Proteolytic effect of starter culture during ripening of smoked horse sausage

Lu Shiling¹ · Han Xianna¹ · Yang yanbin¹ · Li Baokun¹ · Xu Chengjian¹ · Wang Qingling¹

Received: 18 October 2016/Revised: 31 March 2017/Accepted: 13 June 2017/Published online: 10 October 2017
© The Korean Society of Food Science and Technology and Springer Science+Business Media B.V. 2017

Abstract In this study, we assessed the effect of bacterial and endogenous enzymes on the proteolysis of smoked horse sausage. Commercial starter culture (*Staphylococcus xylosus* + *Lactobacillus sakei*) was used in smoked horse sausage. Cathepsin B + L and cathepsin B activities, microbiological growth, pH, and water activity (a_w) were measured. Based on PCR-DGGE fingerprint analyses, the starter culture inhibited endogenous bacterial growth. During ripening, the residual activity of cathepsin B + L and cathepsin B was higher in batch C (control) than in batch S (containing starter cultures). The starter and endogenous enzymes promote the degradation of sarcoplasmic and myofibrillar proteins; however, the degradation of these proteins was higher in batch S than in batch C. Therefore, bacterial enzymes played a major role in the degradation of proteins during the ripening of smoked horse sausage.

Keywords Smoked horse sausage · Starter culture · Proteolysis · Cathepsins

Introduction

Horsemeat has a low content of fat and cholesterol and a high content of heme-iron [1]. Smoked horse sausage is traditionally consumed in China, especially in Xinjiang. Dry, ripe sausage contains horsemeat, salt and spices. During fermentation, several biochemical reactions take

place, which ultimately contribute to the characteristic flavors and texture of the product. These biochemical reactions include lipolysis, proteolysis, and synthesis of carbonyls and volatile flavor compounds [2, 3].

In fermented sausage, proteolysis is affected by the product formulation, ripening conditions and starter culture [4]. Initially, meat proteins are hydrolyzed by endogenous enzymes (cathepsins and calpains) into polypeptides, which are subsequently hydrolyzed by peptidases into peptides. The smaller peptides are hydrolyzed into amino acids by starter and endogenous enzymes [4, 5].

Small peptides and free amino acids are the main components of the non-protein nitrogen (NPN) fraction of fermented meats. Certain amino acids are directly or indirectly converted into volatile and nonvolatile flavor compounds in dry and semi-dry sausages [6].

Amino acid degradation yields high levels of flavor compounds. However, studies have reported that high levels of free amino acids does not increase aroma compounds level. In fermented foods, aroma compounds are produced by transamination and deamination reactions catalyzed by endogenous enzymes and microbial enzymes from lactic acid bacteria (LAB), fungi, and staphylococci [7].

Commonly used starter cultures include *Lactobacillus sakei*, *Pediococcus acidilactici*, *L. curvatus*, and *Staphylococcus xylosus*. These bacteria produce an enzyme with potential proteolytic roles and improve sensory properties of fermented sausage [8]. However, the presence of endogenous bacteria in raw meat, e.g., lactic acid bacteria and *Staphylococcus*, make it difficult to assess the proteolytic effect of starter cultures by conventional microbiological methods.

During fermentation, the microbial diversity of smoked sausage can be determined by polymerase chain reaction-

✉ Wang Qingling
qingling1100@163.com

¹ College of Food Science, Shihezi University,
Shihezi 832003, People's Republic of China

denaturing gradient gel electrophoresis (PCR-DGGE). Additionally, the microorganisms can be identified through the amplification of distinctive genes. Nevertheless, few studies have evaluated the effect of microbial fermentation on endogenous enzymes and proteolysis. In this study, we determined the effect of starter and endogenous enzymes on the proteolysis of smoked horse sausage.

Materials and methods

Sausage production and sampling

Two batches of smoked horse sausages were produced, a control batch (C, devoid of starter culture) and a batch with starter culture (S). The sausages contained horse meat (80%), horse fat (20%), salt (2.5%), sugar (2%), sodium nitrate (0.02%), pepper powder (0.1%), Chinese red pepper (0.15%), ginger powder (0.2%), aniseed (0.1%), and monosodium glutamate (0.1%). The production of smoked horse sausages was performed as reported by Lu et al. [21].

Water activity and pH measurements

The samples (20 g; three replicates) were homogenized in 180 mL distilled water (pH 7.00). The pH value of the homogenate was measured using a Hanna HI9025c Microprocessor pH meter (Detroit, MI, USA). Water activity (a_w) was determined with a HYGROLAB water activity meter (CH-8303, Rotronic, Bassersdorf, Switzerland).

Nonprotein nitrogen (NPN) concentration

Nonprotein nitrogen (NPN) concentration was measured by the method reported by Zhao [9], with slight modifications. Sausage (4 g) was homogenized with 20 mL of 0.05 M citric acid buffer (pH 6.0) X at 10,000 rpm for 2 min (Ultra Turrax T25), allowed to equilibrate for 2 h at 4 °C, and centrifuged at 10,000g for 20 min. The resulting supernatant was passed through a Whatman filter. The filtrate was incubated overnight with 20 mL of 10% TCA at 4 °C and centrifuged at 2500g for 10 min. The supernatant was used for the determination of NPN.

DNA extraction

Total bacterial DNA was extracted from duplicate 10-g samples collected at each sampling time. The samples were homogenized at room temperature in a stomacher tube containing 90 mL of saline peptone water for 30 min. The homogenates were subsequently centrifuged at 2000g for 10 min at 4 °C. The resulting supernatant was transferred

into two sterile centrifuge tubes and centrifuged at 10,000g for 20 min at 4 °C. The pellet was transferred into a sterile 2.0-mL tube and re-suspended in 200 µL GA buffer containing TIANamp Bacteria DNA Kit reagents (Tiangen Biotech, Beijing, China) and 10 µL lysozyme (20 mg/mL). Bacterial DNA was extracted following the Protocol for Purification of Genomic DNA from total bacteria [10]. Finally, DNA was eluted with TE buffer (100 µL; TIANGEN) and stored at −20 °C.

PCR-DGGE analysis

The primers U968-GC (5' CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC), which contained a GC clamp, and L1401 (5' GCG TGT GTA CAA GAC CC) were used for the amplification of the V6–V8 regions of the bacterial 16S rRNA gene [11]. The PCR reaction system (25 µL) consisted of 12.5 µL Go Taq Green Master Mix, 1 µL of U968-GC and L1401, 9.5 µL ddH₂O, and 1 µL DNA. The PCR conditions consisted of one cycle at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and one cycle at 72 °C for 10 min. The PCR products were analyzed by 1.2% agarose gel electrophoresis.

DGGE was performed according to the method by Hu et al. [12], with a slight modification. Electrophoresis was performed at 200 V for 10 min and at 85 V for 16 h at 60 °C on a 8% polyacrylamide gel (acrylamide: bisacrylamide 37.5:1), which contained a denaturing gradient (40 to 60% urea-formamide). The DGGE gel was stained with SYBR Green for 30 min and rinsed with Milli-Q water.

Sequencing of DGGE bands

Fragments of the DGGE bands were excised using a sterile scalpel, and the DNA from each band was eluted overnight in 20 µL of TE at 4 °C. An aliquot of DNA (2 µL) was re-amplified by PCR with the primer a lack of the GC clamp, purified, and sequenced by Sanbo Company (Beijing, China). The sequences (approximately 420 bp) were identified using advanced BLAST. BLAST searches from GenBank were used to determine the closest relatives of the partial 16S rRNA sequences obtained [13]. Sequences with 96% or higher similarity were considered to be from the same species.

Enzymatic activities

Lysosomal enzyme extracts were obtained by homogenizing 5 g of sample devoid of fat and connective tissue with four volumes of 50 mM sodium acetate buffer (pH 6.0) containing 1 mM EDTA, 100 mM NaCl, and 0.2% (v/v)

Triton X-100 at 4 °C. The extract was stirred at 4 °C for 1 h, centrifuged at 10,000g for 30 min, and passed through glass wool, previously deionized with ultrapure water [14, 15]. The supernatant, containing the enzymes, was collected.

The enzymatic activities of cathepsin B + L and cathepsin B were measured with fluorescent-binding peptides as substrates as reported by Zhao et al. [13], Li et al. [16.] and Buckow et al. [17], with a slight modification. Cathepsins B and L were assayed in the presence of their common substrate, N-CBZ-L-phenylalanyl-L-arginine-7-amino-4-methylcouarin (Z-PHE-ARG-AMC; Sigma), and cathepsin B was assayed with N-CBZ-L-arginyl-L-arginine-7-amino-4-methylcouarin (Z-ARG-ARG-AMC; Sigma) [14, 18]. The release of 7-amino-4-methylcoumarin (AMC; Sigma) was monitored using a fluorescent spectrophotometer at 380 nm (excitation) and 460 nm (emission). Results were expressed as units (U) per gram of muscle (U = nmol AMC released at 40 °C/min).

Preparation of sarcoplasmic and myofibrillar proteins

Sausage (4 g) was homogenized (4000g at 4 °C) with 40 mL of 0.03 M potassium phosphate buffer (pH 6.5, 1:10 dilution). Following centrifugation at 10000 g for 20 min at 4 °C, the resulting supernatant was passed through a 0.45- μ m filter membrane. The filtrate contained the sarcoplasmic protein fraction.

Myofibrillar proteins were extracted by the method reported by Han [19]. Sausage (5 g) was homogenized with four volumes of titanic buffer (100 mM Tris and 10 mM EDTA, pH 8.3) and centrifuged at 2500g for 20 min. The resulting pellets were mixed with four volumes of standard salt solution (SSS; 100 mM KCl, 20 mM K_2HPO_4/KH_2PO_4 , 2 mM $MgCl_2$, 1 mM EGTA, and 1 mM NaN_3 , pH 7.0) and centrifuged at 2000g for 10 min. This procedure was performed three times. The resulting pellets were dispersed in four volumes of SSS and centrifuged at 4000g for 10 min. The pellets (two replicates were performed) were dispersed in four volumes of 0.1 mM KCl and centrifuged at 4000g for 10 min. Finally, the pellets (two replicates were performed) were dispersed in four volumes of 0.1 mM NaCl and centrifuged at 4000g for 10 min. The resulting pellets contained the myofibrillar protein fraction.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Changes in sarcoplasmic and myofibrillar proteins during sausage ripening were analyzed by SDS-PAGE. The extracted protein was diluted 4:1 with SDS-PAGE sample

buffer and heated at 100 °C for 5 min prior to electrophoresis. SDS-PAGE was performed in a vertical gel electrophoresis system (DYCZ-24DN), as reported by Casaburi et al. [2]. A 12% separating gel and a 5% stacking gel were used for sarcoplasmic and myofibrillar proteins. Sample (5 μ L) was loaded into each well, and electrophoresis was performed at 80–100 V. The gel was stained for 30 min with Coomassie Brilliant Blue R-250 (1 g/L) containing 40% (v/v) methanol and 10% (v/v) acetic acid and destained with 10% (v/v) methanol and 10% (v/v) acetic acid. The molecular weights (MWs) of the proteins were calculated. The standard proteins (PM Mid-Range MMWprotein Marker, CWBIO) for sarcoplasmic protein comparisons included lysozyme (14.4 kDa), trypsin inhibitor (20.0 kDa), triose phosphate isomerase (27.0 kDa), lactate dehydrogenase (35 kDa), actin (45 kDa), bovine serum albumin (66 kDa), and heat shock protein (90 kDa). The standard proteins (Premixed Protein Marker, Broad, TaKaRa) for myofibrillar protein comparisons included aprotinin (6.5 kDa), lysozyme (14.3 kDa), trypsin inhibitor (20.1 kDa), carbonic anhydrase (29 kDa), ovalbumin (44.3 kDa), BDA (66.4 kDa), b-phosphatase (97.2 kDa), and myosin (116 kDa).

Statistical methods

The data were analyzed using Statistical Analysis System software (SAS 9.2). Analysis of variance (ANOVA) was used to assess any significant differences ($P < 0.05$).

Results and discussion

pH

Batch C sausages were devoid of starter culture (control), and batch S sausages contained starter culture. Prior to fermentation, the pH of both batches was approximately 5.76. The pH of batch S decreased after 2 days and was 4.67, 4.57, and 4.5 on days 2, 7, and 21, respectively. Compared to batch S, batch C had a slower pH decline. The pH of batch C was 5.68, 5.49, and 5.4 on days 2, 7, and 21, respectively. The starter culture had a significant acidification effect. During the initial stages of fermentation, the rapid growth of LAB in S, which decreased pH, prevented the growth of undesirable bacteria, such as *enterobacteriaceae* as previously reported [4, 20]. From day 21, a slight increase in pH was observed in batches C and S probably due to the production of ammonia and biogenic amines as a result of enzymatic activity [21].

A_w

A_w decreased in all samples and was 0.91 and 0.73 at 0 and 28 days, respectively. Throughout the ripening period, there were no significant differences in a_w values between batches C and S ($P > 0.05$), consistent with the findings of Ikonic [22].

NPN

The changes in NPN are presented in Fig. 1. NPN concentration increased in both batches throughout the ripening period and was higher in batch S than in batch C ($P < 0.05$). Similar results have been reported by Ikonic et al. [23] and Flores et al. [24]. However, Leroy et al. [25] reported no significant effects of starter culture on NPN concentration in fermented sausage. The discrepancy in the results is probably due to the processing technique, starter culture, and raw meat used.

DGGE analysis

The V6–V8 region of the bacterial 16S rRNA gene was amplified using primers U968-GC and L1401 (420 bp), which were designed to amplify the 16S rRNA gene fragments (~420 bp). DGGE was performed in 8% polyacrylamide gels containing 37.5:1 acrylamide–bisacrylamide and with a 40–60% denaturing gradient for the separation of PCR products. The results are shown in Fig. 2.

The genes of the individual bands were re-amplified and identified. The results are presented in Table 1. The sequences exhibited more than 98% similarity with sequences obtained from the GenBank database. It was challenging to assess the microbial ecosystem using

Fig. 1 Changes in NPN concentration during the ripening of smoked horse sausage

Fig. 2 DGGE profiles of the bacterial DNA extracted from control (C) and starter culture-inoculated (S) samples at 0, 2, 7, 11, 21, and 28 days of ripening. Lines C0–C28 represent C. Lines S0–S28 represent S

conventional methods. The results of PCR-DGGE analysis revealed interactions between starter cultures and endogenous bacteria.

In addition to the starter culture (bands 5, 6, 12, and 13), there were endogenous microorganisms such as uncultured *Staphylococcus* sp., *S. saprophyticus*, *S. saprophyticus* subsp. *bovis*, *S. saprophyticus*, *Weissella hellenica*, *L. sakei* subsp. *carneus*, *P. pentosaceus* and *L. sakei* subsp. *sakei* (bands 1, 2, 3, 4, 7, 8, 9, 10, 11, 13, and 14, respectively; Fig. 2). *Staphylococcus* sp., *S. saprophyticus*, and *S. saprophyticus* subsp. *Bovis* were inhibited, and *S. saprophyticus* and *P. pentosaceus* were the most predominant bacteria during fermentation. In this study, batch S underwent a rapid acidification process; antimicrobials such as bacteriocins were probably produced by the starter culture [25, 26]. In batch S, bands 7, 8, 9, and 11 were not detected on day 0 because the detection threshold of different template DNAs in a mixture by PCR-DGGE was approximately 1% of the total DNA [27]. *W. hellenica*, *Enterococcus faecalis*, *L. sakei* subsp. *sakei*, and *L. sakei*

Table 1 Strains and DGGE bands identified by means of 16S rRNA gene sequencing

Bands ^a	Closest relative	ID (%)	Accession no. ^b
1	<i>Uncultured Staphylococcus</i> sp.	98	KC331209
2	<i>Staphylococcus saprophyticus</i>	99	KC331210
3	<i>Staphylococcus saprophyticus</i> subsp. <i>bovis</i>	99	KC331211
4	<i>Staphylococcus saprophyticus</i>	100	KC331212
5,6	<i>Staphylococcus xylosus</i>	99	KC331213
7	<i>Weissella hellenica</i>	99	KC331214
8,9	<i>Lactobacillus sakei</i> subsp. <i>carneus</i>	99	KC331215
10	<i>Pediococcus pentosaceus</i>	99	KC331216
11	<i>Lactobacillus sakei</i> subsp. <i>sakei</i>	94	KC331217
12	<i>Lactobacillus sakei</i>	99	KC331218
13	<i>Lactobacillus sakei</i>	99	KC331219
14	<i>Enterococcus faecalis</i>	99	GU385496

ID represents the similarity with sequences in the GenBank databases

^aBands on the DGGE gels are shown in Figs. 1 and 2

^bAccession number of the sequence of the closest relative obtained by BLAST searches

subsp. *carneus* were inhibited in batch S during ripening. Starter bacteria, which were the most predominant, inhibited the growth of endogenous bacteria in batch S.

Enzymatic activity analysis

The role of cathepsin is crucial during the ripening of fermented meat products. The activity of cathepsin is affected by temperature, NaCl concentration, and pH value [14]. The changes in cathepsin B + L and cathepsin B are presented in Fig. 3(A) and (B). Initially, cathepsin B + L activity in batch S was higher, while residual cathepsin B + L activity in batch C was slightly higher than in batch S. At 7 day, cathepsin B + L activity was 83 and 76% in batch C and batch S, respectively (i.e., 3.12 U/g in batch C and 2.85 U/g in batch S). At 28 days, the residual enzymatic activity was 2.35 and 1.41 U/g in batch C and S, respectively. Therefore, the residual enzymatic activity was 62% in batch C and 38% in batch S on day 28 relative to that of day 0.

The trend of cathepsin B activity was similar to that of cathepsin B + L. Cathepsin B activity was higher in batch C than in batch S during ripening (Fig. 3B). The residual enzymatic activity was 84% in C and 20% in S on day 28 compared to that of day 0, consistent with the findings of Larrea et al. [28]. The activities of cathepsin B and L were significantly affected by pH value. During ripening, pH was 5.4–5.76 and 4.5–4.67 in batch C and S, respectively. The optimum pH of cathepsin B is approximately 5.5 [29].

Fig. 3 Evolution of (A) cathepsin B + L and (B) cathepsin B during ripening of smoked horse sausage. Results are expressed as nmol AMC/min/g meat

Fig. 4 SDS-PAGE electrophoretograms of sarcoplasmic proteins throughout the ripening of smoked horse sausage. Lanes St, molecular weight standard ranging from 14.4 to 90 kDa. (C) Control and (S) and starter culture-inoculated samples. Lanes 0–28, sarcoplasmic proteins after 0, 2, 7, 11, 21, and 28 days of ripening

Fig. 5 SDS-PAGE electrophoretograms of myofibrillar proteins throughout the ripening of smoked horse sausages. Lanes St, molecular weight standard ranging from 6.5 to 200 kDa. (C) Control and (S) and starter culture-inoculated samples. Lanes 0–28, sarcoplasmic proteins after 0, 2, 7, 11, 21, and 28 days of ripening

Proteolysis of sarcoplasmic proteins

The SDS-PAGE of sarcoplasmic proteins in batches C and S is shown in Fig. 4. Significant changes in sarcoplasmic proteins occurred in the starter culture sausages. Protein bands corresponding to 90, 66, 35, 27, and 14.4 kDa decreased between days 0 and 2. The 90- and 66-kDa bands were completely degraded in S between days 0 and 2. The intensity of the 45-kDa band decreased between days 0 and 2. New bands emerged from the 45-kDa band due to the hydrolysis of myofibrillar proteins [8].

The hydrolysis of sarcoplasmic proteins was affected by the presence of bacterial and endogenous enzymes. Compared to endogenous enzymes, starter culture enzymes were more important in proteolysis during the ripening of smoked horsemeat sausages [4, 30].

Proteolysis of myofibrillar proteins

The SDS-PAGE of myofibrillar proteins are shown in Fig. 5. In batch S, the intensity of the bands decreased gradually during ripening and 97- and 200-kDa accumulated.

The main difference was observed in the 20.1 kDa band. The intensity of this band gradually decreased and disappeared at the end of ripening. Additionally, the 29-kDa band decreased during ripening. As reported by Díaz et al. [30] and Toldrá [8], during processing of dry fermented sausages, several biochemical reactions are catalyzed by muscle endopeptidases (calpains and cathepsins B, D, H, and L). The changes of sarcoplasmic and myofibrillar proteins detected by SDS-PAGE reveals that bacterial and endogenous enzymes contributed to the degradation of these proteins. However, bacterial enzymes played a major role in the degradation of the proteins, confirmed by the SDS-PAGE profiles.

Acknowledgements This work was supported by grants from the National Natural Science Foundation of China (Grant Nos. 31360392 and 2014-2017). We thank the staff of the Animal Product Lab, College of Food, Shihezi University, for their technical assistance.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Lorenzo JM, Pateiro M. Influence of type of muscles on nutritional value of foal meat. *Meat Sci.* 93:630–638 (2013)
- Casaburi A, Aristoy MC, Cavella S, Di Monaco R, Ercolini D, Toldra F, Villani F. Biochemical and sensory characteristics of traditional fermented sausages of vallo di diano (Southern Italy) as affected by use of starter cultures. *Meat Sci.* 76: 295–307 (2007)
- Kargozari M, Moini S, Akhondzadeh Basti A, Emam-Djomeh Z, Gandomi H, Revilla Martin I, Ghasemlou M, Carbonell-Barachina ÁA. Effect of autochthonous starter cultures isolated from Siahmazgi cheese on physicochemical, microbiological and volatile compound profiles and sensorial attributes of sucuk, a Turkish dry-fermented sausage. *Meat Sci.* 97(1): 104–114 (2014)
- Hughes MC, Kerry JP, Arendt EK, Kenneally PM, McSweeney PLH, O'Neill EE. Characterization of proteolysis during the ripening of semi-dry fermented sausages. *Meat Sci.* 62(2): 205–216 (2002)
- Casaburi A, Di Monaco R, Cavella S, Toldrá F, Ercolini D, Villani F. Proteolytic and lipolytic starter cultures and their effect on traditional fermented sausages ripening and sensory traits. *Food Microbiol.* 25(2): 335–347 (2008)
- Candogan K, Wardlaw FB, Acton James C. Effect of starter culture on proteolytic changes during processing of fermented beef sausages. *Food Chem.* 116: 731–737 (2009)
- Bruna JM, Fernández M, Hierro EM, Ordóñez JA, de la Hoz L. Combined use of pronase E and a fungal extract (*Penicillium aurantiogriseum*) to potentiate the sensory characteristics of dry fermented sausages. *Meat Sci.* 54(2): 135–145 (2000)

8. Toldrá F, Rico E, Flores J. Cathepsins B, D, H and L activities in the processing of dry-cured ham. *J. Sci. Food Agr.* 62(2): 157–161 (2006)
9. Zhao GM. Studies on the effects of muscle proteolytic enzymes in the processing of Jinhua ham. PhD thesis, Nanjing Agricultural University, Nanjing, China (2004)
10. Zhou XQ, Wang YF, Cai Y, Huang XZ, Hao YB, Tian JQ, Chai TY. PCR-DGGE detection of the bacterial community structure in the Inner Mongolia steppe with two different DNA extraction methods. *Acta Ecologica Sinica*, 27(5): 1684–1689 (2007)
11. Zhu WY, Williams BA, Konstantinov SR, Tamminga S, De Vos, WM, Akkermans ADL. Analysis of 16 s rDNA reveals bacterial shift during in vitro fermentation of fermentable carbohydrate using piglet faeces as inoculum. *Anaerobe*. 9(4): 175–180 (2003)
12. Hu P, Zhou G, Xu X, Li C, Han Y. Characterization of the predominant spoilage bacteria in sliced vacuum-packed cooked ham based on 16 s rDNA-DGGE. *Food Control*. 20(2): 99–104 (2009)
13. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSIBLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25(17): 3389–3402 (1997)
14. García-Garrido JA, Quiles-Zafra R, Tapiador J, Luque de Castro MD. Activity of cathepsin B, D, H and L in Spanish dry-cured ham of normal and defective texture. *Meat Sci.* 56(1): 1–6 (2000)
15. Zhao GM, Zhou GH, Wang YL, Xu XL, Huan YJ, Wu JQ. Time-related changes in cathepsin B and L activities during processing of Jinhua ham as a function of pH, salt and temperature. *Meat Sci.* 70:381–388 (2005)
16. Li SH, Zhou XQ, Zhang N, Liu H, Ma CW. Purification and characterization of cathepsin L2 from dorsal muscle of silver carp (*Hypophthalmichthys molitrix*). *Food Chem.* 111: 879–886 (2008)
17. Buckow R, Truong BQ, Versteeg C. Bovine cathepsin D activity under high pressure. *Food Chem.* 120(2): 474–481 (2009)
18. Blanchard P, Mantle D. Comparison of proteolytic enzyme levels in chicken, Pig, lamb and rabbit muscle at point of slaughter: role in meat tenderization post mortem. *J. Sci. Food Agr.* 71: 83–91 (1996)
19. Han MY. Study on relationship between the pork myofibrillar protein structure and it's functionality of heat-induced gelation by low field NMR and raman spectroscopy. MS thesis, Nanjing Agricultural University, Nanjing, China. (2009)
20. Castano A, Fontan G, Fresno JM, Tornadijo ME, Carballo J. Survival of Enterobacteriaceae during processing of Chorizo de cebolla, a Spanish fermented sausage. *Food Control*. 13(2): 107–115 (2002)
21. Lu S, Ji H, Wang Q, Li B, Li K, Xu C, Jiang C. The effects of starter cultures and plant extracts on the biogenic amine accumulation in traditional Chinese smoked horsemeat sausages. *Food Control*. 50: 869–875 (2015)
22. Ikonc P, Tasic T, Petrovic L. Proteolysis and biogenic amines formation during the ripening of Petrovska klobasa, traditional dry-fermented sausage from Northern Serbia. *Food Control*. 30: 69–75 (2013)
23. Ikonc P, Jokanovic M, Tasic T, Skaljic S, Sojic B, Tomovic V, Dzinic N, Petrovic L. The Effect of Different Ripening Conditions on Proteolysis and Texture of Dry-fermented Sausage Petrovska klobasa. *Procedia Food Sci.* 5: 97–100 (2015)
24. Flores J, Marcus JR, Nieto P, Navarro JL, Lorenzo P. Effect of processing conditions on proteolysis and taste of dry-cured sausages. *Zeitschrift Für Lebensmittel-Untersuchung und-Forschung A* 3: 168–172 (1997)
25. Leroy F, Verluyn J, de Vuyst L. Functional meat starter cultures for improved sausage fermentation. *Int. J. Food Microbiol.* 106: 270–285 (2006)
26. Salim Ammor M, Mayo B. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. *Meat Sci.* 76(1): 138–146 (2007)
27. Muyzer EC, Waal D, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16 s rDNA. *Appl Environ Microb.* 59(3): 695–700 (1993)
28. Larrea V, Hernando I, Quiles A, Lluch MA, Perez-Munuera I. Changes in proteins during Teruel dry-cured ham processing. *Meat Sci.* 74: 586–593 (2006)
29. Zhao GM, Zhou GH, Wang YL, Xu XL, Huan YJ, Wu JQ. Time-related changes in cathepsin B and L activities during processing of Jinhua ham as a function of pH, salt and temperature. *Meat Science* 70(2): 381–388 (2005)
30. Díaz O, Fernandez M, De Fernando GDG, de la Hoz L, Ordoñez JA. Proteolysis in dry fermented sausages: the effect of selected exogenous proteases. *Meat Sci.* 46(1): 115–128 (1997)