ELSEVIER

Food Chemistry 72 (2001) 105–112

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Species identification of formed fishery products and high pressure-treated fish by electrophoresis: a collaborative study

Monique Etienne [a,*], Marc Jérôme [a], Joël Fleurence [a], Hartmut Rehbein [b], Rainer Kündiger [b], Rogério Mendes [c], Helena Costa [c], Iciar Martínez [d]

[a] *Institut Français de Recherche pour I'Exploitation de Ia Mer (Ifremer), Laboratoire BPQ/VP, BP 21105, F-44311 Nantes cedex 03, France*

[b] *Bundesforschungsanstalt für Fischerei, Institut für Biochemie und Technologie, Pamaille 9, D-22767 Hamburg, Germany*

[c] *Instituto de Investigação das Pescas e do Mar (IPIMAR), Avenida de Brasília, 1449-006 Lisboa, Portugal*

[d] *Norwegian Institute of Fisheries and Aquaculture Ltd. (NIFA), 9291 Tromsø, Norway*

Received 5 April 2000; received in revised form 12 June 2000; accepted 12 June 2000

Abstract

The suitability and reliability of three electrophoretic methods of fish species identification, urea isoelectric focusing (IEF), sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) and native IEF, were evaluated on formed fish fillets and high pressure fish flesh by a collaborative study among four institutes. By following optimized standard operation procedures, the protein patterns of processed fish were compared to patterns of raw reference samples. The method to use depended of the effect of processing on the protein pattern. The proteins obtained from formed products were not denatured and therefore any of the three methods proved to be adequate, with a preference for native IEF which had a better discriminatory power for the species used. The high pressure process altered the proteins, and so only urea IEF and SDS-PAGE methods could be used. For these products, the chosen method should then be the one with the better discriminating power for the species being examined. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Electrophoresis; Urea IEF; SDS-PAGE; Identification; Formed fish; High pressure; Protein

1. Introduction

With the increasing diversity of seafood products, due in part to the greater number of species being traded, but also to new processing possibilities, there is a need for suitable methods of identification to ensure compliance with the labeling regulations and thereby to prevent the substitution of fish species. Two main techniques can be used for species identification when the fish has lost its biological characteristics: protein electrophoresis (An, Wei, Zhao, Marshall & Lee, 1989; Durand, Landrein & Quero, 1985, Mackie, 1980, 1996; Rehbein, 1990,1992; Sotelo, Piñeiro, Gallardo & Perez-Martin, 1992,1993) and molecular biological methods (bibliographic review: Bossier, 1999).

For authentication of fish fillet or fish muscle, classic electrophoretic methods have proved to be reliable, easy to apply by food control laboratories and, at present, still less sophisticated and cheaper than molecular biological methods (Piñeiro et al., 1999), despite these latter methods representing the future in food control laboratories.

The fish species identification of raw fillets, using isoelectric focusing (IEF) of water-soluble proteins is now a well-established procedure which was validated by collaborative exercises (Lundstrom, 1980; Rehbein et al., 1995). Methods of authentication of cooked fish and shellfish have been described by many authors (An, Marshall, Otwell & Wei, 1988; Civera & Parisi, 1991; Craig, Ritchie & Mackie, 1995; Scobbie & Mackie, 1988). Recently, in a European study, two techniques have been optimized, a sodium dodecylsulfate gel electrophoresis (SDS-PAGE) method (Piñeiro et al., 1999) and a urea IEF method (Etienne et al., 1999); the resulting standard operation procedures (SOP) are described below. The suitability of these optimized procedures, to authenticate cooked fish, was evaluated by two collaborative studies within nine laboratories (Etienne et al., in press; Rehbein et al., 1999).

As part of an additional study into the effects of different forms of processing on protein profiles, the same standard operation procedures (SOPs) were tested on formed fish fillet and fish mince treated by high pressure

* Corresponding author. Tel.: +33-2-4037-4062; fax: +33-2-4037-4071.

0308-8146/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0308-8146(00)00205-3

processing. As the proteins were denatured, to different extents, depending on the processing conditions, it was important to know to what degree the protein profiles were altered, or not, and whether any error of species identification might result.

2. Materials and methods

2.1. Fish samples

The list of the processed fish samples with the corresponding raw reference samples used in the study are presented in Table 1. The formed fish products were produced by Pickenpack, Lüneburg, Germany. The corresponding reference species samples were collected, either on research cruises of the Institute of Biochemistry and Technology, Hamburg, Germany, or were bought as wet fish at the local market in Hamburg-Altona. The high pressure (HP) treated fish were prepared by Ifremer, Nantes, France from Atlantic salmon and saithe bought at the local market. The fish were filleted, the fish flesh was minced and vacuum-packed in plastic bags before HP treatment at Aistoni, Nantes; sub-samples of minced fish were kept frozen to be used as reference samples. The HP treatment conditions were 400 megapascal at 15°C during 15 min for Atlantic salmon and 350 megapascal at 15°C during 15 min for saithe. All the samples were stored deep-frozen at about −20°C.

Frozen samples, provided with dried ice, were delivered by air freight and arrived in good condition within 36 h at each participating laboratory. All institutes participated in the collaborative study of formed fish fillet identification, whereas HP-treated products were analysed by two laboratories, NIFA, Tromsø and Ifremer, Nantes.

2.2. Analytical methods

Standard operating procedures, defined previously in EC projects, were used: two methods developed for the authentication of processed fish, urea isoelectric focusing (urea IEF) on CleanGel (Etienne et al., 1999) and sodium dodecyl suiphate-polyacrylamide gel electrophoresis (SDS-PAGE) on ExcelGel (Piñeiro et al., 1999). Isoelectric focusing (IEF), a classic method for identifying raw fish flesh, applied to H_2O-soluble fish protein extract (Rehbein et al., 1995) was also tested.

The same basic equipment was used, a flat-bed electrophoresis apparatus (Multiphor II Electrophoresis System from Pharmacia Biotech or equivalent), an electrophoresis power supply to be run at least at 2000 V, a thermostatic circulator, a homogenizer (Polytron or Ultraturrax), a centrifuge to be used at 20,000 g, a spectrophotometer capable of measurement at 280 nm with quartz cuvettes, a rocking platform, a gel air dryer and an image analysis system.

The protein determination of the extracts was done using the OD_{280}-procedure. The principle of this determination is based on the assumption that if a solution gives an absorbance at 280 nm of 1, this means that the protein concentration is 1 mg/ml. Fish muscle extracts, bovine serum albumin (BSA) standard solution [10 mg/ml in 0.2% (w/v) SDS] and reagent control without protein (extraction solution) were diluted 20-fold with 0.2% (w/v) SDS. The absorbance was measured at 280 nm in a spectrophotometer using quartz cuvettes and the protein content of the extracts (mg protein/ml) was calculated using the equation:

$$P_{\text{sample}} = [A_{\text{sample}} - A_{\text{blank}}]^{*}20; \quad \text{(20 is the dilution factor).} \quad (1)$$

As a control, the absorbance was also read for the BSA standard solution and the 0.2% SDS solution; the difference ($A_{\text{BSA}} - A_{\text{SDS}}$) should be close to 0.33. This protein determination was done on the extracts prior to freezing.

2.2.1. Urea IEF (CleanGel) analysis

2.2.1.1. Protein extraction. Fish flesh samples (500 mg) were homogenised with 4 ml of extraction solution [8 M

Table 1
Fish samples used for the collaborative study

Description of sample	Fish species	Provider
Formed fillet, battered	Hoki *(Macruronus novaezelandiae)*	Pickenpack/IBT
Raw fillet (reference)	Hoki *(Macruronus novaezelandiae)*	IBT
Formed fillet, battered	Cod *(Gadus morhua)*	Pickenpack/IBT
Raw fillet (reference)	Cod *(Gadus morhua)*	IBT
Formed fillet, battered	Red fish *(Sebastes* spp.*)*	Pickenpack/IBT
Raw fillet (reference)	Red fish *(Sebastes marinus)*	IBT
Formed fillet, battered	Alaska pollock *(Theragra chalcogramma)*	Pickenpack/IBT
Raw fillet (reference)	Alaska pollack *(Theragra chalcogramma)*	IBT
HP treated mince	Atlantic salmon *(Salmo salar)*	Aistom/Ifremer
Raw mince	Atlantic salmon *(Salmo salar)*	Ifremer
HP treated mince	Saithe *(Pollachius virens)*	Alstom/Ifremer
Raw mince	Saithe *(Pollachius virens)*	Ifremer

urea, 0.1 M 1,4-dithiothreitol (DDT), 20 mM sodium phosphate, pH 6.5]. The mixture was kept at room temperature for at least 30 min, and undissolved material was then removed by centrifugation (20°C, 15 min, 20,000 *g*). The protein concentrations of the sample extracts were measured and adjusted to 8 mg/ml with the extraction solution.

2.2.1.2. Preparation of the pI calibration kit. The pI markers used were parvalbumin dry matters (PADM) prepared according to a procedure described by Rehbein, Kündiger, Piñero and Pérez-Martín (2000). The PADM mixture gave 7 bands in urea LEF.

2.2.1.3. Electrophoresis conditions

2.2.1.3.1. Rehydration of CleanGel IEF. CleanGel (Pharmacia Biotech, 18-1035-32) was rehydrated in 21 ml of 8 M urea, 0.5% (w/v) Servalyte 2–4, 2% (w/v) Servalyte 4–6 and 0.5% (w/v) Servalyte 4–9T.

2.2.1.3.2. Instrument and gel preparation. The gel support plate was cooled to 15°C. Electrode wicks (Boehringer Ingelheim Bioproducts, 42942) were then cut to a suitable length for the gel and soaked with an appropriate volume of anode fluid 3 (0.025 M aspartic acid, 0.025 M glutamic acid, 10 mM $CaCl_2$) or cathode fluid 10 (2 M ethylenediamine, 0.025 M arginine, 0.025 M lysine). The gel was placed on the cooling plate, and the soaked electrode wicks were applied.

2.2.1.3.3. Sample application and running conditions. The IEF settings were: pre-focusing (500 V, 8 mA, 8 W, 30 min), sample entrance (500 V, 8 mA, 8 W, 20 min), and focusing (2000 V, 14 mA, 14 W, 5000 Vh). After pre-focusing, the applicator strip [7×1 mm, silicon rubber (Boehringer Ingelheim Bioproducts, 42989)] was positioned 2 cm in front of the cathodic wicks; 7.5 μl of each sample extract were placed into the slots of the strip, and 10 μl of pI marker solution were applied under the same conditions.

2.2.1.4. Fixation and Coomassie staining. At the end of the run, at 5000 Vh, the proteins were fixed and stained with Coomassie Serva Violet 17 dye (Boehringer Ingelheim Bioproducts, 35072). The gel was placed in fixing solution [20% (w/v) TCA] for 30 min, washed in de-staining solution [methanol/acetic acid/water (25/10/65) (v/v/v)] for 30 min, and stained with [0.1% (w/v) SERVA Violet 17 dissolved in de-staining solution] for 10 min. After a destaining step, the gel was soaked in the preserving solution [1% (w/v) glycerol (87%)] for 10 min, covered with a cellophane preserving sheet and dried in a gel air-drying system. The gels were then scanned.

2.2.2. SDS-PAGE analysis

2.2.2.1. Protein extraction. Fish flesh samples (300 mg) were homogenised in 4 ml of extraction solution [2% (w/v) SDS, 0.1 M DTT, 60 mM Tris-HCl, pH 7.5]. Samples were then boiled in a water bath (100°C) for 2 min, homogenised for 30 s while hot, and centrifuged (20,000 *g* at 20°C for 15 min). The protein concentration of the samples was adjusted to 0.3 mg/ml with Laemmli buffer [4.8% (w/v) SDS, 1 mM EDTA, 0.1 M DTT, 20% (v/v) glycerol, 125 mM Tris-HCl, pH 6.8, 0.05% (w/v) bromophenol blue].

2.2.2.2. Preparation of the molecular weight calibration kit. Novex Mark 12 (LC 5677), BioRad Polypeptide SDS-PAGE molecular weight standards (161-0326) and Pharmacia Biotech molecular weight markers [MW range 2512-16949 (80-1129-83)] were used for calibration. A mixed solution was prepared according to the following steps: (i) 80-fold dilution of the BioRad marker with Laemmli buffer, (ii) reconstitution of the Pharmacia Biotech Marker with 2 ml Laemmli buffer and 10-fold dilution with Laemmli buffer, and (iii) mixture of the BioRad and Pharmacia Biotech solutions, Novex Mark 12 and Laemmli buffer in the proportions 7+7+8+34.

The staining indicator [1.6 mg bovine plasma albumin (Sigma, A 7517) and 1.6 mg egg white lysozyme (Sigma, L 4631) in 1 ml of extraction solution] was diluted 784 times with Laemmli buffer in two steps (2 times 1/28).

2.2.2.3. Electrophoresis conditions

2.2.2.3.1. Instrument and gel preparation. The gel support plate was cooled to 15°C and the gel [ExcelGel SDS Homogeneous, 15% (Pharmacia Biotech, 80-1262-01)] was positioned on the plate with the wells on the cathode side. The cathode and anode buffer strips (Pharmacia Biotech, 17-1342-01) were positioned, respectively, above the wells and the on the other side of the gel.

2.2.2.3.2. Sample application and running conditions. Aliquots (10 μl) of sample extracts, together with the molecular weight marker mixture and the staining indicator, were dropped into the wells of the gel. Running conditions were 600 V, 30 mA and 30 W. Once the bromophenol front began to enter the anode electrode strip, electrophoresis was continued for another 20 min and then stopped.

2.2.2.4. Silver staining. The proteins were fixed and stained using the Silver Staining Kit, Protein (Pharmacia Biotech, Plusone 17-1150-01). The gel was soaked in preserving solution [1%(w/v) glycerol (87%)] for 20 sheet and dried in a gel air-dryung system. The gels were then scanned.

2.2.3. Isoelectric focusing analysis of water-soluble proteins

2.2.3.1. Extraction of water-soluble proteins. Fish flesh samples (5 mg) were homogenized in 10 ml of pre-

cooled distilled water in an ice bath. The mixture was clarified by centrifugation (20 min, 20,000 *g*, 4°C), and the supernatant was collected and stored at +4°C until analysis (24 h maximum). Protein concentrations of the sample extracts were measured and adjusted to 8 mg/ml with distilled water.

2.2.3.2. Preparation of the pI calibration kit. The content of one vial of broad pI kit, pH 3.5/9.3 (Pharmacia Biotech, 17-0471-01) was solubilised in 100 µl of distilled water.

2.2.3.3. Electrophoresis conditions

2.2.3.3.1. Instrument and gel preparation. The gel support plate was cooled to 15°C. IEF electrode strips (Pharmacia Biotech, 18-1004-40) were soaked in the following solutions: anode 1 M H_3PO_4, cathode 1 M NaOH. The gel [Ampholine PAG plates 3.5/9.5 (Pharmacia Biotech, 80-1124-80)] was placed on the cooling plate, and the soaked electrode strips were applied to the gel.

2.2.3.3.2. Sample application and running conditions. Aliquots (10 µl) of sample extracts or of pI marker were applied 10 mm from the cathode using sample application pieces (Pharmacia Biotech, 80-1129-46). Running conditions were 1500 V, 50 mA, 30 W and 1.5 h.

2.2.3.4. Fixation and Coomassie staining. The gel was placed in fixing solution [11.6% (w/v) TCA, 3.4% (w/v) sulphosalicylic acid] for 30–60 min maximum, washed in destaining solution for 5 min [ethanol/acetic acid/water (50/16/134) (v/v/v)], stained for 10 min in staining solution [0.1% (wfv) Coomassie Brilliant blue R-250 (Sigma, B-0149) dissolved in destaining solution] preheated to 60°C. After a further destaining step, the gel was soaked in the preserving solution [1% (w/v) glycerol in destaining solution] for 1 h, covered with a cellophane preserving sheet and dried in a gel air-drying system. The gels were then scanned.

3. Results and discussion

3.1. Protein content of the extracts

The results of the protein determination performed in a participating laboratory, NIFA, Tromsø, Norway are compiled in Table 2. In general, 2% SDS extraction solution rendered extracts of higher protein content than water, which was in turn more efficient for extracting protein than 8 M urea solution. The power of solubilisation of urea is less than that of SDS for denatured protein, as shown by An et al. (1988).

When the protein concentration was lower than that required, the volume applied to the gel was proportionally higher, in order to load the same amount of protein on all the lanes.

3.2. Urea IEF (CleanGel)

It can be seen (Fig. 1) that, using CleanGels, protein patterns were characterised by strong bands in the acidic part of the gel. The protein fraction showing an apparent pI less than 5.6 afforded approximately five sharp and intensive coloured discriminating bands. As noticed by Rehbein et al. (1999), urea IEF is a reliable technique for differentiating closely related species, rich in parvalbumins, such as *Gadidae* and *Merlucidae*. In other respects, closely-related species, characterised by few proteins in the acidic zone, such as some salmon species or tuna species, are more difficult to authenticate using urea IEF in this range of pH values (Etienne et al., in press).

All laboratories reported that each species had a characteristic profile. The formed samples of *Macruronus novaezelandiae, Gadus morhua, Sebastes* spp and *Theragra chalcogramma* were indistinguishable from those of the reference samples (Fig. 1). The raw and high pressure-processed sample (*Salmo salar* and *Pollachius virens*) gave almost identical profiles, only some

Table 2
Protein content of the extracts of raw and treated fish

Fish species	Processing	Protein content (mg/ml) in extracts		
		H_2O	Urea	SDS
Macruronus novaezelandiae	Formed fillet	8.05	6.77	16.6
Macruronus novaezelandiae	Raw fillet	13.4	7.18	16.0
Gadus morhua	Formed fillet	8.98	7.54	16.4
Gadus morhua	Raw fillet	8.76	7.79	14.2
Sebastes spp	Formed fillet	9.62	8.36	16.1
Sebastes marinus	Raw fillet	11.6	8.08	12.0
Theragra chalcogramma	Formed fillet	7.15	5.37	15.3
Theragra chalcogramma	Raw fillet	9.68	7.50	16.5
Salmo salar	HP treated mince	32.1	10.8	20.7
Salmo salar	Raw mince	32.8	8.45	50.3
Pollachius virens	HP treated mince	7.85	5.45	13.3
Pollachius virens	Raw mince	13.4	9.18	19.2

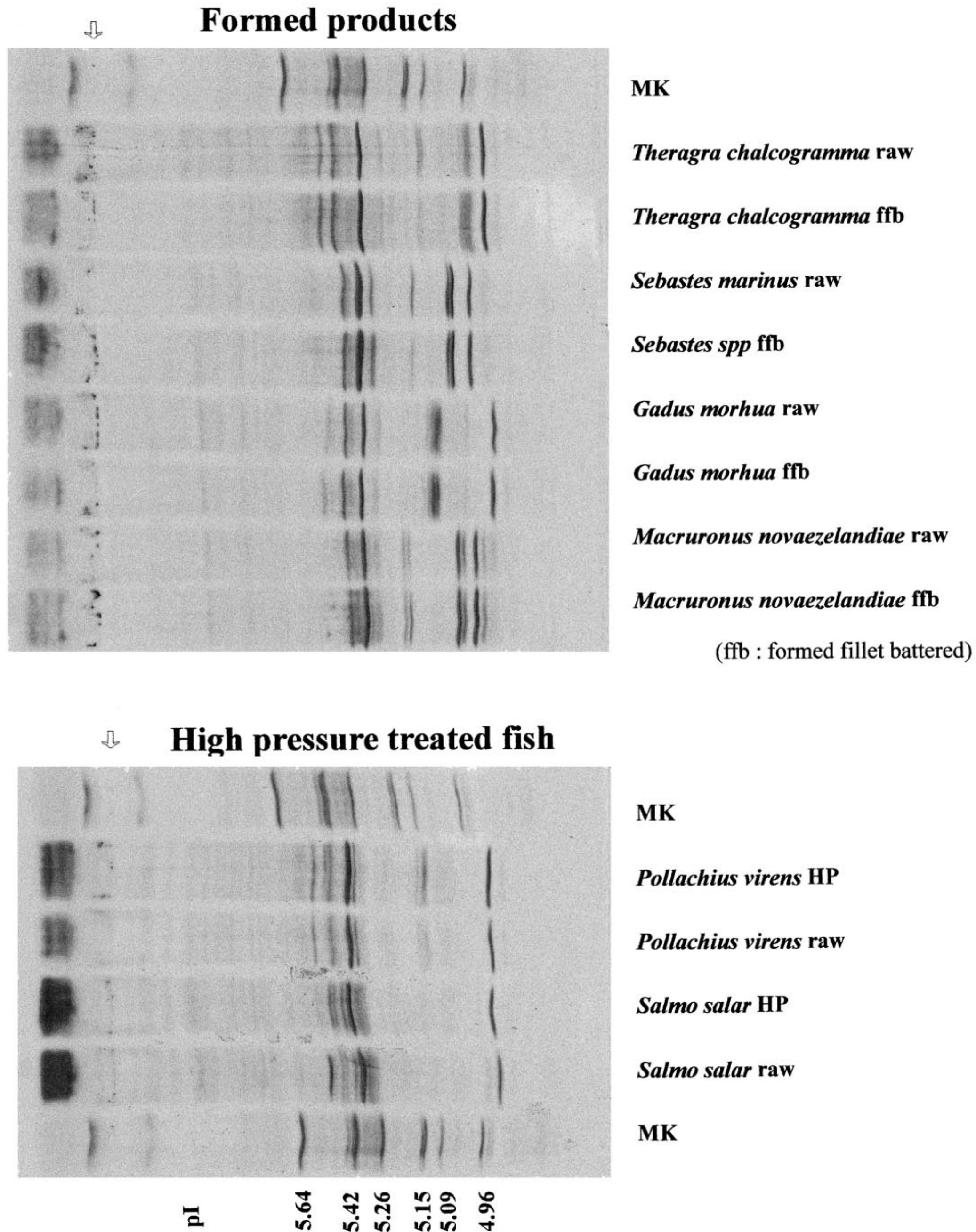

Fig. 1. Urea IEF pattern. Urea extracts of raw and processed fish run on CleanGel rehydrated with 8 M urea, 0.5% (w/v) Servalyte 2–4, 2% (w/v) Servalyte 4–6 and 0.5% (w/v) Servalyte 4–9T. The pI markers were parvalbumin dry matters (PADM) prepared according to Rehbein (in press). The anode is on the right side of the figure and the position of sample application is indicated by an arrow.

slight modifications of intensity of a few bands were inferred by this processing. These results are in conformity with previous data obtained from other processing techniques such as cooking and smoking (Etienne et al., 1999; Mackie et al., 2000; Rehbein et al., 1999).

Thus, in principle, the identification of formed and high pressure-processed fish can be made by urea IEF (CleanGel) using raw authentic samples.

3.3. SDS-PAGE

Fig. 2 shows that the SDS-PAGE protein patterns were characterized by numerous bands spread out over the whole gel in a large range of molecular weight, from less than 10 kDa to more than 200 kDa. However, only the low molecular weight region, with protein fractions under 30 kDa, afforded discriminatory bands among

the species that permitted the samples to be authenticated (Civera & Parisi, 1991; Mackie et al., 2000; Piñeiro et al., 1999; Rehbein et al., 1999; Scobbie & Mackie, 1988; Seki, 1976; Seki, Takayasu & Kokuryo, 1980; Sotelo et al., 1992). The small differences in the patterns, sometimes observed in the high molecular weight region, from actin (42 kDa) to myosin heavy chain (200 kDa) were considered too weak to be used for species identification in this gel system (Rehbein et al., 1999).

Using SDS-PAGE, the patterns of formed fillets and high pressure-processed sample were identical to those of raw samples (Fig. 2).

Using raw samples as reference material, the differentiation of numerous fish species was possible using SDS-PAGE, by considering proteins of molecular weight lower than 30 kDa. The exceptions were *Gadus morhua* and *Theragra chalcogramma*, which were undistinguishable (Fig. 2), though the SDS-PAGE technique allows discrimination among other closely related *Gadidae* species, such as *Gadus morhua, Merlangius merlangus, Pollachius virens* and *Melanogrammus aeglefinus* (Rehbein et al., 1999).

3.4. Isoelectric focusing analysis of water-soluble proteins

The IEF profiles of water-soluble proteins of the raw samples (Fig. 3) displayed many species-specific bands, that were spread out over almost the whole gel in a large range of pI values, in contrast to urea-IEF profiles of 8M urea extracts (Fig. 1) which showed fewer species-specific bands, situated only in the acidic zone. IEF also had a greater resolving power than SDS-PAGE, as noticed by Sotelo et al. (1992).

The formed products, prepared from *Macruronus novaezelandiae, Gadus morhua, Sebastes* spp and *Theragra chalcogramma* gave species-specific patterns, iden-

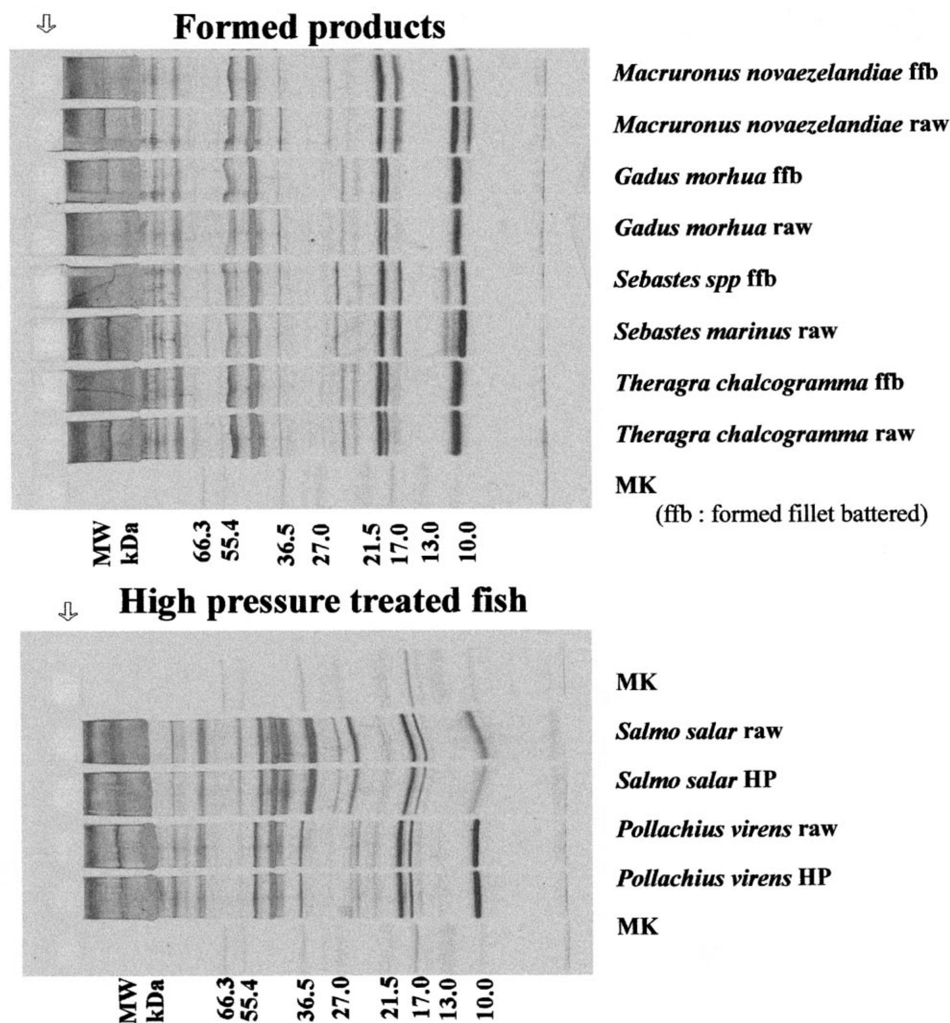

Fig. 2. SDS-PAGE pattern. Buffer extracts of raw and processed fish run on ExcelGel Homogenous 15%. The molecular weight marker was a mix of Novex Mark 12, BioRad Polypeptide SDS-PAGE MW standards and Pharmacia Biotech MW molecular weight markers. The anode is on the right side of the figure and the position of sample application is indicated by an arrow.

tical to those obtained from the respective raw reference samples (Fig. 3). It seems, therefore, that the processing conditions have not been severe enough to significantly alter the native IEF pattern of the formed fish.

High pressure treatment had an important effect on the pattern of the processed samples; many bands were lost and, especially in the case of *Salmo salar,* at pH above 5.85, there were no bands (Fig. 3). Therefore, IEF of water-soluble extracts does not seem to be an appropriate method for evaluation of these samples.

4. Conclusion

For species identification of formed products, any of the three methods, urea IEF, SDS-PAGE or IEF of H_2O-soluble protein, using precast gels proved to be adequate, because the processing does not alter the protein patterns. On the other hand, for high pressure processed fish, only the urea-IEF and SDS-PAGE methods can be used for discriminating among species; high pressure processing is more severe and it alters the native IEF pattern.

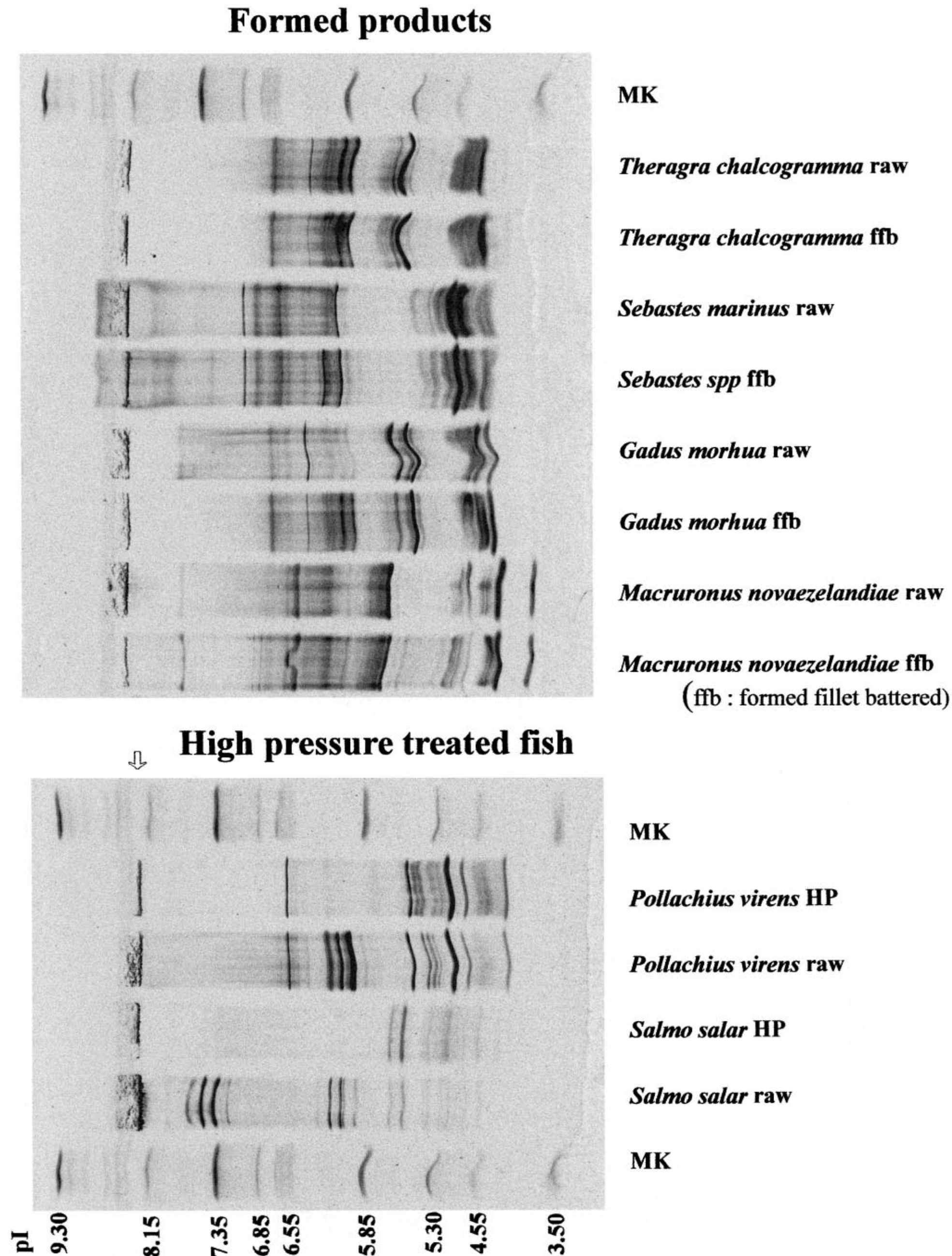

Fig. 3. Native IEF pattern. Aqueous extracts of raw and processed fish run on Ampholine PAG plate. The pI marker used is broad pI marker used is a broad pI kit, pH 3.5/9.3 from Pharmacia Biotech. The anode is on the right side of the figure and the position of sample application is indicated by an arrow.

The method selected should be the one that discriminates best among species being examined. Thus, when the processing denatures fish muscle proteins, urea IEF should be preferred for differentiation of closely-related species which are rich in parvalbumins, such as the *Gadidae* and the *Merlucidae*. On the other hand, urea IEF (CleanGel) is less powerful than SDS-PAGE for the discrimination of processed species characterized by neutral and basic protein bands, such as those of the tuna and salmon families. Nevertheless, in contentious cases, it is preferable to use both methods of analysis.

When the proteins are not denatured (formed products), native IEF, which gives patterns with many discriminatory protein bands, remains an efficient and easy-to-apply technique for authentication.

Acknowledgements

The study has been carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT95-1227, "Advanced methods for identification and quality monitoring of (heat) processed fish". Authors wish to thank Dominique Chevalier, ENITIAA, Nantes, for her advice and help in the preparation of the high pressure products.

References

An, H., Marshall, M. R., Otwell, W. S., & Wei, C. I. (1988). Electrophoretic identification of raw and cooked shrimp using various protein extraction system. *Journal of Food Science, 53*, 313–318.

An, H., Wei, C. I., Zhao, J., Marshall, M. R., & Lee, C. M. (1989). Electrophoretic identification of fish species used in surimi products. *Journal of Food Science, 54*, 253–257.

Bossier, P. (1999). Authentication of seafood products by DNA patterns. *Journal of Food Science, 64*(2), 189–193.

Civera, T., & Parisi, E. (1991). The use of sodium dodecyl sulphate polyacrylamide gel electrophoresis for the identification of raw and cooked shrimps and crabs. *Italian Journal of Food Science, 3*, 149–157.

Craig, A., Ritchie, A., & Mackie, I. (1995). Determining the authenticity of raw reformed breaded scampi (*Nephrops norvegicus*) by electrophoretic techniques. *Food Chemistry, 52*, 451–454.

Durand, P., Landrein, A., & Quero, J. C. (1985). *Catalogue électrophorétique des poissons commerciaux*. Nantes, France: Institut français de recherche pour l'exploitation de la mer.

Etienne, M., Jérôme, M., Fleurence, J., Rehbein, H., Kondiger, R., Malmhedem-Yman, I., Ferm, M., Craig, A., Mackie, I., Jessen, F., Smelt, A., & Luten, J. (1999). A standardized method of identification of raw and heat-processed fish by urea isoelectric-focusing: A collaborative study. *Electrophoresis, 20*, 1923–1933.

Etienne, M., Jérôme, M., Fleurence, J., Rehbein, H., Kündiger, R., Mendes, R., Costa, H., Pérez-Martin, R., Piñeiro-González, C., Craig, A., Mackie, I., Malmhedem-Yman, I., Ferm, M., Martínez I., Jessen F., Smelt, A., & Luten, J. (in press). identication of fish species after cooking by SDS-PAGE and urea IEF: a collaborative study. *Journal of Agricultural and Food Chemistry*.

Lundstrom, R. (1980). Fish species identification by thin-layer polyacrylamide gel isoelectric focusing: Collaborative study. *Journal of the Association of Official Analytical Chemists, 63*(1), 69–73.

Mackie, I. M. (1980). A review of some recent applications of electrophoresis and iso-electric focusing in the identification of species of fish in fish and fish products. In J. J. Connell, *Advances in fish science and technology* (pp. 444–450). Farnham, UK: Fishing News Books.

Mackie, I. M. (1996). Authenticity of fish. In P. R. Ashurst, & M. J. Dennis, *Food authentication* (pp. 140–170). London: Blackie Academic and Professional.

Mackie, I., Graig, A., Etienne, M., Jérôme, M., Fleurence, J., Jessen, F., Smelt, A., Kruijt, A., Malmheden-Yman, I., Herm, M., Martinez, I., Pérez-Martín, R., Piñero, C., Rehbein, H., Kündiger, R. (2000). Species identification of smoked and gravad fish products by sodium dodecylsuphate polyacrylamide gel electrophoresis, urea isoelectic focusing and native isoelectric focusing: a collaborative study. *Food Chemistry* (in press).

Piñero, C., Barros-Velázquez, J., Pérez-Martín, R., Martínez, I., Jacobsen, T., Rehbein, H., Kündiger, R., Mendes, R., Etienne, M., Jérôme, M., Craig, A., Mackie, I., & Jessen, F. (1999). Development of a sodium dodecyl sulfate-polyacrylamide gel electrophoresis reference method for the analysis and identification of fish species in raw and heat-processed samples. A collaborative study. *Electrophoresis, 20*, 1425–1432.

Rehbein, H. (1990). Electrophoretic techniques for species identification of fishery products. *Zeirschrift für Lebensmitte 1-Untersuchung und- Forschung, 191*, 1–10.

Rehbein, H. (1992). Parvalbumins as marker proteins for the fish species in fishery products. In H. H. Huss, M. Jakobsen, & J. Liston, *Quality assurance in the fish industry* (pp. 399–405). London: Blackie Academic and Professional.

Rehbein, H., Etienne, M., Jérôme, M., Hattula, T., Knüdsen, B., Jessen, F., Luten, J. B., Bouquet, W., Mache, I. M., Ritchie, A. H., Martin, R., & Mendes, R. (1995). Influence of variation in methodology on the reliability of the isoelectric focusing method of fish species identification. *Food Chemistry, 52*, 193–197.

Rehbein, H., Kündiger, R., Malmhedem-Yman, I., Ferm, M., Etienne, M., Jérôme, M., Craig, A., Mackie, I., Jessen, F., Martínez, I., Mendes, R., Smelt, A., Luten, J., Piñeiro, C., & Pérez-Martin, R. (1999). Species identification of cooked fish by urea isoelectric focusing and sodium dodecylsulfate polyacrylamide gel electrophoresis: a collaborative study. *Food Chemistry, 67*, 333–339.

Rehbein, H., Kündiger, R., Piñeiro, C., & Pérez-Martin, R. (2000). Fish muscle parvalbumins as marker proteins for native and urea isoelectric focusing. *Electrophoresis 21*, 1458–1463.

Scobbie, A. E., & Macide, I. M. (1988). The use of sodium dodecyl sulphate polyacrylamide gel electrophoresis in fish identification — a procedure suitable for cooked and raw fish. *Journal of Food Science and Agriculture, 44*, 343–351.

Seki, N. (1976). Identification of fish species by SDS-polyacrylamide gel electrophoresis of the myofibrillar proteins. *Bulletin of the Japanese Society of Scientific Fisheries, 42*, 1169–1176.

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. *Bulletin of the Japanese Society of Scientific Fisheries, 46*, 1001–1006.

Sotelo, C. G., Piñeiro, C., Gallardo, J. M., & Pérez-Martín, R. I. (1992). Identification of fish species in smoked products by electrophoresis and isolelectric focusing. *Zeitschrift für Lebensmitte l-Untersuchung und- Forschung, 195*, 224–227.

Sotelo, C. G., Piñeiro, C., Gallardo, J. M., & Pérez-Martín, R. I. (1993). Fish species identification in seafood products. *Trends of Food Science and Technology, 4*, 395–401.