IDENTIFICATION OF ANTIOXIDATIVE PEPTIDES FROM DRY-CURED XUANWEI HAM

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Abstract – This study was aimed to identify antioxidative peptides generated in dry-cured Xuanwei ham. Based on the scavenging effects on DPPH, ·OH and O_2^{-}·, peptides generated during the ripening period of Xuanwei ham showed strong antioxidant ability. By using anion exchange column and reversed-phase HPLC, fractions with strong antioxidant activity were separated based on their polarity differences. The fractions with strong antioxidant effects were further identified by LC-MS/MS. The results suggest that antioxidative peptides could be produced during the long processing of Xuanwei ham. Asp-Leu-Glu-Glu, Ser-Pro-Gly-Pro, Ser-Gly- Tyr and Pro-Leu-Pro were the main peptides contributing to the antioxidant activity of Xuanwei Ham.

Key Words –Antioxidant activity; Peptide sequence; Purification.

I. INTRODUCTION

Xuanwei ham belongs to China traditional meat product with more than 1000 year history from the generation of dry-curing technology to preserve raw meat[1]. It is produced in Xuanwei city of Yunnan province where the average temperature is between 13℃~14℃. Combined with mountainous region, the special climate conditions in Xuanwei city provide superior environment for ham ripening naturally. During the ripening period, proteins and fats are hydrolyzed by endoenzymes to produce small peptides and aliphatic acids contributing to the unique flavor of Xuanwei ham[2]. Recent study showed that the peptides extracted from traditional Jinhua ham could scavenge free radicals and the sequence of most active peptide was determined as Gly-Lys-Phe-Asn-Val[3]. However, no studies have investigated the antioxidative peptides in dry-cured Xuanwei ham. Thus, the current study was aimed to purify the peptides with high antioxidant activity and identify the peptide sequence to explore the antioxidant ability of Xuanwei ham.

II. MATERIALS AND METHODS

Xuanwei hams were purchased from Puji after natural ripening for 6~8 months (Xuanwei, China). The hams were salted in cold room at 2~4 ℃ and 58%~72% relative humidity for 2 months. Fat and muscle tendon of ham were cut manually and the biceps femoris was used to extract the peptides.

According to Toldrá[4], 20 g of biceps femoris muscle from the processed Xuanwei hams were minced and homogenized with 80 mL of phosphate buffer (0.2 mM, pH 7.2) for three times (10 s each at 22,000 rpm). The homogenate was centrifuged at 12,000 g for 20 min. Filtering through filter paper, three volumes of ethanol were added in the supernatant and maintained at 4 ℃ for 120 min and then centrifuged again. After filtering through 0.45 μm membrane filter, the supernatant was dried in rotary evaporator and then stored at -20 ℃.

The antioxidant activity of Xuanwei ham peptides was evaluated by measuring the scavenging ability of DPPH, ·OH and O_2^{-}·, respectively[3,5,6]. The peptides were purified by protein liquid chromatography on a HiPrep 16/10 DEAE anion-exchange column. According to the scavenging effect on free radicals, the fractions with strong activity were separated by reversed-phase high performance liquid chromatography (RP- HPLC, Waters, MA, USA) on a Primesphere BEH 130 C_{18} (Waters, 1.7um, 2.1×150 mm) column. The sequence of peptides was analyzed by LC-MS/MS. SAS software (version 9.0) was used to analyze data and significant differences ($P<0.05$) were detected by Duncan's multiple-range test in one-way analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

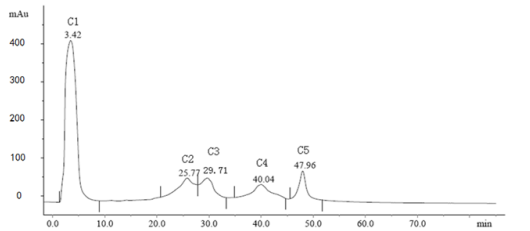
The antioxidant activity of peptides from Xuanwei ham on scavenging free radicals was shown in Table 1. Peptides had relatively less DPPH and

·OH scavenging activity than GSH while the O_2^{-}· scavenging effect was greater than GSH at same concentration (*P<0.05*).

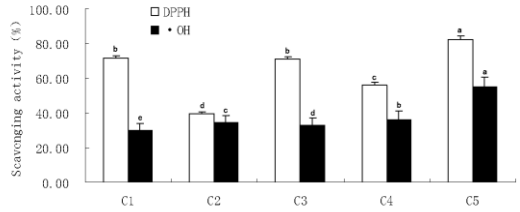
Table 1 The antioxidant activities of peptides from Xuanwei ham

	DPPH radical	Superoxide radical	Hydroxyl radical
XHP	35.34±2.11^b	78.05±5.33^a	23.45±1.65^b
GSH	85.24±3.23^a	56.33±1.34^b	71.24±2.97^a

Scavenging effects were tested at concentration of 1.0 mg/mL; Different letters (*a, b*) in the same column indicate significant differences by Duncan's multiple range test (*P*<0.05).



(A)



(B)

Figure 1. A. Anion-exchange chromatography of peptides from Xuanwei ham; B. The antioxidant activities of different peptide fractions.
C1~C5 represents different fractions in XHP; Scavenging effects were tested at concentration of 1.0 mg/mL; Different letters in same column indicated significant differences (*P*<0.05).

Depending on the polarity differences, the peptides were separated by anion exchange chromatography. As shown in Fig.1A, the peptides were separated to five different fractions. Among these fractions, the first fraction showed weak ability to bind with anions which was eluted within short time. The scavenging effect on DPPH and ·OH of fraction C5 reached to 82.14% and 54.95%, respectively (*P<0.05*), which were much higher than other four fractions (Fig. 1B). Compared with the scavenging effects before separation, the purified fractions have stronger antioxidant ability on scavenging DPPH and ·OH radicals.

In order to get the main antioxidative peptides, the fraction C5 was subjected into RP-HPLC. As shown in Fig.2, the fraction C5 was separated to seven peaks. The peak values of C5-1, C5-2 and C5-4 were slightly lower than the other four peaks. Comparing the antioxidant activity of each fraction, C5-3, C5-5, C5-6 and C5-7 showed higher DPPH scavenging ability at the concentration of 0.5 mg/mL (Table 2, P<0.05). The particle signals of C5-3, C5-5, C5-6 and C5-7 were analyzed by LC-MA/MS which were further sequenced as Pro-Leu-Pro, Ser-Gly-Tyr, Ser-Pro-Gly-Pro, and Asp-Leu-Glu-Glu, respectively.

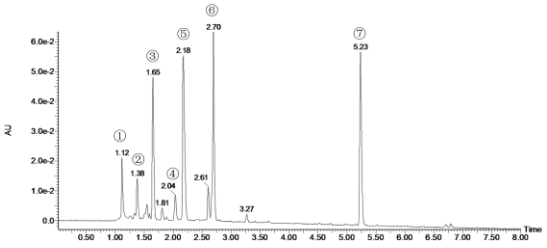


Figure 2. Separation of fraction C5 by RP-HPLC

Table 2 The antioxidant activities of different fractions purified from C5

Fractions	Scavenging effect on DPPH free radical
C5-3	69.98±3.48^b
C5-5	49.33±3.45^d
C5-6	51.26±2.36^c
C5-7	74.45±3.26^a

C5-3,5,6,7 represents different fractions in C5; Scavenging effect was tested at concentration of 0.5 mg/mL; Different letters in the same column indicate significant differences by Duncan's multiple range test (*P*<0.05).

The antioxidant activity of peptides is known to be associated with the sequence, structure molecular weight and amino acid composition[7]. It was identified that the presence of hydrophobic amino acid played important role in scavenging free radicals including Leu, Val, Met, Phe, Pro, Tyr, Ala and Trp[3]. In this study, the main antioxidative peptides were consisted by three or four amino acids with the molecular weight

ranging from 324.9 to 504.2 Da. Moreover, there was at least one of hydrophobic amino acid and the Pro showed high percentage in antioxidative peptides. Thus, Pro, Leu, Tyr and Glu probably were the main amino acid residues to possess the antioxidant effect in Xuanwei ham. The most active peptide of Asp-Leu-Glu-Glu with m/z 505.2 $(M+H)^+$ showed 74.45% DPPH scavenging effect at the concentration of 0.5 mg/mL.

IV. CONCLUSION

Peptides purified from Xuanwei ham possess antioxidant activity based on the free radical scavenging ability. Pro-Leu-Pro, Ser-Gly-Tyr, Ser-Pro-Gly-Pro and Asp-Leu-Glu-Glu are identified to be the main peptides with strong antioxidant activity in Xuanwei ham. According to the scavenging effect on DPPH radical, the peptide with strongest antioxidant activity is identified as Asp-Leu-Glu-Glu.

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