PEPTIDE FRAGMENTS IN WATER SOLUBLE FRACTIONS EXTRACTED FROM HANWOO BEEF AS INFLUENCED BY CHILLER AGEING AND HEATING

Jieun Yang, Touseef Amna, Inho Hwang*

Department of Animal Science, Chonbuk National University, Jeon-ju, South Korea

*inho.hwang@jbnu.ac.kr

Abstract – The major taste-relevant components in meat are water soluble compounds such as amino acids, peptides. Since little is known about water soluble compounds in heat-treated post-mortem Hanwoo beef, we investigated the taste-relevant components present in Hanwoo *longissimus dorsi* muscle, heated at 77°C after 3, 14 days of post-mortem ageing. After treatment, each sample was chopped, centrifuged and ultra-filterated (< 10 kDa). Analysis of peptide fragments was determined by LC/MS, Hybrid Quadrupole-TOF LC/MS/MS Mass Spectrometer and De-novo sequencing. The specific peptide sequences (EVPEVHEEVH) only found in samples, heat-treated after 14 days, not 3 days of post-mortem ageing. In addition, these peptide fragments could be matched on bovine troponin-T fast skeletal muscle (residue 29-38). This finding indicate that the peptide fragments present in the water soluble fractions of Hanwoo beef was not only stable during heating at 77°C but also have possibility to possess taste properties.

I. INTRODUCTION

Meat taste is one of the important sensory characteristic of muscle foods with many other attributes such as texture and odor [1]. Nishimura [2] reported that meat taste have a great effect with aroma as components of the flavor. Same author suggested that water soluble compounds such as free amino acids, peptides, nucleotides, lactic acid, sugars, and minerals are the key factor of meat taste.

It is well known that beef immediately after slaughter go through post-slaughter processes such as chiller ageing, cooking and so on. This can make muscle edible in the form of meat, particularly numerous chemical and physical changes occur in compounds present in muscle such as sarcoplasmic proteins, myofibrillar contractile proteins, polypeptides, and nucleotides [3]. Moreover, a number of publications have demonstrated the importance of chiller ageing on the enhancement of meat taste attributes. As described above, the formation of taste-relevant components is based on proteolytic activity during the conversion of muscle to meat, in other words, chiller ageing and heating.

Therefore it is elucidated that combined effect of chiller ageing and heating, via proteolysis can influence on the formation of taste-relevant components in meat.

II. MATERIALS AND METHODS

Animals and sampling

A total of ten (10) 36-month-old korean native Hanwoo beef were sampled from commercial herds, and cattle were slaughtered at a commercial abattoir. Carcasses were placed in a chilling room for approximately 35 min and chilled at 4°C with wind speed of 2 m/sec at 85% humidity overnight at for 24 h. Following day, left side carcass was ribbed at 13th rib and the first lumber vertebrate, and carcass quality grade were determined. After grading, *longissimus dorsi* (LD) muscle were immediately taken from the right side of the carcasses, then vacuum packed and transferred to the Meat Science Laboratory of the Chonbuk National University under chilling condition. Subsequently, each LD muscle sample was divided into two portions (two ageing groups, 3 and 14 days) and then stored at 4°C chilling room until designated ageing periods.

Experimental design, Water soluble fractions extracted from Hanwoo longissimus dorsi muscle

The sample reaching designated ageing periods (3 or 14 days) was subjected to heat in water bath until core temperature of each sample reached at 77°C. After heating, each muscle sample was cooled in flowing water (approximately 18°C) for 30 min and then chopped (5mm or less in size). Chopped meat samples were centrifuged at 14,000 xg and 4°C for 20 min, and the resulting supernatant was

also collected. These water soluble fractions were filtered through whatman paper (No. 541) and finally were subjected to further fractionation into peptide sizes of < 10 kDa using membrane ultra-filtration and lyophilized and finally stored in -40°C.

Determination of amino acid sequence after general screening of peaks

Separation of peptide was performed using a HPLC (Agilent 1100 series, Agilent technology, USA). Thirty µl of fraction (< 10 kDa) were injected; absorbance was measured using DAD detector at 240 nm. ZORBAZ B-C18 column (1×150mm, 3.5µm, agilent, USA) was used. The mobile phase consisted of solvent A (0.2% formic acid in water (v/v)) and solvent B (0.2 formic acid in acetonitrile (v/v)). Analysis of LC/MS was operated in ESI mode. The LC/MS data was collected in the range of m/z 200-2000 with scan rate of 1 sec/scan, source temperature was 120°C. Accurate molecular weights of identified peptides were determined with Hybrid Quadrupole-TOF LC/MS/MS Spectrometer (AB Science Instruments, CA 94404, USA) coupled with positive mode electrospray ionization (ESI) source. For N-terminal amino acid sequencing, the quadrupole was operated in deconvolution method of Bayesian Peptide Reconstruct with the scan range m/z 50-2,000 and sequenced using de novo sequencing program of peptide sequence (AB Sciex Instruments, CA 94404, USA).

III. RESULTS AND DISCUSSION

Effect of chiller ageing and heating on the formation of specific peptide fragments

Small peptides and free amino acids formed by proteolysis display a tendency to improvement of taste in meat [4]. When designated chiller ageing day reached, the samples were given for heating at 77°C respectively. The resulting extracts were subjected to analysis by LC/MS analysis as described earlier in the materials and methods section. We selected 2 different kinds of water soluble fractions on the basis of chiller ageing periods (3 or 14 days). Subsequently, to know the peak pattern in the water soluble fractions extracted from Hanwoo beef which heated at 77°C after 3 and 14 days of chiller ageing, we did general screening through base peak chromatogram (BPC). In general, the HPLC profile of water soluble fractions aged for 3 days seems to be similar to 14 days (data not shown). But, there are two major peaks found in the water soluble fraction which was heated at 77°C after 14 days of chiller ageing at retention time 1-20 min. We labeled these two major peaks as peak 1 and peak 2 at retention time (RT) 14.29 and 19.99 respectively (Fig. 1). Moreover, these major peaks were given to further investigation on the basis of mass ion peak. The peak 2 has molecular ion peak at 578.6 [M+3H] (Fig. 2). The metabolite through protein breakdown was troponin-T fast skeletal muscle fragments and sequence was Glu-Val-Pro-Glu-Val-His-Glu-Glu-Val-His (Fig. 2). On the contrary, Peak 1 couldn't match with any protein fragments. Noguchi *et al*. [5] stated that some glutamic acid-containing peptides have an umami taste like MSG. Even troponin-T fragments found in our study have a four glutamic acid residue. Moreover, Nishimura (2001) stated that a peptide formed by degradation of troponin-T influences on enhancement of beef taste. Concluded that troponin-T fragments presented in water soluble fractions extracted from heat-treated (77°C) post-mortem aged (14 days) Hanwoo beef have the possibility to contribute to meat taste.

IV. CONCLUSION

In summary, we examined specific peptide fragments which roles in attributing meat taste according to post-slaughter process such as chiller ageing and heating. As a result, troponin-T fast skeletal muscle fragments (residue 29-38) was only found in water soluble fractions of Hanwoo beef heated at 77°C after 14 days not 3 days post-mortem ageing. By this is meant troponin-T fragments formed via proteolysis by aforementioned factors in Hanwoo beef is considered of a great role in meat taste.

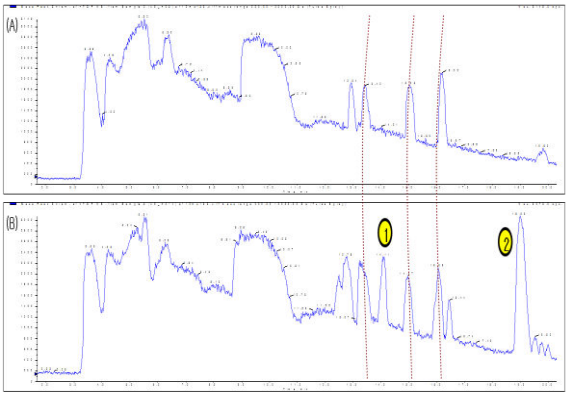

Figure 1. General screening of peak patterns of water soluble fractions extracted of Hanwoo beef as affected by ageing periods under identical heating temperature (77°C) at retention time 0-20 min. (A) 3 days post-mortem (B) 14 days post-mortem ageing

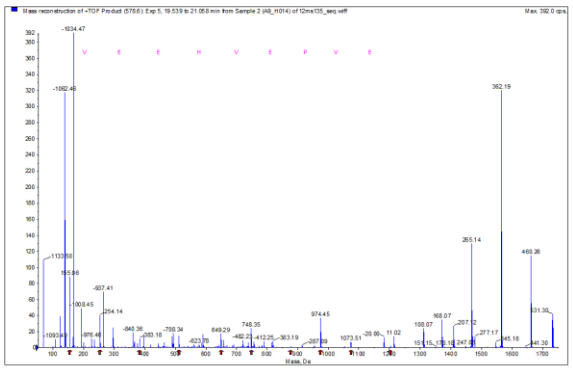

Figure 2. Identification of peptide fragments. Following sequence was matched to the troponin-T fast skeletal muscle type of Bos taurus (residues 29-38)

REFERENCES

1. Sasaki, K., Motoyama, M., & Mitsumoto, M. (2007). Changes in the amounts of water-soluble umami-related substances in porcine longissimus and biceps femoris muscles during moist heat cooking. Meat science, 77:67-172.
2. Nishimura, T. (1998). Mechanism Involved in the Improvement of Meat Taste during Postmortem Aging. Food Science and Technology International, Tokyo, 4:241-249.
3. Davis, C. E., & Anderson, J. B. (1984). Size exclusion/HPLC of heated water soluble bovine and porcine muscle proteins. Journal of Food Science, 49:598-602.
4. Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. Critical Reviews in Food Science, 38: 331-352.
5. Noguchi, M., Yamashita, M., Arai, S., & Fujimaki, M. (1975). On the bitter-masking activity of a glutamic acid-rich oligopeptide fraction. Journal of Food Science, 40: 367-369.
6. Nishimura, T. (2001). Palatability on texture, aroma, and taste of meat. Japanese Journal of Taste and Smell Research, 8, 161-168.