Sourness-suppressing Peptides in Cooked Pork Loins

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This study was conducted to identify the sourness-suppressing peptides in cooked pork and to clarify the mechanism of sour taste suppression by the peptides. An extract prepared from pork loins vacuum-cooked at 60 °C for 6 hours after conditioning at 4 °C for 20 days was separated into three fractions: under MW 500 (Fraction I), MW 500–1,000 (Fraction II), and over MW 1,000 (Fraction III). The Fraction I content was largest. As judged by sensory evaluation, the addition of Fraction II was capable of suppressing stronger sourness than the other fractions. Fraction II also enhanced umami and saltiness. Three peptides (APPPPAEV-HEVV, APPPPAEVHEVVE, and APPPPAEVHEV-HEEVH) in Fraction II increased greatly during conditioning. A common peptide, APPPPAEVHEV, in the amino acid sequences of the three peptides suppressed the sour taste. The mechanism of sourness suppression by the peptide was concluded to comprise inhibition of the binding of sour taste substances to the membranes of the tongue.

Key words: sourness-suppressing peptide; postmortem conditioning; vacuum cooked

It is well known that meat becomes more palatable with postmortem conditioning, which not only causes tenderization but also improves the flavor. The free amino acids (FAA) and peptides that increase during postmortem aging play an important role in the improvement of meat taste.1–4 Some reports have indicated that there are peptides that change taste perception. It has been shown that carnosine and anserine exhibit a buffering activity,5 and that the dipeptides Gly-Glu, Pro-Glu, and Val-Glu have a buffering action and improve the taste of Japanese sake.6 Glutamic acid-rich oligopeptides7 and casein hydrolysate8 have been reported to mask a bitter taste. Ishii et al.9 have reported that the addition of a MW 500–1,000 fraction of hydrolyzed wheat gluten to a umami taste solution enhanced its strength, and the addition of a peptide fraction of MW 1,000–10,000 to an extract of un-aged beef has been reported to improve the taste by suppressing its sourness.10

A “delicious peptide” in beef hydrolyzed by papain has been isolated11,12 and an amino acid-derivative which imparts a favorable “broth taste” has been isolated from beef.13 On the other hand, in the case of pork, the molecular weights and structures of peptides that improve taste have not been determined and the mechanisms of sourness suppression by the peptides have not been clarified.

Hence, we did the present work to identify the peptides that suppressed the sourness and improved the taste of vacuum-cooked pork loins after postmortem conditioning for 20 days at 4 °C, and to clarify the mechanism of sour taste suppression by the peptides.

Materials and Methods

Materials. Pork loins were obtained from the carcasses of porcine hybrids (Landrace × Large white × Hampshire). The loins were stored in vacuum packing at 4 °C for 20 days after slaughter. Some stored loin muscles were heated at 60 °C in a water bath for 6 hours in the vacuum-packed state. After storage and further heating, they were frozen until used. They were used after being thawed at 4 °C.

Preparation of peptide fractions. The LD (Longissimus dorsi) muscles of pork loins were ground through a 3-mm plate. The ground LD muscle was freeze-dried and then grease was removed by adding 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, California, USA), and then freeze-dried. The various peptide fractions, Fraction I (under MW 500), Fraction II (MW 500–1,000), and Fraction III (over MW 1,000), were obtained.

Abbreviations: FAA, free amino acids; LD, Longissimus dorsi; Car, carnosine; Ans, anserine

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Assaying of binding to epithelial tissue of porcine tongues. Fresh porcine tongues were obtained from a local slaughterhouse and stored at 1 °C for 2 days after slaughter. The epithelial tissue of the porcine tongues was prepared according to a modification of the Ca$^{2+}$ precipitate refining method.14) The epithelial tissue containing foliate papillae was removed from the underlying dermis with a scalpel and fine forceps. After mincing with scissors, the epithelial tissue was homogenized with a ten-fold volume of 10 mM Tris–HCl buffer (pH 7.4) containing 10 mM CaCl$_2$ and 30 mM mannitol, and the homogenate was centrifuged at 30,000 $\times$ g for 20 min. The precipitate was further homogenized with 10 mM Tris–HCl buffer (pH 7.4) containing 100 mM mannitol, and the homogenate was centrifuged at 40,000 $\times$ g for 20 min. The precipitate was used as the epithelium of porcine tongues.

The binding of radioactively labeled L-lactic acid to the epithelium was measured by a modification of the method of Torii and Cogan.15) L-[U-$^{14}$C] lactic acid, sodium salt (120 Ci/mmol), purchased from Amersham Bioscience Co. (London, UK), was used as labeled L-lactic acid. To 0.15 ml (approximately 0.17 mg protein/ml) of epithelium solution were added 0.15 ml of a 0.5% lactate sodium solution containing labeled L-lactic acid and 0.04 ml of 0.5 mM K-phosphate buffer (pH 7.2). Then, 0.16 ml of a peptide fraction solution or deionized 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 0.45 μm 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 7.2) and the filter disc was placed in a bottle containing 5 ml of scintillation fluid (Aquasol$^{TM}$, New England Nuclear, Montpelier, Vermont, USA), then the radioactivity (C-DPM) in 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 lipid species, dioctylphosphate and trioctylmethylammonium chloride, was measured with a taste sensor (SA401, Anritsu Co., Atsugi, Japan).16–18) A tartaric acid solution (1–10 mM) containing 10 mM KCl was used as the sour taste solution. Using the taste sensor system, the binding of tartaric acid to the membrane was relatively well reflected by the electric potential (mV) of the taste sensor. The electrical potential of the tartaric acid solution with or without the peptide fraction was measured.

Results and Discussion

Recovery of peptide fractions of pork

Table 1 shows the changes in the amounts of peptide fractions in extracts of pork loins on conditioning at 4 °C with vacuum-packed heating at 60 °C for 6 hours. The Fraction I (under MW 500) content in pork gradually increased as the storage period became longer, and that of peptide fraction II (MW 500–1,000) also increased up to 20 days. However, that of peptide fraction III (over MW 1,000) was unchanged throughout storage for 30 days. The peptides increased during storage for 20 days coincided with the results of our previous paper.19) With heating at 60 °C for 6 hours after postmortem conditioning, the amount of each of the peptide fractions of pork stored for 2 days increased, on the other hand, that of the...
pork stored for 20 days was almost unchanged. Although we did not investigate the reason, peptides did not seem to increase by inactivation of endogenous after post-mortem aging for 20 days.

Sensory properties of peptides fraction

Sensory properties were examined by sensory evaluation. Figure 1 shows that the sourness of a lactic acid solution after adding Fraction II of an extract of pork loins stored at 4°C for 20 days was weaker than in the case of Fraction I or III. This indicated that Fraction II suppressed sourness. The increase in these peptides during conditioning appeared to play an important role in the change in the sourness of unaged pork to mildness. 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. Such a mild taste induced by conditioning has been reported to be similar to those of the peptides in the 1,000–10,000 fraction of beef.

Ishii et al. investigated the effect of adding peptide fractions obtained from aged beef cooked at 60°C for 6 hours on the taste of the under 500 fraction solution prepared from non-aged beef cooked at 100°C for 10 min. They showed that the addition of the MW 1,000–10,000 fraction significantly suppressed the sourness and enhanced overall preference, although the addition of the MW 500–1,000 or over MW 10,000 fraction had no effect. The element of MW 1,000–10,000 fraction suppressing sourness have not yet been identified and isolated. However, in case of pork, the MW 500–1,000 fraction showed the suppression of sourness. The structure of these peptides in pork appears to be similar to those of the peptides in the 1,000–10,000 fraction of beef.

The taste of a 0.08% Fraction II solution was hardly noticed by most of the panel on sensory evaluation (data not shown). Figure 2 shows the effect of adding Fraction II on the basic taste solution, including sourness. It was also clarified that the umami of the monosodium glutamate solution and the saltiness of the sodium chloride solution both became stronger on adding 0.08% Fraction II, but bitterness and sweetness were unchanged. These results showed that Fraction II caused the umami and salty taste reinforcement as well as sourness suppression. It has been reported that the increase in free amino acids and oligopeptides on prolonged conditioning of pork was identical with that

Table 1. Amounts of Peptide Fractions Obtained on Extraction of Cooked Pork Loins

<table>
<thead>
<tr>
<th>Fraction (molecular weight)*</th>
<th>Amount** (mg/g meat)</th>
<th>Conditioning (days)</th>
<th>Heating after postmortem conditioning (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>I (less than MW500)</td>
<td>9.84</td>
<td>16.82</td>
<td>37.60</td>
</tr>
<tr>
<td>II (MW500–1,000)</td>
<td>1.48</td>
<td>4.18</td>
<td>7.48</td>
</tr>
<tr>
<td>III (more than 1,000)</td>
<td>1.39</td>
<td>1.44</td>
<td>1.49</td>
</tr>
</tbody>
</table>

Extract was prepared from pork loins stored under vacuum-packing at 4°C and by vacuum-packed heating at 60°C for 6 hours.

* Three fractions were obtained from pork loin extracts with an ultrafiltration membrane of MW 500 or MW 1,000 (Amicon Co.).
** The weights of fractions after freeze-drying per weight of wet meat are presented as the amounts.
of taste intensity and total palatability.\textsuperscript{3,21)} Tamura \textit{et al.}\textsuperscript{19}) reported that the umami of the under MW 500 fraction obtained from beef cooked at 60 °C for 6 hours was significantly enhanced by the addition of the MW 500–1,000 fraction. However, our results showed that Fraction II (MW 500–1,000) of pork suppressed the sour taste and enhanced the umami or salty taste. Peptides possessing the enhancement of umami of inosinic acid were isolated from chicken muscle hydrolysate by bromelain, their structure being Glu-Glu, Glu-Val, Asp-Glu-Glu, and Glu-Glu-Asn.\textsuperscript{22)} The MW 500–1,000 fraction of pork was also thought to contain these peptides identified in chicken muscle hydrolysate.

**Purification and identification of the peptides suppressing sourness**

In order to identify peptides suppressing sourness, an analysis of peptides in Fraction II of pork loins stored for 2 and 20 days at 4 °C was performed. Figure 3 shows that the main peaks indicated by arrows, peptides (1), (2), and (3), were greatly increased during storage. On amino acid analysis of the peptides with an amino acid sequencer and a mass spectrometer, peptides (1), (2), and (3) were identified as APPPPAEVHEV, APPPPAEVHEVVE, and APPPPAEVHEVHEEV respectively. These peptides were homologous to the peptide APPPPAEVHEVHEEV, which was generated from troponin T in pork during postmortem conditioning.\textsuperscript{23)} The structure of these peptides was similar to that of the peptide that increased during postmortem aging of beef.\textsuperscript{24)} Many researchers have reported that troponin T was degraded by the activity of proteases during postmortem aging of chicken,\textsuperscript{25)} porcine,\textsuperscript{23)} and bovine\textsuperscript{26)} muscles at low temperatures. The purified troponin T was also shown to be degraded by calpain and cathepsin,\textsuperscript{27,28)} indicating that the peptide APPPPAEVHEVHEEV was produced by the action of calpain or/and cathepsin. The clarification of the mechanism is the next problem to be solved.

Using synthetic peptide, APPPP or APPPPAEVHEV, the 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, but the addition of APPPPAEVHEV suppressed it (Fig. 4). From our results, it was concluded that the three peptides containing common sequences in Fraction II suppressed the sour taste, and increases in these peptides during conditioning imparted a mildness to the meat taste. This is the first report to discover a peptide suppressing sourness.

**Mechanism of suppression of sourness on the addition of peptides**

Binding assays were performed using epithelium of porcine tongue and synthetic lipid membrane in order to examine the mechanism of suppression of sourness on the addition of peptide.

i) Binding assay of peptide using 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, the lower was the amount of lactic acid binding to the epithelium (Fig. 5). The effect of pH on lactate binding was also examined. The amount of lactic acid bound in the presence of Fraction II was arbitrarily taken to be 100%. Each value is the average for ten samples. The bar indicates the standard deviation.

**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 (Fig. 7). These results indicated that Fraction II inhibited the interaction of sour taste substances to the membranes of tongues. Furthermore, this inhibitory action depended on the concentration of Fraction II and was higher at neutrality.

It has been reported that the causative agents of sourness are protons, and proton sensitive ion channels located in taste cells have been considered to act as sour-taste receptors.29,30 It was concluded that the peptides (Fraction II) derived from pork extract suppressed sourness through their interaction effects on proton sensitive ion channels or sour taste substances. That is, sour taste substances cannot reach the proton sensitive ion channels of sour taste due to their interaction with sour taste substances or their covering of the entrance of proton sensitive ion channels, caused by the proposed mechanism shown in Fig. 8. The next problem to be resolved is the clarification of the relationship between the suppressing activity and structure of peptides.
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References


