Kasetsart J. (Nat. Sci.) 46 : 98 - 106 (2012)

Proteolytic Capability of *Staphylococcus xylosus* and *Candida zeylanoides* Isolated from Chinese Xuanwei Ham

Aixiang Huang[1,*], Sarote Sirisansaneeyakul[2,3], Zongdao Chen[4], Zhixia Wu[1] and Yusuf Chisti[5]

ABSTRACT

The bacterium *Staphylococcus xylosus* and the yeast *Candida zeylanoides*, originally isolated from dry-cured Chinese Xuanwei ham, were investigated for proteolytic activity towards myofibrillar protein extracts of fresh and salted semimembranosus muscle of pork. Both microorganisms were proteolytically active and caused progressive generation of shorter peptides and free amino acids through the hydrolysis of myofibrillar proteins, which were analyzed by gel electrophoresis (SDS-PAGE)/a high-performance liquid chromatography and an automated amino acid analyzer, respectively. *C. zeylanoides* had a more pronounced proteolytic activity compared with *S. xylosus*. Hydrophilic peptides that have been associated with flavor development were detected in chromatograms of hydrolyzed samples of myofibrillar proteins.

Keywords: *Candida zeylanoides*, Chinese Xuanwei ham, myofibrillar protein, proteolysis, *Staphylococcus xylosus*

INTRODUCTION

Xuanwei ham is a famous, dry-cured ham of China. This uncooked ham is produced in Xuanwei, a city in Yunnan province, southwestern China. Xuanwei ham is well known for its characteristic rose-red color and unique flavor. Xuanwei ham has been produced since the Qing Dynasty (1727 AD) (Yu *et al*., 2005), but microbial and biochemical changes that accompany its production are only now being understood.

Xuanwei ham is produced from the hind legs of pigs. The traditional process uses legs of the local Wujin pig breed, but legs of crossbred pigs are also used. Hind legs from slaughtered and skinned animals are cut and trimmed. The legs (or 'green ham' at this stage) are squeezed by hand to remove blood. The green ham is then held under cool conditions for 24 hr to age the meat. The ripened leg is hand-rubbed with curing salt, (sodium chloride mixed with about 0.15% by weight of sodium nitrate) for about 10 min (Huang *et al*., 2009; Huang *et al*., 2011). This is followed by a 40-day 'drying' stage which involves hanging the legs from bamboo supports in a room at a temperature of about 13 °C. A 120-

1 Faculty of Food Science and Technology, Yunnan Agricultural University, Kunming 650201, P.R. China.

2 Department of Biotechnology Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.

3 Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University, Kasetsart University, Bangkok 10900, Thailand. (CASTNAR, NRU-KU, Thailand)

4 Faculty of Food Science, Xinan University, Chong Qing 400716, P.R. China.

5 School of Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand.

* Corresponding author, e-mail: aixianghuang@126.com

Received date : 10/08/11 Accepted date : 26/12/11

day fermentation stage then follows (Huang *et al*., 2009). The entire process takes nearly 190 d (Huang *et al*., 2009). The production process has been described in detail in the literature (Huang *et al*., 2009; Huang *et al*., 2011). The literature has discussed the microbiology of production of Xuanwei ham (Jiang *et al*., 1990; Li *et al*., 2003; Wang *et al*., 2006; Huang *et al*., 2009), but barely any information exists on flavor development during its production.

Development of flavor in Xuanwei ham is a complex process that remains unclear. Microorganisms are known to contribute to flavor development in many European dry-cured meats (Lücke, 1986; Rodríguez *et al*., 1994; Hinrichsen and Pedersen, 1995; Núñez *et al*., 1996; Martín *et al*., 2004). Flavors arise as a consequence of the partial decomposition of meat and fat to aldehydes, alcohols, esters, amino acids and other organic compounds. Enzymatic and chemical reactions involved in the flavor development include lipid oxidation, Maillard reaction, and Strecker degradation (Toldrá, 1998). Both the endogenous enzymes in the meat and the microbial action contribute to the flavor development (Toldrá, 1998; Martín *et al*., 2004; Zhao *et al*., 2006).

Molds are involved in the ripening of some dry-cured meat products where they have a positive effect on the flavor and appearance (Lücke, 1986; Martín *et al*., 2004). According to the traditional view, high quality Xuanwei ham must have a 'green growth' (that is, molds) on it, but superior quality Xuanwei ham has been produced in the absence of molds, by inoculating the meat with some yeasts isolated from the ham (Wang *et al*., 2006). Although pure starter cultures are not used in the traditional production of Xuanwei ham, the production hygiene and product quality may be improved if the microorganisms that are essential for its production are identified and used as starters. For example, starter cultures have been proposed for producing Spanish dry-cured ham (Carrascosa and Cornejo, 1991), although they are not in use.

In certain dry-cured hams, the proteolytic activities of lactic acid bacteria and *Staphylococcus xylosus* are known to be important in the flavor development (Molina and Toldrá, 1992; Fadda *et al*., 1998; Fadda *et al*., 1999a). *S. xylosus* and the yeast *Candida zeylanoides* are among the dominant microorganisms found in dry-cured Iberian hams (Rodríguez *et al*., 1994; Núñez *et al*., 1996). *S. xylosus* and *C. zeylanoides* have been isolated also from Xuanwei ham, as predominant microbial species (Huang *et al*., 2009). The current study reports on *S. xylosus* and *C. zeylanoides* isolated from dry-cured Xuanwei ham and their proteolytic activity on the myofibrillar protein of fresh and salted pork, to assess the potential of these isolates as starter cultures for producing dry-cured Xuanwei ham.

MATERIALS AND METHODS

Microorganisms and culture conditions

Staphylococcus xylosus and *Candida zeylanoides* isolated during processing of Chinese dry-cured Xuanwei ham (Huang *et al*., 2009; Huang *et al*., 2011) were used. *S. xylosus* was grown in mannitol salt agar (MSA) broth (1 g beef extract, 75 g NaCl, 10 g mannitol, 10 g peptone, 1 L distilled water, pH 7.5) at 37 °C for 48 hr. *C. zeylanoides* was grown in yeast peptone D-glucose (YPD) broth (5 g yeast extract, 20 g glucose, 20 g peptone, 1 L distilled water, pH 5.0) at 28 °C for 5 d (Chen, 1995).

Preparation of cell suspension

S. xylosus and *C. zeylanoides* were grown separately in the media specified above at the specified temperatures for 20 hr in 250 mL shake flasks at 150 rpm. Each flask had a culture volume of 150 mL. Cells were harvested by centrifugation (Anke TGL-16G-A, Shanghai, China) at 10,000×*g* for 20 min at 4 °C (Fadda *et al*., 1999a), washed twice with 100 mL of NaCl (8.5 $g.L^{-1}$) containing

20 mM $CaCl_2$. Washed cells were separately resuspended in 20 mL of 50 mM Tris-HCl, pH 6.5.

Preparation of muscle protein extracts

Fresh and salted (with NaCl (40 $g.L^{-1}$) for 3 d at 4 °C) semimembranosus (SM) muscle from 8-month-old pigs (Yorkshire × Duroc × local Wujin variety) were used for myofibrillar protein extraction (Molina and Toldrá, 1992; Fadda *et al*., 1999a). Each 18 g muscle fetched under sterile conditions was homogenized with 30 mL phosphate buffer (20 mM, pH 6.5) at 6,500 rpm for 3 min using a stomacher (IKA T25 Basic, Germany). The resulting suspension was centrifuged (10,000×*g*, 20 min, 4 °C; Anke 64R Centrifuge, UK), the pellet was collected and washed three times with the phosphate buffer specified above to remove muscle proteinases. The washed pellet was resuspended in 27 mL of 0.1 N phosphate buffer (pH 6.5) that contained 0.7 M KI and 0.02 g/100 mL sodium azide. The resulting suspension was homogenized at 7,000 rpm for 3 min at 4 °C. The suspension was then centrifuged (10,000×*g*, 20 min, 4 °C), the supernatant was recovered and diluted tenfold with the above specified phosphate buffer. The dilute supernatant was filter sterilized by passing through a 0.22 µm membrane filter (Whatman, USA) and used as the protein extract.

The prepared protein extracts were tested for sterility by the plate count method. The concentration of protein in the extract was determined using the Coomassie brilliant blue (G-250, Sigma, USA) method (Li, 1994). Albumin (fraction V) purchased from Kunming Jie-Hui Biotechnology Co., Ltd., was used as the protein standard. The protein content of the fresh and salted muscle extracts was 0.924 and 0.874 $mg.mL^{-1}$, respectively.

Microbial counts

A 10 mL cell suspension of *S. xylosus* or *C. zeylanoides* was inoculated in 50 mL myofibrillar protein extracts and incubated at 30 °C in 150 mL shake flasks held at 150 rpm. Samples were taken at 0, 48 and 96 hr. *S. xylosus* and *C. zeylanoides* counts were made on mitis salivarius agar (MSA) and YPD agar plates, respectively, (Liu, 2003). The MSA plates were incubated at 37 °C for 48 hr and the YPD plates were incubated at 28 °C for 5 d.

Gel electrophoresis

The hydrolysis of muscle proteins after incubation with microbial cells was monitored by sodium dodecyl sulfate gel (SDS)-polyacrylamide electrophoresis (PAGE) analysis (PowerPac Basic, BIO-RAD, USA). The polyacrylamide gels contained (g/100 mL) 12% separating gel and 5% stacking gel. Protein standards were run in parallel lanes for characterizing the molecular weight of the hydrolysates. The high molecular weight protein standards (Amersham Biosciences, UK) used were: myosin (220 kDa), α-2-macroglobulin (170 kDa), β-galactosidase (116.3 kDa), transferrin (76 kDa) and glutamate dehydrogenase (53 kDa). The standards for low molecular weight proteins (Promega, USA) were: phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), actin (42.7 kDa), carbonic anhydrase (31.0 kDa) and lysozyme (14.4 kDa). Proteins were visualized by staining with Coomassie brilliant blue (R-250, Sigma, USA). Destaining involved soaking in several changes of a mixture of 50% methanol, 10% acetic acid and water by volume, until a clear background resulted.

Peptide analyses

The various peptides present in protein extracts after incubation with microbial cells were determined using a high-performance liquid chromatograph (Hewlett-Packard-1100, USA) equipped with a multi-wavelength UV detector and an autoinjector (Rodríguez *et al*., 1998; Sanz *et al*., 1999). Each 0.8 mL sample was deproteinized

with 1.6 mL acetonitrile. The supernatant obtained was dried by evaporation and resuspended in 200 µL solvent A (0.1% by vol trifluoroacetic acid in MilliQ water). Samples of 20 µL were applied to a Symmetry $ODSC_{18}$ column (3.5 µm, 4.6 × 75 mm). The mobile phases were solvent A and solvent B (acetonitrile:water:trifluoroacetic acid, 60:40:0.04 by vol). The elution was performed as follows: an isocratic phase of 1 % solvent B for 5 min followed by a linear gradient from 1 to 100% of solvent B for 25 min. The flow rate was constant at 0.8 $mL.min^{-1}$. The column temperature was 40 °C. Peptides were detected at 214 nm wavelength.

Amino acid analyses

The concentrations of free amino acids in fresh and salted muscle myofibrillar protein extracts after incubation with microbial cells, were monitored as follows: a 2.0 mL sample was adjusted to pH 2.1 with 3% sulfonic salicylic acid. The sample was centrifuged (15,000×*g*, 20 min, 4 °C; Anke 64R Centrifuge, UK) and 1.5 mL of the supernatant was filtered through a 0.45 µm membrane filter (Whatman, USA). A 1.0 mL sample of the filtrate was analyzed for amino acids by an automated amino acid analyzer (Hitachi L-8800, Japan). All analyses followed the Chinese standard for amino acid analysis (Chinese National Standard, GB/T 18246-2000). The amino acid concentration was detected spectrophotometrically at 570 nm.

RESULTS AND DISCUSSION

Microbial growth on myofibrillar protein extracts

Colony counts of *S. xylosus* and *C. zeylanoides* on inoculated myofibrillar protein extracts after various durations of incubation are shown in Table 1. No microorganisms were detected in uninoculated control extracts at any time (Table 1). Both the bacteria and yeast counts in the inoculated extracts declined progressively with incubation time. Neither extract supported microbial growth because of the presence of sodium azide and a lack of any nutrients other than myofibrillar proteins (Glaasker *et al*., 1998). Extracts had been formulated to suppress growth (Molina and Toldrá, 1992; Fadda *et al*., 1999b), as the intention was to assess the ability of nongrowing cells to effect hydrolysis of myofibrillar proteins. This aspect is discussed next.

Electrophoretic and chromatographic analyses

The SDS-PAGE profiles of the myofibrillar protein extracts incubated with *S. xylosus* and *C. zeylanoides* for various periods are shown in Figure 1. For any given ham extract (that is, fresh or salted ham), the control profiles (0 hr incubation) in lanes P_0 (*S. xylosus*) and J_0 (*C. zeylanoides*) were quite similar, as no proteolysis had occurred and the starting extracts were the same. After 48 hr (lanes P_{48} and J_{48}) and 96 hr (lanes P_{96} and J_{96}) of incubation with *S. xylosus* (P-lanes) and *C. zeylanoides* (J-lanes), the number

Table 1 Microbial growth on fresh and salted myofibrillar protein extracts.

Fresh tissue extract				Extract of salted tissue			
Incubation time (hr)	Control ($CFU.mL^{-1}$)	*S. xylosus* ($CFU.mL^{-1}$)	*C. zeylanoides* ($CFU.mL^{-1}$)	Incubation time (hr)	Control ($CFU.mL^{-1}$)	*S. xylosus* ($CFU.mL^{-1}$)	*C. zeylanoides* ($CFU.mL^{-1}$)
0	0	7.10×10^{9}	3.40×10^{8}	0	0	1.43×10^{9}	2.01×10^{8}
48	0	2.42×10^{7}	3.70×10^{6}	48	0	1.18×10^{7}	5.50×10^{6}
96	0	6.30×10^{5}	6.17×10^{4}	96	0	1.30×10^{5}	1.45×10^{4}

of protein bands had greatly diminished compared with the number in the control lanes P_0 and J_0. This confirmed that both *S. xylosus* and *C. zeylanoides* hydrolyze proteins that are originally present in myofibrillar extracts of fresh and salted ham. With one exception, all proteins of molar mass greater than about 50 kDa were essentially completely hydrolyzed after 48 hr of incubation with *S. xylosus* (Figure 1a, b). The sole exception was the 220 kDa band that was greatly diminished but not entirely eliminated after 48 hr.

In lanes J_{48} and J_{96} (Figure 1a), the appearance of low molecular weight bands that did not exist in the original extract (lane J_0) was due to the hydrolysis of the larger peptides (that is, with a molar mass of more than 116.3 kDa, as the standard protein of β-galactosidase). Of the peptides originally present in the extract, the low molecular weight polypeptides (molar mass less than or equal to about 43 kDa) were

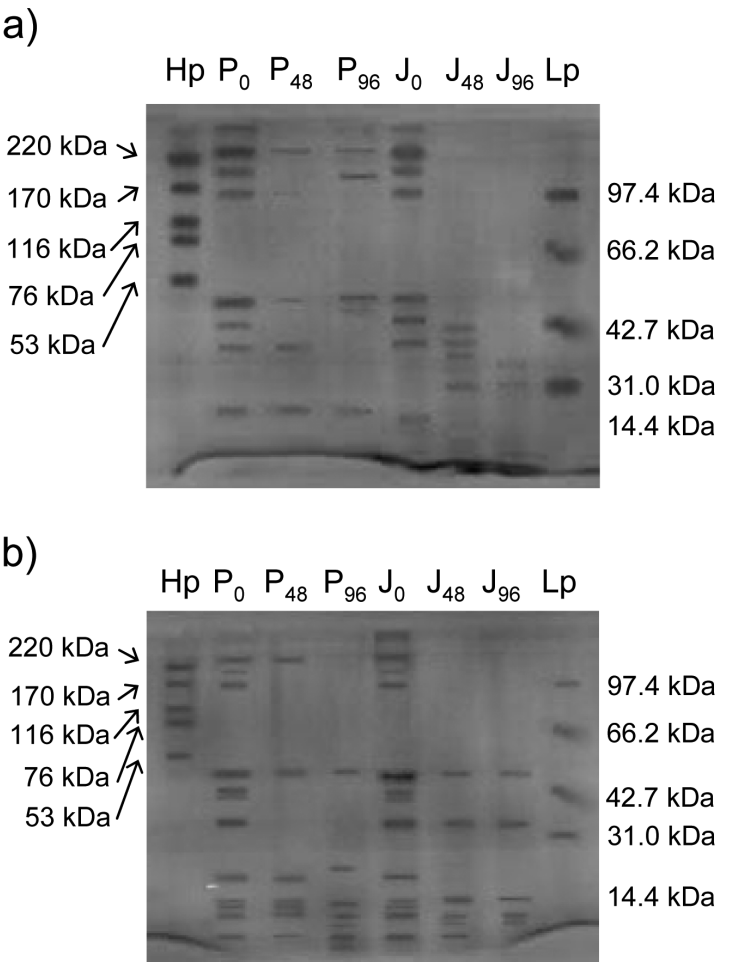


Figure 1 SDS-PAGE of muscle myofibrillar protein hydrolyzed by *S. xylosus* and *C. zeylanoides*. Fresh muscle extract (a) and salted muscle extract (b). Lanes Hp, Lp are high- and low-molecular weight standards, respectively. P and J represent myofibrillar protein inoculated with *S. xylosus* and *C. zeylanoides*, respectively. Subscripts 0, 48 and 96 are incubation times in hours after inoculation.

less susceptible to hydrolysis by *C. zeylanoides* as these bands were the last to disappear (Figure 1a, lanes J_{0-96}). With extracts of fresh ham, *C. zeylanoides* appeared to have a superior hydrolytic capability compared with *S. xylosus*.

The hydrolysis of extracts of salted ham was generally similar to that of fresh ham. Similar results have been reported for the hydrolysis of pork muscle proteins by *Lactobacillus curvatus* and *Lactobacillus sake* and *Lactobacillus plantarum* (Fadda *et al*., 1998; Fadda *et al*., 1999a).

Based on the SDS-PAGE of myofibrillar protein (fresh and salted SM), hydrolysis with *S. xylosus* and *C. zeylanoides* showed that high molecular weight proteins, likely myosin (200 kDa) and actin (42.7 kDa), were hydrolyzed eventually, whereas the bands of intermediate molecular weight proteins (53 to 31 kDa) were partially hydrolyzed. As a result, faint bands of low molecular weight protein (31 to 14.4 kDa) appeared. Compared to *S. xylosus*, the proteolytic activity of *C. zeylanoides* was superior.

Chromatographic peptide profiling of ham extracts incubated with *S. xylosus* and *C. zeylanoides* confirmed the kind of changes that were qualitatively revealed by SDS-PAGE. Based on the retention times of the peaks on the chromatograms (data not shown), the hydrolysis of myofibrillar protein extracts by both microorganisms generated some hydrophilic peptides. Hydrophilic peptides have been attributed to the development of a desirable cured-meat taste in other studies (Aristoy and Toldrá, 1995). An increase in peptide fractions is quite common in meat fermentations and some of these peptides have been linked to specific tastes such as bitter and savory (Díaz *et al*., 1997; Sentandreu et *al*., 2003).

Amino acid analyses

Free amino acid profiles of muscle myofibrillar protein extracts incubated for various periods (0, 48 and 96 hr) with *S. xylosus* and *C. zeylanoides* are shown in Tables 2 and 3, respectively. In addition to the amino acids shown (Tables 2 and 3), the muscle extracts contained polypeptides (see previous section), at least initially (that is, at 0 hr). As shown in Table 2, the incubation of the fresh muscle extract with *S. xylosus* caused a progressive decline with time in the concentration of every amino acid, except cysteine. The concentration of cysteine increased from 0.56 mg/100 mL at 0 hr to 1.18 mg/100 mL at 96 hr (Table 2). These results suggest that the free amino acids in the extract of fresh muscle were mostly being consumed by *S. xylosus*, as also reported by Silvina *et al*. (1999). With *Lactobacillus plantarum*, no substantial proteolysis of any polypeptides occurred, with the exception of cysteine, none of the amino acids increased in concentration as a result of incubation with *S. xylosus*. (The very slight increase in methionine between 0 hr and 48 hr (Table 2) is readily explained as being within the expected error for these kinds of measurements.)

A comparison of the amino acid concentration data for the extract of salted muscle incubated with *S. xylosus* revealed a quite different picture. Between 48 hr and 96 hr of incubation, the concentration of 13 amino acids actually increased quite substantially, as shown by the shaded data in Table 2. Clearly, *S. xylosus* was much more effective in hydrolyzing polypeptides in the extract of salted muscle than it was in the extract of fresh muscle.

Table 3 shows that for incubation of the fresh muscle extract with the yeast *C. zeylanoides*, the concentrations of 10 amino acids increased after 48 hr of incubation compared to the values at 0 hr (see Table 3, shaded data in the fresh muscle extract column). Clearly, the yeast was more effective in hydrolyzing the fresh extract polypeptides than was the bacterium *S. xylosus* in the same extract. Table 3 further reveals that *C. zeylanoides* was as effective in hydrolyzing the salted muscle extract as it was in hydrolyzing the fresh muscle extract. Thus, between 0 and 48

Table 2 Free amino acid profiles (mg/100 mL) of myofibrillar extracts after various times of incubation with *S. xylosus*. (Between 48 hr and 96 hr of incubation, the concentration of 13 amino acids actually increased quite substantially as shown by the shaded data.)

	Fresh muscle myofibrillar extract			Salted muscle myofibrillar extract		
Amino acid	0 hr	48 hr	96 hr	0 hr	48 hr	96 hr
Asp	11.91	1.32	0.00	10.28	0.28	1.02
Thr	0.84	0.00	0.00	0.00	0.00	0.99
Ser	0.73	0.16	0.12	0.33	0.12	0.98
Glu	18.98	3.41	0.64	22.03	2.70	2.37
Gly	1.17	0.10	0.00	0.50	0.85	0.66
Ala	1.19	0.42	0.33	0.95	0.28	1.18
Cys	0.56	0.90	1.18	0.00	0.56	1.07
Val	1.23	0.54	0.27	0.96	0.24	1.33
Met	0.57	0.58	0.20	0.54	0.00	0.63
Ile	1.66	0.00	0.00	1.89	0.67	0.67
Leu	0.52	0.00	0.00	0.00	0.00	1.22
Tyr	0.00	0.00	0.00	0.00	0.00	0.58
Phe	2.45	1.46	1.13	1.79	0.86	2.33
Lys	2.46	1.97	0.91	2.43	1.59	2.49
His	1.04	0.44	0.71	0.87	0.32	0.93
Arg	0.71	0.00	0.00	0.63	0.00	1.08
Pro	3.98	0.00	0.00	1.91	0.00	0.00

Asp = Asparagine; Thr = Threonine; Ser = Serine; Glu = Glutamic acid; Gly = Glycine; Ala = Alanine; Cys = Cysteine; Val = Valine; Met = Methionine; Ile = Isoleucine; Leu = Leucine; Tyr = Tyrosine; Phe = Phenylalanine; Lys = Lysine; His = Histidine; Arg = Arginine; Pro = Proline.

Table 3 Free amino acid profiles (mg/100 mL) of myofibrillar extracts after various times of incubation with *C. zeylanoides*. (Between 0 hr and 48 hr of incubation, the concentration of 10 amino acids actually increased quite substantially as shown by the shaded data.)

	Fresh muscle myofibrillar extract			Salted muscle myofibrillar extract		
Amino acid	0 hr	48 hr	96 hr	0 hr	48 hr	96 hr
Asp	1.14	6.38	7.23	1.09	5.72	3.20
Thr	2.09	1.33	0.52	2.33	1.13	0.43
Ser	1.32	1.21	1.48	1.37	0.76	0.58
Glu	9.92	10.77	4.93	10.57	11.36	3.98
Gly	1.80	0.30	0.17	1.81	0.17	0.12
Ala	22.79	3.86	1.87	24.26	3.38	1.05
Cys	0.87	0.47	1.12	1.94	1.83	1.39
Val	0.77	2.19	1.37	1.19	2.03	1.08
Met	0.00	2.54	0.56	0.27	0.33	0.24
Ile	0.37	2.08	0.58	0.41	1.44	0.39
Leu	0.70	3.45	0.95	0.86	1.70	0.65
Tyr	0.26	1.22	0.99	0.72	0.91	0.70
Phe	0.99	4.68	3.96	1.44	3.57	2.64
Lys	3.36	11.63	4.49	3.57	3.61	2.31
His	0.92	1.49	1.65	0.93	1.44	1.05
Arg	5.10	2.17	0.69	5.61	3.01	0.42
Pro	0.46	0.00	0.00	0.46	0.00	0.00

Asp = Asparagine; Thr = Threonine; Ser = Serine; Glu = Glutamic acid; Gly = Glycine; Ala = Alanine; Cys = Cysteine; Val = Valine; Met = Methionine; Ile = Isoleucine; Leu = Leucine; Tyr = Tyrosine; Phe = Phenylalanine; Lys = Lysine; His = Histidine; Arg = Arginine; Pro = Proline

hr of incubation, the concentrations of 10 amino acids increased as a consequence of the hydrolysis of polypeptides (see Table 3, shaded data in the salted muscle extract column). The amino acids that increased in concentration were exactly the same as those that had increased in concentration in the fresh muscle extract.

Similar changes in the concentration of free amino acids with incubation time have been observed during the hydrolysis of muscle protein with other bacterial strains isolated from European dry-cured hams (Fadda *et al*., 1999a; Fadda *et al*., 1999b). Free amino acids are known to contribute to the flavor of the cured ham directly and as precursors of other flavoring agents (Toldrá and Aristoy, 1993; Hinrichsen and Pedersen, 1995; Toldrá, 1998).

CONCLUSIONS

Nongrowing *S. xylosus* and *C. zeylanoides* were shown to be able to progressively hydrolyze myofibrillar protein extracts of fresh and salted ham to produce polypeptides and free amino acids. *C. zeylanoides* had a generally greater proteolytic activity compared with the bacterium *S. xylosus*. In view of their demonstrated proteolytic activity and known occurrence in various dry-cured hams, *S. xylosus* and *C. zeylanoides* isolated from Chinese Xuanwei ham are worth investigating further as potential starters for Xuanwei ham fermentation.

ACKNOWLEDGEMENTS

This work was conducted under the Greater Mekong Subregion Joint Research and Development Project, supported by the Ministry of Science and Technology of the People's Republic of China and the Thailand International Development Cooperation Agency. The authors are grateful to the Faculty of Food Science and Technology, Yunnan Agricultural University and the Faculty of Food Science, Xinan University, for further supporting this work.

LITERATURE CITED

Aristoy, M.C. and F. Toldrá. 1995. Isolation of Flavor Peptides from Raw Pork Meat and Dry Cured Ham, pp. 1323–1343. *In* G. Charalambous, (ed.). **Food Flavors: Generation, Analysis and Process Influence.** Elsevier. London.

Carrascosa, A.V. and I. Cornejo. 1991. Characterization of *Micrococcaceae* strains selected as potential starter cultures to Spanish dry-cured ham process. 2. Slow process. **Fleischwirtschaft** 71: 1187–1188.

Chen, T.Sh. 1995. **Production and Application of Microbial Medium.** China Agricultural Press. Beijing. 642 pp.

Chinese National Standard. 2000. **Determination of Amino Acids in Feeds,** GB/T 18246-2000, China Standard Press. Beijing.

Díaz, O., M. Fernández, G.D. García de Fernando, L. de la Hoz and J.A. Ordóñez. 1997. Proteolysis in dry fermented sausages. The effect of selected exogenous proteases. **Meat Sci.** 46: 115–128.

Fadda, S., G. Vignolo, A. Holgado and G. Oliver. 1998. Proteolytic activity of *Lactobacillus* strains isolated from dry cured sausages on muscle sarcoplasmic proteins. **Meat Sci.** 49: 11–18.

Fadda, S., Y. Sanz, G. Vignolo, M.C. Aristoy, G. Oliver and F. Toldrá. 1999a. Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. **Appl. Environ. Microbiol.** 65: 578–584.

Fadda, S., Y. Sanz, G. Vignolo, M.C. Aristoy, G. Oliver and F. Toldrá. 1999b. Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. **Appl. Environ. Microbiol.** 65: 3540–3546.

Glaasker, E., S.B.T. Frans, P.F.T. Steeg, W.N. Konings and B. Poolman. 1998. Physiological response of *Lactobacillus plantarum* to salt and nonelectrolyte stress. **J. Bacteriol.** 180: 4718–4723.

Hinrichsen, L. And S.B. Pedersen. 1995. Relationship among flavor, volatile compounds, chemical changes, and microflora in Italian-type dry-cured ham during processing. **J. Agric. Food Chem.** 43: 2932–2940.

Huang, A.X., S. Sirisansaneeyakul, S., Z.D. Chen, Sh.Ch. Liu and Y. Chisti. 2009. Microbiology of Chinese Xuanwei ham production. **Fleisch. Int.** 24(3): 64–67.

Huang, A.X., S. Sirisansaneeyakul, Ch.R. Ge, Z.D. Chen, Q.Ch. Huang, W. Qin and Y. Chisti. 2011. Physicochemical changes during processing of Chinese Xuanwei ham. **Kasetsart J. (Nat. Sci.)** 45: 539–550.

Jiang, D.F., R.L. Duan and P. Ma. 1990. The study of chemical compounds and microflora in Xuanwei style ham, (in Chinese). **J. Yunnan Univ.** 3: 71–75.

Li, J.W. 1994. **Methods and Principles of Biochemical Experiments.** Beijing University Press, Beijing, 465 pp.

Li, P.L., Q.W. Shen and Y.N. Lu. 2003. Investigation of major microbial composition of Xuanwei ham, (in Chinese). **J. China Microecol.** 15(5): 262–263.

Liu, Y.D. 2003. **Tests of Microbiology,** 2nd ed. Peoples Hygiene Press. Beijing. 565 pp.

Lücke F.K. 1986. Microbiological processes in the manufacture of dry sausage and raw ham. **Fleischwirtschaft** 66: 1505–1509.

Martín, M., J.J. Córdoba, F. Núñez, M.J. Benito and M.A. Asensio. 2004. Contribution of a selected fungal population to proteolysis on dry-cured ham. **Int. J. Food Microbiol.** 94: 55–66.

Molina, I. and F. Toldrá. 1992. Detection of proteolytic activity in microorganisms isolated from dry-cured ham. **J. Food Sci.** 57: 1308–1310.

Núñez, F., M.M. Rodríguez, J.J. Córdoba, M.E. Bermúdez and M.A. Asensio. 1996. Yeast population during ripening of dry-cured Iberian ham. **Int. J. Food Microbiol.** 29: 271–280.

Rodríguez, M., F. Núñez, J.J. Córdoba, C. Sanabrin, E. Bermúdez and M.A. Asensio. 1994. Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. **Int. J. Food Microbiol.** 24: 329–335.

Rodríguez, M., F. Núñez, J.J. Córdoba, M.E. Bermúdez and M.A. Asensio. 1998. Evaluation of proteolytic activity of microorganisms isolated from dry cured ham. **J. Appl. Microbiol.** 85: 905–912.

Sanz, Y., S. Fadda, G. Vignolo, M.C. Aristoy, G. Oliver and F. Toldrá. 1999. Hydrolytic action of *Lactobacillus casei* CRL705 on pork muscle sarcoplasmic and myofibrillar protein. **J. Agric. Food Chem.** 47: 3441–3448.

Sentandreu, M.A., S. Stoeva, M.C. Aristoy, K. Laib, W. Voelter and F. Toldrá. 2003. Identification of small peptides generated in Spanish dry-cured ham. **J. Food Sci.** 68: 161–166.

Silvina, F., Y. Sanz, G. Vignolo, M.C. Aristoy, G. Oliver and F. Toldrá. 1999. Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. **Appl. Environ. Microbiol.** 65: 3540–3546.

Toldrá, F. and M.C. Aristoy. 1993. Availability of essential amino acids in dry cured ham. **Int. J. Food Sci. Nutr.** 44: 215–219.

Toldrá, F. 1998. Proteolysis and lipolysis in flavor development of dry-cured meat products. **Meat Sci.** 49: 101–110.

Wang, X.H., P. Ma, D.F. Jiang, Q. Peng and H. Ya. 2006. The natural microflora of Xuanwei ham and the no-mouldy ham production. **J. Food Eng.** 77: 103–111.

Yu, Z.S., W.X. Jiang, C.Y. Qiu and M.Y. Fan. 2005. History of development of Xuanwei ham. **Proceedings of the 5th Chinese Congress of Meat Science,** Beijing. 264–265.

Zhao, G.M., Y.L. Wang, W. Tian, G.H. Zhou, X.L. Xu and Y.X. Liu. 2006. Changes of arginyl and leucyl aminopeptidase activities in biceps femoris along Jinhua ham processing. **Meat Sci.** 74: 450–458.