# THE PROTEOME HOMOLOGY OF PEPTIDES EXTRACTED FROM DRY-CURED XUANWEI HAM

Lujuan Xing, Xiaoge Gao, Guanghong Zhou and Wangang Zhang*

□Jiangsu Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control; Nanjing Agricultural University, Nanjing, Jiangsu, 210095, China.

*Corresponding author email: wangang.zhang@njau.edu.cn

**Abstract –The objective of this study was to investigate the proteome homology of peptides purified from dry-cured Xuanwei ham. The Xuanwei ham peptides (XHP) were extracted and then LC-ESI-Q-TOF-MS/MS connected with Proteome Discoverer was used to analyze the peptide compositions. The results showed that there were 93 peptides identified in Xuanwei ham. The proteome homology results showed that myosin was the main protein for the generation of peptides accounting for 39% of all peptides. Hydrophobic amino acids accounted for 21% of free amino acids, among which Glu and His were the main amino acids. The abundant composition of peptides and free amino acid may endow the special flavor and characteristic for Xuanwei ham.**
**Key Words – Peptides; Proteome homology; Amino acid composition.**

## I. INTRODUCTION

Xuanwei ham is produced in Xuanwei city of Yunnan province and the special climatic condition contributes to the unique flavor and texture for dry-cured hams. During the long ripening time, intense proteolysis is formed in ham muscles and 10% of muscle proteins including soluble and insoluble proteins could be degraded[1]. Intense protein degradation results in the accumulation of peptides with different sizes and free amino acids at the end of processing. Many studies have reported that bioactive peptides could be produced in dry-cured hams, while no studies have studied the proteome homology of these bioactive peptides in Xuanwei ham.

## II. MATERIALS AND METHODS

### 2.1. Peptide extraction.

Xuanwei hams were purchased from Puji Food Company (Xuanwei, Yunan, China). Following by the research of Escudero et al[2], 20 g of biceps femoris muscle from the processed Xuanwei hams were minced and homogenized with 80 mL of hydrochloric acid (0.01 mmol/L) by homogenizer (IKA T25 digital ultraturrox, Germany) for three times (10 s each at 22,000 rpm) and then the homogenate was centrifuged (12,000 g) at 4 ℃ for 20 min. Filtering through filter paper, three volumes of ethanol (40%, V:V) were added in the supernatant to combine protein and maintained at 4 ℃ for 120 min and then following with centrifuge again (12,000 g, 20 min, 4 ℃). After filtering through 0.45 μm membrane filter paper (Millipore, Bedford, MA, USA), the supernatant was dried in rotary evaporator, and then stored at -20 ℃ until being used.

### 2.2. Characterization of peptide sequences.

The fraction derived from ultrafiltration intercept with 3,000 Da was first desalted using an OASIS HLB cartridge (Waters Inc., USA). Nano-LC-MS/MS coupled with a Dionex Ultimate 3000 nano-LC system (Thermo Fisher Scientific Ltd., Fremont, CA, USA) and a linear quadrupole ion trap Orbitrap (LTQ Orbitrap XL) mass spectrometer (Thermo Electron, Bremen, Germany) equipped with a nano electrospary ion source was used. The acquired MS/MS data were analyzed using Proteome Discoverer 14.0 (Thermo Fisher Scientific Ltd., Fremont, CA, USA). Basic parameter settings included a precursor mass tolerance of 10 ppm, a product mass tolerance of 0.02 Da, and a “no-enzyme” constraint. The proteome of pig (Sus) was downloaded from the Uniprot protein database.

### 2.3 Statistical analysis.

SAS software (version 9.0) was used to analyze all the data and significant differences ($P$<0.05) were detected by Duncan’s multiple-range test (20) in one-way analysis of variance (ANOVA).

## III. RESULTS AND DISCUSSION

During the fermentation of dry-cured ham, many polypeptides, small peptides and free amino acids were generated through the hydrolysis of protein by endogenous enzyme including cathepsins, calpains and exopeptidases[3]. In current study, the crude peptides were extracted from hams and the ultrafiltration throttling tube was used to retain the part of peptides with the molecular weight less than 3,000 Da. After desalination, the crude peptides were injected into the LC–MS/MS mass spectrometry for identifying their peptide content.

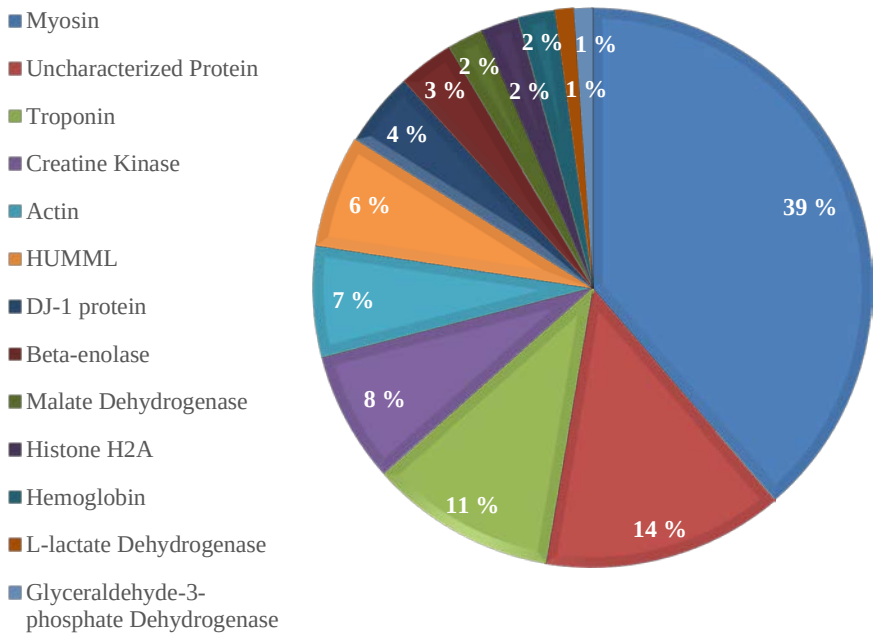


Figure 1. Distribution in percentages of identified peptides according to the origin protein

Table 1 The composition of amino acid in XHP

| Amino acid | Content（g/100g） | Amino acid | Content（g/100g） |
|---|---|---|---|
| Asp | 1.34±0.17$^{bc}$ | Cys | 0.07±0.01$^{g}$ |
| Glu | 3.33±0.70$^{a}$ | Val | 1.19±0.14$^{bcd}$ |
| Ser | 0.56±0.02 $^{defg}$ | Met | 0.38±0.06$^{fg}$ |
| His | 3.54±1.18$^{a}$ | Phe | 0.67±0.04$^{defg}$ |
| Gly | 0.80±0.09 $^{cdef}$ | Ile | 0.80±0.11$^{cdef}$ |
| Thr | 1.19±0.07$^{bcd}$ | Leu | 1.07±0.03$^{bcde}$ |
| Arg | 0.86±0.09$^{cdef}$ | Lys | 1.46±0.28$^{b}$ |
| Ala | 1.07±0.07$^{bcde}$ | Pro | 0.28±0.02$^{fg}$ |
| Tyr | 0.42±0.06$^{feg}$ | Total | 19.04 |

Different letters a ~ g in the same column indicate significant differences by Duncan's multiple range test ($P$<0.05).

A total of 93 peptides in Xuanwei ham were identified and the homologous proteins were also matched as shown in Fig.1. This figure lists the parent proteins with 99.9% similarity in traceable sequences. The 39% of these peptides were hydrolyzed from myosin, followed by tyoponin, creatine kinase, actin, and DJ-1

protein. Among all the hydrolyzed proteins, the metabolism related enzymes accounted for low proportion as among which malate dehydrogenase and L-lactate dehydrogenase were 2% and 1%, respectively. In the study of Mora[4], the molecular weight of antioxidant peptides was mainly concentrated in 400-2,500 Da in Spanish dry cured ham. Most of those peptides were hydrolyzed from actin which was 23% in all of the proteins. The result of amino acid composition in XHP showed that hydrophobic amino acids accounted for 21% of free amino acids (Table.1), among which Glu and His had higher content while the content of Cys was 0.07 g/100g ($P$<0.05). In a previous study to investigate antioxidant activity of amino acid, it was concluded that Cys, Tyr and Trp were the most reactive amino acids in scavenging $ABTS^{+}$ while Phe, His, Lys and Met showed lower trolox equivalent antioxidant capacity (TEAC). The Asp and Glu would play important role on the scavenging effect on superoxide radicals, which was associated with the antioxidant activity of functional peptides[5].

## IV. CONCLUSION

Based on the detection of proteome homology, there were 93 peptides hydrolyzed from 12 proteins in XHP. The main protein for the generation of peptides was myosin which accounting for 39% in all proteins. The free amino acid composition was 19.04 g/100g and hydrophobic amino acids accounted for 21% in Xuanwei ham. The abundant peptides and free amino acids could contribute to the special flavor and characteristics of Xuanwei ham.

## ACKNOWLEDGEMENTS

The current research was supported by the National Natural Science Foundation of China (Grant No: 31571853).

## REFERENCE

1. Mora, L., Sentandreu, M. A., Koistinen, K. M., Fraser, P. D., Toldrá, F., & Bramley, P. M. (2009). Naturally generated small peptides derived from myofibrillar proteins in Serrano dry-cured ham. Journal of Agricultural and Food Chemistry 57: 3228-3234.
2. Escudero, E., Aristoy, M.C., Nishimura, H.. Arihara, K., & Toldrá, F. (2012). Antihypertensive effect and antioxidant activity of peptide fractions extracted from Spanish dry-cured ham. Meat Science 91: 306-311.
3. Mora, L., Valero, M., Del Pino, M. S., Sentandreu, M. & Toldrá, F. (2011). Small peptides released from muscle glycolytic enzymes during dry-cured ham processing. Journal of Proteomics 74: 442-450.
4. Mora, L., Escudero, E., Fraser, P. D., Aristoy, M.-C., &Toldrá, F. (2014). Proteomic identification of antioxidant peptides from 400 to 2500Da generated in Spanish dry-cured ham contained in a size-exclusion chromatography fraction. Food Research International 56: 68-76.
5. Wang, Y., Li, D., Cheng, N., Gao, H., Xue, X., Cao, W. & Sun, L. (2015). Antioxidant and hepatoprotective activity of vitex honey against paracetamol induced liver damage in mice. Food & Function 6: 2339-2349.