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学 位 論 文 題 名

# Mechanisms Involved in the Formation of Characteristic Taste and Flavor during the Production of Dried Herring Fillet

（身欠きニシン製造過程中におけるこくの発現機構に関する研究）

## 学位論文内容の要旨

Dried herring fillet ("Migaki-nishin" in Japanese) is a traditionally popular food item in Japan due to its remarkable flavor enhancing properties. In particular, addition of dried herring fillet to noodle soup enhances flavor characteristics such as thickness, mouthfulness, and taste continuity. These flavor characteristics are often called *koku* in Japanese. It is thought that herring fillets obtain their unique taste and flavor during drying process. During drying of herring fillet, a large number of biochemical reactions occur and some of them can lead to the formation of characteristic taste and flavor of the final product. The aim of this study was to investigate the mechanisms responsible for the generation of characteristic taste and flavor during the production of dried herring fillet.

In chapter 1, physicochemical and sensory changes in herring fillet during drying are described. Herring fillet was obtained from five different drying stages (2, 4, 6, 8 and 10 days) and analyzed for proximate composition, color, browning intensity, available lysine, and free amino groups. Water-soluble extracts (WSE) were prepared and analyzed by HPLC and fluorescence spectrophotometer to determine Maillard reaction products. Sensory evaluation was carried out to determine the flavor characteristics of WSE in the Japanese noodle soup. Chemical analyses showed that extractive nitrogen and amount of peptides increased significantly ($P < 0.05$) up to the 8th day of drying and then slightly decreased by the 10th day. Glutamic acid + glutamine, alanine, glycine, and histidine were the most abundant free amino acids and the largest increase was found in samples dried for 10 days. A decrease in Hunter's $L^*$ value (lightness) and increase in $b^*$ value

(yellowness) as well as browning intensity suggested that nonenzymatic browning occurred in herring fillet during drying. Fluorescence spectrophotometric determination also revealed that Maillard reactions progressed throughout the drying period. In addition, available lysine content and free amino groups decreased significantly ($P < 0.05$) as drying progressed. Sensory evaluation showed that addition of WSE to Japanese noodle soup significantly enhanced the flavor characteristics such as thickness, mouthfulness and continuity with the increased length of drying time. These results suggest that during the drying period, proteolysis should occur to cleave off the taste enhancers. At the same time Maillard reaction products must increase markedly, which might partially contribute to the characteristic taste and flavor of dried herring fillet.

In chapter 2, experiments were conducted to investigate the changes in lipids and their contribution to the taste of dried herring fillet. Lipid was extracted from herring fillets following different drying stages to measure the degree of lipid oxidation, and changes in lipid composition and fatty acid profile. Flavor characteristics of dried herring lipid and refined docosahexaenoic acid (DHA) were evaluated on sensory perception, because DHA was exclusively released from phospholipids during drying. Peroxide value, carbonyl value and acid value of the lipids were significantly ($P < 0.05$) increased during the drying period. Marked increase in free fatty acids, with decreases in triacylglycerol and phospholipid content were observed in proportion to drying time. This result suggested that hydrolysis of triacylglycerol and phospholipid should have take place, caused by lipases and phospholipases. The decreases in polyunsaturated fatty acids (PUFAs), especially DHA, were observed in the total lipid and phospholipid fraction. On the contrary, significant increase in PUFAs, especially DHA was found in the free fatty acid fraction. Sensory evaluation showed that addition of DHA to Japanese noodle soup significantly ($P < 0.05$) enhanced the intensities of thickness, mouthfulness and continuity of the soup flavor. These results suggest that during the drying period, not only lipid oxidation occurs but also lipolysis predominantly releases DHA, which should have at least in part contribute to the characteristic taste and flavor of dried herring fillet.

In chapter 3, flavor enhancing *koku* components of dried herring fillet were isolated and

evaluated for their effects on sensory perception. Sensory evaluation revealed that addition of dried herring fillet water-soluble extracts to Japanese noodle soup significantly ($P < 0.05$) enhanced the soup flavor characteristics such as thickness, mouthfulness and continuity. The extracts were fractionated by dialysis and chromatography. Fractions containing flavor enhancers were isolated by sensory perception. Results from instrumental analyses showed that the *koku* flavor enhancers in dried herring fillet were creatine and creatinine. Creatine and creatinine content in dried herring fillet were determined to be 1068.77 and 170.06 mg/100 g dry matter, respectively.

Mechanisms responsible for the characteristic taste and flavor of dried herring fillet were elucidated in chapter 4. This chapter also describes *in situ* chemical changes in lipid during drying of herring fillet. Dialyzed water-soluble fractions (DWSF) were prepared from herring fillet dried for 4- (DHF4) and 10-days (DHF10), and subjected to the sensory evaluation. Addition of the DWSF from DHF10 to Japanese noodle soup resulted in pronounced mouthfulness and complex body (thickness) as well as more long lasting taste sensation when compared to the DWSF from DHF4. This suggests that DWSF from DHF10 might contain some of the *koku* imparting compounds that are generated during the drying process. To elucidate the mechanism responsible for the characteristic *koku* in dried herring fillet, DWSF from DHF4 was interacted with DHA and the reaction products were evaluated for their effects on sensory perception. The addition of the reaction products of DWSF from DHF4 with DHA to Japanese noodle soup significantly ($P < 0.05$) enhanced the intensities of thickness, mouthfulness, and continuity of the Japanese noodle soup as compared to the DWSF from DHF4. Furthermore, to investigate the *in situ* chemical changes in dried herring lipid, ESI-MS analysis was done using phosphatidylcholine molecular probe. ESI-MS analyses showed that lyso-derivatives were the most abundant compounds in the lipid fraction of dried herring fillet. In addition, a small amount of lipid oxidation products and their reaction products were also observed in dried herring fillet. These results demonstrated that during the drying period, partial hydrolysis should occur to release free fatty acids. These free fatty acids or their oxidation products (also generated during drying) might react with amino acid related compounds to generate the characteristic taste and flavor of dried herring fillet.

In conclusion, at least four aforementioned factors should be involved in generating the characteristic *koku* of dried herring fillet.

# 学位論文審査の要旨

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学位論文題名

# Mechanisms Involved in the Formation of Characteristic Taste and Flavor during the Production of Dried Herring Fillet

## (身欠きニシン製造過程中におけるこくの発現機構に関する研究)

身欠きニシンは、その特有のこくが好まれている日本の伝統的な食品の一つである。本研究はそのこくの本体を解明し、こくの不足しがちな加工食品の品質改善のための基礎的知見を得ることを目的として行ったものである。

第1章では身欠きニシン製造中の乾燥工程における成分組成変化、色調の変化、有効性リジン量及び遊離アミノ酸量の変化について調べた。また、水抽出物量及びメラノイジン量の変化についても分析した。その結果、色調変化においては白色度に相当する $L^*$値の減少、黄色度に相当する $b^*$値の増大、褐色度の増大を認め、また、メラノイジン量の増大も蛍光分光分析法によって認めた。有効性リジン量は乾燥の進行とともに減少したのに対し、遊離のグルタミン酸、グルタミン、アラニン、ヒスチジンは増大を示した。可溶性窒素量及びペプチド量は乾燥過程の中盤過ぎまでは増加したが、最終日には僅かな減少が認められた。乾燥経過日ごとに水抽出を行い、抽出物をめんつゆに入れてプロのパネリストによる官能評価を行った結果、乾燥の終盤に近づくにつれて、味の厚み、持続性に代表されるこくが増すことを認めた。これらのことからShahは味の濃厚さに寄与するといわれるヒスチジンの増加に加え、メラノイジン及びリジンとの反応物の増加が身欠きニシンのこくの発現に一部関与している可能性が高いことを示した。

身欠きニシンは脂質含量が高いことから、第2章では脂質の変化がこくの発現に大きく影響していると考え、身欠きニシン製造の乾燥工程中における脂質組成及び脂肪酸組成の変化、さらに脂質酸化度の変化についても調べた。その結果、脂質組成の変化においては、トリアシルグリセロール及びリン脂質の減少、遊離脂肪酸の増加を認めた。リン脂質画分にはドコサヘキサエン酸(DHA)が多く、遊離脂肪酸画分においてもDHAは最も組成比の高い不飽和脂肪酸であった。そこで、遊離脂肪酸形態のDHAをめんつゆに分散させて官能評価を行ったところ、明らかなこくの向上を認めた。一方、リノール酸や中鎖の脂肪酸について同様に調べた結果においては、こくへの影響を認め得なかった。このことから、Shahは身欠きニシン製造の乾燥工程中に、特にリン脂質から遊離したDHAがこくの発現に強く関与していることを証明するに至った。

続く第3章では先ず加熱水抽出物及び室温水抽出物双方にめんつゆに対するこくの附与効果があることを認め、次いで透析膜を用いて室温水抽出物を分子量1,000以下、1,000～5,000、5,000以上の3画分に分けて、それぞれのめんつゆに対するこくの附与効果について検討した。その結果、分子量1,000～5,000の画分にめんつゆに対するこく附与効果があることが判明したので、さらに高速ゲル浸透クロマトグラフィー、逆相高速液体クロマトグラフィーで分画精製を進め、最終的に二つのピークにめんつゆに対するこくの附与効果があることを突き止めた。質量分析及び核磁気共鳴スペクトル分析の結果、Shah はそれらがそれぞれクレアチン及びクレアチニンであることを明らかにすることに成功した。クレアチン及びクレアチニンの比率を種々変えて、めんつゆに対するこくの附与効果を調べた結果、クレアチンとクレアチニンが 95:5(w/w)から70:30(w/w)の範囲にあるときに、明らかなこくの附与効果を持つことが判明した。

最後の第4章では身欠きニシンのこく物質の形成に至るプロセスの再現モデル実験を先ず行った。すなわち、身欠きニシン製造中の乾燥工程初期段階のニシン筋肉より水抽出物を得、それを遊離脂肪酸形態の DHA と 40℃で2時間相互作用させた。相互作用生成物をめんつゆに加えたところ、より明確なこく附与効果を認めた。このことから Shah は、身欠きニシン製造中の乾燥工程で、特にリン脂質から DHA が遊離し、乾燥期間中に水溶性抽出物と相互作用して、独特のこくの形成に至るものと考えた。リン脂質から DHA が遊離すると、リゾリン脂質が残る筈である。そこで Shah は、質量分析計で分子構造の変化を容易に追える DHA 結合型ジアシルリン脂質プローブを合成し、それを原料開きニシンの筋肉内に注入して、身欠きニシン製造の乾燥工程中のリン脂質の変化を再現させた。その結果、明らかなリゾリン脂質のイオンピーク生成を認め、また同時に各種の少量酸化生成物を確認するに至った。

以上の知見より、Shah は身欠きニシン製造の乾燥工程において、特にリン脂質から DHA が遊離し、その DHA もしくは DHA の初期酸化物と、分子量 1000-5000 の水溶性画分とが相互作用して生成した物質が、こくの形成に最も大きく寄与している可能性が高いことを明らかにした。またこのことに加え、Shah はヒスチジン、メラノイジン及びリジンとの反応物、さらにはクレアチンとクレアチニンが一定の範囲の比率で存在していること等が複合的に身欠きニシン特有のこく発現に寄与していることも明らかにした。斯様に複雑な水産食品のこくの解明の先鞭をつけた同氏の功績は極めて大きいと判断される。申請者 Shah の研究成果は、これまで未解明であった伝統的な代表的水産食品の一つである身欠きニシンのこくの発現機構の一端を明らかにし、水産食品学の分野において重要な知見を見い出したものと位置づけられる。

よって審査員一同は申請者が博士（水産科学）の学位を授与される資格のあるものと判定した。