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Analysis of Pork Extracts by Taste Sensing System and the Relationship between *Umami* Substances and Sensor Output

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We investigated the application of a taste sensing system to the analysis and evaluation of a water extract prepared from the *longissimus* muscles of six breeds of pork. We selected sensor probes that discriminate among the different pork breeds and applied the output data from selected sensor probes to principal component (PC) analysis. Then the PC scores were used in a correlation analysis with the concentrations of the *umami*-related substances in pork extracts. We found that sensor output was related to the concentrations of the *umami*-related substances.

1. Introduction

In Japan, the evaluation and classification of pork carcasses have been carried out mainly with regard to their yield and visual properties such as lean meat color, fineness of muscle fiber, fat color, and physical properties of the fat. These properties, especially visual properties, are currently important for consumers' choice of muscle foods in the market.(1) On the other hand, analysis and improvement of pork taste have been necessary for the satisfaction of varied consumers' requirements. The visual properties of pork carcasses are related to the quality of muscle. However, actual taste is not analyzed in the evaluation and classification of pork. For evaluation of the taste of the pork or other species

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of meat, sensory evaluation has been used, but such sensory evaluation has not been adapted for the routine evaluation of pork carcasses because it has been difficult in the market to retain a sensory panel and to maintain objectivity and repeatability of evaluation.

In recent years, a biomimetic taste sensing system has been developed. Toko and co-workers have developed this taste sensing system using the electrical potential of a lipid-containing membrane[2] and applied it to the analysis and evaluation of taste in various kinds of foods.[3] We examined the lipid-membrane taste sensing system to see if it could be used to discriminate and to evaluate the actual taste of pork with objectivity and/or repeatability.

In this study, we applied the taste sensing system to the discrimination and evaluation of pork. First, we analyzed the water extracts of pork *longissimus* muscles for discrimination of the taste of pork meat. In addition, we examined the relationship between sensor output data and *umami*-related substances, which are considered important for the taste of the water-soluble fraction of pork.

2. Materials and Methods

2.1 *Sample preparation*

Pork *longissimus* muscles were harvested from Landrace, Duroc, Large White, Meishan, Berkshire and Three-way cross (Duroc × Landrace × Large White) pork carcasses. Duroc carcasses were obtained from two different producers to investigate the variability in taste by producer, and these samples were designated Duroc(A) and Duroc(B). All samples were cut into cubes (each side was approximately 1.0 cm) and extracted by boiling them in distilled water for 1 h. These extracts were filtered with Advantec No. 131 filter paper and were one-tenth the volume of the sample muscles. The extract solution was then used for analysis by the taste sensing system.

2.2 *Analysis by taste sensing system*

We used a taste sensing system SA402 (Anritsu Co. and Intelligent Sensor Technology, Inc.) equipped with eight different lipid membrane probes for the analysis. The composition of each lipid membrane is presented in Table 1. The measurement procedure was previously described by Ikezaki *et al.*[4] Briefly, zero-adjusted probes were applied to a sample solution, and the sample output was collected. After that, a blank solution was measured again before the probe was washed. The output was called the change of membrane potential caused by adsorption (CPA), which was fit for an aftertaste.[4] Then, probes were washed and zero-adjusted for measurement of the next sample.

Commercial beef extract (F-6359, Ariake Japan Co., Tokyo) was used to calibrate the sensor output. Differences in the sample output and CPA output between samples and commercial beef extract were used as the measured output. Two sets of output data, such as sample output and CPA output, were collected by the taste sensing system from pork extracts. The output data were then subjected to statistical analysis.

Table 1
Lipids in sensor probes used in this study.

Lipid
Dioctyl phenyl phosphonate / Hexadecanoic acid
Potassium tetrakis 4-tert-butylphenyl borate / Bis 1-butylpentyl adipate
Dioctyl phenyl phosphonate / Phosphoric acid di-n-decyl ester
2-Nitrophenyl octyl ether / Phosphoric acid di-n-decyl ester
2-Nitrophenyl octyl ether / Tetradodecylammonium bromide
Dioctyl phenyl phosphonate / Tetradodecylammonium bromide
Tetradodecylammonium bromide
Dioctyl phenyl phosphonate / Phosphoric acid di-n-hexadecyl ester / Teteradodecylammonium bromide

2.3 *Determination of umami-related substances*

Inosin monophosphate (IMP), oligopeptides, glutamate, and total free amino acids were analyzed for *umami*-related substances as described previously.[5]

IMP, glutamate, and total free amino acids were determined by high-performance liquid chromatography (HPLC). For IMP determination, pork extracts were treated with perchloric acid to remove proteins and neutralized to pH 6.5–6.8 with a potassium hydroxide solution. Then the filtrate of neutralized samples was assayed using HPLC. The assay conditions have been described previously for a JASCO Filepak SIL C18T column (4.6×250 mm; JASCO, Tokyo). IMP was measured with an ultraviolet detector (UV-1570; JASCO) set at 254 nm. The mobile phase was 40 mM potassium dihydrogenphosphate – 60 mM dipotassium hydrogenphosphate (pH not adjusted), and the flow rate was 1.0 mL / min.

For the analysis of free amino acids and glutamate, the extract solution was treated with a final addition of 5.0% (w/v) trichloroacetic acid to remove proteins and applied to an amino acid auto-analyzer L-8500 (Hitachi, Co., Tokyo).

Oligopeptides were evaluated by Lowry's method in a 2% (w/v) trichloroacetic acid soluble fraction of pork extracts.

2.4 *Statistical analysis*

Sensor output (sample and CPA output) data were applied to the general linear model (GLM) for selection of the probes that to discriminate differences among pork breeds.

Subsequently, output data from the selected probes were analyzed by principal component (PC) analysis. Then the PC scores and the concentrations of the *umami*-related substances were applied to correlation analysis. The differences in *umami*-related substances among the samples were analyzed using the GLM and Duncan's multiple range test.

All statistical analyses were carried out by the SAS system ver 6.12 (SAS Institute, Cary, NC).

3. Results and Discussion

First we analyzed the differences between normal and CPA output of each sensor probe among the pork breeds. Table 2 indicates the probability of the differences in sensor output were significant among the breeds of pork analyzed by the GLM procedure. Sensors No. 1 and 2 did not detect any difference between normal and CPA output. The differences in normal output of sensors No. 5, 6, 7, and 8, and CPA output of sensors No. 3, 4, and 7 were statistically significant among the breeds ($P < .05$). These seven data points were applied to the following PC analysis.

The results of PC analysis are presented in Table 3. Eigenvalues of PCs 1 and 2 were over 1.0. The cumulative proportion of PCs 1 and 2 was .7656. Therefore, we applied the scores of PCs 1 and 2 to the following analysis.

Mean values of *umami*-related components in extracts prepared from each breed are shown in Table 4. Concentrations of IMP, total amino acids, and oligopeptides were significantly different among the breeds ($P < .001$). Glutamate concentration, however, was not different among the breeds ($P = .976$). IMP concentration was the highest in Duroc(A), the second highest in Meishan, and the lowest in Duroc(B). Oligopeptide concentration was the highest in Meishan, and the second highest in Landrace, and the

Table 2
Probability that the differences in sensor output were significant among the breeds of pork. The indication 'ns' means not significant ($P > .05$).

	Probability	
Sensor No.	Sample output	CPA outout
1	ns	ns
2	ns	ns
3	ns	$P < .001$
4	ns	$P < .001$
5	$P < .001$	ns
6	$P < .001$	ns
7	$P < .001$	$P < .001$
8	$P < .001$	ns

Table 3
Principal component analysis of seven sets of output data of the taste sensing system applied to pork extracts.

Principal component	Eigenvalue	Proportion	Cumulative
1	4.0876	0.5840	0.5840
2	1.2714	0.1816	0.7656
3	0.8011	0.1144	0.8800
4	0.3541	0.0506	0.9306
5	0.2794	0.0399	0.9705
6	0.1326	0.0189	0.8985
7	0.0738	0.0105	1.0000

Table 4
Concentrations of *umami*-related components in extracts prepared from each breed. Values are expressed as means ± SD. Values with different superscripts in each measurement differ significantly ($P < .05$).

	N	IMP (nmol/ml)	total amino acids (mmol/ml)	oligopeptides (mg/ml)	glutamate (nmol/ml)
Landrace	8	56.8±5.9 ^{ab}	0.89±0.08 ^{d}	0.42±0.03 ^{b}	65.2±14.7
Duroc(A)	5	47.8±4.6 ^{c}	1.25±0.10 ^{b}	0.38±0.07 ^{bc}	61.2±29.7
Duroc(B)	8	61.9±5.5 ^{a}	1.06±0.18 ^{bcd}	0.35±0.03 ^{c}	74.2±44.6
Large White	5	49.7±5.9 ^{c}	1.04±0.08 ^{bcd}	0.29±0.03 ^{d}	70.3±26.7
Meishan	6	60.1±3.7 ^{a}	0.99±0.07 ^{cd}	0.47±0.03 ^{a}	63.9±11.2
Berkshire	5	51.8±3.1 ^{bc}	1.74±0.43 ^{a}	0.34±0.04 ^{c}	77.5±64.4
Three way cross	5	49.3±5.9 ^{c}	1.21±0.20 ^{bc}	0.29±0.02 ^{d}	74.8±26.7

lowest in Large White. Total amino acid concentration was the highest in Burkshire, but was low in Meishan and Landrace, although their IMP and oligopeptide concentrations were relatively high.

The correlation coefficients between PC scores and concentrations of *umami*-related substances are indicated in Table 5. The score of PC1 was negatively correlated with IMP and oligopeptides at a significance level of $P < .001$. On the other hand, total free amino acid concentration was positively correlated with PC1 ($P < .01$). IMP concentration had positive and significant correlation with oligopeptide concentration ($r = .327$, $P < .05$) and was negatively correlated with total amino acid concentration ($r = –.26$, $P = .088$). The correlations between PC1 and *umami*-related substances were in good agreement with the correlations of total amino acids with IMP and oligopeptides. Sensor probes used in this study include multi-taste-sensitive, *umami*-taste-sensitive, bitter-taste-sensitive, and salty-taste-sensitive. We did not investigate the correlations between sensor sensitivity and each concentration of *umami*-related components, but we considered the taste sensing system useful for evaluation of the taste of pork extracts, especially *umami*-related taste, from the results presented in Table 5.

Figure 1 is an X-Y plot of the pork samples with PCs 1 (horizontal axis) and 2 (vertical axis). Samples were separated to some degree among the breeds. According to the results of the correlation between PCs and concentrations of *umami*-related substances, we concluded that the left side of the plot (negative values of PC1) tended to indicate 'IMP and oligopeptide originated *umami*', and the right side (positive values of PC1) tended to indicate 'amino acids originated *umami*'. In Fig. 1, Meishan and Duroc(B) are located on the left side (IMP and oligopeptide side) of the plot, and Berkshire, Duroc(A), and Large White are mostly located on the right side (amino acid side). These results are in good agreement with the difference in concentrations of the *umami*-related substances among the breeds indicated in Table 4.

The taste of pork and other kinds of muscle foods are believed to be derived from water-soluble taste substances and lipid-soluble taste-active components. In the water-soluble fraction, sourness, sweetness and *umami* all contribute to the taste, with *umami* considered

Table 5
Correlation coefficients between principal component scores and the concentrations of the *umami*-related substances. The indication 'ns' means not significant ($P > .05$).

	Umami relevant substances			
Principal component	IMP	total amino acids	oligopeptides	glutamate
1	–0.606	0.439	–0.652	0.085
	$P < .001$	$P < .01$	$P < .001$	ns
2	–0.180	0.277	–0.057	0.115
	ns	ns	ns	ns
3	0.078	0.034	0.059	–0.114
	ns	ns	ns	ns
4	–0.193	–0.134	–0.160	0.057
	ns	ns	ns	ns
5	–0.220	0.163	–0.289	0.071
	ns	ns	ns	ns
6	0.069	0.104	–0.119	–0.211
	ns	ns	ns	ns
7	–0.106	0.044	0.161	–0.051
	ns	ns	ns	ns

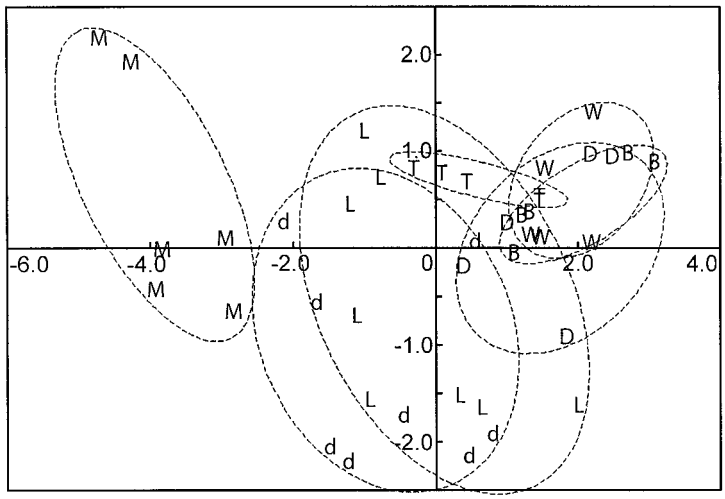

Fig. 1. Plot of the pork samples with scores of principal component 1 (horizontal axis) and 2 (vertical axis). L, D, d, W, M, B and T in the plot indicate Landrace, Duroc(A), Duroc(B), Large White, Meishan, Berkshire, and Three-way cross (Duroc × Landrace × Large White), respectively.

to be especially important to the taste of muscle foods. For example, it was reported that IMP and glutamate addition increased the brothy taste intensity of beef and pork extract.[6] Postmortem conditioning of pork and chicken meat increases intramusclar amounts of IMP and free amino acids, and the intensity of brothy *umami*.[7] Oligopeptides are also considered to relate to the taste of pork.[8] In addition, glutamate and IMP are characterized

as important contributors to the meat-like taste intensity of chicken extract.[9,10] Therefore, the evaluation of *umami* in the water-soluble fraction is important for the evaluation of pork taste. We were not able to evaluate the characteristics of the taste in the water-soluble fraction by only chemical determination of taste-active substances, because there is a synergistic effect of monosodium glutamate and IMP that affects pork taste.[11]

In this study, we tried to apply the taste sensing system in the evaluation of pork taste, and we examined the relationship between sensor output and *umami*-related substances. We indicated the taste characteristics of pork extracts as 'IMP and oligopeptide originated *umami*' and 'amino acid originated *umami*.' Our findings indicate that the taste sensing system may be useful for the evaluation and characterization of the taste of pork extract. However, we could not indicate the characteristics of PCs except for PC1. The species of lipid membrane probes, use of the taste sensing system, and statistical analysis should be investigated to improve the application of the taste sensing system for analysis and characterization of the taste of pork and other kinds of muscle foods.

On the other hand, the differences in concentrations of *umami*-related substances and sensor output among the breeds were not caused by genetic differences. The samples were obtained from different producers; therefore, the feeding, slaughter and storage conditions were different among the pork samples in this study. We considered that the differences in *umami* characteristics indicated in this study did not show a common tendency. The effects of pre- and post-harvest conditions on the output of the taste sensing system and the *umami* characteristics of pork should also be investigated.

In addition, peptides and other water-soluble substances were discovered as taste enhancers in conditioned and cooked beef, pork and chicken.[12,13] We expect that the taste sensing system can be used to analyze the characteristics of meat taste including *umami*-related substances and the effect of such taste-enhancing molecules, resulting in an improved evaluation and classification system for muscle foods.

In conclusion, we indicated that taste sensing system is useful for discrimination of pork extracts, and that principal component scores obtained from sensor output correlated with *umami*-relevant substances. Further investigation is needed about the relationship between sensory evaluation using human panels and on the output of a taste sensing system to characterize sensor output.

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