

Amino Acids and Fatty Acid Composition Content of Fish Sauce

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Abstract: The production of sardine fermented fish sauce was replicated in the laboratory condition in order to determine amino acid and fatty acid composition changes associated with fish sauce processing. Fish sauce was produced by incubating mixtures of sardine (*Sardina pilchardus*) at 6 different concentrations of sodium chloride and glucose at 37°C for 57 days. Changes in amino acid composition and fatty acid composition between groups were observed. Regarding the total lipids and percentages of DHA and EPA, we can postulate that sardines fish sauce is a good source of DHA and EPA. Either with spices or with out spices fish sauce may be a good source for human with high levels of DHA/EPA ratio and essential amino acid contents. The aim of this study was preparing a fish sauce from sardine (*Sardina pilchardus*) and determining the fatty acid and amino acid contents. Either for increasing the consuming rates or increasing the economical values of fish, evaluating new alternative processes have a great importance in Turkey. Fish sauce will be one of those products. And due to the current results of amino acid contents, for an adult a few drops of fish sauce can provide the daily requirement of leucine and isoleucine.

Key words: Fish sauce, fermentation, sardine, fatty acids, amino acid content, *Sardina pilchardus*

INTRODUCTION

Fermentation is one of the oldest techniques in food preservation as it not only extends the shelf-life but also enhances the flavor and nutritional quality of the product (Visessanguan *et al.*, 2004). Fish sauce is a clear brown liquid, obtained as hydrolysis product of salted fish after a period of salting. It is commonly used as a condiment in Southeast Asia and an amino acid source of certain social classes in the region. Fish sauces contain 20 g L⁻¹ of nitrogen, of which 16 g L⁻¹ are in the form of amino acids (Sanceda *et al.*, 1996).

Fish sauce has a characteristic aroma, which often serves as an indicator to measure the quality of fish sauce, since the very salty taste tends to overpower the other flavor constituents. A number of reports revealed that volatile acids were the most abundant group of volatile compounds in fish sauce (Saisithi, 1994). Patis, nuocmam, nampla and shottsuru contained C2-10 straight and branched-chain volatile acids (Dougan and Howard, 1975; Beddows *et al.*, 1979). Several conflicting reports on the formation and development of these compounds have been made. For instance, Nguyen-An-Cu and Vialard-Goudou (1953) identified acetic and n-butyric acids and suggested that lactic acid bacteria could be involved. The findings of Dougan and Howard (1975) on the determination of individual volatile fatty acids showed

that appreciable amounts of straight chain acids were more likely to have been formed by atmospheric oxidation of fish lipids. However, Beddows *et al.* (1980) reported that it seems unlikely that acetic and n-butyric acids could be derived from oxidation of lipid in the manufacture of fish sauce since the quantity of lipid present in the fish was insufficient to account for the amount of Volatile Fatty Acids (VFA). It was found that when fresh fish was mixed with salt and fermented, (no spoilage prior to salting), very little VFA were formed. Saisithi (1994) and Beddows *et al.* (1979) isolated bacterial species that were able to produce VFA when inoculated on hydrolyzed rockfish (*Sebastodes* or *Stolephorus* sp.). Propionic, n-butyric and n-pentanoic acids appeared to be derived from amino acids via bacterial actions using U 14°C labeled protein hydrolysates. An obvious difference in the volatile fatty acids profile in fish sauces fermented in the presence (aerobic) and absence of oxygen (anaerobic fermentation) was reported (Sanceda *et al.*, 1992). The production and consumption of fish sauce could not be done in Turkey up to now. No studies have been conducted on producing such fermented product in Turkey. This study therefore, is aimed at investigating the amino acid content and the fatty acid composition of fermented sardines with a view to using it as a food condiment to flavor soups and stews and as a protein supplement in animal feeds.

MATERIALS AND METHODS

Raw material and sauce formulations: Sardines (*Sardina pilchardus*) caught from Aegean Sea in Turkey. Fish samples were transported to the laboratory with ice packs within 2 h after landing. They were gutted, sorted, prepared as a fillet and washed with fresh water. Approximately 45 kg of sardines were used for this study and shared in 6 groups for different formulations of sauces. Each group was about 5 kg. Laboratory fish sauce production all sardines fillets were cut in to pieces about 1 cm long. On the other hand, at the same time formulations of additives were prepared. The mixture of group A was 10 g NaCl and 100 g fish for each bottle of this group. The formulation of group B was 10 g NaCl + 1 g red pepper + 1 g garlic powder + 100 g fish samples. Group C performed with 10 g NaCl + 5 g glucose + 100 g fish sample. Group D was 10 g NaCl + 5 g glucose + 1 g red pepper + 1 g garlic powder + 100 g fish sample. In group E 10 g NaCl + 10 g glucose + 100 g fish were used. And for the last formulation, group F was prepared with 10 g NaCl + 10 g glucose + 1 g red pepper + 1 g garlic powder + 100 g sardine samples. The ratios were calculated according to these formulas and used in each 5 kg samples pools. For each group 70 and totally 420 bottles were used in the study. Each bottle was filled with mixtures by hand, using plastic gloves. The capacity of the glass bottle was 200 mL, the color was dark brown. The tips of the bottles were plastic and no air could enter when they were closed. When all bottles were filled and closed they were incubated at 37°C in an incubator for 57 days. In the last day of fermentation, 6 bottles were taken for amino acid determination analysis and for determining fatty acid composition.

FAME analysis: Lipid extraction was done according to the Bligh and Dyer method. Methyl esters were prepared by transmethylation, using 2 M KOH in methanol and n-hexane, according to the method described by Ichihara *et al.* (1996). About 10 mg of extracted oil was dissolved in 2 mL hexane, followed by 4 mL of 2 M methanolic KOH. The tube was then vortexed for 2 min at room temperature. The heating process was run at 100°C for 7 min to hidrolize all the fatty acids. On completion of the process, they were cooled. After centrifugation at 4000 rpm for 10 min, the solution formed in two phases. The lower phase, containing the fatty methyl esters was transferred to a clean, 10 mL bottle and dried. Then the hexane layer was taken for GC analysis.

Gas chromatographic conditions: The fatty acid composition was analyzed by a GC IUPAC II D19, equipped with a flame ionization detector and a fused

silica capillary SGE column (30 m, 0.32 mm, ID 0.25 lm BP20, 0.25 UM, USA). The oven temperature was initially set at 140°C, held for 5 min, raised to 200°C at the rate of 4°C min⁻¹, held again at 220°C and then held for a further 10 min, while the injector and the detector temperatures were set at 220 and 280°C, respectively. The sample size was 1 ll and the carrier gas was controlled at 16 ps. The split used was 1:100. Fatty acids were identified by comparing the retention times of FAME with the standard 37 component FAME mixture (Supelco, Poole, Dorset). Two replicate GC analyses were performed and the results were expressed in GC area (%) as mean±SD.

Amino acid composition: Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method (AOAC, 1995). For amino acid analysis, samples were hydrolyzed in 6 N HCl at 110°C for 24 h (AOAC, 1984) in an evacuated sealed ampoule. Excess acid from the hydrolysate was removed by flash evaporation under reduced pressure. The analysis was carried out using an Eppendorf Biotronic LC 3000 Amino acid Analyzer (Eppendorf- Biotronic, Hamburg, Germany), according to standard procedures. The results are given as means of triplicate values.

Statistical analysis: The (SPSS, version 9.0. Chicago, IL, USA) program was used to search for significant differences between mean values of the different results. Differences between means were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey and Duncan tests. The results are presented as means±SD. When a significant difference was detected between the group (p<0.05), either the Tukey or Duncan multiple comparison test was applied in order to a certain more conservative differences using multiple comparison.

RESULTS AND DISCUSSION

The contents of amino acids in prepared sardine fish sauce with fermentation is show in Table 1. All groups contained high amounts of glutamic acid, alanine, lysine, leucine and aspartic acid. The comparison of amino acid composition of fish sauce in different groups showed that lysine was the most abundant essential amino acid in the study. When the contents of non-essential amino acids in the raw material were compared with sauces, the content of aspartic acid was found to be higher in each group. After fermentation, the contents of aspartic acid, glutamic acid, histidine and hidoksil-L-proline were increased, whereas, the content of others were significantly decreased in fish sauce group in a comparison with raw sardine.

Table 1: Comparison amino acid contents of the raw sardine and fish sauce groups

Components	Raw sardine	Group A	Group B	Group C	Group D	Group E	Group F
Protein (g/100 g) (N x 6.25)	13.8±0.1 ^a	14.0±0.2 ^{ab}	14.2±0.1 ^b	13.1±0.1 ^c	14.1±0.1 ^{ab}	12.8±0.1 ^c	11.8±0.1 ^d
Determined amino acids (mg/100 g)							
Alanine	871.1±2.9 ^a	610.4±8.1 ^b	753.1±6.8 ^c	751.4±7.5 ^c	892.9±26.1 ^a	704.5±4.8 ^d	574.6±4.6 ^e
Glycine	629.8±3.0 ^{ad}	425.7±2.7 ^b	566.6±3.1 ^c	606.6±5.8 ^c	659.9±19.9 ^d	542.4±20.2 ^e	449.8±18.3 ^b
Valine*	827.5±16.4 ^a	778.5±19.5 ^b	864.7±14.6 ^c	663.8±5.3 ^d	785.3±12.7 ^b	675.2±6.5 ^d	558.4±8.8 ^e
Leucine*	1482.0±3.0 ^a	1102.7±13.5 ^b	1348.0±41.5 ^c	1066.8±1.6 ^b	1298.6±7.6 ^d	1069.2±3.4 ^b	834.6±0.6 ^e
Isoleucine*	797.7±26.3 ^a	506.5±2.2 ^b	665.5±12.3 ^c	549.9±2.3 ^b	733.5±22.1 ^d	614.5±16.8 ^c	479.2±5.2 ^e
Threonine*	520.2±14.2 ^a	293.2±10.1 ^b	457.5±25.5 ^a	598.2±8.7 ^c	598.6±8.0 ^c	513.1±4.5 ^c	416.1±5.2 ^d
Serine	530.4±5.2 ^a	222.7±5.1 ^b	421.3±7.4 ^c	571.2±9.4 ^d	513.7±9.7 ^a	512.3±3.8 ^a	392.8±8.2 ^e
Proline	505.3±15.8 ^a	340.6±16.7 ^b	505.7±14.9 ^a	480.8±6.6 ^c	468.9±18.9 ^c	410.7±11.5 ^d	324.0±15.7 ^e
Aspartic acid	1058.1±20.5 ^a	4530.2±84.9 ^b	3656.6±132.4 ^c	1949.2±21.7 ^d	1470.8±9.5 ^e	2002.2±14.9 ^d	1549.2±24.7 ^e
Methionine*	426.7±9.3 ^a	147.5±1.1 ^b	280.7±14.1 ^c	227.5±2.1 ^d	247.8±7.6 ^d	280.5±5.9 ^c	198.1±2.7 ^e
Hidoksil-L-prolin (Hyp)	0.0±0.0	465.2±25.1 ^a	231.9±16.4 ^b	28.9±2.4 ^c	0.0±0.0	86.2±2.7 ^d	40.7±3.8 ^e
Glutamic acid	847.8±18.9 ^a	1009.8±4.8 ^b	1094.7±11.8 ^c	1356.6±13.9 ^d	1520.7±33.7 ^e	1452.1±15.8 ^f	1184.1±13.8 ^e
Phenylalanine*	701.4±15.5 ^a	473.9±6.4 ^b	615.9±20.1 ^c	503.5±7.9 ^b	556.1±27.4 ^b	499.7±7.2 ^b	373.7±6.0 ^d
Lysine*	1812.2±42.0 ^a	1230.7±26.1 ^b	1325.5±22.4 ^c	1363.8±9.8 ^c	1539.6±13.4 ^d	1567.0±19.7 ^d	1171.5±19.7 ^b
Histidine	769.8±14.2 ^a	1488.3±46.9 ^b	809.9±24.7 ^c	550.8±13.7 ^d	0.0±0.0	345.9±8.8 ^e	283.6±18.1 ^f
Tyrosine	480.7±37.9 ^a	0.0±0.0	179.8±11.2 ^b	157.9±2.7 ^b	94.7±4.3 ^c	353.7±9.4 ^d	67.5±2.1 ^e
E/EN	1.15	0.49	0.67	0.77	1.02	0.81	0.82

Arithmetic means±SD; Different letters characterize significant differences (p<0.05); n = 3

In the study of Kilinc *et al.* (2006), the fish sauces with spices were determined to be with lower bacteria counts than fish sauces without spices. The addition of glucose to the fish sauces caused a significant increase in the bacterial counts. *Staphylococcus aureus* and yeast mould counts were not detected during fermentation period in the study of Kilinc *et al.* (2006). The increase in the level of each free amino acid during fermentation seemed to be attributable to the results of dynamic balance between the production and breakdown of free amino acids by autolysis and microbial action. The degradation of some muscle proteins into peptides and amino acids. That gives the sauce the proper texture and flavor. Peptides and amino acids serve as precursors for amine formation in the presence of amino acid decarboxylase positive microorganisms. Amino acids formed from the fish protein are transformed in various ways by microbial activity when the acidity level allows such organisms to grow. The aminoacids are largely decarboxylated and biogenic amines such as cadaverine, putrescin, tyramine and histamine were formed (Clucas and Ward, 1996).

The world health organization recommended leucine and isoleucine requirements for adults of 14 and 19 mg amino acid/kg body weight per day (FAO, 1986). Minimum leucine level was determined in Group F, which was 834.6±0.6 mg/100 g and isoleucine level was determined as 479.2±5.2 mg/100 g, so for an adult a few drops of fish sauce can provide the daily requirement. In another report, LAB are reported to be important in food flavor development, the presence of LAB in the sample is also expected to contribute significantly to the flavor of fish

sauces (Gibbs, 1987). Ijong and Ohta (1995) stated that bakasang produced with 100 g kg⁻¹ salt gives more hydrolysed products compared with bakasang produced with 200 g kg⁻¹ salt. The traditional product fermented under variable temperature has lower total amino acid content than the laboratory products. Also glutamic acid, lysine and isoleucine are found to be the predominant amino acids in bakasang.

Fatty acid compositions of prepared fish sauce are shown in Table 2. Fish sauce groups were contained significantly (p<0.05) higher proportions in C14:0, C16:0, C18:1n 9 trans, C18:2, n6cis and C18:3n3. The abundant fatty acids were C16:0, C18:0, C20:5n3 cis and C22:6 n3. The Saturated Fatty Acids (SAFA) and Mono Unsaturated Fatty Acids (MUFA) were significantly higher in Group E, whereas Group B showed a higher content of Poly Unsaturated Fatty Acids (PUFA). Due to those results while the highest PUFA was observed in Group B, the lowest PUFA was in Group E. The ratios of PUFA/SUFA were >0.90 in groups A, B and D. And also the ratios of DHA/EPA were >4.0 in groups A, B, C and F. Besides, the Groups B, D and F showed a great content of linoleic (C18:2n6) and groups A, B and C higher contents of docosahexanoic (C22:6n3) acids, which are considered as essential fatty acids for its beneficial effects for human health. The higher values of eicosapentaenoic, docosahexanoic and n-3 series acids in group A and group B, which make fish sauce more favorable for human consumption. The major fatty acids of fish sauce in both groups were observed to be palmitic acid (16:0), oleic acid (18:1-n9), Eicosapentaenoic Acid (EPA, 20:n5-3) and Docosahexanoic Acid (DHA, 22:6n-3) (Table 2).

Table 2: Comparison of fatty acid contents of the prepared fish sauces

Fatty acids (%)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
C14:0	3.13±0.03 ^a	3.38±0.02 ^b	3.91±0.13 ^c	4.08±0.02 ^c	4.46±0.03 ^d	3.35±0.03 ^b
C15:0	0.68±0.01 ^a	0.99± 0.19 ^b	0.77±0.02 ^a	0.76±0.00 ^a	1.09±0.00 ^b	0.85±0.01 ^c
C16:0	24.77±0.02 ^a	24.77± 0.02 ^a	28.46±0.23 ^b	26.54±0.20 ^f	31.26±0.19 ^d	26.13±0.24 ^{bc}
C17:0	1.17±0.00 ^a	1.10± 0.01 ^a	1.22±0.01 ^a	1.12±0.02 ^a	1.38±0.01 ^b	1.29±0.10 ^{ab}
C18:0	6.98±0.00 ^a	6.61± 0.07 ^b	7.44±0.07 ^c	6.04±0.05 ^d	7.97±0.01 ^e	6.93±0.02 ^a
C20:0	1.00±0.02 ^a	1.06± 0.04 ^a	1.12±0.01 ^b	0.92±0.02 ^a	1.50±0.01 ^c	1.17±0.00 ^b
C21:0	1.25±0.01 ^a	0.62±0.67 ^b	0.99±0.03 ^c	1.17±0.01 ^a	1.17±0.01 ^a	0.90±0.01 ^c
C22:0	0.21±0.00 ^a	0.19±0.00 ^a	0.20±0.01 ^a	0.00±0.00 ^b	0.20±0.01 ^a	0.23±0.01 ^a
C23:0	0.10±0.14 ^a	0.15±0.00 ^a	0.26±0.02 ^b	0.26±0.01 ^b	0.17±0.00 ^a	0.21±0.00 ^b
C24:0	0.65±0.03 ^a	0.53±0.00 ^b	0.59±0.00 ^{ab}	0.50±0.03 ^b	0.57±0.00 ^b	0.57±0.00 ^b
ΣSUFA	39.94	39.40	44.96	41.39	50.77	41.81
C14:1	0.00±0.00 ^a	0.25±0.19 ^b	0.23±0.01 ^b	0.24±0.00 ^b	0.27±0.01 ^b	0.23±0.00 ^b
C16:1	2.88±0.00 ^a	2.85±0.01 ^a	3.07±0.01 ^b	3.43±0.18 ^c	3.66±0.06 ^d	3.16±0.09 ^a
C17:1	0.72±0.00 ^a	0.79±0.02 ^a	0.70±0.02 ^a	0.73±0.02 ^a	1.00±0.00 ^b	0.68±0.01 ^a
C18:1n9t	0.00±0.00 ^a	0.26±0.01 ^b	5.85±8.27 ^c	0.00±0.00 ^a	0.21±0.06 ^b	0.15±0.00 ^d
C18:1n9c	10.18±0.10 ^a	5.81±0.05 ^b	5.88±8.31 ^c	11.24±0.09 ^d	11.04±0.10 ^f	11.52±0.10 ^f
C20:1n9	1.38±0.01 ^a	1.21±0.03 ^b	1.36±0.00 ^a	1.18±0.02 ^b	1.46±0.04 ^c	1.22±0.01 ^b
C22:1n9	1.39±0.10 ^a	1.15±0.03 ^b	1.14±0.02 ^b	1.00±0.02 ^c	1.75±0.66 ^d	0.98±0.02 ^c
C24:1n9	3.68±0.08 ^a	2.85±0.10 ^b	3.22±0.71 ^c	2.20±0.01 ^d	2.47±0.14 ^e	3.07±0.66 ^f
ΣMUFA	20.24	15.17	21.45	20.02	21.86	21.01
C18:2 n6t	0.28±0.00 ^a	0.30±0.02 ^a	0.27±0.00 ^a	0.11±0.16 ^b	0.37±0.04 ^a	0.27±0.00 ^a
C18:2 n6c	2.07±0.04 ^a	8.50±0.10 ^b	3.06±0.21 ^c	9.80±0.07 ^d	2.44±0.05 ^e	10.00±0.05 ^f
C18:3 n6 g	0.00±0.00 ^a	0.08±0.12 ^a	0.00±0.00 ^a	0.13±0.18 ^b	0.00±0.00 ^a	0.00±0.00 ^a
C18:3 n3	0.65±0.03 ^a	0.75±0.03 ^b	0.70±0.01 ^b	0.95±0.00 ^c	0.63±0.01 ^a	0.80±0.00 ^{bc}
C20:2 cis	0.48±0.00 ^a	0.48±0.07 ^a	0.41±0.03 ^b	0.37±0.00 ^b	0.40±0.00 ^b	0.37±0.00 ^b
C20:3 n3	0.00±0.00 ^a	0.07±0.10 ^b	0.00±0.00 ^a	0.22±0.00 ^c	0.08±0.11 ^b	0.14±0.00 ^d
C20:5n3 cis	6.82±0.02 ^a	5.86±0.09 ^b	5.52±0.06 ^c	5.85±0.04 ^b	5.15±0.01 ^c	5.01±0.03 ^d
C22:6 n3	28.54±0.03 ^a	24.34±2.23 ^b	22.65±0.21 ^c	20.23±0.18 ^d	18.86±0.07 ^e	20.37±0.16 ^d
ΣPUFA	38.84	40.38	32.61	37.66	27.93	39.96
PUFA/SUFA	0.98	1.02	0.72	0.90	0.55	0.48
n6	2.35	8.88	3.33	10.04	2.81	10.27
n3	36.01	31.02	28.87	27.25	24.72	26.32
n3/n6	15.32	3.49	8.56	2.71	8.79	2.56
DHA/EPA	4.18	4.15	4.10	3.45	3.66	4.06
Unidentified	0.99	5.07	0.99	0.17	0.0	1.39

Arithmetic means±SD; Different letters characterize significant differences (p<0.05); n = 3

De-Leonardis and Macciola (2004) studied the fatty acid composition values of sardines. Their findings were similar in sardine fish sauce sample groups and the fatty acid profile was equally distributed among saturated fatty acids, on average 38.3 and 31.2% monounsaturated and 30.4% polyunsaturated. The Polyunsaturated Fatty Acid n3 (PUFA-n3) represented on average 20.9%, always higher than PUFA-n6. C20:5n3 Eicosapentaenoic Acid (EPA) and C22:6n3 Docosahexaenoic Acid (DHA) were the most abundant PUFA-n3. This observation was typical because palmitic acid is the key metabolite in fish (Andrade *et al.*, 1995). The levels of the essential C18:2n-6 fatty acid were higher than the levels of C18:3n-3, with variations from 2.07-10.9% and from 0.63-0.95%, respectively. Considering the polyunsaturated DHA and EPA fatty acids, all brands showed high levels of DHA and this is possibly due to retro conversions of EPA to DHA (Aubourg *et al.*, 1990). There are significant differences (p<0.05) between saturated C14:0, C16:0 and C18:0 fatty acids among the different studied groups. In the study of Aquerreta *et al.* (2001) a fermented fish sauce, which tries to imitate the ancient Roman garum,

was developed from tuna (*Tunnus thynnus*) liver and mackerel (*Scomber scombrus*). The authors determined the best conditions which were 10% salt (5% added at the start of the process and the 5% after 24 h of fermentation), 35-37°C and Neutrased (an endoprotease from *Bacillus subtilis*) at 0.125 Anson Units/100 g. And in their study the lipid fraction showed a very interesting fatty acid profile, especially in relation to its n-3 fatty acids (PUFA/SFA ratio 0.98; 4.2 g eicosapentaenoic acid and 11.3 g docosahexaenoic acid/100 g fat), which was similar to current study.

CONCLUSION

Regarding the total lipids and percentages of DHA and EPA, we can postulate that sardines fish sauce is a good source of DHA and EPA. And due to the current results of amino acid contents for an adult a few drops of fish sauce can provide the daily requirement of leucine and isoleucine. Either with spices or without spices fish sauce may be a good source for human with high levels of DHA/EPA ratio and essential amino acid contents.

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