

Identification of fish species in dried fish products by immunostaining using anti-myosin light chain antiserum

Y. Ochiai, S. Watabe*

*Laboratory of Aquatic Molecular Biology and Biotechnology, Graduate School of Agricultural and Life Sciences,
The University of Tokyo, Yayoi, Tokyo 113-8657, Japan*

Received 27 July 2003; accepted 28 July 2003

Abstract

In order to immunologically detect fish species used in boiled and dried fish products (dried fish sticks), the availability of anti-serum against myosin light chains was examined. After dissolving the samples in the presence of 8 M urea and 1% sodium dodecyl sulfate (SDS), the solubilized matters were applied to SDS–polyacrylamide gel electrophoresis and electroblotted onto PVDF membranes. Subsequently, protein bands concerned were stained using anti-myosin light chain (alkali light chain 1) rabbit anti-serum and horseradish peroxidase-conjugated anti-rabbit immunoglobulin. Most of the fish species could be identified by fingerprinting of immunostained patterns even after drastic processing, even though the protein staining patterns were not clear enough for species identification.

© 2003 Elsevier Ltd. All rights reserved.

Keywords: Myosin light chain; Species authentication; Antiserum; Immunodetection; Fish stick

1. Introduction

Species identification of origin of meat and fish in processed muscle foods by biochemical techniques has been of great interest in the field of food science for the last few decades. Recently, authenticity of food labeling, especially for used materials, has been emphasized due to the strengthened labeling regulations in many countries across the world. Tremendous efforts have been made to identify animal species basically by electrophoretic analyses of proteins (Chen & Hwang 2002; Etienne et al., 2000; Hayden, 1977; Mackie, 1969; Pineiro, Barros-Velazquez, Sotelo, Perez-Martin & Gallardo, 1998; Pineiro, Vazquez, Marina, Barros-Velazquez & Gallardo, 2001; Seki, Takayasu & Kokuryo, 1980).

The methods for species identification have greatly advanced in recent years owing to the progress in DNA manipulation techniques such as the polymerase chain reaction (PCR) (Bossier, 1999; Partis, Croan, Guo, Clark, Coldham, & Murby, 2000; Quinteiro et al., 1998;

Ram, Ram, & Baidoun, 1996; Takagi & Taniguchi, 1995) and related techniques (random amplification of polymorphic DNA, restriction fragment length polymorphism), using genomic or mitochondrial DNA, targeting cytochrome *b* or ribosomal RNA (Asensio et al., 2001; Chapela et al., 2002; Colgen, O'Brien, Maher, Shilton, McDonell, & Ward, 2001; Elvevoll, Sorensen, Osterud, Ofstad, & Martinez, 1996; Hold et al., 2001; Lahiff et al., 2001; Martinez & Yman, 1998; Matsunaga et al., 1999; Palumbi & Cipriano, 1998; Quinteiro et al., 2001; Rego, Martinez-Tizon, Vieites, Leira, & Mendez, 2002). These methods have become powerful tools provided that the target meat (fish) of products are raw or only mildly cooked (Downey, McElhinney, & Fearn, 2000; Hunt, Parkes, & Luley, 1997; Mackie, 1969). In the case of intensively processed meat or fish, however, animal species identification is practically difficult. DNA is considered to be more thermostable compared to proteins, and thus DNA techniques have been preferentially applied to drastically processed food such as canned fish (Quintero et al., 1998; Ram, Ram, & Baidoun, 1996). However, there is also a drawback in DNA techniques, namely intraspecies variability in partial nucleotide sequences, causing unexpected digestion patterns by restriction enzymes (Quintero et al., 1998;

* Corresponding author. Tel.: +81-3-5841-7520; fax: +81-3-5841-8166.

E-mail address: awatabe@mail.ecc.u-tokyo.ac.jp (S. Watabe).

Ram, Ram, & Baidoun, 1996). Therefore, it is still necessary to explore better marker proteins for species identification.

During processing of dried fish stick (“fushi”) and boiled and dried whole fish (“niboshi”), fish muscle becomes as hard as a stone and even glassy. Fushi is usually made from middle sized red-fleshed fish such as skipjack, bonito, mackerel, and so on, while niboshi is usually made from small sized red-fleshed fish such as sardine. Boiling time is usually as long as 60–90 min, and moisture is reduced to as low as 14–16%. Especially in case of fushi, drying is reinforced by molding with genera *Penicillium* and *Aspergillus*. The effect of such excessive drying on species identification of material fish has not yet been characterized. On the other hand, adulteration or imitating these products using fish material of low commercial value is against food sanitation laws.

Fish white skeletal muscle myosin contains three kinds of light chain, namely, alkali light chains (A1, A2) in decreasing order of molecular weights and dithio-bis-2-nitrobenzoate (DTNB) light chain (Watabe, Ochiai, & Hashimoto, 1982). Myosin light chains used to be considered a good parameter for animal species identification for highly processed foods because of its species-specific composition and mobility on electrophoretic gels (Seki et al., 1980). However, the protein bands corresponding to the myosin light chain region were ambiguous after protein staining and inadequate for species identification.

Recently, we proposed an immunological method to quantify the content of dark muscle in the mince and processed fish gel (kamaboko) using antisera against myosin light chain of mackerel white and dark muscles (Ochiai, Ochiai, Hashimoto, & Watabe, 2001). In the previous study, myosin light chains were found to be still extractable even after processing to kamaboko. The finding encouraged us to attempt fish species identification in more intensively processed fish. In order to examine simple, fast, and reliable methods to authenticate the fish species used in dried fish products, an immunological technique was attempted using antisera against myosin light chains. In this study, the same antisera were used for the species identification of material fish of fushi and niboshi, both of which are the essential seasonings for Japanese traditional dishes.

2. Materials and methods

2.1. Samples

Fresh specimens of mackerel (*Scomber japonicus*), skipjack (*Katsuwonus pelamis*), bonito (*Auxis tapeinotoma*), sardine (*Sardinops melanostictus*), round herring (*Etrumeus micropus*), walleye pollack (*Theragra chalc-*

gramma), yellowfin tuna (*Thunnus albacares*), and horse mackerel (*Trachurus japonicus*) were purchased from local fish markets. The fish were transported to our laboratory in ice, and white skeletal (ordinary) muscle was immediately excised without a trace of dark muscle. The excised muscle was stored frozen at -40°C until used.

Fushi prepared from skipjack, bonito, mackerel, sardine, horse mackerel, and yellow fin tuna, and niboshi prepared from sardine, round herring, and anchovy were obtained from the local suppliers.

2.2. Preparation of proteins

Myofibrils were prepared according to Yang, Okitani, and Fujimaki (1970) and were finally suspended in 0.16 M KCl. Myosins were prepared according to Stafford, Szentkiralyi, and Szent-Gyorgyi (1979) from white muscles of mackerel, skipjack, round herring, and walleye pollack. Al light chains were isolated by the method consisting of urea treatment and DEAE-cellulose column chromatography according to Gazith, Himmel-farb, and Harrington (1970), Weeds and Lowey (1971), Lowey and Holt (1972), and Watabe, Dinh, and Hashimoto (1984) except that the slope of linear gradient (potassium phosphate concentration) was in the range of 50 mM–0.4 M.

2.3. Preparation of antisera

Antisera against Al light chains (from the white skeletal muscles of mackerel, skipjack, sardine, horse mackerel, and walleye pollack) were raised in adult female rabbits as previously reported by Watabe, Dinh, Ochiai, and Hashimoto (1983). The sera were kept frozen at -80°C until they were used.

2.4. Solubilization of protein in fish stick

White skeletal muscles, myofibrils, and the products were dissolved in 10 vol of 0.01 M sodium phosphate buffer (pH 7.2) containing 8 M urea, 1% SDS, 1% 2-mercaptoethanol, and 0.1 mM phenylmethanesulfonyl fluoride (PMSF), and were allowed to stand overnight at room temperature. The supernatant obtained by centrifugation ($10,000\times g$, 10 min) was exhaustively dialyzed against 0.01 M sodium phosphate buffer (pH 7.2) containing 0.1% SDS, 0.1% 2-mercaptoethanol, and 0.1 mM PMSF to remove urea, and further dialyzed against the same buffer without SDS.

2.5. Electrophoresis

SDS-PAGE was performed using polyacrylamide gradient gels (10–20%) according to Laemmli (1970) using slab gels (2 mm in thickness). The amount of proteins applied to each lane was adjusted to 15–20 μg .

After the run, protein bands were stained with Coomassie brilliant blue 250-R.

2.6. Immunoblotting

After the run, the proteins on SDS gels were electrophoretically transblotted to polyvinylidene fluoride (PVDF) membranes (Millipore Co., Bedford, MA, USA). After blocking with bovine serum albumin, the membranes were treated with tris(hydroxymethyl)aminomethane-buffered saline (TBS) containing 0.1% anti-A1 light chain antiserum, and stained by treatment with anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (Sigma Chemical Co., St. Paul, MO, USA), and stained with diaminobenzidine (Dojin, Kumamoto, Japan) and hydrogen peroxide (Wako, Osaka, Japan).

2.7. Other analytical methods

A double immunodiffusion test was carried out according to [Ouchterlony \(1958\)](#). Protein concentration was determined according to [Lowry, Rowsbrough, Farr, and Randall \(1951\)](#).

3. Results and discussion

[Etienne et al. \(2000\)](#) claimed that urea extraction is less effective than SDS treatment. In the present experiment, however, proteins were extracted in the presence of both urea and SDS. Efficiency of extraction of proteins was therefore considered to be very high. In this study, A1 light chain was chosen among three molecular species of myosin light chains, because it is easy to be isolated ([Watabe, Dinh, & Hashimoto, 1984](#)) and gives sufficient level of cross-reaction among different fish species ([Ochiai, Watabe, & Hashimoto, 1988](#); [Watabe, Dinh, Ochiai, & Hashimoto, 1983](#)). Anti-A1 antisera formed single precipitation lines irrespective of the combination of antisera and antigens (myosin light chains) from different species. [Fig. 1](#) shows the precipitation lines formed when anti-skipjack A1 antiserum was diffused in 1% agar gel plate against A1 light chains from several fish species. Strong cross-reactivity was observed even between phylogenetically remote species (such as skipjack and pollack). This is in good accordance with our previous report ([Ochiai et al., 1988](#)).

As shown in [Fig. 2](#), when white skeletal muscle from several fish species was solubilized in the presence of urea and SDS, run on SDS gel, and immunostained with several antisera against A1 light chain, strong cross-reaction was observed not only among 3 molecular species of myosin light chains (A1, A2, and DTNB light chain, refer to [Watabe, Ochiai, & Hashimoto, 1982](#)), but also with other proteins of higher molecular weight.

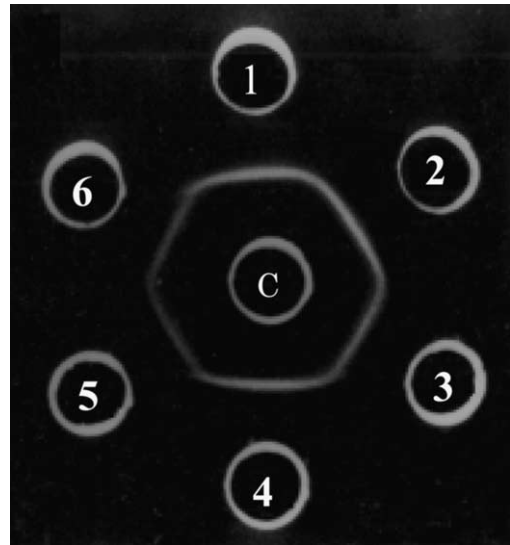


Fig. 1. Double immunodiffusion in 1% agar gel against homologous and heterologous light chains from various fish species. In the central well (C), 1 μ l of each antiserum was poured (anti-skipjack myosin A1 light chain antiserum in this figure). Into the peripheral wells was poured 1 μ g of A1 light chain purified from the white skeletal muscle of each species. The agar plate was then left in a humid box at 25 °C for 12 h, and the precipitation lines formed were observed. 1, skipjack; 2, bonito; 3, round herring; 4, skipjack; 5, sardine; 6, walleye pollack.

We observed strong cross-reactivity of myosin light chains among different fish species when myosin light chain fractions from various fish species were reacted with anti-myosin light chain antisera ([Ochiai, Kobayashi, Handa, Watabe, & Hashimoto, 1989](#)).

It was suggested that these stained proteins (including those of higher molecular weight) possess, in part, the same or similar epitopes as in A1 light chain. It is not likely that such cross-reaction was due to contaminating proteins in the myosin light chain fraction used for antisera preparation, because the mobility of stained bands other than those of A1 light chains was clearly different among the antisera tested. It is possible that the stained proteins have EF hand motifs (helix–loop–helix structures typical for Ca^{2+} -binding proteins), which are also present in myosin light chains. Since muscle itself contains myofibrillar proteins as well as sarcoplasmic proteins, it is difficult to identify those stained proteins. Besides, these cross-reacting proteins made the species identification easier. It would be a benefit for the muscle products, which retain whole proteins. On the other hand, based only on the protein-stained patterns, it was difficult to identify fish species. However, anti-A1 light chain antisera facilitated the identification by amplifying the differences in the low-molecular-weight region including myosin light chains. Different antisera gave clearly different stained patterns. Fidelity of species identification is thus enhanced by the combination of a few sets of antisera.

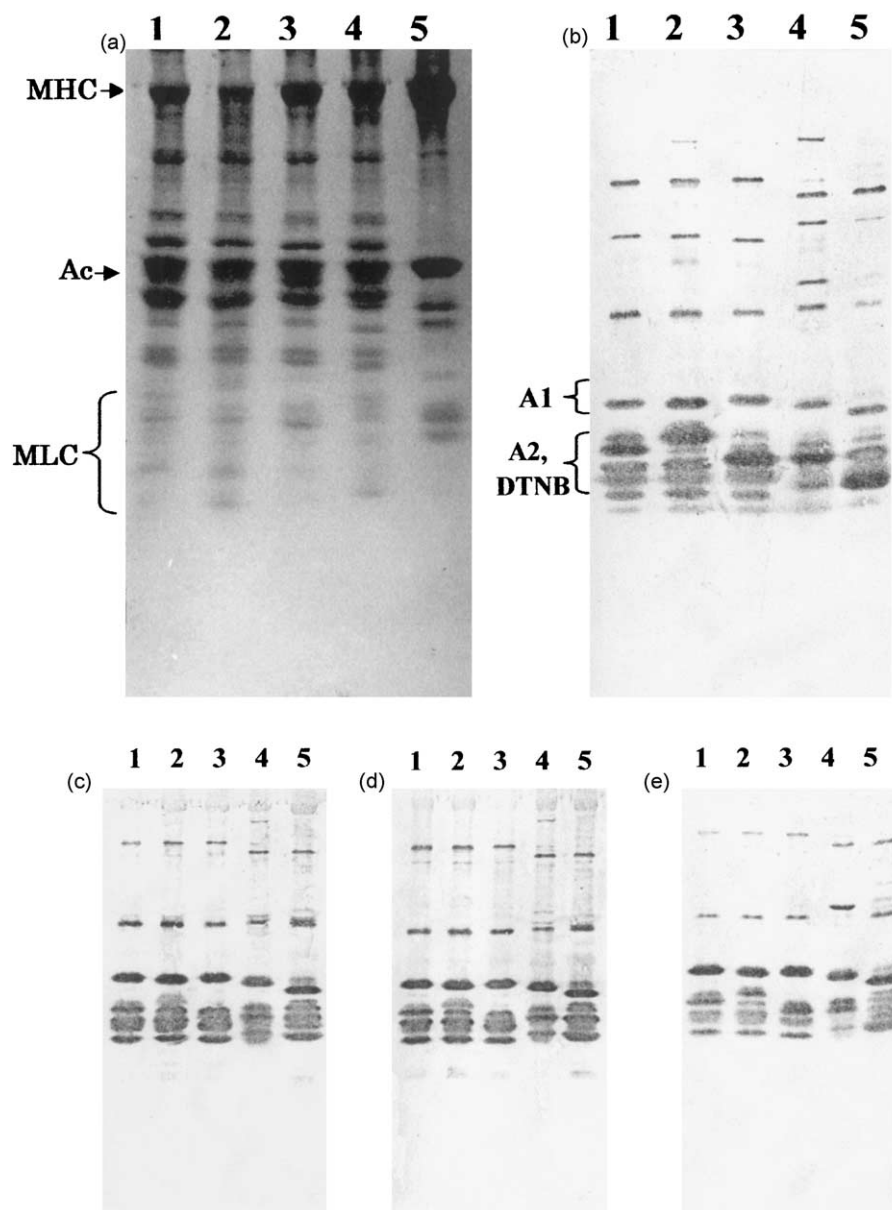


Fig. 2. Protein staining and immunostaining patterns of solubilized fish white muscle after SDS-PAGE. (a), Coomassie brilliant blue staining; Lane 1, skipjack; 2, bonito; 3, mackerel; 4, sardine; 5, walleye pollack. (b) through (e), immunostained using anti-A1 antisera (b), anti-mackerel A1; (c), anti-skipjack A1; (d), anti-round herring A1; e, anti-walleye pollack A1. Abbreviations: MHC, myosin heavy chain; Ac, actin; MLC, myosin light chains, A1 and A2, alkali light chains 1 and 2, respectively; DTNB, DTNB light chain. Myosin heavy chain and actin were also used as the inner molecular weight marker (205 and 44 kDa, respectively).

Then, myofibrils were run on SDS gel, and were stained likewise using several anti-A1 antisera. As shown in Fig. 3, myosin light chains were strongly stained, and the patterns were different enough for species identification. In the case of myofibril, the bands of higher-molecular-weight range were poorly stained as expected, because water-soluble proteins were removed almost completely through the preparation procedure.

In the next place, such species-dependent staining patterns were applied to dried fish products. In Fig. 4 are shown the protein staining and immunostained patterns of solubilized fractions from fushi and niboshi. The protein bands were so ambiguous that species could not be identified. It is because muscle proteins (both myofibrillar and sarcoplasmic ones) denatured through drastic heating and drying and became insoluble against urea and SDS. Even the bands corresponding to myosin

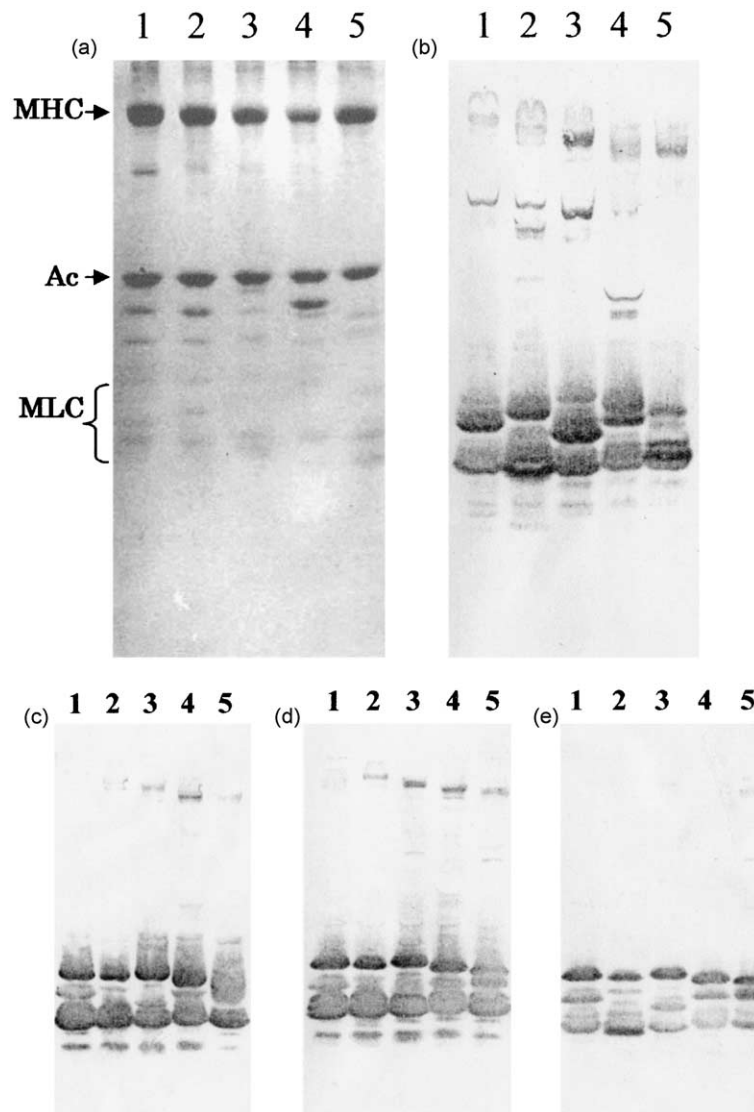


Fig. 3. Protein staining and immunostaining patterns of solubilized fish myofibrils after SDS-PAGE. (a), Coomassie brilliant blue staining; Lane 1, skipjack; 2, bonito; 3, mackerel; 4, sardine; 5, walleye pollack. (b) through (e), immunostained using anti-A1 antisera (b), anti-mackerel A1; (c), anti-skipjack A1; (d), anti-round herring A1; (e), anti-walleye pollack A1. Refer to the legend of Fig. 2 for the abbreviations used.

light chains could not be recognized. When the same gels were visualized with anti-mackerel A1 antiserum, myosin light chains were clearly stained. Because the material fish for fushi and niboshi are limited to several species (as examined in the present experiment), the stained patterns were clear enough to specify fish species.

The present study showed that species identification is still possible, even in the case when muscle proteins are intensively denatured and become insoluble. Myosin light chains, due to their small size (mol. wt. about 30,000) and high content of hydrophilic amino acid residues, remained partly soluble after prolonged processing under drastic conditions, and were resistant to decomposition. Accordingly, even in the case when no

protein bands are visible on the gel, they can be detected by immunoblotting. This method is considered to be effective when all the protein components are denatured and insolubilized through intensive processing.

DNA is much more resistant to heat treatment compared to proteins. Actually, Quintero et al. (1998) were able to identify canned tuna species which had been treated at 115 °C for 55 min. Under such conditions, most proteins aggregate and become insoluble, and SDS-PAGE patterns become smear as in Fig. 4A (left panel). However myosin light chains also survived even after boiling and subsequent prolonged drying. It is therefore worth using these proteins as a species marker. According to Pineiro et al. (1998), parvalbumin isoforms show species-specific patterns,

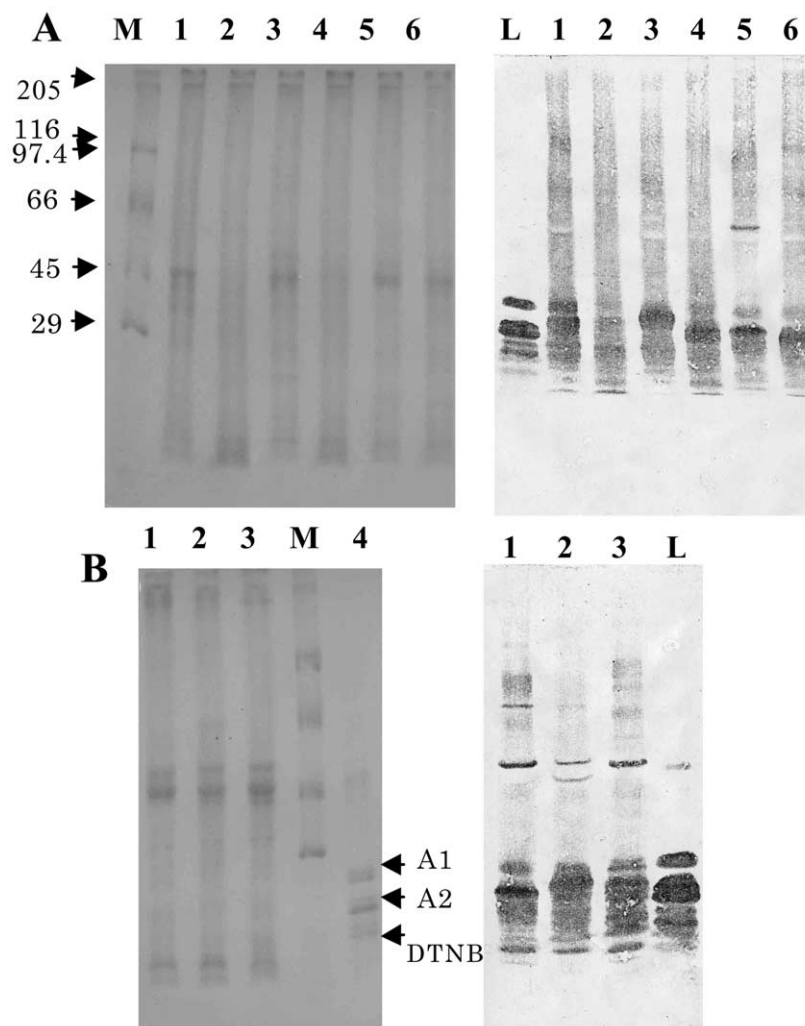


Fig. 4. Protein staining and immunostaining patterns of solubilized fushi and niboshi. A (fushi): left, Coomassie brilliant blue staining: lanes M, molecular weight marker (the figures on the left indicate the molecular weight in kDa); 1, yellowfin tuna; 2, skipjack; 3, bonito; 4, mackerel; 5, horse mackerel; 6, sardine. Right, immunostained with anti-mackerel-A1 antiserum, lane L, partially purified fraction of myosin light chains from mackerel white muscle; lanes 1 through 6 are the same as those in the left figure. B (niboshi), left, Coomassie blue staining: 1, sardine; 2, round herring; 3, anchovy; M, molecular weight marker (the same as Lane M in A); 4, myosin light chain fraction of mackerel white muscle. Right, immunostained patterns with anti-mackerel A1, lanes 1, sardine; 2, round herring; 3, anchovy; L, myosin light chain fraction of mackerel white muscle. Refer to the legend of Fig. 2 for the abbreviations used.

which were sufficient for species identification of gadoid fish on two-dimensional gels. This protein resembles myosin light chains in molecular weight, solubility, and even the partial amino acid sequence. In collaboration with immunological techniques, parvalbumin might also become a hopeful marker protein for species authentication by protein techniques.

In conclusion, for fish products processed under drastic conditions, species identification is very difficult because of crucial decomposition and intensive aggregation of proteins. However, myosin light chains are extractable even after prolonged processing due to their small molecular sizes and water solubility. Taking advantage of such characteristics, species identification was attempted using anti-myosin light chain antiserum. The results obtained showed that the immunostaining

patterns facilitated species identification of material fish even for drastically processed fish products such as fushi and niboshi.

Acknowledgements

This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Science, Sport, and Technology of Japan.

References

- Asensio, L., Gonzalez, I., Fernandez, A., Rodrigues, M. A., Hernandez, P. E., Garcia, T., & Martin, R. (2001). PCR-SSCP: a simple method for the authentication of grouper (*Epinephelus guaza*),

- wreck fish (*Polyprion americanus*), and Nile perch (*Lates niloticus*) filets. *Journal of Agricultural and Food Chemistry*, 49, 1720–1723.
- Bossier, P. (1999). Authentication of seafood products by DNA patterns. *Journal of Food Science*, 64, 189–193.
- Chapela, M. J., Sotelo, C. G., Calo-Mata, P., Perez-Martin, R. I., Rehbein, H., Hold, G. L., Quintero, J., Rey-Mendez, M., Rosa, C., & Santos, A. T. (2002). Identification of cephalopod species (*Pmmastrephidae* and *Loliginidae*) in seafood products by forensically informative nucleotide sequencing (FINS). *Journal of Food Science*, 67, 1672–1676.
- Chen, T. Y., & Hwang, D. F. (2002). Electrophoretic identification of muscle proteins in puffer species. *Journal of Food Science*, 67, 936–942.
- Colgen, S., O'Brien, L., Maher, M., Shilton, N., McDonnell, K., & Ward, S. (2001). Development of a DNA-based assay for species identification in meat and bone meal. *Food Research International*, 34, 409–414.
- Downey, G., McElhinney, J., & Fearn, T. (2000). Species identification in selected raw homogenized meats by reflectance spectroscopy in the mid-infrared, near-infrared, and visible range. *Applied Spectroscopy*, 54, 894–899.
- Elvevoll, E. O., Sorensen, N. K., Osterud, B., Ofstad, R., & Martinez, I. (1996). Processing of marine foods. *Meat Science*, 43, S265–275.
- Etienne, M., Jerome, M., Fleurence, J., Rehbein, H., Kundiger, R., Mendes, R., Costa, H., Perez-Martin, R., Pineiro-Gonzalez, C., Craig, A., Mackie, I., Yman, I. M., Ferm, M., Martinez, I., Jessen, F., Smelt, A., & Luten, J. (2000). Identification of fish species after cooking by SDS-PAGE and urea IEF: a collaborative study. *Journal of Agricultural and Food Chemistry*, 48, 2653–2658.
- Gazith, J., Himmelfarb, S., & Harrington, W. F. (1970). Studies on the subunits of myosin. *Journal of Biological Chemistry*, 245, 15–22.
- Hayden, A. R. (1977). Detection of chicken flesh in sausages. *Journal of Food Science*, 42, 1189–1192.
- Hold, G. L., Russell, V. J., Pryde, S. E., Rehbein, H., Quintero, J., Vidal, R., Rey-Mendez, M., Sotelo, C. G., Perrez-Martin, R. I., Santos, A. T., & Rosa, C. (2001). Development of a DNA-based method aimed at identifying the fish species present in food products. *Journal of Agricultural and Food Chemistry*, 49, 1175–1179.
- Hunt, D. J., Parkes, H. C., & Luley, I. D. (1997). Identification of the species of origin of raw and cooked meat products using oligonucleotide probes. *Food Chemistry*, 60, 437–442.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- Lahiff, S., Glennon, M., O'Brien, L., Lyng, J., Smith, T., Maher, M., & Shilton, N. (2001). Species-specific PCR for the identification of ovine, porcine and chicken species in meat and bone meal (MBM). *Molecular and Cellular Probes*, 15, 27–35.
- Lowey, S., & Holt, J. C. (1972). An immunochemical approach to the interaction of light and heavy chains in myosin. *Cold Spring Harbor Symposium on Quantitative Biology*, 37, 19–28.
- Lowry, O. H., Rowsbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
- Mackie, I. M. (1969). Species identification of cooked fish by disk electrophoresis. *Analyst*, 93, 458–460.
- Martinez, I., & Yman, I. M. (1998). Species identification in meat products by RAPD analysis. *Food Research International*, 31, 459–466.
- Matsunaga, T., Chikuni, K., Tanabe, R., Muroya, S., Shibata, K., Yamada, J., & Shinmura, Y. (1999). A quick and simple method for the identification of meats and meat products by PCR assay. *Meat Science*, 51, 143–148.
- Ochiai, Y., Kobayashi, T., Handa, A., Watabe, S., & Hashimoto, K. (1989). Immunological cross-reactivity of myosin light chains from various species of fish. *Nippon Suisan Gakkaishi*, 55, 2151–2156.
- Ochiai, Y., Ochiai, L., Hashimoto, K., & Watabe, S. (2001). Quantitative estimation of dark muscle content in the mackerel meat paste and its products using antisera against myosin light chains. *Journal of Food Science*, 66, 1301–1305.
- Ochiai, Y., Watabe, S., & Hashimoto, K. (1988). Physicochemical and immunological properties of myosin light chains from the ordinary muscle of marine teleost fishes. *Comparative Biochemistry and Physiology*, 90B, 347–353.
- Ouchterlony, O. (1958). Diffusion-in-gel methods for immunological analysis. In P. Kallos (Ed.), *Progress in allergy*, vol. V (pp. 1–78). Basel: Karger.
- Palumbi, S. R., & Cipriano, F. (1998). Species identification using genetic tools: the value of nuclear and mitochondrial gene sequences in whale conservation. *Journal of Heredity*, 89, 459–464.
- Partis, L., Croan, D., Guo, Z., Clark, R., Coidham, T., & Murby, J. (2000). Evaluation of a DNA fingerprinting method for determining the species origin of meats. *Meat Science*, 54, 369–386.
- Pineiro, C., Barros-Velazquez, J., Sotelo, C. G., Perez-Martin, R. I., & Gallardo, J. M. (1998). Two-dimensional electrophoretic study of the water-soluble protein fraction in white muscle of gadoid fish species. *Journal of Agricultural and Food Chemistry*, 46, 3991–3997.
- Pineiro, C., Vazquez, J., Marina, A. I., Barros-Velazquez, J., & Gallardo, J. M. (2001). Characterization and partial sequencing of species-specific sarcoplasmic polypeptides from commercial hake species by mass spectrometry following two-dimensional electrophoresis. *Electrophoresis*, 22, 1545–1552.
- Quinteiro, J., Sotelo, C. G., Rehbein, H., Pryde, S. E., Medina, I., Perez-Martin, R. I., Rey-Mendez, M., & Mackie, I. M. (1998). Use of mtDNA direct polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. *Journal of Agricultural and Food Chemistry*, 46, 1662–1669.
- Quinteiro, J., Vidal, R., Izquierdo, M., Sotelo, C. G., Chapela, M. J., Perez-Martin, R. I., Rehbein, H., Hold, G. L., Russell, V. J., Pryde, S. E., Rosa, C., Santos, A. T., & Rey-Mendez, M. (2001). Identification of hake species (*Merluccius genus*) using sequencing and PCR-RFLP analysis of mitochondrial DNA control region sequences. *Journal of Agricultural and Food Chemistry*, 49, 5108–5114.
- Ram, J. L., Ram, M. L., & Baidoun, F. (1996). Authentication of canned tuna and bonito by sequence and restriction site analysis of polymerase chain reaction products of mitochondrial DNA. *Journal of Agricultural and Food Chemistry*, 44, 2460–2467.
- Rego, I., Martinez, A., Gonzalez-Tizon, A., Vieites, J., Leira, F., & Mendez, J. (2002). PCR technique for identification of mussel species. *Journal of Agricultural and Food Chemistry*, 50, 1784–1789.
- Seki, N., Takayasu, M., & Kokuryo, H. (1980). Preparation of myosin light chains as an index for identification of fish species from raw muscle and cooked products. *Nippon Suisan Gakkaishi*, 46, 1001–1006.
- Stafford, W. F. III, Szentkiralyi, E. M., & Szent-Gyorgyi, A. G. (1979). Regulatory properties of single-headed fragment of scallop myosin. *Biochemistry*, 18, 5273–5280.
- Takagi, M., & Taniguchi, N. (1995). Random amplified polymorphic DNA (RAPD) for identification of three species of *Anguilla*, *Anguilla japonica*, *A. australis* and *A. bicolor*. *Fisheries Science*, 61, 884–885.
- Watabe, S., Ochiai, Y., & Hashimoto, K. (1982). Identification of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) and alkali light chains of piscine myosin. *Nippon Suisan Gakkaishi*, 48, 827–832.
- Watabe, S., Dinh, T. N. L., Ochiai, Y., & Hashimoto, K. (1983). Immunological specificity of myosin light chains from mackerel ordinary and dark muscles. *Journal of Biochemistry*, 94, 1409–1419.
- Watabe, S., Dinh, T. N. L., & Hashimoto, K. (1984). Isolation of myosin light chains from ordinary and dark muscle myosins of mackerel. *Nippon Suisan Gakkaishi*, 50, 71–77.
- Weeds, A. G., & Lowey, S. (1971). Substructure of the myosin molecule. II. The light chains of myosin. *Journal of Molecular Biology*, 61, 701–725.
- Yang, R., Okitani, A., & Fujimaki, M. (1970). Studies on myofibrils from the stored muscle. Part 1. Post-mortem changes of adenosine triphosphatase activity of myofibrils from rabbit muscle. *Bioscience, Biotechnology and Biochemistry*, 34, 1765–1772.