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ORIGINAL RESEARCH

Reduced-salt shrimp sauces from Alaskan pink shrimp and nonglutinous rice cultivar *Tsuyahime* koji: Preparation and characterization

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Abstract The objective of this study was to develop high-quality shrimp sauce using Alaskan pink shrimp and nonglutinous rice cultivar *Tsuyahime* koji. The liquefaction rates of mashes were high at approximately 75.1-81.2% after fermentation for 8 months. The salt contents of sauces were remarkably low at approximately 6.5-7.2 g/100 g in comparison with those of commercially available (CA) fish sauces. The tested sauces showed not only significantly high antioxidative activities and scavenging abilities against reactive oxygen species (ROS) but also powerful angiotensin I-converting enzyme (ACE) and hyaluronidase inhibitory activities. Sensory analysis revealed that the sauce No. 7 prepared using 30 wt.% koji to broiled shrimp exhibited superior taste strength, taste balance, first taste, and aftertaste and had strong sweetness and umami and weak bitterness and saltiness among the tested sauces. In addition, it was rich in essential amino acids. Therefore, reduced-salt alaskan pink shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji could be utilized as alternatives of CA fish sauces, which is demanded for consumers and fish sauce industries. Moreover, these may be contributed to the prevention and cures of lifestyle-related diseases and allergy.

Keywords Alaskan pink shrimp . Development . Nonglutinous rice cultivar *Tsuyahime* koji . Physicochemical and functional properties . Reduced-salt sauce

Introduction

The self-sufficiency food rate based on calories in Japan was the lowest at approximately 38% among the developed countries, such as Canada, Australia, and America (Ministry of Agriculture, Forestry and Fisheries 2019). There are some theories of the cause of the lowest rate. Rice as a staple food is a domestic foodstuff that is almost 100% self-sufficient, however, its consumption is decreasing every year. Annual consumption of rice per person is 54.4 kg in 2018, which is less than half that in 1965 (Ministry of Agriculture, Forestry and Fisheries 2019). Therefore, an increasing self-sufficiency food rate remains among the most significant issues for food problems in Japan. The nonglutinous rice cultivar *Tsuyahime* is a rice variety that developed as one of high-quality rice cultivar in Yamagata, Japan. However, many kinds of high-quality rice cultivar,

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such as nonglutinous rice cultivars *Yumepirika*, *Koshihikari*, and *Hitomebore*, are distributed around Japan. Therefore, it is strongly desired the development of new application of the staple food rice.

Malted rice (koji) has been used for the production of various fermented foods, such as miso, soy sauce, and sake, from ancient times. Koji could be improved the properties of foods (appearance, taste, and flavor) and nutritional values of foods. Among some fermented foods, fish sauces are fermented seasonings and produced using various aquatic organisms. The consumption is limited due to not only the distinctive, complicate, and unacceptable odors but also high salt contents in Japan. However, fish sauces are preferably used as main seasonings in Southeast Asian countries. It is possible to produce high-quality fish sauces using koji. So far there are few reports of shrimp sauce to the best of our knowledge (Funatsu et al. 2019). Therefore, the present study aimed to develop low-salt shrimp sauce with good sensory acceptability using Alaskan pink shrimp (*Pandalus eous*, Makarov) and nonglutinous rice cultivar *Tsuyahime* koji. Moreover, we tried to elucidate the physicochemical and functional properties of these sauces for further applications.

Materials and methods

Samples

Fresh Alaskan pink shrimp (length: 11.5-14.5 cm, body weight: 6.5-11.0 g) was purchased from Yamagata Fisheries Cooperative Association Yura Branch (Yamagata, Japan). Brown rice (nonglutinous rice cultivar *Tsuyahime*) and salt were obtained from a local supermarket. A koji mold (No.1 bacteria) for soy sauce production was purchased from Akita Konno Shoten Co., Ltd. (Akita, Japan). All chemicals were of reagent grade.

Koji preparation

The brown rice was polished and then washed with running water. After soaking in water overnight, the rice was drained with a sieve. These were steamed for 90 min and then cooled. The koji mold (0.035 wt.%) was sprinkled, and then these were fermented at temperature of 33 °C and relative humidity of 90%. The koji obtained was cooled at 15 °C overnight.

Determination of enzyme activity of koji

According to Revised National Tax Administration Agency Analysis Method commentary (The Brewing Society of Japan 1993), α-amylase, glucoamylase, α-glucosidase, and acid carboxypeptidase activities of koji were determined.

Shrimp sauce preparation

Fresh shrimp and lightly broiled shrimp on the surface until lightly browned were individually ground using food processor. The ingredients (Table 1) were mixed, incubated at approximately 23 °C, and gently mixed once a day. The mashes were fermented for 8 months and then heated at 90 °C for 30 min. After that, samples were left to stand at room temperature for 2 days, followed by centrifuged at 22,200 x g at 20 °C for 1 h. The supernatants obtained were filtrated using No.1 filter paper. The liquefaction rates of the mashes were calculated as follows: Liquefaction rate (%)= (the weight of mash after centrifugation/the weight of mash before centrifugation) x 100.

Table 1 Ingredient composition for the shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji

	1	2	3	4	5	6	7	8
Fresh shrimp (g)	1000	1000			1000	1000		
Broiled shrimp (g)			1000	1000			1000	1000
Tsuyahime koji (g)	100	100	100	100	300	300	300	300
21.6% (w/w) saline solution (g)	421	421	421	421	498	498	498	498
Alcalase 2.4L FG (ml)	0	4.274	0	4.274	0	4.274	0	4.274

Physicochemical properties of fish sauces

The proximate analysis was performed as described by Kagawa (2020). The color and the color difference were evaluated as described by Nagai et al. (2019a). The pH at 20 °C, water activity at 20 °C, total nitrogen, formol nitrogen, soluble solids excluding salts, Brix% at 20 °C, alcohol at 20 °C, total sugars, direct reducing sugars, acidity-I and acidity-II, titratable acidity, and specific gravity at 20 °C were analyzed according to Soy Sauce Test (Japan Soy Sauce Research Institute 1985). Total phenols, total flavonoids, and total flavonols were evaluated as described by Slinkard and Singleton (1977), Kim et al. (2003), and Jimoh et al. (2010), respectively. Histamine was measured using a kit 'Checkcolor Histamine' (Kikkoman Biochemifa Company, Tokyo, Japan).

Functional properties of fish sauces

The antioxidative activity, radical [superoxide anion radicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, and hydroxyl radicals] scavenging activity, as well as ACE and hyaluronidase inhibitory activities were measured as described by Nagai et al. (2019b). Ascorbic acid (AA), *tert*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-4-methylphenol (BHT), α-tocopherol (TP), and trolox (TL) as positive controls and distilled water as negative control were used for the determination of antioxidative activities and radical scavenging activities.

Sensory analyses of fish sauces

Sensory evaluation was performed at 25 °C in seven-point scale (-3 = weak, 0 = neither weak nor strong, 3 = strong) by the panel of four panelists. The panelists are trained specialist that works in Yamagata Fisheries Cooperative Association Yura Branch, Yamagata, Japan. They have excellent sensory discrimination abilities. Therefore, they can make the correct judgment. The quality characteristics (color, smell, taste strength, taste balance, first taste, aftertaste, sweetness, umami, sourness, bitterness, saltiness, and overall acceptance) were evaluated.

Amino acid analysis

Free amino acid analysis was performed using an amino acid analyzer (L-8900, Hitachi High-Technologies Corp., Tokyo, Japan) by on-line post-column derivatization with ninhydrin.

Statistical analysis

Except for color analysis and sensory analysis, the assay was repeated three times independently. These results were indicated as means ± standard deviation. Significant differences were tested by one-way analysis of variances with the Tukey's test ($P < 0.05$). Minitab Statistical Software was used for statistical analyses.

Results and discussion

Shrimp sauces preparation

Acid carboxypeptidase is an exopeptidase that cleavage the peptide bond of protein from the C-terminus and liberates the amino acids. α-Amylase is an endopeptidase that break α-1,4 bonds of the starch and glycogen in a random manner. Glucoamylase is an exopeptidase that hydrolyze α-1,4 bond of the sugar chain from non-reduced terminal of the starch and produce one molecule of glucose. α-Glucosidase is an enzyme that hydrolyze α-1,4 bonds of the sugar chains. First, the enzyme activity of the koji was measured. As a result, α-amylase, glucoamylase, α-glucosidase, and acid carboxypeptidase activities were 1898±3.8, 70.2±0.9, 0.0068±0.0006, and 270.2±3.1 U/g koji, respectively. These results indicated that nonglutinous rice cultivar *Tsuyahime* koji could be fully digested the starches and proteins in ingredients for the shrimp sauces. It is a possibility to cause decomposition of the mashes during fermentation at room temperature under low salt condition, if the content of the koji is low. In addition, the materials can effectively digest using 30 wt.% koji in comparison with the addition of 10 wt.% koji, resulting the sauces with good eating qualities. Therefore, the sauces were prepared using 10 and 30 wt.% koji to the shrimp. During fermentation, the pH values (initial pH values: 6.62 - 7.10) of the mashes linearly decreased to the range of 5.02-5.80 after 2 months, and then the overall trend was flat (Fig. 1). Thus, it was suggested that the enzymes contained in the ingredients acted actively till after 2 months and subsequently caused the decrease of these enzyme activities. The liquefaction rates of the mashes were high at approximately 75.1-81.2%. The rates were similar to those of fish sauces prepared using by-products from kamaboko processing (Takano et al. 2012). However, the rates were much higher than those of sakura shrimp sauces (Funatsu et al. 2019). The polysaccharide chitin, which is the main component of shrimp shells, possesses the strong crystal structures by hydrogen bonds. Therefore, the shells of Alaskan pink shrimp can't digest by the digestive enzymes contained in the shrimp and by the enzymes released from the koji. Thus, it is necessary to digest using chitinase that hydrolyze the glycoside bonds of the chitin to completely liquefy the mashes. Alaskan pink

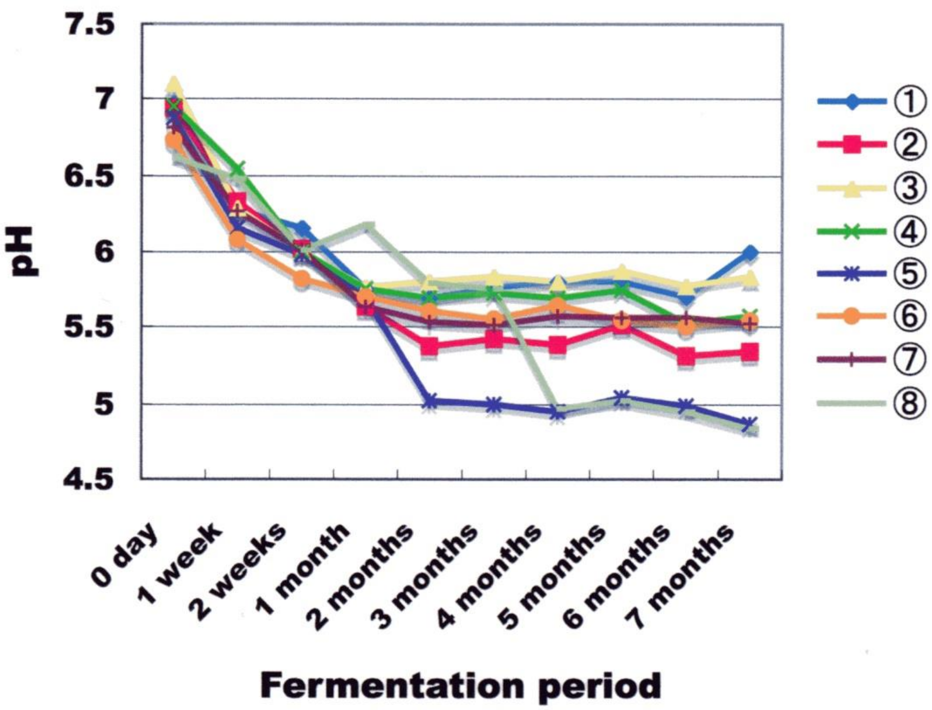

Fig. 1 The pH changes of the mashes during the fermentation

Fig. 2 The shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji

shrimp sauces are shown in Fig. 2.

Proximate composition

The proximate compositions and the energy values of the shrimp sauces are presented in Table 2. The sauces showed higher water contents than CA fish sauces. High water contents may affect the shelf life of shrimp sauces because it is easy to grow the pathogenic microorganisms, such as *Escherichia coli* and *Staphylococcus aureus*. The crude protein content of sauce No. 2 was highest as well as those of CA *ikanago shoyu* and *ishiru*. In contrast, the contents of the sauces No. 5, 7, and 8 were low as well as that of CA nam pla. Thus, the protein degradation in the sauces prepared using 30 wt.% koji accelerated in comparison with that in the sauces prepared using 10 wt.% koji because of high contents of rice koji. In contrast, the cause of low protein degradation in only sauce No. 2 is still unknown. The crude lipid contents were low at approximately 0.1-0.2 g/100 g, excluding the sauces No. 2 and 8. It suggested that the lipids in the sauces No. 2 and 8 could not remove by centrifugation and filtration using filter paper. There were significant differences in carbohydrate contents of the tested sauces ($P < 0.05$). The crude ash contents were remarkably low when compared with those of CA fish sauces. The salt contents were significantly low at approximately 6.5-7.2 g/100 g. The energy was highest in the sauce No. 2, followed by the sauces No. 5 and 8. In contrast, those of sauces No. 1, 3, 4, 6, and 7 were low in the range of 54.2-62.2 kcal/100 g. In general, the salt contents of CA fish sauces are noticeably high at approximately over 20 % (Kagawa 2020). In fact, some researchers reported that the salt contents of fish sauces were in the range of 14.9-26.9 % (Dohmoto et al. 2001; Ikeda et al. 2018; Taira et al. 2007; Uchida et al. 2005). These results indicated that Alaskan pink shrimp sauces were reduced-salt sauces, as these sauces fell into reduced-salt grade according to Japan Soy Sauce Research Institute (1985).

Physicochemical properties

As shown in Table 3, the physicochemical properties of the shrimp sauces were investigated. The L^* and b^* values of sauce No. 1 were high, however those of the sauce No. 7 were low among the tested sauces. In contrast, the sauce No. 8 showed high a^* value, however the sauce No. 1 exhibited fairly low value. In general, the colors of the sauces No. 5-8 were dark-brown when compared with those of the sauces No. 1-4. It suggested that the browning on the sauces No. 5-8 progressed in comparison with that on the sauces No. 1-4. In fact, the absorbance at 440 nm, as an indication of the browning (Shimohashi 2013), of the sauces No. 5-8 (A_{440} = 4.200-6.310) was significantly higher than that of the sauces No. 1-4 (A_{440} = 2.060-2.540). The pH values of the sauces No. 1 and 3 were high at 6.14-6.33, while those of the sauces No. 5 and 8 were low at 4.92-5.01. Generally, except for the sauce No. 6, the pH values of the sauces No. 5-8 were low when compared with those of the sauces No. 1-4. It suggested that low pH levels of the

Table 2 Proximate compositions and energy values of the shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji

	Water (g/100 g)	Crude proteins (g/100 g)	Crude lipids (g/100 g)	Carbohydrates (g/100 g)	Crude ashes (g/100 g)	Salts (g/100 g)	Energy (kcal/100 g)
1	79.2±0.2^{a}	11.5±0.3^{b}	0.2^{c}	1.9^{e}	7.2±0.2^{b}	7.2±0.1^{b}	55.4^{c}
2	73.3±0.2^{ab}	13.0±0.3^{a}	1.0^{a}	5.6^{b}	7.1±0.2^{b}	7.1±0.1^{b}	83.4^{a}
3	78.6±0.3^{a}	10.1±0.2^{c}	0.1^{c}	3.9^{d}	7.3±0.3^{b}	6.6±0.1^{b}	56.9^{c}
4	79.6±0.3^{a}	10.1±0.3^{c}	0.2^{c}	3.0^{d}	7.1±0.1^{b}	7.1±0.1^{b}	54.2^{c}
5	77.5±0.2^{a}	8.9±0.2^{d}	0.1^{c}	6.2^{ab}	7.3±0.2^{b}	6.5±0.1^{b}	69.4^{b}
6	77.5±0.2^{a}	10.3±0.2^{c}	0.2^{c}	4.8^{c}	7.2±0.2^{b}	6.5±0.1^{b}	62.2^{bc}
7	78.4±0.3^{a}	8.3±0.2^{d}	0.1^{c}	6.2^{ab}	7.0±0.2^{b}	6.5±0.1^{b}	58.9^{c}
8	76.0±0.2^{a}	9.2±0.2^{d}	0.4^{b}	7.2^{a}	7.2±0.2^{b}	6.7±0.1^{b}	69.2^{b}
A*	63.0^{b}	13.9^{a}	0^{d}	2.1^{e}	20.8^{a}	21.2^{a}	65^{b}
B*	61.2^{b}	12.8^{a}	0^{d}	4.2^{c}	21.8^{a}	21.9^{a}	68^{b}
C*	69.4^{b}	6.1^{e}	0^{d}	1.1^{e}	23.3^{a}	24.3^{a}	29^{e}
D*	65.5^{b}	9.1^{d}	0.1^{c}	2.7^{d}	22.7^{a}	22.9^{a}	48^{d}

A: *ikanago shoyu*, B: *ishiru* (*ishiri*), C: shotturu, D: nam pla. *Data was quoted from Standard Tables of Food Composition in Japan 2020 sauces were due to more amino acids produced in 30 wt.% koji to the shrimp and in adding alcalase, in particular, acidic amino acids such as aspartic acid and glutamic acid. Several studies showed that the pH values of fish sauces ranged from 4.5-6.7 (Funatsu et al. 2019; Ikeda et al. 2018; Kilinc et al. 2006; Puat et al. 2015). The water activities of the sauces were approximately 0.74-0.81. These were in agreement with the result of Kilinc et al. (2006) on sardine sauces and with the result of Puat et al. (2015) on *kecap ikan* and nam pla. Overall, the sauces No. 1-4 showed high total nitrogen (1.61-2.08%) and formol nitrogen (1.32-1.36%) contents when compared with the sauces No. 5-8 ($P < 0.05$). The protein degradation rates of the sauces No. 3-5, 7, and 8 were high at 0.81-0.84, however that of the sauces No. 2 was low at 0.65. A correlation was observed between the protein contents of the sauces and the formol nitrogen/total nitrogen ($R^2 = 0.7587$). The sauces No. 5 and 8 showed the soluble solid contents excluding salts at approximately 20 %. In contrast, the contents of freshwater silver carp sauces prepared using soy sauce koji and lactic acid bacteria were notably low at approximately 6.2-12.7% (Uchida et al. 2005). Among the tested sauces, the sauces No. 1-3 fell into special grade of dark soy sauce and tamari, according to Japanese Agricultural Standard (2009). Except for the sauce No. 4, the Brix% ranged from approximately 22.0-27.1%. It is possible that the yeasts exist in the environments for producing shrimp sauces. If the yeast is contained in the mashes, alcohol is produced during the fermentation. The yeasts affect the qualities of shrimp sauces. Therefore, alcohol contents of the sauces were measured. As a result, alcohol did not detect in all tested sauces. Dohmoto et al. (2001) reported that the alcohol contents of fish sauces from hoki, Southern bluefin tuna, and spear squid were 0.98-2.85 g/100 ml. On the whole, the sauces No. 5-8 exhibited especially higher total sugars and direct reducing sugars contents than the sauces No. 1-4 due to higher contents of rice koji in the ingredients. That is, it suggested that the degradation of carbohydrates into sugars on the sauces No. 5-8 accelerated in the mashes when compared with that on the sauces No. 1-4. The acidity-I of the sauces No. 5 and 8 was high scores in the range of 6.7-7.5 ml, however those of the sauces No. 1 and 3 were low one. No significant differences were found in acidity-II, excluding the sauce No. 1. Titratable acidity ranged from approximately 9.3-17.6 ml. According to Funatsu et al. (2019), sakura shrimp sauces showed acidity-I and -II and titratable acidity of 8.0-10.7, 7.7-8.7, and 16.2-19.4 ml, respectively. Overall, the contents of total phenols, total flavonoids, and total flavonols of the sauces No. 5-8 were much higher than those of the sauces No. 1-4, depending on higher contents of rice koji in the ingredients. High contents of phenol compounds may affect the functionalities of shrimp sauces, such as antioxidative activity and ROS scavenging activity. Histamine has been incriminated as the causative agent of allergy-like food poisoning. Therefore, high

Table 3 Physicochemical properties of the shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji

Parameters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Color *L**	7.530±0.351^{a}	3.730±0.155^{c}	2.598±0.120^{d}	4.723±0.193^{b}	2.470±0.111^{d}	2.778±0.163^{d}	1.653±0.102^{e}	3.790±0.179^{c}
*a**	2.585±0.119^{c}	3.617±0.124^{b}	2.835±0.133^{bc}	3.778±0.164^{b}	2.907±0.145^{bc}	1.990±0.082^{cd}	1.245±0.077^{d}	4.793±0.224^{a}
*b**	6.540±0.286^{a}	3.870±0.176^{c}	1.323±0.091^{e}	5.555±0.269^{b}	2.273±0.102^{d}	3.130±0.187^{c}	1.467±0.085^{e}	4.760±0.202^{bc}
*ΔE*ab*	-	Appreciable	Much	Appreciable	Much	Appreciable	Much	Appreciable
pH at 20°C	6.33±0.01^{a}	5.55±0.01^{ab}	6.14±0.01^{a}	5.88±0.01^{ab}	5.01±0.01^{b}	5.66±0.01^{ab}	5.66±0.01^{ab}	4.92±0.01^{b}
Water activity (Aw) at 20°C	0.77±0.01^{a}	0.77±0.01^{a}	0.74±0.01^{a}	0.78±0.01^{a}	0.76±0.01^{a}	0.77±0.01^{a}	0.78±0.01^{a}	0.81±0.01^{a}
Total nitrogen (%)	1.83±0.03^{b}	2.08±0.03^{a}	1.62±0.02^{c}	1.61±0.02^{c}	1.43±0.02^{d}	1.64±0.03^{c}	1.32±0.02^{d}	1.47±0.03^{d}
Formol nitrogen (%)	1.32±0.02^{a}	1.36±0.02^{a}	1.36±0.01^{a}	1.33±0.01^{a}	1.16±0.01^{b}	1.19±0.01^{b}	1.10±0.01^{b}	1.21±0.02^{b}
Formol nitrogen/Total nitrogen	0.72±0.01^{ab}	0.65±0.01^{b}	0.84±0.01^{a}	0.82±0.01^{a}	0.81±0.01^{a}	0.73±0.01^{ab}	0.83±0.01^{a}	0.82±0.01^{a}
Soluble solids excluding salts (%)	16.7^{bc}	17.0^{b}	17.4^{b}	10.5^{d}	20.6^{a}	15.5^{c}	16.1^{bc}	19.9^{a}
Brix% at 20°C	23.9±0.1^{b}	24.1±0.1^{b}	24.0±0.1^{b}	17.6±0.1^{d}	27.1±0.1^{a}	22.0±0.1^{c}	22.6±0.1^{c}	26.6±0.1^{a}
Alcohol (%) at 20°C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total sugars (g/100 ml)	0.25±0.01^{e}	0.86±0.02^{d}	0.25±0.01^{e}	0.91±0.02^{d}	4.78±0.10^{b}	5.48±0.11^{ab}	6.06±0.13^{a}	3.55±0.06^{c}
Direct reducing sugars (g/100 ml)	0.10^{d}	0.41±0.01^{c}	0.09^{d}	0.73±0.03^{c}	2.58±0.06^{b}	4.62±0.08^{a}	2.94±0.05^{b}	2.34±0.04^{b}
Acidity-I (ml)	1.0±0.1^{c}	3.0±0.1^{b}	1.2±0.1^{c}	1.9±0.1^{bc}	7.5±0.1^{a}	2.3±0.1^{b}	2.7±0.1^{b}	6.7±0.1^{a}
Acidity-II (ml)	8.3±0.2^{b}	9.2±0.2^{a}	9.6±0.2^{a}	9.1±0.2^{a}	10.1±0.2^{a}	9.5±0.1^{a}	9.6±0.2^{a}	9.6±0.2^{a}
Titratable acidity (ml)	9.3±0.2^{c}	12.2±0.2^{b}	10.8±0.2^{c}	11.0±0.2^{c}	17.6±0.2^{a}	11.8±0.1^{bc}	12.3±0.2^{b}	16.3±0.2^{a}
Specific gravity at 20°C	1.086±0.003^{a}	1.097±0.003^{a}	1.090±0.002^{a}	1.091±0.002^{a}	1.108±0.003^{a}	1.113±0.002^{a}	1.106±0.002^{a}	1.109±0.002^{a}
Total phenols (mg/ml)*	6.07±0.12^{b}	5.08±0.10^{c}	4.91±0.09^{c}	5.35±0.10^{bc}	6.31±0.12^{ab}	6.93±0.11^{a}	6.17±0.09^{b}	4.40±0.06^{d}
Total flavonoids (mg/ml)*	0.73±0.03^{c}	0.73±0.02^{c}	0.73±0.02^{c}	0.82±0.01^{b}	0.82±0.01^{b}	1.04±0.03^{a}	0.89±0.02^{b}	1.13±0.03^{a}
Total flavonols (mg/ml)**	0.10±0.01^{b}	0.11±0.01^{b}	0.10±0.01^{b}	0.11±0.01^{b}	0.19±0.01^{a}	0.27±0.02^{a}	0.21±0.01^{a}	0.20±0.01^{a}
Histamine (ppm)	81.4±0.3^{d}	81.4±0.2^{d}	192.2±0.5^{a}	83.1±0.3^{d}	138.5±0.4^{bc}	148.9±0.5^{b}	129.9±0.3^{c}	135.1±0.4^{bc}

Color analysis was repeated ten times independently. *gallic acid equivalent, **rutin equivalent.

levels of histamine may be responsible for allergy-like food poisoning. Histamine is produced through the histidine decarboxylation by histidine decarboxylase. The histamine contents of the sauces No. 5-8 were high at approximately 129.9-148.9 ppm when compared with those of the sauces No. 1, 2, and 4, except for the sauce No. 3. It was suggested that the difference of bacteria adhering to the surface of shrimp, such as halophilic histamine-producing bacteria *Photobacterium phosphoreum*, affected the histamine contents of the shrimp sauces (Kanki et al. 2004). Funatsu et al. (2019) stated that the histamine contents of sakura shrimp sauces were approximately 4.7-18.0 ppm. Histidine content of Alaskan pink shrimp (360 mg/100 g) is almost the same as that of sakura shrimp (330 mg/100 g) and is low when compared with those of red fish meats, such as tuna (2300-2500 mg/100 g), bonito (1200-2400 mg/100 g), and mackerel (1300 mg/100 g) (Kagawa 2020). It was suggested that histidine decarboxylase activities on the mashes of Alaskan pink shrimp sauces were higher than those on the mashes of sakura shrimp sauces. In contrast, other researchers reported the histamine contents of fish sauces at approximately 88-592 ppm (Taira et al. 2007; Takano et al. 2012). Moreover, Thai fish sauces showed the histamine levels at approximately 100-1000 ppm (Brillantes and Samosorn 2001). Its majority was in the range of 200-600 ppm.

Functional properties

To linoleic acid oxidation for 200 min, the antioxidative activities of shrimp sauces were measured. The sauces No. 5 and 6 completely inhibited the oxidation (Table 4). The activities of the sauces No. 4, 7, and 8 were remarkably high as well as those on 1 mM TP and TL. In contrast, the sauce No. 3 possessed the middle activity as well as 1 mM BHA and 0.1 mM TL. It was suggested that high antioxidative activities on the sauces No. 5-8 were due to high contents of phenol compounds derived from the koji, such as flavonoids and flavonols when compared with those on the sauces No. 1-4 (Table 3). Next, the radical scavenging activities of shrimp sauces were investigated (Table 5). The sauce (No. 7) perfectly scavenged superoxide anion radicals. The sauces No. 5 and 8 performed substantially complete inhibition against the radicals, and the sauces No. 3 and 4 exhibited significantly high activities at approximately 94.8 %. In contrast, the activity of the sauce No. 1 showed high level as well as 1 mM TL. The hydroxyl radical scavenging activities of the sauces No. 1, 3, and 4 were fairly high the same as 0.01 mM BHT and TL, while those of the sauces No. 2 and 5-8 ranged from approximately 71.4 to 77.8%. The sauces No. 3-5, 7, and 8 completely inhibited DPPH radicals. Moreover, the sauces No. 1 and 2 showed significantly high activities as well as 1 mM TP and TL. According to Aoshima and Ooshima (2009), fish sauces made from bonito, red sea bream,

Table 4 Antioxidative activities of the shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji

Samples	**50 min**	**100 min**	**200 min**
1	0^{g}	0.047±0.002^{d}	0.064±0.004^{f}
2	0.032±0.002^{d}	0.038±0.002^{e}	0.048±0.003^{fg}
3	0.073±0.005^{bc}	0.074±0.004^{cd}	0.102±0.006^{e}
4	0^{g}	0.010±0.001^{f}	0.027±0.002^{g}
5	0^{g}	0^{g}	0^{h}
6	0^{g}	0^{g}	0^{h}
7	0^{g}	0.011±0.001^{f}	0.021±0.002^{g}
8	0^{g}	0.011±0.001^{f}	0.028±0.002^{g}
1 mM AA	0.022±0.001^{e}	0.135±0.006^{b}	0.469±0.027^{b}
5 mM AA	0.016±0.001^{ef}	0.032±0.003^{e}	0.090±0.008^{ef}
0.01 mM BHA	0.084±0.005^{b}	0.120±0.008^{b}	0.245±0.012^{c}
0.1 mM BHA	0.056±0.003^{c}	0.090±0.006^{c}	0.165±0.010^{d}
1 mM BHA	0.054±0.002^{c}	0.057±0.003^{d}	0.100±0.006^{e}
0.01 mM BHT	0.082±0.003^{b}	0.112±0.009^{bc}	0.248±0.011^{c}
0.1 mM BHT	0.058±0.004^{c}	0.108±0.005^{bc}	0.173±0.008^{d}
1 mM BHT	0.044±0.002^{d}	0.051±0.003^{d}	0.093±0.005^{ef}
1 mM TP	0.006^{f}	0.025±0.001^{ef}	0.028±0.002^{g}
0.01 mM TL	0.084±0.005^{b}	0.094±0.006^{c}	0.262±0.013^{c}
0.1 mM TL	0.038±0.002^{d}	0.051±0.003^{d}	0.123±0.008^{e}
1 mM TL	0.011±0.001^{f}	0.031±0.002^{e}	0.032±0.002^{g}
Control	0.379±0.008^{a}	0.715±0.025^{a}	1.406±0.041^{a}

Values are the absorbance at 500 nm

and shrimp not only scavenged DPPH radicals but also suppressed the production of hydrogen peroxide. All the tested sauces perfectly inhibited ACE activities, indicating excellent suppression effect against blood pressure increase in shrimp sauces. Hyaluronidase inhibitory activities of the sauces were measured. The sauces No. 7 and 8 exhibited powerful activities at approximately 81%. The relatively high activities were shown in the sauces No. 1-3. In contrast, the activity of the sauce No. 6 was approximately 61.3%. Next, the activities of these sauces were expressed as millimoles of sodium cromoglicate (SC) equivalent per kg of sauces. SC has been used for CA anti-allergic and anti-asthmatic drugs. The activities were in the range of 32.2-41.7 mmol/kg sauces. An ampule (2 ml) of 1% SC inhalant liquid 'sawai' (Sawai Pharmaceutical Co., Ltd., Osaka, Japan) is used for CA anti-allergic and anti-asthmatic drug. This contains 20 mg of SC. In contrast, it was estimated to contain approximately 47.3 mg SC equivalents per 2 ml of shrimp sauce No. 7. In addition, it was calculated that these sauces contained approximately 36.7-47.3 mg SC equivalents per 2 ml of the sauces. Thus, it was suggested that the tested sauces had beneficial anti-allergic effects when compared with CA drug 'sawai'.

Sensory analysis

The colors of the shrimp sauces No. 5-8 showed dark-brown in comparison with the sauces No. 1-4, suggesting the progress of browning by the Maillard reaction (Table 6). As described above, the absorbance at 440 nm of the sauces No. 5-8 was approximately 2-3 times as high as those of the sauces No. 1-4. The taste strength, taste balance, first taste, and aftertaste had the highest scores in the sauce No. 7 among the tested sauces. In addition, the sauce No. 7 had strong sweetness and umami and weak bitterness and saltiness. In contrast, the sauce No. 1 showed high score in terms of sourness. There was no relationship in the evaluation of sourness between the pH value and the titratable acidity of the sauce. It was unknown why the sauce No. 1 was evaluated as the sourness shrimp sauce. Finally, the overall acceptability was observed. The sauce No. 7 was overall favorable when compared with all sauces evaluated. From these results, it indicated that the sauce No. 7 prepared using 30 wt.% koji to broiled shrimp was a shrimp sauce with desirable sensory qualities. Hoki, Southern bluefin tuna, and spear squid sauces reported by Dohmoto et al. (2001) had mellow taste and savory aroma when compared with CA nuoc-mam. Takano et al. (2012) evaluated that by-products sauces from kamaboko processing had high saltiness and low bitterness in comparison with deep-sea smelt sauces. Uchida et al. (2005) indicated that sensory quality of freshwater

Table 5 Radical scavenging activities and ACE and hyaluronidase inhibitory activities of the shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji

Samples	Superoxide anion radicals (% inhibition)	Hydroxyl radicals (% inhibition)	DPPH radicals (% inhibition)	ACE (% inhibition)	Hyaluronidase (% inhibition)
1	72.5±1.2[d]	83.8±1.4[c]	88.1±1.0[b]	100[a]	73.4±2.1(38.8)[b]
2	88.6±1.7[c]	77.8±1.3[cd]	87.9±1.1[b]	100[a]	72.8±1.9(38.2)[b]
3	94.8±1.9[b]	81.5±1.4[c]	100[a]	100[a]	76.0±2.0(40.0)[ab]
4	94.8±2.0[b]	79.2±1.4[c]	100[a]	100[a]	68.6±1.8(36.4)[bc]
5	99.5±2.1[ab]	77.1±1.3[cd]	100[a]	100[a]	66.1±1.7(34.7)[bc]
6	88.6±1.8[c]	71.6±1.2[d]	94.7±1.3[ab]	100[a]	61.3±1.3(32.2)[c]
7	100[a]	72.3±1.2[d]	100[a]	100[a]	81.0±2.2(41.7)[a]
8	97.6±1.9[ab]	71.4±1.3[d]	100[a]	100[a]	80.6±2.1(41.4)[a]
1 mM AA	14.7±0.2[h]	13.2±0.2[f]	3.1*[f]		
5 mM AA	89.9±5.3[c]	17.6±0.7[f]	34.1±2.0**[d]		
0.01 mM BHA	29.3±0.5[g]	59.1±0.8[e]	5.5[f]		
0.1 mM BHA	36.4±0.9[g]	93.3±1.4[b]	17.5±0.4[e]		
1 mM BHA	51.9±1.4[e]	95.2±1.4[b]	72.7±3.6[c]		
0.01 mM BHT	11.7±0.2[h]	82.8±0.9[c]	3.9[f]		
0.1 mM BHT	46.6±1.0[f]	97.6±1.6[b]	7.9±0.1[f]		
1 mM BHT	48.4±1.2[f]	100[a]	31.7±0.8[d]		
1 mM TP	52.6±4.2[e]	67.6±4.3[d]	87.6±2.8[b]		
0.01 mM TL	46.4±1.0[f]	81.5±0.6[c]	0.1[g]		
0.1 mM TL	58.1±1.1[e]	91.8±1.2[b]	17.9±0.2[e]		
1 mM TL	76.1±1.9[d]	100[a]	86.3±3.3[b]		

*0.1 mM AA; **1.0 mM AA. Values in brackets are millimoles of sodium cromoglicate equivalents per kg of fish sauces silver carp sauce could be improved by koji.

Free amino acid composition

Free amino acid composition of the shrimp sauce No. 7 is given in Table 7. Total amino acid contents were approximately 5881.0 mg/100 ml. Glutamic acid followed by alanine and glycine was the most dominant amino acids. The sauce contained high amounts of taurine (approximately 119.5 mg/100 ml) and ornithine (approximately 134.1 mg/100 ml) produced by complete decomposition of arginine. In contrast, serine was low level. Cystine, anserine, and arginine were not detected at all. Essential amino acids contributed approximately 43.2% of total amino acids. Umami amino acids (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 881.8, 1342.7, 2207.2, and 2554.7 mg/100 ml, respectively. Excluding CA *shotturu*, total amino acid contents of No. 7 were fairly lower than those of CA *ikanago shoyu*, *ishiru*, and nam pla due to high rate of rice koji in the ingredients. In contrast, it was suggested that *shotturu* was inhibited the fermentation and therefore the protein degradation in the ingredients did not proceed due to high concentration of salts (Table 2). The contents of glutamic acid in raw material fish species as main materials were as follows: shrimp sauces (Alaskan pink shrimp, 2500 mg/100 g), *ikanago shoyu* (Japanese sand lance, 2400 mg/100 g), *ishiru* (Japanese sardine, 2700 mg/100 g), *ishiri* (Japanese flying squid, 2200 mg/100 g), *shotturu* (sailfin sandfish, 2200 mg/100 g), and nam pla (anchovy, 2500 mg/100 g), respectively (Kagawa 2020). Thus, the content of glutamic acid was no significant difference among these materials. It suggested that the proteins in the ingredients were not fully digested under the processing conditions, although the sauce No. 7 showed excellent sensory acceptability. In fact, total amino acid contents of the sauce No. 7 were low when compared with those of CA fish sauces except for *shotturu*. It is desired the processing method for high digestability to improve the quality of the sauce. Then, it could be produced the shrimp sauce with rich amino acids and excellent umami and flavor. Other researchers referred that glutamic acid and lysine were the most abundant amino acids, and leucine, aspartic acid, and alanine were relatively high levels of amino acids in fish sauces (Dohmoto et al. 2001; Taira et al. 2007; Ikeda et al. 2018; Funatsu et al. 2019). These findings were in agreement with our results. Free amino acid composition of fish sauces are largely influenced by the ingredients of sauces and fermentation conditions, such as pH, temperature, water and salt content, and enzyme activities of microorganisms.

Fish sauces have been widely produced and used in Southeast Asian countries, such as China, Indonesia, Cambodia, Laos, Malaysia, Myanmar, Thailand, and Vietnam. In contrast, these have been produced in limited areas, such as Akita, Ishikawa, and Kagawa, in Japan (Mori et al. 2005). In recent year, the demand of fish sauces has been increasing due to the trend of ethnic foods and the diversified eating habits. In spite of high nutritional values, the consumption of fish sauces had been limited in Japan because of distinctive, complicate, and unacceptable odors. In addition, the low-salt policy (World Health Organization 2012) has been internationally taken an approach to decrease the amount of sodium intake in the diet and to minimize sodium contents in foods by reduced-salt and lowered-salt for prevention of chronic diseases, such as high blood pressure, arteriosclerosis, ischemic heart disease, etc (He and MacGregor 2010; Liem et al. 2011; Tsugane et al. 2004). However, high concentration of salts was absolutely essential factor for fish sauce production at room temperature without spoilage. The pathogenic microorganisms, such as *Clostridium botulinum*, *C. perfringens*, *E. coli*, *Salmonella*, *S. aureus*, and *Vibrio parahaemolyticus*, cause food poisoning. Generally, the water activities of CA soy sauces are low at approximately 0.76-0.85 (Japan Soy-sauce Brewers' Association 2019). The growth of these microorganisms is inhibited under these conditions. In contrast, the water activities of CA low-salt and reduced-salt soy sauces are high at approximately 0.86-0.91. Therefore, it is possible that low-salt and reduced-salt soy sauces cause the degradation by the growth of pathogenic microorganisms. In addition, the storage periods of soy sauces with high water activities are short. In contrast, the water activities of the sauces prepared from Alaskan pink shrimp were low at approximately 0.74-0.81. It suggests that these shrimp sauces are hard to be spoiled. In fact, as a result of the total viable bacterial count and coliform tests, the viable bacterial counts were not detected and *E. coli* colony's was negative during the storage periods of 7 months at room temperature, although the browning of the sauces gradually progressed with the passage of storage periods (data not shown). Thus, we could be produced the reduced-salt shrimp sauces with good sensory qualities by the fermentation with nonglutinous rice cultivar *Tsuyahime* koji. These were rich in essential amino acids. Moreover, these sauces showed not only significantly high antioxidative activities and scavenging activities against ROS but also powerful ACE and hyaluronidase inhibitory activities. Nowadays the trends of eating habits have changed more and more. The consumers need to maintain healthy dietary life and tend to require foods with high nutritional values and functionalities for health. Therefore, Alaskan pink shrimp sauces could be effectively utilized as alternatives of CA fish sauces. Moreover, these sauces may have contributed the prevention and cures of lifestyle-related diseases and allergy. It is possible to produce the fish sauces with different properties depending on the ingredients and the fermentation conditions. It is planning to identify the functional constituents of the shrimp sauces and to clarify the mechanisms related to these functionalities.

Conclusion

In summary, it could be produced reduced-salt Alaskan pink shrimp sauces fermented with nonglutinous

Table 6 Sensory evaluations of the shrimp sauces fermented with nonglutinous rice cultivar *Tsuyahime* koji

	Samples							
Factors	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Color	-1	-1	-1	-1	1	2	2	2
Smell	2	1	2	2	1	1	1	1
Taste strength	2	1	1	1	0	-1	3	1
Taste balance	0	0	0	0	0	1	3	2
First taste	2	1	1	1	1	-1	3	1
Aftertaste	2	1	1	1	2	-1	2	0
Sweetness	2	2	2	2	1	0	3	2
Umami	2	2	2	2	1	1	3	2
Sourness	2	1	-1	1	1	-2	1	0
Bitterness	-3	0	-3	-1	-1	-3	-3	-3
Saltiness	1	0	-1	-2	-2	-3	-3	1
Overall acceptance	-0.57	0.22	1.08	0	0.78	0.72	1.92	-0.04

Table 7 Free amino acid composition of the shrimp sauce No. 7 fermented with nonglutinous rice cultivar *Tsuyahime* koji

	Shrimp sauce	**A***	**B***	**C***	**D***
Amino acids	**mg/100 g (mg/100 ml)**	**mg/100 g**	**mg/100 g**	**mg/100 g**	**mg/100 g**
Taurine	108.0^{a} (119.5)	-	-	-	-
Aspartic acid	416.7^{d} (460.9)	1200^{a}	1300^{a}	560^{c}	850^{b}
Threonine	163.2^{d} (180.5)	570^{a}	600^{a}	240^{c}	400^{b}
Serine	6.9^{e} (7.6)	440^{b}	570^{a}	260^{d}	340^{c}
Glutamic acid	797.3^{d} (881.8)	1900^{a}	1500^{b}	860^{d}	1300^{c}
Glycine	630.9^{b} (697.8)	720^{a}	630^{b}	450^{d}	520^{c}
Proline	175.2^{e} (193.3)	490^{b}	570^{a}	270^{d}	350^{c}
Alanine	691.3^{b} (764.6)	850^{a}	650^{b}	370^{d}	560^{c}
Valine	328.6^{d} (363.4)	700^{a}	600^{b}	280^{d}	450^{c}
Cystine	N.D. (N.D.)	100^{a}	100^{a}	35^{c}	54^{b}
Methionine	157.6^{c} (174.3)	300^{a}	220^{b}	140^{c}	180^{bc}
Isoleucine	322.5^{b} (356.7)	430^{a}	380^{ab}	200^{c}	280^{b}
Leucine	459.7^{b} (508.4)	560^{a}	450^{b}	300^{d}	370^{c}
Tyrosine	71.9^{b} (79.5)	160^{a}	71^{b}	44^{c}	60^{bc}
Phenylalanine	244.0^{c} (269.9)	360^{b}	410^{a}	170^{d}	260^{c}
Tryptophan	66.3^{b} (73.3)	85^{a}	39^{c}	12^{d}	57^{b}
Ornithine	121.2^{a} (134.1)	-	-	-	-
Lysine	472.2^{d} (522.3)	1100^{a}	960^{b}	470^{d}	770^{c}
Histidine	84.2^{b} (93.1)	290^{a}	260^{a}	95^{b}	270^{a}
Anserine	N.D. (N.D.)	-	-	-	-
Arginine	N.D.^{d} (N.D.)	490^{a}	310^{b}	260^{bc}	160^{c}
Total	5317.7^{d} (5881.0)	11000^{a}	9600^{b}	5000^{d}	7300^{c}

N.D.: not detected. A: ikanago shoyu, B: *ishiru* (*ishiri*), C: *shotturu*, D: nam pla. *Data was quoted from Standard Tables of Food Composition in Japan 2020.

rice cultivar *Tsuyahime* koji under room temperature condition without spoilage. It can contribute to the reduction of sodium intake for the prevention of chronic diseases, which is recommended by the World Health Organization. These sauces had beneficial properties for health, such as remarkably high antioxidative activities, ROS scavenging activities, and ACE and hyaluronidase inhibitory activities. Among these shrimp sauces, the sauce prepared using 30 wt.% koji to ground broiled shrimp was the sauce with excellent sensory acceptability, rich essential amino acids, and high nutritional value. It may be an alternative of CA high-salt fish sauces, which is demanded for consumers and fish sauce industries.

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Conflict of interest The authors have declared no conflicts of interest for this article.

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