Identification of Fish Species after Cooking by SDS−PAGE and Urea IEF: A Collaborative Study

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A collaborative study, to validate the use of SDS−PAGE and urea IEF, for the identification of fish species after cooking has been performed by nine laboratories. By following optimized standard operation procedures, 10 commercially important species (Atlantic salmon, sea trout, rainbow trout, turbot, Alaska pollock, pollack, pink salmon, Arctic char, chum salmon, and New Zealand hake) had to be identified by comparison with 22 reference samples. Some differences in the recoveries of proteins from cooked fish flesh were noted between the urea and the SDS extraction procedures used. Generally, the urea extraction procedure appears to be less efficient than the SDS extraction for protein solubilization. Except for some species belonging to the Salmonidae family *(Salmo, Oncorhynchus),* both of the analytical techniques tested (urea IEF, SDS−PAGE) enabled identification of the species of the samples to be established. With urea IEF, two laboratories could not differentiate *Salmo salar* from *Salmo trutta*. The same difficulties were noted for differentiation between *Oncorhynchus gorbuscha* and *Oncorhynchus keta* samples. With SDS−PAGE, three laboratories had some difficulties in identifying the *S. trutta* samples. However, in the contrast with the previous technique, SDS−PAGE allows the characterization of most of the *Oncorhynchus* species tested. Only *Oncorhynchus mykiss* was not clearly recognized by one laboratory. Therefore, SDS−PAGE (Excel gel homogeneous 15%) appears to be better for the identification, after cooking, of fish such as the tuna and salmon species which are characterized by neutral and basic protein bands, and urea IEF (CleanGel) is better for the gadoid species, which are characterized by acid protein bands (parvalbumins). Nevertheless, in contentious cases it is preferable to use both analytical methods.

Keywords: *Electrophoresis; urea IEF; SDS−PAGE; identification; cooked fish; protein*

INTRODUCTION

Identification of fish species after processing and more particularly after cooking is a necessary step in the quality control of seafood products. This analytical approach is justified by the development of trading arrangements which lead to increased fish product diversity available to the consumers.

The use of electrophoresis techniques, especially isoelectric focusing of sarcoplasmic proteins, for fish speciation is well-known (Rehbein, 1990; Mackie, 1996). However, this methodology was mainly validated on raw fish. For the heated products, the isoelectric focusing (IEF) method is only applicable to fish species which show a specific pattern with the heat-stable parvalbumins (Rehbein, 1992). Generally, myofibrillar and sarcoplasmic proteins denatured by heating can be solubilized from fish muscle by the use of a chaotropic agent such as urea or a detergent such as sodium dodecyl sulfate (SDS). The proteins obtained with this procedure can be further analyzed by urea isoelectric focusing (An et al., 1989) or by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). However, the application of these methodologies to the characterization of fish species in cooked products has not been validated by a collaborative study and is still not used routinely by laboratories involved in the control of seafood.

The work described here gives the main results obtained from a collaborative study among nine European laboratories involved in the validation of urea IEF

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Table 1. Fish Species Used for the Collaborative Study with Various Protein Concentrations of Fish Extracts[a]

code	fish species		protein content (mg/g of flesh) urea IEF	SDS−PAGE
	References (Raw)			
R_1	*Oncorhynchus gorbuscha*	pink salmon	182.4	215.6
R_2	*Oncorhynchus keta*	chum salmon	125.8	135.2
R_3	*Salmo trutta*	sea trout	170.3	170.3
R_4	*Oncorhynchus mykiss*	rainbow trout	170.5	153.2
R_5	*Salvelinus alpinus*	Arctic char	204.7	151.1
R_6	*Limanda limanda*	dab	103.4	157.9
R_7	*Reinhardtius hippoglossoides*	Greenland halibut	171.2	155.6
R_8	*Salmo salar*	Atlantic salmon	208.1	167.1
R_9	*Hippoglossus hippoglossus*	halibut	124.8	136.9
R_{10}	*Dicentrarchus labrax*	sea bass	142.9	142.9
R_{11}	*Clarias gariepinus*	African catfish	151.5	122.6
R_{12}	*Psetta maxima*	turbot	88.9	141.9
R_{13}	*Lepidorhombus whiffiagonis*	megrim	187.7	154.2
R_{14}	*Thunnus alalunga*	albacore	185.9	197.3
R_{15}	*Thunnus albacares*	yellow fin tuna	148.8	173.9
R_{16}	*Katsuwonis pelamis*	skipjack tuna	139.6	179.6
R_{17}	*Merluccius australis*	New Zealand hake	115.7	136.0
R_{18}	*Merluccius merluccius*	hake	129.5	140.0
R_{19}	*Merluccius hubbsi*	southwest Atlantic hake	57.7	125.4
R_{20}	*Macruronus magellanicus*	Patagonian whiphake	88.2	142.0
R_{21}	*Theragra chalcogramma*	Alaska pollock	78.2	168.4
	Cooked Samples for Identification			
C_1	*Oncorhynchus gorbuscha*	pink salmon	65.7	180.4
C_2	*Salvelinus alpinus*	Arctic char	215.6	269.9
C_3	*Oncorhynchus keta*	chum	67.8	151.9
C_4	*Psetta maxima*	turbot	111.4	175.4
C_5	*Oncorhynchus mykiss*	rainbow trout	147.5	214.3
C_6	*Pollachius pollachius*	pollack	136.2	158.6
C_7	*Salmo salar*	Atlantic salmon	192.1	391.6
C_8	*Merluccius australis*	New Zealand hake	46.3	145.8
C_9	*Salmo trutta*	sea trout	227	268.1
C_{10}	*Theragra chalcogramma*	Alaska pollock	48.8	138.1

[a] Two extracting solutions were used: 8 M urea, 0.1 M DDT, and 20 mM sodium phosphate, pH 6.5, for CleanGel and 2% (w/v) SDS, 0.1 M DTT, 60 mM Tris-HCl, pH 7.5, for SDS−PAGE.

and SDS−PAGE techniques for fish species identification in cooked products.

MATERIALS AND METHODS

Fish Samples. Fish samples were either collected on research cruises of institutes participating in this study or bought at local fish markets or from fish farms. All the samples were brought together at IFREMER (Nantes, France), and two sets of frozen samples were prepared: the first contained 21 raw fish reference samples and the second 10 unknown cooked samples. Samples of light muscle of the fish species listed in Table 1 were used as reference samples and were stored deep frozen at −20 °C. Ten samples noted C_1−C_{10} (Table 1) were cooked under the following conditions: pieces of fillet (40−50 g) were sealed in plastic bags and heated for 10 min in a boiling water bath until the core temperature of the sample reached 75/80 °C (determined by thermocouples). Subsequently, the samples were removed from the bath, allowed to cool to room temperature, and stored deep frozen at −20 °C.

Table 2. p*I* Values of the Components of the PADM Mixture

no. of band	p*I* value	fish species	no. of band	p*I* value	fish species
1	4.96	conger eel	5	5.42	conger eel
2	5.09	striped red mullet	6	5.50	sole
3	5.15	striped red mullet	7	5.64	sole
4	5.26	striped red mullet			

Frozen samples, packed with dry ice, were delivered by air freight and arrived in good condition within 36 h at the participating institutes. Each laboratory received the list of the reference fish species and a note explaining that the unknown samples might contain samples of species not included in the references, as well as two samples of the same species.

Analytical Methods. The two standard operation procedures, SDS−PAGE and urea IEF (CleanGel), defined previously for analysis of raw and heated fishery products were used (Piñeiro et al., 1999; Etienne et al., 1999).

They required the same basic equipment constituted by a flat-bed electrophoresis (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 20000*g*, a spectrophotometer capable of measurement at 280 nm with quartz cuvettes, a rocking platform, a gel air dryer, and an image analysis system.

For both electrophoretic methods, protein determination of the extracts was by the OD_{280} procedure. The principle of this determination is based on the assumption that if a solution gives an A_{280nm} of 1, this means that the protein concentration is 1 mg/mL. This protein determination requires that the extracts were not frozen before analysis.

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 of protein/mL) was calculated using the equation:

$$P_{sample} = [A_{sample} - A_{blank}] \times 20$$

In the equation, 20 is the dilution factor. As a control, the difference ($A_{BSA} - A_{SDS}$) should be close to 0.33.

Urea IEF (CleanGel) Analysis. *Protein Extraction*. Fish flesh (light muscle) was cut into small pieces, and 500 mg of the flesh was homogenized with 4 mL of extraction solution [8 M urea, 0.1 M 1,4-dithiothreitol (DDT), 20 mM sodium phosphate, pH 6.5] using an Ultraturrax homogenizer equipped with a small rod at high speed for 1 min. The mixture was kept at room temperature for at least 30 min, and then undissolved material was removed by centrifugation (20 °C, 15 min, 20000g_{max}). The extract could be stored at room temperature (≈20 °C) for 4 days.

Preparation of Samples for Urea IEF. The protein concentrations of the sample extracts were adjusted to about 8 mg/mL with the extraction solution.

Preparation of the pI Calibration Kit. The used p*I* markers were parvalbumin dry matters (PADM) prepared at the Federal Research Centre for Fisheries (Hamburg, Germany), following a procedure described by Rehbein et al. (in press). The PADM of three fish species were used: conger eel (*Conger conger*), striped red mullet (*Mullus surmuletus*), and sole (*Solea solea*). The PADM mixture gives seven bands in urea IEF (Table 2).

From each PADM a solution at 10 mg/mL was prepared using the extraction solution (8 M urea, 0.1 M DDT, 20 mM sodium phosphate, pH 6.5). Each solution was mixed using a vortex, kept at room temperature for 30 min, remixed by vortex and subsequently homogenized with the Ultaturrax homogenizer at low speed to avoid foaming, and kept at room temperature for at least 5 min. The undissolved material was

separated by centrifugation. Equal volumes of PADM solutions of conger eel, sole, and striped red mullet were mixed to obtain the PADM p*I* calibration kit. The PADM solutions were stable for at least 3 days at room temperature.

Electrophoresis Conditions. *Rehydration of CleanGel IEF*. A full-size CleanGel (Pharmacia Biotech, code no. 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 according to the following procedure. The rehydration solution was degassed and poured into the large chamber of the Gelpool (Pharmacia Biotech, code no. 18-1031-58). Starting at the edge, the gel film, with the gel surface facing down, was set into the rehydration solution and slowly lowered. The gel was gently agitated several times during the first 15 min. Then the pool was covered with a glass plate (and a wet towel if humidity of the atmosphere was low), and allowed to stand overnight on a horizontal table to enable complete reswelling of the gel. Immediately before use, the rehydrated gel was taken out of the pool, and droplets were wiped off of the gel surface with a sheet of filter paper.

Instrument and Gel Preparation. The gel support plate was cooled to 15 °C. To avoid crystallization of urea during the run within the gel, a small volume of water was poured onto the bottom of the electrophoresis chamber. Electrode wicks (Boehringer Ingelheim Bioproducts, catalog no. 42942) were 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). After evenly dispensing a small volume of kerosene onto the center of the flat-bed electrophoresis apparatus, the gel was placed on the cooling plate, excess kerosene was removed by means of paper towels, and the soaked electrode wicks were applied to the gel.

Sample Application and Running Conditions. The setting conditions for IEF were prefocusing (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 V h).

After prefocusing, the applicator strip [7 × 1 mm, silicon rubber (Boehringer Ingelheim Bioproducts, catalog no. 42989)] was placed approximately 2 cm in front of the cathodic wicks, and 7.5 μL aliquots of each sample extract containing about 8 mg/mL of protein were placed into the slots of the strip; 10 μL of p*I* marker solution was applied under the same conditions.

After about 1000 V h, when the proteins had entered the gel, the applicator strip was removed to avoid bleeding or smearing of proteins.

Coomassie Staining. At the end of the run, at 50 000 V h, electrode strips were removed, and the proteins were fixed and stained with Coomassie dye Serva Violet 17 (Boehringer Ingelheim Bioproducts, catalog no. 35072).

The gel was successively shaken in 200 mL of each solution. It was placed in fixing solution [20% (w/v) TCA] for 30 min, washed in destaining solution [methanol/acetic acid /water (25/10/65) (v/v/v)] for 30 min, stained with 0.1% (w/v) SERVA Violet 17 dissolved in solution for 10 min, destained by changing several times the destaining solution until the background was sufficiently clear and colorless, soaked in the preserving solution [1% (w/v) glycerol (87%)] for 10 min, and then covered with a cellophane preserving sheet and dried at room temperature or in a gel air drying system.

The gels were scanned.

SDS−PAGE Analysis. *Protein Extraction*. Muscle from raw or heated fish was cut into small pieces, and 300 mg of the flesh was homogenized in 4 mL of extraction solution [2% (w/v) SDS, 0.1 M DTT, 60 mM Tris-HCl, pH 7.5] using an Ultraturrax homogenizer equipped with a small rod for 30−60 s at low speed to avoid foaming. Then the samples were boiled in a water bath (100 °C) for 2 min and afterward homogenized while hot for 30 s. Finally, the samples were centrifuged (20000g_{max} at 20 °C for 15 min).

Preparation of Samples for SDS−PAGE. 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].

Three molecular weight markers were used: Novex Mark 12 (catalog no. LC 5677, delivered in solution), Bio-Rad polypeptide SDS−PAGE molecular weight standards (catalog no. 161-0326, delivered in solution), and Pharmacia Biotech molecular weight markers, MW range 2512−16 949 (catalog no. 80-1129-83, delivered dry). A mixed solution was prepared according to the following steps: (i) the Bio-Rad marker was diluted 80 times with Laemmli buffer, (ii) the Pharmacia Biotech marker was reconstituted with 2 mL of Laemmli buffer and diluted 10 times with Laemmli buffer, and (iii) the Bio-Rad solution, the Pharmacia Biotech solution, the Novex Mark 12, and the Laemmli buffer were mixed in the proportion 7 + 7 + 8 + 34.

The staining indicator [1.6 mg of bovine plasma albumin (Sigma A7517) and 1.6 mg of egg white lysozyme (Sigma L4631) in 1 mL of extraction solution] was diluted 841 times with Laemmli buffer in two steps (2 times 1/28).

Electrophoresis Conditions. *Instrument and Gel Preparation*. The gel support plate was cooled to 15 °C by means of a thermostatic circulator, about 1 mL of kerosene was poured onto the plate, and the gel [Excel gel SDS homogeneous 15% (Pharmacia Biotech 80-1262-01)] was positioned on the plate with the wells at the cathodic side. The white cathodic buffer strip (Pharmacia Biotech 17-1342-01) was positioned above the wells with the narrow side against the gel. Similarly, the yellow anodic buffer strip (Pharmacia Biotech 17-1342-01) was positioned at the other edge of the gel.

Sample Application and Running Conditions. Ten microliters of samples, molecular weight marker mixture, and staining indicator was applied in the wells of the gel. The running conditions were 600 V, 30 mA, and 30 W; when the bromophenol front started to enter the yellow electrode strip, the electrophoresis was continued for another 20 min and then stopped. The electrode strips were removed, and the back side of the gel (gel bond) was cleaned using filter paper moistened with ethanol to remove kerosene.

Silver Staining. The proteins were fixed and stained using the Silver Staining Kit Protein (Pharmacia Biotech, Plusone 17-1150-01) with one modification: the stop solution was made with 5% acetic acid to reduce browning of the background. The developing step was stopped when the bands of the staining indicator became visible. The gel was soaked in the preserving solution [1% (w/v) glycerol (87%)] for 20 min, then covered with a cellophane preserving sheet, and dried at room temperature or in a gel air drying system.

The gels were scanned.

RESULTS AND DISCUSSION

The previous studies performed by the same participants (Piñeiro et al., 1999; Etienne et al., 1999) had shown that the protein pattern of raw fish muscle was not significantly changed by cooking the fish. Thus reference material was used raw for identification of cooked fish in the collaborative study. Nine laboratories participated in the exercise; all of the laboratories performed the analysis, eight to identify the unknown samples and the ninth, which had prepared and distributed the samples, to analyze the similarities between patterns using the results of the eight laboratories plus their own results. The participants were informed that the set of unknown samples might contain samples not included in the references, as well as two samples of the same species.

Protein Content of the Extracts. The results of the protein determination performed in the Institute which had prepared the samples are compiled in Table 1. The urea extracts of cooked samples were, in general, less concentrated in protein than raw extracts. With SDS extracts, the protein contents in raw and cooked fish

Table 3. Result of the Collaborative Study Using Urea CleanGel IEF[a]

	fish species	laboratory							
		1	2	3	4	5	6	7	8
C_1	*O. gorbuscha*	+	+	+	*O. gorbuscha* or *S. trutta*	+	+	+	*O. keta* or *O. gorbuscha*
C_2	*S. alpinus*	+	+	+	+	+	+	+	+
C_3	*O. keta*	+	+	+	*O. keta* or *O. gorbuscha*	+	+	ni	*O. keta* or *O. gorbuscha*
C_4	*P. maxima*	+	+	+	+	+	+	+	+
C_5	*O. mykiss*	+	+	+	+	+	+	+	+
C_6	*P. pollachius*	*T. chalcogramma*	*	*	*	*	*	*	*
C_7	*S. salar*	+	+	+	+	*S. trutta*	+	+	+
C_8	*M. australis*	+	+	+	+	+	+	ni	+
C_9	*S. trutta*	+	+	+	+	+	*S. salar*?	ni	*S. salar* or *S. trutta*
C_{10}	*T. chalcogramma*	+	+	+	+	+	+	+	*M. magellanicus*

[a] + = fish species was correctly identified; * = fish was designated as not included in the references; ni = nonidentified.

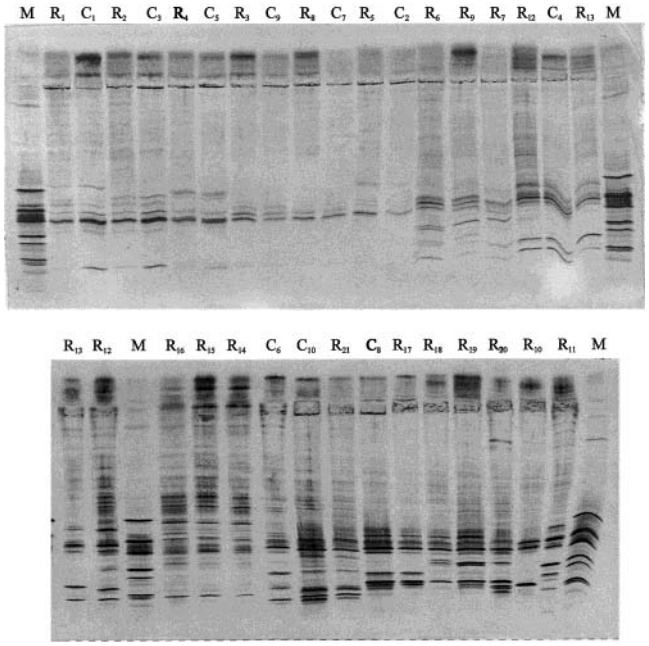

Figure 1. Urea IEF with CleanGel. Extracts of raw (references R_1−R_{21}) and cooked (samples C_1−C_{10}) fish muscle were run on CleanGels rehydrated with 8 M urea, 0.5% (w/v) Servalyte 2-4, 2% (w/v) Servalyte 4-6, and 0.5% (w/v) Servalyte 4-9. M = p*I* calibration proteins. The cathode is at the top of the gel.

were nearly the same. Protein recovery of cooked samples in urea extracts indicates that the power of solubilization of urea is less effective than that of SDS for denatured protein, as shown by An et al. (1988).

Results of the Collaborative Study. *Species Identification by Urea IEF.* The results of the collaborative study using urea IEF (CleanGels) are compiled in Table 3, and two gels corresponding to this exercise are shown in the Figure 1. Some laboratories gave comments with their results: laboratory 3 found similarities between the patterns of *Oncorhynchus mykiss* and *Salmo trutta*, laboratory 6 said the unknown sample C_9 differed from *Salmo salar* by only one single band and CleanGels were difficult to interpret, and laboratory 3 found that *Oncorhynchus keta* and *Oncorhynchus gorbuscha* were impossible to distinguish, as were *S. salar* and *S. trutta*, and it noted the difficulty to differentiate *O. mykiss* and *Salvelinus alpinus*.

One error was made about pollack, the fish that was not included in the references, and in one case Alaska pollock was not correctly identified. Arctic char, turbot, rainbow trout, and New Zealand hake were always identified, as opposed to pink salmon and chum salmon on one hand and Atlantic salmon and sea trout on the other hand. The differences of p*I* values observed between species belonging to different families were sufficient to allow discrimination between them. The differences of p*I* values between species belonging to a same genus may be too faint in some cases: while there were no difficulties in identifying one hake (*Merluccius australis*) among some other hakes (*Merluccius merluccius* and *Merluccius hubbsi*), problems appeared with salmons. Two teams found it difficult or almost impossible to differentiate two salmons species belonging to the genus *Oncorhynchus* (*O. gorbuscha* and *O. keta*), and three encountered the same problem with the genus *Salmo* (*S. salar* and *S. trutta*).

The protein patterns obtained using CleanGel are characterized by strong bands in the acidic part of the gel, as shown in Figure 1. These bands may represent parvalbumins, as well as myosin light chains and troponin C (Rehbein, 1998, and unpublished results). The differentiation between species is made mostly using the acidic bands (p*I* <5). The protein patterns of the hakes show many acidic bands whereas fewer bands characterize the salmons, which can explain the difficulties encountered by some laboratories in identifying these species. Furthermore, the salmon samples may contain different amounts of white and red (pink) tissue, which contain different isoforms of proteins with different p*I* values (Martínez et al., 1991, 1993). Finally, the fact that salmonids display individual myofibrillar protein polymorphisms, possibly stock related (Martínez et al., 1994), may obscure the identification of the species if they affect prominent bands.

Species Identification by SDS−PAGE. The results of the collaborative study obtained with SDS−PAGE are compiled in Table 4, and pictures of gels corresponding to this exercise are shown in Figure 2. The comments were the following: laboratory 1 found similarities between the patterns of *M. australis* and *M. hubbsi* and also between *O. mykiss* and *S. alpinus*, and laboratory 5 said that unknown sample C_8 did not fully match to *M. australis* and that *S. salar* and *S. trutta* were almost indistinguishable.

Most fish species were easily identified by each laboratory. However, there were one mistake and one uncertainty with hake (*M. australis* and *M. hubbsi*), problems with salmon, in particular with *S. salar* and *S. trutta*, and the same error as previously was made about pollack.

The differentiation of fish species was possible by SDS−PAGE considering proteins of molecular weight lower than 30 000. These results confirm those in previous publications (Seki, 1976; Seki et al., 1980; Civera and Parisi, 1991; Scobbie and Mackie, 1988; Sotelo et al., 1992), but some differences in the patterns

Table 4. Result of the Collaborative Study Using SDS−PAGE[a]

		laboratory							
	fish species	1	2	3	4	5	6	7	8
C_1	*O. gorbuscha*	+	+	+	+	+	+	+	+
C_2	*S. alpinus*	+	+	+	+	+	+	+	+
C_3	*O. keta*	+	+	+	+	+	+	+	+
C_4	*P. maxima*	+	+	+	+	+	+	+	+
C_5	*O. mykiss*	+	+	*S. trutta*?	+	+	+	+	+
C_6	*P. pollachius*	*T. chalcogramma*	*	*	*	*	*	*	*
C_7	*S. salar*	+	+	*S. trutta* ?	+	*S. trutta*	+	+	+
C_8	*M. australis*	+	+	*M. hubbsi*	+	*M. australis*?	+	ni	+
C_9	*S. trutta*	+	+	*S. trutta*?	+	*S. trutta*?	*S. salar*?	+	+
C_{10}	*T. chalcogramma*	+	+	+	+	+	+	+	+

[a] + = fish species was correctly identified; * = fish was designated as not included in the references; ni = nonidentified.

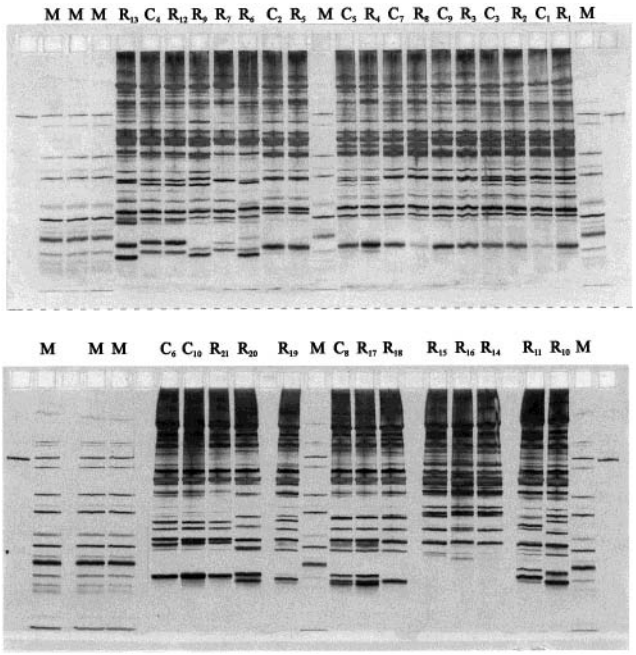

Figure 2. SDS−PAGE. Extracts of raw (references R_1−R_{21}) and cooked (samples C_1−C_{10}) fish muscle were run on Excel gel homogeneous 15%. M = p*I* calibration proteins. The cathode is at the top of the gel.

between MW 30 000 and MW 50 000 could be useful to distinguish closely related species such as *S. salar* and *S. trutta* (difference in the area of MW 40 000).

Concluding Remarks about the Collaborative Exercise. The collaborative exercise was difficult, because the species for identification were closely related. There were five samples belonging to the Salmonidae family, two *Salmo* and three *Oncorhynchus*, and among the reference fish there were also various other closely related species. Despite these difficulties the results were rather good: within the eight participants, using both methods, five laboratories identified all the fish samples that were among the references, two laboratories had encountered difficulty in the differentiation between *S. salar* and *S. trutta* but identified the other samples, and the last laboratory had more difficulties.

The laboratories involved in the study have mostly considered that SDS−PAGE on Excel 15% homogeneous gels was the easiest and cheapest method to perform because the gels were ready to use, there was no need for sample applicators (the wells are already made on the gel), and the cooked samples could be easily identified using raw samples as standards, even if they were not located in adjacent lanes. The silver staining was as fast as the fastest Coomassie staining and the background comparably low. In addition, the samples can be stored frozen in Laemmli buffer. Urea IEF did not provide much additional information to that already obtained by SDS−PAGE. The only problem encountered with some of these gels was the distortion of the migration, which can possibly be corrected by using paper electrode strips instead of the polyacrylamide buffer strips currently used. CleanGels were sometimes considered difficult to interpret because of the few protein bands allowing the discrimination and also faint staining of protein bands. This last difficulty might be overcome by applying more protein on the gels or by using another dye for staining.

Each of the two techniques, SDS−PAGE and urea IEF, can be used for the identification of cooked fish using raw reference samples. Nevertheless, for closely related species characterized by numerous acidic bands, such as hakes, it seems that both methods should be used because they bring complementary information.

Efficiency of SDS−PAGE and Urea IEF for Differentiation of Related Species. The patterns of the reference samples were performed by bringing together (side by side) the fish belonging to a same family or species which are susceptible to be substituted for one another when sold as fish fillets.

Differentiation within Some Flat Fish Species. Turbot (*Psetta maxima*), megrim (*Lepidorhombus whiffiagonis*), dab (*Limanda limanda*), Greenland halibut (*Reinhardtius hippoglossoides*), and halibut (*Hippoglossus hippoglossus*) can be easily achieved by urea IEF and SDS−PAGE, except for turbot and megrim, the patterns of which shows more common protein bands on urea gels; nevertheless, the protein bands between pH 4.9 and pH 5.6 allow differentiation.

Differentiation of Species within the Salmonidae. These include pink salmon (*O. keta*), chum salmon (*O. gorbuscha*), rainbow trout (*O. mykiss*), Arctic char (*S. alpinus*), Atlantic salmon (*S. salar*), and sea trout (*S. trutta*). Using urea IEF the differentiation between the three genera, *Salmo*, *Oncorhynchus*, and *Salvelinus*, is feasible, but the discrimination between the species belonging to the genus *Oncorhynchus* is rather difficult, and the differentiation between the species belonging to the genus *Salmo* is almost impossible. With the SDS−PAGE technique these differentiations are possible. However, Atlantic salmon (*S. salar*) and sea trout (*S. trutta*) are very difficult to discriminate.

Differentiation within Some Tuna Species. These include albacore (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), and skipjack tuna (*Katsuwonis pelamis*). The pattern of skipjack tuna differs slightly from those of albacore and yellowfin tuna with both

Table 5. Efficiency of SDS−PAGE and Urea IEF for Differentiation of Related Species

	SDS−PAGE	urea IEF
flat fish *L. whiffiagonis* *H. hippoglossus* *P. maxima* *R. hippoglossoides* *L. limanda*	easy differentiation	easy differentiation except for *L. whiffiagonis*/*P. maxima* (possible)
Salmonidae *O. gorbuscha* *O. keta* *O. mykiss* *S. trutta* *S. salar* *S. alpinus*	possible differentiation except for *S. trutta*/*S. salar* (difficult)	difficult differentiation for *O. mykiss*/*O. gorbuscha*/*S. alpinus*, extremely difficult for *S. trutta*/*S. salar*
Scombridae *T. albacares* *T. alalunga* *K. pelamis*	possible for *Thunnus*/*Katsuwonus*, extremely difficult for *T. albacares*/*T. alalunga*	difficult for *Thunnus*/*Katsuwonus*, extremely difficult for *T. albacares*/*T. alalunga*
Merlucciidae/Marouridae *M. australis* *M. hubbsi* *M. merluccius* *M. magellanicus*	easy differentiation except for *M. merluccius*/*M. hubbsi* (possible)	easy differentiation
Gadidae *T. chalcogramma* *P. pollachius*	possible differentiation	easy differentiation

methods. The only difference between albacore and yellowfin tuna is one faint band on the SDS gel. They are almost indistinguishable with the urea CleanGel technique.

Differentiation of Species within Some Merluciidae and Macrouridae. Hake (*M. merluccius*), New Zealand hake (*M. australis*), southwest Atlantic hake (*M. hubbsi*), and Patagonian whiphake (*Macruronus magellanicus*) are easily differentiated by both systems. However, SDS−PAGE needs more attention to discriminate the hake from southwest Atlantic hake.

Differentiation between Two Gadoids. Alaska pollock (*Theragra chalcogramma*) and pollack (*Pollachius pollachius)* differentiation can be made without difficulties with either system.

A compilation of all of these results is given in Table 5.

CONCLUSION

The methods described allow the identification of many fish species when cooked. Urea IEF (CleanGel) is less powerful than SDS−PAGE for the discrimination of species characterized by neutral and basic protein bands such as those of the tuna and salmon families. SDS−PAGE seems to be the first method to perform in a control analysis, as it can identify most species conclusively. Nevertheless, in contentious cases, it is preferable to use both methods of analysis.

ABBREVIATIONS USED

SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; IEF, isoelectric focusing; PADM, parvalbumin dry matter.

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