https://doi.org/10.17221/49/2024-CJFS

Quality characteristics of Hokkaido brown bear meat sauces prepared with rice koji mold and food enzymes

*Azusa Takeda[1], Masataka Saito[2], Takeshi Nagai[3,4]**

[1]*Faculty of Agriculture, Yamagata University, Tsuruoka, Japan*
[2]*Department of Applied Nutrition, Kagawa Nutrition University, Sakado, Japan*
[3]*Graduate School of Agricultural Sciences, Yamagata University, Tsuruoka, Japan*
[4]*The United Graduate School of Agricultural Sciences, Iwate University, Morioka, Japan*

**Corresponding author: nagatakenagatake@yahoo.co.jp, tnagai@tds1.tr.yamagata-u.ac.jp*

Citation: Takeda A., Saito M., Nagai T. (2024): Quality characteristics of Hokkaido brown bear meat sauces prepared with rice koji mold and food enzymes. Czech J. Food Sci., 42: 372–381.

Abstract: Hokkaido brown bear (*Ursus arctos yesoensis*) meats are used as ingredients of game cuisines. In contrast, shank meats are unsuitable as edible meats due to gamy tastes and tough meats. Here, new meat sauces were developed using glutinous rice koji and food enzymes for the application of the underutilised meat i.e. shank meat. The proximate analysis showed that the obtained sauces were reduced-salt sauces at approximately 6.4–7.7 g·(100 g)$^{-1}$ as salt equivalent. The sauces had a light colour, no unacceptable odours, and strong sweetness and umami taste. The sauce with good sensory acceptability was rich in glutamic acid, leucine, lysine, and alanine. Besides, the essential amino acid contents were remarkably high at approximately 55.6%. In addition, the tested sauces had good antioxidative activities, scavenging activities against reactive oxygen species such as superoxide anion radicals and hydroxyl radicals, and angiotensin I-converting enzyme and hyaluronidase inhibitory activities. These results suggested that Hokkaido brown bear shank meat sauces, which had positive effects for human health, could be used as one of novel condiment with consumer demands.

Keywords: functionality; game meats; Hokkaido brown bear; meat sauce; underutilised resource

In recent years, the demand for natural condiments has been increasing due to the diversified eating habits and the trend of ethnic foods. At present, there are few studies on the condiments using game meats (Funatsu et al. 2015; Funatsu 2016). These sauces have distinctive, complicate, and unacceptable odours. Additionally, these sauces contain a large amount of sodium at approximately 20% as salt equivalents. Excess sodium intake is known to increase the risk of hypertension (DiNicolantonio and Lucan 2014). Besides, elevated blood pressure is one of the major risk factor for cardiovascular disease, stroke, disability, and death (Mills et al. 2016; Freeman et al. 2023). Foods are the principal source of dietary sodium. Consequently, lowering sodium levels in foods may contribute to reduce the risk of these diseases.

Hokkaido brown bear (*Ursus arctos yesoensis*) lives only in Hokkaido, the northernmost island of Japan. Recently, the damage of agricultural products, such as rice, fruits, vegetables, and feed crops by wild animals including Hokkaido brown bear is a serious problem in Japan. It is estimated to be approximately 15.6 billion yen (119.3 million USD) in 2022 (Ministry of Agriculture, Forestry, and Fisheries 2023). The capture of these animals has been mainly performed for prevention of these damages, resulting in increase

This research was partly supported by a grant from the Egashira Foundation of Japan.

https://doi.org/10.17221/49/2024-CJFS

of captured animals. These meats are called game meats and used as ingredients of game cuisines. However, there is a little demand for the meats, especially the shank meats, due to gamy tastes and tough meats, although hokkaido brown meats are eaten as simmened dish and bear meat hotpot. Therefore, it needs to develop the processing techniques of meats for an effective utilisation of underutilised meats. To the best of our knowledge, there is no information available concerning Hokkaido brown bear meat sauce in the past studies. The main purpose of this study was to develop high-quality meat sauces using Hokkaido brown bear shank meats by short-term fermentation. In addition, we aimed to clarify the physicochemical properties and functional properties of the sauces for their industrial applications.

MATERIAL AND METHODS

Material. Frozen Hokkaido brown bear shank meats were purchased from NNF Co., Ltd. (Japan). Brown rice of non-glutinous rice cv. Haenuki and glutinous rice cv. Himenomochi were obtained from a local wholesale market (Yamagata, Japan). Koji mold (*Aspergillus oryzae*) for soy sauce production was purchased from Akita Konno Shoten Co., Ltd. (Japan). Commercially available salt was obtained from Kobe Bussan Co., Ltd. (Japan). Alcalase 2.4L FG and Flavourzyme 1000L were obtained from Novozymes (Japan).

Chemical analysis. The proximate composition was analysed as described by Kagawa (2024).

Rice koji preparation. Brown rice was polished and then washed with the running water. After soaking in adequate water overnight, the rice was drained with a sieve for 1 h. These were steamed for 30 min and then cooled to approximately at 36 °C. Then, 0.05% (*w*/*w*) of the koji mold was sprinkled, gently mixed, and these were fermented at 36 °C and relative humidity of 90%. The obtained koji was cooled and used for the preparation of meat sauces.

Enzyme activity determination of koji. The α-amylase, glucoamylase, α-glucosidase, acid protease, and acid carboxypeptidase activities of the koji were determined according to Revised National Tax Administration Agency Analysis Method commentary. The enzyme solution of the koji was prepared by the homogenised extraction method (The Brewing Society of Japan 1993).

Meat sauce preparation. The moromi for meat sauces was prepared according to the formulation in Table 1. The vacuum-packed meats with a nylon/polyethylene film were heated for 1 min after the temperature in the central part of the meats reached at 75 °C. After the meats were ground using a meat grinder, these were mixed with koji, saline solution, water, and food enzymes as necessary. The moromi was fermented at 33 °C and gently mixed once a day. After fermentation for 2 months, the moromi was heated for 30 min at 90 °C. These were centrifuged at 30 000 × g for 1 h at 4 °C to remove the lees. The supernatants were filtrated using No. 1 filter paper to remove the precipitates and used in the following experiments. The liquefaction rates of the moromi were calculated as described by Nagai et al. (2020a).

Microbiological analysis. The detection of lactic acid bacteria and yeasts was performed at 35 °C using plate count agar medium with bromocresol purple and potato dextrose agar medium containing 0.01% (*w*/*w*) chloramphenicol, respectively.

Physicochemical property. The physicochemical properties (colour, colour difference, pH, water activity (*aw*), total nitrogen, formol nitrogen, soluble solids excluding salts, Brix %, ethanol, total sugars, direct reducing sugars, acidity-I, acidity-II, titratable acidity, specific gravity, and histamine) of the sauces were evaluated as described by Nagai et al. (2020a).

Functional property. The total phenols, total flavonoids, total flavonols, antioxidative activity, radical scavenging activity, and angiotensin I-converting enzyme (ACE) and hyaluronidase inhibitory activities were determined as described by Nagai et al. (2020a).

Table 1. The formulation of meat sauces A–F prepared using Hokkaido brown bear shank meats

Ingredients	A	B	C	D	E	F
Boiled ground meat (g)	333	333	333	333	333	333
Rice koji (g)	33	33	33	100	100	100
21.6% (*w*/*w*) saline solution (g)	140	140	140	166	166	166
H_2O (mL)	100	100	100	118	118	118
Alcalase 2.4L FG (mL)	–	1.423	1.423	–	1.423	1.423
Flavourzyme 1000L (mL)	–	–	1.423	–	–	1.423

https://doi.org/10.17221/49/2024-CJFS

Sensory evaluation. Sensory quality of the sauces was assessed by nineteen trained panellists (21–60 years old; 1 male and 18 females) selected among staff and students of the department at room temperature. The panellists rinsed their mouths with water at room temperature before the analysis of each sauce. Each panellist was served 1 mL of the sauces and the sensory quality of the sauces was evaluated at 2 min intervals. The panel evaluated the sauces on the bases of the colour, smell (animal stinking), first taste (taste of the moment put in the mouth), aftertaste (taste leave after swallowing), sweetness, umami, sourness, saltiness, and overall acceptance in seven-point scale (–3 = weak, 0 = neither weak and strong, 3 = strong). Bitterness did not evaluate as the sauces had no bitterness in the exploratory experiments.

Free amino acid composition. One millilitre of the sauce E was added 5 mL of distilled water and then homogenised in ice. These were filled up to 10 mL with distilled water and then centrifuged at 30 000 × g for 15 min at 4 °C. The supernatants were diluted with 4 volumes of distilled water and then added the same volumes of 5% trichloroacetic acid. These were centrifuged at the same conditions and the supernatants were filtered with Millipore membrane filter (pore size 0.20 μL). Free amino acid analysis of the sauces were performed using an amino acid analyser (L-8900; Hitachi High-Technologies Corp., Japan) by on-line post-column derivatisation with ninhydrin.

Statistical analysis. The experiments were conducted in triplicate independently and these results were reported as mean ± SD (standard deviation). Statistical analysis of the results was performed by one-way ANOVA (analysis of variance) with the Tukey's test or Dunnett's test ($P < 0.05$) using Minitab Statistical Software (version 17).

RESULTS AND DISCUSSION

The proximate composition of the shank meats was investigated. The water, crude proteins, crude lipids, carbohydrates, and crude ashes contents were approximately 75.4, 20.5, 2.6, 0.5, and 1.0 g·(100 g)$^{-1}$, respectively (data not shown). The energy was calculated to be approximately 107.4 kcal·(100 g)$^{-1}$. Thus, the meats are suitable for the meat sauce production due to high proteins contents and low lipids contents.

Meat sauces preparation. The enzyme activities of the koji are an important factor for producing high-quality meat sauces. The α-amylase, glucoamylase, α-glucosidase, acid protease, and acid carboxypeptidase activities of cv. Haenuki koji were approximately 652.6, 237.3, 0.008, 113.6, and 2 610.9 U·g^{-1} koji, respectively. In contrast, those of cv. Himenomochi koji were approximately 615.7, 247.1, 0.008, 110.2, and 2 356.3 U·g^{-1} koji, respectively. Ito and Yamaguchi (2004) measured the Brix % and direct reducing sugars and glucose contents of rice pastes prepared using steamed rice and koji for miso production. The values of the pastes using cv. Himenomochi koji were high when compared with those of the pastes using cv. Hitomebore and cv. Akitakomachi koji. Murakami (2021) investigated the maltose contents of cooked rice saccharified using barley malt. The content of glutinous rice cv. Koganemochi was significantly higher than those of non-glutinous rice cv. Hinohikari, Tsuyahime, and Milkyqueen. These findings suggest that glutinous rice starches are easily saccharified when compared with non-glutinous rice starches. Therefore, cv. Himenomochi koji was used for meat sauce production. During fermentation, the pH values of the moromi (initial pH values: 5.53–5.91) decreased to the range of 3.70–3.87. The pH values and total nitrogen contents of the moromi had reached approximately 4.2–4.4 and 3.7–4.3 g·(100 g)$^{-1}$, respectively after fermentation for 2 months. In addition, the lactic acid bacteria and yeasts were not detected in the moromi, suggesting the digestion of the moromi by the enzymes released from rice koji and additive-food enzymes.

The liquefaction rates of the moromi were fairly high in the range of 70.1–86.9%. Besides, the addition of food enzymes was effective for the liquefaction of the moromi. Mikami et al. (2007) reported the increase of the yields of pork meat sauces using Alcalase 2.4L and Flavourzyme 500L. In addition, Trang et al. (2005) revealed that the yields of the pork meat sauces increased using Alcalase 2.4L and Pectinase 3S, however, the increase of salt contents resulted in decrease of the yields of the sauces. High contents of salts inhibited the fermentation of the moromi regardless of with or without the addition of enzymes. Hokkaido brown bear shank meat sauces are shown in Figure 1.

Proximate composition. The water contents of the sauces ranged from approximately 76.0–81.6 g·(100 g)$^{-1}$ (Table 2). The crude protein contents were approximately 11.8–15.9 g·(100 g)$^{-1}$. These contents of the sauces were significantly high with the addition of food enzymes, suggesting the acceleration of proteolysis in the moromi. The crude lipid contents were low. The carbohydrate contents of the sauces D–F were remarkably high when compared with those of the sauces A–C. In addition, the energies of the sauces D–F were significantly higher than those of the sauces A–C. The salt contents of the sauces were approximately 6.4–7.7 g·(100 g)$^{-1}$ as salt

https://doi.org/10.17221/49/2024-CJFS

Figure 1. Meat sauces prepared using Hokkaido brown bear shank meats

See Table 1 for the definitions of meat sauces A–F

equivalents. Thus, the tested sauces were reduced-salt sauces, as these sauces fell into reduced-salt grade according to Japan Soy Sauce Research Institute (1985). In contrast, the salt contents of other sauces were approximately 2–3 times as high as those of Hokkaido brown bear shank meat sauces (Abe and Ohnishi 2005; Mikami et al. 2007; Yang et al. 2012; Funatsu et al. 2015). Excessive sodium intake increases the risks of gastric cancer (Liem et al. 2011), arteriosclerosis and ischemic heart disease, chronic kidney disease, stroke, and obesity (He and MacGregor 2010), and diminishes bone mineral density (Tsugane et al. 2004). Besides, high-salt sauces are limited in the use of cooking recipes. Thus, Hokkaido brown bear shank meat sauces are attractive condiments for consumers and its related industries.

Physicochemical parameters. In general, the colours of foods are an important factor to the preference and acceptability of consumers. The colour parameters of the sauces are shown in Table 3. The L^* (lightness) and b^* (yellowness) values of the sauces D–F were relatively low when compared with those of the sauces A–C. In contrast, a^* (redness) values of the sauces D–F were much higher than those of the sauces A–C. Whiteness indexes of the sauces D–F were low in comparison with those of the sauces A–C. That is, the sauces D–F had colours with high degree of redness and low degree of whiteness and yellowness. The results indicated the browning by the Maillard reaction during fermentation on the sauces D–F. The colour differences of the sauces were evaluated to the sauce A as follows: B–D (much) and E and F (very much), respectively.

The pH values of the sauces were approximately 4.94–5.35. These values were almost similar to those of Yezo sika deer hind leg meat sauces (Funatsu et al. 2015) and pork meat sauces (Mikami et al. 2007). The *aw*s of the foods are important in relation to their shelf life. The *aw*s of the sauces were approximately 0.87–0.89. According to Japan Soy-sauce Brewers' Association (2023), the *aw*s of commercially available (CA) soy sauces are approximately 0.76–0.85. Thus, the *aw*s of the tested sauces are slightly higher than those of CA soy sauces. The limit pHs and *aw*s

Table 2. Proximate compositions of meat sauces prepared using Hokkaido brown bear shank meats

Samples	Water	Crude proteins	Crude lipids	Carbohydrates	Crude ashes	Salts	Energy
	g·(100 g)$^{-1}$	g·(100 g)$^{-1}$	g·(100 g)$^{-1}$	g·(100 g)$^{-1}$	g·(100 g)$^{-1}$	g·(100 g)$^{-1}$	kcal·(100 g)$^{-1}$
A	81.6^{a} ± 1.3	12.4^{c} ± 0.0	0.6^{a} ± 0.0	2.9^{c} ± 0.1	2.5^{a} ± 0.2	6.7^{b} ± 0.3	66.6^{c}
B	78.1^{b} ± 0.4	15.5^{a} ± 0.0	0.4^{ab} ± 0.0	3.5^{c} ± 0.1	2.5^{a} ± 0.2	6.5^{b} ± 0.3	79.6^{b}
C	78.2^{b} ± 0.4	15.9^{a} ± 0.3	0.2^{b} ± 0.0	3.0^{c} ± 0.1	2.7^{a} ± 0.1	6.5^{b} ± 0.3	77.4^{b}
D	79.1^{b} ± 0.7	11.8^{c} ± 0.0	0.5^{ab} ± 0.0	6.0^{b} ± 0.1	2.6^{a} ± 0.1	7.7^{a} ± 0.5	75.7^{b}
E	76.4^{c} ± 0.6	13.8^{b} ± 0.3	0.3^{b} ± 0.0	7.1^{a} ± 0.1	2.4^{a} ± 0.1	6.6^{b} ± 0.3	86.3^{a}
F	76.0^{c} ± 0.1	14.2^{b} ± 0.2	0.4^{ab} ± 0.0	7.2^{a} ± 0.1	2.2^{a} ± 0.1	6.4^{b} ± 0.5	89.2^{a}

$^{a–c}$ Different letters in the same lane indicate a significant difference ($P < 0.05$); see Table 1 for the definitions of meat sauces A–F

https://doi.org/10.17221/49/2024-CJFS

Table 3. Physicochemical properties of meat sauces prepared using Hokkaido brown bear shank meats

Parameters		A	B	C	D	E	F
Colour	L^*	$20.26^{a} \pm 0.44$	$16.45^{b} \pm 0.56$	$14.28^{c} \pm 0.92$	$16.28^{b} \pm 1.16$	$13.44^{c} \pm 2.49$	$11.92^{d} \pm 3.10$
	a^*	$0.87^{c} \pm 0.49$	$1.68^{c} \pm 0.58$	$2.72^{c} \pm 1.33$	$2.15^{c} \pm 0.73$	$8.17^{a} \pm 1.46$	$4.19^{b} \pm 1.30$
	b^*	$21.75^{a} \pm 3.15$	$17.96^{b} \pm 1.89$	$15.50^{c} \pm 2.18$	$18.82^{b} \pm 4.34$	$13.97^{cd} \pm 2.38$	$11.17^{d} \pm 1.93$
ΔE^*ab		–	much	much	much	very much	very much
pH at 20 °C		$4.95^{b} \pm 0.01$	$5.28^{a} \pm 0.02$	$5.35^{a} \pm 0.03$	$4.94^{b} \pm 0.01$	$5.10^{ab} \pm 0.01$	$5.10^{ab} \pm 0.01$
Water activity (*aw*) at 20 °C		$0.89^{a} \pm 0.00$	$0.87^{a} \pm 0.01$	$0.89^{a} \pm 0.00$	$0.88^{a} \pm 0.01$	$0.89^{a} \pm 0.00$	$0.88^{a} \pm 0.00$
Total nitrogen (%)		$1.98^{c} \pm 0.00$	$2.48^{a} \pm 0.00$	$2.54^{a} \pm 0.05$	$1.89^{c} \pm 0.00$	$2.21^{b} \pm 0.05$	$2.28^{b} \pm 0.03$
Formol nitrogen (%)		$0.88^{c} \pm 0.01$	$1.10^{b} \pm 0.02$	$1.36^{a} \pm 0.02$	$0.92^{c} \pm 0.01$	$1.14^{b} \pm 0.04$	$1.34^{a} \pm 0.02$
Formol nitrogen/total nitrogen		$0.44^{b} \pm 0.00$	$0.44^{b} \pm 0.01$	$0.54^{a} \pm 0.01$	$0.49^{ab} \pm 0.00$	$0.52^{a} \pm 0.02$	$0.59^{a} \pm 0.01$
Soluble solids excluding salts (%)		$15.9^{c} \pm 0.3$	$19.7^{b} \pm 0.3$	$20.1^{b} \pm 0.3$	$20.4^{b} \pm 0.5$	$23.3^{a} \pm 0.5$	$23.6^{a} \pm 0.5$
Brix % at 20 °C		$22.6^{c} \pm 0.0$	$26.2^{bc} \pm 0.0$	$26.6^{bc} \pm 0.0$	$28.1^{b} \pm 0.1$	$29.9^{a} \pm 0.3$	$30.0^{a} \pm 0.0$
Alcohol (%) at 20 °C		$0.24^{a} \pm 0.00$	$0.24^{a} \pm 0.00$	$0.24^{a} \pm 0.00$	$0.20^{a} \pm 0.00$	$0.18^{a} \pm 0.00$	$0.25^{a} \pm 0.00$
Total sugars [g·(100 mL)$^{-1}$]		$4.75^{c} \pm 1.62$	$2.81^{d} \pm 0.65$	$2.29^{d} \pm 0.87$	$14.29^{a} \pm 12.65$	$11.13^{b} \pm 6.69$	$10.26^{b} \pm 7.71$
Direct reducing sugars [g·(100 mL)$^{-1}$]		$0.53^{c} \pm 0.04$	$1.51^{c} \pm 0.06$	$1.61^{c} \pm 0.02$	$12.74^{b} \pm 0.74$	$12.26^{b} \pm 0.82$	$18.53^{a} \pm 0.48$
Acidity-I (mL)		$0.9^{a} \pm 0.0$	$0.8^{b} \pm 0.0$	$0.7^{b} \pm 0.0$	$0.9^{a} \pm 0.0$	$0.9^{a} \pm 0.0$	$1.0^{a} \pm 0.0$
Acidity-II (mL)		$0.9^{b} \pm 0.0$	$1.3^{a} \pm 0.0$	$1.2^{a} \pm 0.0$	$1.0^{b} \pm 0.0$	$1.4^{a} \pm 0.1$	$1.4^{a} \pm 0.0$
Titratable acidity (mL)		$1.8^{a} \pm 0.0$	$2.1^{a} \pm 0.0$	$1.9^{a} \pm 0.1$	$1.9^{a} \pm 0.0$	$2.3^{a} \pm 0.0$	$2.4^{a} \pm 0.0$
Specific gravity at 20 °C		$1.098^{a} \pm 0.004$	$1.099^{a} \pm 0.003$	$1.099^{a} \pm 0.004$	$1.120^{a} \pm 0.003$	$1.111^{a} \pm 0.005$	$1.116^{a} \pm 0.001$
Total phenols [mg gallic acid equivalent·mL^{-1}]		$3.42^{c} \pm 0.09$	$5.38^{b} \pm 0.10$	$6.13^{a} \pm 0.29$	$4.76^{b} \pm 0.09$	$6.72^{a} \pm 0.18$	$6.51^{a} \pm 0.17$
Total flavonoids [mg quercetin equivalent·mL^{-1}]		$0.82^{c} \pm 0.02$	$0.93^{b} \pm 0.02$	$0.94^{b} \pm 0.03$	$0.75^{c} \pm 0.02$	$0.92^{b} \pm 0.03$	$1.20^{a} \pm 0.05$
Total flavonols [mg rutin equivalent·mL^{-1}]		$0.07^{b} \pm 0.00$	$0.08^{b} \pm 0.00$	$0.10^{b} \pm 0.01$	$0.08^{b} \pm 0.00$	$0.12^{ab} \pm 0.00$	$0.15^{a} \pm 0.00$
Histamine (mg·kg^{-1})		$25.1^{a} \pm 4.1$	$15.6^{b} \pm 5.0$	$28.4^{a} \pm 2.5$	$24.9^{a} \pm 2.8$	$0.5^{c} \pm 0.8$	$2.5^{c} \pm 1.3$

$^{a–d}$ Different letters in the same row indicate a significant difference ($P < 0.05$); the colour measurements of the sauces were performed using a colourimeter (NR-11A; Nippon Denshoku Industries Co., Ltd., Japan); L^* – lightness; a^* – redness; b^* – yellowness; ΔE^*ab – colour difference; see Table 1 for the definitions of meat sauces A–F

for the growth of foodborne disease agents are 5.0 and 0.88 (*Staphylococcus aureus*), 4.2 and 0.92 (*Salmonella*), 4.6 and 0.92 (*Bacillus cereus* and *Clostridium botulinum*), and 5.4 and 0.94 (*C. perfringens*), respectively (Sekine 2017). Therefore, it suggests that the appropriate management to *S. aureus* is required in the sauces C and E during storage at 4 °C.

High total nitrogen contained in the sauces bring to more favourable tastes as umami parameter. The contents of the sauces treated with food enzymes were high at approximately 2.21–2.54. Besides, the formol nitrogen contents of the sauces C and F as umami component parameter were significantly high when compared with those of other sauces. Similar trends were observed in the formol nitrogen contents/total nitrogen contents, indicating effective proteolysis of the meat and rice proteins. Mikami et al. (2007) reported that there were no significant differences in the total nitrogen contents of pork meat sauces regardless of the kinds of enzymes. Additionally, the contents decreased with increasing the salt contents. In contrast, Trang et al. (2005) demonstrated that the contents of ground

https://doi.org/10.17221/49/2024-CJFS

pork sauces without enzyme treatment were influenced by the salt contents. However, there were no significant differences in the contents of the sauces with enzyme treatment regardless of salt contents.

The soluble solids excluding salts contents were approximately 15.9–23.6%. Particularly, the contents were high in the sauces E and F. It was due to the high rates of rice koji and the addition of enzymes. In contrast, the contents of Yezo sika deer hind leg meat sauces were low at approximately 11.9–15.9% (Funatsu et al. 2015), indicating that Hokkaido brown bear shank meat sauces were rich in the extracts. The Brix % of the sauces ranged from 22.6–30.0%. Among the analysed sauces, the Brix % of the sauces E and F was significantly high, suggesting strong sweetness of the sauces E and F. Total sugar contents and direct reducing sugar contents were investigated. The sauces D–F possessed significantly high contents, because the degradation of carbohydrates into sugars was accelerated in the moromi.

The acidity-I and -II are used as the indicators of first taste and aftertaste of the meat sauces. The sauces showed low acidity-I at approximately 0.7–1.0 mL and acidity-II at approximately 0.9–1.4 mL, respectively, suggesting weak first taste and aftertaste. In addition, the titratable acidities as an indicator of sourness of the sauces were approximately 1.8–2.4 mL. Yang et al. (2012) stated that the titratable acidities of the spent hen meat sauces were remarkably high at approximately 13.90–27.14 mL. That is, Hokkaido brown bear shank meat sauces were sauces with low acidity. It was considered that organic acids such as lactic acid did not produce in the meat sauces as lactic acid bacteria and yeasts were not detected in the moromi during fermentation. It is planning to investigate the organic acids of the sauces in detail to clarify why the acidities of the meat sauces were significantly low when compared with those of spent hen meat sauces.

The total phenol contents of the sauces D–F were high at approximately 4.76–6.72 mg gallic acid equivalents·mL^{-1}. It was due to high rates of rice koji of the sauces D–F. In addition, the total flavonoid contents and total flavonol contents of the sauces were approximately 0.75–1.20 mg quercetin equivalents·mL^{-1} and 0.07–0.15 mg rutin equivalents·mL^{-1}, respectively. These components will make a contribution to antioxidative activities of the sauces. Histamine contents of the sauces, which is the causative substance of allergy-like food poisoning, were as low as those of Yezo sika deer hind leg meat sauces (Funatsu et al. 2015), suggesting safe condiment for human consumption.

Functional property. The antioxidative activities of the sauces to linoleic acid oxidation were evaluated. The sauces perfectly inhibited the linoleic acid oxidation (data not shown), suggesting powerful antioxidant capacities of the sauces. The superoxide anion radical scavenging activities of the sauces D–F were higher than those of the sauces A–C (Table 4). Especially, the sauce E performed complete inhibition against these radicals. The trolox equivalents antioxidant capacities (TEAC) against these radicals were 6.98–7.10 mmol TE·mL^{-1} (TE – trolox equivalent). In addition, the sauce C had high hydroxyl radical scavenging activity among the sauces A–C (Table 4). In contrast, the sauces D–F showed the moderate activities at approximately 43.0–60.6%. The TEAC against hydroxyl radicals were 2.99–5.58 mmol TE·mL^{-1}. The measurement of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity is mainly used for the evaluation of the antioxidative activities of foods (Gulcin and Alwasel 2023). The sauces A–E showed remarkably high DPPH radical scavenging activities at approximately 66.1–87.0% (Table 4). Additionally, the sauce F completely inhib-

Table 4. Radical scavenging activities and ACE and hyaluronidase inhibitory activities of meat sauces prepared using Hokkaido brown bear shank meats (% inhibition)

Samples	Superoxide anion radicals	Hydroxyl radicals	DPPH radicals	ACE	Hyaluronidase
A	53.1^{d} ± 0.6	58.4^{b} ± 1.5	66.1^{d} ± 1.3	100^{a}	73.4^{b} ± 5.6 (38.4^{b})
B	57.6^{d} ± 0.7	61.5^{b} ± 1.3	79.9^{c} ± 1.2	100^{a}	82.3^{a} ± 3.7 (42.6^{a})
C	72.8^{c} ± 0.7	78.0^{a} ± 0.5	83.2^{c} ± 1.7	100^{a}	83.8^{a} ± 3.0 (43.3^{a})
D	89.1^{b} ± 1.3	50.4^{c} ± 1.3	82.7^{c} ± 1.7	100^{a}	75.8^{b} ± 5.3 (38.8^{b})
E	100^{a}	60.6^{b} ± 0.6	87.0^{b} ± 0.4	99.5^{a} ± 0.7	85.7^{a} ± 2.4 (43.7^{a})
F	98.3^{a} ± 0.4	43.0^{d} ± 1.1	100^{a}	100^{a}	85.6^{a} ± 0.8 (43.5^{a})

$^{a–d}$Different letters in the same lane indicate a significant difference ($P < 0.05$); values in brackets are millimoles of sodium cromoglicate equivalents per kg of meat sauces; see Table 1 for the definitions of meat sauces A–F; DPPH – 1,1-diphenyl-2-picrylhydrazyl; ACE – angiotensin I-converting enzyme

https://doi.org/10.17221/49/2024-CJFS

ited the DPPH radicals. The TEAC against DPPH radicals were 0.53–0.54 mmol TE·mL^{-1}.

Next, all the tested sauces exhibited significantly high ACE inhibitory activities (Table 4). Particularly, the sauces A–D and F performed complete inhibition of ACE activities. Besides, the IC_{50} (half maximal inhibitory concentration) values against ACE activities of the sauces were calculated to 10.31–33.97 μg protein·mL^{-1}. Thus, Hokkaido brown bear shank meat sauces showed excellent suppression effects against the increase of blood pressure. The hyaluronidase inhibitory activities of the sauces ranged from at approximately 73.4–85.7% (Table 4). Sodium cromoglicate (SC) is used for one of CA anti-allergic and anti-asthmatic drugs. The activities of the sauces were calculated to 38.4–43.7 mmol of SC equivalent (SCE) per kg of the sauces. It was suggested that 2 mL of the sauces (contains approximately 43.2–49.7 mg SCE) exhibited good anti-allergic effects in comparison with CA anti-allergic drug (2 mL of an ampule) that contains 20 mg of SC (1% SC inhalant liquid sawai; Sawai pharmaceutical Co., Ltd., Japan). The protein digestion using enzymes generates various kinds of peptides and amino acids with beneficial effects to human health. In fact, the meat-derived peptides from beef, pork, and chicken possesses antioxidative, antihypertensive, antimicrobial, antithrombotic, opiate-like, and mineral binding effects (Xing et al. 2019). In recent years, we tried to prepare Alaskan pink shrimp sauces using non-glutinous rice cv. Yukiwakamaru and Tsuyahime koji (Nagai et al. 2020a, b). These shrimp sauces perfectly inhibited ACE activities as well as Hokkaido brown bear shank meat sauces. In addition, these sauces exhibited the same inhibition against superoxide anion radicals and hyaluronidase as meat sauces. In contrast, the scavenging activities against hydroxyl radicals and DPPH radicals of shrimp sauces were slightly high when compared with those of meat sauces. Thus, Hokkaido brown bear shank meat sauces had multifunctional health benefits including the prevention of cardiovascular diseases, high blood pressure, and allergy.

Sensory evaluation. The colours of the sauces D–F had high scores (Figure 2). In particular, the scores of the sauces E and F were high due to the nonenzymatic browning reactions. Excluding the sauce F the sauces had low scores in terms of smell (animal stinking), suggesting the sauces with no unpleasant odours. The first taste had high score in the sauces with the addition of food enzymes, however, the sauce E showed low score in terms of aftertaste. The sauces D–F had strong umami and weak sourness. In addition, the analysed sauces showed strong saltiness, although the salt contents of the sauces were fairly low. Therefore, the sauces were aged for 1 month at 4 °C. Consequently, the sauces had strong sweetness and umami and weak saltiness, sug-

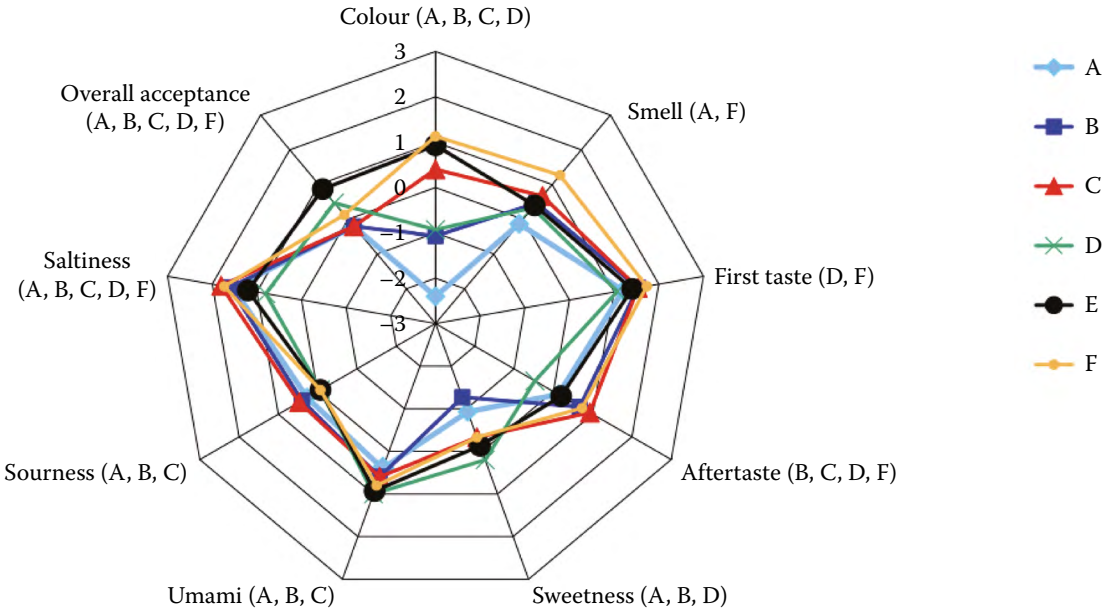

Figure 2. Sensory evaluation of meat sauces prepared using Hokkaido brown bear shank meats

See Table 1 for the definitions of meat sauces A–F; significant difference at $P < 0.05$ indicates between meat sauce E and other sauces in parentheses

https://doi.org/10.17221/49/2024-CJFS

gesting decrease in sharp taste of salt. Additionally, the tastes of sweetness, umami, sourness, and saltiness are accompanied by the first taste or the aftertaste. Mikami et al. (2007) reported that pork meat sauces with 20% salts, Alcalase 2.4L, and Flavourzyme 500L preferred in colour, flavour, taste, and overall evaluation. In contrast, the flavour scores of ground pork meat sauces with 15% salts were high (Trang et al. 2005). In addition, the sauces with the addition of enzymes showed high scores in colour, aroma, flavour, and overall characteristics. Generally, the game meat has distinctive and unacceptable odours. In contrast, Hokkaido brown bear shank meat sauces had no unpleasant smell and taste due to the use of fresh meats and the suppression of lipid oxidation of the moromi during fermentation. Next, we tried to investigate the correlation between the physicochemical properties and sensory attributes of the meat sauces. As a result, there were positive correlation only between the contents of soluble solids excluding salts and the scores in terms of umami of the meat sauces (R^2 = 0.5731). Finally, it was concluded that the sauce E was a sauce with good overall acceptability.

Free amino acid composition. Total free amino acid contents of the sauce E were approximately 5 338.4 mg·(100 g)$^{-1}$ (Table 5). Glutamic acid was the dominant amino acid, followed by leucine, lysine,

Table 5. Free amino acid composition of meat sauce E prepared using Hokkaido brown bear shank meats

Amino acids	Meat sauce E*	Yezo**	Thick***	Thin***	Low-salt thin***	Tamari***
	mg·(100 g)$^{-1}$ [mg·(100 mL)$^{-1}$]	mg·(100 g)$^{-1}$	mg·(100 g)$^{-1}$	mg·(100 g)$^{-1}$	mg·(100 g)$^{-1}$	mg·(100 g)$^{-1}$
Taurine	84.1^{a} (93.4)	42^{a}	–	–	–	–
Aspartic acid	314.5^{e} (349.4)	553^{d}	780^{b}	620^{cd}	710^{c}	1 300^{a}
Threonine	228.5^{c} (253.9)	323^{b}	320^{b}	250^{c}	280^{c}	490^{a}
Serine	20.3^{d} (22.5)	198^{c}	410^{b}	340^{b}	380^{b}	640^{a}
Glutamic acid	747.5^{c} (830.5)	874^{c}	1 600^{b}	1 300^{b}	1 600^{b}	2 700^{a}
Glycine	193.3^{c} (214.8)	163^{c}	310^{b}	260^{b}	290^{b}	620^{a}
Proline	198.4^{d} (220.4)	195^{d}	510^{b}	400^{c}	430^{c}	630^{a}
Alanine	480.9^{b} (534.3)	511^{b}	420^{c}	280^{d}	340^{cd}	580^{a}
Valine	333.0^{c} (370.0)	457^{b}	410^{b}	320^{c}	350^{c}	570^{a}
Cystine	ND	9^{c}	86^{b}	64^{b}	63^{b}	120^{a}
Methionine	222.3^{a} (247.0)	218^{a}	71^{b}	85^{b}	78^{b}	83^{b}
Isoleucine	335.2^{b} (372.4)	433^{a}	380^{ab}	300^{b}	290^{b}	460^{a}
Leucine	676.1^{b} (751.2)	775^{a}	560^{c}	430^{d}	420^{d}	600^{c}
Tyrosine	46.7^{b} (51.9)	80^{a}	89^{a}	60^{ab}	51^{b}	110^{a}
Phenylalanine	321.7^{b} (357.4)	349^{b}	340^{b}	260^{c}	240^{c}	440^{a}
GABA	12.2 (13.6)	–	–	–	–	–
Tryptophan	96.4^{a} (107.1)	20^{b}	18^{b}	13^{b}	16^{b}	23^{b}
Ornithine	100.8^{a} (112.0)	9^{b}	–	–	–	–
Lysine	626.2^{b} (695.7)	810^{a}	420^{c}	320^{d}	380^{cd}	660^{b}
Hydroxylysine	4.8 (5.3)	–	–	–	–	–
Histidine	129.3^{b} (143.7)	152^{b}	170^{ab}	140^{b}	140^{b}	250^{a}
Anserine	121.2 (134.6)	–	–	–	–	–
Carnosine	42.1 (46.8)	–	–	–	–	–
Arginine	2.9^{d} (3.2)	546^{a}	240^{c}	260^{c}	300^{c}	410^{b}
Total	5 338.4^{d} (5 931.1)	6 718^{b}	7 100^{b}	5 800^{c}	6 400^{c}	11 000^{a}

$^{a–d}$ Different lowercase letters in the same row indicate a significant difference (P < 0.05); *values are mean of triplicate assays; **data was quoted from the report of Funatsu (2016); ***data was quoted from Standard Tables of Food Composition in Japan 2024; Yezo – yezo sika deer hind leg meat sauce prepared using rice koji; thick – soy sauce; thin – thin soy sauce; low-salt thin: low-salt thin soy sauce; tamari – tamari soy sauce; ND – not detected; GABA – gamma-aminobutyric acid

https://doi.org/10.17221/49/2024-CJFS

alanine, and valine. These amino acids contributed approximately 53.6% of total amino acids. In addition, the sauce E contained relatively high amounts of taurine, tryptophan, and ornithine. Ornithine improves sleep quality, promotes the secretion of growth hormone, enhances skin strength, and heals wound (Aoki et al. 2010). Additionally, gamma-aminobutyric acid (GABA) was detected at approximately 12.2 mg·(100 g)$^{-1}$. GABA has positive health promoting effects, such as improvement of memory and sleep quality, enhancement of immunity, and regulation of blood pressure and hormone (Sun et al. 2021). It is known the existence of glutamic acid decarboxylase (GAD) that produces GABA from glutamic acid in koji mold (Tsuchiya et al. 2003). It was suggested that GAD in rice koji played an important part in GABA production of sauce E. In addition, anserine [121.2 mg·(100 g)$^{-1}$] and carnosine [42.1 mg·(100 g)$^{-1}$] were also detected in the sauce E. Essential amino acid contents were remarkably high at approximately 2 968.7 mg·(100 g)$^{-1}$ (55.6% of total amino acids). Besides, umami amino acid (glutamic acid), sour amino acids (aspartic acid, glutamic acid), sweet amino acids (alanine, glycine, proline, serine, threonine, valine), and bitter amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, tryptophan, valine) were approximately 747.5, 1 062.0, 1 454.4, and 2 941.5 mg·(100 g)$^{-1}$, respectively (Kubota and Morimoto 2021).

CONCLUSION

In the present study, it could be developed reduced-salt meat sauces from Hokkaido brown bear shank meats as a part of effective use of underutilised meats. The sauces had light colour, no unpleasant smell, and strong sweetness and umami, suggesting the meat sauces with good sensory acceptability. Besides, the sauces were rich in essential amino acids and had good antioxidant capacity, especially scavenging activities against superoxide anion radicals and DPPH radicals, and antihypertensive and anti-allergic effects. Thus, Hokkaido brown bear shank meat sauces were condiments with multifunctional properties for human health. Further research is planning to elucidate the functional constituents of the sauces with relation to these functionalities in the near future.

REFERENCES

Abe M., Ohnishi S. (2005): Manufacture of fermented meat sauce from silky-fowl. Bulletin of the Kagawa Prefecture Livestock Experiment Station, 40: 28–29.

Aoki M., Komatsu M., Ochiai M., Watanabe F., Igarashi M., Nonomura T., Tomita S., Umehara N., Nakai N., Morishita K. (2010): Healing effects of oral administration of L-citrulline or L-ornithine in a rat pressure-ulcer model-comparison with L-arginine. Japanese Pharmacology and Therapeutics, 38: 807–816.

DiNicolantonio J.J., Lucan S.C. (2014): The wrong white crystals: Not salt but sugar as aetiological in hypertension and cardiometabolic disease. Open Heart, 1: e000167.

Freeman M.W., Halvorsen Y-D., Marshall W., Pater M., Isaacsohn J., Pearce C., Murphy B., Nicholas Alp M.P.H., Srivastava A., Bhatt D.L., Brown M.J. (2023): Phase 2 trial of baxdrostat for treatment-resistant hypertension. The New England Journal of Medicine, 388: 395–405.

Funatsu Y. (2016): Quality characteristics of Yezo sika deer *Cervus nippon yesoensis* meat sauce products prepared using different fermentation methods. Journal of the Brewing Society of Japan, 111: 150–159.

Funatsu Y., Miyauchi Y., Kawakami M., Ishioroshi M. (2015): Quality characteristics of Yezo sika deer (*Cervus nippon yesoensis*) meat sauce products prepared using soy sauce production technology. The Japanese Journal of Zootechnical Science, 86: 53–61.

Gulcin I., Alwasel S.H. (2023): DPPH radical scavenging assay. Processes, 11: 2248.

He F.J., MacGregor G.A. (2010): Reducing population salt intake worldwide: From evidence to implementation. Progress in Cardiovascular Diseases, 52: 363–382.

Ito Y., Yamaguchi Y. (2004): Studies on saccharification of *mochigome* using *koji*. Journal of Local Independent Administrative Agency Iwate Industrial Research Institute, 11: 11–14.

Japan Soy-sauce Brewers' Association (2023): HACCP Implementation Support Guide (for Beginners). Available at https://www.mhlw.go.jp/content/11130500/000502990.pdf#search=%27%E6%B0%B4%E5%88%86%E6%B4%BB%E6%80%A7%E3%81%A8pH%E3%81%8B%E3%82%89%E8%A6%8B%E3%82%8B%E5%B8%82%E8%B2%A9%E5%93%81%E3%81%97%E3%82%87%E3%81%86%E3%82%86%E3%81%AE%E5%8D%B1%E5%AE%B3%E5%BE%AE%E7%94%9F%E7%89%A9%E3%83%AA%E3%82%B9%E3%82%AF%E3%81%AB%E3%81%A4%E3%81%84%E3%81%A6%27 (accessed Mar 14, 2024; in Japanese).

Japan Soy Sauce Research Institute (1985): Soy Sauce Test. Tokyo, Japan Soy Sauce Research Institute. (in Japanese)

Kagawa A. (2024): Standard Tables of Food Composition in Japan. 8th Ed. Tokyo, Kagawa Nutrition University Publishing Division. (in Japanese)

Kubota K., Morimoto Y. (2021): Food Science-Nutrition Facts and Functionality. 2nd Ed. Tokyo, Tokyo Kagaku Dozin. (in Japanese)

Liem D.G., Miremad F., Keast R.S. (2011): Reducing sodium in foods: The effect on flavor. Nutrients, 3: 694–711.

https://doi.org/10.17221/49/2024-CJFS

Mikami M., Trang N. H., Shimada K., Sekikawa M., Hukushima M., Ono T. (2007): Properties of 'Shishibishio' fermented pork meat sauce. Nippon Shokuhin Kagaku Kogaku Kaishi, 54: 152–159.

Mills K.T., Bundy J.D., Kelly T.N., Reed J.E., Kearney P.M., Reynolds K., Chen J., He J. (2016): Global disparities of hypertension prevalence and control. Circulation, 134: 441–450.

Ministry of Agriculture, Forestry, and Fisheries (2023): Status of Crop Damage Caused by Wild Birds and Animals Nationwide (2022). Available at https://www.maff.go.jp/j/press/nousin/tyozyu/231128.html (accessed Mar 14, 2024; in Japanese).

Murakami Y. (2021): Effects of rice cultivar characteristics on the constituent profile of *komeame* (rice syrup). Bulletin of the Center for Educational Research and Teacher Development Shizuoka University, 31: 208–218.

Nagai T., Saito M., Tanoue Y., Kai N., Suzuki N. (2020a): Characteristics of low-salt Alaskan pink shrimp sauce prepared using nonglutinous rice cultivar *Yukiwakamaru* koji. Journal of Food Processing and Preservation, 44: e14747.

Nagai T., Saito M., Tanoue Y., Kai N., Suzuki N. (2020b): Reduced-salt shrimp sauces from Alaskan pink shrimp and nonglutinous rice cultivar *Tsuyahime* koji: Preparation and characterization. International Aquatic Research, 12: 279–290.

Sekine Y. (2017): Relationship between water activity and pH and inactivation of pathogenic microorganisms. Journal of Soy Sauce Research and Technology, 43: 333–338.

Sun L., Bai Y., Zhang X., Zhou C., Zhang J., Su X., Luo H., Yao B., Wang Y., Tu T. (2021): Characterization of three glutamate decarboxylases from *Bacillus* spp. for efficient γ-aminobutyric acid production. Microbial Cell Factories, 20: 153.

The Brewing Society of Japan (1993): Revised National Tax Administration Agency Analysis Method Commentary. 4th Ed. Tokyo, Commentary Editorial Committee.

Trang N.H., Shimada K., Sekikawa M., Ono T., Mikami M. (2005): Fermentation of meat with koji and commercial enzymes, and properties of its extract. Journal of the Science of Food and Agriculture, 85: 1829–1837.

Tsuchiya K., Nishimura K., Iwahara M. (2003): Purification and characterization of glutamate decarboxylase from *Aspergillus oryzae*. Food Science and Technology Research, 9: 283–287.

Tsugane S., Sasazuki S., Kobayashi M., Sasaki S. (2004): Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women. British Journal of Cancer, 90: 128–134.

Xing L., Lie R., Zhang W., Guanghong Z. (2019): Meat protein based bioactive peptides and their potential functional activity: A review. International Journal of Food Science and Technology, 54: 1956–1966.

Yang J-H., Kawakami M., Ishioroshi M., Funatsu Y. (2012): The quality of meat sauce prepared from culled hens meats. New Food Industry, 54: 43–49.

Received: March 14, 2024
Accepted: September 24, 2024
Published online: October 24, 2024