02738451
Applied Biochemistry

1st Semester 2020

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1/15

Food Biochemistry II

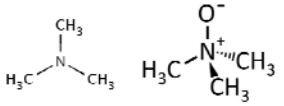
- Seafood enzymes
- "freshness" เป็นพารามิเตอร์สำคัญของคุณภาพในอาหารทะเลมากกว่าเนื้อสัตว์อื่น ๆ
- การเสื่อมคุณภาพไม่ได้เกิดขึ้นจากจุลินทรีย์เพียงอย่างเดียว แต่เกิดจาก endogenous enzymes ด้วย
- บางครั้ง autolytic change ก็ทำให้เกิดความเปลี่ยนแปลงของ texture และ taste ที่พึงประสงค์ด้วย

2/15

- Cold adaptation of enzymes
- อาหารทะเลมาจากสัตว์ที่เป็น poikilotherm
- อุณหภูมิร่างกายเปลี่ยนตาม habitat
- เอนไซม์ทำงานได้ที่อุณหภูมิต่ำ
- การเก็บรักษาอาหารทะเล ยากกว่าสัตว์เลือดอุ่น

3/15

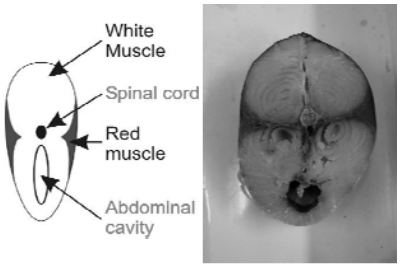
- Effect of pressure
- ปลาทะเลลึก สะสม trimethylamine-N-oxide (TMAO) เพื่อรักษาสภาพของโปรตีนที่ความกดดันสูง



4/15

- Energy metabolism of seafood
- ปลาเนื้อแดงกับเนื้อขาวต่างกันที่หลอดเลือดและจำนวน mitochondria
- เนื้อขาว เกิดการสะสมของเสียที่กำจัดออกไม่ได้

5/15



6/15

- Enzymatic degradation of TMAO
- TMAO เป็นองค์ประกอบที่ harmless และ nontoxic
- แต่เป็น precursor ของ breakdown product ที่ไม่พึงประสงค์
- TMAO ถูกย่อยด้วยเอนไซม์ 2 pathways

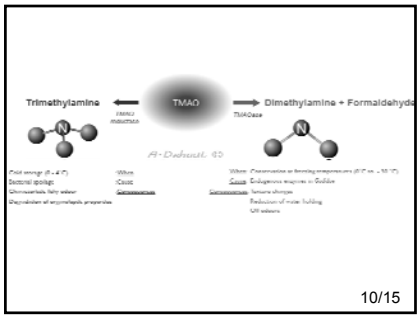
7/15

- TMAO reductase
- แบคทีเรียหลายชนิดรีดิวซ์ TMAO เป็น TMA
- TMA มีกลิ่นคาวจัด (strong fishy odor)
- TMA เป็นดัชนีวัดการ spoilage ของอาหารทะเล

8/15

- TMAO aldolase (TMAOase)
- TMAO เป็น precursor ในการสร้าง dimethylamine (DMA) และ formaldehyde
- DMA มีกลิ่นอ่อนกว่า TMA
- Formaldehyde ส่งผลกระทบต่อ texture ของเนื้อปลา ทำให้เนื้อแข็งกระด้าง มีเส้นใยมากขึ้น

9/15



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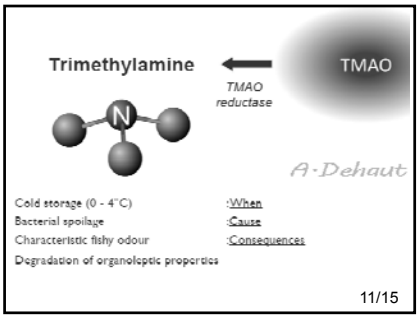
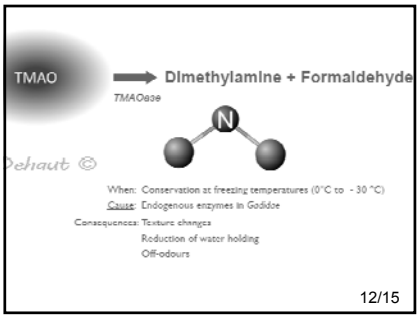


11/15



12/15

- Postmortem proteolysis in fresh fish
- เป็นปัจจัยสำคัญทำให้ texture เสีย กล้ามเนื้ออ่อนตัวลง
- ต่างจากเนื้อวัว หมู ที่การย่อย myofibrillar proteins ช่วยให้เนื้อนุ่มน่ากิน

13/15

- Postmortem hydrolysis of lipids during frozen and cold storage
- เกิด free fatty acid มากขึ้น ทำให้เกิดกลิ่นไม่ดี เมื่อถูก oxidized

14/15

- Endogenous enzymatic reaction during the processing of seafood
- เกิดกลิ่นรสและเนื้อสัมผัสที่ดี ระหว่างการทำปลาเค็ม
- การผลิตน้ำปลา กะปิ เกิดจาก digestive proteases ของปลาเอง กับกิจกรรมของ halotolerant lactic acid bacteria

15/15

ชื่อ _____ รหัสประจำตัวนิสิต _____

02738451 Applied Biochemistry ครั้งที่ 2 27-31 ก.ค. 63

เรื่อง Characterization of fermented seaweed sauce prepared from nori (*Pyropia yezoensis*)

1. ผู้แต่งหลักของบทความนี้ ทำงานอยู่ที่เมือง ..... ประเทศ .....

2. แปล keyword ของบทความนี้ 4 คำ เป็นภาษาไทย

1) ..... 2) .....3) .....4) .....

3. บทความนี้มีงานวิจัยที่สำคัญคือ เป็นครั้งแรกที่มีการผลิต “อาหารหมัก” จาก .....

4. จากข้อ 3. อ้างอิงจากบทความที่ [2] ใน References ซึ่งเกี่ยวกับอาหารหมักจาก .....

5. การหมักเอทานอลจากเมล็ดหญ้าทะเล เป็นงานวิจัยของประเทศ ..... ในปี .....

6. สาหร่ายทั่วไป มีข้อเสียเปรียบถั่วเหลืองในแง่ใด หากนำมาผลิตเป็นซอส

..... แต่ในบทความนี้ ผลิตซอสจาก .....

7. ใช้เอนไซม์ ..... เพื่อย่อยสาหร่ายทั่วไป แต่บทความนี้ใช้เอนไซม์ .....

8. เปรียบเทียบคุณภาพของซอสโนริ กับผลิตภัณฑ์คล้ายกัน 2 ชนิดคือ ..... และ .....

9. ในแง่ความปลอดภัยด้านอาหาร (food safety) ตรวจสอบเรื่อง ..... ในซอสโนริ

10. ตรวจสอบรสชาติด้วยวิธี .....

11. ประโยชน์ของซอสโนริต่อร่างกาย ตรวจสอบเรื่อง .....

12. ซอสโนริก่อให้เกิดภูมิแพ้หรือไม่ อย่างไร .....

13. ในการทดลอง ซื้อโนริมาจากจังหวัดใดของญี่ปุ่น .....

14. Nsa กับ Nsb มีกระบวนการหมักต่างกันอย่างไร .....

15. ใช้เวลาหมักนาน ..... ที่อุณหภูมิประมาณ .....

16. วิธีที่ใช้ในการวิเคราะห์องค์ประกอบ

ไนโตรเจนทั้งหมด ใช้วิธี .....

เกลือ ..... ตามมาตรฐานญี่ปุ่น ตีพิมพ์ ปี ค.ศ. .....

ความเข้มข้นกรดแลกติก ..... ของประเทศ .....

หาปริมาณของแข็งละลายน้ำได้ (ที่ไม่ใช่เกลือ) ด้วยวิธี .....

เพปไทด์ .....

น้ำตาล .....

วิตามิน B1 .....

วิตามิน B12 .....

กรดโฟลิก .....

ฮีสตามีน ..... ของประเทศ .....

แร่ธาตุ Na K Cu Pb .....

แร่ธาตุ P Fe Ca Mg Zn Mn .....

แร่ธาตุ Selenium .....

ไอโอดีน .....

กรดอะมิโนอิสระ .....

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Characterization of fermented seaweed sauce prepared from nori (*Pyropia yezoensis*)

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High-salt content seaweed sauces were prepared for the first time using nori (*Pyropia yezoensis*) by fermentation and characterized. Components and taste of the two nori sauces (NSs) prepared separately were compared with those of soy and fish sauces. The NSs were rich in total nitrogen compounds (1.5 g N/100 ml on average) and potassium (880 mg/100 g), and had a unique free amino acid composition (e.g., taurine 617 mg/100 g), explaining their unique taste as evaluated by a taste sensing system. As for their food function, inhibitory activity of angiotensin-converting enzyme was observed. As for their food safety, arsenic was detected at a 0.8 mg/100 g level in total, but inorganic arsenic was not detected (<0.05 mg/100 g) and not regarded as a problem. Allergy-causing substances contained in wheat, soy beans, and crustaceans were not detected (<0.1 mg/100 g) with NSs. These results suggest that the nori sauce has a high potential as a novel nutritional source for humans.

[**Key words:** Allergen free; Fermented seaweed sauce; Halophilic lactic acid bacteria; Nori; *Pyropia yezoensis*]

Despite the long history of fermentation technology, fermented food items produced from seaweeds have not yet been developed (1,2). Lactic acid fermentation of seaweeds was observed for the first time in 1998 and published in 2004 on a cellulase-treated culture of *Ulva* spp. (Chlorophyta) (3). Lactic acid fermentation has further been demonstrated in Phaeophyta (4–6) and Rhodophyta (7). On the other hand, ethanol fermentation has also been reported in various kinds of seaweeds (3,8,9) and seagrass (10). Among these studies on ethanol fermentation, seagrass seeds have been shown to contain suitable components as a substrate for ethanol production, and demonstrated a produce of 16.5% v/v concentration ethanol (10). These findings in lactic acid and ethanol fermentation open the possibility of developing novel fermented foods and beverage items from the previously underutilized seaweed and seagrass resources (11). The present study focuses on the development of a novel sauce product from seaweeds using lactic acid fermentation. Soy sauce is commonly manufactured from soy beans which contain protein at a 44% level on a dry weight basis (12) and this makes it possible to produce sauces rich in free amino acid components. On the other hand, seaweeds usually contain lower quantities of protein at an average 15% level on a dry weight basis (13) and are expected to be difficult to produce a sauce rich enough in free amino acid components. However, nori, a dried sheet product of *Pyropia yezoensis*, is known to contain an exceptionally high (i.e., 12.5–51.5% w/w) protein content among seaweeds (13) and can be expected to produce a high quantity of free amino acids after degradation. Pretreatment with cellulase was observed to be promotive for performing lactic acid fermentation for many kind of seaweeds (3,4). However, pretreatment with protease was used to promote fermentation as for the case of protein-rich seaweed nori (7). Protocol for performing lactic acid fermentation on seaweeds was developed in low-salt conditions at first using Lactobacilli starter (3,4,6). Then, halophilic lactic acid bacteria were isolated from nori culture (14) and it was expected that lactic acid fermentation on seaweeds can be performed in a high-salt content condition as well. The present study attempted to prepare high-salt seaweed sauce products for the first time from nori. Components of the nori sauces (NSs) prepared in two batches were analyzed and compared with common sauce products such as soy sauce and fish sauce to evaluate their potential as a sauce product. Heavy metal contents of the NSs were measured for food safety. The taste of the NSs was characterized by a sensor technology system, Taste Sensing System SA402B. Physiological function of the NSs was tested as for inhibitory activity of angiotensin-converting enzyme (ACE). Allergy factors contained in the NSs were also checked.

MATERIALS AND METHODS

Sauce products Nori (dried sheets of *P. yezoensis*, which is a common Japanese food item utilized in rolled sushi and other traditional Japanese foods; protein content was 47.8% on a dry weight basis) was purchased from Okayama prefectural

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branch of the Japan Fisheries Cooperative. Two nori sauces (NSa and NSb) were prepared in different batches as follows (Fig. 1): For preparing NSa, a 1.8 kg of the nori was housed into a plastic bag with 360 g of sodium chloride, 10.13 L of distilled water, 21 g of protease (P Amano 3SD, Amano Enzyme Co., Ltd., Nagoya, Japan), 21 g of peptidase (R, Amano Enzyme Co., Ltd.), 21 g of mannanase (BGM, Amano Enzyme Co., Ltd.), and mixed well. The NSb was prepared in the same way as the NSa except that the three kinds of enzyme were added at a level of 10.5 g each, respectively. The cultures were digested for 24 h at 23°C, a further 0.9 kg of nori added, and 1 kg of sodium chloride, and 40 ml of starter culture. The starter culture was prepared from a fresh preculture of *Tetragenococcus halophilus* NBRC 109726 for NSa and *T. halophilus* NBRC 109727 for NSb grown on GAM plates (Nissui Pharmaceuticals Co., Tokyo, Japan) to make a cell suspension at a concentration of optical density at 660 nm of 1.0 (2.0×10^8 CFU/ml for NBRC 109726 and 6.8×10^6 CFU/ml for NBRC 109727) with a sterile 2.5% w/v sodium chloride solution. The mouth of the cultures was banded to contain a little air in the headspace and further incubated for two years in an ambient temperature (conditioned at 23°C for the main part of the culture period) and collected. The cultures were centrifuged for 20 min at 10,000 ×g and the supernatant was filtrated with a glass filter (GF/D, Sigma–Aldrich Co., St. Louis, MO, USA) to obtain the NSa and NSb. The culture mushes before being centrifuged and filtration were designated as moromi. The NSa-moromi and NSb-moromi were used for functional analysis. Commercial products of a soy sauce (SS) prepared from marudaizu-soybean (soybean without extraction for lipid content), koikuchi (common type), tokkyu-grade (special grade) of a common Japanese brand and a fish sauce (FS) prepared from anchovy (a Chinese brand) were purchased in a super market and used for comparative studies.

Component analysis Chemical analysis of the sauce components was conducted according to the method of Japan Agricultural Standards (15) and the Standard Tables of Food Composition in Japan (16). Total nitrogen was measured by the Kjeldahl method. Salt content was measured by the Mohr's method. Lactic acid concentration was measured with a commercial kit (F-kit D-/L-lactic acid, Roche Diagnostics, Basel, Switzerland). The unsalted soluble solid content was calculated by subtracting the salt content value from the Brix value. Peptide content in the supernatant was measured by the biuret method (17). Sugar was measured by the phenol sulfuric acid method using porphyran as a standard substance (18). Low molecular weight sugar content was obtained from the sample filtrated through an ultrafiltration system attached with the MWCO = 3000 Da filter (Amicon Ultra-0.5, Merck Millipore, Billerica, MA, USA). High molecular weight sugar content was calculated by subtracting the low molecular weight sugar content from total sugar content. Vitamin B_1 was measured by high performance liquid chromatography (16). Vitamin B_{12} and folic acid were measured by microbial assay using *Lactobacillus delbrueckii* subsp. *lactic* ATCC 7830 and *Lactobacillus rhamnosus* ATCC 7649, respectively (16). Histamine was measured by a commercial kit (Kikkoman Biochemifa, Company, Tokyo, Japan). As for minerals and metals components, sodium, potassium, copper, lead, arsenic, cadmium, and mercury were measured by atomic absorption spectrophotometer (AA 240FS, Agilent Technologies, Inc., CA, USA and ZA-3000, Hitachi High-Technologies Corporation, Tokyo, Japan) (19,20). Phosphorus, iron, calcium, magnesium, zinc, and manganese were measured by inductively-coupled plasma-optical emission spectrometer (ICP/OES 725-ES, Agilent Technologies, Inc. and HG4500, Hirayama Sangyo Co., Ltd., Ibaragi, Japan) (19). Selenium was measured by fluorescence spectrometry (FP-8200, JASCO, Tokyo, Japan). Iodine was measured by gas chromatography (6890N, Agilent Technologies, Inc.). Inorganic arsenic was extracted after a 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, Inc.) (21). Chromium was measured by the diphenylcarbazide colorimetric method (V-630, JASCO) (19). Cyanide was measured by the pyridine-pyrazolone method (V-630, JASCO) (22). Free amino acid composition was measured by the Amino Acid Analyzer (Model L-8900, Hitachi High-Technologies America Inc., Schaumburg, IL, USA).

Evaluation by a taste sensing system The sauce samples were 10-fold diluted with distilled water and evaluated for taste by the Taste Sensing System SA402B (Intelligent Sensor Technologies, Inc., Kanagawa, Japan) (23–25). Five kinds of lipid membrane sensors were used to measure the eight kinds of taste stimuli: the sensors C00 (phosphoric acid di-n-decyl ester) for bitterness and aftertaste from bitterness, AE1 (tetradodecylammonium bromide) for astringency and aftertaste from astringency, AAE (phosphoric acid di(2-ethylhexyl) ester and trioctylmethylammonium chloride) for umami and umami richness, CT0 (tetradodecylammonium bromide and n-tetradecyl alcohol) for saltiness, and CA0 (phosphoric acid di(2-ethylhexyl) ester and trioctylmethylammonium chloride) for sourness. The estimated intensity of taste was calculated from the sensor output on the basis of Weber's and Weber–Fechner law (26) by using manufacture's application. The normality of the score was checked by comparing the values obtained from 5 fold-, 10 fold-, and 20 fold-diluted samples prepared from each sauce. The result was expressed in a two-dimensioned graph of aftertaste from bitterness and umami richness scores with SS to be plotted as a control position of taste.

ACE inhibition test Angiotensin-converting enzyme inhibition test was conducted as for NSs and soy sauce. Rabbit lung ACE and N-hippuryl-histidyl-leucine hydrate (Hip-His-Leu) were purchased from Sigma–Aldrich Co. Purified ACE was obtained by extracting the rabbit lung ACE with 200 mM sodium borate buffer (pH 8.3) (27). A mixture consisting of 250 µl substrate solution (7.6 mM Hip-His-Leu in 200 mM sodium borate buffer, pH 8.3) and 30 µl test samples (two- and eight-times dilutions) was preincubated for 5 min at 37°C. A 100 µl of the purified ACE was added to the mixture and incubated for 20 min at 37°C. The reaction was stopped by addition of 100 µl of 1N-HCl and 1.5 ml of ethyl acetate. The hippuric acid was separated and quantified by HPLC with UV detection (228 nm). Commercial hippuric acid (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was used as standard. Water solutions of valyl-tyrosine prepared at 25 mM and 125 mM concentrations were used as positive controls.

Allergen test Allergen tests for wheat and soy were conducted by immunochromatography using a commercial kit, the FASTKIT slim (NH Foods Ltd., Tokyo, Japan). Allergen test for crustacean targeting for shrimp and crab substances was conducted by both the immunochromatography and the enzyme immunoassay methods using the FA test, Immunochromato-Kokakurui II (Nissui Pharmaceuticals Co., Tokyo, Japan) and EIA-Kokakurui II (Nissui Pharmaceuticals Co.), respectively.

RESULTS

Comparison of basic characteristics The NSs-moromi treated after centrifugation was observed to contain 17% w/w of floating fraction (lipid-like fraction), 60% of supernatant fraction (sauce fraction, Fig. 2), and 23% of pellet fraction (undecomposed fraction) on average. Basic characteristics of the obtained sauce fractions (NSa and NSb) are shown in Table 1 with that of soy

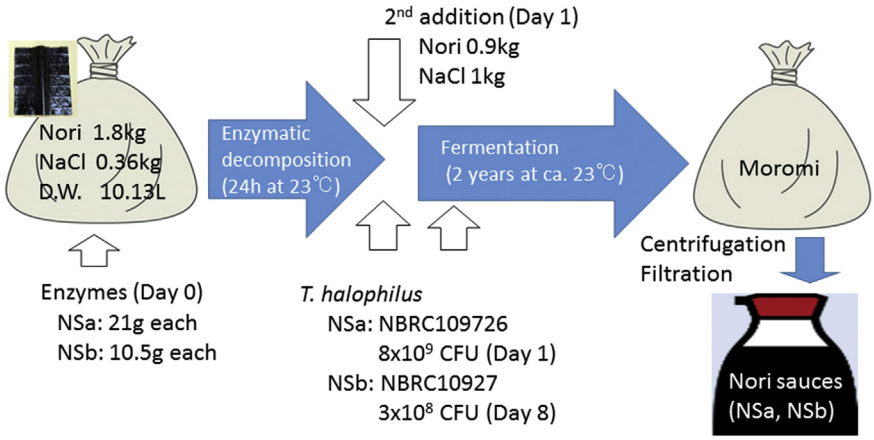

FIG. 1. Scheme for preparing nori sauces, NSa and NSb. Twenty one grams each (for NSa) or 10.5 g each (for NSb) of protease, peptidase, and mannanase, respectively, were added for decomposition of nori on day 0. *Tetragenococcus halophilus* was added on day 1 for the case of NSa, while on day 8 for the case of NSb because growth of the pre-culture was not enough on day 1. The fermented mush before centrifugation and filtration was designated as moromi.

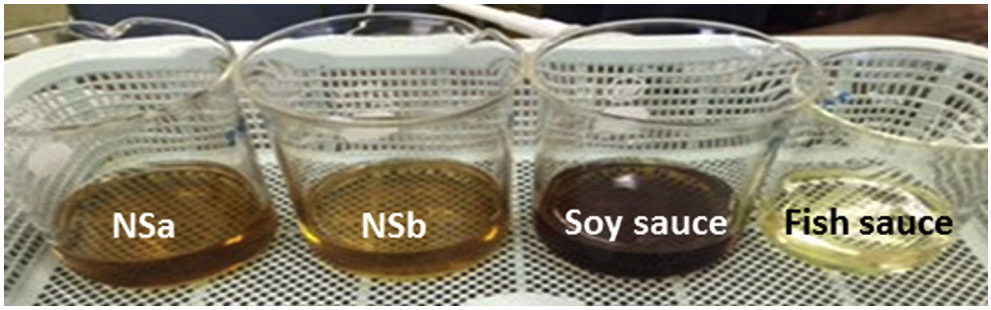

FIG. 2. Appearance of the nori sauces (NSa, NSb) with reference products of soy and fish sauces.

sauce (SS) and fish sauce (FS). Total nitrogen (g N/100 ml) of the NSs, SS, and FS was 1.51, 1.65, and 1.42, respectively. Value of pH was 5.5 (NSs), 4.8 (SS), and 5.3 (FS). Salt concentration (%) was 11.0 (NSs), 14.1 (SS), and 16.9 (FS). Brix value (%) was 37.3 (NSs), 46.5 (SS), and 37.5 (FS). Unsalted soluble solid contents (g/100 g) was 26.3 (NSs), 32.4 (SS), and 20.6 (FS). Lactic acid content (mg/100 ml) was 62 (NSs), 58 (SS), and 75 (FS). Peptide content (mg/ml) was 13.8 (NSs) and 12.7 (SS). Total sugar (mg/ml), low molecular weight sugar (M.W. < 3000 Da) (mg/ml), and high molecular weight sugar (M.W. > 3000 Da) (mg/ml) were 58.0, 6.8, and 51.2 (NSs) and 49.2, 30.4, and 18.8 (SS), respectively. Vitamin B1, B12, and folic acid were 0.22 mg/100 g, 14 μg/100 g, and 79 μg/100 g (NSs), 0.04 mg/100 g, <0.03 μg/100 g, and 6 μg/100 g (SS), and <0.01 mg/100 g, 0.59 μg/100 g, and 3 μg/100 g (FS), respectively. Histamine (mg/100 ml) was detected at 9.0 (NSa) and 0.9 (NSb) levels as for NSs, while not detected (<0.1 mg/100 ml) for SS and FS.

TABLE 1. Comparison of basic characteristics among two nori-sauce samples, soy sauce, and fish sauce.

Characteristics	Nori-sauce samples			Soy sauce (koikuchi)	Fish sauce (anchovy)
	NSa	NSb	Average		
Total nitrogen (g N/100 ml)[a]	1.53	1.49	1.51	1.65	1.42
pH	5.5	5.4	5.5	4.8	5.3
Salt (% w/v)[b]	10.8	11.2	11.0	14.1	16.9
Brix (%)	35.3	39.3	37.3	46.5	37.5
Unsalted soluble solid contents (g/100 g)[c]	24.5	28.1	26.3	32.4	20.6
Lactic acid (mg/100 ml)[d]	60	63	62	58	75
Peptide (mg/ml)[e]	14.9	12.7	13.8	12.7	NT[f]
Sugar (mg/ml)[g]					
Total	56.3	59.7	58.0	49.2	NT[f]
Low molecular size (MW<3000 Da)	8.5	5.1	6.8	30.4	NT[f]
High molecular size (MW>3000 Da)	47.8	54.6	51.2	18.8	NT[f]
Vitamin B1 (mg/100 g)	0.25	0.18	0.22	0.04	<0.01
Vitamin B12 (μg/100 g)	16	12	14	<0.03	0.59
Folic acid (μg/100 g)	78	79	79	6	3
Histamine (mg/100 ml)[h]	9.0	0.9	5.0	<0.1	<0.1

[a] Total nitrogen was measured by Kjeldahl method.
[b] Salt was measured by the Mohr's method.
[c] Unsalted soluble solid contents was obtained by subtracting salt% from soluble solid contents%.
[d] Lactic acid was measured by a commercial kit (F-kit D-/L-lactic acid, Roche Diagnostics).
[e] Peptide was measured by the biuret method.
[f] Not tested.
[g] Sugar was measured by the phenol sulfuric acid method using porphyran as a standard substance. Low molecular size sugar was obtained from the sample filtrated through an ultrafiltration system attached with the MWCO = 3000 Da filter. High molecular weight size sugar was obtained by subtracting low molecular size sugar content from total sugar.
[h] Histamine was measured by a commercial kit (Kikkoman Biochemifa Company).

Comparison of mineral and metal components Mineral and metal components of the sauces are shown in Table 2. Sodium, potassium, phosphorus, calcium, and magnesium (mg/100 g) were 3990, 880, 169, 45.0, and 65.9 (NSs), 5670, 458, 156, 37.6, and 68.1 (SS), and 6760, 156, 11.2, 18.8, and 48.5 (FS), respectively. Iron, zinc, and manganese (mg/100 g) were 0.84, 0.61, and 0.78 (NSs), 2.09, 0.97, and 1.05 (SS), and 1.27, 0.24, and 0.02 (FS), respectively. Copper, selenium, and iodine (mg/100 g) were not detected from any sauces at a level 0.01, 0.005, and 0.5, respectively. Heavy metals were measured only as for NSs. Arsenic was measured at a 0.80 mg/100 g level as for NSs, but inorganic arsenic was not detected (<0.05 mg/100 g). Cadmium, chromium, and cyanide (mg/100 g) were detected from NSs at a trace level of 0.01, 0.025, and 0.035, respectively. Lead and mercury (mg/100 g) were not detected at levels of 0.005 and 0.001 levels, respectively.

Comparison of free amino acid composition Free amino acid composition is shown in Table 3. Total quantity of free amino acid (mg/100 g) was 5592 (NSs), 5528 (SS), and 4661 (FS). Glutamic acid and aspartic acid contents contributing to umami taste were 685 and 400 (NSs), 850 and 140 (SS), and 630 and 450 (FS), respectively. Threonine, alanine, glycine, proline, and serine

TABLE 2. Comparison of mineral and metal components of two nori-sauce samples, soy sauce, and fish sauce.

Components (mg/100 g)	Nori-sauce samples			Soy sauce (koikuchi)	Fish sauce (anchovy)
	NSa	NSb	Average		
Na[a]	3920	4060	3990	5670	6760
K[a]	867	892	880	458	156
P[b]	168	170	169	156	11.2
Ca[b]	53.4	36.5	45.0	37.6	18.8
Mg[b]	65.0	66.8	65.9	68.1	48.5
Fe[b]	0.89	0.79	0.84	2.09	1.27
Zn[b]	0.92	0.30	0.61	0.97	0.24
Mn[b]	0.81	0.74	0.78	1.05	0.02
Cu[a]	<0.01	<0.01	<0.01	<0.01	<0.01
Se[c]	<0.005	<0.005	<0.005	<0.005	<0.005
I[d]	<0.5	<0.5	<0.5	<0.5	<0.5
As (total, as As_2O_3)[a]	0.78	0.81	0.80	NT[e]	NT[e]
As (inorganic)[f]	<0.05	<0.05	<0.05	NT[e]	NT[e]
Pb[a]	<0.005	<0.005	<0.005	NT[e]	NT[e]
Cd[a]	0.01	0.009	0.010	NT[e]	NT[e]
Hg[a]	<0.001	<0.001	<0.001	NT[e]	NT[e]
Cr[g]	0.05	<0.05	0.025	NT[e]	NT[e]
Cn[h]	0.04	0.03	0.035	NT[e]	NT[e]

[a] Measured by atomic absorption spectrophotometer.
[b] Measured by inductively-coupled plasma-optical emission spectrometer (ICP/OES).
[c] Measured by fluorescence spectrometry.
[d] Measured by gas chromatography.
[e] Not tested.
[f] Measured by high performance liquid chromatography-ICP-mass spectrometry.
[g] Measured by the diphenylcarbazide colorimetric method.
[h] Measured by the pyridine-pyrazolone method.

TABLE 3. Free amino acid composition of two nori-sauce samples, soy sauce, and fish sauce.

Amino acids (mg/100 g)	Nori-sauce samples			Soy sauce (koikuchi)	Fish sauce (anchovy)
	NSa	NSb	Average		
Glutamic acid	680	690	685	850	630
Aspartic acid	360	440	400	140	450
Threonine	340	350	345	290	320
Alanine	860	890	875	690	470
Glycine	240	260	250	210	220
Proline	240	260	250	340	140
Serine	340	160	250	390	240
Isoleucine	340	330	335	390	240
Leucine	590	570	580	600	330
Methionine	170	120	145	110	160
Phenylalanine	230	190	210	360	210
Tryptophan	60	40	50	10	450
Valine	500	520	510	410	380
Arginine	50	20	35	490	50
Taurine	323	295	309	14	66
Cystine	20	<10	10	<10	<20
Tyrosine	30	30	30	60	50
Histidine	<10	<10	<10	150	190
γ-Amino boric acid	4	5	4.5	11	<1
Total	5377	5170	5274	5515	4596

Amino acids were measured by the amino acid auto analyzer after being eliminated protein molecules.

contributing to sweet taste were 345, 875, 250, 250, and 250 (NSs), 290, 690, 210, 340, and 390 (SS), and 320, 470, 220, 140, and 240 (FS), respectively. Isoleucine, leucine, methionine, phenylalanine, tryptophan, valine, and arginine contributing to bitter taste were 335, 580, 145, 210, 50, 510, and 35 (NSs), 390, 600, 110, 360, 10, 410, and 490 (SS), and 240, 330, 160, 210, 450, 380, and 50 (FS), respectively. Taurine was measured at 645 (NSa), 589 (NSb), 27 (SS), and 131 (FS). Histidine was not detected (<10 mg/100 g) with NSs. Gamma amino butyric acid was measured at a 4.5 mg/100 g level on average with NSs.

Characterization of taste Among the eight elements of taste, scores of aftertaste from astringency, umami, and sourness showed abnormal values and these three taste elements were not evaluated properly in the present study (data not shown). Scores in bitterness and astringency showed normal values, but the difference of the scores was less than 1.0 among the sauces (data not shown). One unit of the estimated intensity of taste corresponds to 20% difference in concentration of the taste substances in test samples based on the manufacture's manual and the difference of the scores less than 1.0 was regarded not significant. Three elements of taste, aftertaste from bitterness, umami richness, and saltiness showed normal value and significant difference (>1.0 of score) among the sauces. The scores of estimated intensity of aftertaste from bitterness and umami richness was shown on Fig. 3. The NSa and NSb showed higher scores in these two taste elements compared with the soy and fish sauces. The scores of saltiness were 3.9 (NSa), 2.7 (NSb), 0 (SS), and 0.4 (FS) and showed discrepancy with the actual salt concentration (%) 10.8 (NSa), 11.2 (NSb), 14.1 (SS), and 16.9 (FS) (Table 1).

ACE inhibition test Results of ACE inhibition test are shown in Fig. 4. Inhibitory activity against ACE was observed both in two fold- and eight fold-diluted samples of NSa (53% and 26% inhibition to the blank control, respectively) and NSb (58% and 29% inhibition to the blank control, respectively). The ACE inhibition activity of the NSs was the same or a little weaker strength level compared with that of the soy sauce product (71% and 40% inhibition to the blank control, respectively). The inhibitory activity was also observed for NSa-moromi and NSb-moromi samples at the same strength level, suggesting that functional element is contained in soluble fraction of NSa- and NSb-moromi.

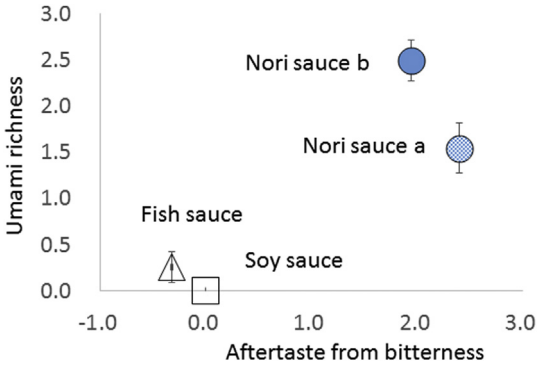

FIG. 3. Results of evaluation of nori-sauce taste by a taste sensing system equipped with lipid membrane sensors. The sample was diluted at 10-fold and measured. The nori-sauces showed significant difference in aftertaste from bitterness (horizontal axis) and umami richness (vertical axis) compared with soy and fish sauces. The data were plotted as the values representing soy sauce to be set on the zero point of the graph. Data is shown as average (bar: SD) of triplicate measurements.

Allergen tests Results of the allergen tests are shown in Table 4. The NSs do not contain wheat flour and soy bean, and the allergen tests for these substances were negative. The nori might contain trace amounts of crustaceans, but the allergen tests for shrimp and crab substances were also negative (<0.1 mg/100 g).

DISCUSSION

High-salt content seaweed sauce was prepared from nori for the first time in the present study and characterized. Progress of fermentation in the nori-sauce tanks was observed from the predominance of halophilic lactic acid bacteria. Numbers of halophilic lactic acid bacteria was 5.0×10^6 cfu/ml after 28 months of culture for the case of NSb-moromi (data not shown). Microbial community analysis in the nori-sauce cultures is currently being analyzed. The NSs obtained after centrifugation showed a slight red-brownish color (Fig. 2) and had an acceptable weak seaweed

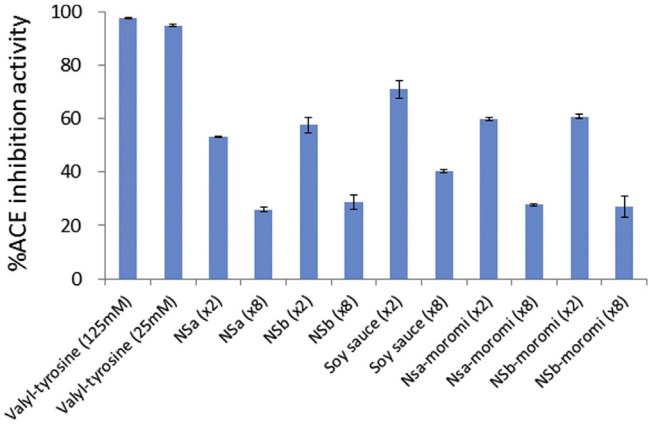

FIG. 4. Results of angiotensin-converting enzyme (ACE) inhibition test for nori-sauce a (NSa), nori-sauce b (NSb), soy sauce, fish sauce, NSa-moromi (NSa without being centrifuged), and NSb-moromi (NSb without being centrifuged). The ACE test was conducted by immunochromatography and enzyme immunoassay methods. The test samples were diluted at two and eight times with distilled water before measurement. Water solutions of valyl-tyrosine prepared at 25 mM and 125 mM concentrations were used as positive inhibition controls. Data is expressed as ACE and blank controls to be 0% and 100% inhibition, respectively. Data is shown as average (bar: SD) of duplicate measurements.

TABLE 4. Results of allergen test with nori-sauce b sample.

Allergen	Results	Methods
Wheat	Negative (<0.1 mg/100 g)	Immunochromatography[a]
Soy	Negative (<0.1 mg/100 g)	Immunochromatography[a]
Crustaceans (shrimp and crab)	Negative (<0.1 mg/100 g)	Immunochromatography[b]
Crustaceans (shrimp and crab)	Negative (<0.1 mg/100 g)	Enzyme immunoassay[c]

[a] Measured by FASTKIT slim, NH Foods Ltd.
[b] Measured by FA test, Immunochromato-Kokakurui II [Nissui], Nissui Pharmaceuticals Co., Ltd.
[c] Measured by FA test, EIA-Kokakurui II [Nissui], Nissui Pharmaceuticals Co., Ltd.

odor. The NSs were shown to contain 1.5 g/100 ml of total nitrogen (Table 1), and this value is comparable to the tokkyu-grade of koikuchi-category in the Japanese Agricultural Standards for soy sauce (28). Component analysis clarified the unique characteristics of the NSs when compared with the SS. The NSs were 1.9 times richer in potassium while 0.7 times less in quantity for sodium (Table 2). As for free amino acids related with umami, glutamic acid content was approximately 20% less for NSs, but aspartic acid was 2.9 times higher. Free amino acids related with sweet taste such as threonine, alanine, and glycine were higher in NSs, while those related with bitter taste such as isoleucine, phenylalanine, and arginine were less in NSs. These profiles in free amino acid composition explained part of the unique taste of NSs. The taste of NSs was slightly saltier probably because of the weakness in umami, while a little sweeter, and having strong aftertaste when compared with soy sauce although sensory evaluation of taste was not conducted quantitatively in the present study. The saltier impression of NSs despite the actually lower salt content compared with SS and FS may be explained by shortage in glutamic acid content, while sweeter impression of NSs can be explained by richness in threonine, alanine, and glycine. The strong aftertaste of the NSs was certificated by a sensor system (Fig. 3). This strong aftertaste may be related with richness of sulfur-containing compounds such as taurine, methionine, cysteine, sulfated polysaccharides (e.g., porphyran) in the NSs compared to soy sauce although this hypothetic idea needs further evidence before being accepted. The present study also provided evidence of a potential functional and safety issues related to NSs. The NSs showed inhibitory activity against ACE (Fig. 2). Peptides of nori are well known to have an inhibitory activity against ACE (29). Therefore, peptides contained in NSs (Table 1) are probably the causative substance of this function. Richness of vitamin B1 (0.22 mg/100 g, Table 1), vitamin B12 (14 μg/100 g, Table 1), folic acid (79 μg/100 g, Table 1), and taurine (617 mg/100 g, Table 3) is also preferable characteristic of the NSs from the viewpoint of function. Richness of the high molecular weight algal sugars of the NSs (Table 1) is another interesting consideration from the viewpoint of function. As for food risk elements, Joint FAO/WHO expert committee on food additives (JECFA) showed a list of regulatory concentrations for food additives (30). According to this list, cadmium and arsenic are planned to be regulated at a concentration under 0.05 mg/100 g (as for one food item) and 0.1–0.3 mg/100 g (as for six food items), respectively. The present study showed that contamination level of cadmium is at a 0.05 mg/100 g (NSa) or less (Table 2) and will not be a significant risk. As for arsenic, although total arsenic compounds were detected at an 0.8 mg/100 g level in NSs, the inorganic form of arsenic was under the detection level (less than 0.05 mg/100 g). The Codex committee is still collecting information before determining the regulatory contamination level of arsenic compounds considering the difference of toxicity strength depending on its chemical form (31). As for chromium, although the regulatory contamination level is not shown by Codex committee (31), actual contamination level in NSs is at a trace level (0.01 mg/100 g or less) and will not be a significant risk. Another risk of fermented sauce is accumulation of histamine. Small quantity (i.e., 9.0 and 0.9 mg/100 ml in NSa and NSb, respectively) of histamine was observed in NSs (Table 1). Histamine is known to be produced from decarboxylation of histidine (32). Histidine was observed not to be remaining in the NSs (<10 mg/100 g, Table 3), therefore, it was expected that a further increase of histamine will not occur with the NSs. The histamine level of NSs was less than the regulation criteria of 40 mg/100 g which was determined by Codex committee for the case of fish sauces (33). Another note-worthy characteristic of NSs is on allergens. Allergy reactions against wheat and soy bean are widespread in children and can cause serious medical problems (34). The NSs do not use wheat flour or soy beans as a raw material and were shown to be negative to these allergen tests (Table 4). Negative results were also obtained as for allergen test against shrimp and crab by immunochromatography and enzyme-linked immunosorbent assays. These kit tests are not enough to assure the safety from allergens, but patients showing allergy reaction against nori have not been published as far as we know (34). As a conclusion, the present study clarified the unique components, food function, and food safety evidences of the nori-sauce, and the fermented sauce prepared from seaweeds has a high potential as a novel nutritional source for humans.

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