isoprenoids found in wines, having an important sensorial impact on wine aroma, and the concentration of vitispirane increases with aging (36). As shown in Fig. 3, the peak areas of vitispirane and benzoic acid changed significantly, whereas that of other volatile compounds did not change significantly during fermentation. The peak area of benzaldehyde was relatively smaller than those of vitispirane and benzoic acid, as shown in Table 1. Therefore, it is not easy to indicate benzaldehyde in Fig. 3, although the peak area of benzaldehyde increased by 191%. Recent reports showed that the quality of prickly pears juice was enhanced after fermentation by *L. fermentum* ATCC 9338 (37). Similar to the results in this study, risky compounds such as furan or furfural decreased during the fermentation of the mixed berry juice by *L. plantarum* LP-115. In conclusion, we showed that the antioxidant activity of mixed berry juice increased significantly after fermentation by *L. plantarum* LP-

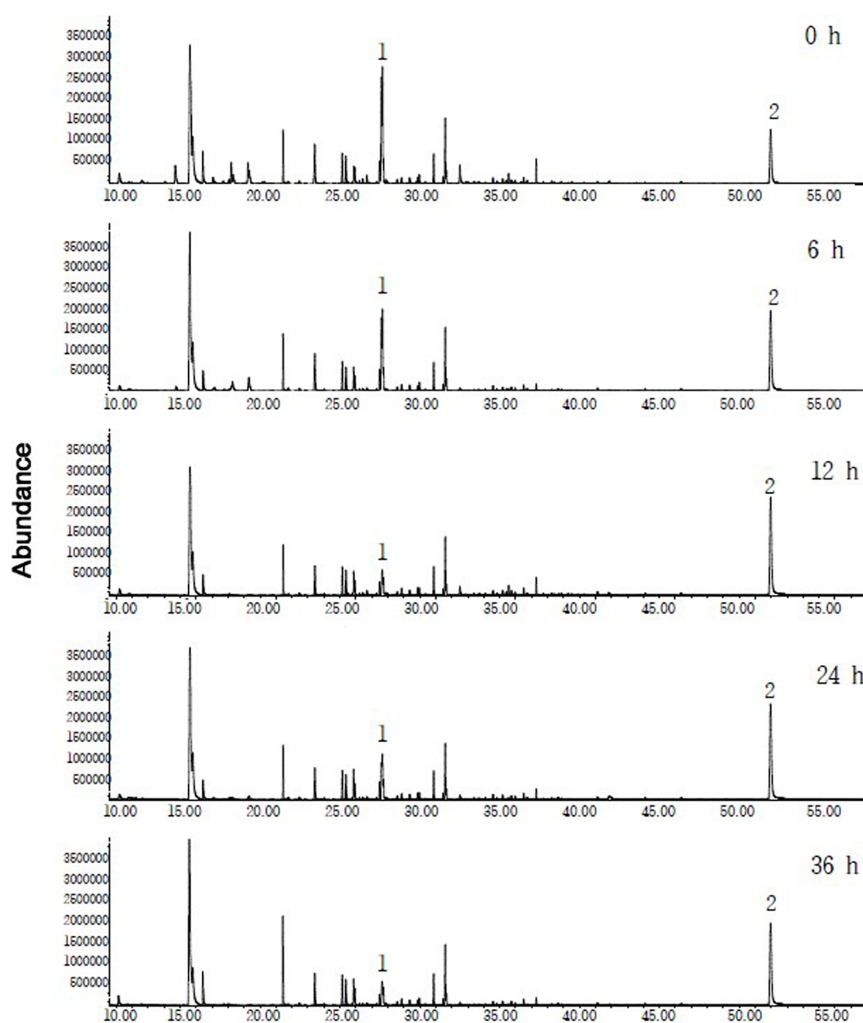
115. For further understanding of the improved antioxidant activity of the mixed berry juice, 34 volatile compounds were identified by SPME and GC-MS. Among them, the peak areas of benzoic acid and benzaldehyde, known to possess antioxidant activities, increased greatly. However, the peak area of vitispirane, which is the most abundant terpene compound in the mixed berry juice, decreased drastically after fermentation.

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**Disclosure** The authors declare no conflict of interest.

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**Fig. 3.** Total ion chromatograms of the mixed berry juice by solid phase microextraction and gas chromatography-mass spectrometry during fermentation by *L. plantarum* LP-115. 1: vitispirane, 2: benzoic acid.

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