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Quality Characteristics of Fish Sauce Produced from Anchovy (*Engraulis encrasicolus*)

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Abstract: Fish sauce is a liquid hydrolysate of salted fish. This product is a popular foodstuff that can be adapted to our changed nutritional understanding due to intensive work life and it can support the closure the deficit animal originated protein demand.

In this study, it was aimed to produce a high nutritious fish sauce which can be an alternative food product from the unevaluated whole and waste of anchovy (*Engraulis encrasicolus*) produced abundant quantities in Turkey. The quality properties and consumption suitability of the obtained products were tried to be determined by physico-chemical, chemical, microbiological and sensory, analyzes.

It was determined that there is no greater quality differences between whole and waste of anchovy sauces. Whole and waste anchovy sauces have; 61 - 63% water, 12 - 8% protein, 4 – 5% fat and 3 – 2% carbohydrate, respectively. Salt

content was found as 18% in both groups and biogenic amines and microorganism count were found at low levels. Both sauce groups were found to be safe for consume.

Fish sauces obtained from whole fish and fish waste were very close to each other in terms of nutritive values. Sauce groups have 8.62 – 13.02% protein, 4.45 – 6.07% fat, 18% salt and low biogenic amine contents (max 84.33 mg/kg) and microorganism loads (max 10^1 cfu/g). It has been found that fish sauce, which was received appreciate by consumer, has an alternative processed fish product potency, which is particularly suitable for the evaluation of wastes.

Keywords: *Engraulis encrasicolus*, anchovy, fish waste, fish sauce, quality characteristics.

Introduction

Seafood are valuable animal origin protein resources which contain all essential amino acids along with being rich omega-3, 6 and others fatty acids. These properties of seafood bring them into the cousins of all countries. However, the reasons of being highly perishable and there are lots of unconsumed product every year cause to economical lose and contributing deficiency of animal origin proteins.

Seafood processing technologies include good ways to save products from being unconsumed or perished as well as evaluating discards and wastes. Fish sauce is one of the greatest way to convert rich protein content from unused fishes or wastes to a valuable food products.

Fish sauce, which has specific characteristic odor, is a liquid hydrolysate of salted fish (Ishige, 1986). It is a popular foodstuff among Asian countries because of high seafood catch, lots of wastes and easy to produce. First fish sauce production is based to 2000 years ago. In Romans, fish sauce named as “Garum” was produced with special spices adding to whole seafood and fermenting in an earthenware pot (Aquerreta *et al*., 2001). Today fish sauce production spread to all over world, especially in Asian, America and Mediterranean countries. However, the production changes in every country and mostly done with traditional methods. Moreover, trying to new raw material to evaluate as fish sauce brings some quality and safety concerns along with. Thus, in this study, we have tried to evaluate whole anchovy, refers to unconsumed fish, which is constituted more than 54% of total seafood production of Turkey and anchovy waste, refers to discards and wastes occurs after fish processing by seafood processing plants, as fish sauce. Some of quality characteristics as well as product safety were tried to determine of produced whole and waste of anchovy sauces.

Material and Methods

Material

Research material, anchovy (*Engraulis engrasicolus*), were purchased from local fish market. Total of 55 kg anchovy were used in experiments. All chemicals used in analysis were analytical grade.

Fish Sauce Production

Anchovy samples were divided into two groups which are named as “whole” and “wastes”. Whole fish states that all parts of the fish without cutting any part and

wastes of anchovy state, viscera, fishbone (separated after filleting), head and gills. Fish sauce production from both group was schemed in Figure 1.

In both sauce production group, 20% salt, 4% sugar, 1% red pepper, 0.5% black pepper, and 0.2% clove were added to minced samples. After mixing spices and minces, the pastes of both group placed into 500 to 2000 ml glass bottles. After closing the lids of glass bottles, samples were incubated at 37°C for two months. At the end of incubation period, fish sauces were filtered. After all samples filtered, the second filtration was performed with using fine filter papers and then they were bottled and prepared for analysis.

Physicochemical Analyses

Colour measurement of sauces was performed via using a Machine Vision (MV) System equipped with Nikon D300 digital camera and LensEye software. L*, a* and b* values with the range of 0 -100 were measured, which symbols indicate white to black (shine – dull), red (+) to green (-) and yellow (+) or (blue (-) respectively (Brimelow and Groesback, 1993).

The pH values of sauce samples were measured with a pH meter (Hanna pH 211) equipped with a glass rod probe (Hanna) (Ludorf and Meyer, 1973). Salt content of sauce samples was determined according to the method of Mohr (AOAC, 2000).

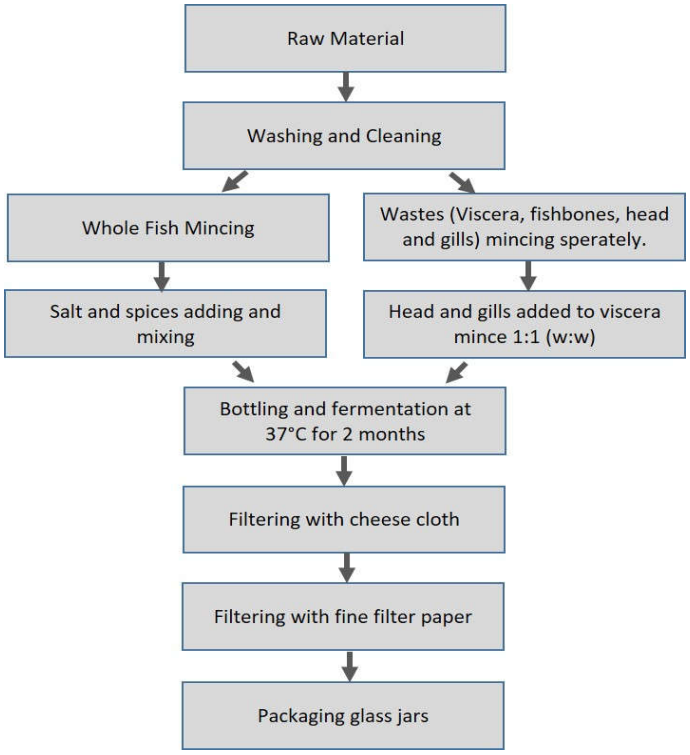

Figure 1. The steps of fish sauce production from anchovy

Proximate Analysis

Water content was measured at dried samples. Drying process was done according to AOAC 2000, with stove at 105ºC. Protein content was determined with Kjeldahl method according to AOAC 2000. Fat analysis was done according to Folch method described by Bligh and Dyer (1959). Ash amount was determined with ash furnace at 600ºC for 6 hours (AOAC, 2000).

Biogenic Amine Analysis

Biogenic amine levels were determined in two steps. First step, extraction, was performed according to the method described by Eerola *et al*. (1993). Briefly, 5 ml sauce sample was centrifuged for 3000 x g after adding 0.4 mol/L perchloric acid. After filtration with whatman 2, the samples mixed again perchloric acid and vortexed for one min., then again centrifuged for 3000 x g and the bright supernatant was collected for further analysis. The second step, derivatization was performed by mixing 0.5 ml extracted sauce sample from step 1 with 100 µl 2N NaOH, 150 µl saturated $NaHCO_3$ and 1 ml $C_{12}H_{12}ClNO_2S$ (dancyl chloride). After vortexing, samples were incubated at 40 C for 45 min. At the end of incubation, samples were cooled to room temperature in dark and then 50 µl 25% ammonium was added and left to incubation at room temperature in dark. After last incubation, 3.2 ml ammonium acetate + acetonitrile was added to reach mixture volume 5 ml. The mixture then was vortexed till melt is seen, and filtered 0.45 µl syringe filter. Filtered sample injected to HPLC (Shimadzu) equipped with Zorbax Eclipse XDB-C18-5 5 µm (4.6x150 mm).

Microbiological Analysis

Total aerobic bacteria (TAB), total halophilic bacteria (THB), Lactobacillus sp., Bacillus sp. counts were performed according to method FDA/BAM (1998). All samples were homogenized with bacterial peptone water and streak plate were used to determine the counts of bacteria. Plate count agar (Merck) for TAB, plate count agar with 7% NaCl (Merck) for THB, MRS agar (Merck) for lactobacillus sp. and Mannitol-Egg-yolk-Polymyxine Agar (Merck) for Bacillus cereus count were used in microbiological analyses.

Sensory Analysis

Sensory analyses were performed with two steps. First step was product sensory profile analyses which performed with 14 trained panelists. For determining the sensorial characteristics of sauces, panelists used a scale, modified from both the form of DLG (Deutsche Landwirtschafts-Gesellschaft) and the form used by Ritthiruangdej and Suwonsichon (2006), which uses 9 (very good) to 1 (inconsumable). Second step was the evaluating the acceptance of consumers. Consumers were selected randomly from random socio-economic status. A total of 80 people were joined to sensory analysis of fish sauces. Asked consumers to choose 1 (dislike extremely) to 9 (like extremely) point to evaluate some criteria such as colour, odor, aroma, saltiness and taste. All results of sensory analyses were given as average point in responded area.

Statistical Analysis

The descriptive statistics (mean, standard error (SE) and range) of the findings of the physicochemical parameters, proximate composition, biogenic amine levels, microbiological counts and the result of sensory analysis were calculated using Microsoft Office Excel 2007 software (Seattle, USA). The differences in results between sauce groups were determined with one-way ANNOVA and Tukey Test. The acceptable of the data for one-way ANNOVA analysis was determined with Anderson-Darling and Levene’s Test. Significance was established at $P<0.05$.

Results and Discussion

Physicochemical Properties

The colour of anchovy fish sauces produced from whole and wastes determined with the values of L*, a* and b*. Results of colour analysis indicate that

fish sauces groups have similar colour except redness. Redness of samples was measured with a* value which was determined higher in whole fish sauces. L* and b* values were determined nearly same in both groups (Table 1).

Table 1. Physicochemical properties of fish sauces produced from whole and wastes of anchovy

	Whole	Waste
L value*	56,74 ± 0,99^{b}	57,08 ± 1,02^{ab}
*a** value	52,10 ± 0,88^{a}	39,67 ± 0,93^{b}
*b** value	66,40 ± 0,86^{a}	65,48 ± 1,00^{a}
pH	5,59 ± 0,01^{b}	5,53 ± 0,02^{c}
Saltness	18,64 ± 0,08^{a}	18,68 ± 0,12^{a}

All results given in average. Different letters in same column show differences (p<0.05)

The pH values (5.59 – 5.53) in this study (Table 1) was slightly lower compared those reported by Ibrahim (2010), Cho *et al*. (1999), Park *et al.* (2001) and Ijong and Ohta (1995) and it is in agreement with those reported by Cho *et al*. (2000) and Aquerreta *et al*. (2001). The pH values of fish sauces generally reflect the bacterial activity during fermentation and probably as a consequence of the accumulation of basic compounds (Aquerreta *et al*., 2001).

The salt content of fish sauces is generally found to be high. Because of salt preservation purpose of minced product and fermentation process, salt is highly demanded additive for fish sauce production. Commercial fish sauces generally have high salt content, which is up to 18% (min. 10%). In our anchovy sauces, salt level was determined at 18% in both groups.

Proximate composition

Nutritive properties of experimentally produced anchovy fish sauces were tried to determine with proximate composition analyses. Result were given in Table 2. It was determined that sauce produced from anchovy wastes are richer in water than whole anchovy sauce group, however protein content was determined lower. Ash, fat and carbohydrate values have shown similarities in both groups. In general, whole fish sauce protein content (12%) nearly same as fresh fish and wastes can be assumed as protein source with 8% value.

Table 2. Proximate compositions of fish sauces produced from whole and wastes of anchovy

	Whole	Waste
Moisture	61,08 ± 0,60^{bc}	63,88 ± 0,98^{a}
Protein	12,22 ± 0,57^{a}	8,62 ± 0,48^{b}
Fat	4,45 ± 0,41^{c}	5,61 ± 0,27^{ab}
Ash	19,24 ± 0,27^{b}	19,16 ± 0,37^{b}
Carbohydrate	3,02 ± 0,18^{a}	2,74 ± 0,14^{ab}

All results given in average. Different letters in same column show differences (p<0.05)

In previous studies, the water content of fish sauces produced experimentally was reported in the range of 60-85% (Bersamin ve Napugan, 1961; Hjalmarsson *et al*., 2007; Gildberg *et al*., 2007). Similarly, we have found at about 60% of water content in anchovy sauces. Protein amount, in fish sauces, is generally calculated with total nitrogen value. Total nitrogen is one of the most important quality parameters and there are such standards which determine the quality classes of fish sauces according to total nitrogen amounts (Hjalmarsson *et al*., 2007). For Thailand fish sauce named as “Nampla”, most realistic quality determination is performed to total nitrogen index (Wilaipan, 1990). According to this index, fish sauces, “nampla” and “patis” should have more than 1.5% total nitrogen to obtain high quality class and more than 2% to obtain best quality class (Wilaipan, 1990; Lopetcharat *et al*., 2001; Lopetcharat and Park, 2002; Hjalmarsson *et al*., 2007). In our study nitrogen amount were determined with Kjeldahl method and results indicated as% total nitrogen. In whole and waste anchovy sauces, total nitrogen was determined as 1.54% and 2.08%, respectively. These values are suitable for nitrogen quality index mentioned above.

Low fat content was main perspective of fish sauces because of staying insoluble lipids in meat and raw materials during the fermentation process. However, ash and carbohydrate are raised because of additives, especially salt, sugar and spices, used in preparation of the sauces. Ash amount was 19% and carbohydrate value was about 3% in both sauce groups. These values show similarity between other sauces reported in prior studies (Cho *et al*., 2000; Park *et al*., 2001; Ibrahim, 2010). Some small differences may be seen because of the rate of ingredients amount used for preparing sauces.

Biogenic Amine Levels

Biogenic amines occur with decarboxylation of free amino acids by the enzyme which is produced by bacteria and named as decarboxylase. Biogenic amines can be seen both fresh and spoiling fishes. Along with this, some of food processing technics, like fermentation, can cause the formation of these amines. In this study, we have determined the levels of 8 biogenic amines, including most important one which named as histamine (Table 3). Histamine levels were determined as 7.98 mg/kg in whole anchovy sauce and 10.78 mg/kg wastes of anchovy sauces at maximum levels ($P<0.05$). In previous studies, researchers have reported histamine levels much more than ours. Zaman *et al*. (2010) have determined histamine levels in 5 different commercial fish sauces as 62.5-393.3 mg/kg. At the beginning of fermentation, the biogenic amine level can raises along with fermentation period. Once fermentation fully controlled by lactic acid bacteria biogenic amine production by other bacteria is restricted. So that, biogenic amine level stays same till product is ready. Yongsawatdigul *et al*. (2004) have reported that the level of histamine was reached to 200 mg/100g in Indian anchovy (*Stolephorus indicus*) sauces after 16 hours of fermentation; Jiang *et al*. (2014) have reported the value as 355 mg/kg for “Yu-lu” at maximum level. In our study, while histamine levels were found to be lower than the values reported by prior studies, cadaverine and putrescine were found to be higher than histamine but still lower than reported biogenic amine values. The differences of biogenic amine levels in our anchovy sauces were found statically important ($P<0.05$), which means salt, spices and sugar addition did not affect, but raw material differences affect the biogenic amine levels in

the products. On the other hand, the reason of resulting low biogenic amine contents in our anchovy fish sauces is addition of high amount of salt, which inhibits the growth of decarboxylase positive bacteria.

Table 3. The biogenic amine levels determined in anchovy sauces.

Biogenic amines	Whole	Waste
Triptamin	ND	ND
Betafeniletilamin	ND	ND
Putresin	9,84 ± 0,04^{d}	21,02 ± 0,02^{b}
Kadaverin	55,22 ± 1,17^{b}	84,33 ± 0,65^{a}
Histamin	7,98 ± 0,01^{b}	10,78 ± 0,08^{a}
Tiramin	11,89 ± 0,07^{d}	29,79 ± 0,05^{a}
Spermidin	ND	ND
Spermin	ND	ND
Total	84,94	145,93

ND: not determined. Different letters (a, b, c) in same lane indicate differences among groups (P < 0.05)

Microbiological Analysis

Microbiological quality is generally underestimated commercial fish sauces because of limiting properties of fermentation process on the bacterial growth. However, in our study, we have tried to determine bacterial content of anchovy sauces. Total aerobic bacteria, total halophilic bacteria, lactobacillus sp. and Bacillus cereus were tried to count in the sauce samples. It was observed that maximum bacterial count of sauces was restricted 10^{1} cfu/ml. Total aerobic bacteria was constituted the majority of the bacterial load with the level of 1.8 to 2.6 x 10^{1} cfu/ml. The reasons of low levels bacterial counts in sauce samples are salt, spices and fermentation process, which are natural inhibitors of microorganisms. These results are in agreements with the reports made by previous studies. Authors have reported that the bacterial load is rapidly decrease to 10^{1} cfu/ml after fermentation process started (Lopetcharat and Park, 2002; Dissaraphong *et al*., 2009).

Sensory Analysis

Sensory analysis has the most important parameters determining the quality of food products, whatever their microbiological or chemical quality is good or not (Huss, 1995; Celik, 2004). Especially in seafood, sensorial evaluation is a better way to determine to freshness and quality than other quality determining methods (Reinceccius, 1990). Along with this, sensorial analysis are mostly consulted method to determine acceptability and product identify by the producers (Meilgaard *et al*., 1999). In this study, the sensory analysis were performed with two steps. Product profile determining with trained 10 panelists was performed before the measuring the acceptability of products by consumers. Second step, consumer liking test, applied to determine the real situation of experimentally produced fish sauces from whole and wastes of anchovy. According to findings of sensory analysis, the most salient property was determined as there is no differences were found between whole and waste sauce groups by consumers. Especially taste of wastes was seen well better than whole fish sauce which indicates the wastes of fishes can be turned into food

products that are highly acceptable by consumers (Figure 2). The colour of fish sauces groups were found to be different by panelists (P<0.05) but not by consumers (P>0.05) (Figure 2, 3). The colour of fish sauces is mainly affected by fermentation process, added species and raw material (Lopetcharat and Park, 2002). Aroma was tried to determine with using eight parameters. These are sweet, caramelized, fermented, roasted, meaty, cheesy, fishy and rancid (Figure 4). All panelist and consumers found the aromas of sauces group similar (P> 0.05). These results has shown similarity between the some of prior studies. Gilberd *et al*. (2007) produced four different sauces from whiting and anchovy with their wastes and compared their sauces with the commercial ones. They have found no greater differences between sauce groups, however they have reported that experimentally produced sauces has some differences between commercial anchovy sauces, especially in aroma and taste properties. The authors emphasized that production process (fermentation, additives) as well as raw material were the major factors of these differences. Similar report was done by Jiang *et al*. (2007) who have determined that the salty, meaty, roasted and cheesy aroma of “Yu-Lu”, a commercial sauce produced from anchovy, is raised with prolonged fermentation time.

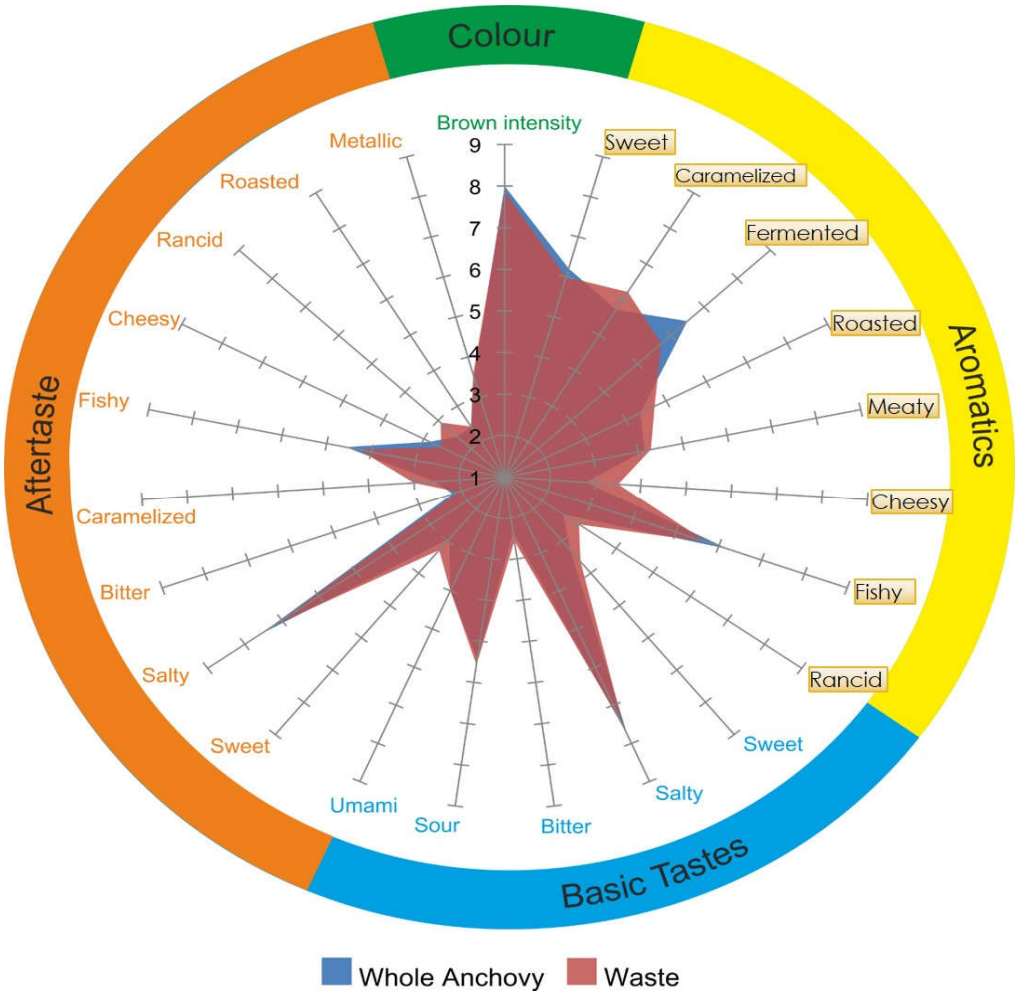

Figure 2. Sensory profile of anchovy sauces determined by trained panelists

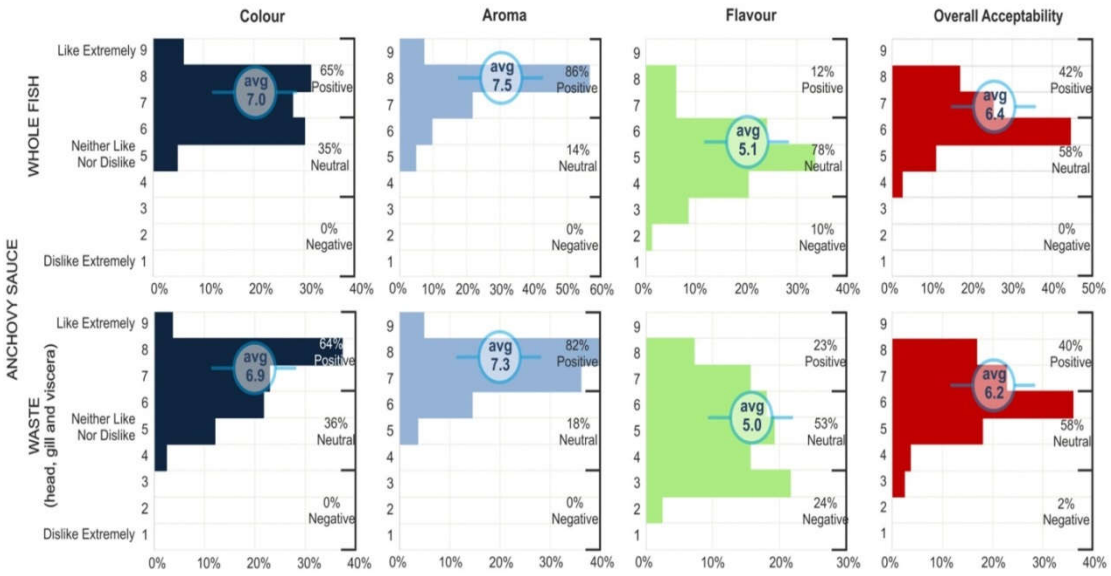

Figure 3. The results of consumer sensory analysis

Conclusion

Fish sauce is easy to produce and it is highly nutritive among same class of food. It economically important also carries the characteristics of raw material and. It is important to recover protein resources to valuable food stuff because of meeting the occurring deficiency animal origin proteins with raising population in the world. Processing seafood cause the large amount of wastes, moreover, there are lots of unconsumed seafood unevaluated every year in all countries. For this reason, fish sauce production from anchovy as well as its wastes, which is most produced seafood in Turkey was evaluated as a fish sauce in this study. It is important to try fish sauce production and determine the quality characteristics produced sauces by all countries, especially with their major seafood and its wastes to obtain high economic incoming and recovery of nutritive protein resource as well.

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