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Development and characterisation of high-quality meat sauces made from wild boar meat

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Abstract

The damage to agricultural crops by wild animals such as wild boars and deer is becoming a serious problem in Japan. Among them, the consumption of wild boar meat is limited owing to its gamy tastes and toughness. Therefore, we aimed to develop a processing technique for meat utilisation. In the present work, meat sauces from underutilised wild boar meat were prepared using rice *koji* and food enzymes. The salt contents of meat sauces were remarkably lower than those of meat sauces from other animals and commercially available soy sauces. Next, the functional properties of meat sauces were investigated for future applications. Meat sauces exhibited powerful antioxidative, radical scavenging, and hyaluronidase inhibitory activities. In addition, ACE activities were completely inhibited by these sauces. Among the tested sauces, the meat sauce prepared with 30% (w/w) rice *koji* and 0.5% (w/w) Alcalase 2.4 L FG to the boiled ground meats had good taste strength and balance, as well as strong sweetness and umami, and weak bitterness and saltiness. In addition, the meat sauce had high nutritional value owing to its rich total and essential amino acids. High-quality meat sauce, which has positive effects on human health, will fulfil the demands of modern consumers and soy sauce-related industries as an upcycling meat sauce owing to its eating quality and health benefits.

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Introduction

Soy sauce originates from the fermented paste of salted foods, *hishio*, produced in China. Three types of *hishio* can be classified as follows: *kokubishio* made from grains; *kusabishio* made from fruits, vegetables, or seaweed; and *shishibishio* made from fish or meats (Funatsu, 2016). Among them, soy sauces made from grains such as soybeans and wheat are important traditional fermented condiments in Japan. Owing to their pleasant umami taste, and distinctive and unique aroma, soy sauces are used in various dishes such as sashimi, simmered foods, grilled fishes, and tempura. Thus, soy sauces are indispensable in Japanese cuisines, *washoku* meals. According to the Japanese Agricultural Standards (JAS, 2019), soy sauces are established in five species, such as *koikuchi*, *usukuchi*, tamari, *saishikomi*, and *shiro*, due to different ingredient compositions.

In recent years, the damage to agricultural products by wild birds and animals, especially wild boars, deer, and monkeys, has become a serious problem on farms, fruit gardens, and woodlots in Japan. The annual damage to these products is estimated to reach approximately 15.6 billion yen in 2022 (MAFF, 2024a). Therefore, these animals have mainly been captured to prevent this damage, resulting in an increase in captured animals. These are called game meats (gibiers), and used as ingredients in game cuisine. Wild boar meat is low-fat and low-energy compared to beef and pork (Kagawa, 2024). However, this meat has little demand due to its gamy tastes and toughness (MAFF, 2024b). The ratios of

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the number of animals used for gibier utilisation to the number of captured animals were fairly low, at approximately 6.1% (wild boars) and 15.2% (deer) (Statistics of Japan, 2024). Therefore, it is necessary to develop new processing techniques for utilisation of game meats. Recently, the demand for natural condiments has increased owing to the trend of ethnic foods and diversified eating habits. However, studies on meat sauces [Yezo sika deer hind leg meats (Funatsu *et al*., 2015; Funatsu, 2016), pork (Trang *et al*., 2005; Mikami *et al*., 2007), silky-fowl meats (Abe and Ohnishi, 2005), and spent hen meats (Yang *et al*., 2012)] are limited. In addition, these meat sauces contain a large amount of sodium (approximately 20%) as salt equivalents, and have distinctive, complicated, and unpleasant smells. Therefore, the consumption of these meat sauces is limited. To the best of our knowledge, no detailed information is available on the preparation and characterisation of wild boar meat sauce. The present work thus aimed to develop an acceptable high-quality and low-salt meat sauce using wild boar meat. In addition, we also elucidated the physicochemical and functional properties of meat sauces for their industrial applications. The results obtained in the present work would provide useful information for consumers and soy sauce-related industries.

Materials and methods

Materials

Frozen wild boar meats (boneless rib, neck, and shoulder mixture) were purchased from Iwataya (Hiroshima, Japan). *Koji* mould (No. 2 bacteria) for soy sauce production was obtained from Akita Konno Shoten Co., Ltd. (Akita, Japan). Brown rice of non-glutinous rice cultivar *Haenuki* and commercially available (CA) salt (Kobe Bussan Co., Ltd., Hyogo, Japan) were purchased from a local wholesale market (Yamagata, Japan). Alcalase 2.4 L FG and Flavourzyme 1000 L were obtained from Novozymes (Chiba, Japan). All chemicals were of analytical grade.

Proximate analysis

The moisture contents were determined using a Moisture Determination Balance (FD-600; Kett Electric Laboratory, Tokyo, Japan). The crude protein contents were measured by the Kjeldahl method using a conversion factor of 6.25. The crude lipid contents were analysed by the Soxhlet extraction method. The crude ash contents were determined using an electric furnace (AMI-II; Nitto Kagaku Co., Ltd., Aichi, Japan). The carbohydrate contents were calculated by difference. The salt contents were determined by the Mohr method. The energy was calculated using the Atwater’s calorie factors (Kagawa, 2024).

Koji preparation

Brown rice was polished using a rice-polishing machine (MR-E520, TWINBIRD Co., Niigata, Japan), and the polished rice was rinsed under running water to remove the bran. The rinsed polished rice was then soaked in adequate water overnight, and then drained with a sieve for 1 h. The rice was then steamed for 90 min, and cooled to approximately 36°C. The *koji* mould [0.035% (w/w)] was sprinkled to the rice, and then gently mixed. The rice was incubated at 33°C and relative humidity of approximately 90%. After 12 h, the rice was gently mixed and then incubated for another 32 h under the same condition. After gentle mixing, the rice was incubated for another 40 h under the same condition, and then mixed in the same manner. After incubation for 48 h under the same condition, the rice was taken out from the incubator. The malted rice (*koji*) was cooled overnight in a cool incubator at 15°C, and used for the preparation of meat sauces.

Measurement of enzyme activity of koji

Enzyme solution was prepared from the *koji* by the homogenised extraction method (The Brewing Society of Japan, 1993). The *koji* was added with five volumes of 10 mM sodium acetate buffer (pH 5.0) containing 85.6 mM NaCl, and then homogenised in ice. The homogenates obtained were centrifuged at 30,000 *g* at 4°C for 5 min. The supernatants obtained were dialysed against 10 mM sodium acetate buffer (pH 5.0) at 4°C for 1 d. The dialysate was diluted twice with distilled water at 4°C. The solution was used for the determination of enzyme activity of the *koji*. The α-amylase, glucoamylase, α-glucosidase, and acid carboxypeptidase activities of the *koji* were measured as described by Revised National Tax Administration Agency Analysis Method commentary (The Brewing Society of Japan, 1993).

Preparation of meat sauces

Six different types of meat sauces were prepared according to Nagai *et al*. (2020). The formulations of the meat sauces are shown in Table 1. The vacuum-packed meats with a nylon/polyethylene

Table 1. Formulation of meat sauces prepared from wild boar meats.

	A	B	C	D	E	F
Boiled ground meat (g)	1000	1000	1000	1000	1000	1000
Haenuki koji (g)	100	100	100	300	300	300
21.6% (w/w) saline solution (g)	421	421	421	498	498	498
Alcalase 2.4 L FG (mL)	-	4.274	4.274	-	4.274	4.274
Flavourzyme 1000 L (mL)	-	-	4.274	-	-	4.274

film were heated for 1 min after the temperature in the central part of meats reached at 75°C to prevent food poisoning. These were ground using a meat grinder. The mashes (*moromi*) were prepared by gently mixing ground meats, *koji*, NaCl solution, Alcalase 2.4 L FG, and Flavourzyme 1000 L. Alcalase 2.4 L FG is an endo-type protease with high protein degradation ability. Flavourzyme 1000 L is an endo- and exo-type protease with salt-tolerance and high hydrolysis ability. Therefore, the use of these enzymes was suitable for the preparation of meat sauces. The *moromi* were filled in each bottle, and then fermented at 25°C. The *moromi* were gently mixed once a day. After fermentation for seven months, the *moromi* were heated at 90°C for 30 min. These were then centrifuged at 22,200 *g* at 20°C for 1 h, and the supernatants obtained were filtered using No. 1 filter paper. The liquefaction rates of the *moromi* were calculated by the following equation: liquefaction rate (%) = (the weight of supernatants after centrifugation / the weight of *moromi* before centrifugation) × 100.

Physicochemical parameters

The colours of meat sauces were analysed using a colorimeter (NR-11A, Nippon Denshoku Industries Co. Ltd., Tokyo, Japan) with illuminant D65 calibrated to black and white standards. The colour difference (ΔE^*ab) was calculated as follows: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Iida, 1956). The pH was measured at 20°C using a pH meter (HI98103, HANNA Instruments Inc., Italy). The water activity (a_w) was determined at 20°C using a water activity meter (SP-W; AS ONE Co., Osaka, Japan). The Brix was measured at 20°C using a refractometer (PAL-Pâtissier, Atago Co. Ltd., Tokyo, Japan). The ethanol contents were determined using an alcoholic meter (Alcomate AL-3, Riken Keiki Co., Ltd., Tokyo, Japan). The total nitrogen, formol nitrogen, soluble solids excluding salts, total sugars, direct reducing sugars, acidity I, acidity II, titratable acidity, and specific gravity at 20°C were evaluated according to the Soy Sauce Test (Japan Soy Sauce Research Institute, 1985). The total phenolic contents (Slinkard and Singleton, 1977), total flavonoid contents (Kim *et al*., 2003), and total flavonol contents (Jimoh *et al*., 2010) were determined using gallic acid, quercetin, and rutin as standards, respectively. The histamine contents were measured using a kit 'Checkcolor Histamine' (Kikkoman Biochemifa Company, Tokyo, Japan).

Microbiological analysis

The determination of total plate counts and total coliforms of the meat sauces were performed using standard methods agar medium and desoxycholate agar medium, respectively. Yeast and mould, and lactic acid bacteria were detected using potato dextrose agar medium containing 0.01% (w/w) chloramphenicol and plate count agar medium with bromocresol purple, respectively. Microbial populations were determined by plating 10-fold serial dilutions of the meat sauces.

Functional properties

The antioxidative activities, radical scavenging activities against superoxide anion radicals, hydroxyl radicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, and angiotensin I-converting enzyme (ACE) and hyaluronidase inhibitory activities of the meat sauces were determined as described by Nagai *et al*. (2020). Ascorbic acid (AA), *tert*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-4-methylphenol (BHT), α-tocopherol (TP), and Trolox (TL) were used as positive controls, and distilled water was used as negative control.

Sensory analysis

The colour, smell, taste strength, taste balance, first taste, aftertaste, sweetness, umami, sourness, bitterness, and saltiness of meat sauces were evaluated by a panel consisting of six trained panellists at 25°C. The panel was instructed to rinse the mouths with water between each sample, and not to make the comments to prevent influencing other panellists during analysis. Seven-point scale was used with -3 = weak (light), 0 = neither weak (light) nor strong (dark), and 3 = strong (dark).

Free amino acid composition

The free amino acid composition of meat sauce was analysed using a HPLC system (L-8900, Hitachi High-Technologies Corp., Tokyo, Japan) by on-line post-column derivatisation with ninhydrin.

Statistical analysis

Each assay was repeated three times independently, except for colour analysis (ten times of independent measurements) and sensory analysis. The results were reported as mean ± standard deviation. Statistical analysis was performed by One-way analysis of variances with the Tukey's test or Dunnett's test ($p < 0.05$). Minitab Statistical Software (version 17) was used for the statistical analysis.

Results and discussion

Preparation of meat sauces

Proximate compositions of raw meats

The chemical compositions of raw wild boar meats were water (59.5 ± 1.0 g/100 g), crude proteins (19.9 ± 0.7 g/100 g), crude lipids (19.2 ± 1.2 g/100 g), carbohydrates (0.7 g/100 g), and crude ashes (0.7 ± 0.1 g/100 g), respectively. Hiraoka (2012) investigated the proximate compositions of round meat of raw wild boars, and reported as follows: water (73.6 g/100 g), crude proteins (18.6 g/100 g), crude lipids (5.4 g/100 g), carbohydrates (0.9 g/100 g), and crude ashes (1.5 g/100 g), respectively. The tested meats were fairly low in water and crude ashes contents, and higher in crude fats contents than round meats. In contrast, Fujimitsu *et al*. (2016) reported that the proximate compositions of the wild boar loin meats, boneless ribs, and round meats captured in winter in Tottori prefecture, Japan were water (29.1, 26.3, and 59.5 g/100 g), crude proteins (7.3, 9.3, and 18.7 g/100 g), crude lipids (61.3, 60.5, and 20.3 g/100 g), carbohydrates (1.8, 3.5, and 0.7 g/100 g), and crude ashes (0.4, 0.5, and 0.9 g/100 g), respectively. Therefore, it was suggested that these compositions depended on the cut of meat, age, body weight, feed, seasons, and other factors. Next, the enzymatic activity of *koji* was determined. The α-amylase, glucoamylase, α-glucosidase, and acid carboxypeptidase activities of *koji* were 4968.7 ± 7.2, 153.9 ± 3.2, 0.053 ± 0.010, and 614.2 ± 9.4 U/g *koji*, respectively. *Koji* has been suggested to digest the proteins and starches in the ingredients of meat sauces.

pH values of moromi

The pH value of *moromi* was observed during the fermentation periods. The initial pH values were 6.79 - 7.04, and the pH values decreased to the range of 4.51 - 4.80 after fermentation for seven months. This indicated the release of peptides and free amino acids during fermentation. It was suggested that the meats and rice could be efficiently digested in *moromi*.

Total plate counts and total coliforms in *moromi* were measured after fermentation for seven months. The total plate count of *moromi* was < 300 CFU/g in all cases. In addition, no total coliforms were detected in *moromi*. Mikami *et al*. (2007) reported that the total plate counts of *moromi* from ground pork using Alcalase 2.4 L with 15, 20, and 30% salts after six months ranged from 3.3×10^2 to 7.0×10^2 CFU/g regardless of salt contents. In contrast, no coliforms were detected in *moromi*. We prepared the wild boar meat sauces with a low salt concentration (approximately 6%). A high salt concentration is required to prevent the growth of spoilage bacteria. However, the growth of spoilage bacteria was inhibited in *moromi* during fermentation for seven months, suggesting that the *moromi* for wild boar meat sauces could be fermented under low salt concentration without spoilage.

Liquefaction rates of moromi

The liquefaction rates of *moromi* were 54.2 ± 2.6% (sauce A), 73.3 ± 5.7% (sauce B), 72.6 ± 4.9% (sauce C), 61.4 ± 3.5% (sauce D), 71.2 ± 4.1% (sauce E), and 74.7 ± 6.3% (sauce F), respectively. These rates markedly increased with the addition of enzymes regardless of the *koji* ratio. However, these rates did not increase using the two enzymes. In contrast, the yields of pork sauces significantly increased with Alcalase 2.4 L and Flavourzyme 500 L compared to those using Alcalase 2.4 L (Mikami *et al*., 2007). The yields of sauces differed depending on the types and cuts of meat. Trang *et al*. (2005) prepared meat sauces using ground pork, *koji*, and commercial enzymes such as Alcalase 2.4 L and Pectinase 3S. The yields of sauces without the addition of enzymes with 15% salts and those with the addition of Alcalase or Pectinase with 15% salts were 30.2, 49.8 and 50.6%, respectively. In addition, the yields of sauces decreased with increasing salt contents. In other words, the fermentation of *moromi* was inhibited by high salt content regardless of the presence or absence of enzymes (Aquerreta *et al*., 2002). In contrast, there was no significant difference in the sauce yield after enzyme treatment. In the present work, the yields of wild boar meat sauces with added enzymes were higher than those of ground pork sauces (Trang *et al*., 2005) and hind leg meat sauces of Yezo sika deer (Funatsu *et al*., 2015). That is, effective proteolysis and liquefaction of *moromi* with the addition of enzymes were accomplished at a low salt concentration of approximately 6%.

Wild boar meat sauces are shown in Figure 1. The colour of sauce D was light brown compared to that of sauce A. In addition, the colours of sauces B, C, E, and F were dark brown compared to sauce D. The absorbance of sauces at 440 nm was used as an indicator of the browning (Shimohashi, 2013). The values were sauce A (1.7941), sauce B (2.5354), sauce C (2.5120), sauce D (2.4428), sauce E (2.5537), and sauce F (2.5493), respectively. The advancement of browning by the Maillard reaction was largely due to the addition of enzymes, and the high ratio of *koji* to the ingredients in the sauces. In contrast, the colour of pork sauce using Alcalase 2.4 L was light yellow after fermentation for one month (Mikami *et al*., 2007). However, the colour of sauce changed to light brown after fermentation for two to three months. In addition, the sauce turned dark brown after fermentation for six months. Thus, it was suggested that the dramatic progression of the browning on the sauce occurred from three to six months.

Figure 1. Meat sauces prepared from wild boar meats.

Physicochemical parameters

Chemical composition

The proximate composition of wild boar meat sauces was investigated. The water contents of sauces ranged from approximately 68.5 to 73.8 g/100 g (Table 2). The contents of sauces with the addition of enzymes were lower than those without the addition of enzymes. The crude protein contents of sauces ranged from approximately 10.5 to 15.4 g/100 g. When the sauces were prepared with the addition of enzymes, the contents were significantly higher than those prepared without the addition of enzymes regardless of the *koji* ratio. The crude lipid contents were low at approximately 0.2 - 0.7 g/100 g. The contents of sauces with the addition of enzymes were lower than those without the addition of enzymes regardless of the ratio of *koji*. These results indicated that the ingredients of sauces could be effectively digested by enzyme treatment. This suggested the acceleration of proteolysis and lipolysis in *moromi* by adding enzymes. The carbohydrate contents of sauces prepared using 300 g *koji* were higher than those prepared using 100 g *koji* because of the high carbohydrate contents of rice *koji*. No significant difference was observed in the crude ash contents (approximately 7.6 - 8.8 g/100 g) among the tested sauces. The energies of sauces ranged from approximately 73.1 to 95.8 kcal/100 g. In addition, the energies of sauces with the addition of enzymes were high regardless of the ratio of *koji*. Thus, wild boar meat sauces were high-protein and low-ash compared to CA soy sauces from grains, such as soybeans and wheat. The salt contents were significantly low at approximately 7.0 - 7.8 g/100 g. According to the Japan Soy Sauce Research Institute (1985), the wild boar meat sauces can be categorised

Table 2. Proximate compositions of meat sauces prepared from wild boar meats.

	Water (g/100 g)	Crude proteins (g/100 g)	Crude lipids (g/100 g)	Carbohydrates (g/100 g)	Crude ashes (g/100 g)	Salts (g/100 g)	Energy (kcal/100 g)
A	73.8 ± 0.1[a]	11.4 ± 0.2[c]	0.7[a]	5.3[d]	8.8 ± 0.1[c]	7.1 ± 0.1[d]	73.1[c]
B	70.5 ± 0.1[b]	15.4 ± 0.2[a]	0.4[b]	6.1[d]	7.6 ± 0.1[c]	7.0 ± 0.1[d]	89.6[b]
C	69.7 ± 0.1[b]	15.4 ± 0.2[a]	0.2[c]	6.3[d]	8.4 ± 0.1[c]	7.5 ± 0.1[d]	88.6[b]
D	72.5 ± 0.1[a]	10.5 ± 0.1[c]	0.5[b]	8.5[c]	8.0 ± 0.1[c]	7.8 ± 0.1[d]	80.5[c]
E	69.6 ± 0.1[b]	12.6 ± 0.2[b]	0.2[c]	9.7[b]	7.9 ± 0.1[c]	7.8 ± 0.1[d]	91.0[b]
F	68.5 ± 0.1[b]	13.2 ± 0.2[b]	0.2[c]	10.3[b]	7.8 ± 0.1[c]	7.5 ± 0.1[d]	95.8[b]
G*	67.1[b]	7.7[d]	0[d]	7.9[c]	15.1[a]	14.5[b]	77[c]
H*	69.7[b]	5.7[e]	0[d]	5.8[d]	16.8[a]	16.0[a]	60[d]
I*	70.9[b]	6.4[e]	Tr	7.6[c]	12.1[b]	12.8[c]	77[c]
J*	57.3[c]	11.8[b]	0[d]	15.9[a]	15.0[a]	13.0[c]	111[a]

Tr: trace amount; G: soy sauce; H: thin soy sauce; I: low-salt thin soy sauce; and J: tamari soy sauce. *Data obtained from Standard Tables of Food Composition in Japan 2024. Different lowercase superscripts in similar column indicate significant difference ($p < 0.05$).

as reduced-salt meat sauces. In contrast, the salt contents of other meat sauces were remarkably high as follows: 16.25% (silky-fowl meat sauce) (Abe and Ohnishi, 2005), 20.5 - 23.5% (*shishibishio* made from pork) (Mikami *et al*., 2007), 18.57 - 19.61% (spent hen meat sauces) (Shimohashi, 2013), and 19.3 - 20.7% (Yezo sika deer hind leg meat sauces) (Funatsu *et al*., 2015). A high concentration of salts is indispensable for the production of meat sauces without spoilage at 25°C, as salts reduce the a_w of foods, and prevent the growth of spoilage bacteria. Salt reduction is a global consumer and government trends around the world. Excessive sodium intake has been reported to increase the risks of gastric cancer (Liem *et al*., 2011), arteriosclerosis, ischemic heart disease, chronic kidney disease, stroke, obesity (He and MacGregor, 2010), and diminish bone mineral density (Tsugane *et al*., 2004). Therefore, recently, positive attempts have been made to decrease the amount of sodium intake in the diet, and to minimise the sodium content in foods by reducing salt. Therefore, wild boar meat sauces could be useful condiments for consumers and related industries compared to other meat sauces.

Colour

Sauce A exhibited significantly higher L^*, a^*, and b^* values than the other tested sauces (Table 3). These values decreased with an increase in the ratio of *koji* and the addition of enzymes. Sauce A had significantly higher whiteness index than sauces B - F. Next, the ΔE^*ab values of sauces B - F were calculated to sauce A. The colour differences of these sauces were evaluated as “much”. Additionally, the values of sauces A - C, E, and F were calculated to sauce D. The colour differences were evaluated as follows: A (much), B (trace), C (slight), E (slight), and F (noticeable), respectively. The metric chroma showed that sauce A had a vivid colour with a high degree of yellowness. However, the higher the ratio of *koji* to the ingredients of sauces, the lower the metric chroma of sauce, resulting in sauces with dull colour. Therefore, it was suggested that colours of the sauces were influenced by the ratio of *koji* and the addition of enzymes.

pH

The pH values of wild boar meat sauces ranged from 4.51 to 4.80 (Table 3). In contrast, the pH values of Yezo sika deer hind leg meat sauces were 4.6 - 5.1 regardless of the kind of *koji*, and with or without the addition of *T. halophilus* and *Z. rouxii* (Funatsu *et al*., 2015). In addition, the pH values of *shishibishio* from pork with 15% salt after fermentation for six months were 4.94 (Alcalase 2.4 L addition) and 4.76 (Alcalase 2.4 L and Flavourzyme 500 L addition), respectively (Mikami *et al*., 2007). In contrast, the pH values of *shishibishio* with 20 and 25% salts were high at 5.01 to 5.07. These results indicated that high salt concentrations slowed the growth of microorganisms such as lactic acid bacteria, and inhibited the enzyme activities of *koji*.

Table 3. Physicochemical properties of meat sauces prepared from wild boar meats.

Parameters	A	B	C	D	E	F
Colour *L**	3.856 ± 0.152[a]	1.473 ± 0.115[b]	1.041 ± 0.102[c]	1.685 ± 0.126[b]	1.365 ± 0.131[b]	1.292 ± 0.100[b]
Colour *a**	5.703 ± 0.286[a]	1.400 ± 0.124[b]	1.480 ± 0.147[b]	1.783 ± 0.139[b]	0.785 ± 0.090[c]	0.171 ± 0.023[d]
Colour *b**	5.300 ± 0.255[a]	1.209 ± 0.159[b]	0.061 ± 0.012[e]	1.266 ± 0.141[b]	0.358 ± 0.078[c]	0.138 ± 0.030[d]
Whiteness	3.54[a]	1.46[b]	1.03[b]	1.66[b]	1.36[b]	1.29[b]
*ΔE*ab*	- Much	Much Trace	Much Slight	Much -	Much Slight	Much Noticeable
Metric chroma	7.79[a]	1.85[b]	1.48[bc]	2.19[b]	0.86[c]	0.22[d]
pH at 20°C	4.67 ± 0.01[a]	4.70 ± 0.01[a]	4.80 ± 0.01[a]	4.55 ± 0.01[a]	4.55 ± 0.01[a]	4.51 ± 0.01[a]
Water activity (a_w) at 20°C	0.85 ± 0.01[a]	0.85 ± 0.01[a]	0.85 ± 0.01[a]	0.85 ± 0.01[a]	0.85 ± 0.01[a]	0.85 ± 0.01[a]
Total nitrogen (%)	1.83 ± 0.01[c]	2.46 ± 0.02[a]	2.46 ± 0.02[a]	1.68 ± 0.01[c]	2.02 ± 0.01[b]	2.12 ± 0.01[b]
Formol nitrogen (%)	1.05 ± 0.01[b]	1.36 ± 0.01[a]	1.48 ± 0.01[a]	1.01 ± 0.01[b]	1.14 ± 0.01[b]	1.18 ± 0.01[b]
Formol nitrogen/ Total nitrogen	0.57 ± 0.01[a]	0.55 ± 0.01[a]	0.60 ± 0.01[a]	0.60 ± 0.01[a]	0.56 ± 0.01[a]	0.56 ± 0.01[a]
Soluble solids excluding salts (%)	21.4[c]	26.4[b]	25.9[b]	28.3[b]	31.6[a]	32.6[a]
Brix% at 20°C	28.5 ± 0.1[c]	33.4 ± 0.1[bc]	33.4 ± 0.1[bc]	36.1 ± 0.1[b]	39.3 ± 0.1[a]	40.1 ± 0.1[a]
Alcohol (%) at 20°C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total sugars (g/100 mL)	1.40 ± 0.01[e]	4.31 ± 0.02[d]	2.65 ± 0.02[e]	13.15 ± 0.04[c]	15.75 ± 0.08[b]	21.26 ± 0.12[a]
Direct reducing sugars (g/100 mL)	0.30 ± 0.01[d]	1.71 ± 0.05[c]	1.71 ± 0.06[c]	10.94 ± 0.09[b]	10.15 ± 0.12[b]	16.26 ± 0.14[a]
Acidity-I (mL)	1.1 ± 0.1[b]	1.4 ± 0.1[a]	1.2 ± 0.1[b]	1.1 ± 0.1[b]	1.4 ± 0.1[a]	1.4 ± 0.1[a]
Acidity-II (mL)	1.3 ± 0.1[b]	1.9 ± 0.2[a]	1.6 ± 0.1[ab]	1.4 ± 0.1[b]	1.7 ± 0.2[a]	1.9 ± 0.1[a]
Titratable acidity (mL)	2.4 ± 0.1[b]	3.3 ± 0.2[a]	2.8 ± 0.1[ab]	2.5 ± 0.1[b]	3.1 ± 0.2[a]	3.3 ± 0.1[a]
Specific gravity at 20°C	1.097 ± 0.001[a]	1.109 ± 0.001[a]	1.112 ± 0.001[a]	1.137 ± 0.001[a]	1.149 ± 0.001[a]	1.148 ± 0.001[a]
Total phenols (mg gallic acid equivalent/mL)	8.91 ± 0.10[b]	8.87 ± 0.11[b]	7.30 ± 0.09[c]	6.52 ± 0.05[d]	9.22 ± 0.11[b]	12.16 ± 0.15[a]
Total flavonoids (mg quercetin equivalent/mL)	0.76 ± 0.03[c]	0.95 ± 0.05[b]	0.89 ± 0.04[b]	0.98 ± 0.06[b]	1.50 ± 0.09[a]	1.53 ± 0.10[a]
Total flavonols (mg rutin equivalent/mL)	0.09 ± 0.01[c]	0.13 ± 0.02[bc]	0.14 ± 0.02[bc]	0.16 ± 0.02[b]	0.29 ± 0.03[a]	0.28 ± 0.03[a]
Histamine (mg/kg)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D.: not detected. Different lowercase superscripts in similar row indicate significant difference ($p < 0.05$).

Water activity

The a_w of foods affects their shelf life. The a_w of wild boar meat sauces were low at approximately 0.85 (Table 3). According to the Japan Soy Sauce Brewers' Association (2023), the a_w of CA soy sauces are approximately 0.76 to 0.85. The growth of microorganisms containing *Clostridium botulinum* ($a_w \geqq 0.94$) is suppressed under these conditions. Therefore, it was suggested that wild boar meat sauces could be preserved without degradation by the growth of pathogenic microorganisms similar to CA soy sauces.

Total nitrogen and formol nitrogen contents

When the total nitrogen contents of meat sauces are high, the tastes are favourable. The total nitrogen contents of wild boar meat sauces were high ranging from approximately 1.68 to 2.46% (Table 3). In addition, the formol nitrogen contents (protein degradation parameter) of sauces ranged from 1.01 to 1.48%. The protein degradation rates (formol nitrogen content/total nitrogen content) were 0.55 to 0.60. The total nitrogen contents and the formol nitrogen contents of sauces A - C were significantly higher than those of sauces D - F, as the ratio of the meat to the ingredients of sauces A - C was higher than those of sauces D - F. In contrast, the total nitrogen contents of Yezo sika deer hind leg meat sauces were 1.7 - 1.9 g/100 mL regardless of the kind of *koji*, and with or without the addition of *T. halophilus* and *Z. rouxii* (Funatsu *et al*., 2015). Mikami *et al*. (2007) reported that the total nitrogen contents of *shishibishio* from pork were 1.7 - 2.0 g/100 mL. In addition, there was no significant difference in the total nitrogen contents between the sauce using Alcalase 2.4 L and that using Alcalase 2.4 L and Flavourzyme 500 L. These contents decreased with an increase in salt content. Trang *et al*. (2005) measured the total nitrogen contents of ground pork sauces with 15, 20, and 25% salts. These contents ranged from 1.4 to 2.6 g/100 mL. In addition, the total nitrogen contents of the untreated sauces decreased with increasing salt contents. However, there was no significant difference in the contents of sauces treated with the enzymes regardless of salt content.

Soluble solid content excluding salts

The soluble solid contents excluding salts of wild boar meat sauces were approximately 21.4 - 32.6% (Table 3). These contents increased with an increase in the *koji* ratio and the addition of enzymes. According to the Japanese Agricultural Standard (JAS, 2019), wild boar meat sauces fall into a special grade of dark soy sauce, tamari, and *saishikomi*. In contrast, the soluble solid contents excluding salts of Yezo sika deer hind leg meat sauces ranged from 11.9 to 15.9% (Funatsu *et al*., 2015). It was suggested that wild boar meat sauces were rich in the extract components compared to Yezo sika deer hind leg meat sauces.

Brix% and alcohol content

The Brix% of wild boar meat sauces were remarkably high in the range of approximately 28.5 - 40.1% (Table 3), suggesting the strong sweetness of the sauces. Alcohol was not detected in any of the tested sauces, because yeasts were not detected in *moromi* during fermentation (Table 3). In contrast, alcohol was detected in spent hen meat sauces (0.01 - 2.50%) (Yang *et al*., 2012) and silky-fowl meat sauce (1.44 g/100 mL) (Abe and Ohnishi, 2005), respectively, suggesting the existence of yeasts in these *moromi*.

Total sugar and direct reducing sugar contents

The total sugar and direct reducing sugar contents of wild boar meat sauces D - F were significantly higher than those of sauces A - C (Table 3). This was due to the high ratio of *koji* to the ingredients of sauces D - F compared with that of sauces A - C. Additionally, it was suggested that the degradation of carbohydrates into sugars in sauce F was accelerated simultaneously using two kinds of enzymes.

Acidity

The acidity I and II are important factors affecting the qualities of sauces, as these indexes are used as indicators of sourness of sauces (acidity I: first taste; and acidity II: aftertaste). These contents of wild boar meat sauces were low at approximately 1.1 - 1.4 mL (acidity I) and 1.3 - 1.9 mL (acidity II), respectively (Table 3). Therefore, the titratable acidities of sauces were low at 2.4 - 3.3 mL, suggesting a weak first taste and aftertaste. In contrast, the titratable acidities of spent hen meat sauces were fairly high at approximately 13.90 - 27.14 mL (Yang *et al*., 2012), suggesting large amounts of organic acids. The specific gravities of the wild boar meat sauces ranged from 1.097 to 1.148

(Table 3). Based on these findings, the Brix% of meat sauces with the addition of enzymes was higher than those of meat sauces without the addition of enzymes regardless of the ratio of *koji*. Total sugar contents of meat sauces with the addition of enzymes were significantly higher than those without the addition of enzymes. In addition, the total nitrogen contents of meat sauces with the addition of enzymes were significantly higher than those of meat sauces without the addition of enzymes. These trends were supported by the fact that the soluble solids contents excluding salts of meat sauces with the addition of enzymes, namely the contents of extract components, were remarkably higher when compared to those without the addition of enzymes. Therefore, it was suggested that the addition of enzymes could produce meat sauces with a strong sweetness and umami taste. Additionally, these results indicated that the first taste and aftertaste of meat sauces became stronger with the addition of enzymes to *moromi*.

Phenolic contents

The total phenolic contents of the wild boar meat sauces A - C and D - F ranged from approximately 7.30 to 8.91 and 6.52 to 12.16 mg gallic acid equivalents/mL, respectively (Table 3). Sauces D - F (approximately 0.98 - 1.53 mg quercetin equivalents/mL) showed high total flavonoid contents compared to sauces A - C (approximately 0.76 - 0.95 mg quercetin equivalents/mL) (Table 3). Additionally, the total flavonol contents of sauces D - F (approximately 0.16 - 0.28 mg rutin equivalents/mL) were much higher than those of sauces A - C (approximately 0.09 - 0.14 mg rutin equivalents/mL) (Table 3). This suggested that these contents of sauces were influenced by the ratio of *koji* to the ingredients. In addition, the flavonoid contents of sauces with the addition of enzymes were significantly higher than those without the addition of enzymes. Therefore, it was suggested that adding enzymes to *moromi* could produce meat sauces with antioxidant properties.

Histamine content

Histamine is the causative agent of allergy-like food poisoning. Funatsu *et al*. (2015) reported that the histamine contents of hind leg meat sauces of Yezo sika deer were approximately 3.7 - 44.9 mg/kg. In contrast, histamine was not detected in the tested wild boar meat sauces (Table 3), suggesting safe condiments without histamine poisoning.

Functional property

First, the antioxidative activities of wild boar meat sauces against linoleic acid oxidation were investigated. The results were expressed as the changes in absorbance at 500 nm for 200 min. Each sauce completely inhibited oxidation after 100 min (Table 4). In addition, sauces B - E showed a complete inhibition of oxidation after 200 min. In contrast, sauces A and F exhibited remarkably high antioxidative activities. The activity of sauce A was higher than those of 5 mM AA, 1 mM BHA and BHT, and 0.1 mM TL. Additionally, sauce F exhibited the same activity as 1 mM TP and TL. These results indicated that wild boar meat sauces showed remarkably high antioxidative activities regardless of the ratio of *koji*, and with or without the addition of enzymes.

Next, the superoxide anion radical scavenging activities of wild boar meat sauces were measured. The activities of sauces A - C ranged from approximately 53.9 to 73.3% (Table 5). In contrast, sauce D showed high activity as well as 5 mM AA, which exhibited the highest activity among the antioxidants used as positive control. In addition, sauces E and F completely scavenged these radicals. The hydroxyl radical scavenging activities of these sauces ranged from approximately 42.6 to 75.2% (Table 5). The wild boar meat sauces had moderate scavenging activities as well as 0.01 mM BHA. Moreover, excluding sauce A, sauces B - F exhibited significantly high DPPH radical scavenging activities of approximately 83.7 - 86.3% (Table 5). These activities were similar to those observed with 1 mM TP and TL. These results indicated that meat sauces with significantly higher superoxide anion radical scavenging activities could be produced with the addition of enzymes compared to the sauces without the addition of enzymes. However, there was no significant difference in the hydroxyl radicals and DPPH radicals scavenging activities of meat sauces regardless of the ratio of *koji*, and with or without the addition of enzymes.

The tested meat sauces completely inhibited ACE activities (Table 5), suggesting their excellent suppressive effects against increased blood pressure. In addition, these sauces showed relatively high hyaluronidase inhibitory activities of approximately 57.3 - 76.3% (Table 5). Sodium cromoglicate (SC) is used as an anti-allergic and anti-asthmatic drug. The activities of sauces were calculated as 30.8 - 39.3 mmol of SC equivalent per kg of sauces. An ampule

Table 4. Antioxidative activities of meat sauces prepared from wild boar meats.

Samples	50 min	100 min	200 min
A	0^{f}	0^{f}	0.051 ± 0.006^{ef}
B	0^{f}	0^{f}	0^{h}
C	0^{f}	0^{f}	0^{h}
D	0^{f}	0^{f}	0^{h}
E	0^{f}	0^{f}	0^{h}
F	0^{f}	0^{f}	0.016 ± 0.002^{f}
1 mM AA	0.022 ± 0.001^{e}	0.135 ± 0.006^{b}	0.469 ± 0.027^{b}
5 mM AA	0.016 ± 0.001^{ef}	0.032 ± 0.003^{e}	0.090 ± 0.008^{ef}
0.01 mM BHA	0.084 ± 0.005^{b}	0.120 ± 0.008^{b}	0.245 ± 0.012^{c}
0.1 mM BHA	0.056 ± 0.003^{c}	0.090 ± 0.006^{c}	0.165 ± 0.010^{d}
1 mM BHA	0.054 ± 0.002^{c}	0.057 ± 0.003^{d}	0.100 ± 0.006^{e}
0.01 mM BHT	0.082 ± 0.003^{b}	0.112 ± 0.009^{bc}	0.248 ± 0.011^{c}
0.1 mM BHT	0.058 ± 0.004^{c}	0.108 ± 0.005^{bc}	0.173 ± 0.008^{d}
1 mM BHT	0.044 ± 0.002^{d}	0.051 ± 0.003^{d}	0.093 ± 0.005^{ef}
1 mM TP	0.006^{f}	0.025 ± 0.001^{e}	0.028 ± 0.002^{f}
0.01 mM TL	0.084 ± 0.005^{b}	0.094 ± 0.006^{c}	0.262 ± 0.013^{c}
0.1 mM TL	0.038 ± 0.002^{d}	0.051 ± 0.003^{d}	0.123 ± 0.008^{e}
1 mM TL	0.011 ± 0.001^{f}	0.031 ± 0.002^{e}	0.032 ± 0.002^{f}
Control	0.379 ± 0.008^{a}	0.715 ± 0.025^{a}	1.406 ± 0.041^{a}

AA: ascorbic acid; BHA: *tert*-butyl-4-hydroxyanisole; BHT: 2,6-di-*t*-butyl-4-methylphenol; TP: α-tocopherol; and TL: trolox. Values are absorbance at 500 nm. Different lowercase superscripts in similar column indicate significant difference ($p < 0.05$).

Table 5. Radical scavenging activities and ACE and hyaluronidase inhibitory activities of meat sauces prepared from wild boar meats.

Samples	Superoxide anion radicals (% inhibition)	Hydroxyl radicals (% inhibition)	DPPH radicals (% inhibition)	ACE (% inhibition)	Hyaluronidase (% inhibition)
A	53.9 ± 0.7^{e}	57.4 ± 1.1^{e}	57.9 ± 1.4^{c}	100^{a}	$57.3 \pm 1.9(30.8)^{c}$
B	73.3 ± 1.0^{c}	58.1 ± 1.0^{e}	85.3 ± 1.7^{a}	100^{a}	$70.3 \pm 2.2(36.6)^{b}$
C	60.9 ± 0.9^{d}	75.2 ± 1.2^{c}	84.2 ± 1.5^{a}	100^{a}	$76.3 \pm 2.4(39.3)^{a}$
D	86.4 ± 1.1^{b}	48.0 ± 0.6^{f}	83.7 ± 1.6^{a}	100^{a}	$60.6 \pm 1.9(31.2)^{c}$
E	100^{a}	62.6 ± 1.3^{d}	86.3 ± 1.8^{a}	100^{a}	$74.2 \pm 2.1(37.1)^{a}$
F	100^{a}	42.6 ± 0.5^{f}	86.2 ± 1.6^{a}	100^{a}	$68.1 \pm 1.8(34.3)^{b}$
1 mM AA	14.7 ± 0.2^{g}	13.2 ± 0.2^{g}	3.1^{f}*		
5 mM AA	89.9 ± 5.3^{b}	17.6 ± 0.7^{g}	34.1 ± 2.0^{d}**		
0.01 mM BHA	29.3 ± 0.5^{f}	59.1 ± 0.8^{e}	5.5^{f}		
0.1 mM BHA	36.4 ± 0.9^{f}	93.3 ± 1.4^{b}	17.5 ± 0.4^{e}		
1 mM BHA	51.9 ± 1.4^{e}	95.2 ± 1.4^{b}	72.7 ± 3.6^{b}		
0.01 mM BHT	11.7 ± 0.2^{g}	82.8 ± 0.9^{c}	3.9^{f}		
0.1 mM BHT	46.6 ± 1.0^{e}	97.6 ± 1.6^{b}	7.9 ± 0.1^{f}		
1 mM BHT	48.4 ± 1.2^{e}	100^{a}	31.7 ± 0.8^{d}		
1 mM TP	52.6 ± 4.2^{e}	67.6 ± 4.3^{d}	87.6 ± 2.8^{a}		
0.01 mM TL	46.4 ± 1.0^{e}	81.5 ± 0.6^{c}	0.1^{g}		
0.1 mM TL	58.1 ± 1.1^{d}	91.8 ± 1.2^{b}	17.9 ± 0.2^{e}		
1 mM TL	76.1 ± 1.9^{c}	100^{a}	86.3 ± 3.3^{a}		

*0.1 mM AA; and **1.0 mM AA. Values in brackets are millimoles of sodium cromoglicate equivalents per kg of meat sauces. Different lowercase superscripts in similar column indicate significant difference ($p < 0.05$).

(2 mL) of 1% SC inhalant liquid Sawai (Sawai pharmaceutical Co., Ltd., Osaka, Japan) for CA anti-allergic and anti-asthmatic drug contains 20 mg of SC. It was calculated that 2 mL of meat sauces contained approximately 34.6 - 44.8 mg SC equivalents. This suggested that wild boar meat sauces showed stronger anti-allergic effects than CA anti-allergic drugs. Wild boar meat sauces may help prevent chronic diseases, such as cardiovascular diseases, high blood pressure, and allergies.

Meat is a rich source of proteins, minerals, and vitamins. Protein digestion increases the digestion and absorption rates of proteins, as well as generates various peptides and amino acids that have functional roles in human health. In recent years, various processing technologies, such as traditional aging (Lee *et al*., 2021), enzymatic hydrolysis (Zhu *et al*., 2018), ultrasound (Li *et al*., 2020), high-pressure processing (Xue *et al*., 2020), and pulsed electric field (Bhat *et al*., 2019) have been reported to improve the protein digestibility of meat and meat products. These techniques can increase the sensory qualities of meat and meat products, and improve their functional qualities. Enzymatic hydrolysates of meat using digestive enzymes, such as pepsin, trypsin, and pancreatin, exhibited antioxidative activities regardless of the type of meat (Takeda *et al*., 2015). In addition, meat-derived peptides showed antioxidative, antihypertensive, antimicrobial, opioid, and antithrombotic effects, as the physiological actions (Xing *et al*., 2019). Most of them have been investigated using animal proteins, such as beef, pork, and chicken. However, there are few reports on the functional properties of game meats hydrolysates from wild boars. A substantial amount of active peptides and amino acids generated from meats with the use of *koji* and the addition of enzymes, and the phenolic compounds contained in rice *koji* contributed to the high functionality of wild boar meat sauces, such as antioxidant properties and ACE and hyaluronidase inhibition.

Sensory evaluation

Sauces E and F had significantly higher scores in terms of colour (Figure 2), suggesting browning due to the Maillard reaction. In contrast, sauce A had the lowest score. The taste strength and balance had high scores with an increase in the ratio of *koji*, and the addition of enzymes. Except for sauces D and F, the sauces had significantly higher scores for first taste. In contrast, the aftertaste had generally low scores for all tested sauces. Sauces E and F had significantly stronger sweetness and umami than sauces A - D. These factors were high, when the *koji* ratio was high. It was suggested that various sugars were produced by starch hydrolysis, contributing to strong sweetness of the sauces. Excluding sauce D, sauces B, C, E, and F had higher sourness scores, suggesting lactic acid fermentation in these *moromi*. In addition, sauce A had the highest sourness score. Bitterness had the lowest scores among all the tested sauces. Except for sauces A and B, sauces C – F exhibited the lowest saltiness scores. Mikami *et al*. (2007) reported that *shishibishio* from pork with 15%

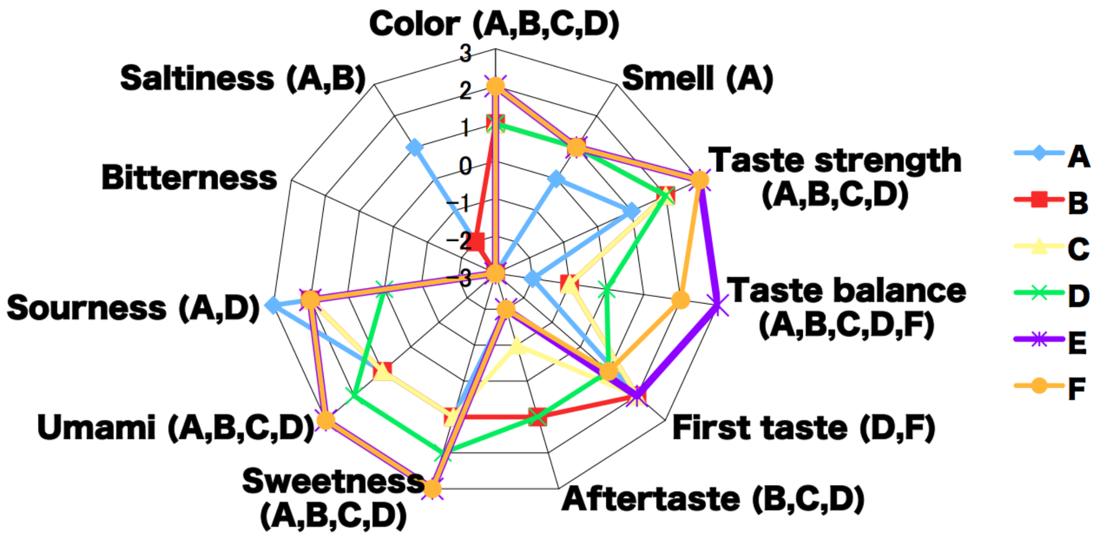

Figure 2. Sensory evaluation of wild boar meat sauces. Significant difference ($p < 0.05$) was found between meat sauce E and other sauces in parentheses.

salts showed low flavour scores owing to the gas generated during fermentation. In contrast, *shishibishio* with 20% salts, particularly with addition of Alcalase 2.4 L and Flavourzyme 500 L, had the highest scores for colour, flavour, taste, and overall evaluation. Trang *et al*. (2005) reported that ground pork sauces showed a clear brown colour with the addition of enzyme. Additionally, the flavours of sauces with 15% salts had high scores; however, the sauces had low scores with increasing salt content. The sauces had low scores in colour, aroma, flavour, and overall characteristics owing to the low content of free amino acids regardless of salt content without the addition of enzymes. Overall, the sauces with the addition of enzymes showed high scores for all items. According to Funatsu *et al*. (2015), Yezo sika deer hind leg meat sauce using soy sauce *koji* without *T. halophilus* and *Z. rouxii* was preferred in terms of colour, flavour, and overall quality among the tested sauces. In contrast, the sauces using soybean *koji* had strong unpleasant odours. Game meats have distinctive and unacceptable odours. It is considered that the generation of these odours is closely related to the oxidation of polyunsaturated fatty acids, such as linoleic and linolenic acids, which are produced by the loss of meat freshness. According to Fujimitsu *et al*. (2016), the crude lipid contents of wild boar loin meats and boneless ribs were approximately three times higher than those of round meats. These contents were much higher than those of pork (Kagawa, 2024). Besides, the linoleic and linolenic acid contents of wild boar meats were high as follows: loin meat (16.8 and 1.5 g/100 g total fatty acid), boneless ribs (16.5 and 1.4 g/100 g total fatty acid), and round meat (21.0 and 1.6 g/100 g total fatty acid), respectively (Fujimitsu *et al*., 2016). Nevertheless, no unpleasant smell or taste was observed in wild boar meat sauces because of the use of fresh meats, although fatty meats were used to prepare the meat sauces. Therefore, it was concluded that sauce E was a meat sauce with good sensory acceptability.

Free amino acid composition

Free amino acid compositions of wild boar meat sauce E are shown in Table 6. Total free amino acid contents were approximately 6349.1 mg/100 g (7291.9 mg/100 mL). The contents were the same as those of CA low-salt thin soy sauce. Additionally, these contents were higher than those of the CA thin soy sauce. In contrast, their contents were significantly lower than those of CA and tamari soy sauce. The glutamic acid content was the highest among these free amino acids, followed by leucine, lysine, aspartic acid, and alanine. These amino acids accounted for approximately 53.7% of the total free amino acids. In contrast, the dominant amino acids in CA soy sauces were glutamic acid, aspartic acid, leucine, lysine, and proline. The glutamic acid content of meat sauce E was significantly lower than those of CA soy sauces. In contrast, leucine and lysine contents were significantly higher than those of CA soy sauces. The essential amino acid contents were calculated to be approximately 3188.4 mg/100 g. These amino acids contributed approximately 50.2% of the total amino acids. This ratio was much higher than those of the CA soy sauces (soy sauce, approximately 37.8%; thin soy sauce, approximately 39.4%; low-salt thin soy sauce, approximately 32.9%; tamari soy sauce, approximately 30.2%). In addition, the umami, sour, sweet, and bitter amino acids contents were approximately 881.4, 1481.1, 1982.3, and 3156.0 mg/100 g, respectively. Taurine (approximately 94.0 mg/100 g), ornithine (approximately 126.3 mg/100 g) as a decomposition product of arginine, and carnosine (approximately 42.1 mg/100 g) as a radical scavenger were detected in sauce E. A small amount of hydroxyproline and hydroxylysine, derived from collagen, and γ-aminobutyric acid (GABA) were detected. According to Mikami *et al*. (2007), the total free amino acid contents of *shishibishio* from pork with 15 % salts were approximately 7.0 g/100 mL. In addition, those of the sauce with the addition of Flavourzyme 500 L increased to approximately 7.8 g/100 mL. In contrast, their contents decreased with increasing salt content. This suggested that high concentrations of salt inhibited the enzyme activities, resulting in low free amino acids contents. Glutamic acid, lysine, leucine, alanine, and aspartic acid were the dominant amino acids in the sauces. Funatsu *et al*. (2015) reported that the total free amino acid contents of Yezo sika deer hind leg meat sauces were strongly affected by the type of *koji* as follows: 8263 - 9001 mg/100 mL (soybean *koji*), 7779 - 8178 mg/100 mL (soy sauce *koji*), 6638 - 7039 mg/100 mL (rice *koji*), 7112 - 8256 mg/100 mL (minced meat *koji*), and 6219 - 6721 mg/100 mL (thin sliced meat *koji*), respectively. Glutamic acid, lysine, and leucine were the dominant

Table 6. Free amino acid composition of meat sauce (E) prepared from wild boar meats.

	Meat sauce (E)	**A***	**B***	**C***	**D***
Amino acids	**mg/100 g (mg/100 mL)**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**
Taurine	94.0 (108.0)	-	-	-	-
Aspartic acid	599.7[d] (688.7)	800[b]	640[cd]	700[c]	1300[a]
Threonine	331.9[b] (381.2)	310[b]	240[c]	260[c]	460[a]
Serine	60.3[c] (69.2)	380[b]	320[b]	340[b]	570[a]
Glutamic acid	881.4[c] (1012.3)	1600[b]	1400[b]	1600[b]	2700[a]
Glycine	289.4[b] (332.4)	320[b]	270[b]	290[b]	610[a]
Proline	292.1[d] (335.5)	520[b]	410[c]	430[c]	620[a]
Hydroxyproline	13.1 (15.0)	-	-	-	-
Alanine	581.0[a] (667.3)	430[b]	290[c]	340[c]	580[a]
Valine	427.6[b] (491.1)	410[b]	330[c]	340[c]	560[a]
Cystine	N.D.	88[b]	67[c]	63[c]	120[a]
Methionine	203.7[a] (234.0)	72[b]	88[b]	77[b]	83[b]
Isoleucine	393.3[ab] (451.7)	380[ab]	300[b]	280[b]	450[a]
Leucine	708.2[a] (813.4)	570[b]	450[c]	420[c]	600[b]
Tyrosine	131.0[a] (150.5)	89[c]	61[d]	49[e]	100[b]
Phenylalanine	328.3[b] (377.0)	360[ab]	410[a]	170[d]	260[c]
GABA	8.5 (9.8)	-	-	-	-
Tryptophan	39.8[a] (45.7)	18[b]	14[c]	16[bc]	23[b]
Ornithine	126.3 (145.0)	-	-	-	-
Lysine	637.6[a] (732.3)	430[b]	340[c]	370[bc]	650[a]
Hydroxylysine	11.6 (13.3)	-	-	-	-
Histidine	118.0[c] (135.5)	170[ab]	150[b]	140[b]	240[a]
Anserine	22.8 (26.2)	-	-	-	-
Carnosine	42.1 (48.3)	-	-	-	-
Arginine	7.4[d] (8.5)	240[c]	260[bc]	300[b]	400[a]
Total	6349.1[c] (7291.9)	7200[b]	5900[c]	6300[c]	11000[a]

N.D.: not detected. A: soy sauce, B: thin soy sauce, C: low-salt thin soy sauce, and D: tamari soy sauce. *Data obtained from Standard Tables of Food Composition in Japan 2024. Different lowercase superscripts in similar row indicate significant difference ($p < 0.05$).

amino acids as well as wild boar meat sauce E and the other meat sauces (Trang *et al*., 2005; Mikami *et al*., 2007). In addition, high ornithine contents were detected in the sauces prepared using soybean *koji*, *T. halophilus*, and *Z. rouxii* (Tanaka, 2012), suggesting the conversion of arginine by decomposition into ornithine through the arginine deiminase pathway. Ornithine converts harmful ammonia to harmless urea *via* the urea cycle in the liver (Harada *et al*., 2019). Ornithine promotes the secretion of growth hormones, improves sleep quality, enhances skin strength, and heals wounds (Aoki *et al*., 2010). In addition, the enhancement of wound-breaking strength and collagen deposition in the dorsal skin has been shown by dietary ornithine supplementation tests using wild-type and Inos knockout mice (Han *et al*., 2002). GABA is an inhibitory neurotransmitter in the central nervous system that plays a role in physiological functions such as regulating blood pressure, improving sleep quality and memory, regulating hormones, and enhancing immunity (Sun *et al*., 2021). Therefore, GABA has positive health-promoting effects that can aid in preventing and treating many diseases (Diana *et al*., 2014). Therefore, GABA is incorporated into many GABA-enriched food products, such as cereal-based products, dairy products, meats, vegetables, legumes, and beverages. In the present work, wild boar meat sauce was shown to be a condiment with multifunctional health-promoting effects. Future studies should investigate the functional constituents and mechanisms underlying the beneficial effects of wild boar meat sauce.

Conclusion

In the present work, meat sauces were prepared from wild boar meats. Meat sauce with no unpleasant smells could be prepared using 30% (w/w) rice *koji* and 0.5% (w/w) Alcalase 2.4 L FG to the boiled ground meats. The salt content of the sauce was remarkably low at approximately 7.8 g/100 g. The sauce exhibited powerful antioxidative, radical scavenging, and hyaluronidase inhibitory activities. In addition, the sauce completely inhibited ACE activity. The sauce had good taste strength and a balance of sweetness, umami, and sourness, as well as strong sweetness and umami, and weak bitterness and saltiness. Additionally, the sauce was rich in total and essential amino acids. Therefore, wild boar meat sauce could be a suitable condiment for modern consumers and soy sauce-related industries owing to its eating quality and health benefits.

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