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Changes in lipids and their contribution to the taste of *migaki-nishin* (dried herring fillet) during drying

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Running title: Changes in lipids and their contribution to the taste of *migaki-nishin*

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Abstract

This study was conducted to investigate the changes in lipids and their effect on the taste of *migaki-nishin* during drying. Lipid was extracted from herring fillets on different drying stages to measure the degree of lipid oxidation and changes in lipid composition, and fatty acid profile. Peroxide value, carbonyl value and acid value of the lipids were significantly increased ($P < 0.05$) during the drying period. Marked increase in free fatty acids, with decreases in triglyceride and phospholipid content were observed in proportion to drying time and this result suggested that hydrolysis was induced by lipases and phospholipases. The decreases in polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were observed in the total lipids and phospholipid fraction. In addition, significant increase in PUFAs especially DHA was found in the free fatty acid fraction. Sensory evaluation showed that an addition of DHA to *mentsuyu* significantly ($P < 0.05$) enhances the intensities of thickness, mouthfulness and continuity. These results suggest that during drying period lipid oxidation was not only occurred but also lipolysis predominantly released DHA, which might have a contribution to *kokumi* enhancement of *migaki-nishin*.

Key words: *Migaki-nishin*; lipid oxidation; lipolysis; phospholipid; DHA; taste; *kokumi*

1. Introduction

In Asia, particularly Japan, China, Korea, Taiwan and Singapore, there is a high market demand for dried seafood products, such as horse mackerel, salmon, cod, herring, flounder, scallops, squid, and skate wings. The consumer preference of these products not only their traditionally desirable taste and flavour, but also their high content of ω -3 polyunsaturated fatty acids especially in fish lipids. These fatty acids seem to have various health benefits, such as decreasing the risk of stroke, reducing serum triacylglycerol levels, reducing blood pressure, and insulin resistance and modulating the glucose metabolism (Li, Bode, Drummond & Sinclair, 2003). On the other hand, intake of lipid

oxidation products are the major causes of many pathological effects such as ageing, heart disease, cancer, and brain dysfunction (Kinsella, 1987).

Lipid is an important component, determining both functionality and sensory properties of processed fish products. Depending on the content, composition, and properties, lipids as well as their fatty acids, contribute to a wide range of quality attributes. The changes in lipid during processing, such as lipolysis and lipid oxidation in fish muscle are associated with quality deterioration (Pacheco-Aguilar, Lugo- Sánchez & Robles- Burgueño, 2000). Moreover, lipolysis induced by lipases and phospholipases, produces fatty acids that undergo further oxidation to produce low-molecular weight compounds that are responsible for the rancid off-flavour and taste of fish and fish products (Toyomizu, Hanaoka & Yamaguchi, 1981). It has been reported that volatile compounds produced by lipid oxidation, which are primarily responsible for the development of typical flavour during ripening of anchovy (Triqui & Reineccius, 1995). Much of the literature has concentrated on degradation of lipid and the effects of storage on fish flavour due to the highly unsaturated nature of fish polyunsaturated fatty acids (Kawai, 1996; Alasalvar, Anthony Taylor & Shahidi, 2005). However, little is known regarding the changes in lipids that occurred during drying in fish, which ultimately contribute to the development of characteristic taste and flavour of the final products.

Fatty fish, especially herring (*Clupea harengus*) is rich in long-chain polyunsaturated fatty acids, which make them susceptible to oxidation (Undeland, Hall & Lingnert, 1999). Furthermore, herring appears to have higher oxidation rates than other fish species, perhaps due to higher activity of enzymatic oxidation (Hultin, 1988). Consequently, the development of lipid oxidation often limits the possibilities for storage and processing of this species. In Japan, processing of herring (*Clupea pallasei*) fillets are usually carried out by drying under controlled conditions. Dried herring fillets are also known as *migaki-nishin* in Japan. It is widely used as an ingredient in savoury dishes including noodles. Although, the primary purpose of drying is preservation, but, at the same time, the drying continues, the product develops characteristic taste and flavour (unpublished data). However, the relationship between lipolysis and lipid oxidation on the development of *migaki-nishin* taste still

remains unclear. Therefore, the aim of this study was to investigate the changes in lipids and their effect on the characteristic taste and flavour of *migaki-nishin* during drying.

2. Materials and methods

2.1. Migaki-nishin samples

Migaki-nishin was obtained from Iwasaki Suisan Ltd. Hakodate, Japan. Samples were collected from different drying stages (2, 4, 6, 8 and 10 days). Frozen herring captured at the coast of Kamchatka Peninsula, Russia in October 2006, which was subjected to the processing. Herring fillets were dried by using a huge electric fan. Room temperature and relative humidity were maintained at approximately 17 °C and 45%, respectively.

2.2. Chemicals

Trimethylsilyl diazomethane was purchased from Aldrich Chemical Co., Inc. USA. Sodium thiosulfate, potassium hydroxide, 2,4-dinitrophenylhydrazine (2, 4-DNPH) were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. All other chemical solvents were of analytical or HPLC grade.

2.3. pH value measurement

To determine pH value, 10 g of each sample was homogenized with 10-fold volume of distilled water and the pH was measured directly by using a pH meter (Beckman Instruments, Inc., Fullerton, CA, USA).

2.4. Lipid extraction

Total lipids were extracted from *migaki-nishin* samples with a solvent combination of chloroform: methanol: distilled water according to the method of Bligh and Dyer (1959) with slight modification

making those final ratios 10:5:3, v/v/v. The extracted lipid was redissolved in chloroform and stored under argon gas in dark at $-50\text{ }^{\circ}\text{C}$ until further analysis.

2.5. Peroxide value measurement

Peroxide value of the extracted lipid was measured according to AOAC (1995) and was expressed as meq/kg of lipid.

2.6. Carbonyl value measurement

Carbonyl value was determined by using a modified method described by Endo et al. (2003). In brief, 2, 4-DNPH solution was prepared by dissolving 50 mg of 2, 4-DNPH in 100 mL 1-BuOH containing 3.5 mL concentrated HCl. Lipid sample (20–200 mg) was weighed into a 10 mL volumetric flask and filled with 1-BuOH. One millilitre of lipid solution was transferred in a 15 mL test tube, and then mixed with 1 mL of 2, 4-DNPH solution. The test tube was stoppered and incubated at 40°C for 20 min. The test tube was cooled, then 8 mL of 8% KOH in 1-BuOH was added, and centrifuged (5 min, $2000 \times g$). The absorption of the upper phase was measured at 420 nm with a Hitachi U-2000 spectrophotometer (Hitachi, Ltd. Tokyo, Japan). The concentration of carbonyl compound in the lipid samples was calculated from a standard calibration curve generated with known amounts of n-octylaldehyde and the value was expressed as $\mu\text{M/g}$ lipid.

2.7. Measurement of oxidized fatty acid in lipids

Oxidized fatty acid contents in lipids were analyzed according to the method described by Nonaka (1957). Briefly, 2-3 g of lipid sample was saponified with alcoholic potassium hydroxide following the standard procedure given in AOAC (1995). After evaporating alcohol, resulting soap was dissolved in hot water and transferred to a separatory funnel, and then decomposed with HCl. After cooling, the liquid was shaken with *n*-hexane and allowed to stand until separated completely into two clear layers. The insoluble oxidized fatty acid was found to adhere to the side of the funnel or forming sediment in

the *n*-hexane layer. The aqueous layer was poured off first then *n*-hexane layer was poured off through filter, and the oxidized fatty acid was washed with *n*-hexane to remove residual fatty acids. The oxidized fatty acid was dissolved in warm methanol, evaporated and the residue was then dried until the weight remains constant.

2.8. Determination of acid value

Acid value was determined according to AOAC (1995). Acid value was analyzed by titration of approximately 0.5 g of lipid, dissolved in a mixture of 100 mL of ethanol and diethyl ether (1:1; v/v), with 0.01N potassium hydroxide. Phenolphthalein was used as indicator. The results were expressed as mg KOH/g lipid.

2.9. Fractionation of total lipids

Polar lipids (PL) and non-polar lipids (NL) were separated from the total lipids by using Sep-Pak Vac 12cc Silica Cartridges (Waters Corporation, Milford, MA, USA) as described by Juaneda and Rocquelin (1985). The lipid samples (200 mg of total lipid) were loaded on the top of the cartridges. Then non-polar and polar lipids were eluted with chloroform and methanol respectively, in sequential order.

2.10. Lipid class composition analysis

The lipid class compositions of non-polar lipids were determined by using a commercial silica gel 60F₂₅₄ (Merck KGaA, Darmstadt, Germany) thin-layer chromatograph (TLC) plate using a single step development system consisting of *n*-hexane: diethyl ether: acetic acid (80:20:1, v/v/v). The plate was sprayed with 50% H₂SO₄ and heated at 150–160 °C for 15 min. Each spot was identified by authentic lipid standards and then lipid compositions were analyzed by using scanner and Scion Image software (Scion Corporation, Frederick, Maryland, USA).

2.11. Analysis of fatty acid composition

Fatty acid methyl ester (FAME) was derived from the total lipids, triglyceride and phospholipid fraction of *migaki-nishin* lipids according to the protocol of Prevot and Mordret (1976). In brief, dried lipid samples were dissolved in 1 mL *n*-hexane, and 0.2 mL of methanolic 2N-NaOH solution was added. The mixture was shaken and kept at 50 °C for 20 seconds and then 0.2 mL of methanolic 2N-HCl solution was added. The *n*-hexane layer was collected, concentrated and subjected to gas chromatographic analysis with a Hitachi 163 Gas Chromatograph (Hitachi, Ltd. Tokyo, Japan) connected with a PEG-20M liquid phase-coated G-300 column (1.2 mm i.d. × 40 m, 0.5 μm; Chemicals Evaluation and Research Institute, Saitama, Japan) with flame ionization detection. The temperature of the column, detector and injector were 170, 250 and 240 °C, respectively. The fatty acids were identified by comparing the peak retention times with authentic standards (GL Sciences Inc. Tokyo, Japan) and by following the theory of linear relationship between the carbon number unit or the number of double bonds of fatty acid and logarithm of the corresponding retention times.

Fatty acid methyl ester of free fatty acid (FFA) fraction was prepared by using trimethylsilyl diazomethane. In brief, dried FFA sample was dissolved in 1 mL of methanol and then trimethylsilyl diazomethane was added slowly until bubble was disappeared. The solutions were left for 30 min at room temperature and then evaporated for dryness. The FAME was then dissolved in *n*-hexane and subjected to gas chromatographic analysis as described above.

2.12. Sensory evaluation

Sensory evaluation was carried out by adding *migaki-nishin* lipid or DHA in the *mentsuyu* (a Japanese noodle soup) following the modified method of Ueda, Yonemitsu, Tsubuku and Sakaguchi (1997). *Mentsuyu* was consisted of 35% soy sauce, 8% “Nishi ki Aji 200” (brewed rice), 3.7% “Sudashi J-1” (dried bonito and mackerel extract), 5% highly concentrated “Katsuoconku” (dried bonito extract), 0.5% highly concentrated “Kombuekisu” (kelp extract), 2% sugar, 10% F-80 (fructose corn syrup), 1.21% salt and 34.59% water. It was diluted with 6 volumes of distilled water and then subjected to

sensory evaluation. *Migaki-nishin* lipid (2.10%) or DHA (0.10%) (maintaining the same amount of DHA) was dissolved in *mentsuyu* and then warmed to 60 °C in a water bath. To dissolve lipid or DHA homogenously in the *mentsuyu*, 0.01% ethanol was also added. Sensory evaluation was performed in separated sensory booths. The panelists were asked to judge the intensities of the test samples using a scale of 1–7, where 3 points was given to the control solution. The test samples were awarded points on the basis of saltiness, umami, thickness, mouthfulness and continuity. The panel was composed of five trained assessors from the Food Creation Center, Kyowa Hakko Food Specialties Co. Ltd., Ibaraki, Japan.

2.13. Statistical analysis

All measurements were carried out in triplicate and the results expressed as mean \pm standard deviation. Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to identify differences among the means at $P < 0.05$ using Statgraphics version 7.0 for Windows (StatPoint, Inc., Virginia, USA). The Q value examination was also performed to minimize the error (Dean & Dixon, 1951). For sensory evaluation analysis, *t*-test was carried out to determine significant differences between test samples and control (Steel & Torrie, 1980).

3. Results and discussion

3.1. Changes in pH

Changes in pH of *migaki-nishin* during drying are shown in Fig. 1. The pH value was slightly decreased from 6.79 to 6.42 during the drying period, which might be due to increasing of free fatty acids that were produced by lipolysis. These results are in agreement with those reported by Nakagawa, Noto, Yasokawa and Kamatani (2007), whom reported that pH was decreased from 6.50 to 6.35 during industrial soft drying of herring fillets. They also stated that decrease in pH correlates with the production of lactic acids and the largest increase of *Staphylococcus*, which might improve

the quality and by optimizing microorganism growth as well as preventing deterioration of soft dried *migaki-nishin*.

3.2. Lipid oxidation

Lipid peroxidation, corresponding to the oxidative deterioration of polyunsaturated fatty acids in fish muscle, leads to the production of off-flavour and off-odour, thereby shortening the shelf-life of food (Ramanathan & Das, 1992). Peroxide value of *migaki-nishin* lipid was increased rapidly from 5.52 to 11.86 meq/kg within 4 days of drying and then gradually increased up to 10 days of drying (16.07 meq/kg) (Fig. 2A). However, the peroxide values observed in the present study were in acceptable limits ($PV \leq 20$ meq/kg fish lipid) (Connell, 1995). Pacheco-Aguilar et al. (2000) also observed an increase in peroxide value of Monterey sardine muscle during storage at 0 °C for 15 days. Moreover, lipid hydroperoxides are readily decomposed into a wide range of carbonyl compounds, hydrocarbons, ketones, and other materials that contribute to off flavour of foods (Frankel, 1991). Therefore, the accumulation of secondary oxidation products was measured by determining the carbonyl compounds. Carbonyl value was varied between 4.66 and 7.27 $\mu\text{M/g}$ lipid, which was initially decreased within 4 days and then increased significantly up to 10 days of drying (Fig. 2B). The decrease in carbonyl value at day 4 was probably due to the decomposition of carbonyl compounds or the interaction with muscle proteins. However, these results are in agreement with those of Takiguchi (1999), who reported that the carbonyl value in pulverized *niboshi* increased gradually from 0 to 10 meq/kg lipid during storage at -20 °C for 60 days.

Oxidised fats are undesirable components of human diets. The most potential sources of oxidised fat in the diet are oily fish and high fat foods that produce during processing or cooking. Oxidised fatty acid contents of lipids were ranged from 0.56% to 1.13%, which was increased significantly up to 4 days drying then slightly thereafter (Fig. 2C). However, there was no significant increase of oxidised fatty acid found from 4 days to 10 days lipid. This result indicated that rusting developed in *migaki-nishin* during the drying period. Takiguchi (1988) reported that oxidised fatty acid contents

were increased during drying with subsequent storage of *niboshi* and *niboshi* powder. He also found that increasing rate of oxidised fatty acid contents in non-smoked control were higher than those of samples smoked for 60 or 360 min.

In addition to oxidative changes in lipid, acid value was measured to determine the degree of lipolysis in *migaki-nishin*. Hydrolysis of glycerol-fatty acid esters is one of the important changes that occur in fish muscle lipids during postmortem with the release of free fatty acids. This is catalysed by lipases and phospholipases (Pacheco-Aguilar et al. 2000). Acid value was ranged from 11.20 to 18.94 mg KOH/g of lipid, which was increased significantly ($P < 0.05$) in various extents during the drying period (Fig. 2D). An increasing of acid value suggested that free fatty acids might be released by partial hydrolysis of lipids during drying of *migaki-nishin*.

3.3. Changes in lipid composition during drying of *migaki-nishin*

Changes to the lipids during drying of *migaki-nishin* may greatly influence the characteristic taste and flavour of the final product. Based on dry weight, total lipid content in *migaki-nishin* was ranged between 39.05% and 41.22% (Table 1). The content of total lipids were similar to those reported for Icelandic herring caught in autumn (Gudmundsdóttir & Stefánsson, 1997). During drying, lipid content was slightly decreased in the 10 days drying sample. However, no significant changes were observed in the total lipids throughout the drying period. Aro, Larmo, Backman, Kallio and Tahvonon (2005) reported that the lipid content of pickled herring decreased significantly from 13.8% to 11.5% during 12 months of storage.

Table 1 shows that triglyceride (TG) is the major lipid class, followed by free fatty acid (FFA), phospholipids (PL), cholesterol and diglyceride. With the increasing of drying time, triglyceride and phospholipid content were decreased while FFA content was significantly increased ($P < 0.05$). This result suggested that the hydrolysis of TG and PL might take place, caused by lipases or phospholipases. Hernández-Herrero, Roig-Sagués, López-Sabater, Rodríguez-Jerez and Mora-Ventura (1999) found that free fatty acid contents increased gradually during the ripening of

salted anchovies. They also reported that salting did not inhibit the action of lipases responsible for the liberation of free fatty acids. However, during separation of neutral lipids and phospholipids by Sep-Pak Vac Silica Cartridges, a significant amount remained in the cartridges, suggesting that oxidative degradation of lipid might have occurred in *migaki-nishin* during drying.

3.4. Changes in fatty acid composition of total lipids

Fatty acid compositions of total lipids in *migaki-nishin* are depicted in Table 2. The most abundant fatty acids of total lipids were oleic (18:1n-9), palmitic (16:0), palmitoleic (16:1n-9), and cetoleic (22:1n-11) acids. Monounsaturated fatty acids clearly constituted the main group ranged from 209.35–230.80 mg/g dry matter of all fatty acids. Fatty acid profiles of the extracted total lipids are in agreement with the values of Atlantic herring (Aro et al., 2005). The total amount of polyunsaturated fatty acids (PUFAs) was ranged between 61.08 and 76.38 mg/g dry matter. Within this group the major fatty acids were eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). However, the total amount of PUFAs was decreased significantly ($P < 0.05$), which suggested that the PUFAs might be degraded by oxidation during drying. These results are more or less similar to those reported in processed herring (Aro et al., 2000, 2005), and in pulverized *niboshi* (boiled and dried anchovy) during storage at 25 °C for 60 days (Takiguchi, 1996).

3.5. Changes in fatty acid composition of triglyceride fraction

Table 3 shows the fatty acid composition of triglyceride fraction of *migaki-nishin* lipid during drying. Monounsaturated fatty acids were the most abundant (136.84–172.17 mg/g dry matter), followed by saturated fatty acids (69.56–76.72 mg/g dry matter) and polyunsaturated fatty acids (45.46–49.04 mg/g dry matter). Oleic (18:1n-9) and palmitic (16:0) acids were found to be predominant fatty acids in the triglyceride fraction. Saturated and monounsaturated fatty acid content were significantly decreased ($P < 0.05$), whilst polyunsaturated fatty acid content remained unchanged during the drying

period. A similar result was also found in the smoked dried products of anchovy during storage at 35 °C for 30 days (Takiguchi, 1988).

3.6. Changes in fatty acid composition of phospholipid fraction

In the phospholipid fraction, monounsaturated fatty acid content was almost unchanged ranging from 4.62 to 6.87 mg/g dry matter, whereas polyunsaturated fatty acid content was decreased from 12.90 to 7.73 mg/g dry matter (Table 4). Docosahexaenoic acid was predominant fatty acid and it was decreased significantly ($P < 0.05$) throughout the drying period. It is well known that PUFAs are especially sensitive to oxidation and degradation phenomena both by enzymatic and chemical oxidation, which produces a great variety of volatile compounds (Coutron-Gambotti & Gandemer, 1999). Oxidative degradation of phospholipids might be one of the reasons for the development of characteristic taste and flavour of *migaki-nishin* during drying. Triqui and Reineccius (1995) reported that lipid oxidation (mainly of n-3 PUFAs) is likely to be responsible for flavour development in anchovy during ripening. They also stated that increases in the concentration of volatile compounds are associated with the development of typical flavour after anchovy ripening.

3.7. Changes in fatty acid composition of free fatty acid fraction

Changes in fatty acid composition of free fatty acid fraction during drying in the *migaki-nishin* lipid are shown in Table 5. The most abundant fatty acids of free fatty acid fraction were docosahexaenoic (22:6n-3), palmitic (16:0), oleic (18:1n-9) and eicosapentaenoic (20:5n-3) acids. In this fraction, saturated and monounsaturated fatty acid content were almost similar, ranging between 13.37 and 19.59 mg/g dry matter, and between 10.22 and 17.96 mg/g dry matter, respectively. Polyunsaturated fatty acids were the most abundant (17.76–37.72 mg/g dry matter) fatty acids and the content was significantly increased ($P < 0.05$) throughout the drying period. In this group, the most abundant fatty acids were EPA and DHA, and the largest increase was observed in DHA increasing up to 20.34 mg/g from 9.16 mg/g dry matter during drying. This result suggests that DHA might originate from partial

hydrolysis of phospholipids during drying. The changes observed in EPA and DHA contents were in accordance with those reported by Takiguchi (1988).

3.8. Sensory evaluation

In this study, it was suggested that free fatty acids, especially DHA, should be released by partial hydrolysis of phospholipids during drying of *migaki-nishin*. Free fatty acids found in food itself and those derived from oils by lingual lipase may play an important direct role in taste perception (Gilbertson, Fontenot, Liu, Zhang & Monroe, 1997). Thus, we speculated that the released DHA should enhance the characteristic taste and flavour of *migaki-nishin*. Based on this hypothesis, sensory evaluation was carried out to determine the effect of *migaki-nishin* lipid or DHA in *mentsuyu*. Sensory evaluation showed that an addition of *migaki-nishin* lipid to *mentsuyu* significantly enhances ($P < 0.05$) the intensity of mouthfulness (Fig. 3). However, no significant influence was observed on the intensities of thickness and continuity compared to the control. Furthermore, addition of DHA significantly ($P < 0.05$) enhances the intensities of thickness, mouthfulness and continuity, so-called *kokumi* (a Japanese term). These results suggested that the intensities of thickness and continuity was dependent on the total amount of free fatty acids, particularly DHA. The influence of DHA is in accordance with the *migaki-nishin* oil specific effects. Koriyama, Wongso, Watanabe and Abe (2002) reported that an enhancing effect of DHA on the umami when added to the basic tastants (IMP + MSG). It has also been reported that fat has a taste as well as a viscosity stimulus, which could also influence perceived flavour (Mattes, 2003).

4. Conclusion

Drying of herring fillets causes large changes in fatty acid composition simultaneously with an increase in lipid oxidation. It was found that free fatty acids especially DHA content increased significantly during the drying period, indicating partial hydrolysis of phospholipids. Moreover, sensory evaluation showed that an addition of DHA to *mentsuyu* significantly enhances the intensities of

thickness, mouthfulness, and continuity. These results suggest that during the drying period lipid oxidation not only occurred, but also lipolysis predominantly released DHA, which might have a contribution to *kokumi* enhancement of *migaki-nishin*.

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Figure Captions

Fig. 1. Changes in pH during drying of *migaki-nishin*.

Fig. 2. Changes in peroxide value (A), carbonyl value (B), oxidized fatty acid (C), and acid value (D) of *migaki-nishin* lipid during drying.

Fig. 3. Taste profile of *migaki-nishin* lipid and DHA in *mentsuyu* by sensory evaluation. Data are mean \pm standard deviation (n = 5). * $P < 0.05$ vs. control.

Table 1

Changes in lipid composition (g/100 g dry matter) during drying of *migaki-nishin*

| Composition | Drying time (days) | | | | |
|-----------------|--------------------|----------------|---------------|----------------|----------------|
| | 2 | 4 | 6 | 8 | 10 |
| Total lipid | 40.85 ± 1.67a* | 39.84 ± 2.14a | 40.38 ± 1.46a | 41.22 ± 1.52a | 39.05 ± 0.65a |
| Triglyceride | 30.28 ± 0.31a | 29.22 ± 0.44ab | 28.81 ± 0.75b | 28.26 ± 1.23b | 26.36 ± 0.74c |
| Free fatty acid | 4.55 ± 0.27c | 5.12 ± 0.41bc | 6.27 ± 0.75ab | 6.78 ± 0.91a | 6.86 ± 0.67a |
| Cholesterol | 1.07 ± 0.36c | 1.12 ± 0.04c | 1.30 ± 0.09bc | 1.47 ± 0.34ab | 1.71 ± 0.12a |
| Diglyceride | 0.053 ± 0.01c | 0.058 ± 0.01c | 0.071 ± 0.01b | 0.080 ± 0.003b | 0.092 ± 0.001a |
| Phospholipids | 2.66 ± 0.20bc | 2.80 ± 0.18c | 2.43 ± 0.25b | 2.35 ± 0.16ab | 2.03 ± 0.08a |

*Different characters in the same row denote significantly different ($P < 0.05$).

Table 2

Changes in fatty acid composition (mg/g dry matter) of total lipids in *migaki-nishin* during drying

| Fatty acid | Drying time (days) | | | | |
|-------------------|--------------------|----------------|----------------|----------------|----------------|
| | 2 | 4 | 6 | 8 | 10 |
| 14:0 | 38.59 ± 0.63b* | 44.64 ± 1.92a | 40.46 ± 1.62b | 43.07 ± 1.20a | 34.46 ± 0.99c |
| 16:0 | 63.72 ± 0.90b | 69.62 ± 0.55a | 60.71 ± 0.47c | 59.67 ± 2.07c | 59.28 ± 1.67c |
| 18:0 | 5.90 ± 0.29a | 4.55 ± 1.02b | 5.41 ± 0.30ab | 4.30 ± 0.58b | 4.38 ± 0.70b |
| Σ saturated | 108.21 ± 1.28b | 118.81 ± 1.47a | 106.58 ± 2.16b | 107.04 ± 2.82b | 98.12 ± 1.38c |
| 14:1n-9 | 1.88 ± 0.10c | 2.07 ± 0.07bc | 2.33 ± 0.19a | 2.09 ± 0.01b | 1.88 ± 0.01c |
| 16:1n-9 | 43.83 ± 0.82ab | 37.73 ± 1.16d | 45.08 ± 1.18a | 41.03 ± 1.56c | 42.20 ± 1.44bc |
| 18:1n-9 | 98.14 ± 1.45d | 101.39 ± 1.93c | 99.56 ± 0.23cd | 110.00 ± 0.67a | 105.82 ± 0.33b |
| 20:1n-9 | 38.90 ± 0.33a | 30.13 ± 0.10d | 31.95 ± 0.41c | 36.34 ± 0.38b | 36.31 ± 0.15b |
| 22:1n-11 | 41.59 ± 0.82a | 38.03 ± 1.16b | 32.58 ± 1.28c | 41.34 ± 0.93a | 38.46 ± 0.51b |
| Σ monounsaturated | 224.34 ± 2.76b | 209.35 ± 4.16c | 211.50 ± 1.72c | 230.80 ± 4.13a | 224.67 ± 0.67b |
| 16:2 | 9.25 ± 0.04b | 8.35 ± 0.17d | 8.91 ± 0.21c | 9.45 ± 0.09b | 9.94 ± 0.15a |
| 16:3 | 3.01 ± 0.07c | 2.70 ± 0.21d | 3.59 ± 0.07a | 3.29 ± 0.09b | 2.75 ± 0.01d |
| 18:2n-6 | 3.64 ± 0.02c | 4.40 ± 0.60ab | 4.94 ± 0.79a | 4.04 ± 0.42ab | 4.02 ± 0.25c |
| 18:3n-3 | 1.69 ± 0.10bc | 1.79 ± 0.31ab | 2.13 ± 0.26a | 1.68 ± 0.06bc | 1.35 ± 0.10d |
| 18:4n-3 | 5.92 ± 0.19b | 5.85 ± 0.36b | 7.03 ± 0.46a | 6.20 ± 0.19b | 4.71 ± 0.09c |
| 20:2n-6 | 1.27 ± 0.49a | 0.64 ± 0.21b | 1.05 ± 0.12ab | 0.82 ± 0.11ab | 0.76 ± 0.01b |
| 20:3n-6 | 1.20 ± 0.07ab | 1.11 ± 0.31b | 1.53 ± 0.32a | 1.38 ± 0.15ab | 1.17 ± 0.09ab |
| 20:4n-6 | 1.68 ± 0.24b | 1.82 ± 0.21ab | 2.18 ± 0.39a | 1.94 ± 0.17ab | 1.68 ± 0.09b |
| 20:5n-3 | 24.35 ± 1.67a | 21.81 ± 0.68ab | 25.04 ± 4.78a | 20.70 ± 1.46ab | 18.22 ± 3.37b |
| 22:6n-3 | 19.23 ± 0.27a | 19.35 ± 1.12a | 19.98 ± 1.43a | 18.24 ± 1.82ab | 16.48 ± 0.96b |
| Σ polyunsaturated | 71.21 ± 3.11ab | 67.82 ± 3.56b | 76.38 ± 2.85a | 67.74 ± 2.61b | 61.08 ± 3.27c |
| Others | 4.74 ± 0.81ab | 2.42 ± 2.06b | 9.34 ± 2.47a | 6.62 ± 1.58ab | 6.63 ± 3.69ab |

*Different characters in the same row denote significantly different ($P < 0.05$).

Table 3

Changes in fatty acid composition (mg/g dry matter) of triglyceride fraction in *migaki-nishin* lipid during drying

| Fatty acid | Drying time (days) | | | | |
|-------------------|--------------------|----------------|----------------|----------------|----------------|
| | 2 | 4 | 6 | 8 | 10 |
| 14:0 | 27.66 ± 0.67b* | 27.85 ± 0.31b | 34.02 ± 0.87a | 28.31 ± 0.92b | 26.14 ± 0.62c |
| 16:0 | 44.16 ± 1.02a | 43.01 ± 0.48a | 40.64 ± 1.58b | 40.83 ± 1.15b | 40.61 ± 1.08b |
| 18:0 | 3.19 ± 0.11a | 3.14 ± 0.17a | 2.06 ± 0.47b | 2.65 ± 0.34ab | 2.81 ± 0.36a |
| Σ saturated | 75.01 ± 1.60ab | 74.00 ± 0.85bc | 76.72 ± 1.25a | 71.79 ± 1.78cd | 69.56 ± 1.52d |
| 14:1n-9 | 1.37 ± 0.03b | 1.60 ± 0.04ab | 1.88 ± 0.54a | 1.54 ± 0.13ab | 1.59 ± 0.28ab |
| 16:1n-9 | 35.31 ± 0.67a | 28.12 ± 0.37b | 23.97 ± 0.04d | 26.44 ± 1.43c | 28.77 ± 0.85b |
| 18:1n-9 | 76.73 ± 1.36a | 77.26 ± 1.11a | 62.53 ± 1.77b | 75.95 ± 4.83a | 66.71 ± 3.28b |
| 20:1n-9 | 31.03 ± 0.33a | 26.74 ± 0.19b | 29.84 ± 1.26a | 25.81 ± 0.81b | 21.05 ± 1.18c |
| 22:1n-11 | 27.73 ± 3.90b | 28.12 ± 0.61b | 35.74 ± 2.79a | 26.90 ± 3.63b | 18.71 ± 3.77c |
| Σ monounsaturated | 172.17 ± 1.72a | 161.84 ± 1.27b | 153.95 ± 4.52c | 156.64 ± 3.34c | 136.84 ± 2.10d |
| 14:2 | 0.89 ± 0.06b | 1.11 ± 0.08ab | 1.30 ± 0.37a | 1.21 ± 0.11ab | 1.26 ± 0.26ab |
| 16:2 | 6.46 ± 0.06a | 6.19 ± 0.05a | 4.78 ± 0.43d | 5.72 ± 0.04b | 5.29 ± 0.18c |
| 16:3 | 1.29 ± 0.05a | 1.37 ± 0.04a | 1.22 ± 0.39a | 1.48 ± 0.13a | 1.61 ± 0.29a |
| 18:2n-6 | 2.12 ± 0.04a | 2.53 ± 0.03a | 2.69 ± 0.41a | 2.21 ± 0.13a | 2.41 ± 0.23a |
| 18:3n-3 | 0.78 ± 0.07b | 1.21 ± 0.05a | 1.10 ± 0.38ab | 0.96 ± 0.12ab | 1.17 ± 0.31ab |
| 18:4n-3 | 2.95 ± 0.05a | 2.37 ± 1.63a | 2.64 ± 0.41a | 2.96 ± 0.19a | 3.43 ± 0.17a |
| 20:2n-6 | 0.38 ± 0.16b | 0.62 ± 0.07ab | 0.79 ± 0.14a | 0.58 ± 0.15ab | 0.34 ± 0.38ab |
| 20:4n-6 | 0.68 ± 0.03a | 0.91 ± 0.12a | 1.00 ± 0.14a | 0.89 ± 0.15ab | 0.52 ± 0.46a |
| 20:5n-3 | 17.82 ± 0.23a | 16.33 ± 0.03b | 17.44 ± 0.84a | 15.67 ± 0.38b | 18.20 ± 0.81a |
| 22:5n-6 | 1.02 ± 0.06b | 1.10 ± 0.08ab | 1.25 ± 0.04a | 1.00 ± 0.15b | 0.95 ± 0.10b |
| 22:6n-3 | 12.97 ± 0.28b | 13.29 ± 0.09ab | 14.83 ± 0.30a | 12.78 ± 0.39b | 13.74 ± 1.85ab |
| Σ polyunsaturated | 47.36 ± 0.54ab | 47.03 ± 1.12ab | 49.04 ± 2.73a | 45.46 ± 1.36b | 48.92 ± 1.98a |
| Others | 8.26 ± 0.73a | 9.33 ± 2.39a | 8.38 ± 1.58a | 8.71 ± 5.10a | 8.29 ± 2.73a |

* Different characters in the same row denote significantly different ($P < 0.05$).

Table 4

Changes in fatty acid composition (mg/g dry matter) of phospholipid fraction in *migaki-nishin* lipid during drying

| Fatty acid | Drying time (days) | | | | |
|-------------------|--------------------|---------------|-----------------|---------------|---------------|
| | 2 | 4 | 6 | 8 | 10 |
| 14:0 | 0.63 ± 0.02b* | 0.66 ± 0.02b | 0.78 ± 0.06a | 0.82 ± 0.05a | 0.65 ± 0.03b |
| 16:0 | 7.02 ± 0.14b | 7.92 ± 0.11a | 5.84 ± 0.40cd | 6.05 ± 0.19c | 5.49 ± 0.17d |
| 18:0 | 1.16 ± 0.01b | 1.43 ± 0.03a | 0.92 ± 0.09d | 1.04 ± 0.04c | 0.94 ± 0.01d |
| Σ saturated | 8.81 ± 0.15b | 10.01 ± 0.13a | 7.54 ± 0.56cd | 7.92 ± 0.17c | 7.08 ± 0.17d |
| 14:1n-9 | 0.03 ± 0.01bc | 0.08 ± 0.02a | 0.02 ± 0.01c | 0.05 ± 0.01b | 0.04 ± 0.03bc |
| 16:1n-9 | 0.73 ± 0.01c | 0.68 ± 0.05c | 1.08 ± 0.05a | 0.94 ± 0.06b | 1.05 ± 0.04a |
| 18:1n-9 | 3.12 ± 0.04d | 3.50 ± 0.17c | 5.07 ± 0.13a | 3.99 ± 0.22b | 3.81 ± 0.13b |
| 20:1n-9 | 0.41 ± 0.02a | 0.36 ± 0.01b | 0.37 ± 0.01ab | 0.41 ± 0.01a | 0.26 ± 0.04c |
| 22:1n-11 | 0.33 ± 0.01b | 0.32 ± 0.02b | 0.33 ± 0.12ab | 0.48 ± 0.06a | 0.17 ± 0.06c |
| Σ monounsaturated | 4.62 ± 0.04e | 4.94 ± 0.24d | 6.87 ± 0.05a | 5.87 ± 0.25b | 5.33 ± 0.04c |
| 14:2 | 0.06 ± 0.01b | 0.11 ± 0.01a | 0.05 ± 0.02b | 0.06 ± 0.04b | 0.07 ± 0.02b |
| 16:2 | 0.27 ± 0.01c | 0.37 ± 0.03b | 0.48 ± 0.04a | 0.34 ± 0.05b | 0.33 ± 0.02b |
| 16:3 | 0.03 ± 0.01b | 0.08 ± 0.01a | 0.03 ± 0.01b | 0.04 ± 0.01ab | 0.07 ± 0.03a |
| 18:2n-6 | 0.19 ± 0.01a | 0.16 ± 0.01a | 0.09 ± 0.01b | 0.10 ± 0.03b | 0.09 ± 0.01b |
| 18:3n-3 | 0.02 ± 0.00b | 0.07 ± 0.01a | 0.02 ± 0.01b | 0.02 ± 0.00b | 0.02 ± 0.01b |
| 18:4n-3 | 0.04 ± 0.01b | 0.08 ± 0.01a | tr ⁺ | 0.03 ± 0.01c | 0.03 ± 0.02c |
| 20:4n-6 | 0.20 ± 0.02a | 0.23 ± 0.01a | 0.04 ± 0.01c | 0.09 ± 0.01b | 0.07 ± 0.03bc |
| 20:5n-3 | 2.25 ± 0.02a | 1.93 ± 0.05b | 1.41 ± 0.07c | 1.53 ± 0.13c | 1.39 ± 0.03c |
| 22:6n-3 | 9.84 ± 0.06a | 9.55 ± 0.29a | 7.61 ± 0.53b | 7.27 ± 0.33b | 5.66 ± 0.21c |
| Σ polyunsaturated | 12.90 ± 0.12a | 12.58 ± 0.16a | 9.73 ± 0.52b | 9.48 ± 0.35b | 7.73 ± 0.11c |
| Others | 0.27 ± 0.01b | 0.47 ± 0.05a | 0.16 ± 0.08bc | 0.23 ± 0.02bc | 0.16 ± 0.12c |

*Different characters in the same row denote significantly different ($P < 0.05$). ⁺tr: trace

Table 5

Changes in fatty acid composition (mg/g dry matter) of free fatty acid fraction in *migaki-nishin* lipid during drying

| Fatty acid | Drying time (days) | | | | |
|-------------------|--------------------|---------------|----------------|---------------|---------------|
| | 2 | 4 | 6 | 8 | 10 |
| 14:0 | 6.87 ± 0.08a* | 4.67 ± 0.17c | 8.34 ± 0.06a | 7.42 ± 0.62b | 3.96 ± 0.19d |
| 16:0 | 8.61 ± 0.96cd | 7.85 ± 0.22d | 10.14 ± 0.25ab | 10.90 ± 0.05a | 9.33 ± 0.36bc |
| 18:0 | 0.68 ± 0.05d | 0.85 ± 0.04bc | 1.11 ± 0.06a | 0.93 ± 0.01b | 0.83 ± 0.05c |
| Σ saturated | 16.16 ± 0.99b | 13.37 ± 0.25c | 19.59 ± 0.21a | 19.25 ± 0.63a | 14.12 ± 0.56c |
| 16:1n-9 | 2.56 ± 0.20d | 3.72 ± 0.06c | 4.01 ± 0.08b | 4.51 ± 0.08a | 3.98 ± 0.12b |
| 18:1n-9 | 6.52 ± 0.44e | 7.04 ± 0.03d | 8.62 ± 0.09c | 11.41 ± 0.16a | 9.06 ± 0.23b |
| 20:1n-9 | 1.02 ± 0.07d | 1.46 ± 0.01c | 1.41 ± 0.02c | 1.89 ± 0.08b | 2.02 ± 0.06a |
| 22:1n-11 | 0.12 ± 0.03a | 0.06 ± 0.03a | 0.15 ± 0.06a | 0.15 ± 0.07a | 0.16 ± 0.09a |
| Σ monounsaturated | 10.22 ± 0.74e | 12.28 ± 0.10d | 14.19 ± 0.22c | 17.96 ± 0.18a | 15.22 ± 0.24b |
| 14:2 | 0.65 ± 0.02a | 0.39 ± 0.06c | 0.55 ± 0.03b | 0.42 ± 0.01c | 0.43 ± 0.07c |
| 16:3 | 0.67 ± 0.04c | 0.69 ± 0.01c | 0.91 ± 0.05a | 0.86 ± 0.03a | 0.77 ± 0.04b |
| 18:2n-6 | 0.46 ± 0.04b | 0.39 ± 0.03b | 0.64 ± 0.09a | 0.66 ± 0.04a | 0.72 ± 0.03a |
| 18:3n-3 | 0.26 ± 0.03b | 0.23 ± 0.02b | 0.37 ± 0.04a | 0.36 ± 0.05a | 0.40 ± 0.03a |
| 18:4n-3 | 0.45 ± 0.03e | 0.61 ± 0.02d | 0.80 ± 0.03c | 0.90 ± 0.05b | 1.37 ± 0.04a |
| 20:2n-6 | 0.12 ± 0.01a | 0.08 ± 0.01a | 0.08 ± 0.01a | 0.11 ± 0.03a | 0.11 ± 0.06a |
| 20:3n-6 | 0.30 ± 0.02c | 0.35 ± 0.02c | 0.48 ± 0.05b | 0.42 ± 0.04b | 0.58 ± 0.05a |
| 20:4n-6 | 0.31 ± 0.02c | 0.31 ± 0.01c | 0.47 ± 0.05b | 0.48 ± 0.03b | 0.75 ± 0.06a |
| 20:5n-3 | 5.38 ± 0.26e | 8.58 ± 0.15c | 8.22 ± 0.08d | 9.84 ± 0.18b | 12.25 ± 0.18a |
| 22:6n-3 | 9.16 ± 0.47e | 12.81 ± 0.26d | 13.73 ± 0.29c | 15.09 ± 0.15b | 20.34 ± 0.75a |
| Σ polyunsaturated | 17.76 ± 0.67e | 24.44 ± 0.25d | 26.25 ± 0.05c | 29.14 ± 0.33b | 37.72 ± 0.91a |
| Others | 1.35 ± 0.58b | 1.11 ± 0.07b | 2.67 ± 0.09a | 1.45 ± 0.25b | 1.54 ± 0.29b |

*Different characters in the same row denote significantly different ($P < 0.05$).

Fig. 1 (Shah et al.)

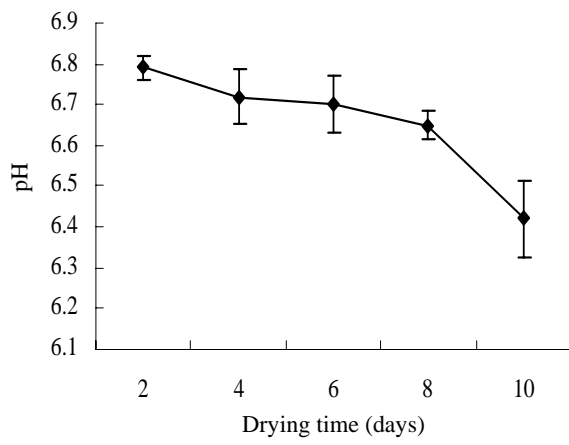


Fig. 2 (Shah et al.)

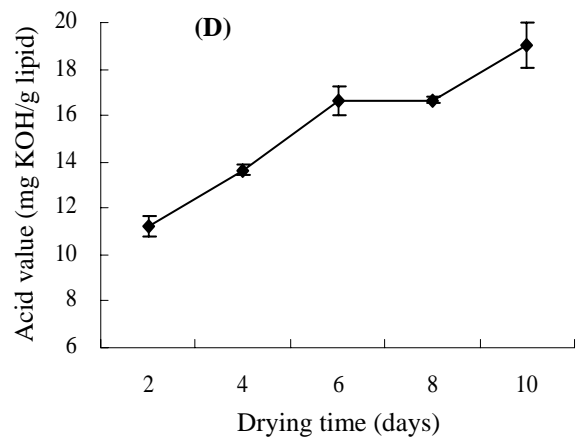
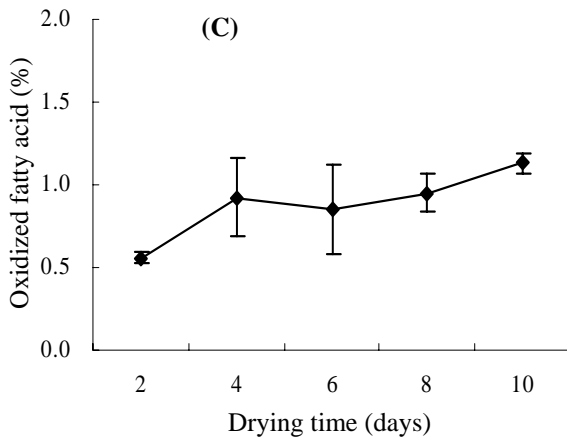
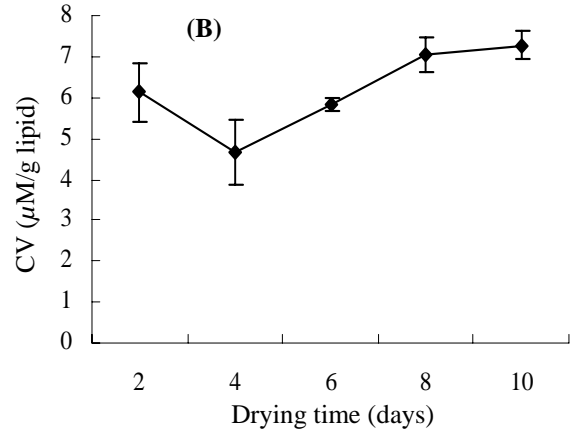
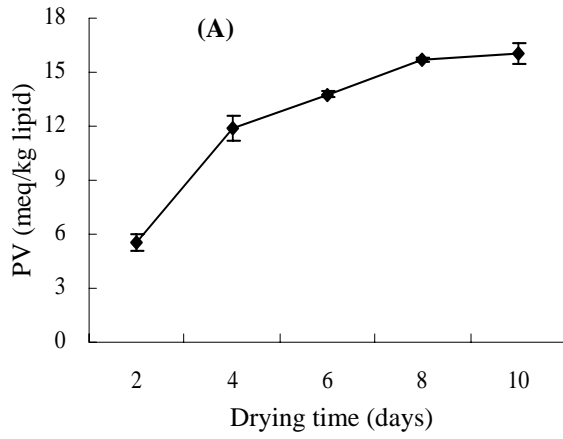


Fig. 3 (Shah et al.)

