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Technical Paper

Effect of soy sauce yeast inoculation and ureter removal on the quality characteristics of meat sauce prepared from pig kidneys

Yasuhiro FUNATSU[1*], Kanami AZEKAWA[1], Takefumi ONO[2], Hirofumi HAYASAKA[3], Wataru MATSUDA[4], Naoyuki MAEDA[1], Akira TANAKA[5], and Shuji YOSHIKAWA[5]

[1] *Department of Food Science and Human Wellness, College of Agriculture, Food and Environmental Sciences, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501*

[2] *Hidaka Meat Center Co., ltd., Niikappu, Hokkaido 059-2418*

[3] *Taste &Aroma Strategic Research Institute, Chuo-ku, Tokyo 061-1104*

[4] *Otaru Kaiyo Suisan Co., Ltd., Otaru, Hokkaido 047-0048*

[5] *Food Processing Research Center, Industrial Technology and Environment Research Department, Hokkaido Research Organization, Ebetsu, Hokkaido 069-0836*

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This study investigated the development of fermented meat sauce from pig kidneys (PKs) to promote the use of underutilized livestock byproducts. To this end, we examined the effect of ureter removal and soy sauce yeast (SSY) inoculation on the physicochemical characteristics of the PK sauce mashes (*moromis*) and the quality of the final products. Ureter removal resulted in a decrease in the *L value of the *moromis* and an increase in the *a** and *b** values during the fermentation process. In contrast, SSY inoculation caused the opposite effect in CIE Lab color parameters. A principal component analysis with taste sensor data from the final products after heat treatment and filtration showed that the differences in taste among the four final products were distinguishable. The umami and bitterness tastes were derived from the fermentation of pig kidney sauce and enhanced with SSY inoculation and ureter removal, respectively.**

Keywords: pig kidney, ureter, meat sauce, fermentation, *koji* mold, soy sauce yeast

Introduction

In the slaughter process, livestock pass through dressed carcasses to become cut meat, which is processed into meat and meat products. Animal byproducts such as blood, internal organs, and excess fat, are obtained when processing the meat after slaughter into a dressed carcass (Ito, 2001). Various kinds of intestines, bone, blood, and hooves have been supplied as food, medical, industrial, agricultural, and food materials. However, the intestines rot easily, and putrefaction also progresses quickly. Therefore, the products have a high wastage rate based on microbial inspection, and their value is lost depending on how they are stored[i)]. There are large amounts of livestock in Japan due to the high consumption of pork. Pig tongue, liver, heart, uterus, stomach, small intestine, large intestine, kidney, and feet are considered to be edible and are consumed in Japan. The pig kidney is rarely used as a food material owing to its strong ammonia odor. The odor of the kidney may be reduced by the excision of the ureter and by boiling the kidney in hot water with potherbs. It can then be used in fried and boiled foods[ii)]. The development of methods to utilize pig kidneys as food materials is required in the meat processing industry.

There has been a rise in the consumption of low fat and low salt diets in Japan recently, and there is an increasing demand for flavorful and functional seasonings that can compensate for the dissatisfaction associated with the consumption of such diets (Editorial Office of Food Processing and Ingredients, 2016). The raw pig kidney contains approximately 14 % crude protein (Resource Survey Subcommittee, Council for Science and Technology, Ministry

*To whom correspondence should be addressed. E–mail address: funatsu@rakuno.ac.jp

of Education, Culture, Sports, Science and Technology, 2018); thus, pig kidneys could be used as a raw material for fermented seasoning. However, to obtain high-quality fermented kidney sauce, the ammonia odor from the main raw materials during the manufacturing process must be eliminated. The objective of this study was to elucidate the effect of ureter removal and soy sauce yeast (SSY) inoculation on the quality characteristics of the pig kidney sauce mashes (*moromis*) during fermentation and of the final products.

Materials and Methods

Kidney samples Thirty Landrace pigs (live weight: ~ 115 kg) were slaughtered at Hidaka Meat Center Co., Ltd. on August 23, 2016. The fresh kidneys were removed from the dressed carcasses before the meat manufacturing process and stored at −30 °C before starting the experiment.

Preparation of four kinds of pig kidney sauce Approximately 47 kg of frozen pig kidney was partially thawed at 4 °C overnight. After removing the fat tissue, the kidney was treated separately, with and without ureter removal. Each section was boiled at 85 °C for 30 min in a boil-processing apparatus (KRS-2633, Kajiwara Inc.) and drained well. After cooling to 25–30 °C, the kidneys were cut and minced with meat choppers (82 mm grinder, Higashimoto kikai Co., Ltd.). Approximately 10 kg of the ground meat was placed in a plastic bag and frozen at −30 °C. The next day, four kinds of pig kidney sauce *moromis* (Nos.1–4) were prepared using 40 % thawed boiled kidney sample, 35 % tap water, 1% commercial proteinase powder (CPP) (Sumizyme LP50, SNBL, Ltd.), 15 % salt and 10 % rice *koji* (Fukuyama jozou Co., Ltd.). The rice *koji* was rehydrated with 20 % tap water and stirred by hand for 40 min at 25–30 °C. After preparation, the surface of each *moromi* was covered with a top sheet. Regular stirring and replacement of the sheet were conducted during fermentation at 35 °C. After two days of fermentation, pig kidney *moromis* with and without ureter were divided into two parts, and one part was inoculated with 10^6 cfu/g of SSY (*Zygosaccharomyces rouxii*, Akita Konno Co., Ltd.). Furthermore, on the seventh day of fermentation, 1% CPP was added to each *moromi*. Thus, four types of *moromis* were prepared, which were as follows: No. 1, prepared using boiled kidney without ureter removal and with SSY inoculation; No. 2, prepared using boiled kidney without ureter removal or SSY inoculation; No. 3, prepared using boiled kidney with SSY inoculation after ureter removal; and No. 4, prepared using boiled kidney without SSY inoculation after ureter removal. After fermentation for 10 weeks, these *moromis* were compressed, and the obtained liquid was heated at 85 °C for 30 min. The final products were obtained after the addition of 0.025 % clarifying agent (Koporock SA, Otsuka Foods Co., Ltd.) to the *moromis*, which were then filtered with 0.2 % diatomaceous earth (Musashinolite No. 1, Musashino Chemical Laboratory, Ltd.). The final products were refrigerated and analyzed within six months.

Preparation of the assay sample A portion of the *moromi* was collected over time during fermentation and centrifuged (10 000 × *g*, 30 min, 4 °C). After centrifugation, the supernatant was filtered with filter paper (No. 5C, Toyo Roshi Kaisha, Ltd.), and the filtrate obtained was used as the analytical sample.

Physicochemical analysis The color (L^*, a^*, and b^* values) of the analytical sample was measured using a spectrophotometer (SA4000, Nippon Denshoku Industries Co., Ltd.) employing the transmission method with a glass cell (2 mm × 40 mm × 50 mm). The pH of the sample was measured with a pH meter (HM-30R, DKK-TOA Cooperation) at room temperature. Soluble solids excluding salt (SSES) values of the sample were calculated after subtracting the salt content from the Brix value (Japan Soy Sauce Institute, 1985). The total nitrogen content was determined by the Kjeldahl method (Tsutsumi and Yasui, 1996). The formol nitrogen level was determined according to the soy sauce test method. The protein degradation ratio was calculated as the ratio (%) of the formol nitrogen to the total nitrogen (Okazaki and Noguchi, 2008). The histamine level of the sample was determined with an enzymatic method using a histamine dehydrogenase from *Rhizobium* sp. (Sato *et al.*, 2005). The ammonia level of the sample was determined using the ninhydrin method (Ito *et al.*, 1991).

Antioxidative activity The hydrophilic-oxygen radical absorbance capacity (H-ORAC) value of the sample was determined using Trolox as a standard reagent according to the H-ORAC standard analysis procedure [iii].

Free amino acid The crude protein in the sample was removed by the addition of sulfosalicylic acid, and then filtered through a 0.22 μm nylon filter. The amino acid composition of each filtered sample was measured using an auto amino acid analyzer (L-8900, Hitachi High-Tech Corporation).

Organic acid Each analytical sample was diluted five-fold with ultrapure water. The diluted sample was mixed with a 5% perchloric acid solution at a ratio of 1:1 (v/v). The sample was filtered using a 0.45 μm cellulose acetate filter after centrifugation (9 167 × *g*, 15 min, 4 °C). Organic acids were determined with post-column labeling using HPLC. The HPLC analysis conditions were as follows: guard column, RSpak KC-G6B (Showa Denko K.K.); separation column, RSpak KC-811 (Showa Denko K.K.) × 2; eluent, 3 mM perchloric acid solution (pH 2.5); flow rate, 1.0 mL/min; reaction liquid, 0.2 mM BTB-15 mM sodium phosphate solution; column oven temperature, 60 °C; detection wavelength, 445 nm; injection volume, 10 μL.

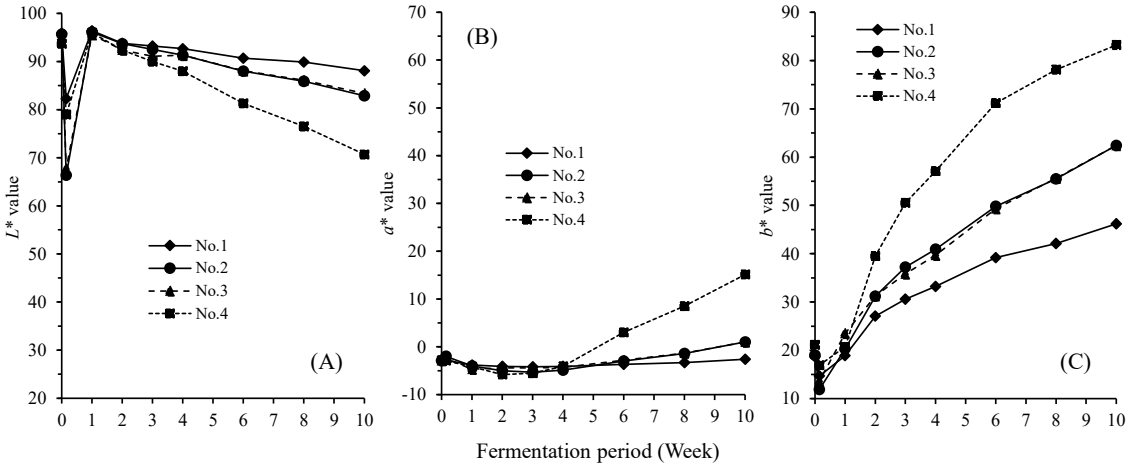

Fig. 1. Changes in lightness (A), redness (B), and yellowness (C) of four kinds of pig kidney sauce mashes (*moromis*) during fermentation. No.1: prepared using boiled kidney without ureter removal and with SSY inoculation, No.2: prepared using boiled kidney without ureter removal or SSY inoculation, No.3: prepared using boiled kidney with SSY inoculation after ureter removal, and No.4: prepared using boiled kidney without SSY inoculation after ureter removal.

Taste sensor analysis The final products were diluted 10 times with distilled water. Multiple taste tests of the diluted products were conducted using a taste sensor (TS5000Z, Inteligent Sensor Technology Inc.). The taste of the measurement sample was interpreted with six kinds of first tastes (bitterness/food, bitterness/medicine, astringency, umami, saltiness, and sourness) and four kinds of after tastes (bitterness/food, bitterness/medicine, astringency, and umami). Principal component analysis (PCA) was carried out using the taste sensor data obtained in this study. Taste axes for the positive and negative directions of the horizontal and vertical axes were selected according the magnitude of the eigenvector. Differences in taste induced by ureter removal and SSY inoculation were identified with a scatter diagram, which was written in a concrete language that shows the characteristics of taste.

Statistical analysis Statistical analysis of the data obtained in the taste sensor analysis was performed by one-way analysis of variance using JMP 11 (SAS Institute Japan Ltd.), and the statistical significance of the mean differences was determined using the Tukey-Kramer HSD test with a significance level of 95 %.

Results and Discussion

The differences in the color changes among the four types of moromis during fermentation The changes in the color of the four types of *moromis* during fermentation are shown in Fig.1. The *L** values (lightness) of all samples decreased on the first day and quickly recovered to the same level at the start of fermentation after the seventh day, then gradually decreasing to the end of fermentation (A). The magnitude of the decrease in the *L** value from the first week to the tenth week followed this order among the samples: No. 1 > No. 2 ≒ No. 3 > No. 4. The *a** values (redness) of all samples slowly decreased until the third week and increased after the fourth week (B). The magnitude of the increase rate of the *a** value followed this order among the samples: No. 4 > No. 2 ≒ No. 3 > No. 1. The *b** values (yellowness) of all samples decreased at day one, but increased after the first week. The differences among the samples were extremely high compared to the trend of increasing *a** values, although the increasing rate of *b** values after the first week among the four samples followed the same trend as that of the *a** values. The decrease in the *L** and *b** values of all samples immediately after fermentation may have been due to the fact that a portion of hemoproteins such as hemoglobin, remained in the samples after boiling [iv]. Ohmata (1972) reported that the generated pigment could be eluted in salt water when preparing ingredients for the brewing process. The details are under consideration. The color variation of all samples after the first week could be related to the amino-carbonyl reaction in the foods as described by Usui (2015). The lightness (*L**) value was higher in the glass cell Nos. 1 and 3 than in Nos. 2 and 4, while the *a** and *b** values yielded contrary results. This tendency might repress the amino-carbonyl reaction due to the consumption of free sugars in the *moromis* by SSY during fermentation (Yoshikawa *et al.*, 2010a). In particular, the lightness (*L**) value of No. 4 was the lowest among all samples, while in contrast, the *a** and *b** values of No. 4 were higher than those of Nos. 1–3. This may be one of the causes of the advanced amino-carbonyl reaction during fermentation because the increase in the formol nitrogen level was higher in

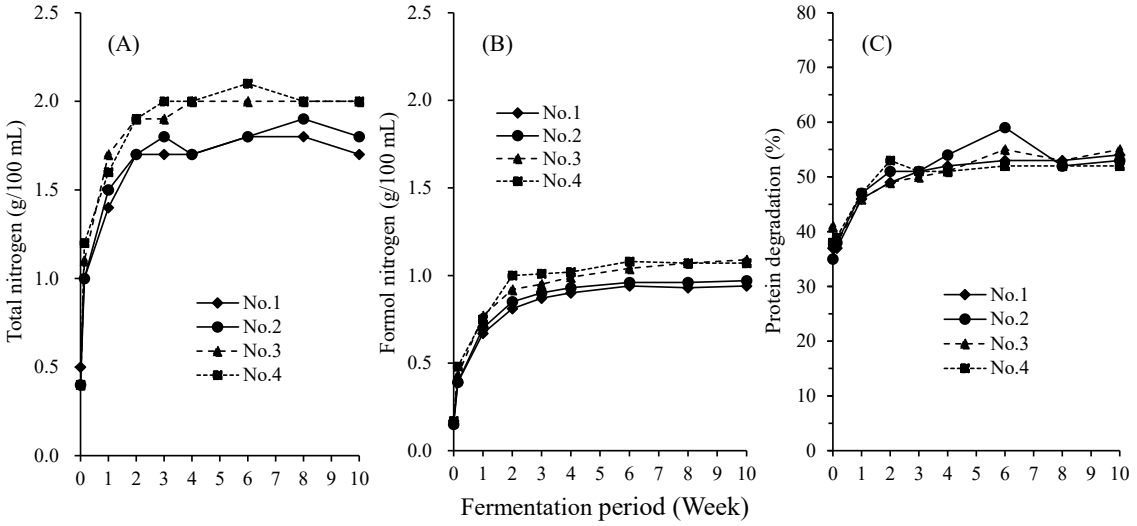

Fig. 2. Changes in total nitrogen (A) and formol nitrogen (B) contents, and protein degradation ratio (C) of the pig kidney sauce *moromis* during fermentation.

No. 4 than in the other samples until week 2 (Fig. 2B), and the D-glucose levels were higher in No. 4 (approximately 3–5 g/100 mL) than in the other samples (approximately 1–4 g/100 mL).

Differential changes in the chemical and extractive components among the four types of moromis during fermentation Changes in total and formol nitrogen levels as well as the protein degradation ratio of the four types of *moromis* during fermentation are shown in Fig. 2. The total nitrogen levels sharply increased in the first two weeks in all samples, and the increase in the total nitrogen level followed this order among the samples: No. 3 > No. 4 > No. 2 > No. 1 (A). The total nitrogen levels were higher in the samples that underwent ureter removal (Nos. 3 and 4) than in the untreated samples (Nos. 1 and 2). This might be due to the fact that Nos. 3 and 4 were rich in proteins such as collagen, reticulin, and elastin, which form connective tissue fibers in the ureter (Editorial Committee of Japan Society for Meat Science and Technology, 2010). Formol nitrogen levels rose rapidly in the first two weeks in all samples and then increased gradually (B). These tendencies were similar to those of total nitrogen levels, except for differences in the absolute values. The protein degradation ratio of all samples rapidly increased in the first two weeks and increased gradually throughout the fermentation (C). The protein degradation ratios of all samples were 52–54 %, and there was no significant difference between the samples.

The changes in pH, total organic acids, and acidic and basic amino acids of the four *moromis* during fermentation are shown in Fig. 3. In all samples, the pH decreased from 6.0 to 5.0 in the first four weeks and then increased gradually, although the pH values were approximately 6.0 at week zero (A). The organic acid levels of all samples rapidly increased in the first four weeks during fermentation and increased gradually thereafter until the end of fermentation (B). The rate of increase was higher in the SSY-inoculated samples (Nos. 1 and 3) than in the SSY-free samples (Nos. 2 and 4). The main organic acid detected in all samples was pyroglutamic acid and the final acid content was 328–403 mg/100 mL (51–69 % of total organic acids).

The acidic amino acid levels of all samples rapidly increased during the second week of fermentation and increased gradually thereafter until the end of the fermentation process (C), following this order among the samples: No. 4 > No. 3 > No. 2 > No. 1. The changes in basic amino acid content showed the same tendency throughout the fermentation period; however, the amino acid content increased more rapidly in the first two weeks, and the rate of increase thereafter was slower than that of the acidic amino acids. According to the relationship between the pH and the levels of organic acids and the acidic and basic amino acids during fermentation, the pH decrease rate was slightly higher in the SSY-inoculated samples (Nos. 1 and 3) than in the SSY-free samples (Nos. 2 and 4), as the organic acid levels were higher in the former than in the latter; however, the basic amino acid levels were slightly higher than the acidic amino acid levels in all samples until week two. The rate of pH decrease was higher in the ureter sample without SSY inoculation (No. 4) than in the other samples (Nos. 1–3) after the fourth week. It could be presumed that the increase in the acidic amino acid levels was higher than that of the basic amino acid levels, although the organic acid levels of all samples were slightly increased after the second week. Therefore, the decrease in pH of the *moromis* could be influenced not only by the organic acid content but

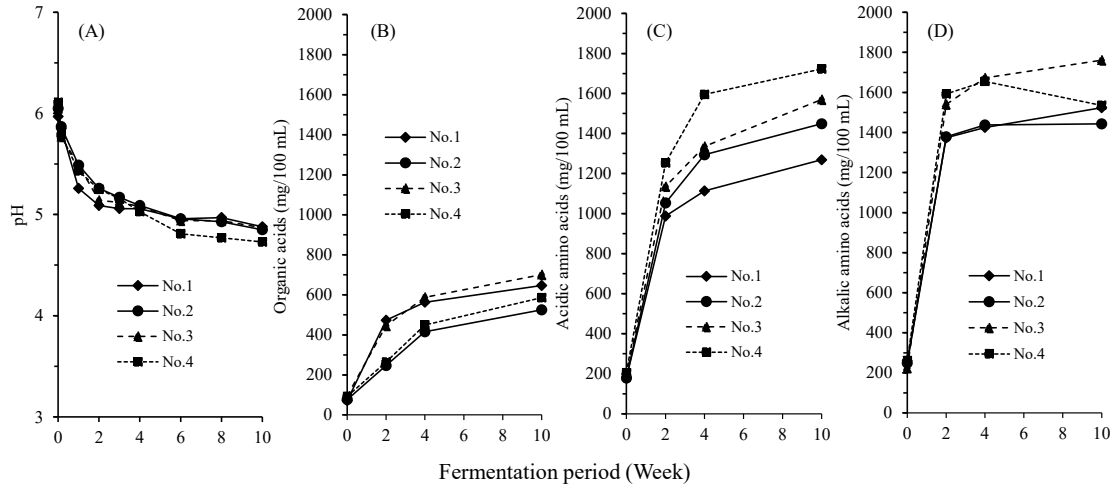

Fig. 3. Changes in pH (A), organic acids (B), acidic (C), and basic (D) amino acid contents of the pig kidney sauce *moromis* during fermentation.
Organic acids: the sum of malic, succinic, lactic, acetic, and pyroglutamic acid contents; Acidic amino acids: the sum of Glu and Asp levels; basic amino acids: the sum of Lys, His, and Arg contents.

also by the acidic and basic amino acid balance.

The kidney sauce has a characteristic organic acid content, low total and lactic acid content, and high pyroglutamic acid content. For fermented seasonings such as fish sauce and miso, the excess growth of halotolerant lactic acid bacteria was repressed by soy sauce yeast (*Z. rouxii*) in salmon sauce *moromi* during fermentation (Yoshikawa *et al.*, 2010b), and lactic acid fermentation was inhibited remarkably by the presence of more than 1 % ethanol in ripening miso (Matsumoto *et al.*, 1980). Yoshikawa *et al.* (2010a) also reported that approximately 2% ethanol was detected in the salmon sauce *moromi* inoculated with *Z. rouxii* at the early stages of fermentation. Hence, the acid levels might be lowered due to the repression of acid formation attributed to the formation of alcohol via SSY inoculation and the lack of the inoculation of halotolerant lactic acid bacteria. Pyroglutamic acid can be accumulated through non-enzymatic conversion from Glu generated through multiple paths and from Gln produced by peptidases (Ichijima, 2002). Ren *et al.* (1993b) reported that the pH value of the peak shown in the β-buffer curve for several organic acids was in the order: succinic (5.0) > acetic (4.7) > citric (4.5) > lactic (4.0) > pyroglutamic (3.0). Therefore, the β-buffer capacity of the *moromis* could be affected by succinic, acetic, and lactic acids compared to pyroglutamic acid due to the pH values of the *moromis* at the end of the fermentation, which were in the range of 4.7–4.9. In contrast, the pH value of the peak shown in the β-buffer curve for several amino acids was reported (Ren *et al.*, 1993a). Two pH peaks were detected in aspartic acid (3.8 and 10.2), glutamic acid (3.9 and 9.9), histidine (6.1 and 9.5) and lysine (9.7 and 11.1), while only one pH peak was detected in arginine (9.3). Therefore, the degree of pH decrease among the samples during fermentation could also be affected by the β-buffer capacity of each amino acid mentioned above. This is currently under further investigation.

The differences in the physicochemical properties and extractive components among the final products The physicochemical properties of the final products are shown in Table 1. The *L** values were lower in the products obtained from the kidneys where the ureters were removed (Nos. 3 and 4) than in those of the intact kidneys (Nos. 1 and 2), while the opposite was observed for the *a** and *b** values. Moreover, the *L** values were higher in the SSY-inoculated samples (Nos. 1 and 3) than in the inoculation-free samples (Nos. 2 and 4), while the opposite was observed for the *a** and *b** values. The degree of dark coloration of the sample was in the following order: No. 4 > No. 2 ≒ No. 3 > No. 1. The pH values of the samples ranged from 4.79 to 4.94. The salt contents of the samples were higher in the samples from which the ureter was removed (Nos. 3 and 4) than in the intact kidney samples (Nos. 1 and 2). The total nitrogen and SSES levels of all samples were 1.59–1.65 g/100 mL and 16–18%, respectively, and these levels were higher than the standard values of special grade soy sauce [v)] in the Japanese Agricultural Standard (JAS). The protein degradation rates of all samples were in the range of 53.2 %–56.3 %. Total and formol nitrogen and the SSES levels of *moromis* were different from the final products, which might be ascribed to diatomaceous earth absorption. The histamine levels of all samples were in the range of 11–12 ppm and these levels were below the CODEX [vi)] limit in fish sauce. Therefore, there was no problem concerning histamine accumulation (Satomi, 2016) in *moromis* during fermentation.

Table 1. Physicochemical characteristics of the four pig kidney seasonings

		No.1	No.2	No.3	No.4
Color	*L**	88.00	82.05	84.33	73.78
	*a**	−2.32	2.37	0.27	12.05
	*b**	47.24	65.67	59.31	80.30
pH		4.86	4.83	4.94	4.79
Salt content (g/100 mL)		17.4	18.3	15.9	15.1
Total nitrogen (g/100 mL)		1.63	1.65	1.62	1.59
Formol nitrogen (g/100 mL)		0.91	0.93	0.88	0.85
Protein degradation ratio (%)		55.8	56.3	54.4	53.2
Soluble solids excluding salt (%)		16	18	17	18
Histamin (ppm)		12	12	11	11
H-ORAC value (μmol TE/100 mL)		3 081	2 844	3 172	3 460

Soluble solids excluding salt = Brix − Salt content. The data are expressed as the mean (n = 2).

Table 2. Organic acids content of the four pig kidney seasonings

(mg/100 mL)

Organic acid	No.1	No.2	No.3	No.4
Citric	< 10	< 10	< 10	< 10
Malic	15	ND	15	ND
Succinic	97	39	87	27
Lactic	48	52	55	55
Acetic	133	83	112	48
Pyroglutamic	305	334	324	326
Total	599	508	593	456

ND: not detected. Sum of organic acids: malic, succinic, lactic, acetic, and pyroglutamic acids. The data are expressed as the mean (n = 2).

Harada *et al.* (2010) reported the antioxidant capacity of commercial fish sauces and found that the ORAC values were affected by the type and quantity of raw materials; the values were high for products containing soybean, wheat, and *koji*. In this study, the ORAC values of the final products determined using the H-ORAC method were in the range of 2 844–3 460 μmol TE/100 mL, regardless of ureter removal and SSY fermentation. These values were similar to those of commercial soy sauce (2 633 μmol TE/100 mL). Thus, the fermented sauce prepared with pig kidney as the main material has the same hydrophilic antioxidant capacity as the Japanese commercial soy sauce.

The organic acid compositions of the final products are listed in Table 2. Six kinds of organic acids were detected in the range of 498–649 mg/100 mL. The main organic acid in the final products was pyroglutamic acid in all samples, regardless of whether the ureter was removed or not and whether the sample was fermented with SSY or not. Malic acid was only detected in the samples with SSY (Nos. 1 and 3), while succinic and acetic acid levels were higher in SSY-free samples (Nos. 2 and 4) than in the inoculated samples (Nos. 1 and 3). Presumably, these organic acids were produced by SSY during fermentation. According to a case study of fish sauces prepared using several kinds of starter cultures, the malic, acetic, and succinic acid levels were higher in barley *koji* with SSY than in SSY-free barley *koji* samples (Yoshikawa, 2012). These results were similar to the results of this study, except that lactic acid was the main organic acid and pyruvate and formic acids were detected in the samples. These differences may be due to the differences in raw materials and fermentation conditions.

The free amino acids composition of the final products is listed in Table 3. Twenty-two free amino acids were detected. Amino acid levels were detected in the range of 7 741–8 841 mg/100 mL, and the main amino acids were Asp, Glu, Ala, Val, Leu, Lys, and Arg, regardless of ureter removal or SSY fermentation. The free amino acid levels and main amino acids of pig and Yezo sika deer sauce products with the same salt content and rice *koji* addition were similar to those obtained in this study. The total amino acid content was slightly different from the levels in pig and Yezo sika deer sauce products; however, the content of the main amino acid was similar (Mikami *et al.*, 2007; Funatsu *et al.*, 2015). According to the ninhydrin method, urea was not detected, and the ammonia

Table 3. Free amino acid compositions of the four pig kidney seasonings

(mg/100 mL)

Amino acid	No.1	No.2	No.3	No.4
Tau	39	50	46	58
Asp	574	749	605	748
Thr	470	483	470	442
Ser	473	506	472	462
Asn	261	259	271	258
Glu	773	840	766	766
Gln	11	14	10	ND
Gly	331	384	327	338
Ala	646	681	640	627
Pro	294	338	309	336
Val	649	650	630	583
Cys	39	12	15	ND
Met	216	214	185	169
Ile	525	518	467	424
Leu	921	920	723	670
Tyr	111	133	124	118
Phe	482	473	448	400
Trp	41	22	36	ND
Orn	14	11	12	9
Lys	713	694	693	606
His	215	202	202	169
Arg	700	688	679	592
Urea	ND	ND	ND	ND
NH_3	76	79	76	73
Total	8 497	8 841	8 131	7 774

ND: not detected. See Fig.1 for Nos.1–4. Total free amino acid levels of the kidney seasonings were calculated excluding the urea and ammonia content.

levels were 73–79 mg/100 mL in all final products. Urea was not detected in any *moromis*, and the range of ammonia content was 20–76 mg/100 mL throughout the fermentation. Moreover, the ammonia levels in the final products were in the range of 82–98 mg/100 mL, as detected with the enzymatic method (Cheuk and Finne, 1984) using an F-kit (J. K. International Inc.). These ammonia contents were low compared to those of commercial soy sauce (183 mg/100 mL). Therefore, boiling pretreatment of pig kidneys before fermentation could be effective for ammonia odor repression.

Differences in the taste of the final products due to ureter removal and SSY inoculation Taste analyses of the final products were conducted using a taste sensor (TS) to investigate taste differences between the samples. Six types of first taste, namely bitterness/medicine, bitterness/food, astringency, umami, salty, and sourness, of the diluted final products were 1.73 to 2.22, 0.17 to 0.59, –0.07 to 0.10, 0.90 to 1.57, –1.45 to –0.63, and –4.80 to –3.90, respectively (Table 4). Four types of after taste, namely bitterness/medicine, bitterness/food, astringency, and umami, were 1.05 to 1.33, –0.01 to 0.12, 0.13 to 0.19, and 0.81 to 1.32, respectively. Therefore, the difference between the maximum and minimum values was approximately 0.5 (concentration difference: approximately 10 %), for first tastes such as bitterness/medicine, umami, salty, and sourness, and after taste, such as umami. As seen in Table 4, the four tastes mentioned above, except for sourness, were significantly different ($p < 0.05$) in No. 4 than in the other samples (Nos. 1–3), while three tastes, except for umami, contributed to the first and after tastes and were significantly different ($p < 0.05$) among the samples (Nos. 1–3). Therefore, changes in taste caused by ureter removal and SSY inoculation were identified in the PCA (Fig.4). The names of the vertical (PC1) and horizontal (PC2) axes were determined, respectively, which considered the magnitude of the eigenvector and the large concentration difference mentioned above. The plus and minus directions of PC1 indicated the strength of umami and the strength of sourness and bitterness, respectively, while the plus and minus directions of PC2 indicated the strength of saltiness and strength of bitterness originating from fermentation,

Table 4. Taste properties of the four pig kidney seasonings estimated using taste sensor analysis

	First taste					
	Bitterness/medicine	Bitterness/food	Astringency	Umami	Salty	Sourness
No.1	1.73^{d}	0.21^{c}	−0.06^{b}	1.44^{a}	−0.79^{b}	−4.38^{b}
No.2	1.87^{c}	0.24^{b}	−0.07^{b}	1.16^{b}	−0.63^{a}	−3.98^{a}
No.3	2.00^{b}	0.17^{d}	−0.03^{b}	1.57^{a}	−1.26^{c}	−4.80^{c}
No.4	2.22^{a}	0.59^{a}	0.10^{a}	0.90^{c}	−1.45^{d}	−3.90^{a}

The data are expressed as the mean (n = 3). The soy sauce synthetic standard solution was set as zero. Different superscript letters show statiscally significant differences ($p < 0.05$).

	After taste			
	Bitterness/medicine	Bitterness/food	Astringency	Umami
No.1	1.08^{b}	0.06^{ab}	0.16^{ab}	1.32^{a}
No.2	1.05^{b}	0.08^{ab}	0.17^{ab}	1.30^{a}
No.3	1.16^{b}	−0.01^{b}	0.13^{b}	1.24^{a}
No.4	1.33^{a}	0.12^{a}	0.19^{a}	0.81^{b}

The data are expressed as the mean (n = 3). The soy sauce synthetic standard solution was set at zero. Different superscript letters show statiscally significant differences ($p < 0.05$).

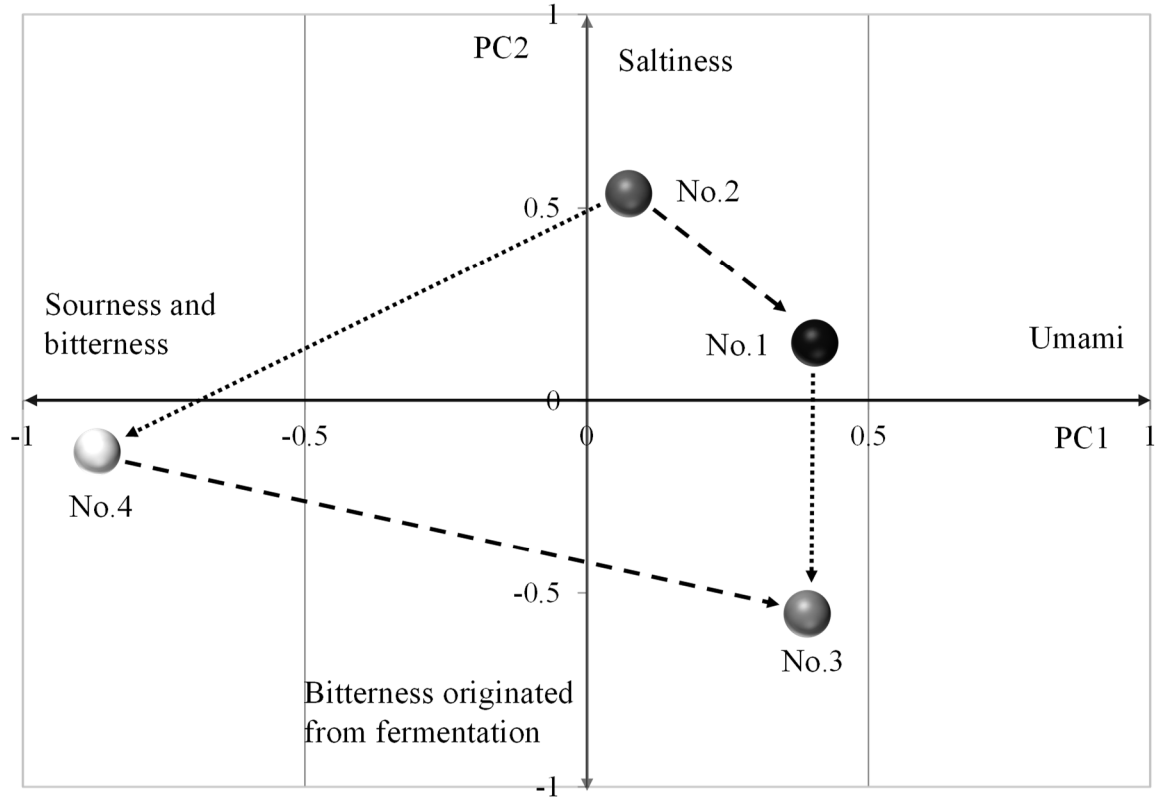

Fig. 4. Scatter plot of the taste obtained in a principal component analysis using taste sensor data.
The broken and dotted lines represent the movements of SSY inoculation and ureter removal from pig kidneys, respectively.

respectively. The contribution ratios of PC1 and PC2 were 62.2 % and 36.8 %, respectively and the cumulative contribution was 99.0 %. The strength of the bitterness originating from fermentation could be increased by moving in the negative direction of PC2 by ureter removal, and moving from No. 1 to No. 3 was similar to moving from No. 2 to No. 4, regardless of whether it was fermented with SSY or not (dashed line in Fig. 4). In contrast, the strength of the umami taste could be increased by moving in the plus direction of PC1 for SSY by inoculation, and moving from No. 4 to No. 3 in the samples prepared after ureter removal resulted in a larger difference than moving from No. 2 to No. 1 in the intact kidney samples (broken line in Fig. 4). In a previous report, the strength of sourness of a Yezo sika deer sauce was increased by the addition of *koji* mold and *Tetragenococcus halophilus*, while the strength of the peculiar taste was increased by SSY-inoculation (Funatsu *et al.*, 2015). The differences in fermentation effects of SSY between the Yezo sika deer sauce and pig kidney sauce could be due to the differences in pH and salt content between the samples.

Based on the above results, we determined that inoculating with SSY starter and *koji* mold in *moromi* made with boiled-minced pig kidney as the main raw material is effective in producing an umami enriched pig kidney sauce of the optimal quality. This sauce has a reduced ammonia odor and this preparation avoids the time-consuming ureter removal step during the manufacturing process.

Conclusions

The effects of ureter removal and SSY inoculation on the fermentation process were investigated to develop a method to use pig kidneys as a main fermented sauce ingredient. With regard to the quality of the *moromi*, the *L** values decreased, while the *a** and *b** values increased throughout the fermentation process upon ureter removal. These values showed opposite changes upon SSY inoculation. The rate of decrease in pH during fermentation was slightly different among the samples and affected the rate of increase in organic acids, and acidic and basic amino acids. In contrast, with regard to the quality of the final product, the main organic acid was pyroglutamic acid. Malic, succinic and acetic acid levels increased with SSY inoculation. Free amino acid levels were in the range of 7 800–8 800 mg/100 mL, and the main amino acids were Asp, Glu, Ala, Leu, and Arg. The ammonia levels were considerably low (73–79 mg/100 mL), as determined by the ninhydrin method. According to the results from the PCA with TS data, umami was enhanced with SSY inoculation. Therefore, we concluded that the meat sauce product, which not only gives off less ammonia odor but also possesses a moderate umami flavor, could be obtained by fermenting *moromi* prepared with pig kidney and SSY inoculation, without ureter removal.

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