IDENTIFICATION OF SOURNESS-SUPPRESSING PEPTIDES IN COOKED PORK LOINS

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Background

It is well known that meat becomes more palatable with postmortem conditioning, which brings about not only tenderization but also improvement of flavor. The free amino acids and peptides that increase during postmortem aging play an important role in the improvement of meat taste (Nishimura, T., 1998). Some reports have indicated that there are peptides that change taste perception. It has been shown that dipeptides such as Gly-Glu, Pro-Glu and Val-Glu have a buffering action and improve the taste of Japanese sake. Glutamic acid-rich oligopeptides and casein hydrolysate have been reported to mask a bitter taste (Noguchi et al., 1975; Tamura et al, 1990). It has been reported that the addition of hydrolyzed wheat gluten to an umami taste solution enhanced its strength. A peptide fraction of MW 1,000-10,000 from beef vacuum-cooked at 60 ^{o}C for 6 hours has been reported to improve the taste of un-aged beef soup by suppressing its sourness（Ishii et al, 1995. Peptides enhancing umami in the presence of inosinic acids were also isolated from chicken hydrolysate by protease (Maehashi, et al, 1998). However, in the case of pork, there is little information on peptides that improve meat taste.

Objectives

The present work was conducted to identify the peptides that suppressed the sourness and improved the taste of vacuum-cooked pork loins after *post mortem* conditioning of 20 days at +4^{o}C. This study was also carried out to clarify the mechanism of sour taste suppression by the peptides.

Materials and methods

Preparation of peptide fractions

Pork loins were stored in the vacuum-packages at +4^{o}C for 20 days after slaughter, and the packages were heated in a water bath at 60^{o}C for 360 min. The ground pork loin was freeze-dried and the lipids were extracted with n-hexane. The ground lean muscle was then homogenized with a ten-fold volume of de-ionized water. The homogenate was centrifuged at 10,000×g for 20 min and the supernatant was collected. Ethanol was added (final concentration, 80%) to this supernatant, followed by centrifugation and then filtration. After evaporation and further freeze-drying of the filtrate, the LD muscle powder was dissolved in de-ionized water. The solution was ultrafiltrated through MW500 and 1,000 cut-off membranes (Amicon Co., Beverly, USA), and then freeze-dried. Each peptide fraction, Fraction Ⅰ (MW < 500), Fraction Ⅱ (MW 500-1,000), or Fraction Ⅲ (MW > 1,000), was obtained.

Sensory evaluation

Using a modification of the paired-preference tests, the changes in the strength of basic tastes on the addition of the peptide fractions were evaluated by a well-trained panel. The relative difference was expressed within a scale of -5 to 5 points, the point in the absence of a peptide fraction being taken as zero. Solutions of 0.2% lactic acid (sourness), 0.04% monosodium glutamate (umami), 0.001% quinine hydrochloride (bitterness), 0.5% sodium chloride (saltiness), and 1.0% sucrose (sweetness) were used as basic taste solutions for sensory evaluation.

Peptide analysis

Peptides were analyzed by HPLC on a reversed-phase column (Senshupack VP-318, Senshu Sci. co., Tokyo, Japan). The filtrate was applied on the column and eluted with a linear concentration (0-40%) gradient of acetonitrile containing 0.1% trifluoroacetic acid at 40 ^{o}C for 80 min. The flow rate was 1.0 ml/min and peptides were detected at 220 nm. The amino acid sequences of the peptides were determined with an ABI amino acid sequencer (Model 477A, Applied Biosystem Japan, Tokyo, Japan), and the molecular weights were measured with a mass spectrometer (TSQ 7000, Thermo Finnigan, San Jose, USA).

Assaying of binding to epithelial tissue of porcine tongues

The epithelial tissue containing foliate papillae was removed from the underlying dermis of fresh porcine tongue with a scalpel and fine forceps. After mincing with scissors, the epithelial tissue was homogenized with a ten-fold volume of 10mM Tris-HCl buffer (pH7.4) containing 10 mM $CaCl_2$ and 30 mM mannitol, and the homogenate was centrifuged at 30,000×g for 20 min. The precipitate was further homogenized with 10mM Tris-HCl buffer (pH 7.4) containing 100 mM mannitol, and the homogenate was centrifuged at 40,000×g for 20 min. This precipitate then served as the epithelium of porcine tongues.

The binding of radioactively labeled L-lactic acid (L-[U-^{14}C] lactic acid, sodium salt (120 Ci /m mol)) to the epithelium was measured. Sodium lactate solution (0.15 ml; 0.5%) containing labeled L-lactic acid and K-phosphate buffer (pH 7.2; 0.04 ml; 0.5M) was then added to 0.15 ml of epithelium solution (approximately 0.17mg protein/ml). Then, 0.16 ml of a peptide fraction solution or de-ionized water was added to this mixture, the final total assay volume being 0.4 ml. The same volume of 10 mM Tris-HCl buffer (pH 7.4) instead of the epithelium solutions was used as a blank. Each sample was incubated at room temperature for 10 min and then filtered rapidly through a 0.45μm cellulose acetate filter (Nihon Millipore Ltd., Tokyo, Japan). After filtration, the filter was immediately rinsed with 10 ml buffer 20 mM Tris-HCl buffer, pH） and the filter disc then placed into a bottle containing 5 ml of scintillation fluid (Aquasol^{TM}, New England Nuclear Co., Montpellier, USA). Then the radioactivity (C-DPM) of the samples was counted with a liquid scintillation counter (LSC-5100, Aloka Co., Mitaka, Japan). Each binding value was calculated by subtraction of the C-DPM value for the blank without epithelium from that with epithelium.

Assaying of binding to a synthetic lipid membrane with a taste sensor

The binding of sour taste substances to a synthetic lipid membrane composed of two types of lipids, dioctylphosphate and triocthylmethyl ammonium chloride, was measured with a taste sensor (SA401, Anritsu Co., Atsugi, Japan). A tartaric acid solution (1-10mM) containing 10 mM KCl was used as the sour taste solution. Using the taste sensor system, binding of tartaric acid to the membrane was relatively well reflected by the electric potential (mV) of the taste sensor. The electric potential of the tartaric acid solution with or without the peptide fraction was measured.

Results and discussion

Recovery of peptide fractions of pork

The amount of Peptide fraction I (MW < 500) in pork loins gradually increased as the storage period became longer, as did that of Fraction II (MW 500-1,000) up to 20 days. However, the amount of Peptide fraction III (MW > 1,000) remained unchanged throughout storage for 30 days. The result that peptides increased during storage for 20 days is in accordance with our previous study (Okumura, 1996). The amounts of peptides in fractions I, II and III from pork loins stored for 20 days were 30.2, 7.5 and 1.6 mg/g meat, respectively. With heating at 60°C for 6 hours after *post mortem* conditioning of two days, the amount of all three peptide fractions increased. However, in the pork stored for 20 days the fractions remained almost unchanged. Although we did not examine the reason for this, the fact that the activities of endogenous proteases in porcine muscle were lost during *post mortem* aging for 20 days may be responsible for the phenomenon.

Effect of addition of peptides fraction on basic tastes

The effect of added peptide fractions on the basic tastes were examined by sensory evaluation. Figure 1 shows that the sourness of a lactic acid solution by addition of Fraction II (0.074%) from pork loins stored at 4°C for 20 days was weaker than that without peptide fraction, while the addition of Fraction Ⅰ (0.37%) or III (0.016%) had little effect on sourness suppression. This indicated that some peptides in Fraction II suppressed sourness. The increase of these peptides during conditioning seemed to play an important role in the change of the sourness of unaged pork. Although the taste of beef taken from the carcass immediately after slaughter is sour and bloody, meat after conditioning at a low temperature has no such taste. Mild taste created during conditioning may be related to an increase in peptides. Therefore, it seemed that Fraction II masked the sourness or metallic taste of immediately *post mortem* pork meat and improved the taste during conditioning.

Purification and identification of the peptides suppressing sourness

In order to identify peptides suppressing sourness, the analysis of peptides in Fraction II of pork loins stored for 2 and 20 days at 4°C was performed. Figure 2 showed that the main peaks indicated by arrows presenting peptides (1), (2) and (3), were greatly increased during storage. On amino acid analyses of the peptides with

an amino acid sequencer and a mass spectrometer, peptides (1), (2), and (3) were identified as APPPPAEVHEVV, APPPPAEVHEVVE and APPPPAEVHEVHEEVH. These peptides were homologous to the peptide, APPPPAEVHEVHEEVH that was generated from troponin T in pork during *post mortem* conditioning. The structure of these peptides was similar to that of the peptide increasing during *post mortem* aging of beef. Many researchers have reported that troponin T was degraded by the activity of proteases during *post mortem* aging of chicken, porcine and bovine muscles at low temperature. The purified troponin T has also been shown to be degraded by calpain and cathepsin, indicating that the peptide APPPPAEVHEVHEEVH seems to be produced by the action of calpain and / or cathepsin. The clarification of the mechanism is the next problem to be resolved.

Using synthetic peptide, APPPP or APPPPAEVHEV, which are common amino acid sequences in the three peptides, the effect of these peptides on sourness was examined by sensory evaluation. The addition of APPPP to a lactic acid solution did not change the sour taste, whereas the addition of APPPPAEVHEV suppressed the sour taste. From these results, it was concluded that the three peptides containing common sequences in Fraction II suppressed the sour taste, and the increases in these peptides during conditioning delivered the mildness to the taste of meat. This is the first report that discovered the peptide suppressing sourness.

Mechanism of suppression of sourness on the addition of peptides

i) Binding assay of peptide using the epithelium of porcine tongue: The effect of concentration of peptide on the lactate binding to the epithelium was examined. The higher the concentration of Fraction Ⅱ added to the sample solution at pH 5 was, the lower was the amount of lactic acid binding to the epithelium (Fig. 3).

ii) Binding of tartaric acid to a synthetic lipid membrane: The binding of tartaric acid to a synthetic lipid membrane was also inhibited by the addition of peptide (3) in Fraction II, suggesting that Fraction II inhibited the binding of sour taste substances to the membranes of tongues. Furthermore, this inhibitory action depended on the concentration of Fraction II. It was concluded that the peptides of Fraction II derived from pork extract suppressed sourness through its interaction with sour taste channel or sour taste substances. That is, sour taste substances can not reach sour taste ion channel, because the peptide is either binding with sour taste substances or covering the entrance of sour taste ion channel (Fig. 4).

Conclusions

A peptide fraction (Fraction II; molecular weight 500-1000) from pork loins were cooked in vacuum-packages at 60°C for 6 hours suppressed sourness. Three peptides (APPPPAEVHEVV, APPPPAEVHEVVE and APPPPAEVHEVHEEVH) were isolated from Fraction II. These peptides seemed to suppress sourness through their interaction with sour taste channels or sour taste substances.

References

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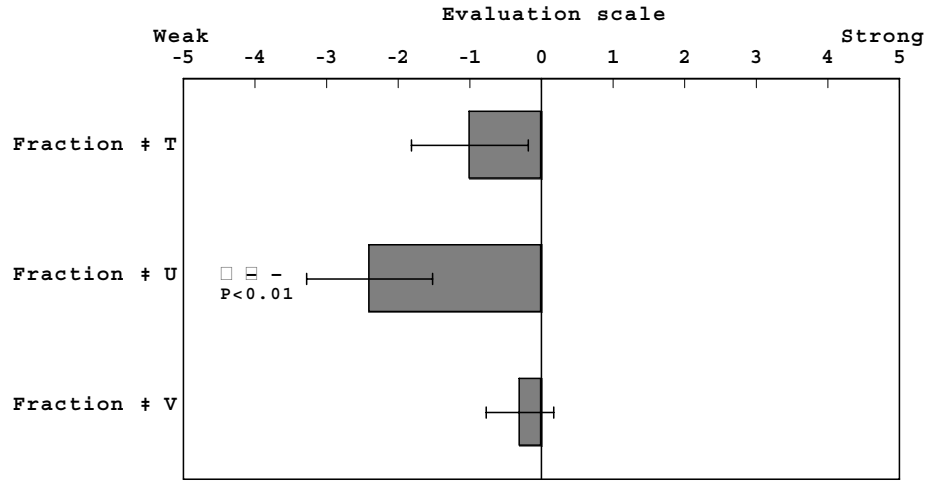

Figure 1. Effects of addition of peptide fraction on sourness intensity

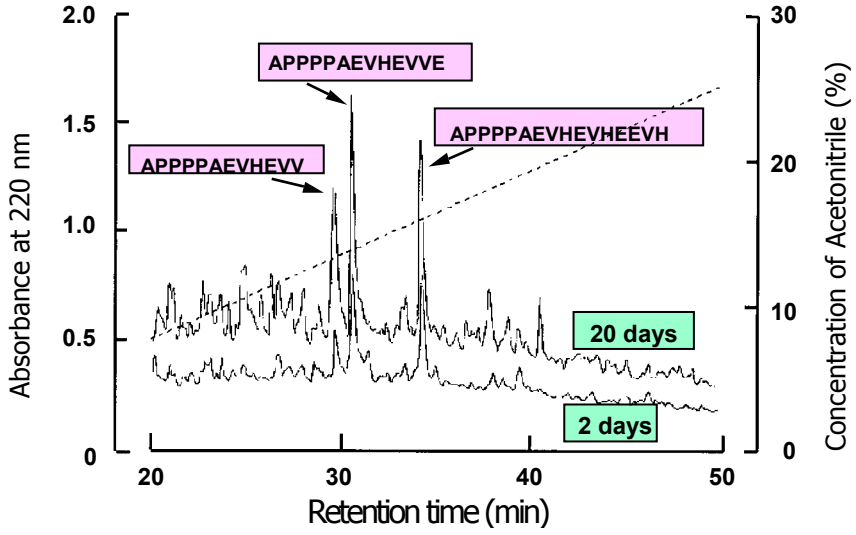

Figure 2. HPLC profile of Fraction II (MW 500-1000) in pork loins stored at 4 °C for 2 or 20 days.

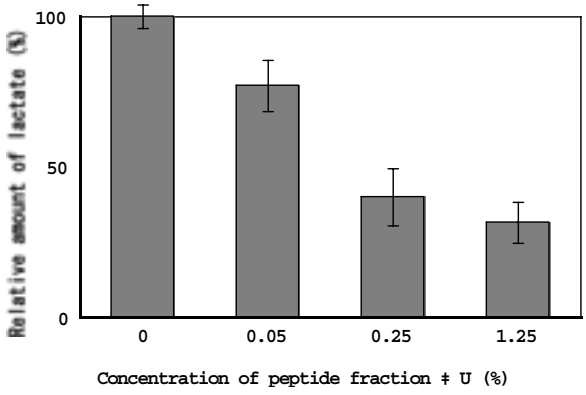

Figure 3. Effect of Fraction II on lactic acid binding to tongue epithelium

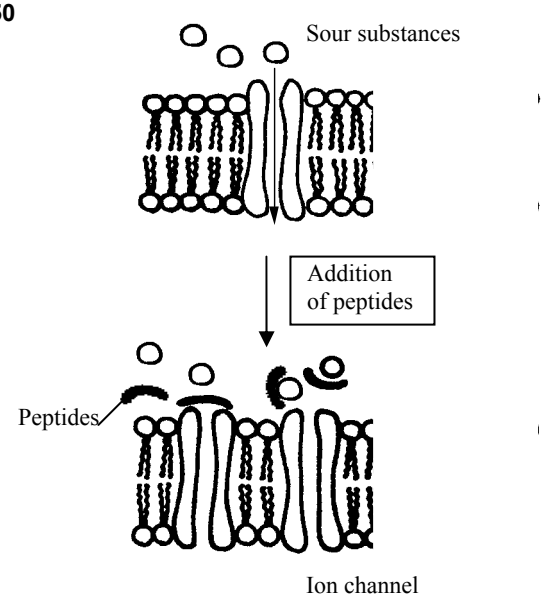

Figure 4. Proposed mechanism of suppression of sourness by addition of peptides