Mechanism involved in the formation of characteristic taste and flavor during the production of dried herring (*Clupea pallasii*) fillet

A.K.M. Azad SHAH [1], Tomoe ISHIHARA [2], Masashi OGASAWARA [3], Hideyuki KURIHARA [1], Naomichi BABA [2] and Koretaro TAKAHASHI [1, *]

[1] Division of Marine Life Science, Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041-8611, Japan

[2] Division of Bioscience, Graduate School of Natural Science and Technology, Okayama University, 1-1-1 Tsushimanaka, Okayama 700-8530, Japan

[3] Food Research & Development Laboratory, Kirin Kyowa Food Co. Ltd., 4041-Ami, Ami-machi, Inashiki-gun, Ibaraki 300-0398, Japan

Running title: Flavor generation of dried herring fillet

*Corresponding author: Tel. & Fax: +81-138-40-5560; E-mail: kore@fish.hokudai.ac.jp

Abstract

The objective of this study was to identify the mechanisms responsible for the characteristic taste and flavor of dried herring fillet (DHF, migaki-nishin in Japanese). Dialyzed water-soluble fractions (DWSF) obtained from the herring fillet dried for 4 days were mixed with fatty acids and the reaction products were evaluated for their effects on sensory perception. Further, to clarify the mechanisms of *in situ* chemical changes in DHF lipid, ESI-MS analysis was done using phosphatidylcholine probe. Sensory evaluation revealed that addition of the reaction products of DWSF with docosahexaenoic acid to Japanese noodle soup significantly ($P < 0.05$) enhanced the soup flavor characters such as thickness, mouthfulness, and continuity, compared to the reaction products of DWSF with linoleic acid or capric acid. ESI-MS analyses showed that lyso-derivatives were the most abundant compounds in the lipid fraction of DHF. A small amount of lipid oxidation products and their reaction products were also observed in DHF. This study demonstrated that during the drying period, partial hydrolysis of lipids released free fatty acids. These free fatty acids or their oxidation products might react with amino acid related compounds to generate the characteristic taste and flavor of DHF.

Keywords: Herring, fatty acid, water-soluble fraction, sensory perception

Introduction

Dried herring (*Clupea pallasii*) fillet (DHF, migaki-nishin in Japanese) is a traditionally popular food item in Japan due to its remarkable flavor enhancing properties. It is widely used as an ingredient in savory dishes including noodles. In particular, addition of DHF to noodle soup enhances flavor characteristics, such as thickness, mouthfulness, and continuity. It is thought that herring fillets obtain their unique taste and flavor during the drying period. Preliminary experiments showed that addition of the dialyzed water-soluble fraction (MW 1000–5000) of DHF to Japanese noodle soup enhanced the soup flavor characteristics such as thickness, mouthfulness and continuity, so-called *kokumi* in Japanese (unpublished results). In another study, we observed a substantial increase of free fatty acids, especially docosahexaenoic acid (DHA), during drying of herring fillet and a correlation between the sensory perception and the level of free fatty acids (Shah *et al*., 2009a). Furthermore, Koriyama *et al*. (2002a) reported that the increase of DHA content (up to 59%) in oil linearly enhanced umami and flavor (continuity and richness) of synthetic tuna extract.

Although oil has no taste of its own, it alters taste perception by increasing the viscosity or fluidity of food, which affects the diffusion coefficients and retention time of taste substances in the mouth (Mela *et al.*, 1994; Pikielna *et al.*, 1994). In addition, long-chain fatty acids (LCFA, number of carbons > 16) seem to be responsible for lipid perception in the oral cavity (Smith *et al*., 2000; Fukuwatari *et al*., 2003). This observation might seem paradoxical since dietary lipids are mainly comprised of triglycerides (TG). It is known that lingual lipase is especially efficient at releasing LCFA from TG in rodents (Kawai and Fushiki, 2003). In humans, the detection threshold for LCFA is particularly low (Chale-Rush *et al*., 2007) in comparison to TG (Schiffman *et al.*, 1998), despite the lack of an efficient TG hydrolysis by lingual lipase (Hamosh, 1990). However, addition of free fatty acids to tastants

(His + lactic acid, quinine sulfate, Leu, and IMP + MSG) as oil-in-water emulsions significantly changes taste intensity (Koriyama *et al.*, 2002b). Moreover, free fatty acids found in food itself and those derived from oils by lingual lipase may play a direct role in taste perception (Gilbertson *et al*., 1997).

Marine lipids, which contain higher quantities of n-3 polyunsaturated fatty acids, are susceptible to oxidation following successive degradation (Gardner, 1983). It is known that lipid oxidation takes place in fatty fish species during processing and storage, e.g. herring lipid is susceptible to oxidation both *in situ* in the tissues and when extracted from the tissues (Shahidi and Spurvey, 1996; Undeland *et al.*, 1999). Moreover, non-conjugated all-*cis* olefinic structures in polyunsaturated fatty acyl groups in phospholipids are very sensitive to oxidation allowing hydroperoxides occurrence as a primary product (Kappus, 1985). Further degradation of them is well documented to afford aldehydes (Sun *et al*., 2002), which are reactive to biological nucleophiles such as ε-amino group in lysine. However, little is known regarding *in situ* chemical changes of phospholipid during drying of herring fillet. A combination of molecular probe and mass spectrometric analysis has proved to be a useful method in identifying the chemical changes in a specific lipid (Shimizu *et al.*, 2009). This novel approach led to the structural and functional identification of molecular species of lipid and their oxidation products such as structural changes of phospholipid hydroperoxides in human blood (Shimizu *et al.*, 2009) and tracing of phospholipases D activity (Oda *et al.*, 2009).

The objective of this study was to investigate the ability of fatty acids (capric acid, linoleic acid and DHA) to enhance flavor of dialyzed water-soluble fractions of DHF4 in Japanese noodle soup. To enable a better understanding of *in situ* chemical changes in lipid during drying of herring fillet, a synthesized phospholipid probe was inserted in the herring

fillet and chemical changes in the lipid were analyzed on ESI-MS.

Materials and Methods

Chemicals Fatty acids (capric acid, C10:0; purity: >99%, peroxide value (PV): 0.42 meq/kg and carbonyl value (CV): <0.10 meq/kg, and linoleic acid, C18:2; purity: >99%, PV: 0.80 meq/kg and CV: <0.10 meq/kg) were purchased from Nu-Chek-Prep (Elysian, MN, USA). Docosahexaenoic acid (C22:6; purity: 98.6%, PV: 1.29 meq/kg, CV: 0.17 meq/kg) was obtained from Nippon Chemical Feed Co. Ltd., Hakodate, Japan. All other chemical solvents were of analytical or HPLC grade. The phosphatidylcholine (PC) probe, 2-[(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-4,7,10,13,16,19-docosahexaenoyl]-1-heptadecanoyl-*sn*-glycero-3-phosphoethyl(*N*,*N*,*N*-dimethylethyl)ammonium (HD-DHA-PC/Et) was synthesized according to methods reported by Baba *et al*. (1990) and Shimizu *et al*. (2009).

Materials Dried herring fillets were obtained from a fishery processing company in Hakodate, Japan. Herring (*Clupea pallasii*) was captured at the coast of Kamchatka Peninsula, Russia in October 2007, and kept frozen until it was processed. Upon arrival at the factory, herring was thawed, gutted, washed and then filleted for drying. Herring fillets were dried using huge electric fans. Room temperature and relative humidity were maintained at approximately 14 °C and 45%, respectively. The herring fillets dried for 4 and 10 days were designated as DHF4 and DHF10, respectively.

Preparation of dialyzed water-soluble fractions (DWSF) Dialyzed water-soluble fractions (DWSF) were prepared from DHF4 and DHF10 following a method described by Okumura *et al.* (2004). In brief, DHF was cut into small pieces, freeze-dried and then

defatted using *n*-hexane. The defatted fillets were then homogenized in a ten-fold volume of de-ionized water. The homogenate was centrifuged at 10,000 × g for 20 min at 4 °C and the resulting supernatant was collected. Ethanol was added (final concentration, 80%) to the supernatant, followed by centrifugation and then filtration. After evaporation and further freeze-drying of the filtrate, the lyophilized powder obtained was dissolved in de-ionized water. The solution was then dialyzed against de-ionized water using 1000 and 5000 Da cut-off membranes (Spectrum Laboratories, Inc., California, USA). The DWSF (MW 1000–5000) was freeze-dried and stored at –50 °C.

Preparation of reaction products or mixture of the DWSF and free fatty acids Fatty acids such as capric acid, linoleic acid, and docosahexaenoic acid (DHA) were dissolved into 90% ethanol, then mixed with the DWSF of DHF4 at a concentration of 0.10%. The aqueous ethanol was evaporated using a rotary evaporator at 37 ºC for two hours to give reaction products of the DWSF and fatty acids. This temperature was selected because it is widely employed in accelerating non-enzymatic browning reactions of model systems. In parallel, DHA in ethanol was mixed with the DWSF of DHF4 at a concentration of 0.10%, and ethanol was instantly evaporated using a rotary evaporator at 30 ºC for 5–10 minutes to give a mixture of the DWSF and DHA.

Sensory evaluation The flavor-enhancing activity of DWSF from DHF4, DWSF from DHF10 as well as reaction products and mixture of DWSF and fatty acids on Japanese noodle soup was evaluated following the method of Ueda *et al.* (1997) with some modifications. Japanese noodle soup was prepared according to a method by Shah *et al.* (2009a). It was diluted with six volumes of distilled water and used as a control solution. Test

samples were dissolved into Japanese noodle soup at a concentration of 0.05% (w/v). After addition of the test samples to Japanese noodle soup, the solution was heated at 60 °C in a water bath. Approximately 50 mL of the test and control solutions were served in opaque disposable plastic cups at the same time. Panel members were instructed to put an adequate volume in the mouth, and then to expectorate. The panelists were asked to judge the intensities of the test samples using a scale of 1 to 7, where "3" was assigned to the control solution. Scoring was done on the basis of saltiness, umami, thickness, mouthfulness, and continuity. Sensory evaluation was performed in the separated sensory booths. The panel was composed of five trained assessors (3 male and 2 female; ages between 26 and 37 years) from the Food Research and Development Laboratory, Kirin Kyowa Food Co. Ltd., Ibaraki, Japan. All the panel members had extensive experience in tasting and agreed on the intensities of saltiness, umami, thickness, mouthfulness, and continuity in Japanese noodle soup.

In situ chemical changes of synthesized phospholipid probe in DHF Ten micro-liter of phosphatidylcholine probes, HD-DHA-PC/Et (**Fig. 1**) (11.9 mM in ethanol) were injected to a fresh herring fillet using micro-syringe. The herring fillet was then placed into a Humidic Chamber IG 400 (Yamato, Tokyo, Japan) and dried under constant temperature (14 ºC) and relative humidity (45%). After drying for 10 days, the parts of the fillet containing HD-DHA-PC/Et were cut into small pieces (10 mm × 10 mm). A mixture of chloroform/methanol (5 mL, 1:2) with a trace amount of BHT was added to each piece and filtration was done using a filter paper. After evaporating the solvent under reduced pressure, the residue was dissolved in a mixture of acetonitrile/methanol/water (215:194:16) with 0.1% ammonium acetate and infused directly into the positive ion mode tandem electrospray

ionization-mass spectrometer (ESI-MS) for spectral acquisition. ESI-MS was conducted using a Perkin-Elmer SCIEX (Thornhill, ON, Canada) API-III tandem quadrapole mass spectrometer. The scan range was *m/z* 510–850. In the product ion scan mode, derivatives from the PC probe HD-DHA-PC/Et showed a single peak at *m/z* 198 as a product ion in ms/ms scan mode, which was (*N*,*N*,*N*-dimethylethyl)ammonioethyl phosphate ion.

Statistical analysis The significant difference between the test samples was assessed using the Wilcoxon test for two independent samples and the Kruskal-Wallis test for k-independent samples ($k > 2$) at a 95% confidence level ($P < 0.05$) (Zar, 1984). This was followed by Mann-Whitney *U* test to allow identification of between-group differences. Results are presented as means, but differences were calculated with the mean ranked scores. All the analyses were performed using STATGRAPHICS *Plus* version 2.1 (StatPoint, Inc., Virginia, USA).

Results and Discussion

Taste characteristics of DWSF The DWSF were prepared from DHF4 and DHF10, and subjected to sensory analysis to evaluate their flavor-enhancing activity on Japanese noodle soup. DWSF in distilled water at a concentration of 0.10% were evaluated as nearly tasteless but acquired a faint aroma (data not shown). However, addition of the DWSF from DHF10 to Japanese noodle soup significantly ($P < 0.05$) increased the intensities of thickness and continuity of the soup flavor when compared to the DWSF from DHF4, whereas the basic taste qualities such as saltiness and umami remained almost unaffected (**Fig. 2A**). These results suggest that the DWSF from DHF10 might contain some of the flavor enhancing substances that are generated during the drying period. In our previous study, we found that

addition of DHF water-soluble extracts to Japanese noodle soup enhanced the soup flavor characters such as thickness, mouthfulness and continuity, and enhancement of these flavor characters increased with drying time (Shah *et al.*, 2009b). Therefore, we postulated that during the drying period, low molecular weight compounds such as free amino acids and peptides are formed, which might interact with free fatty acids that were released as a result of lipid hydrolysis or with lipid oxidation products such as carbonyl compounds. The interaction of these compounds would ultimately generate characteristic taste and flavor of the final product. To explore this hypothesis, the DWSF of DHF4 was reacted with DHA and then subjected to sensory evaluation. A free fatty acid form of DHA was used for the interaction reaction because significant amounts of DHA are liberated especially from polar lipids during drying of herring fillet (Shah *et al.*, 2009a). Moreover, increased level of DHA in the dried herring fillet was almost similar to the level of DHA (0.10%) added to DWSF. The addition of the reaction products of the DWSF of DHF4 and DHA to Japanese noodle soup significantly ($P < 0.05$) enhanced the intensities of thickness, mouthfulness, and continuity of Japanese noodle soup as compared to DWSF of DHF4 (**Fig. 2B**). These results suggest that DHA interacts with water-soluble compounds during the drying period giving reaction products that enhance *kokumi* in DHF. Koriyama *et al*. (2002a) also reported that increase of DHA content in oil linearly enhanced umami and flavor (continuity and richness) of synthetic tuna extract. However, results from studies on goat cheese differ from findings in this study. Engel *et al.* (2000) reports that peptides (<MW 500 Da), as well as lipid have no impact on the gustatory characteristics of reconstituted water-soluble extract in goat cheese.

Taste enhancing effects of the reaction products or mixture of DWSF and DHA Taste profile of Japanese noodle soup containing the reaction products or mixture of DHA and

DWSF from DHF4 are shown in **Fig. 3**. Sensory evaluation showed that the reaction products of the DWSF and DHA significantly ($P < 0.05$) enhanced the intensities of thickness, mouthfulness, and continuity of the Japanese noodle soup as compared to the mixture of the DWSF and DHA. These results suggest that during interaction reaction of DWSF with DHA, some reaction products are generated that enhances the flavor properties of the Japanese noodle soup. It has been reported that pyrrole formation and polymerization taking place when an unoxidized fatty acid is incubated at 37 ºC in the presence of an amino acid (Zamora *et al*., 2000). In addition, many studies have shown that lipid oxidation products are able to react with amines, amino acids, and proteins, producing various compounds which influence food quality such as browning reaction, odor and flavor formation, loss of nutritional quality, and production of compounds with antioxidant effects (Gardner, 1979; Friedman, 1996; Belitz and Grosch, 1987).

Comparison of the effect of fatty acids on the flavor enhancement of DWSF To investigate the effect of fatty acids, DWSF was reacted with capric acid (C10:0), linoleic acid (C18:2) and docosahexaenoic acid (DHA, C22:6), and sensory evaluation was performed in the Japanese noodle soup. The addition of the reaction products of DWSF and unsaturated fatty acids (linoleic and docosahexaenoic acids) resulted in a pronounced increase of flavor intensities such as thickness, mouthfulness, and continuity of the Japanese noodle soup (**Fig. 4**). The reaction products of DWSF with DHA showed significantly ($P < 0.05$) higher flavor-enhancing activity compared to the reaction products of DWSF with linoleic or capric acid. The low flavor-enhancing effect of the reaction products with capric acid might be attributed to the low affinity for fatty acid receptor expressed in the tongue. These results suggest that the flavor-enhancing properties of fatty acid interacted with DWSF depends on a

number of factors, such as carbon chain length, degree of unsaturation of the fatty acids and their interacted compounds. It has been reported that low concentrations of oxidation products are detectable and preferred by animals (Ramirez, 1992). Detection threshold of oxidized linoleic acid was found to be lower than that of linoleic or desensitization linoleic acid on human taste perception (Chale-Rush *et al*., 2007). Furthermore, taste receptor cells isolated from buds in the rat fungiform papillae depolarize in the presence of *cis*-polyunsaturated fatty acids but did not respond to monounsaturated or saturated fatty acids (Gilbertson *et al*., 1997). We also assumed that the reaction products of DWSF of DHF4 with DHA might directly affect human taste perception.

In situ chemical changes of synthesized phospholipid probe in dried herring fillet In order to investigate the *in situ* chemical changes in lipid that occurred during drying of herring fillet, ESI-MS analysis was done using phosphatidylcholine molecular probe. Polyunsaturated olefinic structures are very sensitive to oxidation and ester bonds are susceptible to hydrolysis; both are common structures for phospholipids and triglycerides (Koizumi, 1992). Therefore, we assumed that similar chemical reactions might occur both for the phospholipids and triglycerides in the herring muscle during the production of dried herring fillet. A synthetic molecular probe for triglycerides is not yet available to identify such chemical changes; therefore, phosphatidylcholine probe was selected for this study. A typical ESI-MS spectrum of the reaction products of PC probe (HD-DHA-PC/Et) in the lipid fraction of DHF is shown in **Fig. 5**. Based on the information obtained by positive-ion ESI-MS, presumed chemical structures of the compounds **1**–**6** obtained from DHF lipids are shown in **Fig. 6**. The major peak at *m/z* 834.84 (**1**, calcd. *m/z* 834.6 $[M + H]^+$) was remaining PC probe, HD-DHA-PC/Et. The most abundant peaks at *m/z* 524.46 (**2**, calcd. *m/z* 524.37 [M

+ H]$^+$) and *m/z* 582.54 (**3**, calcd. *m/z* 582.36 [M + H]$^+$) were tentatively identified as lyso-PC/Et species, suggesting that lipolysis occurred in herring fillet during the drying period (Shah *et al.*, 2009a). Another small peak was observed at *m/z* 808.62 (**4**, calcd. *m/z* 808.55 [M + H]$^+$), tentatively identified as oxidation product of DHA residue. During the oxidation of lipids, formation of carbonyl compounds such as aldehydes and ketones through the degradation of lipid hydroperoxides can therefore be predicted in DHF. In the DHF lipid, however, there is a possibility of formation of various imines (compounds **5**–**6**) during the drying period. Presumed structure of compound **5** (calcd. *m/z* 561.33 [M + H]$^+$) might be the interacted compounds i.e. amine conjugated with aldehydes. These results indicate that during the drying period, products of lipid oxidation might interact with amino acid related compounds to generate the characteristic taste and flavor of the DHF. It has been reported that some lipid oxidation products are able to react with the ε-amino groups of the lysine residues producing pyrrole amino acids, and these compounds may be in part responsible for some of the changes produced in foods as a consequence of oxidized lipid/protein reactions (Hidalgo and Zamora, 1993; Zamora *et al.*, 2000). Previous work showed that available lysine content in herring fillet decreased significantly ($P < 0.05$) as drying progressed indicating that non-enzymatic browning occurred due to the interaction between carbonyl compounds formed by lipid oxidation and ε-amino groups of lysine (Shah *et al.*, 2009b).

Results of this study demonstrated that the reaction products of DWSF of DHF4 with DHA strongly enhanced the soup flavor characteristics, such as thickness, mouthfulness, and continuity, so-called *kokumi*. Moreover, *in situ* chemical changes of lipid revealed that a small amount of secondary oxidation products and their reaction products were generated during drying of herring fillet. Thus, it can be concluded that during the drying period, partial hydrolysis of lipids released free fatty acids, which directly or their oxidation products might

react with amino compounds (such as lysine) to generate the characteristic taste and flavor of DHF. Identification of the *kokumi* imparting compounds as well as structure-taste activity relationships of these compounds are currently under investigation and will be published elsewhere.

Acknowledgment

The authors thank the members of the sensory panel of Food Research and Development Laboratory, Kirin Kyowa Food Co. Ltd., Ibaraki, Japan, for their help with sensory assessments.

References

Baba, N., Yoneda, K., Tahara, S., Iwasa, J., Kaneko, T. and Matsuo, M. (1990). A regioselective, stereoselective synthesis of a diacylglycerophosphocholine hydroperoxide by use of lipoxygenase and lipase. *J. Chem. Soc. Chem. Commun.*, 1281–1282.

Belitz, H.D. and Grosch, W. (1987). "Food Chemistry". Springer-Verlag, Berlin.

Chale-Rush, A., Burgess, J.R. and Mattes, R.D. (2007). Evidence for human orosensory (taste?) sensitivity to free fatty acids. *Chem. Senses*, **32**, 423–431.

Engel, E., Nicklaus, S., Garem, A., Septier, C., Salles, C. and Le Quere, J.L. (2000). Taste active compounds in a goat cheese water-soluble extract. 1. Development and sensory validation of a model water-soluble extract. *J. Agric. Food Chem.*, **48**, 4252–4259.

Friedman, M. (1996). Food Browning and its prevention: an overview. *J. Agric. Food Chem.*, **44**, 631–653.

Fukuwatari, T., Shibata, K., Iguchi, K., Saeki, T., Iwata, A., Tani, K., Sugimoto, E. and Fushiki, T. (2003). Role of gestation in the recognition of oleate and triolein in anosmic rats. *Physiol. Behav.*, **78**, 579–583.

Gardner, H.W. (1979). Lipid hydroperoxide reactivity with proteins and amino acid: A review. *J. Agric. Food Chem.*, **27**, 220–229.

Gardner, H.W. (1983). Effect of lipid hydroperoxides on food components. In "Xenobiotics in Foods and Feeds" ed. by J.W. Finley and D.E. Schwass. American Chemical Society, Washington, DC, pp. 63–84.

Gilbertson, T.A., Fontenot, D.T., Liu, L., Zhang, H. and Monroe, W.T. (1997). Fatty acid modulation of K^+ channels in taste receptor cells: gustatory cues for dietary fat. *Am. J. Physiol.*, **272**, C1203–1210.

Hamosh, M. (1990). Lingual and gastric lipases. *Nutrition*, **6**, 421–428.

Hidalgo, F.J. and Zamora, R. (1993). Fluorescent pyrrole products from carbonyl-amine reactions. *J. Biol. Chem.*, **268**, 16190–16197.

Kappus, H. (1985). "Oxidative Stress". Academic Press, London, New York.

Kawai, T. and Fushiki, T. (2003). Importance of lipolysis in oral cavity for orosensory detection of fat. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **285**, R447–454.

Koizumi, C. (1992). Lipid. In "Fisheries Utilization Chemistry", ed. by S. Konosu & K. Hashimoto. Kouseisha Kouseikaku, Tokyo, pp. 75–102.

Koriyama, T., Kohata, T., Watanabe, K. and Abe, H. (2002a). Effects of docosahexaenoic acid content in triacylglycerol on human taste perception. *J. Food Sci.*, **67**, 2352–2356.

Koriyama, T., Wongso, S., Watanabe, K. and Abe, H. (2002b). Fatty acid compositions of oil species affect the 5 basic taste perceptions. *J. Food Sci.*, **67**, 868–873.

Mela, D.J., Langley, K.R. and Martin, A. (1994). Sensory assessment of fat content: effect of emulsion and subject characteristics. *Appetite*, **22**, 67–81.

Oda, K., Imura, M., Ueda, Y., Hosokawa, M., Kobayashi, M., Matsubara, J., Sato, M., Kumura, N., Izumi, M., Nakajima, S., Sugio, T. and Baba, N. (2009). A combination of

unnatural phosphatidyl acceptor and tandem electrospray ionization mass spectrometry for tracing phospholipase D activity. *Biosci. Biotechnol. Biochem*., **73**, 1233–1237.

Okumura, T., Yamada, R. and Nishimura, T. (2004). Sourness-suppressing peptides in cooked pork loins. *Biosci. Biotech. Biochem.,* **68**, 1657–1662.

Pikielna, N.B., Martin, A. and Mela, D.J. (1994). Perception of taste and viscosity of oil-in-water and water-in-oil emulsions. *J. Food Sci.,* **59**, 1318–1321.

Ramirez, I. (1992). Chemoreception for fat: do rats sense triglycerides directly. *Appetite,* **18**, 193–206.

Schiffman, S.S., Graham, B.G., Sattely-Miller, E.A. and Warwick, Z.S. (1998). Orosensory perception of dietary fat. *Curr. Dir. Psychol. Sci*., **7**, 137–143.

Shah, A.K.M.A., Tokunaga, C., Kurihara, H. and Takahashi, K. (2009a). Changes in lipids and their contribution to the taste of *migaki-nishin* (dried herring fillet) during drying. *Food Chem.*, **115**, 1011–1018.

Shah, A.K.M.A., Tokunaga, C., Ogasawara, M., Kurihara, H. and Takahashi, K. (2009b). Changes in chemical and sensory properties of *migaki-nishin* (dried herring fillet) during drying. *J. Food Sci.*, **74**, S309–314.

Shahidi, F. and Spurvey, S.A. (1996). Oxidative stability of fresh and heated-processed dark and light muscles of mackerel (*Scomber scombrus*). *J. Food Lipids*, **3**, 13–25.

Shimizu, R., Nagai, A., Tominaga, H., Imura, M., Onyango, A.N., Izumi, M., Nakajima, S., Tahara, S., Kaneko, T. and Baba, N. (2009). A combination of molecular probe-tandem electrospray ionization mass spectrometry: A technique for tracing structural changes in phospholipid hydroperoxides. *Biosci. Biotechnol. Biochem.*, **73**, 781–784.

Smith, J.C., Fisher, E.M., Maleszewski, V. and McClain, B. (2000). Orosensory factors in the ingestion of corn oil/sucrose mixtures by the rat. *Physiol. Behav.,* **69**, 135–146.

Sun, M., Deng, Y., Batyreva, E., Sha, W. and Salomon, R.G. (2002). Novel bioactive phospholipids: practical total syntheses of products from the oxidation of arachidonic and linoleic esters of 2-lysophosphatidylcholine. *J. Org. Chem.*, **67**, 3575–3584.

Ueda, Y., Yonemitsu, M., Tsubuku, T., Sakaguchi, M. and Miyajima, R. (1997). Flavor characteristics of glutathione in raw and cooked foodstuffs. *Biosci. Biotech. Biochem.*, **61**, 1977–1980.

Undeland, I., Hall, G. and Lingnert, H. (1999). Lipid oxidation in fillets of herring (*Clupea harengus*) during ice storage. *J. Agric. Food Chem.*, **47**, 524–532.

Zamora, R., Alaiz, M. and Hidalgo, F.J. (2000). Contribution of pyrrole formation and polymerization to the nonenzymatic browning produced by amino-carbonyl reactions. *J. Agric. Food Chem.*, **48**, 3152–3158.

Zar, J.H. (1984). Biostatistical Analysis, 2nd edition. Prentice-Hall, New Jersey.

Figure Captions

Fig. 1. Chemical structure of synthesized phosphatidylcholine probe.

Fig. 2. Taste profile of Japanese noodle soup containing **(A)** the dialyzed water-soluble fractions from the herring fillets dried for 4 and 10 days, and **(B)** reaction products of the dialyzed water-soluble fraction of the herring fillet dried for 4 days and DHA. Taste intensity was scored on a 7-point scale where 3 points were assigned to the Japanese noodle soup. Means with different letters in each attribute indicate significant differences ($P < 0.05$).

Fig. 3. Taste profile of Japanese noodle soup containing DHA and the dialyzed water-soluble fraction (DWSF) from the herring fillet dried for 4 days. Taste intensity was scored on a 7-point scale where 3 points were assigned to the Japanese noodle soup. Means with different letters in each attribute indicate significant differences ($P < 0.05$).

Fig. 4. Taste profile of Japanese noodle soup added with the reaction products of the dialyzed water-soluble fraction from 4 days-dried herring fillet and fatty acids. Taste intensity was scored on a 7-point scale where 3 points were assigned to the Japanese noodle soup. Bars represent mean ± SD (n = 5). Means with different letters in each attribute indicate significant differences ($P < 0.05$).

Fig. 5. A typical ESI-MS spectrum of the reaction products of phosphatidylcholine probe recovered from dried herring fillet. Precursor ion scan mode was selected using *m/z* 198 as product ion.

Fig 6. Presumed chemical structures of the compounds identified from dried herring fillet lipids by ESI-MS.

Fig. 1 (Shah et al.)

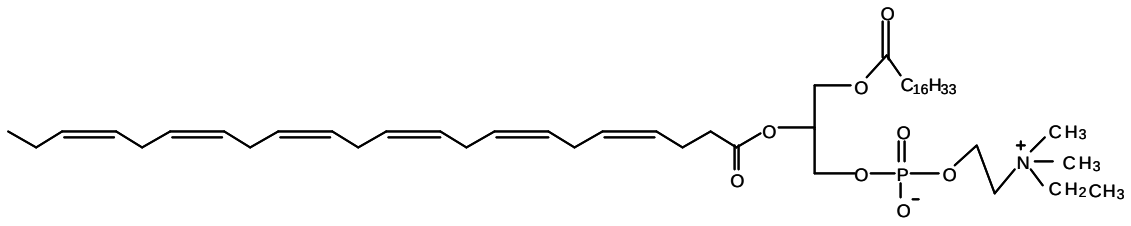
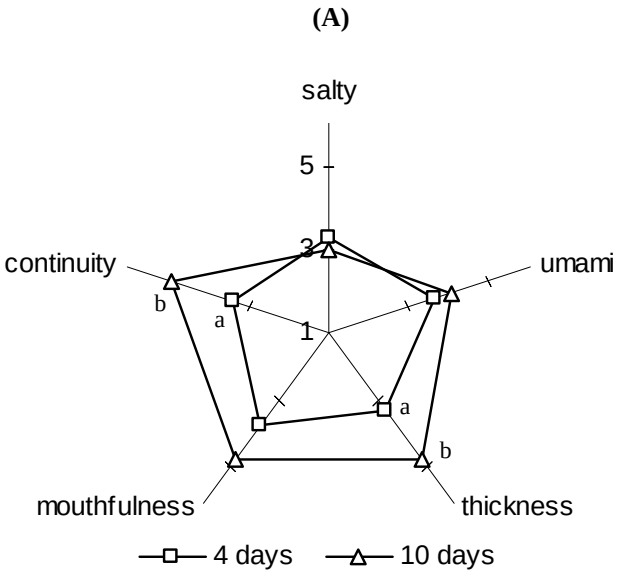


Fig. 2 (Shah et al.)



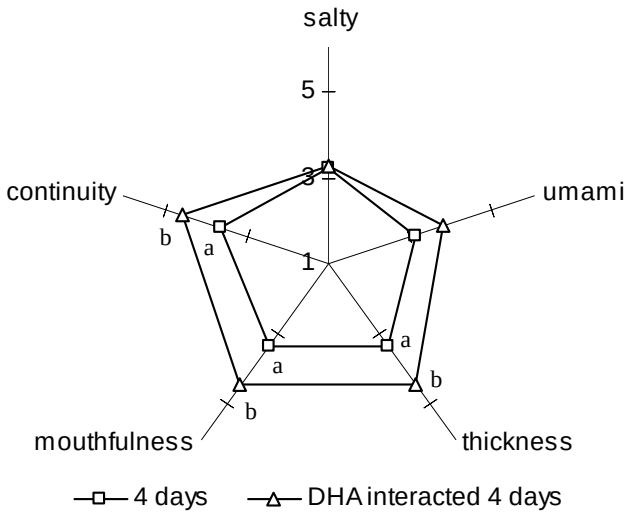


Fig. 3 (Shah et al.)

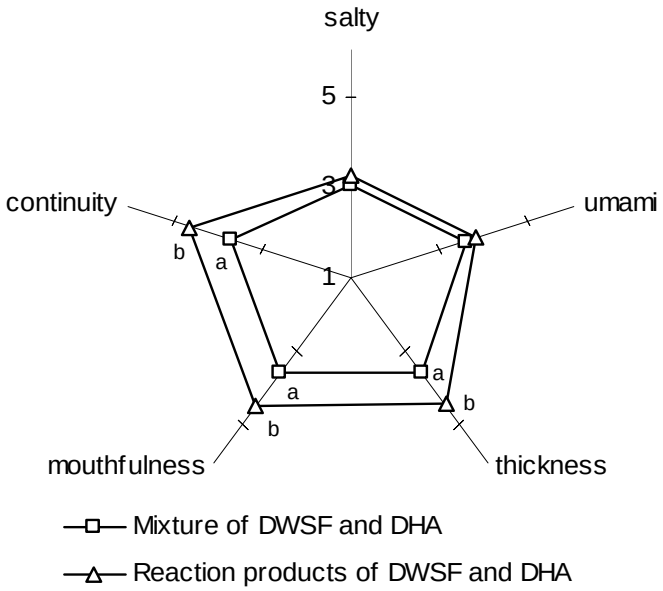


Fig. 4 (Shah et al.)

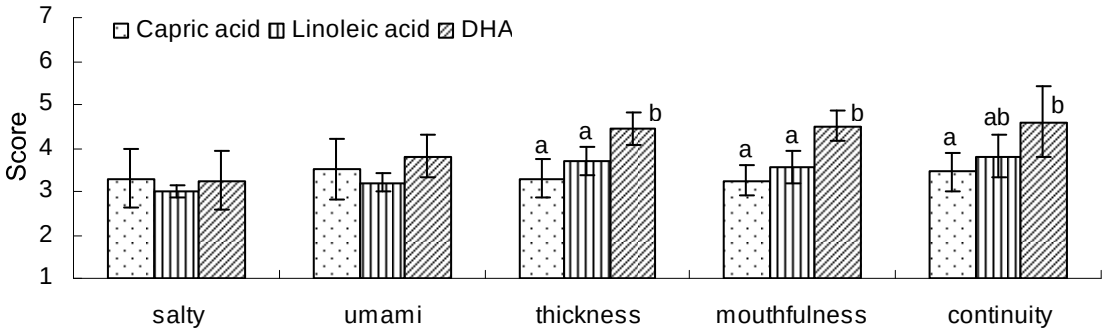


Fig. 5 (Shah et al.)

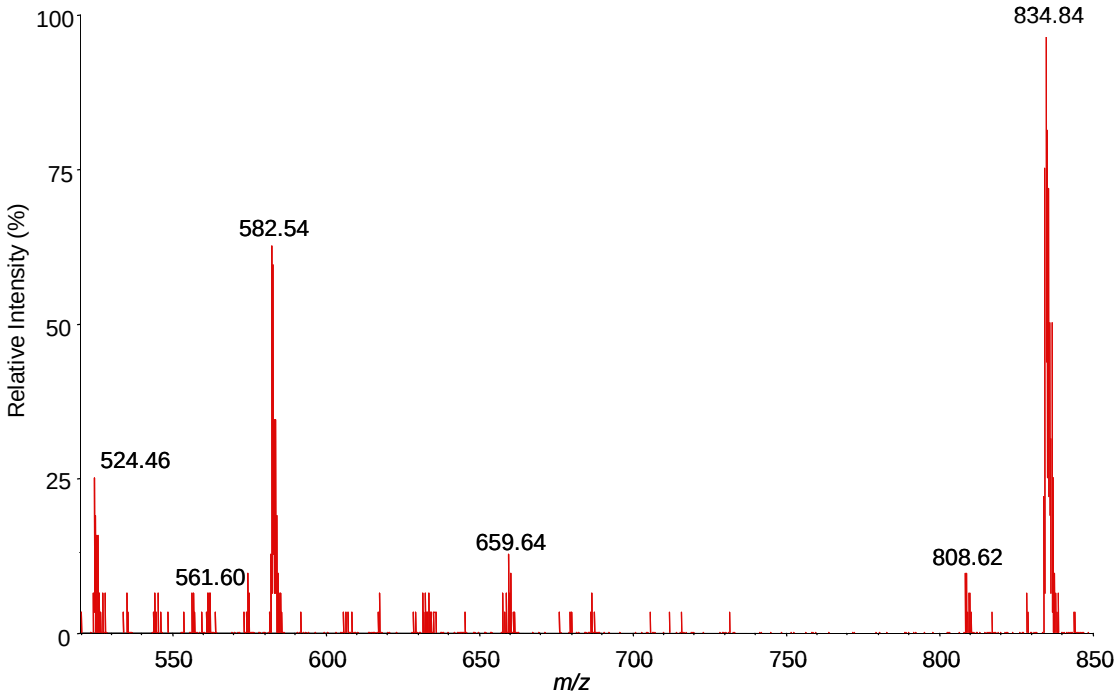


Fig. 6 (Shah et al.)