ANTIOXIDANT POTENTIAL OF PEDIOCOCCUS PENTOSACEUS AND ITS INFLUENCE ON FLAVOUR IN HARBIN DRY SAUSAGE

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Abstract – The lactic acid bacteria *Pediococcus pentosaceus* was evaluated for their potential antioxidant activity. The results revealed that the inoculation of *P. pentosaceus* in dry sausage significantly decreased the quantities of thiobarbituric acid-reactive substance and carbonyl formation, while it also reduced the sulfhydryl loss in sausages ($P < 0.05$). Furthermore, the lower content of volatile compounds that are related to lipid-oxidation, such as aldehydes, ketones and hydrocarbons, was observed in the *P. pentosaceus* inoculated sausage ($P < 0.05$). These results demonstrate that *P. pentosaceus* has the potential to be employed as an antioxidant starter culture in fermented meat products.

Key Words –*Pediococcus pentosaceus*; Antioxidant potential; Harbin dry sausage

I. INTRODUCTION

Oxidation reactions occur widely in meat and meat products, and moderate oxidation of lipids plays an important role in flavour formation of fermented meat products. However, excessive lipid and protein oxidation are closely associated with deteriorative processes that can affect the entire quality traits of meat and meat products.

Lactic acid bacteria (LAB) are essential agents during meat fermentation because they contribute to final product quality and safety. Most researchers are focused on the antioxidant activity of LAB in vitro, however, scant attention has been paid to on fermented meat products. Harbin dry sausage has distinct characteristics of a unique texture, fermented flavour, and short production cycle (approximately 15 days). The objective of the current study was to evaluate the antioxidant effect of *P. pentosaceus* was further tested in vitro in the dry sausage model by detecting lipid and protein oxidation and volatile compounds.

II. MATERIALS AND METHODS

2.1. Bacterial cultures and growth media

P. pentosaceus was stored at the College of Food Science, Northeast Agricultural University, China. The strain was kept on Man Rogosa Sharp (MRS) agar plates at 4 °C and thereafter incubated in MRS broth for 24 h at 30 °C and then maintained at 4 °C.

2.2. Preparation of Harbin dry sausage

Lean pork and pork back fat were obtained from a local packing plant. Two batches of dry sausages were manufactured: the control batch without *P. pentosaceus*, and another batch inoculated with *P. pentosaceus*. Sausages were prepared with lean pork (90%) and pork back fat (10%) minced through orifice plate with additives. The *P. pentosaceus* suspension was inoculated in meat batter while the control sample was not inoculated. For each fermentation time (0, 3, 6, and 9 days), three sausages of each treatment were used to analyse lipid oxidation, protein oxidation, and volatile compounds.

2.3. Measurement of lipid oxidation

The lipid oxidation level in dry sausages was evaluated by thiobarbituric acid reactive substances (TBARS) according to the method of Wang *et al* [1].

2.4. Measurement of protein oxidation

The protein oxidation in dry sausages was assessed by measuring the formation of carbonyls and the loss of sulfhydryl groups.

2.4.1. Preparation of myofibrillar protein

Myofibrillar protein (MP) was prepared from dry sausages according to the method employed by Park *et al* [2], stored at 4 °C, and utilised within 24 h.

2.4.2. Carbonyl content
The carbonyl groups were detected by reaction with DNPH to form protein hydrazones according to the method of Oliver *et al* [3] with slight modifications.

2.4.3 Sulphydryl content
Sulfhydryl (SH) content was determined using Ellman's [4] method.

2.5. Analysis of volatile compound
Volatile compounds in the headspace of a vial with the dry sausages were extracted by solid phase micro-extraction (SPME) according to the method of Chen *et al* [5]. Quantification and identification of volatile compounds were performed by a gas chromatography/mass spectrometry (GC/MS) system.

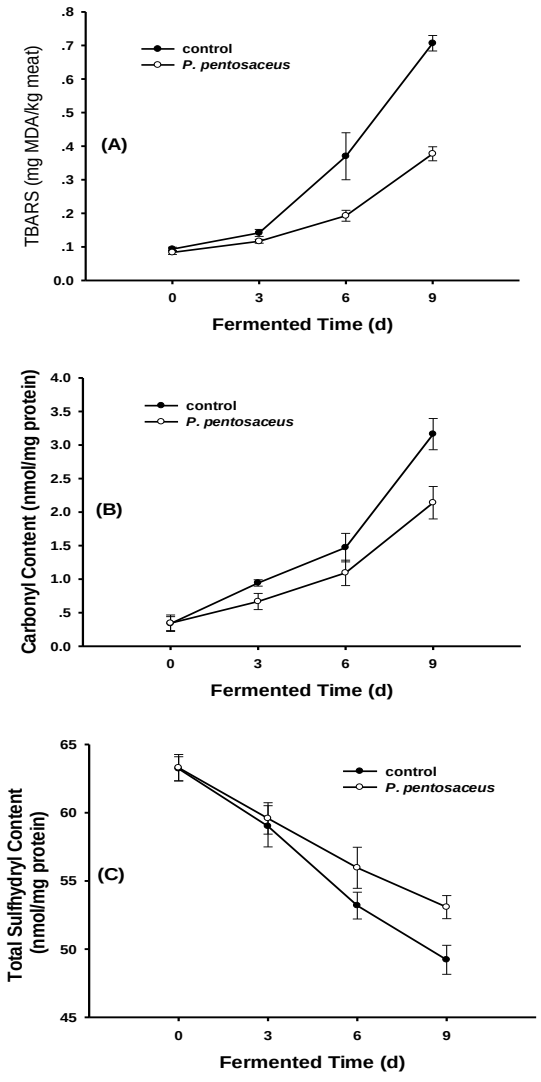
2.6. Statistical analysis
All specific experiments were repeated at least three times and analyzed statistically using the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St Paul, MN, USA).

III. RESULTS AND DISCUSSION

3.1. Inhibition of lipid and protein oxidation in dry sausages
The antioxidant effect of *P. pentosaceus* was evaluated by determining the level of lipid and protein oxidation in dry sausage during fermentation. Lipid oxidation, expressed as TBARS, in control increased rapidly during fermentation, reaching 0.71 mg/kg at 9 days (Fig. 1A). The TBARS production was significantly inhibited in sausage inoculated with *P. pentosaceus* ($P < 0.05$), especially at 6 days and 9 days. Compared with the control, the TBARS level in inoculated sausage decreased by 46.5% at nine days. Sunesen *et al* [6] have previously reported the inhibition of the lipid oxidation by observing a reduction in the generation of volatile compounds that were derived from lipid oxidation reactions in fermented sausages inoculated with *P. pentosaceus*.

Measuring both the formation of carbonyls and the loss of sulfhydryl groups assessed the extent of protein oxidation. As shown in Fig. 1B, the content of carbonyls increased significantly from 0 to 9 days, and inoculation of *P. pentosaceus* significantly decreased the carbonyl formation ($P < 0.05$). The carbonyl content in inoculated sausages reached to 2.14 nmol/mg protein, which decreased by 32.4% at 9 days compared with the control. Additionally, the loss of sulfhydryl groups as a consequence of disulphide formation is another well-described marker of oxidation reactions in muscle protein. The oxidation of cysteine sulfhydryl groups to form disulphide bonds, inducing protein cross-linking.

Figure 1. Thilbarbituric acid reactive substance (TBARS) (A), carbonyl content (B) and total sulfhydryl content (C) of Harbin dry sausages inoculated and non-inoculated with P. pentosaceus during fermentation. Error bars refer to the standard deviations obtained from triplicate sample analysis.



As shown in Fig. 1C, the loss of sulfhydryl groups in the control sample was significantly higher than that in the inoculated sample throughout the fermentation ($P < 0.05$). Sulfhydryl groups could convert into disulphides and other oxidised species, which is one of the earliest observable events during the radical-mediated protein oxidation

3.2. Volatile compound analysis

A total of 48 types of volatile compounds were extracted in control and inoculated sausages throughout the fermentation (Table 1). These volatile compounds mainly originated from lipid autoxidation, bacterial metabolism and spices.

The generation of aldehydes increased during fermentation in both groups of sausages ($P < 0.05$), except for (E)-cinnamaldehyde which originated from spices. Among them, hexanal, heptanal, decanal and nonanal derived from lipid autoxidation in control samples, which were significantly higher in content than that in inoculated sausage ($P < 0.05$), indicating that inoculation of *P. pentosaceus* could inhibit the lipid oxidation. The hexanal is considered to be a characteristic product in lipid oxidation. Heptanal and nonanal are generated from n-6 and n-9 polyunsaturated fatty acid oxidation, respectively. Phenylalanine can be converted to phenylpyruvic acid by the action of LABs, and subsequently oxidised to benzaldehyde.

Ketones can be generated from lipid autoxidation, microbial fermentation and carbohydrate catabolism. 2-pentanone and 2-heptanone were derived from microbial β-oxidation, which commonly occurred in the presence of staphylococcus. Thus, there were no significant differences detected at the same fermentation process in these two methyl ketones. The level of 3-hydroxy-2-butanone was higher in the inoculated sausage ($P < 0.05$), which could be attributed the action of *P. pentosaceus*. 3-hydroxy-2-butanone originated from carbohydrate catabolism by LABs.

Eight types of alcohols were identified, only ethanol exhibited a decrease during the fermentation, especially in the inoculated sausage ($P < 0.05$). This could be due to the esterification of ethanol and acids. Hexanol, was higher in the control samples than in *P. pentosaceus* inoculated sausage, indicating an inhibition of lipid oxidation by *P. pentosaceus*. In addition, the promoted influence of the inoculation with *P. pentosaceus* on the formation of alcohols originated from the amino acid catabolism was clear in Table 1.

Acid generation increased during fermentation and the level of both hexanoic acid and octanoic acid, which arise from the oxidation of corresponding alcohols, was higher in the control group than inoculated sausage ($P < 0.05$). In addition, the levels of acetic acid and benzoic acid in inoculated sausage were higher than control sausage. Esters arise from the esterification of several alcohols and carboxylic acids in meat. The levels of all the esters increased during the fermentation, and a higher level of esters occurred in inoculated sausage ($P < 0.05$).

Hydrocarbons also presented in the volatiles of sausages. Four types of alkanes and ten types of alkenes were detected. They are not major contributors to the flavour due to their high detection threshold. The ten types of alkenes and other compounds have no significant difference between control and inoculated sausage ($P < 0.05$).

According to the correlation analysis of volatile compounds and TBARS values in dry sausages inoculated and non-inoculated with *P. pentosaceus*, positive and significant correlations were found between these compound levels and TBARS values in both groups of sausages (Table 2). The aldehydes, ketone, alcohol and acids were related to the lipid autoxidation.

IV. CONCLUSION

The *P. pentosaceus* was evaluated for their potential antioxidant activity in sausage model. The results showed that inoculation of *P. pentosaceus* lowered the level of lipid and protein oxidation and increased total sulfhydryl content. Furthermore, a reduction of volatile compounds, such as aldehydes, ketones and hydrocarbons, which are derived from lipid oxidation, was observed in *P. pentosaceus* inoculated sausage. Therefore, *P. pentosaceus* could be considered to

be a potential antioxidant starter culture for applications in fermented meat products.

Table 1 Volatile compounds identified and quantified (expressed as AU × 106 extracted by HS-SPME) by GC-MS in the headspace of dry fermented sausages inoculated and non-inoculated with P. pentosaceus during fermentation

Volatile compounds	Day 0		9 days	
	Control	*P. pentosaceus*	Control	*P. pentosaceus*
Aldehydes				
Hexanal	n. d.	n. d.	68.4±1.2^{a}	45.0±1.6^{b}
Heptanal	n. d.	n. d.	31.6±1.8^{a}	13.2±1.0^{b}
Decanal	n. d.	n. d.	4.9±0.4^{a}	2.1±0.4^{c}
Nonanal	12.3±0.6^{e}	12.6±0.2^{e}	59.2±0.6^{a}	48.8±3.5^{b}
Benzaldehyde	n. d.	n. d.	21.5± 3.1^{b}	26.9±1.5^{a}
(E)-Cinnamaldehyde	47.8±1.4^{ab}	50.0±1.4^{ab}	47.8±1.9^{ab}	48.7±2.2^{ab}
Ketones				
2-Pentanone	n. d.	n. d.	20.3±0.9^{a}	21.3±1.8^{a}
2-Heptanone	n. d.	n. d.	52.8±1.2^{a}	52.9±3.0^{a}
2-Nonanone	n. d.	n. d.	10.2±1.0^{a}	4.3±0.9^{bc}
3-hydroxy-2-butanone	n. d.	n. d.	187.5±2.8^{b}	235.9±4.4^{a}
Alcohols				
Ethanol	87.1±1.5^{a}	87.5±2.5^{a}	27.9±1.9^{d}	35.0±3.0^{c}
Hexanol	n. d.	n. d.	27.5±1.1^{a}	22.6±1.1^{b}
2,3-Butanediol	n. d.	n. d.	30.1± 1.1^{b}	40.5±2.5^{a}
3-Phenylpropanol	n. d.	n. d.	1.4±0.4^{c}	5.8±0.4^{a}
Phenylethy Alcohol	n. d.	n. d.	29.7±1.1^{b}	36.9±1.6^{a}
3,7-dimethyl-1,6-Octadien-3-ol	40.5±1.3^{a}	38.4±1.9^{a}	39.4±1.0^{a}	41.1±2.5^{a}
Terpinen-4-ol	26.3±1.0^{a}	24.0±3.7^{a}	26.2±3.0^{a}	26.7±3.0^{a}
Terpineol	15.8±0.2^{a}	17.3±1.4^{a}	16.5±1.17^{a}	16.4±1.3^{a}
Acid				
Acetic acid	n. d.	n. d.	190.5±4.1^{b}	283.0±3.7^{a}
Hexanoic acid	n. d.	n. d.	35.4±1.6^{a}	22.9±1.4^{b}
Octanoic acid	5.5±0.2^{de}	4.7±0.5^{e}	15.4±0.8^{a}	12.6±1.5b^{c}
Benzoic acid	n. d.	n. d.	13.2±0.7^{b}	16.9±0.4^{a}
Esters				
Ethyl acetate	11.6±1.3^{f}	15.7±0.5^{ef}	36.8±2.4^{c}	61.3±3.6^{a}
Hexanoic acid, ethyl ester	52.9±1.3^{e}	49.4±3.2^{e}	113.6±2.3^{b}	175.1±3.0^{a}
Octanoic acid, ethyl ester	n. d.	n. d.	9.5±0.4^{b}	12.8±0.9^{a}
Decanoic acid, ethyl ester	n. d.	n. d.	4.8±0.9^{b}	7.5±0.5^{a}
Nonanoic acid, ethyl ester	n. d.	n. d.	5.5±0.5^{b}	9.8±0.4^{a}
Benzoic acid, ethyl ester	n. d.	n. d.	n. d.	14.7±0.8^{a}
3-methyl-Butanoic acid, ethyl ester	n. d.	n. d.	n. d.	10.6±0.8^{a}
Hydrocarbons				
Hexane	n. d.	n. d.	9.1±0.3^{a}	7.4±0.4^{b}
Heptane	n. d.	n. d.	14.7±0.7^{a}	7.8±0.3^{c}
Tetradecane	n. d.	n. d.	3.2±0.4^{a}	2.7±0.2^{ab}
Hexadecane	n. d.	n. d.	3.4±0.4^{a}	2.2±0.4^{bc}
.alpha.-Calacorene	6.9±1.4^{a}	3.9±0.8^{a}	4.6±0.9^{a}	5.6±1.5^{a}
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	20.9±1.5^{a}	22.1±3.3^{a}	21.3±0.9^{a}	22.4±2.5^{a}
.gamma.-Terpinene	30.3±1.8^{a}	31.6±1.6^{a}	32.0±0.6^{a}	31.5±3.1^{a}
trans-.beta.-Ocimene	9.6±1.0^{a}	10.4±2.0^{a}	9.9±1.0^{a}	11.2±1.4^{a}
2-Carene	23.3 ±2.0^{a}	23.0±1.9^{a}	21.4±3.4^{a}	23.0±3.7^{a}
.alpha.-Muurolene	26.7±1.9^{a}	23.8±3.2^{a}	24.6±4.8^{a}	24.0±3.7^{a}
.gamma.-Muurolene	11.2±1.7^{a}	12.0±2.1^{a}	12.6±0.6^{a}	11.0±0.6^{a}
Caryophyllene	34.7±1.4^{a}	35.3±4.8^{a}	33.7±1.0^{a}	34.0±3.2^{a}
Isosativene	3.3±1.4^{a}	3.2±0.5^{a}	3.3±0.1^{a}	3.4±1.3^{a}
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	10.9±0.9^{a}	11.5±1.8^{a}	9.9±1.7^{a}	11.3±1.0^{a}
Other compounds				
Xanthoxylin	7.9±0.4^{a}	8.2±2.2^{a}	8.3±1.7^{a}	9.1±1.1^{a}
.beta.-Pinene	48.3±2.8^{a}	51.7±3.5^{a}	50.9±3.0^{a}	51.9±1.7^{a}
p-Cymene	18.5±0.7^{a}	16.7±2.7^{a}	16.7±2.2^{a}	19.7±1.5^{a}
Estragole	15.6±1.1^{a}	15.9±2.4^{a}	15.6±1.4^{a}	17.6±2.0^{a}
Anethole	17.6±1.0^{a}	16.0±3.0^{a}	16.1±1.8^{a}	16.4±0.9^{a}

a–f Means within the same row with different superscript letters differ significantly ($P < 0.05$); n.d.: not detected.

Table 2 Correlation coefficients (r) between several volatile compounds and TBARS in dry fermented sausages inoculated and non-inoculated with *P. pentosaceus*

Item	control	*P. pentosaceus*
Hexanal	0.965**	0.899**
Heptanal	0.985**	0.884**
Decanal	0.880**	0.651*
Nonanal	0.858**	0.843**
2-Nonanone	0.844**	0.763**
Hexanol	0.968**	0.972**
Hexanoic acid	0.968**	0.783**
Octanoic acid	0.903**	0.908**

$^{*}P < 0.05$; $^{**}P < 0.01$.

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