Research article

Open Access

Production and component analysis of "*Shirasu*" (whitebait) fish sauce from Kamakura, Japan

Abstract

Introduction: Whitebait ("*shirasu*" in Japanese) is the collective name for immature fry of sardines, herring, mackerel, and various other fish of ~30 mm in length. Kamakura coast is blessed with a temperate climate, where much whitebait is caught. However, no studies of whitebait-derived fish sauce from Kamakura have been reported. Fish sauce is liquid seasoning made by adding salt to fish and allowing it to ferment via bacterial activity. It has a rich umami taste because of abundant peptides and amino acids, the products of protein digestion by enzymes from internal organs.

Method: We used *shirasu* collected in March 2018 along Kamakura coast. To prepare fish soy sauce, 100 g *shirasu* and 300 g salt were aged for 2 months at 25°C and then centrifuged at 4,000 rpm for 10 min. Fish sauce composition was then analyzed, including total nitrogen, formol nitrogen, amino acids, carbohydrate, acidity, and pH. Additionally, the molecular weight distribution of proteins was examined by electrophoresis. β-Amylase and protease activity were also measured periodically during aging.

Results: The composition of shiitake fish soy sauce was as follows: total nitrogen 4.37%, formol nitrogen 0.28%, acidity 2.6%, pH 4.8, total sugars 4.87%, reducing sugars 3.60%, and glutamic acid 290 mg/100 g.

From these results, fish soy sauce aged for 2 months consists of decomposed protein and carbohydrate created by the digestive enzymes of *shirasu*, muscle enzymes, etc. The final composition included amino acids, peptides, and low-molecular-weight sugars. The umami component glutamic acid is produced, and organic acid levels are raised. This sauce is especially rich in minerals, especially iron, copper, magnesium, and zinc, and has high nutritional value.

Significance: *Shirasu* fish sauce was confirmed to have high total nitrogen and strong umami compared with soy sauce. Using this seasoning for cooking confirmed its high food functionalities such as minerals.

Keywords: *shirasu* (whitebait), fish sauce production, general food ingredient analysis, food functionality, kamakura food

Volume 13 Issue 3 - 2024

Akiko Yamada Taniguchi, Machiko Kazami, Haruko Noguchi
Department of Agricultural Innovation for Sustainability, Faculty of Agriculture, Tokyo University of Agriculture, Japan

Correspondence: Akiko Yamada Taniguchi, Department of Agricultural Innovation for Sustainability, Faculty of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 243-0034, Email akiko@nodai.ac.jp

Received: November 28, 2024 | **Published:** December 18, 2024

Introduction

Whitebait (called "*shirasu*" in Japanese) is a general term for the young fish of the marine fish anchovy, Japanese pilchard, and other sardines with white bodies.[1] Kamakura is located on the coast of Sagami Bay, which is blessed with a warm climate, and because it is connected to the deep sea, an abundance of seafood is landed there.[2] Whitebait is especially abundant in spring and fall, and is a local specialty commonly eaten raw or boiled. Many types of seafood high in minerals are caught along the Kamakura coast, and the whitebait is also is expected to have high nutritional value. However, little literature about whitebait caught along the Kamakura coast has been published, and it has not been the focus of much study. Fish sauce is a liquid seasoning made by pickling fish with salt and fermenting it via autolysis and bacteria. It is rich in peptides and amino acids created by the breakdown of proteins in the fish's internal organs enzymatically, and also rich in nucleic acids contained in fish meat. This gives it a rich umami flavour, which it confers on dishes to which it is added, along with saltiness. It also contains substantial minerals and vitamins. In Southeast Asia, fish sauces such as "*nhoc mam*" in Vietnam, "*nang pla*" in Thailand, and "*patis*" in the Philippines are commonly used as seasonings3). In Japan, there are "*shottsuru*" in Akita, "*ishiru*" on the Noto Peninsula, "*ikanago* soy sauce" in Setouchi, and "*kusa-ya* soup" on the Izu Islands.[3] The purpose of this study was to investigate the general components of whitebait caught off the coast of Kamakura, and further, to effectively use such whitebait to produce fish sauce, investigate its general components, and examine its characteristics.

Materials and methods

1) Sample

The sample used was *shirasu* caught off the coast of Kamakura, Japan, in 2018.

2) Method of manufacturing fish sauce

One kilogram of *shirasu* was supplemented with 300 g of salt, and fermented and aged at 25°C for 2 months. Then, it was centrifuged at 4000 rpm for 10 min and the supernatant was collected as fish sauce.

3) Method of measuring the general components of *shirasu* and fish sauce

The general components of *shirasu* and fish sauce produced in Kamakura were measured according to the Food Composition Tables of Japan. Moisture was measured using the normal pressure heat drying method,[4] ash was measured using the direct ashing method,[5] protein was measured using the Kjeldahl method[6] and formol nitrogen,[7] lipids were measured using the Soxhlet extraction method,[8] carbohydrates were measured using the subtraction method,[9] and sugars were measured using the phenol–sulfuric acid method[10] and Somogyi–Nelson method.[11] Acidity was also measured using titratable acidity[12] and hydrogen ion concentration index.[13]

4) Measurement of minerals

Mineral content was measured by atomic absorption spectrometry.[14] *Shirasu* samples were prepared by dissolving the ash obtained by the direct ashing method in 0.1N hydrochloric acid solution to prepare samples for atomic absorption spectrometry. Sodium (589.0 nm), calcium (422.7 nm), magnesium (285.2 nm), iron (248.3 nm), zinc (213.9 nm), and copper (324.8 nm) were measured using an atomic absorption spectrometer (AA-6300; Shimadzu Corporation).

5) Measurement of glutamic acid

Measurement of L-glutamic acid in fish sauce was performed using F-kit L-glutamic acid (product number 139 092; J.K. International Co., Ltd.).

6) Measurement of β-amylase activity

β-Amylase activity was measured using the Somogyi–Nelson method11). That is, 0.5 mL of 1% soluble starch at 40°C was added to 0.5 mL of sample solution, and the solution was heated at 40°C for 10 min, after which 1.0 mL of copper reagent was added, heated in a boiling bath for 20 min, and then rapidly cooled. After adding 1.0 mL of Nelson's reagent, the solution was left to stand for 15 min, after which 5.0 mL of distilled water was added and the absorbance was measured at 660 nm. The enzyme activity was defined in terms of units, with 1 unit representing the titer that liberates 1 μmol of maltose per minute at 40°C.

7) Measurement of protease activity

Protease activity was measured using the azocasein method.[15] A total of 0.5 mL of the sample solution was added to 0.5 mL of 1% azocasein solution, and after 10 min of reaction at 40°C, 0.1 mL of 10 M sodium hydroxide was added and the absorbance was measured at 400 nm. The titer required to increase the absorbance by 0.1 in 1 min at 40°C was defined as 1 unit.

8) Molecular weight distribution of proteins

The gel was placed in an electrophoresis apparatus containing electrophoresis buffer, and a sample containing a marker dye (BPB) was added. Electrophoresis was performed using an electrophoresis apparatus (AF-7300 Compact PEGE; ATTO Corporation). After electrophoresis, the gel was removed and immersed in Coomassie Brilliant Blue staining solution for 10 min, and then immersed in a decolorizing solution for decolorization.

9) Colour measurement

The colour of the whitebait fish sauce was measured using a colour difference meter (CR-400; Konica Minolta Japan, Inc.). The L^* of the colour difference meter indicates the brightness, a^* indicates red to green, and b^* indicates yellow to blue.

Results and discussion

1) General components of *shirasu* from Kamakura

The general components of *shirasu* were measured, and the values shown in Table 1 were obtained. For comparison with general *shirasu*, data from the 2015 Japanese Food Composition Tables are shown. Compared to *shirasu* listed in the Japanese Food Composition Tables, *shirasu* from Kamakura contained 1.1 times more protein and 1.4 times more ash, and was confirmed to be rich in minerals.

Table 1 Food components of Kamakura "*shirasu*" (%)

	Moisture	Protein	Fat	Carbohydrate	Ash
Kamakura *Shirasu*	78.6	16.8	1.2	0.1	3.3
Ingredients *Shirasu*	81.8	15	1.3	0.1	2.4

2) Mineral content of *shirasu* from Kamakura

The mineral content of *shirasu* was measured, the results of which are shown in Table 2. *Shirasu* from Kamakura was characterized by containing 4.8 times more iron, 2 times more copper, approximately 1.5 times more magnesium and zinc, and 1.2 times more calcium than *shirasu* listed in the Japanese Food Composition Tables, and was confirmed to be rich in minerals.

Table 2 Mineral content of Kamakura "*shirasu*" (mg/100g)

	Mg	Na	Ca	Cu	Zn	Fe
Kamakura Shirasu	502	282	244	0.26	1.6	1.9
Ingredients Shirasu	340	380	210	0.13	1.1	0.4

3) Production of fish sauce

The appearance of the fish sauce during aging changed, as shown in Figure 1. As the maturation process progressed, the *shirasu* became less recognizable in terms of their shape, and 1 month after preparation the fish sauce became a thick, gel-like substance. After 2 months it became a liquid, and a brown liquid began to separate from it.

Figure 1 Kamakura "*shirasu*" and "*shirasu*" fish sauce.

4) Changes in fish sauce components over time during aging

For the analysis of each item in Kamakura-produced *shirasu* fish sauce, samples were used immediately after brewing, and after 1 and 2 months. The fish sauce was stirred and 10 samples were measured, from which the average values are shown (Figure 2).

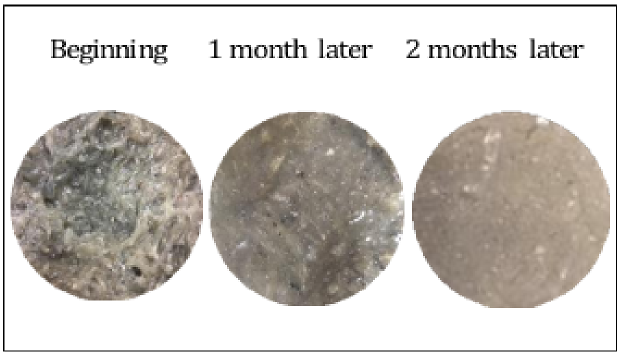

Figure 2 Changes in "*shirasu*" fish sauce over time during aging.

1) Protein

a) Changes in the total protein content of fish sauce during aging

Table 3 shows the changes in the total protein content of fish sauce. The protein content increased 1.4 times from immediately after brewing to 2 months later (Figure 3).

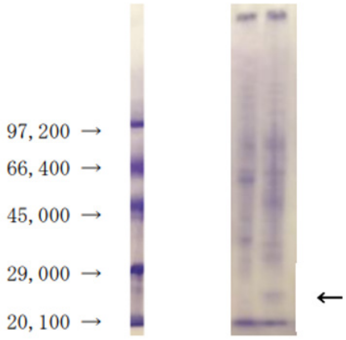

Figure 3 Molecular weight distribution of "*shirasu*" fish sauces as determined by electrophoresis.

Table 3 Changes in protein content in "*shirasu*" fish sauce over time (g/100g)

Beginning	1 month later	2 month later
3.11	4.21	4.37

b) Formol nitrogen content

As shown in Table 4, the formol nitrogen content in fish sauce increased rapidly 1 month after brewing and continued to increase after 2 months, suggesting the production of amino acids and peptides. It is thought that the proteases contained in the *shirasu* promoted the decomposition of proteins.

Table 4 Changes in the amount of formol nitrogen in "*shirasu*" fish sauce over time (%)

Beginning	1 month later	2 month later
0.01	0.25	0.28

2) Sugar content

a) Total sugar content

As shown in Table 5, the total sugar content in fish sauce was 2.2 times higher after 2 months than immediately after brewing. *Shirasu* itself has low carbohydrate content, but when it was made into fish sauce, the sugar content increased to 4.9%. It was assumed that, as it aged, amylase broke it down and produced sugar.

Table 5 Changes in total sugar content in "*shirasu*" fish sauce over time (%)

Beginning	1 month later	2 month later
2.26	3.25	4.87

b) Amount of reducing sugar

As shown in Table 6, the amount of reducing sugar in the fish sauce was 2.3 times higher (at 3.6%) after 2 months compared with that immediately after preparation. From the ratio of total sugar to reducing sugar, it was thought that sugars of the order of glucose to maltose (monosaccharide to disaccharide) were produced.

Table 6 Changes in reducing sugar content in "*shirasu*" fish sauce over time (g/100g)

Beginning	1 month later	2 month later
1.55	2.25	3.60

3) pH

As shown in Table 7, the pH of fish sauce gradually became more acidic from immediately after preparation to 1 and 2 months later. As it aged, the levels of the umami components glutamic acid and organic acids increased, which is thought to have caused the pH to become more acidic. It was also thought that the umami of the fish sauce increased as it aged.

Table 7 Changes in pH in "*shirasu*" fish sauce over time

Beginning	1 month later	2 month later
pH 5.53	pH 4.95	pH 4.77

4) Change in colour of fish sauce

The change in colour of whitebait fish sauce with aging is shown in Table 8. With aging of the whitebait fish sauce, the whitebait components decomposed to produce low-molecular-weight components, and it was inferred that amino acids and sugars reacted to cause an amino-carbonyl reaction, producing browning substances. As a result, the colour of the fish sauce became darker, the brightness decreased, and the red and yellow colours became darker, turning to brown as it aged. From 1 to 2 months, the gradient of brightness and colour (b^*) became particularly large, which suggested that the colour of the fish sauce became darker after 1 month.

Table 8 Changes in color (*L*a* b*star*) over time during aging of "*shirasu*" fish sauce

	Shirasu	Beginning	1 month later	2 month later
L^*	49.29	47.17	46.49	45.16
a^*	-1.37	- 1.26	-0.20	0.77
b^*	-0.89	0.72	2.12	5.70

5) Glutamic acid

After 2 months, the amount of glutamic acid in *shirasu* fish sauce was 290 mg/100 g. The amount of glutamic acid in fish sauce is approximately 0.3%, while the amount of glutamic acid in soy sauce is approximately 1%. However, compared with soy sauce, fish sauce also contains more peptides, suggesting that glutamic acid is not the only umami component.

6) Titratable acidity

The titratable acidity was 0.26 for acidity I and 0.69 for acidity II. Acidity I indicates the amount of organic acid, while acidity II indicates basic amino acids and peptides. It was suggested that fish sauce contains more basic amino acids and peptides than organic acids. It was inferred that fish sauce has a rich umami flavor because it contains substantial amino acids.

7) β-Amylase activity

The β-amylase activity of fish sauce was approximately 2.00 × 100 units/100 g. As the fish sauce matured, the total sugar and reducing sugar contents increased, but it was thought that the amylase presents in the *shirasu* promoted the decomposition of sugar. It is thought that sugar-decomposing enzymes such as glucosidase are also present, which will be considered for future measurements.

8) Protease activity

The protease activity of fish sauce was 5.23 × 106 units/100 g. It was speculated that, as fish sauce matures, the breakdown of proteins is accelerated, resulting in the production of amino acids and peptides, which are the umami components.

9) Distribution of protein molecular weights by electrophoresis

Immediately after preparation of the fish sauce and 2 months later, the protein bands shifted to lower molecular weights, and after 2 months, peptide bands with molecular weights of about 25,000 to 20,000 were observed, indicating protein breakdown. These results suggested that peptides and amino acids were produced during the maturation process.

10) General components of whitebait fish sauce

The general components of whitebait fish sauce are shown in Table 9. The amount of formol nitrogen (amino nitrogen) relative to the total nitrogen was 6.4%, and it was inferred that the fish sauce had been sufficiently decomposed in 2 months, amino acids and peptides were produced, and a fish sauce with umami flavor was produced. However, if aging had been performed for slightly longer, it is possible that a fish sauce with more umami flavor would have been produced. Meanwhile, it is also possible that other flavors such as bitterness may have been produced by long-term aging. The acidity was 2.6% and the pH was 4.77, which was thought to have been due to the production of amino acids. The sugar was thought to have been due to the production of low-molecular-weight sugar.

Table 9 Characteristics of ingredients in "*shirasu*" fish sauce

Total nitrogen	4.37%
Formal nitrogen	0.28%
Proteolysis rate	6.4%
pH	pH 4.77
Acidity	2.6%
Total sugar	4.87%
Reducing sugar	3.60%
Glutamic acid	0.29%

Conclusion

Analysis of the general components of whitebait produced in Kamakura confirmed that it was rich in protein and minerals. This study also determined other characteristics of whitebait produced in Kamakura. In terms of the mineral content, the whitebait was particularly high in iron, copper, magnesium, and zinc, confirming that it was highly nutritious. Fish sauce was produced using this whitebait. Upon maturing the fish sauce for 2 months, the proteins and sugars were broken down by the action of endogenous digestive enzymes and enzymes in the muscles, producing amino acids, peptides, and low-molecular-weight sugars, and the umami components increased. Efforts to develop a fish sauce made from Kamakura whitebait will continue, which will be considered for use in processed foods such as seasonings for raw whitebait in fish sauce and boiled whitebait sauce. There is also a plan to devise dishes featuring whitebait from Kamakura, develop a new food brand using Kamakura's local resources, and promote it both within and outside the region as a new Kamakura product, helping to revitalize the region.

Acknowledgments

None

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Tetsujiro O. Shirasu, a simple food dictionary. 2nd Ed. Tokyo: Kimurabo. 1997.
2. Yoshiro W. Want to know more! Sea creatures: the surprising truth about sardines, Chapter 2: The life of sardines. Tokyo: Koseisha Kosei-Kaku. 2012.
3. Tatsuro T. (ed.). New and expanded edition: The science and technology of soy sauce, Chapter 18: Soy sauces of the world 5 Fish Sauce. Tokyo: Brewing Society of Japan. 1994.
4. Tsutsumi T. Food analysis handbook (ed. Ohara T), analysis of food components 1. Moisture. Tokyo: Kenpakusha. 1972.
5. Iwao H. Food analysis handbook (ed. Ohara T), analysis of food components 5.A. Quantitative analysis of ash content. Tokyo: Kenpakusha. 1972.
6. Yanagida T. Brewing and food science experiment book, 3.3.2 protein. Tokyo: Food Research Society. 1985.
7. Taniguchi A, Furusho R, Matsumoto K. Food chemistry experiment textbook for learning from the basics, calculation by the subtraction method, Tokyo: Kenpakusha. 2014.
8. Tsutsumi T. Food analysis handbook (supervised by Ohara T), analysis of food components 3.B. quantitative determination of fat. Tokyo: Kenpakusha. 1972.
9. Taniguchi A, Furusho R, Matsumoto K. Food chemistry experiment textbook for learning from the basics, quantitative determination of amino nitrogen by formol titration method. Tokyo: Kenpakusha. 2014.
10. Fukui S. Biochemistry experiment methods 1 quantitative method for reducing sugars, phenol-sulfuric acid method. 2nd ed. Tokyo: Academic Press Center. 1990.
11. Fukui S. Biochemical experimental methods 1: Quantitative method for reducing sugars, Somogyi-Nelson method. 2nd ed. Tokyo: Academic Press Center; 1990.
12. Yanagida T. Brewing and food science experimental book, 4.2.9 Titratable acidity. Tokyo: Food Research Society. 1990.
13. Yanagida T. Brewing and food science experiment book, 4.2.8 hydrogen ion concentration (pH). Tokyo: Food Research Institute. 1990.
14. Maekawa A, Sugawara T. New Food analysis handbook, 2.5.5. Quantitative method of inorganic substances. Tokyo: Kenpakusha; 1999.
15. Takano K, Watanabe T. New food experiment book, protease activity, Azocasein method. Sankyo Publishing. 2000.