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Changes in Fatty Acid Composition of *Maiwashi* (Sardine) Added with Linoleic Acid during Fermentation Process in Fish Sauce Making

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This study was conducted to measure temporal changes of fatty acids in fish (*Maiwashi*, sardine family) sauce during fermentation.

In the fish mixture, the significant differences of the total amount of fatty acids in both the control and the linoleic acid added samples were not observed during fermentation. The carbon number 22 : 6 (DHA) and 20 : 5 (EPA) decreased during fermentation process while carbon number 16 : 0 increased. The tendency of the fatty acid compositions of the linoleic acid added sample and the control sample were similar except for the linoleic acid, which significantly degraded in the linoleic acid added sample. The added linoleic acid was in a free form, so it was easy to oxidize and oxidation proceeded as fermentation progress.

In the fish sauce, the total fatty acid was significantly lower than in fish mixture suggesting that the fatty acids might still remain in the fish body during fermentation in both samples. After 12 months fermentation, the composition of other fatty acids in the linoleic acid added sample showed almost the same tendency as the control sample.

From these results, it was clear that the carbon number of some of the long chain fatty acids were cut shorter during fermentation. Linoleic acid did not affect major changes in the composition of fatty acid. This would suggest that addition of linoleic acid could have converted to short chain fatty acids since the composition of fatty acids after 12 months tended to be similar with the control.

Keyword：fish sauce, linoleic acid, short chain fatty acid

INTRODUCTION

Rice is a staple food in Japan and has a flat taste and must be eaten with strong flavor side dishes such as pickles and other fermented or salted foods especially fish.

Most of the fish are rich in Omega 3 fatty acids that can help to protect the body against heart disease. They protect the heart in several ways: Help to protect irregular heart beat, and reduce the risk of clotting by making the blood less sticky and/or help to improve the cholesterol balance of the blood by increasing levels of HDL and decreasing levels of LDL. The main sources of Omega 3 fatty acids are oily fish such as mackerel sardines and many others. Fish is a highly perishable food and the unconsumed catch needs to be preserved. Fermentation is one of the oldest techniques in food preservation, since it not only elongates the shelf-life but also enhances the flavor and nutritional quality of the product.

Fish sauce is a clear brown liquid obtained as a hydrolysis product of salted fish after 1 year of salting. It is a popular traditional fermented fish product in Southeast Asia and called *Noucmam* in Vietnam, *Nampla* in Thailand, *Patis* in the Philipines, *Shotsuru* in Japan etc. It is commonly used not only as a condiment in Southeast Asia but also as a protein source for certain social classes in the region. It is similarly used as soy sauce. *Shottsuru* (fish sauce produced in Akita, Japan) has been popularly used in traditional fish soup in Japan called *Shotturu nabe*, to improve the flavor of the cuisine, without it, the dish could not be considered a special food.

Fish contains saturated and unsaturated fatty acids of which the latter is effective for human health. A number of reports revealed that short chain volatile fatty acids like formic, acetic, propionic and n-butyric acids were most abundant group of volatile compounds in fish sauce identified by some researchers (Truong Van-Chom, 1992; Saisithi *et al.*, 1966; Sanceda *et al.*, 2001). Volatile fatty acids (VFA) are also known as short- chain fatty acids (SCFA and their dissociated ions are sometimes abbreviated as SCFA). It has been reported that straight VFAs (SCFA) developed from fish fats. Addition of a fixed quantity of free fatty acid to fish mixture in the process of fish sauce manufacture during fermentation led to the increase in the SCFAs (Sanceda *et al.*, 2001).

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Objectives

More clear information on the changes in fatty acids during fermentation in the manufacture of fish sauce was investgated. In this study, linoleic acid was added to the fish mixture before fermentation to determine whether it plays a role in the changes of fatty acids during the manufacturing process.

MATERIALS AND METHODS

Materials

Sardinops melanostictus (*Maiwashi* in Japanese) and sodium chloride (salt) were used. Linoleic acid and standard fatty acids used were purchased from GL Science Ltd. Tricosanoic acid as internal standard was purchased from Sigma-Aldrich Co.

Sample Preparation

Head of *Maiwashi* was removed and discarded, and the whole body was cut crosswise into half and minced including intestine. Five hundred grams minced fish was added with 150 g salt and mixed (control). For fish sauce making, linoleic acid (2%) was added to the minced fish salt mixture. The mixture was placed in layers in an enamelware, put weight on top of the mixture and covered with plastic. The mixture was left to ferment at 37℃. Liquid was harvested at 2 months and 12 months. After fermentation, fish sauce was filtered with cheese cloth and further filtered with filter paper (No. 2, ADVANTEC Co.).

Chemical component analysis

Crude fat was analyzed by the Soxhlet extraction method. Crude protein by Khelhdahl method, water was measured at 105℃ and ash by direct incineration method.

Fatty acid analysis

Fatty acid composition was analyzed after the lipids were extracted from the fish mixture and fish sauce by the method of Folch (Folch, 1957). Crude fat was methyl esterified by boron trifluoride methanol and analyzed by GLC under the following conditions to determine the fatty acid composition (Kuwamori, 1997). Gas chromatography was accomplished using a gas chromatograph Shimadzu 9A (Gasukuro Kogyo Inc, Kyoto, Japan) equipped with a flame ionization detector. Separation of fatty acids was done in an Omegawax M320 Column, 30 m×0.32 mm I.D., 0.25 μm (Film Supelco Co.). The initial column temperature was 150℃, then increased to 210℃ at a rate of 1℃/min^{-1}, held for 5 min and to 230℃ at a rate of 4℃/min^{-1}. The injection and detector ports were kept at 260℃. The carrier gas was nitrogen. A multifunction data processor (C-R6A Chromatopac, Shimadzu, Kyoto, Japan) connected to the gas chromatograph was used for a relative quantitative calculation. Fatty acids were identified using the linear relationship between the homologous carbon number and by comparing the retention time of the tested compounds with these of the reference standard.

Statistical analysis

For the fatty acids measurement, experiments were performed in triplicate and the results were expressed as means±SD. A statistical analysis was performed using the SPSS version 19.0. Data were analysed using Student's *t*-test. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Fresh *Maiwashi*

Table 1 shows the chemical composition of *Maiwashi* (*Sardine family*), which was very close to the table of contents listed in Standard Tables of Food Composition in Japan 2010 (Ministry of education, culture, sports, science and technology, 2010), with the exception of crude fat. It was reported that *Maiwashi* caught during summer, have the highest content of fat while those used in our experiment were caught around March suggesting that climate or season has an influence on the fat composition of *Maiwashi*.

Table 1. Chemical composition of *Maiwashi* (*sardine family*)

	%
Carbohydrate	3.9
Fat	5.3
Protein	19.1
Ash	1.8
Water	69.9

Fish Mixture

Table 2 shows the changes in fatty acids composition of fresh and fermented *Maiwashi*. Fatty acids 16:0, 22:6 (DHA) and 20:5 (EPA) were high in fresh sardines totaling to more than 50% of the whole amount of the fish. These fatty acids also conform to the Standard Tables of Food Composition in Japan (Fifth Revised and Enlarged Edition, Fatty acids section, Ministry of education, culture, sports, science and technology, Japan). Although, fatty acids 14:1 and 22:0 were not listed in the Standard Tables of Food Composition in Japan, they registered a little high in our experiment which was similar to that reported by Iyota and Noguchi (1972), and that they revealed that fatty acid contents of sardines depended on season and location harvested.

Table 2. Changes in fatty acid concentration of fresh and fermented *Maiwashi*

(μmol/g)

Fatty acids	Fresh *Maiwashi***	2 months		12 months	
		Control	Linoleic acid added	Control	Linoleic acid added
	μmol S.D. (%)	μmol S.D. (%)	μmol S.D. (%)	μmol S.D. (%)	μmol S.D. (%)
12 : 0	0.19±0.01 (0.2)	0.21±0.03 (0.3)	0.15±0.01* (0.2)	0.27±0.05 (0.4)	0.27±0.02 (0.3)
14 : 0	5.85±0.05 (7.7)	6.95±0.63 (9.2)	5.32±0.34* (6.2)	9.48±0.67 (12.8)	8.85±0.48 (10.9)
14 : 1	2.90±0.12 (3.8)	3.30±0.27 (4.3)	2.40±0.15* (2.8)	4.30±0.26 (5.8)	4.27±0.17 (5.3)
16 : 0	16.16±0.39 (21.2)	17.10±0.89 (22.5)	13.36±0.82* (15.6)	23.30±1.08 (31.4)	22.81±1.11 (28.0)
16 : 1	4.29±0.04 (5.6)	4.96±0.33 (6.5)	4.01±0.34* (4.7)	6.00±0.28 (8.1)	5.75±0.29 (7.1)
17 : 0	0.68±0.01 (0.9)	0.74±0.04 (1.0)	0.67±0.10 (0.8)	1.08±0.07 (1.5)	1.03±0.06 (1.3)
18 : 0	3.66±0.04 (4.8)	4.20±0.18 (5.5)	3.44±0.26* (4.0)	5.89±0.33 (7.9)	5.88±0.29 (7.2)
18 : 1	6.34±0.14 (8.3)	6.19±0.28 (8.1)	7.25±0.40* (8.4)	9.05±0.45 (12.2)	11.89±0.63* (14.6)
18 : 2 (n-6)	1.96±0.01 (2.6)	1.95±0.09 (2.6)	23.60±1.27* (27.5)	1.96±0.04 (2.6)	9.79±0.61* (12.0)
18 : 3 (n-3)	0.44±0.02 (0.6)	0.42±0.04 (0.6)	0.52±0.02* (0.6)	0.21±0.02 (0.3)	0.14±0.00* (0.2)
20 : 0	0.44±0.03 (0.6)	0.51±0.03 (0.7)	0.42±0.04* (0.5)	0.77±0.03 (1.0)	0.84±0.05 (1.0)
20 : 1	1.32±0.08 (1.7)	1.33±0.08 (1.7)	1.06±0.09* (1.2)	2.13±0.09 (2.9)	2.38±0.16 (2.9)
20 : 4 (n-6)	1.51±0.08 (2.0)	1.26±0.06 (1.7)	1.06±0.07* (1.2)	0.49±0.04 (0.7)	0.28±0.01* (0.3)
20 : 5 (n-3)	9.09±0.12 (11.9)	8.46±0.53 (11.1)	7.21±0.45* (8.4)	2.52±0.15 (3.4)	1.60±0.04* (2.0)
22 : 0	4.86±0.20 (6.4)	4.59±0.32 (6.0)	3.87±0.24* (4.5)	1.43±0.05 (1.9)	2.04±0.40 (2.5)
22 : 1	0.25±0.04 (0.3)	0.30±0.06 (0.4)	0.35±0.05 (0.4)	0.81±0.08 (1.1)	0.22±0.01* (0.3)
22 : 6 (n-3)	15.51±0.03 (20.4)	12.39±0.66 (16.3)	10.40±0.44* (12.1)	3.72±0.26 (5.0)	2.38±0.06* (2.9)
24 : 1	0.71±0.08 (0.9)	1.09±0.12 (1.4)	0.75±0.10* (0.9)	0.84±0.06 (1.1)	0.94±0.07 (1.1)
Total fatty acids	76.16±0.08 (100.0)	75.96±4.12 (100.0)	85.82±4.35* (100.0)	74.26±3.16 (100.0)	81.35±3.88 (100.0)

Values are average of triplicates±standard deviation. *Significantly different from Control.
**Fresh *Maiwashi*: Whole fish body except head

The total amount of fatty acid in the samples added with linoleic acid and fermented for 2 and 12 months showed high tendency than the control sample. In both the control and the linoleic acid added samples, the significant differences of the total amount of fatty acids were not observed during fermentation. However in the linoleic acid sample, linoleic acid was significantly degraded ($P<0.05$, the statistical analysis results are not shown). It was thought that since the added linoleic acid was in a free form, it was easy to oxidize and oxidation proceeded as fermentation progress. Sanceda *et al.* (2001) reported that addition of linoleic acid in fish mixture during fermentation process in the manufacture of fish sauce increased the VFA and it could also be possible that the decrease in the amount of linoleic acid in this study might have been due to the conversion of its double bond, and cutting chain into SCFA (VFA).

During the fermentation process, double bond fatty acids are transformed into SCFAs due to the participation of microorganism and/or oxidation of lipid in the aerobic fermentation. The biggest change observed is in the 22 : 6 (DHA), followed by 20 : 5 (EPA). Even in the linoleic acid added samples, the amount of these two fatty acids decreased and it was suspected that they were transformed into saturated fatty acids or changed into other shorter carbon chain fatty acids.

Palmitic acid increased in both the control and in the linoleic acid added samples and the increase might be due to microorganism during fermentation. It has been reported that palmitic acid has toxic effect, thus causes apotosis in many cell types including endothelial cells (Stenz, 2006). However, to date, no casualties have been reported by ingesting fish sauce and since palmitic acid in fish sauce was found to be in a very minimal amount in our study, the amount of palmitic acid could be too small to cause toxicity when taken in a minimal amount, therefore, the negative impact on human health could be ignored. It seemed that linoleic acid had no direct influence on the amount of palmitic acid

Fish Sauce

Table 3 shows the changes in fatty acid composition of fish sauce during fermentation.

The total fatty acid in fish sauce was significantly lower than in fish mixture suggesting that hydrolysis was not complete and the fatty acids might still remain in the fish body during fermentation in both the control and linoleic acid added samples. In this study, some fatty acids were hardly detected. It was considered that since the liquid was filtered first with cheese cloth and finally filtered with filter paper, we suspected that some of the fish oil in

Table 3. Changes in fatty acid composition of fish sauce** during fermentation

(μmol/1ml)

Fatty acids	2 months				12 months			
	Control		Linoleic acid added		Control		Linoleic acid added	
	μmol S.D.	(%)	μmol S.D.	(%)	μmol S.D.	(%)	μmol S.D.	(%)
12 : 0	0.05±0.02	(2.2)	0.02±0.00	(0.5)	0.04±0.01	(3.5)	0.08±0.06	(6.7)
14 : 0	0.20±0.17	(8.3)	0.16±0.01	(3.8)	0.04±0.04	(3.4)	0.05±0.03	(4.2)
14 : 1	0.20±0.17	(8.3)	0.10±0.00	(2.4)	0.03±0.01	(2.6)	0.03±0.00	(3.0)
16 : 0	1.07±0.98	(44.5)	1.15±0.04	(28.1)	0.55±0.09	(45.3)	0.56±0.04	(48.2)
16 : 1	0.03±0.02	(1.1)	0.01±0.01	(0.2)	0.04±0.03	(2.9)	0.02±0.00	(1.4)
17 : 0	0.04±0.04	(1.7)	0.04±0.01	(1.1)	0.02±0.00	(1.7)	0.02±0.00	(1.8)
18 : 0	0.53±0.48	(22.1)	0.60±0.01	(14.5)	0.30±0.02	(24.5)	0.31±0.01	(26.5)
18 : 1	0.14±0.05	(5.7)	0.06±0.01	(1.5)	0.07±0.00	(5.4)	0.05±0.00*	(4.0)
18 : 2 (n-6)	0.01±0.00	(0.3)	0.12±0.01*	(3.0)	tr± −	−	tr± −	−
18 : 3 (n-3)	nd± −	−	nd± −	−	nd± −	−	nd± −	−
20 : 0	nd± −	−	nd± −	−	nd± −	−	nd± −	−
20 : 1	nd± −	−	nd± −	−	0.01±0.00	(1.2)	0.01±0.00	(1.1)
20 : 4 (n-6)	nd± −	−	nd± −	−	nd± −	−	nd± −	−
20 : 5 (n-3)	0.06±0.05	(2.4)	1.78±0.17*	(43.2)	tr± −	−	tr± −	−
22 : 0	nd± −	−	nd± −	−	nd± −	−	nd± −	−
22 : 1	nd± −	−	nd± −	−	nd± −	−	tr± −	−
22 : 6 (n-3)	0.08±0.08	(3.3)	0.07±0.01*	(1.7)	0.02±0.03	(2.0)	0.01±0.00	(0.9)
24 : 1	nd± −	−	nd± −	−	0.09±0.10	(7.5)	0.03±0.01	(2.3)
Total fatty acids	2.42±2.07	(100.0)	4.12±0.14*	(100.0)	1.22±0.31	(100.0)	1.17±0.07	(100.0)

Values are average of triplicates ± standard deviation.
tr: Values are less than 0.01μmol
nd: Not detected.
*Significantly different from Control.
**fish sauce: liquid after 2 and 12 months fermentation

the remaining fish body could not be measured together with some remaining particles of fish body mixture.

In both the control and the linoleic acid added samples, 16 : 0 was contained most followed by 18 : 0 after 12 months fermentation. In the linoleic acid added sample, the composition of other fatty acids showed almost the same tendency as the control sample. In addition, although 18 : 2 (linoleic acid) was detected in a very small amount after 2 month fermentation, it was hardly detected after 12 month fermentation in both samples.

Another possibility is that linoleic acid could be degraded and converted into SCFAs thus the amount in the fish sauce sample after fermentation was not different from the control mixture. Sanceda *et al.* (2001) reported that when 18 : 2 (linoleic acid) was added to the fish mixture before fermentation, the amount of SCFA increased, suggesting that SCFAs were derived from fish fats. Furthermore, they reported that the 18 : 2 added samples contain high ratio of SCFAs after one month fermentation compared with control. They suspected that SCFAs were generated from unsaturated fatty acid. From our result and the result of that of Sanceda's (Sanceda *et al.*, 2001), it is surmised that linoleic acid added to the sample was changed to SCFAs rapidly because the added linoleic acid was a free fatty acid. Therefore, the amount of fatty acids in the 12 months fermentation tended to be similar between the control and the linoleic acid added samples.

The fermentation of fish sauce and fatty acids conversion were extremely related with microorganisms, Fujii *et al.* (1980, 1994). Chihara (2002) identified the kinds of microorganism in *budu* to be mainly *Bacillus* strains. Natteewan (2010) reported that the result of gene sequences during fermentation of fish sauce was due to the microorganism was *T. halophilus* (salt-tolerant lactic acid bacteria). They also reported that fat plays an important role in the generation of SCFAs. The SCFAs, such as acetic acid, propionic acid and lactic acid were reported as energy source of large intestine epithelial cells (Scheppach, 1994). SCFAs have antibacterial action which occurred due to reducing pH in an intestinal tract (Cherrington *et al.*, 1991). Hofmanová *et al.* (2012) demonstrated an important role of fatty acid-induced lipid alterations including butyrate acids in the different apoptotic/differentiation response of colon cells with various carcinogenic potential.

CONCLUSION

In this study, it was clear that the carbon number of

some of the long chain fatty acids were cut shorter during fermentation process of fish sauce. Unsaturated fatty acids were converted to saturated fatty acids when the double bond reduced to single bond. This would suggest that addition of linoleic acid could have converted fatty acids to SCFA since the composition of fatty acids after 12 months tended to be similar with the control. Linoleic acid did not bring major changes in the composition of fatty acid.

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リノール酸を添加した魚醤発酵過程における脂肪酸の経時的変化

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和文抄録

本研究は，魚醤発酵過程における脂肪酸の経時的変化について検討した。

発酵過程におけるろ過前試料中の総脂肪酸含有量は，リノール酸添加試料とコントロール試料間で有意な差は認められなかった。脂肪酸のうち 22:6（DHA）と 20:5（EPA）は発酵過程で減少したが，16:0 は増加が認められた。リノール酸添加試料とコントロール試料の脂肪酸組成は，リノール酸以外は同様の傾向を示した。発酵過程における，リノール酸添加試料中のリノール酸含有量は顕著に減少した。添加したリノール酸は遊離脂肪酸であったため酸化され易く，発酵の進行とともに酸化されたと考えられる。

魚醤に含有される脂肪酸は，ろ過前試料よりも顕著に少なかった。このことは両試料ともに，ろ過過程において脂肪酸が試料中に残存したことを示している。12ヵ月経過後のリノール酸添加試料における脂肪酸組成は，コントロール試料とほぼ同様の傾向を示した。

これらの結果から，いくつかの長鎖脂肪酸は発酵過程において炭素鎖が切断されたと考えられる。添加したリノール酸は脂肪酸組成に大きな影響を及ぼさず，12ヵ月経過後のリノール酸添加試料とコントロール試料間での脂肪酸組成は同様の傾向を示した。そのことから添加したリノール酸は，短鎖脂肪酸へと変換されたのではないかと考えられた。

キーワード：魚醤，リノール酸，短鎖脂肪酸

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