FLAVOUR FORMATION OF HARBIN DRY SAUSAGES BY INOCULATION WITH LACTIC ACID BACTERIA AND *STAPHYLOCOCCUS XYLOSUS*

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I. INTRODUCTION

Harbin dry sausage is a naturally fermented sausage and it is famous in the northeast of China for its distinctive properties, such as flavor, color, and texture. Lactic acid bacteria (LAB), together with coagulase-negative staphylococci, are the primary bacteria found in fermented sausages, contributing to the final organoleptic and hygienic properties of the products. To better illustrate the effect of bacterial fermentation on the flavour development in Harbin dry sausages, the volatile compounds identified and quantified in the present study.

II. MATERIALS AND METHODS

1. Bacterial cultures Two LAB strains (*P. pentosaceus* and *L. curvatus*) and one *Staphylococcus* strain (*S. xylosus*) were used in this study.
2. Harbin dry sausage preparation A total of seven batches of dry sausages were prepared. A control batch was not inoculated with starter culture, and the other batches were inoculated with mixed strains, including *P. pentosaceus*, *S. xylosus* and *L. curvatus* (Pp+Sx+Lc). Sausages were prepared according to the method of Chen *et al*. [1].
3. Volatile compound analysis The volatile compounds of the dry sausages were extracted by solid phase micro-extraction (SPME) and analyzed by GC/MS as described by Chen et al. [2].

III. RESULTS AND DISCUSSION

After a nine-day fermentation, sausage inoculated with the mixed starter culture of Pp+Sx+Lc contained fifty-nine volatile compounds comprising ten aldehydes, four ketones, eight alcohols, six acids, seven esters, four alkanes and eighteen alkenes (shown in Table 1). These compounds were generally derived from lipid oxidation, protein hydrolysis, carbohydrate metabolism, and wine and spices.

Table 1 Volatile compounds identified and quantified (expressed as AU × 10^6) by gas chromatography/mass spectrometry in the head space of the control and sausages inoculated with Pp+Sx+Lc over a nine-day fermentation

Volatile compounds	day 0		day 3		day 6		day 9	
	control	Pp+Sx+Lc	control	Pp+Sx+Lc	control	Pp+Sx+Lc	control	Pp+Sx+Lc
Aldehydes								
Hexanal	n. d.	n. d.	16.56 ± 0.29^{e}	9.30 ± 0.15^{f}	44.69 ± 0.83^{b}	35.54 ± 0.38^{d}	66.96 ± 0.53^{a}	42.64 ± 0.50^{c}
Heptanal	n. d.	n. d.	6.45 ± 0.30^{e}	5.48 ± 0.33^{e}	14.32 ± 0.22^{c}	9.31 ± 0.22^{d}	31.30 ± 1.11^{a}	19.51 ± 0.77^{b}
Decanal	n. d.	n. d.	2.36 ± 0.05^{d}	1.55 ± 0.29^{e}	3.53 ± 0.27^{b}	2.62 ± 0.15^{cd}	4.97 ± 0.32^{a}	3.03 ± 0.16^{bc}
Nonanal	n. d.	n. d.	37.62 ± 0.96^{c}	31.32 ± 1.17^{d}	45.40 ± 0.88^{b}	39.18 ± 0.87^{c}	58.74 ± 0.50^{a}	47.47 ± 0.85^{b}
Benzaldehyde	12.67 ± 0.21^{d}	12.40 ± 0.22^{d}	n. d.	n. d.	10.18 ± 0.74^{c}	17.91 ± 0.61^{b}	19.26 ± 0.83^{ab}	23.69 ± 5.43^{a}
Phenylacetaldehyde	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	4.57 ± 0.06^{b}	6.67 ± 0.40^{a}
2-methyl-propanal	n. d.	n. d.	n. d.	n. d.	2.59 ± 0.55^{c}	6.29 ± 0.17^{b}	5.28 ± 0.06^{b}	12.33 ± 0.94^{a}
2- methyl-butanal	n. d.	n. d.	n. d.	n. d.	5.96 ± 0.47^{d}	21.00 ± 0.97^{b}	13.97± 1.40^{c}	30.83 ± 1.11^{a}
3- methyl- butanal	n. d.	n. d.	n. d.	n. d.	n. d.	9.12 ± 0.56^{b}	4.69 ± 0.56^{c}	17.65 ± 0.87^{a}
(E)-Cinnamaldehyde	47.03 ± 0.56^{a}	43.14 ± 5.60^{a}	45.92± 1.41^{a}	46.47 ± 1.01^{a}	46.67 ± 1.50^{a}	46.44 ± 0.82^{a}	47.20 ± 1.68^{a}	45.76 ± 0.97^{a}
Ketones								
2-Pentanone	n. d.	n. d.	10.88 ± 0.44^{e}	23.85 ± 1.80^{c}	13.73 ± 1.21^{e}	37.37 ± 1.60^{b}	18.91 ± 0.49^{d}	44.11 ± 1.27^{a}
2-Heptanone	n. d.	n. d.	26.84 ± 0.93^{e}	33.66 ± 1.60^{cd}	31.26 ± 1.06^{d}	40.26 ± 2.11^{b}	35.32 ± 0.99^{c}	45.04 ± 1.46^{a}
2-Nonanone	n. d.	n. d.	5.98 ± 0.62^{d}	4.58 ± 0.54^{e}	7.36 ± 0.08^{c}	5.62 ± 0.15d^{e}	10.01 ± 0.48^{b}	11.26 ± 0.42^{a}
3-hydroxy-2-butanone	n. d.	n. d.	120.48± 2.60^{e}	152.23 ± 3.49^{d}	153.62 ± 2.75^{d}	183.06 ± 2.19^{b}	170.44 ± 1.88^{c}	248.25 ± 5.39^{a}
Alcohols								
Ethanol	109.70 ± 3.92^{a}	111.53 ± 1.89^{a}	85.07 ± 2.48^{b}	76.25 ± 2.89^{c}	63.38 ± 0.17^{d}	53.93 ± 2.07^{e}	45.73 ± 1.48^{f}	37.92 ± 1.58^{g}

2,3-Butanediol	n. d.	n. d.	n. d.	n. d.	3.04 ± 0.62^{d}	9.01± 0.57^{c}	26.44 ± 0.99^{b}	29.38 ± 1.67^{a}
Hexanol	n. d.	n. d.	7.25 ± 0.28^{de}	5.69 ± 0.33^{e}	13.27 ± 0.76^{c}	10.44± 0.32^{cd}	23.40 ± 3.04^{a}	16.73 ± 1.01^{b}
Phenylethyl alcohol	n. d.	n. d.	6.04 ± 1.22^{d}	6.11 ± 0.80^{d}	17.36 ± 0.56^{c}	26.19 ± 1.11^{b}	26.59 ± 2.79^{b}	42.51 ± 0.67^{a}
3-Phenylpropanol	n. d.	n. d.	0.64 ± 0.15^{de}	2.32 ± 0.30^{c}	1.07 ± 0.11^{d}	4.90 ± 0.30^{b}	1.77 ± 0.21^{c}	6.51 ± 0.41^{a}
Linalool	40.33 ± 1.25^{e}	39.70 ± 0.64^{e}	41.02 ± 1.49^{e}	40.80 ± 1.32^{e}	40.18 ± 1.23^{e}	38.76 ± 0.98^{e}	39.51 ± 0.83^{e}	39.91 ± 0.62^{e}
Terpinen-4-ol	25.66 ± 1.15^{e}	26.36 ± 1.33^{e}	26.34 ± 0.51^{e}	25.89 ± 0.94^{e}	26.28 ± 1.24^{e}	25.74 ± 1.78^{e}	26.22 ± 1.34^{e}	25.40 ± 0.98^{e}
Terpineol	15.55 ± 0.56^{e}	16.51 ± 1.17^{e}	14.79 ± 0.96^{e}	15.91 ± 0.44^{e}	16.16 ± 1.29^{e}	14.71 ± 0.75^{e}	15.42 ± 0.54^{e}	15.23 ± 1.00^{e}
Acids								
Acetic acid	n. d.	n. d.	71.01 ± 2.61^{e}	73.64 ± 1.60^{e}	96.32 ± 4.35^{d}	172.41 ± 2.00^{c}	185.97 ± 1.42^{b}	289.52 ± 4.57^{a}
Propanoic acid	n. d.	n. d.	n. d.	n. d.	n. d.	10.47 ± 0.27^{b}	n. d.	17.13 ± 0.66^{a}
Hexanoic acid	n. d.	n. d.	17.44 ± 0.99^{c}	12.84 ± 0.45^{d}	21.21 ± 0.98^{b}	15.41 ± 1.05^{c}	33.74 ± 0.49^{a}	21.48 ± 1.43^{b}
Octanoic acid	4.10 ± 0.85^{f}	4.44 ± 0.37^{f}	7.51 ± 0.17^{d}	5.97 ± 0.27^{e}	13.42 ± 0.63^{b}	9.35 ± 0.23^{c}	16.83 ± 0.40^{a}	12.35 ± 0.44^{b}
Benzoic acid	n. d.	n. d.	n. d.	2.45 ± 0.37^{e}	6.83 ± 0.34^{d}	9.25 ± 0.07^{c}	13.50 ± 0.61^{b}	17.58 ± 0.70^{a}
Phenylacetic acid	n. d.	n. d.	n. d.	n. d.	1.70 ± 0.28^{b}	1.91 ± 0.30^{b}	4.59 ± 0.63^{a}	4.93 ± 0.33^{a}
Esters								
Ethyl acetate	52.06 ± 0.87^{f}	51.15 ± 1.22^{f}	103.30 ± 2.89^{e}	116.82 ± 2.02^{d}	123.54 ± 2.82^{c}	137.47 ± 2.74^{b}	126.17 ± 1.97^{c}	166.24 ± 3.02^{a}
Ethyl butyrate	n. d.	n. d.	4.21 ± 0.34^{c}	5.45 ± 0.29^{c}	6.26 ± 1.22^{bc}	8.29 ± 1.81^{ab}	8.14 ± 0.79^{ab}	8.74 ± 0.63^{a}
Ethyl hexanoate	14.99 ± 0.67^{f}	14.88 ± 1.35^{f}	20.57 ± 0.98^{e}	25.91 ± 1.38^{d}	20.74± 0.73^{e}	48.49 ± 0.90^{b}	36.03 ± 1.62^{c}	62.66 ± 1.49^{a}
Ethyl decanoate	n. d.	n. d.	2.24 ± 0.31^{e}	5.27 ± 0.63^{c}	3.62 ± 0.26^{d}	7.43 ± 0.52^{b}	4.74 ± 0.26^{cd}	10.57 ± 0.63^{a}
Ethyl octanoate	n. d.	n. d.	6.46 ± 0.32^{e}	7.25 ± 0.73^{e}	10.00± 0.41^{d}	14.47 ± 1.31^{c}	16.81 ± 0.93^{b}	19.84 ± 0.53^{a}
Ethyl nonanoate	n. d.	n. d.	2.53 ± 0.34^{e}	3.66 ± 0.39^{d}	3.73 ± 0.17^{d}	6.05 ± 0.23^{b}	4.83 ± 0.11^{c}	9.32 ± 0.21^{a}
Ethyl benzoate	n. d.	n. d.	n. d.	n. d.	n. d.	6.44 ± 0.87^{b}	2.41 ± 0.36^{c}	10.88 ± 0.33^{a}
Alkanes								
Hexane	n. d.	n. d.	2.49 ± 0.33^{d}	2.44 ± 0.46^{d}	6.32 ± 0.11^{b}	4.73 ± 0.34^{c}	9.40 ± 0.35^{a}	6.54 ± 0.33^{b}
Heptane	n. d.	n. d.	6.47 ± 0.24^{c}	5.40 ± 0.26^{d}	5.40 ± 0.26^{b}	6.62 ± 0.32^{c}	6.62 ± 0.32^{a}	8.66 ± 0.48^{b}
Tetradecane	n. d.	n. d.	2.82 ± 0.08^{c}	1.98 ± 0.07^{e}	1.98 ± 0.07^{b}	2.08 ± 0.07d^{e}	2.08 ± 0.07^{a}	2.59 ± 0.22^{cd}
Hexadecane	n. d.	n. d.	2.73 ± 0.22^{b}	1.60 ± 0.06^{d}	1.60 ± 0.06^{a}	2.03 ± 0.07^{c}	2.03 ± 0.07^{a}	2.30 ± 0.16^{c}
Alkenes								
Laurene	5.58 ± 0.43^{a}	5.95 ± 0.59^{a}	5.43 ± 0.54^{a}	6.19 ± 0.24^{a}	5.74 ± 0.25^{a}	5.66 ± 0.44^{a}	5.52 ± 0.31^{a}	5.48 ± 0.45^{a}
α-Terpinene	21.52 ± 1.06^{a}	21.99± 1.11^{a}	21.70 ± 1.40^{a}	22.26± 1.77^{a}	21.42 ± 1.04^{a}	22.32 ± 1.87^{a}	21.62 ± 1.17^{a}	23.77 ± 0.55^{a}
γ-Terpinene	31.94 ± 0.52^{a}	32.91 ± 1.41^{a}	33.47± 0.19^{a}	32.69 ± 0.94^{a}	33.60 ± 1.01^{a}	32.12 ± 0.97^{a}	33.03 ± 1.63^{a}	32.69 ± 0.51^{a}
Ocimene	11.27± 0.44^{a}	11.27 ± 1.14^{a}	10.59 ± 0.88^{a}	11.19 ± 1.18^{a}	10.53 ± 0.96^{a}	10.57 ± 0.74^{a}	11.09 ± 0.62^{a}	11.35 ± 1.34^{a}
3-Carene	10.11± 1.03^{a}	11.22 ± 0.77^{a}	12.52 ± 1.71^{a}	11.37± 1.42^{a}	13.00 ± 1.45^{a}	11.69 ± 1.52^{a}	11.60 ± 1.23^{a}	11.78 ± 1.54^{a}
α-Muurolene	25.04± 1.66^{a}	22.55 ± 1.78^{a}	22.53± 0.99^{a}	22.89± 2.12^{a}	22.76 ± 2.02^{a}	23.69 ± 0.66^{a}	25.62 ± 0.74^{a}	22.49 ± 2.47^{a}
γ-Muurolene	13.32 ± 0.50^{a}	14.03± 0.89^{a}	13.41± 1.40^{a}	12.86 ± 2.10^{a}	12.66 ± 0.48^{a}	12.47 ± 0.67^{a}	11.81 ± 1.51^{a}	11.30 ± 1.05^{a}
Caryophyllene	23.04 ± 1.32^{a}	23.71 ± 1.59^{a}	24.64± 2.25^{a}	23.15± 2.04^{a}	22.75± 2.52^{a}	24.11 ± 1.49^{a}	23.07 ± 1.29^{a}	22.15 ± 1.14^{a}
Isosativene	3.81 ± 0.76^{a}	3.19± 0.11^{a}	4.29 ± 0.38^{a}	3.33 ± 0.69^{a}	3.92 ± 0.68^{a}	3.24 ± 1.08^{a}	2.85 ± 0.25^{a}	3.18 ± 0.24^{a}
α-Pinene	5.89± 0.95^{a}	6.81 ± 0.81^{a}	6.41 ± 0.06^{a}	7.02 ± 1.44^{a}	6.69 ± 0.86^{a}	5.62 ± 0.94^{a}	5.33 ± 0.69^{a}	6.84 ± 0.61^{a}
β-Pinene	52.86± 1.54^{a}	51.04 ± 0.95^{a}	52.10 ± 1.99^{a}	51.99± 1.43^{a}	52.07 ± 2.06^{a}	51.56 ± 0.96^{a}	51.16 ± 0.78^{a}	51.60 ± 1.48^{a}
p-Pinene	16.41 ± 1.80^{a}	18.41± 0.99^{a}	18.29 ± 1.24^{a}	15.92 ± 1.33^{a}	16.15 ± 1.08^{a}	14.63 ± 0.52^{a}	15.45 ± 2.73^{a}	16.88 ± 0.96^{a}
Limonene	14.82± 0.57^{a}	15.15 ± 0.51^{a}	15.10± 0.77^{a}	14.55 ± 0.88^{a}	14.63 ± 0.37^{a}	14.97 ± 0.95^{a}	14.78 ± 0.61^{a}	14.18 ± 0.27^{a}
Phellandrene	9.57 ± 0.79^{a}	10.67± 1.34^{a}	10.29 ± 1.07^{a}	12.58± 1.20^{a}	11.26 ± 1.70^{a}	9.67 ± 0.67^{a}	10.44 ± 0.99^{a}	10.63 ± 1.05^{a}
Sabinene	11.15± 1.06^{a}	13.03± 1.06^{a}	12.85 ± 1.21^{a}	11.52± 2.13^{a}	11.22 ± 0.74^{a}	11.22 ± 0.88^{a}	11.62 ± 1.40^{a}	11.82 ± 1.85^{a}
Xanthoxylin	9.15± 1.10^{a}	8.39± 0.53^{a}	10.99 ± 1.05^{a}	12.95 ± 1.51^{a}	11.69 ± 0.91^{a}	12.17 ± 1.78^{a}	10.86 ± 1.88^{a}	10.28 ± 0.74^{a}
Estragole	15.34 ± 1.00^{a}	15.23± 0.45^{a}	15.70± 1.59^{a}	15.77 ± 0.58^{a}	15.33 ± 1.86^{a}	15.47 ± 1.06^{a}	14.55 ± 0.69^{a}	15.62 ± 0.74^{a}
Anethole	15.55 ± 1.17^{a}	16.59 ± 0.84^{a}	15.52 ± 0.74^{a}	16.66± 0.81^{a}	15.55 ± 0.78^{a}	14.89 ± 1.409^{a}	15.93 ± 1.32^{a}	15.66 ± 0.98^{a}

$^{a-g}$ Means within the same column with different superscript letters differ significantly ($P < 0.05$). n. d.: not detected.

IV. CONCLUSION

The levels of compounds derived from lipid oxidation were significantly lower in the inoculated sausage, indicating an inhibition of lipid oxidation by bacterial fermentation. Furthermore, incomplete β-oxidation, amino acid metabolism, and carbohydrate catabolism by the inoculated Pp+Sx+Lc contributed to the flavour development.

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