

IMPROVEMENT OF THE QUALITY OF CURED RABBIT MEAT PRODUCT (CHAN SI TU) USING STAPHYLOCOCCUS XYLOSUS AS STARTER CULTURE

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Abstract: Starter cultures can help improve the quality and safety of traditional fermented meat products. This work was conducted to evaluate the effects of the inoculum of *Staphylococcus xylosus* on the quality characteristics of a Sichuan cured rabbit product (Chan Si Tu). Physicochemical analyses showed that meats inoculated with *S. xylosus* had a significant increase in lightness, redness, cohesiveness and chewiness (P<0.05). In addition, a lower content of nitrite, diethylnitrosamine and histamine, was observed in the inoculated samples (P<0.05). The thiobarbituric acid reactive substances (TBARS) value in the *S. xylosus* incubation group was 0.108±0.004 mg/kg, which was significantly lower than that in the control group (P<0.01). Interestingly, a lower TBARS value was observed in the incubation samples through storage. Furthermore, solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) analysis identified a total of 73 volatiles, and the species and abundance of theses volatile compounds were higher in the samples with added *S. xylosus*, and 12 with odour activity value>1 were proposed as the most odour-active compounds that enhanced the complexity of the product. Thus, inoculation with *S. xylosus* in the cured rabbit meat product (Chan Si Tu) can effectively enhance the product quality.

Key Words: cured rabbit product, starter culture, *Staphylococcus xylosus*, physicochemical properties, volatile flavour compounds.

INTRODUCTION

Cured meat is a popular traditional uncooked meat product prepared since the earliest civilisations. In China, cured meats have a production history of hundreds of years. These products traditionally involve the use of raw meat and meat by-products as the ingredient, blended and mixed with salt, spices and other ingredients, and then dried in air-conditioned rooms during winter (Zeng *et al.*, 2016). In general, the climatic conditions of the production area had an influence on the manufacturing procedure. The final meat has a very strong, attractive flavour and texture, and a great number of products are produced worldwide. In southwest China, cured pork, fermented sausages, dried salted duck and cured rabbit product represent the most popular dry-cured meat spices (Zhou *et al.*, 2008), which belong to intermediate moisture foods (IMF) with water activity (a,) of 0.70 to 0.88 and have good non refrigerated storability.

China accounted for roughly 60% of the world's total rabbit meat production, and this figure has steadily increased in recent years (Li *et al.*, 2018). Although most rabbit meats are sold as cut-up parts or as whole carcass, an increasing number of primarily and further processed products have been developed in recent years. Among them, a dry-cured rabbit meat product called Chan Si Tu is one of the well-known traditional Sichuan cured rabbit meat products, which has significantly contributed to the development of the Chinese rabbit industry (Li *et al.*, 2018, Szendrő *et al.*, 2020).

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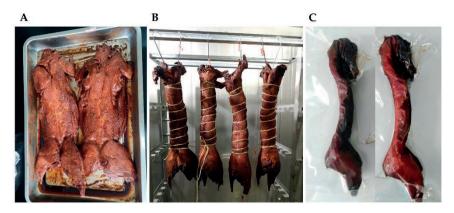


Figure 1: The manufacturing processes of Chan Si Tu. (A) The curing period; (B) tied with hemp twist and hung under air drying fermentation device period; (C) Stored in vacuum package period.

In general, the production of Chan Si Tu involves three main steps. The first is the curing period, during which the meats are processed and curing ingredients are rubbed onto the carcass surfaces, and this lasts about two days. In the second phase, meats are tied with hemp twist and then hung under an air drying fermentation device at 10 to 16°C for about 10 d. The third phase includes air drying for three days, after which the meats are stored in vacuum packaging and kept at room temperature for several days. The final product is the result of microbiological, biochemical, flavour and texture changes occurring during ripening (Ji *et al.*, 2016). There are many factors, such as raw meat properties, additives, processing parameters and air conditioning affecting the quality of products. Among these factors, temperature and humidity vary depending on natural climate conditions and are not controlled, which makes it difficult to guarantee the quality and safety of the final products.

With most fermented meats, safety is generally achieved by controlling or preventing growth of pathogens during processing and reducing contamination (Barbuti and Parolari, 2002), and nowadays the need for safe foods with standard and desirable technological properties has resulted in research focused on starter cultures to control the fermentation and ripening processing. *Lactobacillus* spp. and *Staphylococcus* spp. are the most common microorganisms in fermented meats and are involved in the development of colour, texture and flavour. Thus, there has been an abundance of studies conducted with different strains of these bacteria in various meat species, such as fermented meats, cooked meats, vacuum-stored or raw meats (Sun *et al.*, 2016). *Lactobacillus* spp. have been widely used as a favourable starter culture in the manufacture of fermented sausages and cured meats due to their biochemical functions, such as inhibition of pathogenic and spoilage microorganisms, conversion of metmyoglobin into red myoglobin derivatives and depletion of residual nitrite, as well as contribution to flavour formation (Xiao *et al.*, 2020). In addition, *Staphylococcus* spp. usually participate in the formation and stabilisation of red colour by nitrate reductase activity, inhibition of oxidative rancidity and flavour development of fermented meat products (Cruxen *et al.*, 2017, Xiao *et al.*, 2020). Moreover, the combined use of *Lactobacillus* spp. and *Staphylococcus* spp. can achieve desirable properties of fermented meat products.

Our previous study showed that a mixed-strain starter culture that includes *Pediococcus pentosaceus, Latilactobacillus sakei, Staphylococcus xylosus, Staphylococcus carnosus* and *Debaryomyces hansenii* makes a certain contribution to flavour improvement of wire tied rabbit (Bai *et al.*, 2018). Among them, species belonging to the former *Lactobacillus* genus have the function of reducing the pH of the matrix through production of lactic acid from the fermentation of sugars (Cruxen *et al.*, 2019), which has a direct impact on sensory product quality by providing a mild acidic taste, which has not been widely accepted by Chinese people. Thus, *Staphylococcus* spp. are normally used as an alternative starter culture in dry-cured meat product manufacturing (Cebrián *et al.*, 2020). Therefore, it is necessary to study the effect of *Staphylococcus xylosus* on the quality during the ripening of the cured rabbit product.

MATERIALS AND METHODS

Sample preparation and manufacture

A total of 50 fresh carcasses (weight 1.0 ± 0.05 kg) of Hyla commercial rabbits were sourced from Hawuye Food Co., Ltd., Sichuan, China. Each whole-body was trimmed of visible fat and connective tissue, followed by washing and draining. Meats were randomly divided into two groups: case group (S) samples were mixed in a vacuum tumbling machine, with 600 g of compound seasonings (Meat Processing Key Lab of Sichuan Province) and 2 g of *S. xylosus* (Chr. Hansen), while the other samples were mixed in the vacuum tumbling machine, with the same compound seasonings but without *S. xylosus*, to act as control group (D). The same tumbling protocol was conducted for a period of 2 h (10 min tumbling, 10 min rest, 6 rpm) at 6°C. All meats were subjected to a 48 h salt-curing process and then placed in an air drying fermentation device (Aibo Machinery, Hangzhou, China) under $12~16^{\circ}$ C with $70~75^{\circ}$ RH and slight wind (0.1~0.2 m/s) for 48 h. Then, whole-body meats were tied with hemp twist, hung on iron wires and fermented under $12~13^{\circ}$ C with $75~80^{\circ}$ RH and slight wind (0.1~0.2 m/s) for one week. After that, all the samples were placed in an air condition room for 72 h, and then stored in vacuum packages. Three randomly-chosen rabbit products from both S and D group were taken for physicochemical, textural and volatile flavour compounds profile analyses. All analyses were performed in triplicate.

Water activity, pH, colour and nitrite determination

Water activity (a_w) value was measured with a water activity device (Hygrometer-Lufft, Fellbach, Germany). The meat sample was chopped and placed in the instrument cup and the values were recorded at the end of 10 min. All measurements were carried out at room temperature. The pH value of all samples was evaluated using an inserted pH-star electrode (Matthäus, Pöttmes, Germany). The surface colour of wire tied rabbit samples was determined in terms of L* (lightness), a* (redness), and b* (yellowness) values using a colour measurement device (Minolta, CR-300). Thiobarbituric acid reactive substances (TBARS) analysis was performed according to a procedure previously described (Coutinho de Oliveira *et al.*, 2012) and the values were expressed as mg of malondialdehyde (MDA) per kg of sample. Nitrite content was determined following the national standard method (GB 5009.33-2016, PR China). Nitrosamine contamination with dimethylnitrosamine (DMNA) and diethylnitrosamine (DENA) concentrations were assessed by Varian 3400 gas chromatograph coupled with Finnigan MAT ITD 800 spectrometer following the national standard method (GB/T 5009.26-2003, PR China). The histamine level was determined by high-performance liquid chromatography with diode-array detection according to Michalski and co-workers (Michalski *et al.*, 2021).

Texture profile analysis

Texture profile analysis was determined with a Brookfield CT-3 texture analyser (Brookfield Engineering Laboratories, Inc., USA). The working parameters of this instrument were set according to a protocol recently developed in our laboratory. Briefly, left leg muscles were cut into pieces measuring about $2.0 \text{ cm} \times 1.0 \text{ cm} \times 1.0 \text{ cm}$. The setting was as following: pre-test speed: 2.0 mm/s; test speed: 1.0 mm/s; post-test speed: 1.0 mm/s; target mode distance: 5.0 mm; trigger force: 5.0 g; trigger type: auto; data acquisition rate: 200 points per second.

Volatile flavour compounds identification

The volatile flavour compounds extraction was carried out on a headspace solid-phase microextraction (HS-SPME), followed by identification and quantification on an Agilent 7890B-5977A gas chromatography-mass spectrometry (GC-MS) system. For HS-SPME extraction, 3.0 g of each sample was placed in a 15 mL glass vial and subsequently exposed in the HS for 45 min at 75°C. After exposure, the fibre was retracted into the holder and exposed for 20 min at the injector temperature (220°C). After this procedure, the extraction was transferred to the GC-MS system. The Ultra-Inert Intuvo GC column (HP-5MS UI, 30 m×250 μ m; Agilent Technologies) was used for separation. Screening procedures were as follows: the helium carrier gas flow rate was 1.0 mL min⁻¹; injection port temperature was 220°C; column temperature was 50°C for 2 min and increased to 110°C for 2 min and then increased to 275°C for 5 min. Subsequently, the temperature was increased to 280°C to eliminate impurities from the column. The electron-impact

(EI) ionisation energy was 70 eV, the ion source temperature was 230°C. The detector voltage 350 V, and mass spectra were recorded in total ion monitoring mode (scan range 30-300 m/z).

Data processing and statistics

The data were coded and entered in Microsoft Excel 2019, and the Student's t-test depending on the type of variable was performed using the SPSS 26.0 version statistics software (IBM, Armonk, NY, USA). In GC-MS data analysis, odour activity value (OAV) is often applied to evaluate the contributions of aroma compounds. Numerically, OAV is equal to the ratio of the compound concentration to the odour threshold in water, and compounds with an OAV>1 are generally considered to greatly contribute to aroma characteristics (Wang *et al.*, 2020).

RESULTS AND DISCUSSION

Physicochemical properties of cured rabbit meat

The results of physicochemical properties determination are listed in Table 1: no significant differences are found in either the a_w or pH values between S and D samples. The TBARS value is 0.108 ± 0.004 mg/kg in these meats, compared to 0.298 ± 0.005 mg/kg in the control samples (P<0.01). Although both were well below 0.5 following the national standard method (GB2730-2015, PR China) for cured meat products, the lower value in S group indicates that the degree of lipid oxidation is low and the quality of cured rabbit meats is good. This is similar to the results from Sun *et al.* (2016). The nitrite content of products from S group was apparently lower than those from D group (P<0.01), which showed that *S. xylosus* incubation leads to low nitrite content of wire tied rabbit in a certain range. This is consistent with the previous publication studied on *S. xylosus* inoculated sausages (Essid and Hassouna, 2013). In addition, it can be seen that the nitrosamine of DENA and histamine content of samples in S and D groups changed significantly. The reason for this may be due to the fact that *S. xylosus* could compete with other bacteria containing amino acid decarboxylases and oxidases (Lee *et al.*, 2016), and which may be also involved in nitrosamines formation by reducing nitrates to nitrites and degrading proteins to amines or amino acids.

pH and TBARS values during storage

Changes in the pH and TBARS values of cured rabbit meat product during storage are shown in Table 2. The initial pH value, which was 5.87 ± 0.02 and 5.91 ± 0.02 , decreased to 5.78 ± 0.02 and 5.60 ± 0.01 at 90 d for the S group and D group, respectively. Over the course of storage, the pH values fell sharply during the 60 to 90 d of storage in meats of D group, while the values of the meats in S group decreased more slowly. The pH decrease in the cured meat products could be attributed to the carbohydrates that were metabolised by lactic acid bacteria to produce acids such as lactic acid, and the different changes in pH values between S and D group may be due to the *S. xylosus* competing

Table 1. Physicochemical parameters of cured rabbit meat.

	Grou	Groups			
Items	S	D			
Water activity (a)	0.842±0.007	0.853±0.009			
pH	5.87±0.02	5.91±0.02			
TBARS mg malondialdehyde/kg	0.108±0.004 ^A	0.298±0.005 ^B			
Nitrite content mg/kg	2.03±0.01 ^A	7.06±0.078 ^B			
DMNA content µg/kg	2.99±0.44	3.26±0.67			
DENA content µg/kg	18.70±0.87 ^A	26.10±1.57 ^B			
Histamine content mg/kg	18.90±0.98ª	25.02±1.76 ^b			

Data are expressed as least-squares means \pm standard errors (mean \pm standard error). In the same row, different lowercase letters mean significant difference (*P*<0.05); whereas different capital letters mean extremely significant difference (*P*<0.01). S, case group meat inoculated with *S. xylosus*; D, control group meat; TBARS, thiobarbituric acid reactive substances; DMNA, dimethylnitrosamine; DENA, diethylnitrosamine.

			Time of s	torage (d)	
Items	Groups	0	30	60	90
pН	S	5.87±0.02 ^B	5.86±0.01 ^B	5.83±0.01 ^B	5.78±0.02 ^{bA}
	D	5.91±0.02 ^c	5.85±0.01 ^B	5.80±0.01 ^B	5.60 ± 0.01^{aA}
TBARS	S	0.108±0.004 ^{aA}	0.102±0.006 ^{aA}	0.135±0.007 ^{aB}	0.188±0.004 ^{aC}
	D	0.298 ± 0.005^{bA}	0.312±0.004 ^{bA}	0.382±0.005 ^{bB}	0.503±0.008 ^{bC}

Table 2: Changes in pH and thiobarbituric acid reactive substances (TBARS; mg malondialdehyde/kg) values of cured rabbit meats during storage for 90 d.

All values are given in mean \pm standard error. Values with the different capital letters (A-C) in a row and lower letters (a-b) in a column within variable are statically different (*P*<0.05). S, case group meat inoculated with *S. xylosus*; D, control group meat.

with other bacteria with acid-producing activities (Nie *et al.*, 2014). In addition, the change trend of TBARS values of wire tied rabbit in two groups during storage was the same, which increased sharply from 30 to 90 d (P<0.05). The TBARS value of products from S group was significantly lower than those from D group at each storage stage (P<0.0.5) (Table 2), which showed that inoculation with *S. xylosus* can effectively control the lipid oxidation of wire tied rabbit during storage.

Colour and texture parameters of cured rabbit meat:

To evaluate the effect of a *S. xylosus* starter on the colour of products, the L*, a* and b' were determined on products from both S and D groups. As shown in Figure 2A, lightness (L* values) and redness (a* values) of meats formulated with *S. xylosus* are significantly higher (P<0.05) than those of the control group. Generally, the redness of cured meat can be improved because NO-myoglobin that has bright reddish colour can be generated by nitric oxide from nitrite and myoglobin (Kim *et al.*, 2017). In addition, *S. xylosus* contains nitric oxide synthase, which could promote the formation of nitrosylmyoglobin, and thereby induce the products to appear redder (Sun *et al.*, 2016). Regarding the lightness, meats inoculated with *S. xylosus* showed a significant increasing compared to the control (P<0.05, Figure 2A), and the result is similar to that in meat batters inoculated with *S. xylosus* (Li *et al.*, 2016). We further conducted a texture analysis to study whether the inoculation with *S. xylosus* can improve texture characteristics of meat products. Our result showed that products from S group had a significant increase in cohesiveness and chewiness when compared to those from D group (P<0.05, Figure 2B). Thus, *S. xylosus* has the potential to be used to improve the product colour, but not texture characteristics.

Volatile compounds in cured rabbit meat

The volatile flavour substances detected in S and D group were complex, with a total of 73 volatiles identified in all meat products, including 5 aldehydes, 8 alcohols, 5 esters, 2 acids, 8 ketones, 15 phenols, 12 olefins, 4 hydrocarbons and 14 other substances (Table 3). Among them, the types of volatile compounds of S and D were 64 and 56

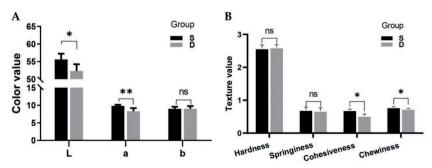


Figure 2: Effects of *S. xylosus* on cured rabbit meat colour (A) and texture (B). Note: S, case group meat inoculated with *S. xylosus*; D, control group meat.

ZHANG et al.

Chemistry					dance (µg/kg)
family	Name	CAS#	Identification	S group	D group
Aldehydes	Hexanal	66-25-1	MS, RI	22.02±10.20	15.43±8.09
	Heptanal	111-71-7	MS, RI	6.99±1.68	5.42±1.02
	3,3-dimethyl-5-oxocyclohexane-1- carbaldehyde	65080-66-2	MS, RI	19.65±0.86	ND
	marine decadienal	762-26-5	MS, RI	17.63±3.46	ND
	2-Ethyl-2-hexenal	645-62-5	MS, RI	11.99±1.06	ND
lcohols	trans-1,2-Cyclopentanediol	5057-99-8	MS, RI	13.41±5.01	ND
	Furfuryl alcohol	98-00-0	MS, RI	185.32±10.13	ND
	11-hexadecyn-1-ol	65686-49-9	MS, RI	25.65±0.97	ND
	3-Furancarbinol	4412-91-3	MS, RI	259.31±7.86	192.55±14.20
	cis 2,3-epoxycyclopentan-1-ol	16326-97-9	MS, RI	12.98±1.06	13.85±1.69
	8-hydroxylinalool	64142-78-5	MS, RI	ND	86.54±2.64
	acetol	116-09-6	MS, RI	ND	18.32±1.64
	UNII:319R5C7293	536-59-4	MS, RI	ND	61.84±3.52
sters	Sabinyl acetate	3536-54-7	MS, RI	15.33±2.72	12.44±2.02
	2-Ethylcyclohexyl 3-chloropropionate	1000282-65-7	MS, RI	83.88±10.24	90.21±6.65
	Ethyl caprylate	106-32-1	MS, RI	30.32±3.88	ND
	2,5-Octadecadiynoic acid methyl ester	57156-91-9	MS, RI	10.54±1.09	4.98±0.32
	Carbobenzoxyhydrazide	5331-43-1	MS, RI	56.32±10.55	59.62±9.00
cids	2-Picolinic acid	98-98-6	MS, RI	22.77±1.77	ND
10100	4-hydroxybutyric acid	591-81-1	MS, RI	13.65±0.42	35.11±6.92
etones	1-Bromo-3-phenyl-1-propanone	10500-29-5	MS, RI	68.22±4.15	62.10±3.77
6101162	2-methylcyclopentenone	1120-73-6	MS, RI	191.02±10.40	198.10±3.77
	3-Methyl-2-cyclopenten-1-one	2758-18-1	MS, RI	156.94±10.13	131.10±6.63
	5 5 1		MS, RI		129.65±5.46
	Methyl cyclopentenolone	80-71-7	MS, RI	111.40 ± 3.55	
	1-Indanone	83-33-0	,	20.99±2.01	27.77±1.95
	Cyclopentanone	120-92-3	MS, RI	ND	16.86±2.87
	2,3-dimethyl-2-cyclopenten-1-one	1121-05-7	MS, RI	294.54±6.86	304.14±9.76
No	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	21835-01-8	MS, RI	ND	42.55±2.06
henols	o-cresol	95-48-7	MS, RI	302.24±17.09	249.15±15.04
	p-Cresol	106-44-5	MS, RI	531.54±15.54	453.70±14.01
	Guaiacol	90-05-1	MS, RI	590.40±10.72	563.33±9.05
	2,6-Xylenol	576-26-1	MS, RI	52.24±2.86	40.02±2.34
	3,5-Dimethylphenol	108-68-9	MS, RI	87.65±3.02	67.25±8.29
	2,5-Dimethylphenol	95-87-4	MS, RI	80.03±2.50	69.66±10.44
	syringol	91-10-1	MS, RI	55.32±3.01	46.75±3.20
	2-Methoxy-5-[(E)-1-propenyl]phenol	19784-98-6	MS, RI	21.03±0.63	13.98±1.10
	4-Ethyl-2-methoxyphenol	2785-89-9	MS, RI	132.65±4.08	155.30±5.02
	Creosol	93-51-6	MS, RI	176.66±1.87	ND
	4-Ethylphenol	123-07-9	MS, RI	75.22±1.88	65.65±3.06
	Phenol,2,3,5,6-tetramethyl-phenol	527-35-5	MS, RI	22.66±1.04	23.33±1.23
	5-Tert-Butylpyrogallol	20481-17-8	MS, RI	4.55±0.46	5.99 ± 0.32
	2,3,5-Trimethylphenol	697-82-5	MS, RI	6.32±0.07	5.98±0.13
	Phenol	108-95-2	MS, RI	454.67±8.67	414.32±9.97
Olefins	Spiro[2.4]hepta-4,6-diene	765-46-8	MS, RI	37.00±1.65	ND
	1,3-dimethylcyclohexene	2808-76-6	MS, RI	25.72±0.88	18.65±0.93
	1-ISO-Propylcyclopentene	1462-07-3	MS, RI	11.55±0.76	12.33±0.44
	6-(Dimethylamine)fulvene	696-68-4	MS, RI	223.09±5.08	169.66±3.03
	m-cymene	535-77-3	MS, RI	18.92±0.76	ND
	D-Limonene	5989-27-5	MS, RI	51.90±1.10	54.12±3.00
	1,3-Hexadiene, 3-ethyl-2-methyl-, (Z)-	74752-97-9	MS, RI	42.44±1.36	44.11±2.02
	1,4-Dihydrothujopsene-(I1)	159087-74-8	MS, RI	9.38±0.33	11.55±0.76

Table 3: Contents and aroma descriptions of cured rabbit meat.

(Table 3, continued on next page)

STAPHYLOCOCCUS	<i>XYLOSUS</i> TO	IMPROVE	CURED	RABBIT	MEAT
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Chemistry				Relative abun	idance (µg/kg)
family	Name	CAS#	Identification	S group	D group
Olefins	Benzocycloheptatriene	264-09-5	MS, RI	17.23±0.63	20.64±1.42
	Caryophyllene	87-44-5	MS, RI	32.30±2.45	20.30±1.87
	Bicyclo[2.2.1]heptane, 2-chloro-1,7,7-	30462-53-4	MS, RI	ND	13.21±0.88
	trimethyl-, (1R-endo)-				
	humulene epoxide ii	19888-34-7	MS, RI	4.65±0.32	ND
Hydrocarbon	Hexane, 3-ethylhexane	619-99-8	MS, RI	5.05±0.09	ND
	Tridecane	629-50-5	MS, RI	17.32±1.03	ND
	Tetradecane	629-59-4	MS, RI	169.66±1.08	170.45±3.05
	3-(2-chloro-phenyl)-6-[2-(1-methyl-	1000154-25-5	MS, RI	33.70±0.91	ND
	pyrrolidin-2-yl)-ethoxy]-imidazo[1,2-b]				
	pyridazine				
Other	Allyl Methyl Sulfide	10152-76-8	MS, RI	5.13±0.94	ND
substances	Pyridine	110-86-1	MS, RI	23.06±1.09	28.08±2.87
	Ethylbenzene	100-41-4	MS, RI	26.77±0.87	10.55±0.68
	p-Xylene	106-42-3	MS, RI	155.87±4.33	105.32±3.44
	1-(3H-Imidazol-4-yl)-ethanone	61985-25-9	MS, RI	64.66±10.42	55.06±1.04
	9-Oxabicyclo[3.3.1]non-6-en-2-one,	63827-16-7	MS, RI	9.01±0.43	10.66±0.32
	oxime				
	4-(2,5-Dihydro-3-methoxyphenyl)	77515-67-4	MS, RI	30.45±1.59	35.55±0.56
	butylamine				
	trans-Z-a-epoxybisasurram	1000131-71-1	MS, RI	7.45±0.65	ND
	2-ethyl-5-methyl furan	1703-52-2	MS, RI	40.87±1.07	33.65±1.23-
	2-Methoxy-5-methylphenol	1195-09-1	MS, RI	199.54±0.15	344.76±1.98
	2,6-Dimethoxytoluene	5673-07-4	MS, RI	36.72±1.87	39.66±0.18
	Oxime-, methoxy-phenyl-	1000222-86-6	MS, RI	ND	28.68±1.55
	1H-Indene, octahydro-; Indan,hexahydro- (6Cl,8Cl); Bi	496-10-6	MS, RI	ND	77.34±0.44
	Phenol, 3,5-dimethyl-	4179-19-5	MS, RI	ND	4.54±0.13

(Table 3, continued from previous page)

Note: All values are given in mean±standard error. ND, not detected; MS, identified by mass spectrometry; RI, identified by retention index. S, case group meat inoculated with *S. xylosus*; D, control group meat.

(Figure 3A), suggesting that the addition of *S. xylosus* to meat affected the production of volatile compounds. These compounds were primarily generated from lipid oxidation, amino acid catabolism, carbohydrate fermentation and microbial activities (Xiao *et al.*, 2020). Among them, phenols, olefins and ketones accounted for a higher proportion of all compounds (Figure 3B). In particular, a higher abundance of these compounds was found in the products from

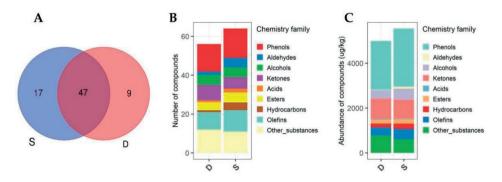


Figure 3: Analysis of volatile flavour substances. (A) Venn diagram of identified volatile compounds; (B) Composition of volatile compounds; (C) Abundance of volatile compounds. Note: S, case group meat inoculated with *S. xylosus*; D, control group meat.

ZHANG et al.

			OAV	OAV value		
NO	Name	Threshold/ (µg/kg)	S group	D group		
1	Furfuryl alcohol	1 000	0.1853	ND		
2	acetol	200	ND	0.0916		
3	UNII:319R5C7293	7 000	ND	0.0088		
4	Hexanal	7.5	2.9361	2.0584		
5	Heptanal	10	0.6992	0.5421		
6	2-Picolinic acid	620 000	3.6729	ND		
7	4-hydroxybutyric acid	>10400000	NF	NF		
8	Ethyl caprylate	2	15.1619	ND		
9	2-methylcyclopentenone	26	7.3470	7.6193		
10	Cyclopentanone	840 000	ND	2.0075		
11	3-Ethyl-2-hydroxy-2-	52	ND	0.8183		
	cyclopenten-1-one					
12	o-cresol	45	6.7165	5.5367		
13	p-Cresol	2	265.7708	226.8507		
14	Guaiacol	0.17	3472.9475	3313.7591		
15	2,6-Xylenol	400	0.1306	0.1001		
16	3,5-Dimethylphenol	5000	0.0175	0.0134		
17	2,5-Dimethylphenol	500	0.1601	0.1393		
18	syringol	400	0.1383	0.1169		
19	4-Ethyl-2-methoxyphenol	25	5.3062	6.2122		
20	4-Ethylphenol	130	0.5786	0.5050		
21	2,3,5-Trimethylphenol	2 300	0.0027	0.0026		
22	Creosol	10	17.6662	ND		
23	Phenol	55 000	0.0083	0.0075		
24	Pyridine	2000	0.0115	0.0140		
25	Ethylbenzene	16	1.6732	0.6594		
26	p-Xylene	8700	0.0179	0.0121		
27	m-cymene	800	0.0237	ND		
28	D-Limonene	34	1.5265	1.5919		
29	Tetradecane	1 000	0.1697	0.1705		
30	Caryophyllene	64	0.5047	0.3172		
31	Allyl Methyl Sulfide	0.5	10.2619	ND		

Table 4: Odour activity value (OAV) of volatile compounds of cured rabbit meat.

Note: ND, not detected; NF, OVA cannot be calculated. S, case group meat inoculated with S. xylosus; D, control group meat.

S group than those from D group (Figure 3C). Throughout cured meat fermentation, lipid hydrolysis and oxidation are important process contributing to flavour development. Moreover, volatile compounds such as aldehydes, ketones, alcohols and esters are generated from free fatty acids autoxidation (Chen *et al.*, 2017). Additionally, lipolytic activity of *S. xylosus* has been detected in fermented meat products (Flores and Toldra 2011, Chen *et al.*, 2017). Thus, our results showed that inoculum of *S. xylosus* with the ability to regulate lipid oxidation is an effective method of improving the flavour of cured rabbit meat.

Aroma contribution to cured rabbit meat:

Since OAV can reflect the contribution of each volatile compound to the product's characteristic flavour, we calculated the OAVs of 31 compounds by dividing the concentrations found in this study by the reported odour threshold from the literature (Table 4). On the basis of the results obtained, 12 with OAV>1 in S group and 8 in D group were likely the most important odour compounds in wire tied rabbit. Furthermore, guaiacol displayed the biggest OAV (3472.9475), followed by p-Cresol (OAV=265.7708), creosol (OAV=17.6662), ethyl caprylate (15.1619), allyl methyl sulfide (OAV=10.2619), 2-methylcyclopentenone (OAV=7.3470), o-cresol (OAV=6.7165), 4-Ethyl-2-methoxyphenol (OAV=5.3062), 2-Picolinic acid (OAV=3.7629), hexanal (OAV=2.9361), ethylbenzene (OAV=1.6732) and D-Limonene

(1.5265) in the S group. Guaiacol has sweet and smoky aroma notes, and was one of the key flavours accounting for bacon aroma (Xie *et al.*, 2008). Likewise, p-Cresol is a potential off-flavour compound characterised by faecal notes, which was commonly produced in fermented food, such as wine (Ji *et al.*, 2020) and ham (Liu *et al.*, 2014). These compounds can be proposed as the most odour-active compounds, as well those that enhance the complex flavour of rabbit meat.

CONCLUSIONS

The role of commercial starter culture of *S. xylosus* in the product quality of cured rabbit meat product (Chan Si Tu) was determined in our study. Our results showed that the inoculum of meat with *S. xylosus* promoted an increase in lightness, redness and cohesiveness. Moreover, the starter culture led to low nitrite content, nitrosamine content and histamine content, and also controlled the lipid oxidation of products during storage. In addition, the level of volatile compounds was higher in the incubation samples. Therefore, the use of *S. xylosus* in cured rabbit meat can promote product quality and safety, while also improving flavour development.

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REFERENCES

- Bai T., Li X., Hou B., Chen L., Wang W. 2018. Effects of complex microbial strains on volatile flavor components of Airdried rabbits. (In Chinese). *Chin. Condiment, 43: 38-43.* https://doi.org/10.3969/j.issn.1000-9973.2018.11.007
- Barbuti S., Parolari G. 2002. Validation of manufacturing process to control pathogenic bacteria in typical dry fermented products. *Meat Sci.*, 62: 323-329. https://doi.org/10.1016/ S0309-1740(02)00124-9
- Cebrián E., Núñez F., Gálvez F.J., Delgado J., Bermúdez E., Rodríguez M. 2020. Selection and evaluation of *Staphylococcus xylosus* as a biocontrol agent against toxigenic moulds in a dry-cured ham model system. *Microorganisms, 8:* 793. https://doi.org/10.3390/microorganisms8060793
- Chen Q., Kong B., Han Q., Xia X., Xu L. 2017. The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development. *LWT-Food Sci. Technol.*, 77: 389-396. https://doi.org/10.1016/j.lwt.2016.11.075
- Coutinho de Oliveira T.L., Malfitano de Carvalho S., de Araújo Soares R., Andrade M.A., Cardoso M. das G., Ramos E.M., Piccoli R.H.. 2012. Antioxidant effects of *Satureja montana* L. essential oil on TBARS and color of mortadella-type sausages formulated with different levels of sodium nitrite. *LWT-Food Sci. Technol.* 45: 204-212. https://doi.org/10.1016/j. *lvt.2011.09.006*
- Cruxen C.E. dos S., Funck G.D., Dannenberg G. da S., Haubert L., Marques J. de L., Kroning I.S., Chaves F.C., da Silva W.P., Fiorentini Â.M. 2017. Characterization of *Staphylococcus xylosus* LQ3 and its application in dried cured sausage. LWT-Food Sci. Technol., 86: 538-543. https://doi.org/10.1016/j.lwt.2017.08.045

- Cruxen C.E. dos S., Funck G.D., Haubert L., Dannenberg G. da S., Marques J. de L., Chaves F.C., da Silva W.P., Fiorentini Â.M. 2019. Selection of native bacterial starter culture in the production of fermented meat sausages: Application potential, safety aspects, and emerging technologies. *Food Res. Int.*, 122: 371-382. https://doi.org/10.1016/j.foodres.2019.04.018
- Essid I., Hassouna M. 2013. Effect of inoculation of selected Staphylococcus xylosus and Lactobacillus plantarum strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. Food Control, 32: 707-714. https://doi.org/10.1016/j.foodcont.2013.02.003
- Flores M., Toldra F. 2011. Microbial enzymatic activities for improved fermented meats. *Trends Food Sci. Technol. 22(2-3): 81-90. https://doi.org/10.1016/j.tifs.2010.09.007*
- Ji L., Wang W., Zhang J., Bai T., Wang Z., Li J. 2016. Study on effects of automatic drying and use of fermentation agents on the physiochemical and microbial properties of wrapped rabbit meat. *Science and Technology of Food Industry* 38(2): 200-204. https://doi.org/10.13386/j.issn1002-0306.2017.02.030
- Ji M., Du H., Xu Y. 2020. Structural and metabolic performance of p-cresol producing microbiota in different carbon sources. *Food Res. Int.* 132: 109049. https://doi.org/10.1016/j. foodres.2020.109049
- Kim T.K., Kim Y.B., Jeon K.H., Park J.D., Sung J.M., Choi H.W., Hwang K.E., Choi Y.S. 2017. Effect of fermented spinach as sources of pre-converted nitrite on color development of cured pork loin. *Korean J. Food Sci. Anim. Resour.*, 37: 105-113. https://doi.org/10.5851/kosta.2017.37.1.05

- Lee Y.C., Kung H.F., Huang C.Y., Huang T.C., Tsai Y.H. 2016. Reduction of histamine and biogenic amines during salted fish fermentation by *Bacillus polymyxa* as a starter culture. J. Food Drug Anal., 24: 157-163. https://doi.org/10.1016/j.jfda.2015.02.002
- Li P., Luo H., Kong B., Liu Q., Chen C. 2016. Formation of red myoglobin derivatives and inhibition of spoilage bacteria in raw meat batters by lactic acid bacteria and *Staphylococcus xylosus*. LWT-Food Sci. Technol., 68: 251-257. https://doi.org/10.1016/j.lwt.2015.12.035
- Li S., Zeng W., Li R., Hoffman L.C., He Z., Sun Q., Li H. 2018. Rabbit meat production and processing in China. *Meat Sci.*, 145: 320-328. https://doi.org/10.1016/j. meatsci.2018.06.037
- Liu X.S., Liu J.B., Yang Z.M., Song H.L., Liu Y., Zou T.T. 2014. Aroma-active compounds in jinhua ham produced with different fermentation periods. *Molecules*, 19: 19097-19113. https://doi.org/10.3390/molecules191119097
- Michalski M., Pawul-Gruba M., Madejska A. 2021. Histamine contents in raw long-ripening meat products commercially available in Poland. J. Vet. Res., 65: 477-481. https://doi.org/10.2478/jvetres-2021-0062
- Nie X., Zhang Q., Lin S. 2014. Biogenic amine accumulation in silver carp sausage inoculated with *Lactobacillus plantarum* plus *Saccharomyces cerevisiae*. Food Chem., 153: 432-436. https://doi.org/10.1016/j.foodchem.2013.12.093
- Sun Q., Chen Q., Li F., Zheng D., Kong B. 2016. Biogenic amine inhibition and quality protection of Harbin dry sausages by inoculation with Staphylococcus xylosus and Lactobacillus plantarum. Food Control, 68: 358-366. https://doi.org/10.1016/j.foodcont.2016.04.021

- Szendrő K., Szabó-Szentgróti E., Szigeti O. 2020. Consumers' attitude to consumption of rabbit meat in eight countries depending on the production method and its purchase form. *Foods*, 9: 654. https://doi.org/10.3390/foods9050654
- Wang M.Q., Ma W.J., Shi J., Zhu Y., Lin Z., Lv H.P. 2020. Characterization of the key aroma compounds in Longjing tea using stir bar sorptive extraction (SBSE) combined with gas chromatography-mass spectrometry (GC–MS), gas chromatography-olfactometry (GC-0), odor activity value (OAV), and aroma recombination. Food Res. Int., 130: 108908. https://doi.org/10.1016/j.foodres.2019.108908
- Xiao Y., Liu Y., Chen C., Xie T., Li P. 2020. Effect of Lactobacillus plantarum and Staphylococcus xylosus on flavour development and bacterial communities in Chinese dry fermented sausages. Food Res. Int., 135: 109247. https://doi.org/10.1016/j.foodres.2020.109247
- Xie J.C., Sun B.G., Wang S.B. 2008. Aromatic constituents from Chinese traditional smoke-cured bacon of Mini-pig. Food Sci. Technol. Int., 14: 329-340. https://doi.org/10.1177/1082013208098331
- Zhou G., Luo X., Xu X., Zhao G., Liu D., Ma H., Huan Y., Sun J., Li C., Huang M., Chen T. 2008. The classification of Chinese meat products. (In Chinese). *Meat Res.*, 10: 3-5. https://doi.org/10.3969/j.issn.1001-8123.2008.10.004
- Zeng W., Wen W., Deng Y., Tian Y., Sun H., Sun Q. 2016. Chinese ethnic meat products: Continuity and development. *Meat Sci.*, 120: 37-46. https://doi.org/10.1016/j.meatsci.2016.04.007