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ORIGINAL ARTICLE

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Preparation and characterization of fermented seaweed sauce manufactured from low-quality nori (dried and fresh fronds of *Pyropia yezoensis*)

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Abstract
The present study tested processes to manufacture fermented sauce from low-quality nori (dried and fresh fronds of *Pyropia yezoensis*). The nori sauce was prepared using three tanks with fresh or dried nori cultured in different conditions. In the present study enzymes were not added for the promotion of the degradation of nori, while in a previous study they were. The supernatants of culture mashes obtained from the three tanks were combined, and this low-quality nori sauce (LNS) was characterized and compared with sauces manufactured from high-quality nori, soy, and fish. The LNS showed low concentrations of total nitrogen compounds (0.20 g/100 ml) and free amino acids, and its taste showed a high sourness score as evaluated by a taste-sensing system. On the other hand, the LNS was rich in polysaccharides, which were observed to be readily degraded to lower molecular weight size sugars by heat treatment. The LNS showed little risk for heavy metal or allergen contamination. The obtained sauce product is expected to be commercially utilized as a component of low allergen-risk sauce products after blending with other seasonings without wheat or soy elements.

Keywords Fermentation · Iroochi-nori · Laver · Allergen · Aftertaste

Introduction

Seaweed sauce was recently developed by fermentation from nori (laver or dried sheets of *Pyropia yezoensis*) (Uchida et al. 2017). This nori sauce was reported to have a unique taste with a strong aftertaste, was rich in functional compounds such as taurine and vitamins, and has potential as a new source of nutrition for humans. Despite the long history of fermentation technology (reviewed by Steinkraus 1997), this product can be regarded as the first fermented food product manufactured from seaweed. In a previous paper (Uchida et al. 2017), however, the nori sauce was manufactured from nori of high quality (protein content 51.6%), and this technology has not been tried using nori of low quality and commercial value, yet.

There are many types of nori categorized as low quality, and so-called iroochi-nori or discolored nori is the most common. Iroochi-nori is formed when cultured nori does not receive a sufficient quantity of nitrogen from environmental water and accumulates a low quantity of phycobiliproteins in its fronds. As a result, the iroochi-nori is characterized as having a low protein (usually less than 30% on a dry weight basis) but high carbohydrate content (Akimoto and Shimizu 2005). The iroochi-nori has a poor taste and, when used as a feed for domestic animals, does not contribute

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to a fisherman's income. It is very difficult to estimate the actual annual production of iroochi-nori because this is not reported in official statistics. However, we collected information suggesting that ca. 0.5–2% of the total nori production is regarded as iroochi-nori based on interviews with members of the Japan Fisheries Cooperative Association. The total annual nori production was 276,129 t on a wet weight basis in Japan 2014 (Statistics Department, http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001141864 Accessed 23 November 2017). Therefore, it is estimated that more than 1380 t (or 138 t on a dry weight basis) of iroochi-nori was produced in Japan 2016, although the actual annual quantity of iroochi-nori is highly variable. Furthermore, it is believed that much more of the iroochi-nori is abandoned in the water without being harvested, thus potentially is a source of organic pollution of fishing grounds. Therefore, to contribute both to an increase in fishermen's incomes and for the remediation of fishing grounds, there is strong commercial interest in the effective utilization of iroochi-nori. Besides iroochi-nori, there are other types of low-quality nori including sumi-nori (abnormal appearance caused by microbial infection), ebi-nori (contaminated with small crustaceans), and yabure-nori (broken sheets of nori), etc.

The present study attempted to prepare nori sauce from these types of low-quality nori. Only nori, salt, and water were added, and enzymes and starter microorganisms, which were used in a previous study (Uchida et al. 2017), were not added. Fermentation was conducted at a large scale (0.5–0.65 t), while the previous fermentations were conducted on a laboratory scale (15 kg) (Uchida et al. 2017). Furthermore, both dried and fresh fronds of valueless nori were tested as raw materials, and this study reports on the use of fresh fronds of nori for the first time. Nutritional components of the nori sauce prepared from the low-quality nori (LNS) were analyzed and compared with those of sauces prepared from high-quality nori (HNS), soy (SS), and fish (FS). Heavy metal contents of the LNS were measured to assess any potential food risks. Allergens in the LNS were also checked. The effect of a heat treatment was examined on the molecular weight distribution of sugar components of the LNS. The taste of the LNS was characterized by sensor technology (Taste Sensing System SA402B).

Materials and methods

Preparation of nori sauce products

Three types of low-quality nori (harvested in Saga Prefecture, 2014) were purchased from the Kashima Branch of the Saga Prefectural Branch of the Japan Fisheries Cooperative Association and used in the present study. The first one was fresh fronds of iroochi-nori with 11.7% protein content on a dry weight basis. The second type was dried and baked sheets of nori with 38.1% protein content on average, but a mixture of various types of commercially valueless ones such as iroochi-nori, sumi-nori, yabure-nori, etc. The third type was dried and baked sheets of nori categorized as second class with 33.9% protein content on a dry weight basis. Three tanks (T1–T3) were used for preparing LNS in different batches as follows:

1. To prepare T1, 388 kg fresh iroochi-nori was added to a 0.8-t-volume plastic tank with 45.3 kg of sodium chloride and 64.7 L of tap water.
2. To prepare T2 and T3, 76.95 kg of dried nori was added to 81 kg of sodium chloride and 490.05 L of tap water, respectively.

The surface of each culture was covered with a plastic sheet to maintain semi-aerobic conditions. The contents of the tanks were mixed well with a shovel at the start of the experiment, then after 1 month, and again after 2 months. The time necessary to perform the mixing treatment was 5–20 min for each tank. The tanks were cultured at ambient temperature for 10–11 months at Hatsukaichi (T1; from 21 April 2015 to 15 March 2016) and Saga (T2 and T3, from 27 May 2015 to 14 March 2016). The average local monthly temperature during the culture period was 16.5 °C (maximum 31.2 °C, minimum 2.1 °C) at Ohtake (located near Hatsukaichi) and 17.2 °C (maximum 32.1 °C, minimum 2.6 °C) at Saga based on monthly climate statistics (Japan Meteorology Agency: http://www.data.jma.go.jp/obd/stats/etrn/index.php?prec_no=44&block_no=47662&year=2016&month=&day=&view=p1 Accessed 23 November 2017). The culture mash before harvesting the supernatant was designated as 'moromi' in the present study. Each 8-kg portion of moromi from T1, T2 and T3 was wrapped with filter cloth and squeezed by a traditional squeezer equipped with a pressing plate (hand-made, 300-L volume, made of stainless steel) to recover the supernatant. Recovery efficiency (%) was calculated for each tank on a volume basis from moromi and the obtained supernatant. The supernatant from the three tanks was combined, designated as LNS, and used for component analysis.

Component analysis

Chemical analysis of the sauce components was conducted as described in Uchida et al. (2017) and the Japan Soy Sauce Research Institute (1985). Total nitrogen was measured by the Kjeldahl method. Salt content was measured by Mohr's method. The soluble solids excluding salt were obtained by subtracting salt (%) from Brix (%). Protein and peptide contents in the supernatant were measured by a commercial kit (DC Protein Assay; BioRad, CA) based on the Lowry

method (Peterson 1979) using bovine serum albumin as a standard. Sugar was measured by the phenol–sulfuric acid method using glucose as a standard (DuBois et al. 1956). Low molecular weight sugar content was obtained from the sample filtered through an ultrafiltration system with a molecular weight cut-off of 5000 Da [Ultrafree-MC-PLHCC (5 K) filter; Merck Millipore, Billerica, MA]. The high molecular weight sugar content was calculated by subtracting the low molecular weight sugar content from the total sugar content. Vitamin B_1 was measured by high performance liquid chromatography (Council for Science and Technology, Ministry of Education, Culture, Sports, Science, and Technology, Japan 2004). Vitamin B_{12} and folic acid were measured by microbial assay using *Lactobacillus delbrueckii* subsp. *lactic* ATCC 7830 and *Lactobacillus rhamnosus* ATCC 7649, respectively (Council for Science and Technology, Ministry of Education, Culture, Sports, Science, and Technology, Japan 2004). Histamine was measured by a commercial kit (Kikkoman Biochemifa, Tokyo). Organic acids were measured by a high performance liquid chromatography system (JASCO, Tokyo). Briefly, the organic acids were separated with ion exclusion chromatography columns (8 mm × 300 mm × 2 columns; Shodex RSpac KC-811; SPELCO Japan, Tokyo), derivatized with bromothymol blue, and detected with an ultraviolet detector (JASCO 2015; absorbance at 445 nm). For mineral and metal components, sodium, potassium, copper, arsenic, lead, cadmium, and mercury were measured by atomic absorption spectrophotometer (AA 240FS; Agilent Technologies, CA; ZA-3000; Hitachi, Tokyo) (Japanese Society for Food Science and Technology 1996; Japan Food Hygiene Association 2005). Phosphorus, calcium, magnesium, iron, and zinc were measured by inductively coupled plasma-optical emission spectrometer (ICP/OES 725-ES; Agilent; HG4500; Hirayama Sangyo, Ibaragi, Japan) (Japanese Society for Food Science and Technology 1996). Inorganic arsenic was extracted after 0.1 mg of sample was partially digested with 2 mL of 0.2 M nitric acid at 80 °C for 1 h and measured by high performance liquid chromatography-ICP-mass spectrometry (Agilent 1200 series and Agilent 7500ce; Agilent Technologies) (Hamano-Nagaoka et al. 2008). Chromium was measured by the diphenylcarbazide colorimetric method (V-630; JASCO) (Japanese Society for Food Science and Technology 1996). Cyanide was measured by the pyridine-pyrazolone method (V-630; JASCO) (Japanese Industrial Standard Committee 2013). Free amino acid composition was measured by an amino acid analyzer (model L-8900; Hitachi, Schaumburg, IL). The data of sauces prepared from HNS (2-year-old product), SS, and FS are cited from a previous study (Uchida et al. 2017) excluding data on proteins and peptides, sugars, and organic acid composition. The values measured on a weight basis were converted to values on a volume basis using the specific gravity of 1.10 g/ml for LNS and HNS and 1.17 g/ml for SS and FS. The data of HNS are expressed as average values of the two batch products except for the heavy metals. The data on heavy metals of HNS represent the values obtained from a single batch sample. The SS is a common commercial Japanese brand (Koikuchi, Super Grade). The FS is a Chinese high-quality brand manufactured from anchovy, which was purchased at a Japanese market.

Allergen test

Allergen tests for wheat and soy were conducted by immunochromatography using a commercial kit (FASTKIT slim; NH Foods, Tokyo). The allergen test for crustaceans targeting shrimp and crab substances was conducted using the FA test (Immunochromato-Kokakurui II; Nissui Pharmaceuticals, Tokyo).

Gel permeation chromatography of LNS

The LNS was further heated for 20 min at 90 °C to cease enzyme activities. The LNS before and after the heat treatment was subject to gel permeation chromatography. A 0.15-g aliquot was mixed with 20 ml of 0.1 M $NaNO_3$, left overnight at room temperature, and filtered through a 0.45-µm membrane filter. The filtrate was analyzed by high performance liquid chromatography (Shodex GPS-101 and RID-71S; Showa Denko, Tokyo) equipped with a gel permeation column (TSKgel G3000PW_{XL}, 7.8 × 300 mm × 2 columns; Tosoh, Tokyo) set at 50 °C with flow rate 1.0 ml/min of 0.1 M $NaNO_3$.

Evaluation by a taste-sensing system

Evaluation of the taste of the sauce products was conducted as described in Uchida et al. (2017). The sauce samples were diluted with distilled water and evaluated by the Taste Sensing System SA402B (Intelligent Sensor Technologies, Kanagawa, Japan) (Tahara and Toko 2013; Hayashi et al. 1989, 2006). At first, the normality of the score was checked by comparing the values obtained from tenfold-, 20-fold, and 40-fold diluted samples prepared from each sauce. Five kinds of lipid membrane sensors were used to measure the eight kinds of taste stimuli: the sensors C00 for bitterness and aftertaste from bitterness, AE1 for astringency and aftertaste from astringency, AAE for umami and umami richness, CT0 for saltiness, and CA0 for sourness. The estimated intensity of taste was calculated from the sensor output on the basis of Weber's and Weber-Fechner laws (Ross and Murray 1996) by using the manufacturer's recommendation: one unit of score was defined to express a 20% difference in taste intensity

(Intelligent Sensor Technology 2011). The result was expressed on a radar chart with the SS score plotted as a standard score (i.e., zero).

The data are expressed as means of duplicate measurements (Tables 1, 2 and 4) or triplicate measurements (Fig. 1) excluding single measurements (Table 3) in this study.

Results

Recovery of nori sauce

At first, a 213-L (240 kg) aliquot of moromi from the 6-month-old supernatant tank T1 was subject to a preliminary squeezing test to confirm the performance of the squeezer designed for the SS. This 6-month-old supernatant was discarded without component analysis. Then, the remaining 224 kg (200 L) aliquot of moromi from T1 was squeezed to obtain 145 L of supernatant after 10 months of fermentation. From the 11-month-old tanks of T2 and T3, 480 kg (429 L) and 504 kg (450 L) of moromi was squeezed to obtain 305 and 225 L of supernatant, respectively. The recovery efficiency of the supernatants was 72.5% (T1), 71.1% (T2), and 50.0% (T3). These three supernatants were combined into one sample, heated for 20 min at 90 °C to cease enzyme activity, and designated as LNS.

Table 1 Comparison of basic characteristics of sauces prepared from low- (*LNS*) and high-quality nori (*HNS*), soy sauce (*SS*), and fish sauce (*FS*)

Characteristics	Nori sauces		SS[a]	FS[a]
	LNS	HNS[a]		
Total nitrogen (g/100 ml)	0.20	1.51	1.65	1.42
pH	4.3	5.5	4.8	5.3
Salt (g/100 ml)	14.6	11.0	14.1	16.9
Soluble solids excluding salt (g/100 ml)	8.9	19.3	23.0	15.2
Protein and peptide (g/100 ml)	0.95	3.28	3.50	1.35
Sugar (g/100 ml)				
Total	7.8	4.0	4.7	4.6
Low molecular weight (MW < 5000 Da)	1.0	1.5	2.2	4.3
High molecular weight (MW > 5000 Da)	6.8	2.5	2.5	0.2
Vitamin B1 (mg/100 ml)	0.04	0.24	0.05	< 0.01
Vitamin B12 (μg/100 ml)	4.18	15.40	<0.03	0.69
Folic acid (μg/100 ml)	39.2	86.9	7.0	3.5
Histamine (p.p.m.)	< 1	55	< 1	< 1

[a]Data are cited from Uchida et al. (2017) excluding data on proteins, peptides and sugars

Table 2 Mineral and metal components of LNS and HNS, SS, and FS

Components (mg/100 ml)	Nori sauces		SS[a]	FS[a]
	LNS	HNS[a]		
Na	6981	4389	6634	7909
K	494	968	536	183
P	46	186	183	13
Ca	48.1	49.5	44.0	22.0
Mg	108.7	72.5	79.7	56.7
Fe	1.21	0.92	2.45	1.49
Zn	0.25	0.67	1.13	0.28
Mn	NT	0.86	1.23	0.02
Cu	< 0.01	< 0.01	< 0.01	< 0.01
As (total, as As_2O_3)	2.5	0.9	NT	NT
As (inorganic)	< 0.06	< 0.06	NT	NT
Pb	< 0.006	< 0.006	NT	NT
Cd	0.04	0.01	NT	NT
Hg	< 0.001	< 0.001	NT	NT
Cr	< 0.06	0.03	NT	NT
Cn	0.12	0.04	NT	NT

NT Not tested; for other abbreviations, see Table 1

[a]Data are cited from Uchida et al. (2017) and shown on a volume basis

Comparison of basic characteristics

Basic characteristics of the LNS are shown in Table 1 with comparative data for HNS, SS, and FS. The characteristics of LNS were: total nitrogen 0.20 g/100 ml, pH value 4.3, salt concentration 14.6 g/100 ml, soluble solids excluding salt

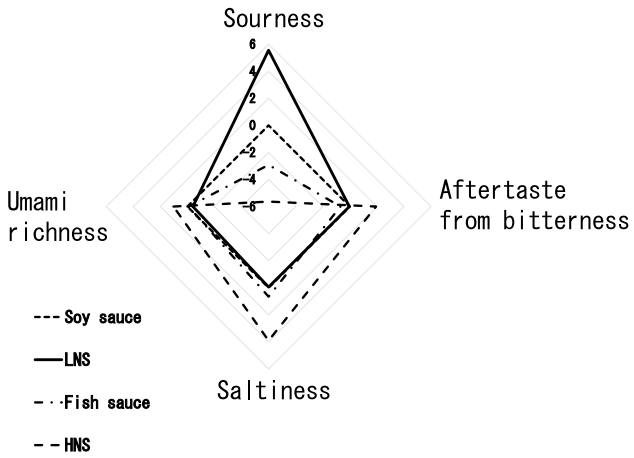

Fig. 1 Results of evaluation of low-quality nori sauce (*LNS*) taste by a taste-sensing system equipped with lipid membrane sensors and compared to high-quality nori sauce (*HNS*), soy sauce, and fish sauce. Samples were diluted tenfold with distilled water then tested. The axis has arbitrary units. One unit score is regarded as a 20% difference in taste intensity

Table 3 Free amino acid composition of LNS and HNS, SS, and FS

Amino acids (mg/100 ml)[a]	Nori sauces		SS[b]	FS[b]
	LNS	HNS[b]		
Glutamic acid	36	754	995	737
Aspartic acid	8	440	164	527
Threonine	7	380	339	374
Alanine	97	963	807	550
Glycine	8	275	246	257
Proline	7	275	398	164
Serine	6	275	456	281
Isoleucine	6	369	456	281
Leucine	15	638	702	386
Methionine	4	160	129	187
Phenylalanine	8	231	421	246
Lysine	13	486	460	785
Tryptophan	< 10	55	12	527
Valine	11	561	480	445
Arginine	4	39	573	59
Cystine	< 10	11	< 10	< 20
Tyrosine	3	33	70	59
Histidine	1	< 10	176	222
Taurine	66	340	16	77
γ-Amino boric acids	42	5	13	< 1
Total	341	6287	6912	6162

For abbreviations, see Table 1

[a]Amino acids were measured by amino acid auto analyzer after reduction of protein molecules

[b]Data are cited from Uchida et al. (2017) and shown on a volume basis

Table 4 Organic acid composition of LNS and HNS, SS, and FS

Organic acids (mg/100 ml)	Nori sauces		SS	FS
	LNS	HNS		
Lactic acid	350	339	1518	2687
Acetic acid	131	2591	601	3935
Pyruvic acid	34	670	475	147
Pyroglutamic acid	5	443	1523	2440
Malic acid	1	51	77	93
Succinic acid	4	67	164	473
Citric acid	< 3	26	307	< 3
Formic acid	8	47	135	186
Propionic acid	< 1	6.0	< 1	402
Isobutyric acid	1	10	7	< 1
n-Butyric acid	< 1	11	124	156

Organic acids were measured by high performance liquid chromatography (JASCO). For abbreviations, see Table 1

8.9 g/100 ml, protein and peptide contents 0.95 g/100 ml, total sugar 7.8 g/100 ml, low molecular weight sugar (< 5000 Da) 1.0 g/100 ml, high molecular weight sugar (> 5000 Da) 6.8 g/100 ml, vitamin B1 0.04 mg/100 ml, vitamin B12 4.18 μg/100 ml, and folic acid 39.2 μg/100 ml. Histamine was not detected (< 1 p.p.m.) in LNS, SS or FS. The LNS contained less total nitrogen and other valuable components such as vitamin B1, B12, and folic acid, although it was richer in sugar components than HNS.

Mineral and metal components

Mineral and metal components are shown in Table 2. Sodium, potassium, phosphorus, calcium, magnesium, iron, and zinc (mg/100 ml) levels in LNS were 6981, 494, 46, 48.1, 108.7, 1.21, and 0.25, respectively. Cadmium and cyanide (mg/100 ml) levels were 0.04 and 0.12, respectively. Copper, lead and, mercury (mg/100 ml) were not at detectable levels of 0.01, 0.006 and 0.001, respectively. Arsenic was 2.5 mg/100 ml for LNS, but inorganic arsenic was not detected (< 0.06 mg/100 ml).

Free amino acid composition

Free amino acid, taurine, and γ-amino boric acid (GABA) levels are shown in Table 3. The total quantity of free amino acid (mg/100 ml) was 341 (LNS), 6287 (HNS), 6912 (SS), and 6162 (FS). The taurine content (mg/100 ml) of LNS was 66, less than that of HNS but richer than that of SS. GABA was 42 mg/100 ml in LNS and higher than that of HNS, SS, and FS. Other kinds of amino acid were also remarkably low in LNS compared with HNS, SS, and FS.

Organic acid composition

Organic acid composition is shown in Table 4. Lactic acid (350 mg/100 ml) and acetic acid (131 mg/100 ml) are major components of LNS as well as HNS, SS, and FS. Pyruvic acid (34 mg/100 ml), pyroglutamic acid (5 mg/100 ml), malic acid (1 mg/100 ml), and succinic acid (4 mg/100 ml) levels were relatively low in LNS compared to HNS, SS, and FS. Volatile organic acids such as formic acid (8 mg/100 ml), propionic acid (< 1 mg/100 ml), isobutyric acid (1 mg/100 ml), and n-butyric acid (< 1 mg/100 ml) were low in LNS as well as in HNS, SS, and FS.

Allergen tests

Results of the allergen tests are shown in Table 5. The LNS does not contain wheat flour and soybean as raw materials, and the allergen tests for these substances were negative. Nori is cultured in water and therefore might contain some crustaceans. However, the allergen tests for shrimp and crab substances were also negative.

Table 5 Results of allergen test on LNS and HNS

Allergen	Nori sauces	
	LNS	HNS[a]
Wheat	Negative[b]	Negative
Soy	Negative[b]	Negative
Crustaceans (shrimp and crab)	Negative[c]	Negative

[a]Data cited from Uchida et al. (2017)

[b]Measured by FASTKIT Slim, NH Foods

[c]Measured by the FA test (Immunochromato-Kokakurui II; Nissui Pharmaceuticals)

Table 6 Molecular weight distribution of polysaccharides contained in the LNS before and after heat treatment

Molecular weight (Da)	Peak area (%)	
	LNS (before heat treatment)	LNS (after heat treatment)
> 100,000	11	5
30,000–100,000	11	11
10,000–30,000	6	7
3000–10,000	3	3
1000–3000	4	2
500–1000	36	42
< 500	29	30

LNS before and after heat treatment was analyzed by gel permeation chromatography

Molecular size distribution of polysaccharides before and after heat treatment of LNS

The supernatant of LNS obtained just after squeezing showed very high viscosity. High molecular weight sugars were predominant in the supernatant before heat treatment, as shown in Table 1. However, the viscosity of the LNS was remarkably reduced after heat treatment. Therefore, the molecular size distribution of polysaccharides was compared before and after heat treatment of LNS (Table 6). The polysaccharide fraction in the molecular weight size range > 100,000 Da decreased and low molecular weight size sugars comparable to oligosaccharides increased after heat treatment: sugars in the size range of molecular weight 500–1000 Da (36%) and < 500 Da (29%) increased to 42 and 30%, respectively.

Characterization of taste

Among the eight elements of taste, the score of estimated intensity of umami showed abnormal values, thus this taste element was not evaluated properly in the present study (data not shown). Scores for bitterness, astringency, and aftertaste from astringency showed normal values, but the difference between the scores was smaller than 1.0 unit and negligible (data not shown). The remaining four scores, for sourness, aftertaste from bitterness, saltiness and umami richness showed normal values and differences larger than 1.0 unit score among the sauces. A difference larger than 1.0 unit score is comparable to a 20% difference in taste intensity and regarded as a level which can be discriminated by a sensory test (Nakamura et al. 2010). The scores of four elements of taste are shown in Fig. 1, with the score of SS set as the zero position. The LNS showed a higher score (5.55) for sourness among the sauces, but lower scores for aftertaste from bitterness and umami richness than those of HNS.

Discussion

Fermented nori sauce was prepared from low-quality nori for the first time in the present study and characterized. It was demonstrated that low-quality nori, including discolored nori, can be used as a raw material for sauce manufacture, as well as high-quality nori. It was also demonstrated that the degradation process during the culture of nori can proceed with the addition of salt alone, although it has been observed that the addition of enzymes such as protease and mannanase promoted the degradation of nori fronds (Uchida et al. 2017). The recovery of the supernatant from moromi was 50.0–72.5% after 10–11 months of culture, but a higher rate of recovery can be expected when culturing for a longer period.

The reason for the low recovery ratio of T3 is unknown. The undegraded precipitates of T1–T3 were observed through a microscope and compared. However, all of the precipitates contained undegraded nori tissue and their appearance could not be discriminated among the three tanks (data not shown). Therefore, one possible explanation for the low recovery of T3 is that the mixing treatment might not have been effective for it. Fresh fronds of nori can be used for sauce manufacture as well as dried sheet nori. However, the fiber component of the fresh fronds was resistant to biodegradation, and intensive labor was required to mix the culture with a shovel even after 3 months of culture. For dried sheet nori, since the fiber component was partially broken down in advance during the sheet-forming and drying process, less labor was required to mix the culture.

Another problem was the production of a microbial film on the culture surface. This could be partially resolved by covering the culture surface with a plastic sheet to keep semi-aerobic conditions. The microbial film was occasionally discarded in the present study.

The comparison of basic characteristic among the sauces clarified that the LNS contained lower quantities of total

nitrogen compounds and other valuable components, such as vitamins B1, B12, and folic acid, while it was richer in sugar components when compared with HNS (Table 1).

Trace metal components were measured to check the food risk of LNS. According to the Joint FAO/WHO Expert Committee on Food Additives (2007) list of regulatory concentrations for food additives, cadmium and arsenic should be at a concentration under 0.05 mg/100 g (for one food item) and 0.1–0.3 mg/100 g (for six food items), respectively. The present study showed that the contamination level of cadmium in LNS was 0.04 mg/100 g (Table 2) so it should not pose a significant risk. Total arsenic compounds were at 2.3 mg/100 g in LNS, but inorganic forms of arsenic were under the detection level (< 0.05 mg/100 g). The Codex Committee is still collecting information before determining the regulatory contamination level of arsenic compounds, and is taking into consideration the difference in strength of toxicity depending on chemical form (Codex Alimentarius Commission 1997). Cyanide was at a level of 0.11 mg/100 g. The regulatory contamination level of cyanide has not been determined by the Codex Committee (Codex Alimentarius Commission 2011) for FS, and neither is it given in the Japan Agricultural Standards for soy sauce (Ministry of Agriculture, Forest, and Fisheries 2009). The contamination level for cyanide is set at 0.001 mg/100 ml for drinking water in Japan (Ministry of Health, Labour and Welfare: http://www.mhlw.go.jp/stf/seisakunitsuite/bunya/topics/bukyoku/kenkou/suido/kijun/kijunchi.html Accessed 23 November 2017). Therefore, it may be necessary to take care regards the contamination level of cyanide. However, the regulatory levels for drinking water and sauce should be different considering the difference in daily intake between them. Contamination with the other tested metals was at trace levels and thus negligible.

Histamine was not detected in LNS (< 1 p.p.m.).

Another noteworthy characteristic of nori sauce is its allergens. Allergic reactions against wheat and soybean are widespread in children and can cause serious illness (Turnbull et al. 2015). The LNS does not contain wheat flour or soybean as raw materials, and was shown to be negative for these allergens (Table 5). Negative results were also obtained for allergen tests for shrimp and crab. These kit tests are not enough to assure the safety of the sauces regards their allergen contents, but medical studies showing patient allergic reactions to nori have not been published, as far as we know, so at present it can be said that LNS is a low-risk food for allergens.

The recovered supernatant showed a high viscosity, the major factor of which was suggested to be porphyran. However, a remarkable decrease of viscosity was observed after the heat treatment, suggesting that porphyran was degraded to compounds of a lower molecular weight, including oligosaccharides.

As for taste, the LNS showed a high score for sourness by a taste-sensing system (Fig. 1). A difference of more than one unit score was regarded as a level which can be discriminated by taste (Intelligent Sensor Technology 2011). However, the LNS actually did not show a sour taste in a preliminary sensory tasting trial (data not shown). The reason for this rather contradictory observation is not known. One possible explanation is the influence of coexisting substances: as the LNS contained a large quantity of polysaccharides, these might have produced a masking effect and reduced the intensity of the sour taste of the LNS.

In conclusion, the present study prepared nori sauce from low-quality nori and clarified its unique characteristics, i.e., richness in sugar components. Therefore, the demonstration of the function of these sugar components is a significant research target to promote the consumption of LNS. Although the taste of the LNS was not satisfactory, this product is expected to be commercially utilizable as a component of low allergen-risk sauces after it is blended with other seasonings that do not contain wheat and soy elements.

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References

Akimoto T, Shimizu N (2005) The measure of effective use of low quality *Pyropia* laver. Bull Fukuoka Fish Mar Technol Res Cent 15:123–131 **(in Japanese)**

Codex Alimentarius Commission (1997) Report of the twenty-ninth session of the Codex Committee on Food Hygiene, ALINORM 97/13A. Food and Agriculture Organization of the United Nations, Geneva

Codex Alimentarius Commission (2011) Standard for fish sauce, CODEX STAN 302-2011. Food and Agriculture Organization of the United Nations, Geneva

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Phenol colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Hamano-Nagaoka M, Hanaoka K, Usui M, Nishimura T, Maitani T (2008) Nitric acid-based partial-digestion method for selective determination of inorganic arsenic in hijiki and application to soaked hijiki. J Food Hyg Soc Jpn 49:88–92

Hayashi K, Matsuki Y, Toko K, Murata T, Yamafuji K, Yamafuji K (1989) Sensing of umami taste and synergistic effects with synthetic lipid membranes. Sens Mater 1(6):321–334

Hayashi N, Chen R, Ikezaki H, Yamaguchi S, Maruyama D, Yamaguchi Y, Ujihara T, Kohata K (2006) Techniques for universal evaluation of astringency of green tea infusion by the use of a taste sensor system. Biosci Biotechnol Biochem 70:626–631

Intelligent Sensor Technology (2011) Taste sensing system SA402B kaiseki application. Intelligent Sensor Technology, Kanagawa **(in Japanese)**

Japan Food Hygiene Association (2005) Standard method of analysis in food safety regulation. Rikagaku-hen. Japan Food Hygiene Association, Tokyo **(in Japanese)**

Japan Soy Sauce Research Institute (1985) Shoyu shikenho. Syouyu Tsuusinsha, Tokyo **(in Japanese)**

Japanese Industrial Standard Committee (2013) Japan industrial standards (JIS) K0102. Japanese Industrial Standard Committee, Tokyo

JASCO (2015) Analysis of organic acids including levulinic acid in soy Sauce. LC application data, no. 220060H. JASCO, Tokyo, p 1–2 **(in Japanese)**

Joint FAO/WHO Expert Committee on Food Additives (2007) JECFA 63rd meeting summary and conclusions. Food and Agriculture Organization of the United Nations, Rome

Ministry of Agriculture, Forestry, and Fisheries (2009) Japanese agricultural standards for soy sauce. Ministry of Agriculture, Forestry, and Fisheries-Kokuji Dai-1218-go, Tokyo **(in Japanese)**

Nakamura M, Sato F, Yoshida S, Kumagai M, Suzuki Y (2010) Evaluation of taste change resulting from thickening of food with instant food thickeners. Nippon Shokuhin Kagaku Kogaku Kaishi 57:380–388 **(in Japanese with English abstract)**

Peterson GL (1979) Review of the folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. Anal Biochem 100:201–220

Ross HE, Murray DJ (1996) Weber on the tactile senses, 2nd edn. Taylor & Francis, Oxford

Steinkraus KH (1997) Comparison of fermented foods of the east and west. In: Lee CH et al (eds) Fish fermentation technology. United Nations University Press, Tokyo, pp 1–12

Tahara Y, Toko K (2013) Electronic tongues-a review. IEEE Sens J 13:3001–3011

The Council for Science and Technology, Ministry of Education, Culture, Sports, Science, and Technology, Japan (2004) Analytical manual for standard tables of food composition in Japan—reference materials for the subdivision on resources. Revised and enlarged edn. Kenpakusha, Tokyo **(in Japanese)**

The Japanese Society for Food Science and Technology (1996) Shinshokuhin bunsekiho. Korin, Tokyo **(in Japanese)**

Turnbull JL, Adams HN, Gorard DA (2015) The diagnosis and management of food allergy and food intolerances. Aliment Pharmacol Ther 41(1):3–25

Uchida M, Kurushima H, Ishihara K, Murata Y, Touhata K, Ishida N, Niwa K, Araki T (2017) Characterization of fermented seaweed sauce prepared from nori (*Pyropia yezoensis*). J Biosci Bioeng 123(3):327–332