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ORIGINAL ARTICLE

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New sauces produced by koji fermentation of kamaboko show acceptable sensory characteristics, improved antioxidant activities, and high angiotensin-converting enzyme inhibition

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Abstract
In our previous study, kamaboko was fermented in koji to prolong its shelf life and to increase its health-promoting effects. After fermentation, the texture of the kamaboko became cheese- or tofuyo-like. In the present study, sauces were produced by the fermentation of kamaboko in fermentation beds of wheat (WS), rice (RS), brown rice (BS), or soybean koji (SS), and their sensory qualities examined. WS, RS and BS had similar qualities to commercial oyster sauce. Fried cabbage with RS had significantly better overall acceptance than fried cabbage with oyster sauce. SS had significantly higher ash, protein and fat contents, and significantly lower carbohydrate contents than WS, RS, and BS. Free amino acid contents increased following fermentation for all sauces, and were highest in SS. The sugar contents of WS, RS, and BS were significantly higher than that of SS. SS had the highest phenolic content followed by BS, WS and RS. Antioxidant activity and angiotensin-converting enzyme (ACE) inhibition were highest in SS. All the new sauces had significantly higher free amino acid contents than the equivalent fermentation beds without kamaboko.

Keywords Koji fermentation sauce · Kamaboko · Chemical composition · Radical scavenging activity · Angiotensin-converting enzyme inhibition · Sensory analysis

Introduction

Numerous types of fermented foods, e.g., fermented products of milk, beans, fruits, grains, vegetables, fish, and meat, are consumed worldwide. Various methods are used to produce fermented foods, and koji fermentation is one of the traditional methods used in Asia. Koji is made from grain inoculated with *Aspergillus oryzae*. Vegetables, meat, and seafood undergo solid-state fermentation (where the fermentation microorganisms grow on the surface of the food) with koji. Koji fermentation is used to produce seasonings/sauces for cooking, and typical examples of these in Japan are miso and soy sauce. A great deal of research has been carried out for the production of new, fermented seasonings/sauces, e.g., from wheat flour (Gao et al. 2018), pork meat (Ohata et al. 2016), insects (Cho et al. 2018), catfish (Gao et al. 2019), and shrimp (Funatsu et al. 2019). We previously reported that koji fermentation of kamaboko can prolong its shelf life (Yoshioka et al. 2020; Nemoto et al. 2020). During the koji fermentation of kamaboko, the kamaboko loses its elastic texture and becomes soft, like cheese. The potential use of the fermentation bed, a by-product of this process which contains mainly fish-derived proteins and grain-derived carbohydrates and salt, as a seasoning/sauce was tested in the present study. The sauces produced in this study were found to have certain similarities to commercial oyster sauce produced by the fermentation of oyster and salt. Thus, to examine whether it is possible to utilize such by-products of the koji fermentation of kamaboko as seasoning sauces, the sauces produced here were tested by sensory analysis to see if they were as acceptable to consumers as oyster sauce.

Koji fermentation is reported to lead to the release of free amino acids and peptides (Yasuda et al. 2012; Kuba

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et al. 2005), to improve antioxidant activity (Lioe et al. 2018; Kuba et al. 2005; Li et al. 2010; Lee et al. 2007), and to increase the inhibition of angiotensin-converting enzyme (ACE), which is of importance for the regulation of blood pressure (Boschin et al. 2014). In addition to analyzing the taste and quality acceptance of the sauces, it was important to analyze their chemical compositions to ascertain their valuable nutritional features. Previous chemical analyses have indicated that kamaboko stimulates fermentation, which is expected to increase the level of released free amino acids. We report the effect of four different types of koji (wheat, rice, brown rice, and soybean) which have been previously used for kamaboko fermentation (Nemoto et al. 2020), and the differences in chemical composition of the fermentation products depending on the grain used.

The chemical composition, antioxidant and anti-hypertensive effects of the four koji sauces (wheat, rice, brown rice, and soybean) were analyzed, and a sensory analysis of fried cabbage served with each carried out.

Materials and methods

Materials

Surimi (produced from Alaska Pollock, FA grade) was purchased from Nippon Suisan Kaisha (Tokyo). Frozen commercial wheat koji (steamed wheat mixed with *Aspergillus oryzae*), rice koji (steamed rice mixed with *A. oryzae*), brown rice koji (steamed brown rice mixed with *A. oryzae*) and soybean koji (steamed soybean mixed with *A. oryzae*) were obtained from Suzuki-Kojiten (Shizuoka, Japan). ACE from rabbit lung, hippuryl-L-histidyl-L-leucine (Hip-His-Leu), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Trolox, and captopril were purchased from Sigma–Aldrich (St. Louis, MO). All the other reagents used for the experiments were of analytical grade.

Kamaboko preparation

Kamaboko was prepared as described by Yoshioka et al. (2020).

Fermentation of kamaboko and handling of the koji fermentation beds and sauces

The commercial kojis (750 g), 900 ml of shochu [Japanese rice liquor, 35% ethanol (volume/volume; White-Takara; Takara Shuzo, Kyoto, Japan)], and 132 g of salt were mixed in a mortar to make the fermentation beds, as described by Onodera and Kikuchi (2003) and in our former report (Yoshioka et al. 2020). The kamaboko was immersed in the individual fermentation beds and kept at 20 °C under darkness for 8 weeks. The ratio of kamaboko:fermentation bed was 1:2 (weight/weight). After 8 weeks of fermentation at 20 °C, the kamaboko (solid product) was removed, and then the wheat, rice, brown rice, or soybean koji-fermented sauces homogenized individually using a food processor to make wheat-koji sauce (WS), rice-koji sauce (RS), brown rice-koji sauce (BS), and soybean-koji sauce (SS). For comparison, fermentation beds with wheat (WB), rice (RB), brown rice (BB), and soybean (SB) koji, but without kamaboko, were maintained at 20 °C for 8 weeks then homogenized and analyzed in the same way as the sauces.

Sensory characteristics of the koji sauces

WS, RS, and BS were selected from preliminary tests for sensory analysis. Oyster sauce obtained from a local supermarket, and salt, were used for comparison. Koji sauce (30 g of WS, RS, or BS), oyster sauce (20 g), or salt (1.5 g) were added to 150-g shredded packaged (commercial) cabbage to make fried cabbage. The cabbage was fried under fixed conditions (high heat for 2 min) without the addition of oil. Fried cabbage with each different sauce was served to a panel for sensory evaluation. Sensory evaluation attributes were selected from a preliminary experiment. A scoring method was used for the sensory analysis to evaluate the differences in qualities among the fried cabbage with different sauces. Two trials were performed, the first to compare the quality of the fried cabbage with WS, RS or BS; the second to examine the difference in the fried cabbage served with RS, oyster sauce or salt. The fried cabbage with either WS, RS, or BS was tested by a panel of 13 individuals (20–24 years old; eight males, five females) for the evaluation of appearance, strength of smell, grassy smell, saltiness, sweetness, umami, aftertaste, and overall acceptability. The panel was asked to evaluate the test samples on a linear scale of: 0 (not detected or strongly dislike), -5 (intermediate), -10 (strong sensation or like a lot). Fried cabbage with RS, oyster sauce or salt were served to a panel of 29 individuals (20-24 years old; 19 males and ten females) to evaluate the sensory characteristics in the same manner as above. The study was conducted in accordance with the Ethical principles regarding human experimentation outlined in the Declaration of Helsinki.

Proximate analysis of the koji fermentation bed and sauce

The proximate compositions (moisture, ash, protein, and fat contents) were determined following the Association of Official Analytical Chemists (1995) method.

Mineral composition of koji fermentation beds and sauces

Mineral compositions were determined using wet-ashed samples, as described in our former report (Yoshioka et al. 2020). Phosphorous (P) content was measured by the molybdenum blue method as described by Briggs (1924). The other minerals were analyzed according to Matsuo et al. (2019) and Yoshioka et al. (2020); sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) contents were measured using an AA-7000 atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan) with an air-acetylene flame; iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) were determined by graphite furnace atomic absorption, with an AA-7000 spectrophotometer (Shimadzu), GFA-7000 graphite furnace atomizer and ASC-7000 auto sampler. A pyrocoated graphite tube was used for the analysis.

Fatty acid composition of koji fermentation beds and sauces

Lipid extraction was based on the method described by Bligh and Dyer (1959) with some modifications. The extracted lipids were transesterified following Matsuo et al. (2019) and Yoshioka et al. (2020). The esterified lipids were subjected to separation, identification and quantification by a gas chromatography mass spectrometry (GC–MS) system (Thermo Fisher Scientific, MA). The GC–MS consisted of a Trace GC ultra, DSQ II MS, AS3000 auto sampler, capillary column (InertCap Pure-WAX, 0.25 µm internal diameter × 0.25 mm × 30 m; GL Sciences, Tokyo) and a split injector. The results were calculated from the relative peak area of each fatty acid detected, and are expressed as percent of total fatty acids.

Preparation of extracts from koji fermentation bed and sauce

Methanolic extracts were prepared as described by Yoshioka et al. (2020).

Sugar, amino acid, and phenolic contents, antioxidant activities, and ACE inhibition of methanolic extracts

The total sugar content of the extracts was analyzed spectrophotometrically using a phenol–sulfuric acid method with glucose as a standard (Dubois et al. 1951). The free amino acid profile was obtained using an amino acid analyzer (High-speed Amino Acid Analyzer L-8900; Hitachi High-Tech, Tokyo) with a Hitachi custom ion exchange column (Hitachi). The phenolic content was determined following the Folin-Ciocalteu method using gallic acid as a standard (Sheikh et al. 2009).

DPPH and ABTS radical scavenging activities of the extracts were analyzed using the protocol reported by Nemoto et al. (2020) and Yoshioka et al. (2020). The results are expressed as the median effective dose, i.e., the concentration of antioxidant required to quench 50% of the initial radicals (ED_{50}) under the given experimental conditions. Trolox, which is a strong radical scavenger, was also tested, and the ED_{50} value converted to Trolox equivalents (eq.) (mg Trolox eq./g DM). ACE inhibitory activity was measured spectrophotometrically using Hip-His-Leu as a substrate according to the method described by Cushman and Cheung (1971), with minor modifications as described by Nakajima et al. (2009). Captopril, which is a strong ACE inhibitor, was also tested. The results are given as inhibition ratio (%), and the different concentrations of the extracts further tested to determine the concentration of samples required to inhibit 50% of the ACE activity (IC_{50}) under the given experimental conditions. The results are expressed as IC_{50} [mg dry matter (DM)/sample; DM/milliliters) or captopril equivalents.

Statistical analysis

The results are presented as mean ± SD. ANOVA and Tukey's test were used to calculate significant differences at $p < 0.05$ (SPSS version 15.0 for Windows; SPSS, Chicago, IL).

Results

Sensory analysis

The results of the sensory analysis of fried cabbage with the different koji sauces (WS, RS, and BS) are shown in Table 1. In terms of overall acceptability, fried cabbage with WS was significantly more favorable than that with BS, while the acceptability of cabbage with RS was not significantly different to that with BS. Fried cabbage with RS was considered to have a significantly better appearance and stronger sweetness than cabbage with WS and BR.

The results of the sensory analysis of fried cabbage with RS, oyster sauce, or salt are shown in Table 2. Fried cabbage with RS, or with salt, had significantly better overall acceptability than fried cabbage with oyster sauce. Fried cabbage with RS, or with salt, was also considered to have significantly stronger sweetness and aftertaste, and to be significantly less salty, than fried cabbage with oyster sauce.

Table 1 Sensory analysis of fried cabbage with the different koji sauces

Perceptions	Score 0	Score 5	Score 10	Fried cabbage with WS	Fried cabbage with RS	Fried cabbage with BS
Appearance	Dislike extremely	Intermediate	Like extremely	3.46 ± 1.51^a	7.46 ± 0.97^c	6.15 ± 1.91^b
Smell	Weak	Intermediate	Strong	4.46 ± 1.94^a	4.31 ± 2.18^a	6.08 ± 1.66^b
Grassy smell	Weak	Intermediate	Strong	3.23 ± 1.96^a	4.85 ± 1.91^b	5.92 ± 2.10^c
Sweetness	Not feel	Intermediate	Feel strongly	5.69 ± 1.89^a	6.62 ± 1.56^b	5.08 ± 2.06^a
Saltiness	Not feel	Intermediate	Feel strongly	4.23 ± 2.39^a	3.38 ± 1.56^a	4.23 ± 2.45^a
Umami	Not feel	Intermediate	Feel strongly	5.46 ± 2.79^b	5.31 ± 1.93^b	4.00 ± 1.73^a
Overall taste	Dislike extremely	Intermediate	Like extremely	5.23 ± 2.31^{ab}	5.69 ± 2.25^b	4.38 ± 1.80^a
Aftertaste	Not feel	Intermediate	Feel strongly	5.15 ± 2.67^a	4.69 ± 1.89^a	4.31 ± 2.39^a
Overall acceptability	Dislike extremely	Intermediate	Like extremely	5.69 ± 2.36^b	5.15 ± 2.34^{ab}	4.38 ± 1.98^a

Values are mean ± SD ($n = 13$; eight males and five females). *Different lowercase letters* within the *same row* indicate significant difference at $p < 0.05$

WS Wheat sauce, *RS* rice sauce, *BS* brown rice sauce

Table 2 Sensory analysis of fried cabbage with RS, oyster sauce, or salt

Perceptions	Score 0	Score 5	Score 10	Fried cabbage with RS	Fried cabbage with oyster sauce	Fried cabbage with salt
Appearance	Dislike extremely	Intermediate	Like extremely	6.21 ± 1.61^b	4.00 ± 1.85^a	7.55 ± 1.55^c
Smell	Weak	Intermediate	Strong	5.86 ± 2.28^b	6.66 ± 2.27^c	4.55 ± 2.26^a
Grassy smell	Weak	Intermediate	Strong	5.72 ± 2.17^b	2.41 ± 1.68^a	6.17 ± 2.09^b
Sweetness	Not feel	Intermediate	Feel strongly	7.07 ± 1.94^c	5.45 ± 2.15^b	4.10 ± 2.18^a
Saltiness	Not feel	Intermediate	Feel strongly	3.76 ± 2.12^a	4.55 ± 1.92^b	5.72 ± 2.59^c
Umami	Not feel	Intermediate	Feel strongly	5.79 ± 2.26^b	5.62 ± 1.54^b	3.93 ± 2.03^a
Overall taste	Dislike extremely	Intermediate	Like extremely	5.17 ± 2.41^a	4.93 ± 1.98^a	5.52 ± 2.03^a
Aftertaste	Not feel	Intermediate	Feel strongly	6.34 ± 1.40^c	5.69 ± 2.31^b	3.93 ± 2.28^a
Overall acceptability	Dislike extremely	Intermediate	Like extremely	5.14 ± 2.55^b	4.48 ± 1.88^a	5.31 ± 2.12^b

Values are mean ± SD ($n = 29$; 19 males and ten females). *Different lowercase letters* within the *same row* indicate significant difference at $p < 0.05$

Proximate composition

The proximate compositions of the koji fermentation beds without kamaboko (WB, RB, BB, and SB) and the kamaboko koji-fermented sauces (WS, RS, BS, and SS) are presented in Table 3. The moisture contents of the fermentation beds at day 0 were 61.9 (WB), 60.9 (RB), 59.1 (BB), and 71.7 g/100 g (SB) (without kamaboko, koji mixed with salt and alcohol). After fermentation with kamaboko they were 64.8 (WS), 65.8 (RS), and 64.3 (BS), 70.5 g/100 g (SS), respectively. At day 0 and at 8 weeks, SB had significantly higher ash, protein and fat contents and lower carbohydrate contents than WB, RB and BB. At day 0 and at 8 weeks, there was no significant difference in ash and protein contents among WB, RB, and BB. The ash content significantly decreased from 20.2 (WB), 18.0 (RB), 19.0 g/100 g DM basis (BB) to 18.7 g (WS), 18.7(RS), 17.3/100 g DM (BS). Adding kamaboko to the fermentation significantly increased the protein content, from 7.29 (WB), 6.03 (RB), 7.14 g/100 g DM (BB) to 11.4 (WS), 10.4 (RS) and 11.8 /100 g DM (BS). Kamaboko fermented koji sauces (WS, RS, and BS) had significantly higher protein content at 8 weeks than the fermentation beds without kamaboko (WB, RB, and BB). The same tendency was not seen for SB and SS. The fat contents of WB, RB, BB, WS, RS, and BS ranged from 1.23–3.72 g/100 g DM, while SB and SS had 12.6 and 13.5 g/100 g DM of fat, respectively. SS had a significantly higher protein content, 35.6 g/100 g DM, at 8 weeks than SB, 35.3 g/100g DM. The proximate composition of SS differed from those of WS, RS, and BS.

Mineral composition

The mineral compositions of the fermentation beds (WB, RB, BB, and SB) and koji sauces (WS, RS, BS, and SS) are presented in Table 4. Na was the major

Table 3 Proximate compositions (%) of the four types of koji fermentation beds and koji sauces

Sample		Time	Moisture	Ash	Protein	Fat	Carbohydrate
Wheat koji	Fermentation bed	Day 0	61.9 ± 0.71 c	7.70 ± 0.25 c	2.61 ± 0.06 e	0.47 ± 0.01 a	27.3
				(20.2 ± 0.23 b)	(7.29 ± 0.29 d)	(1.23 ± 0.06 a)	(71.3)
		8 Weeks	61.8 ± 0.56 c	7.90 ± 0.03 bcd	2.53 ± 0.15 e	0.74 ± 0.05 d	27.1
				(20.7 ± 0.07 b)	(7.04 ± 0.39 d)	(1.94 ± 0.12 cd)	(70.4)
	Sauce	8 Weeks	64.8 ± 0.74 de	6.61 ± 0.04 f	4.01 ± 0.12 b	0.60 ± 0.02 c	24.0
				(18.7 ± 0.43 d)	(11.4 ± 0.20 bc)	(1.69 ± 0.06 b)	(68.2)
Rice koji	Fermentation bed	Day 0	60.9 ± 1.02 ac	7.01 ± 0.28 a	2.19 ± 0.02 cd	0.51 ± 0.01 b	29.4
				(18.0 ± 1.28 a)	(6.03 ± 0.21 abc)	(1.30 ± 0.08 a)	(74.7)
		8 Weeks	59.8 ± 1.07 ab	7.26 ± 0.05 b	2.32 ± 0.01 d	0.70 ± 0.08 cd	29.9
				(18.1 ± 0.13 a)	(6.18 ± 0.02 b)	(1.69 ± 0.19 bc)	(74.1)
	Sauce	8 Weeks	65.8 ± 0.83 e	6.38 ± 0.15 e	3.54 ± 0.02 a	0.61 ± 0.04 c	23.7
				(18.7 ± 0.15 c)	(10.4 ± 0.23 c)	(1.75 ± 0.14 bc)	(69.2)
Brown rice koji	Fermentation bed	Day 0	59.1 ± 0.30 b	7.76 ± 0.10 cd	2.77 ± 0.09 e	0.91 ± 0.07 e	29.5
				(19.0 ± 0.08 b)	(7.14 ± 0.29 b)	(2.23 ± 0.15 d)	(71.7)
		8 Weeks	58.4 ± 1.39 ab	7.98 ± 0.12 d	2.74 ± 0.03 e	1.49 ± 0.36 f	29.4
				(19.2 ± 0.29 b)	(6.97 ± 0.07 b)	(3.72 ± 0.90 e)	(70.1)
	Sauce	8 Weeks	64.3 ± 0.89 de	6.16 ± 0.09 g	4.23 ± 0.07 a	1.25 ± 0.04 f	24.1
				(17.3 ± 0.52 e)	(11.8 ± 0.09 a)	(3.53 ± 0.10 e)	(67.4)
Soybean koji	Fermentation bed	Day 0	71.7 ± 0.29 h	7.91 ± 0.05 h	10.7 ± 0.45 e	3.72 ± 0.17 g	5.97
				(28.0 ± 0.48 g)	(38.5 ± 1.51 g)	(13.1 ± 0.69 f)	(18.5)
		8 Weeks	70.5 ± 0.39 fg	8.79 ± 0.09 h	10.2 ± 0.30 f	3.98 ± 0.52 g	6.49
				(29.8 ± 0.30 h)	(35.3 ± 1.03 e)	(13.5 ± 1.77 f)	(27.8)
	Sauce	8 Weeks	70.9 ± 0.97 gh	6.80 ± 0.04 h	10.3 ± 0.18 c	3.66 ± 0.06 g	8.28
				(23.4 ± 0.65 f)	(35.6 ± 0.92 f)	(12.6 ± 0.48 f)	(23.9)

Values are mean ± SD ($n = 3$–4). Values *in parentheses* are contents on a dry matter (DM) basis. *Different lowercase letters* within the *same column* indicate significant difference at $p < 0.05$

mineral in the fermentation beds and koji sauces, followed by K, P, Mg and Ca. SS had a significantly higher content of Na (4713 mg/100 g DM), K (1254 mg/100 g DM), Mg (203 mg/100 g DM), Ca (109 mg/100 g DM), Zn (2.53 mg/100 g DM), Mn (1.76 mg/100 g DM), Fe (14.3 mg/100 g DM), Cu (0.67 mg/100 g DM) and P (539 mg/100 g DM) than the other koji sauces (WS, RS and BS). The concentrations of Na, Mg and Mn in the fermented sauces (WS, RS, BS, and SS) were significantly lower than those of the fermentation beds maintained for 8 weeks (WB, RB, BB, and SB).

Fatty acid composition

The fatty acid compositions of the total lipids (TL) of the fermentation beds and koji sauces are presented in Table 5. The major fatty acids of the fermentation beds were linoleic acid (C18:2n-6; 32.2–41.2%), palmitic acid (C16:0; 12.5–27.1%), and oleic acid (C18:1; 18.0–32.5%). WB at day 0 contained 41.2% C18:2, but no EPA (C20:5), or DHA (C22:6). After fermentation with kamaboko, WS contained 38.1% linoleic acid, 2.06% EPA, and 3.04% DHA. After kamaboko was immersed in the fermentation beds, all the koji sauces had EPA and DHA derived from kamaboko. WS, RS, and BS contained 28.7–36.7% saturated fatty acids (SFA), 17.8–33.5% monounsaturated fatty acids (MUFA), and 35.3–46.8% polyunsaturated fatty acids (PUFA). Soybeans are considered to be oilseeds, and part of them are cultivated due to gain their oil; therefore, the fat content and fatty acid composition of the SS differed from those of the other koji sauces, and contained 18.2% SFA, 32.8% MUFA, and 47.2% PUFA.

Sugar, phenolic and free amino acids contents, antioxidant activities, and ACE inhibition of methanolic extracts

Extracted sugar and total phenolic contents of the fermentation beds and koji sauces are presented in Fig. 1. At day 0, the sugar concentration in WB was 17.3 g/100 g DM), and after 8 weeks had significantly increased to 27.3 g/100 g DM. Following addition of kamaboko to the fermentation bed, the sugar level of WS was 45.5 g/100 g DM of sugar after 8 weeks. The concentration of sugar increased for

Table 4 Mineral composition (mg/100 g DM) of the four types of koji fermentation beds and koji sauces

Sample		Time	Na	K	Mg	Ca	Zn	Mn	Fe	Cu	P
Wheat koji	Fermentation bed	Day 0	4970 ± 107 c	164 ± 19.4 c	53.5 ± 1.82 c	32.9 ± 0.53 b	1.08 ± 0.03 c	0.87 ± 0.03 d	4.61 ± 0.43 d	0.35 ± 0.04 d	138 ± 3.87 c
		8 Weeks	5381 ± 203 d	184 ± 2.00 cd	55.1 ± 1.62 c	39.3 ± 1.65 e	1.22 ± 0.03 d	0.90 ± 0.01 d	4.53 ± 0.13 d	0.31 ± 0.01 cd	125 ± 3.28 b
	Sauce	8 Weeks	3627 ± 298 a	190 ± 21.2 cde	44.3 ± 0.43 b	34.8 ± 0.88 d	1.01 ± 0.01 b	0.71 ± 0.02 c	3.51 ± 0.05 c	0.30 ± 0.03 cd	182 ± 5.08 d
Rice koji	Fermentation bed	Day 0	4864 ± 205 bc	65.6 ± 14.6 a	45.1 ± 2.04 b	32.5 ± 3.20 abd	1.19 ± 0.07 cd	0.60 ± 0.02 b	1.82 ± 0.11 a	0.24 ± 0.02 b	83.8 ± 4.46 a
		8 Weeks	4722 ± 84.8 b	76.5 ± 1.75 a	43.8 ± 0.25 b	30.3 ± 0.93 a	1.27 ± 0.04 d	0.58 ± 0.02 b	2.17 ± 0.60 ab	0.18 ± 0.02 a	84.8 ± 2.79 a
	Sauce	8 Weeks	4170 ± 195 a	105 ± 10.0 b	38.9 ± 1.12 a	34.9 ± 2.72 bcde	0.82 ± 0.05 a	0.40 ± 0.02 a	2.72 ± 0.51 bc	0.24 ± 0.04 abc	127 ± 8.52 bc
Brown rice koji	Fermentation bed	Day 0	4807 ± 143 bc	208 ± 19.8 cde	119 ± 2.21 de	30.0 ± 0.79 a	1.79 ± 0.13 ef	1.76 ± 0.12 ef	4.55 ± 0.29 d	0.31 ± 0.05 bcd	256 ± 8.36 e
		8 Weeks	4586 ± 138 b	217 ± 3.57 e	124 ± 2.73 e	32.8 ± 0.78 ac	2.13 ± 0.18 fg	2.03 ± 0.08 g	4.46 ± 0.58 d	0.28 ± 0.07 abcd	276 ± 3.17 f
	Sauce	8 Weeks	3876 ± 198 a	228 ± 31.8 de	109 ± 6.18 d	32.6 ± 0.63 b	1.77 ± 0.06 e	1.78 ± 0.09 e	3.03 ± 0.53 bc	0.24 ± 0.03 b	313 ± 20.2 g
Soybean koji	Fermentation bed	Day 0	6899 ± 137 f	1619 ± 83.8 g	242 ± 4.06 g	124 ± 2.66 g	2.45 ± 0.13 g	1.97 ± 0.04 fg	16.2 ± 0.65 e	0.81 ± 0.03 f	527 ± 9.62 h
		8 Weeks	6560 ± 159 e	1739 ± 31.3 g	244 ± 1.19 g	121 ± 0.94 g	2.71 ± 0.06 i	2.01 ± 0.11 fg	16.0 ± 0.85 e	0.80 ± 0.01 f	528 ± 7.11 h
	Sauce	8 Weeks	4713 ± 238 bc	1254 ± 52.5 f	203 ± 4.23 d	109 ± 3.13 f	2.53 ± 0.02 h	1.76 ± 0.04 e	14.3 ± 1.08 e	0.67 ± 0.03 e	539 ± 10.3 h

Values are mean ± SD ($n = 3$). *Different lowercase letters* within the *same column* indicate significant difference at $p < 0.05$

all the tested grains, but WB and WS showed the greatest increase in extracted sugar. A significant increase in extracted sugar from day 0 to 8 weeks was observed for WB, RB, and BB; however, there was no significant increase in that of SB. The extracted sugar contents in SB and SS were significantly lower than in the other fermentation beds and koji sauces.

At day 0, the phenolic contents in WB, RB, and BB ranged from 53.4 to 85.9 mg gallic acid eq./100 g DM, while that of SB was 352 mg gallic acid eq./100 g DM. After 8 weeks, WB, RB, BB and SB had phenolic contents of 86.7, 82.8, 92.4, and 381 mg gallic acid eq./100 g DM, respectively. When kamaboko was immersed into these fermentation beds for 8 weeks, the phenolic contents increased to 185 mg (WS), 185 mg (RS), 213 mg (BS), and 520 mg gallic acid eq./100 g DM (SS).

The free amino acid compositions of the fermentation beds and koji sauces are presented in Table 6. WS, RS, BS, and SS, had total free amino acid contents of 2800, 3250, 3300, and 10,500 mg/100 g DM, respectively. Sauces WS, RS, and BS contained higher concentrations of free amino acids than the fermentation beds WB, RB, and BB maintained at 20 °C for 8 weeks. SB had the highest percentage of protein of all the fermentation beds; the increase in free amino acids, i.e., that in SS following immersion of kamaboko for 8 weeks, was approximately 1.4-fold, which was smaller than that recorded for WS, RS, and BS. SS had the highest concentration of total free amino acids of all the koji sauces, i.e., three times higher than those of the others. After 8 weeks of fermentation, SS contained glutamic acid (1666 mg/100 g DM), leucine (1187 mg/100 g DM), aspartic acid (1003 mg/100 g DM), lysine (985 mg/100 g DM), and phenylalanine (939 mg/100 g DM), while WS, RS, and BS contained glutamic acid (range 394-407 mg /100 g DM), leucine (range 312-365 mg /100 g DM), aspartic acid (range 222-311 mg/100 g DM), lysine (range 340-397 mg /100 g DM), and phenylalanine (range 178–206 mg /100 g DM).

The DPPH and ABTS radical scavenging activity of the fermentation beds and koji sauces are presented in Fig. 2. The Trolox (molecular weight 250.3) ED_{50} was 5.02 μg/ml for the quenching of DPPH, and 3.50 μg/ml for ABTS. WB and BB showed a significant decrease in DPPH and ABTS radical scavenging activities from day 0 to 8 weeks. RB did not show any difference in scavenging activity between day 0 and 8 weeks. There was no increase in DPPH radical scavenging activity in SB from day 0 to 8 weeks, but there was a significant increase in ABTS radical scavenging activity in SB over this period. Radical scavenging activities of WS, RS, BS, and SS were 1.06, 0.52, 1.10, and 2.10 mg Trolox eq./g DM for DPPH, and 2.59, 1.92, 2.75 and 9.01 mg Trolox eq./g DM for ABTS, respectively. The highest radical scavenging activity was seen in SS for both DPPH and ABTS, and was statistically significant for both. The radical

Table 5 Fatty acid composition [area under the curve (%)] of the four types of koji fermentation beds and koji sauces

	Wheat koji			Rice koji			Brown rice koji			Soybean koji		
	Fermentation bed		Sauce	Fermentation bed		Sauce	Fermentation bed		Sauce	Fermentation bed		Sauce
	Day 0	8 Weeks	8 Weeks	Day 0	8 Weeks	8 Weeks	Day 0	8 Weeks	8 Weeks	Day 0	8 Weeks	8 Weeks
Myristic acid, C14:0	0.57 ± 0.00 c	0.78 ± 0.04 d	0.79 ± 0.03 d	1.49 ± 0.14 g	1.63 ± 0.17 g	1.69 ± 0.10 g	1.21 ± 0.10 f	0.79 ± 0.03 d	0.94 ± 0.03 e	0.20 ± 0.03 ab	0.16 ± 0.01 a	0.19 ± 0.01 b
Palmitic acid, C16:0	24.8 ± 0.13 g	2.65 ± 0.40 i	25.2 ± 0.11 h	25.2 ± 0.17 h	27.1 ± 0.19 i	26.7 ± 0.33 i	20.5 ± 0.44 d	22.6 ± 0.12 f	22.0 ± 0.21 e	12.5 ± 0.19 a	13.8 ± 0.12 c	13.2 ± 0.20 b
Palmitoleic acid, C16:1	0.30 ± 0.03 ab	0.50 ± 0.03 f	0.40 ± 0.04 cde	0.48 ± 0.04 ef	0.36 ± 0.03 cd	0.51 ± 0.03 f	0.68 ± 0.08 g	0.38 ± 0.03 cd	0.42 ± 0.04 de	0.34 ± 0.02 bc	0.27 ± 0.02 a	0.30 ± 0.01 b
Stearic acid, C18:0	5.10 ± 0.09 d	6.29 ± 0.08 h	4.70 ± 0.05 ac	7.83 ± 0.11 h	7.73 ± 0.15 g	7.78 ± 0.11 h	5.67 ± 0.30 f	4.15 ± 0.13 b	4.81 ± 0.12 ac	3.95 ± 0.71 ab	5.16 ± 0.06 de	3.95 ± 0.71 ef
Oleic acid, C18:1	18.0 ± 0.30 b	19.0 ± 0.24 c	16.6 ± 0.13 a	25.8 ± 0.16 e	26.4 ± 0.31 f	24.9 ± 0.17 d	32.2 ± 0.54 gh	32.5 ± 0.26 g	32.2 ± 0.38 g	31.8 ± 1.59 h	30.2 ± 0.59 h	31.8 ± 1.59 h
Linoleic acid, C18:2 (n-6)	41.2 ± 0.18 f	37.9 ± 0.53 e	38.1 ± 0.56 e	33.5 ± 0.11 c	32.2 ± 0.46 b	31.8 ± 0.08 b	30.3 ± 0.68 a	33.3 ± 0.26 c	30.3 ± 0.09 a	34.7 ± 0.52 d	34.1 ± 0.64 cd	33.6 ± 0.26 c
α-Linolenic acid, C18:3 (n-3)	3.74 ± 0.09 g	3.19 ± 0.03 f	3.60 ± 0.03 g	1.30 ± 0.07 b	1.10 ± 0.08 a	1.28 ± 0.06 b	2.51 ± 0.12 e	2.18 ± 0.11 d	1.90 ± 0.03 c	10.2 ± 0.20 h	11.7 ± 0.17 j	11.3 ± 0.15 i
Arachidic acid, C20:0	0.31 ± 0.02 a	0.38 ± 0.02 b	0.29 ± 0.01 a	0.52 ± 0.01 c	0.56 ± 0.06 c	0.56 ± 0.02 c	1.53 ± 0.18 h	0.82 ± 0.05 de	0.94 ± 0.06 f	1.05 ± 0.08 g	0.76 ± 0.03 d	0.86 ± 0.02 ef
Eicosenoic acid, C20:1	0.54 ± 0.01 c	0.46 ± 0.01 b	0.81 ± 0.04 f	0.33 ± 0.01 a	0.33 ± 0.03 a	0.50 ± 0.02 b	1.37 ± 0.17 h	0.77 ± 0.05 ef	0.91 ± 0.01 g	0.79 ± 0.05 ef	0.59 ± 0.02 d	0.71 ± 0.01 e
Eicosapentaenoic acid, C20:5 (n-3)	ND	ND	2.06 ± 0.28 d	ND	ND	1.13 ± 0.09 b	ND	ND	0.90 ± 0.04 a	ND	ND	1.34 ± 0.04 c
Docosahexaenoic acid, C22:6 (n-3)	ND	ND	3.04 ± 0.37 d	ND	ND	1.65 ± 0.08 b	ND	ND	2.23 ± 0.23 c	ND	ND	0.96 ± 0.03 a
Others	5.46 ± 0.26 f	5.05 ± 0.21 f	4.40 ± 0.02 e	3.67 ± 0.04 d	2.67 ± 0.24 ab	3.11 ± 0.16 bc	4.70 ± 0.53 ef	2.51 ± 0.06 a	2.48 ± 0.05 a	4.50 ± 0.18 e	3.30 ± 0.09 c	3.05 ± 0.14 bc

Values are mean ± SD ($n = 3$). *Different lowercase letters* within the *same row* indicate significant difference at $p < 0.05$

ND Not detected

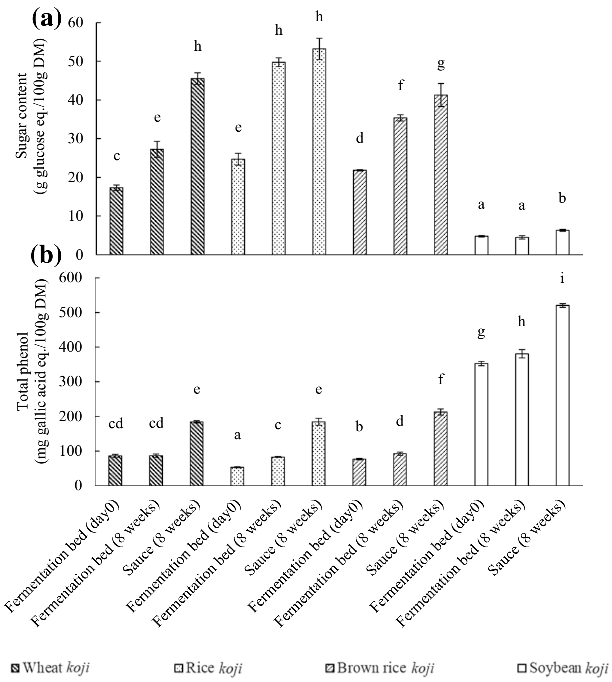

Fig. 1 Sugar contents (**a**) and total phenolic contents (**b**) of the four types of koji fermentation beds and koji sauces. Values are mean ± SD ($n = 3$–4). **a**, **b** *Different lowercase letters* indicate significant difference at $p < 0.05$. *DM* Dry matter

scavenging activities were the same for BS and WS, and significantly greater than that in RS.

The ACE inhibitory activity of the fermentation beds and koji sauces are presented in Fig. 3. At day 0, the ACE inhibitory activities were 0.008 mg for WB, 0.004 mg for RB, 0.003 mg for BB, and 0.039 mg captopril eq./g DM for SB. The koji sauces showed stronger ACE inhibitory activities than the fermentation beds [0.044 mg (WS), 0.019 mg (RS), 0.036 mg (BS), and 0.481 mg captopril eq./g DM (SS)]. SS showed the strongest activity followed by WS, BS, and RS.

Discussion

Sensory characteristics

In our former report (Nemoto et al 2020), wheat-, rice-, and brown rice-koji fermented kamaboko showed significantly better overall acceptance than soybean-koji fermented kamaboko, and preliminary sensory tests with koji sauces also showed better overall acceptance of WS, RS, and BS than SS. In the present study, we compared fried cabbage prepared with three of the koji sauces (WS, RS, and BS) to compare the acceptability of each, and to assess their future potential use. There was significantly favorable overall acceptability of fried cabbage with WS compared to BS, which had a significantly stronger grassy smell and significantly weaker umami taste than the other sauces. The quality of fried cabbage with RS was considered intermediate between that with WS or BS; RS had the strongest sweetness among the tested sauces. When we compared fried cabbage served with RS, oyster sauce or salt, fried cabbage with RS showed significantly better overall acceptability than that with oyster sauce. This was probably because fried cabbage with RS had a significantly weaker smell and saltiness, but a stronger grassy smell, sweetness, and aftertaste than fried cabbage with oyster sauce. When comparing the composition of free amino acids and their concentrations, RS had significantly higher contents of sweet tasting amino acids such as glycine and alanine than WS and BS. In addition, RS had the highest sugar content (Fig. 1), thus both this and its higher contents of sweet tasting amino acids probably accounted for its significantly stronger sweet taste compared to the other sauces. Fermented catfish paste was reported to have a total free amino acid content of around 1000–1300 mg/100 g (Gao et al. 2019). In the present study, RS had a total amino acid content of 1120 mg/100 g FM, thus similar to that of catfish paste, but higher contents of sweet-tasting amino acids such as glycine and alanine. In the present study, koji fermentation produced a cooking sauce that tasted sweeter than the commercial sauce tested (oyster sauce) or other seafood-based sauces. The NaCl content of 20 g of oyster sauce was calculated as 2.28 g using data from a food database [Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) 2015] (it was also calculated to contain 1.54 g of protein, 0.06 g of fat, 3.66 g of carbohydrate using data from the same source). In the present study, 30 g of RS contained 1.08 g NaCl (and 1.91 g of protein, 1.06 g of fat, 7.11 g of carbohydrate). Thus when RS is used instead of oyster sauce as a seasoning, on a weight for weight basis, it leads to a lower NaCl intake, which is considered beneficial for health, as a reduction in dietary NaCl is recommended to prevent high blood pressure. Thirty grams of RS also contained 65.8 g/100 g moisture and 0.52 mg Trolox eq./g DM of DPPH radical scavenging activity; thus one recommended serving would contain 5.34 mg Trolox eq./30 g FM, which is much higher than that of fermented fish or meat sauces (7.5-14 mg Trolox eq./100 mL) as reported by Ohata et al. (2016).

Chemical composition

The proximate compositions of WB, RB and BB did not change when they were maintained at 20 °C for 8 weeks. However, when kamaboko was immersed in these fermentation beds for 8 weeks, their proximate compositions did change. During the fermentation process, most of the kamaboko-derived protein was degraded and the products of this process moved into the koji fermentation beds, thus the

Table 6 Free amino acid concentrations (mg/100 g DM) of the four types of koji fermentation beds and koji sauces

	Wheat koji			Rice koji			Brown rice koji			Soybean koji		
	Fermentation bed		Sauce	Fermentation bed		Sauce	Fermentation bed		Sauce	Fermentation bed		Sauce
	Day 0	8 Weeks	8 Weeks	Day 0	8 Weeks	8 Weeks	Day 0	8 Weeks	8 Weeks	Day 0	8 Weeks	8 Weeks
Asp	47.1 ± 1.46 c	64.1 ± 1.60 d	222 ± 19.7 e	39.2 ± 0.68 b	62.6 ± 0.51 d	311 ± 22.9 f	30.1 ± 1.78 a	44.9 ± 0.63 c	271 ± 19.7 f	432 ± 5.90 g	802 ± 9.36 h	1003 ± 31.6 i
Thr	42.3 ± 2.16 e	44.8 ± 1.33 e	121 ± 7.17 f	33.1 ± 1.00 c	37.4 ± 0.38 d	148 ± 9.03 g	24.8 ± 1.59 a	27.6 ± 0.22 b	132 ± 7.64 fg	232 ± 2.95 h	318 ± 3.97 i	468 ± 14.5 j
Ser	44.3 ± 0.62 c	45.8 ± 1.24 c	130 ± 9.88 d	32.4 ± 0.75 b	44.1 ± 0.30 c	177 ± 12.0 e	26.3 ± 1.68 a	33.0 ± 0.38 b	167 ± 11.6 de	263 ± 3.78 g	421 ± 2.10 g	557 ± 15.7 h
Glu	109 ± 1.52 d	140 ± 4.40 e	396 ± 28.9 f	44.4 ± 1.03 a	82.6 ± 1.19 c	394 ± 27.1 f	46.9 ± 2.84 a	70.9 ± 0.69 b	407 ± 33.7 f	1021 ± 13.5 g	1314 ± 21.8 h	1666 ± 110 i
Gly	34.5 ± 0.50 e	35.1 ± 0.91 e	85.2 ± 6.91 f	25.5 ± 0.55 c	33.2 ± 0.34 d	134 ± 9.77 h	19.5 ± 1.25 a	23.6 ± 0.26 b	116 ± 8.62 g	173 ± 2.91 i	244 ± 4.34 j	335 ± 12.2 k
Ala	70.5 ± 1.25 e	73.6 ± 1.93 e	217 ± 15.0 f	51.1 ± 1.14 b	67.7 ± 0.46 d	288 ± 19.7 g	43.3 ± 2.83 a	54.3 ± 0.53 c	271 ± 17.4 g	379 ± 4.40 h	510 ± 8.34 i	740 ± 27.0 j
Cys	15.4 ± 0.60 c	15.6 ± 1.23 c	27.2 ± 1.23 e	8.19 ± 0.60 a	11.8 ± 3.96 abc	23.9 ± 1.27 d	13.2 ± 0.63 b	15.3 ± 0.62 c	26.7 ± 1.29 de	62.8 ± 1.25 f	73.6 ± 1.50 g	87.5 ± 2.30 h
Val	46.5 ± 1.16 e	48.2 ± 1.10 e	138 ± 6.53 f	29.6 ± 0.78 b	41.7 ± 0.32 d	140 ± 8.27 f	25.2 ± 1.47 a	32.3 ± 0.44 c	143 ± 6.13 f	264 ± 3.16 g	368 ± 5.19 h	496 ± 17.4 i
Met	14.0 ± 0.50 c	14.2 ± 0.32 c	96.5 ± 7.26 d	11.0 ± 0.18 b	14.0 ± 0.11 c	117 ± 8.38 e	9.67 ± 0.64 a	10.5 ± 0.04 a	130 ± 5.02 f	100 ± 1.31 d	141 ± 2.39 g	262 ± 10.5 h
Ile	39.3 ± 0.73 e	40.3 ± 1.15 e	104 ± 6.66 f	19.1 ± 0.32 b	30.6 ± 0.62 d	103 ± 5.42 f	17.0 ± 0.98 a	23.5 ± 0.25 c	101 ± 5.96 f	256 ± 3.37 g	370 ± 5.01 h	469 ± 17.4 i
Leu	64.3 ± 1.14 d	71.5 ± 2.51 e	312 ± 15.4 f	36.5 ± 1.05 b	65.0 ± 0.66 d	353 ± 24.2 fg	32.6 ± 1.93 a	54.1 ± 0.50 c	365 ± 14.3 g	403 ± 4.48 h	772 ± 12.3 i	1187 ± 44.2 j
Tyr	40.2 ± 0.45 c	45.5 ± 0.58 e	175 ± 5.55 g	32.2 ± 0.47 b	53.9 ± 0.54 f	199 ± 8.96 h	27.7 ± 1.68 a	42.5 ± 0.45 d	218 ± 12.2 hi	214 ± 0.45 i	372 ± 2.62 j	636 ± 15.1 k
Phe	43.2 ± 0.66 c	48.6 ± 1.46 d	178 ± 7.20 e	21.0 ± 0.43 a	46.4 ± 0.83 d	178 ± 8.62 e	19.8 ± 1.35 a	38.5 ± 0.14 b	206 ± 6.48 f	300 ± 2.97 g	690 ± 11.9 h	939 ± 27.5 i
Lys	51.2 ± 0.84 c	58.3 ± 1.97 d	340 ± 15.7 f	42.3 ± 1.02 b	63.6 ± 0.81 e	368 ± 21.4 fg	41.0 ± 2.37 a	59.8 ± 0.61 d	397 ± 16.8 g	384 ± 4.46 g	581 ± 5.78 h	985 ± 38.4 i
His	17.2 ± 0.26 c	18.2 ± 0.31 d	46.5 ± 2.27 e	11.5 ± 0.26 e	17.8 ± 0.18 a	59.0 ± 3.26 d	10.7 ± 0.67 f	15.0 ± 0.12 a	57.3 ± 3.36 b	87.5 ± 0.48 g	126 ± 0.67 h	213 ± 7.68 i
Trp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Arg	38.7 ± 0.66 b	45.9 ± 0.59 c	215 ± 8.81 f	21.7 ± 0.51 a	48.9 ± 0.42 d	261 ± 16.1 gh	35.5 ± 2.28 b	58.1 ± 0.90 e	288 ± 14.0 h	250 ± 2.08 g	467 ± 4.83 i	504 ± 26.0 i
Total	717 ± 10.6 c	810 ± 20.8 d	2803 ± 159 e	459 ± 10.7 a	721 ± 2.92 c	3253 ± 205 f	423 ± 25.8 a	604 ± 5.95 b	3296 ± 180 f	4822 ± 55.0 g	7569 ± 48.0 h	10,549 ± 386 i

Values are mean ± SD ($n = 3–4$). *Different lowercase letters* within the *same row* indicate significant difference at $p < 0.05$

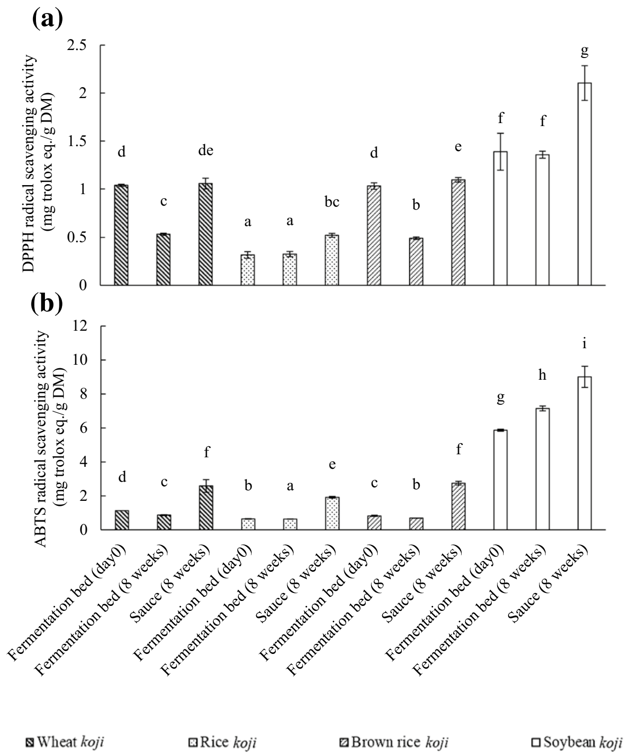

Fig. 2 2,2-Diphenyl-1-picrylhydrazyl (*DPPH*) radical scavenging activity (**a**) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (*ABTS*) radical scavenging activity (**b**) of the four types of koji fermentation beds and koji sauces (mean ± SD, $n = 3–4$). **a**, **b** *Different lowercase letters* indicate significant difference at $p < 0.05$

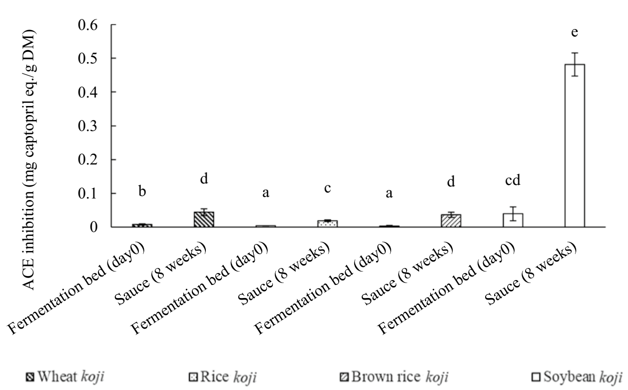

Fig. 3 Angiotensin-converting enzyme (*ACE*) inhibitory activity of the four types of koji fermentation beds and koji sauces (mean ± SD, $n = 3–4$). **a**, **b** *Different lowercase letters* indicate significant difference at $p < 0.05$

protein contents of the WS, RS, and BS significantly increased. The mineral and fatty acid compositions of the four fermentation beds varied, and reflected the mineral and fatty acid compositions of the wheat, white rice, brown rice, and soybean as given in the standard tables of food composition in Japan (MEXT 2015). Moy and Chou (2010) fermented tofu with *A. oryzae* rice-soybean (mixed) koji. At day 0, the koji had 16.1 g/100 g DM of extracted sugar, but after incubation at 37 °C for 16 days the extracted sugars had increased to 61.9 g/100 g DM; the extracted sugar concentration was 55.5 g/100 g DM when the koji was kept at room temperature for 30 days. In our study, kamaboko was used instead of tofu, and was fermented for 8 weeks at 20 °C. At day 0, the extracted sugar concentrations of WB, RB and BB ranged from 17.3–24.7 g/100 g DM, whereas after fermentation with kamaboko the concentrations were 45.5, 53.2, and 41.3 g/100 g DM, respectively. These values and trends are similar to those reported by Moy and Chou (2010), who found that extracted sugar represented 66.7% of the total carbohydrates (based on proximate composition) of WS, 71.8% of RS, and 58.9% of BS. In the present study, the proximate composition of soybean koji differed from that of the other kojis; its extracted sugar content was 6.31 g/100 g DM, which corresponding to 26.4% of the proximate composition of carbohydrate. Seo et al. (2018) analyzed the metabolite contents of wheat, rice, and soybean kojis inoculated with *A. oryzae*. After fermentation, amino nitrogen (equivalent to total free amino acids) was highest in soybean koji followed by the wheat and rice koji. The sugar content was highest in wheat koji followed by rice and soybean koji. Total phenolics were highest in soybean followed by rice and wheat koji (Seo et al. 2018). Similarly, we also found that SS had the highest free amino acid and phenolic contents, and lowest sugar content of the sauces. RS had a significantly higher total free amino acid content than WS, but there was no significant difference in the sugar and phenolic contents of WS and RS. In this study, 24.6–31.3% of proximate proteins were determined as free amino acids after production of the koji sauces. The 8-week fermented SS in particular showed a significantly higher level of free amino acids (total 10,550 mg/100 g DM) in comparison to the other sauces. The protein contents of soybean, wheat, rice, and brown rice are 38.6, 7.67, 7.17 and 7.99 g/100 g DM, respectively (MEXT 2015). Soybean has a much higher protein content than the other grains, which was reflected in the different chemical compositions of the koji sauces in the present study. We reported an increase in the total free amino acid concentrations of kamaboko fermented in wheat, rice, brown rice, and soybean koji, which reached a final range of 2800-12300 mg/100 g DM (Nemoto et al. 2020). Zhao et al. (2018) undertook a solid-state fermentation (with *A. oryzae*) of defatted soybean meal, and found a decrease in moisture and an increase in amino nitrogen (which is equivalent to an increase in free amino acids). In the present study, the free amino acid content of WS, RS, BS, and SS ranged from 2800 to 10550 mg/100 g DM after 8 weeks of fermentation at 20 °C. Yoshioka et al. (2020) reported a free amino acid content of 980 mg/100 g DM for kamaboko fermented in rice koji for 3 months at room temperature. Our results indicate that controlling the temperature at 20 °C for 8 weeks provides effective conditions for fermentation. The aspartic acid, glutamic acid, leucine, and lysine contents of WS, RS and BS were 1.3–6.0 times, 1.3–5.7 times, 1.5–6.7 times, and 1.7–5.8 times greater, respectively than those of WB, RB, and BB at 8 weeks. In SS, these amino acid levels were 1.3–1.7 times higher at 8 weeks of fermentation than those of SB. Zhou et al. (2014) reported that free histidine, phenylalanine, glutamic acid and glycine increased tenfold following the fermentation of surimi with *A. elegans* for 48 h at 25 °C. Yang et al. (2016) fermented silver carp muscle at 30 °C for 48 h, and Kasankala et al. (2012) produced a fish sauce from the fermentation of silver carp muscle at up to 35 °C for up to 30 days. These studies showed a significant increase (5–20 times) in free amino acids after the various fermentation conditions had been applied. In the present study, even though levels of all of the detected amino acids increased after fermentation, at no time was a 20-fold increase seen. Lee et al. (2017) analyzed compounds released into the methanolic extracts of rice koji fermentation. Although they did not immerse additional protein sources such as kamaboko into the fermentation, they did report an increase of sugar and free amino acids, such as methionine, isoleucine, and phenylalanine. In comparison to the results of Lee et al. (2017), although an increase was seen in the free amino acid levels in WB, RB, BB after 8 weeks of incubation in the present study, there was not a significant increase in methionine, isoleucine, or phyenylalanine. The total free amino acid contents of the koji sauces (WS, RS, and BS) were 3.5–5.6 times higher than those of the corresponding fermentation beds maintained for 8 weeks, while the total free amino acid contents of SS were only 1.4 times higher than those of SB at 8 weeks. The koji sauces in this study were made by fermentation, with seafood protein and koji-derived carbohydrate as the main substrates, a similar process to that used for oyster sauce, which is produced from the fermentation of oyster and salt. One of our research goals is to produce new seasonings from waste products of the surimi gel (kamaboko) industry. Thus, we compared the results of our analyses to those for oyster sauce, a commonly used seasoning. Je et al. (2005) reported the free amino acid composition of oyster sauce as 8.5–8.9% (w/w) glutamic acid, 7.00–7.26% alanine, 5.88–6.55% leucine, 5.61–6.14% proline, 5.31–5.76% glycine and 5.05–5.52% lysine. Nguyen and Wang (2012) found that the found that the free amino acids of commercial oyster sauce included 10.5–96.5% glutamic acid. Glutamic acid is well known as a compound that enhances the umami taste, thus glutamate is frequently added to commercial sauces. Our koji sauces, which were also fermented with seafood-derived protein and koji, had 12–16% glutamic acid, 10–11% leucine, 9.3–12% lysine, 7.9–9.5% aspartic acid and 7.0–7.3% alanine. The koji sauces had higher percentages of glutamic acid than those reported by Je et al. (2005). The koji-kamaboko-fermented sauces were richer in umami than the oyster sauce. We could separate the koji sauces into two groups according to their chemical composition: WS, RS, and BS; and SS. The mineral profiles of WS, RS and BS in terms of Na, K, Mg, Ca, Zn, Mn, Fe, Cu and P ranged from 3630 to 4170 mg, 105-228 mg, 38.9-109 mg, 32.6–34.9 mg, 0.82–1.77 mg, 0.40–1.78 mg, 2.72–3.03 mg, 0.24–0.30 mg, and 127-313 mg/100 g DM, respectively; while the oyster sauce contained Na, K, Mg, Ca, Zn, Mn, Fe, Cu and P at 11700 mg, 677 mg, 164 mg, 677 mg, 4.16 mg, 1.04 mg, 3.13 mg, 0.44 mg, and 313 mg/100 g DM, respectively (converted to DM) (MEXT 2015). The sauces had lower Na and K contents than the oyster sauce, thus the servings of the koji sauces could be increased to contain the same levels of Na and K as the latter. The increased serving size accounts for the more umami-rich taste due to the amino acids in the seasonings. The Na and K contents of SS were 4700 mg and 1250 mg/100 g DM, respectively, much higher than those of the other koji sauces, but still lower than that of oyster sauce. The contents of sugars, the release of which from the fermentation beds (especially in WS, RS, and BS) increased with time, can also affect the taste of koji sauces. However, the taste threshold for NaCl is much smaller than that for sugar, thus the Na content is more important when deciding serving size.

The fatty acid compositions of the koji sauces comprised a mixture of compounds derived from kamaboko and koji, and differed from that of oyster sauce. The fat contents of these sauces (1.69–12.6 g/100 g DM) were much higher than that of oyster sauce (0.78 g/100 g DM) (MEXT 2015). The contents of EPA and DHA in the koji sauces ranged from 0.90 to 2.06% and 0.96–3.04%, respectively, while oyster sauce had 21% EPA and 12% DHA. Seo et al. (2018) observed that soybean koji fermentation produced more amino acids, phenolics and flavonoids than wheat and rice koji because of higher contents of these compounds in natural soybean than in wheat and rice. Despite the fact that Seo et al. (2018) did not add a protein source such as kamaboko to the fermentation, there was a clear increase in released compounds when koji was maintained with different substrates. Shin et al. (2019) found an increase in the phenolic content following the solid-state fermentation of black rice bran with *A. oryzae*. However, they also found that, after it reached a maximum level, the phenolic content started to decrease. Shin et al. (2019) explained that this increase in phenolic compounds was due to their release from the rice bran. Degraded grain starch (sugar) and released phenolic compounds from the grain were shown to penetrate not only the kamaboko but also the sauces during the protein degradation process of the fermentation (Shin et al. 2019). In the present study, not only was an increase in free amino acids content observed, but also an increase in phenolic contents and extracted sugars. Taken together with the results of previous studies, the results presented here indicate that 8 weeks of fermentation of kamaboko in koji at 20 °C can promote the degradation of protein and starch to produce free amino acids or peptides, and lead to an increase in extracted sugar, all of which result in koji sauces that are rich in umami and have a sweet taste.

Antioxidant and ACE inhibitory activities

Phenolic compounds, amino acids and peptides, which are released during fermentation by hydrolysis, are reported to have antioxidant and ACE inhibitory activities. Gao et al. (2019) reported an increase in free amino acids and DPPH radical scavenging activity following the fermentation of by-products (fish paste). Nakajima et al. (2009) reported that hydrolyzed fish proteins with total free amino acid contents of 3500–4600 mg/g DM showed DPPH radical scavenging activity of 1.22–2.49 mg Trolox eq./g DM. Umayaparvathi et al. (2014) reported that a hydrolysate of oyster released bioactive peptides with an increase in DPPH radical scavenging activity. Zhang et al. (2019) reported that hydrolyzed oyster meat had enhanced antioxidant activity, based on the tetraethylammonium chloride equivalent value, due to an increase in free amino acids. We cannot compare the antioxidant values of Zhang et al.'s (2019) study to those of ours as they were calculated in different ways. Nevertheless, it is clear that the samples that underwent hydrolysis and released low molecular weight fractions as a consequence showed more antioxidant activities than samples without hydrolysis. Koji sauces were found to have higher free amino acid contents due to fermentation in Zhao et al. (2018) and Gao et al. (2019). The highest DPPH radical scavenging activity in the present study was similar to that of a hydrolysate of fish muscle reported by Nakajima et al. (2009). In Shin et al.'s (2019) study, the DPPH radical scavenging activity of black rice bran fermented with *Aspergillus awamori* and *Aspergillus oryzae* was approximately 20 μmol Trolox eq./g (= 5 mg Trolox eq./g DM). In Lee et al. (2007) the highest DPPH radical scavenging activity of black bean koji inoculated with *A. awamori* had an ED_{50} of 0.69 mg/ml. Different antioxidant values were recorded in these two reports, which used the same microorganisms but different grains. In the present study, the WS, RS, BS, and SS DPPH radical scavenging activities were 0.52–2.39 mg Trolox eq./g DM (= ED_{50} of 2.39–9.65 mg/ml DM). Thus the antioxidant activities were lower than those recorded by Shin et al. (2019) and Lee et al. (2007).

Proteins, their hydrolysates and peptides are known to have ACE inhibitory activity. ACE inhibitory activity also increases with increases in free amino acids, polyphenolics and sugar. The ACE inhibitory activities of WS, RS, BS, and SS fermented for 8 weeks were 0.044, 0.019, 0.036, 0.481 mg captopril eq./g DM (i.e., IC_{50} of 0.123, 0.284, 0.168, and 0.011 mg DM/ml), respectively. In Nakajima et al. (2009), the highest IC_{50} was 0.078 mg DM/mL. Oh et al. (2019) reported an IC_{50} of 0.05 mg/ml for ACE inhibitory peptides of olive flounder surimi. Je et al. (2005) reported ACE inhibition of oyster sauce at an IC_{50} of 0.71–2.75 mg DM/ml. SS showed a stronger level of ACE inhibitory activity (IC_{50} of 0.011 mg DM/ml, 0.481 mg captopril eq./g DM) than the substances reported in the previous studies. Some peptides that are effective in inhibiting ACE are reported to have a bitter taste (Nakajima et al. 2009; Pripp and Ardo 2007). In the present study, SS had a significantly more bitter taste than that the other sauces in a preliminary sensory evaluation. WS, RS, and BS were found to be more suitable as food seasonings, and SS more useful as a source of bioactive compounds.

In conclusion, koji sauces (WS, RS, and BS) with their enhanced sweetness, umami tastes, and relatively low salt content can be used as food seasonings. Koji sauces might be useful replacements for oyster sauce due to their relatively low NaCl content, which can help to achieve a reduction in blood pressure, and stronger ACE inhibitory effects. Soybean koji sauce might also be a useful source of bioactive compounds with antioxidant or ACE inhibitory effects.

In future studies, these types of sauces need to be evaluated by sensory, microbial, and organic acid analyses to obtain the tastiest sauces with the highest antioxidative properties.

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