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ISOELECTRIC FOCUSING OF WATER SOLUBLE FISH PROTEIN AS A MEANS OF FISH SPECIES DIFFERENTIATION

By

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ABSTRACT

Soluble protein from drip water of fillets of cod *Gadus morhua* and saithe *Pollachius virens* have been analysed by isoelectric focusing in polyacrylamid gels. Processing procedures in the fish industry such as cold storage of raw fish, freezing storage and blood residues in the fillets had little influence on the protein patterns.

In mixed minces of cod and saithe, protein bands specific for saithe could be observed at the 1% level in the cod mince, and was distinct at the 3% level.

A distinct difference in the protein patterns was found between cod specimens from the Baltic and from the Barents Sea, and therefore geographical type variations have to be considered when using the method as a check against labelling regulations for fish products.

INTRODUCTION

In the interest of fair dealing in international trade and for the protection of the consumer, reliable analytical methods are needed to check that the specifications of fish or fish products comply with labelling regulations. The method of isoelectric focusing offers a favourable method to be used for the identification of fish species. It is then essential to know whether the fish processing methods may influence the protein patterns and thereby complicate the identification of fish products, and also how far one may trust the species identity of the protein patterns.

The technique of isoelectric focusing is used for the separation of ampholytes in stable pH gradients. The ampholytes are separated and charaterized by determining their isoelectric point (pI). Proteins migrate to a point in the gel corresponding to the pH of their isoelectric point, creating a species-specific protein band pattern.

Isoelectric focusing of sarcoplasmic proteins has been used in protein taxonomic studies of fish and tapeworms. (DJUPSUND, 1976; KUMARATILAKE and THOMSON, 1979; LUNDSTRØM, 1979.)

The development of this method has provided a means of separation of high resolving power creating distinct protein bands superior to conventional electrophoretic methods. Small differences in the isoelectric point of proteins, insufficient for resolution in conventional electrophoresis, give clearly separated zones in the isoelectric focusing method.

Conventional electrophoresis can be used effectively for the identification of raw fish fillets. For closely related species and sub-species the isoelectric focusing system gives readily distinguishable patterns according to MACKIE (1980), who found protein patterns from Atlantic and Pacific cod better differentiated by isoelectric focusing than by conventional electrophoresis (MACKIE and RITCHIE, 1981). The aim of the present work was to test the influence of fish handling procedures as cold storage, freezing storage and of blood residues in the fillets on the protein patterns obtained by isoelectric focusing in polyacrylamid gels. Further was studied the detection level of mixtures of different fish species in minced fish using the same method, as well as intraspecific differences in the protein patterns within the same species.

MATERIAL AND METHODS

Samples of fish

Cod, *Gadus morhua*, from the Barents Sea and from the Baltic, and saithe, *Pollachius virens* from the North Sea were used in this investigation. The samples of saithe represented both bled and unbled fish. The fish were kept frozen at – 18°C for one week before preparing the samples.

The influence of cold storage and freezing storage on the protein patterns was investigated on samples from cod, kept in ice for one day (nos 1–6) and for fourteen days (nos 7–12). The fishes were filleted, and 200 grams of the fillets were frozen at −25°C. After storage at −25°C for one week, the samples were kept at different temperatures and time intervals as listed in Table 1.

Table 1.

	−60°C	−25°C	−13°C
Sample nos 1 and 7	7 months	0 months	0 months
» » 2 » 8	6 »	0 »	1 »
» » 3 » 9	3 »	3 »	1 »
» » 4 » 10	3 »	2 »	2 »
» » 5 » 11	3 »	1 »	3 »
» » 6 » 12	3 »	0 »	4 »

To estimate the detection limit of mixtures of different species in minced fish products, cod from the Barents Sea and saithe from the North Sea were thoroughly minced and mixed to give 1, 3, 5 and 10 percent of saithe in cod.

Preparation of aquous extracts

About 50 g of the fish muscle was taken from the dorsal region and after completely thawing 1 ml of the drip water was collected and centrifuged at 20.000 g for 15 min. The supernatant was transferred to small tubes and stored frozen at −18°C.

Preparation of polyacrylamid gel slabs

The chemicals were supplied by LKB, Bromma, Sweden, and the polyacrylamid gels were prepared according to the manufacturer's (LKB) instructions. Only commercially prepared gels from LKB were used.

Isoelectric focusing procedures

Isoelectric focusing was carried out using a LKB power supply model 2103, and LKB multiphor basic unit model 2117. The cooling platform of the multiphor basic unit was connected to a refrigerated water bath keeping a constant temperature in the gel of 2°C. Sample application was performed by soaking a Whatman Filter 1×5×5 mm in the solution, and placing it on the gel near the cathode. Using a constant power of 24 W and a voltage of 1400 V the focusing was completed in 2 hrs. at the pH range of 3.5 to 9. For some experiments at a pH range of 4 to 6.5 the same power was used, but with a voltage of 1800 V and a focusing time of 3 hrs.

The pH gradient was measured using a 3 mm diameter micro combination surface pH electrode. Fixing, staining, destaining and preserving of the gels was performed as described by DJUPSUND (1976).

RESULTS AND DISCUSSION

Fig. 1 shows the protein patterns in fillets from 10 saithe, the first 5 gutted (no 1–5) and the last 5 ungutted (no 6–10). Except for a weak protein band at pH 7.3 in the samples from unbleeded saithe (indicated by an arrow), no distinct differences could be observed between the two groups. Blood residues in unbled fish seemed to have little influence on the protein patterns.

Fig. 2 shows the influence of the time of cold storage and freezing storage on the protein patterns in cod from the Barents Sea. The freezing storage temperature program was the same for samples of fish kept in ice for 1 day and for 14 days (Table 1). The total storage time for all samples was 7 months.

No difference in the protein patterns could be observed between samples nos 1–5 representing cod stored at 0°C for 1 day, and samples nos 6–11 representing cod stored at 0°C for 14 days. For the two samples stored under

the most unfavourable freezing condition (Table 1), a few bands at pH 4.8 were more faint in sample no 6 than in the other samples and had completely disappeared in sample no 12 (indicated by an arrow). One may conclude that the normally used storage temperatures of fish and fish products have only slight effects on the protein patterns obtained by isoelectric focusing and further that the reproducibility of the method is good provided a careful standardization of sample preparation, application and running conditions.

Special problems concerning labelling regulations arise when fish products consist of morphologically indistinguishable fishes having different protein patterns. A test on two stocks of herring from the Irish Sea showed that minor differences in the overall protein patterns do exist. (MACKIE 1980). Closely related species or sub-species of hake (*Merluccius sp.*) also have different protein patterns obtained by electrophoretic techniques. (MACKIE and JONES 1978).

The cod species *Gadus morhua* has an extensive geographical distribution, and a wide range of morphological features are found within the species. Some morphological criteria are affected by the environment, and therefore morphological data may be inadequate for the identification of a particular form or variant of cod. Isoelectric focusing of the soluble protein may be used as an additional means for the identification by individual fish, since protein patterns are independent of sex, age and physiological condition. (TSUYUKI et al. 1965.)

The Norwegian fishing industry import cod from the Baltic and the Pacific. As taste and texture of the flesh from cod caught in these regions are within the range found in the Atlantic cod, no precautions are taken to keep cod caught in different regions separate in the production.

The protein patterns from ten individual specimens of cod are given in Fig. 3. Nos 1 to 5 represent cod from the Barents Sea, and nos 6 to 10 represent cod caught in the Baltic. A significant difference in the protein patterns was observed between the two groups. This observation may complicate the identification of fish products from cod, and must be taken into consideration in the labelling control.

The pH-range 3.5–9 is preferred for most analyses. For certain problems it was found advantageous to use a more narrow pH range giving better resolution of the protein bands (GJERDE 1982). Fig. 4 shows the isoelectric focusing pattern of 5 samples of cod from the Barents Sea carried out at the pH range 4 to 6.5. The resolution of the protein bands increased, but the bands were broader and less distinct than those in Fig. 3. Compared to isoelectric focusing at the pH range 3.5 to 9, the protein patterns obtained at the pH range 4 to 6.5 gave no advantage for the identification of cod.

Fig. 5 shows protein patterns of minced cod mixed with 1 to 10% of saithe. In the 1% sample (no 1) some weak protein bands at pH 4.5 and pH 6,3–6,5,

characteristic for saithe, were detectable (indicated by arrows). These protein bands were more distinct in the 3% sample. Therefore, even with such small percentages as 1–3% of saithe mixed with cod, one can detect protein bands specific for saithe. However, for mixtures of closely related species with nearly identical protein patterns, it may be impossible to state positively that a fish product consists of more than one fish species.

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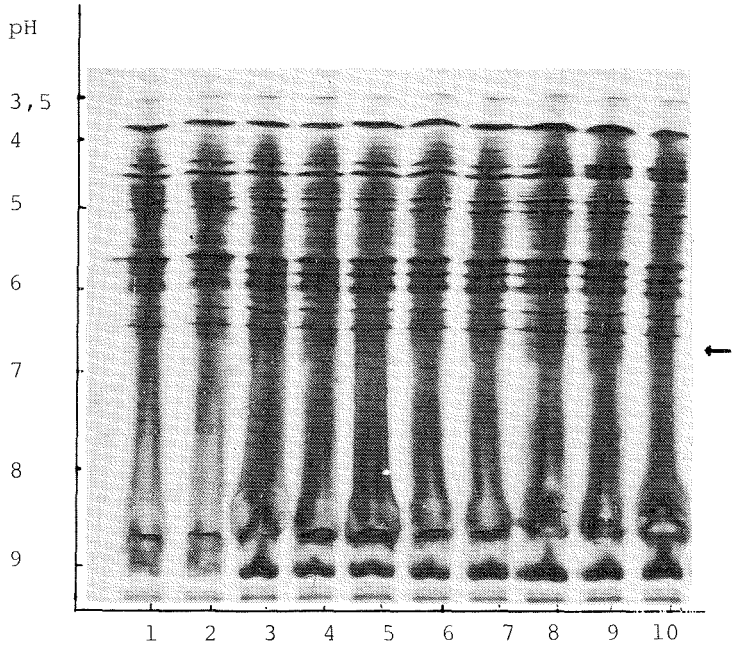

Fig. 1. The electrofocusing protein patterns of bled and unbled saithe, stated pH range 3.5–9.
Nos 1 – 5 Bled saithe
Nos 6 – 10 Unbled saithe

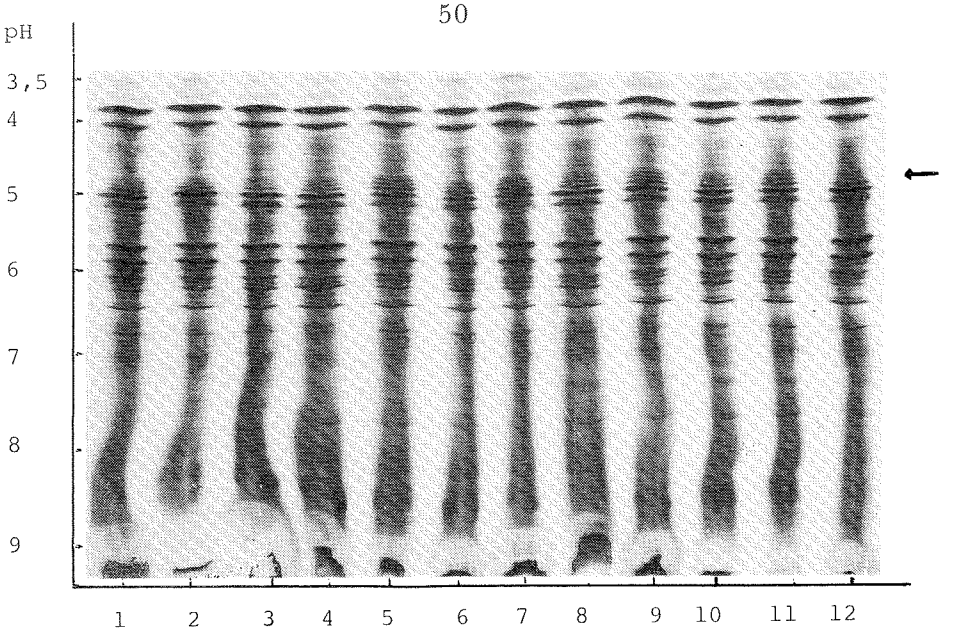

Fig. 2. Influence of cold storage and freezing storage on the protein patterns of cod, stated pH range 3.5–9. Table 1 describes the treatment of the samples.

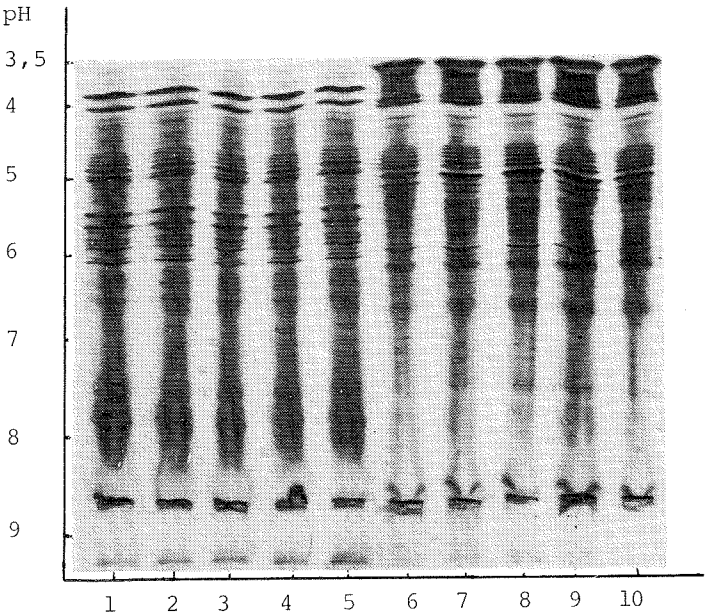

Fig. 3. The electrofocusing protein patterns of cod caught in the Barents Sea and cod caught in the Baltic.

Nos 1 – 5 Cod caught in the Barents Sea

Nos 6 – 10 Cod caught in the Baltic

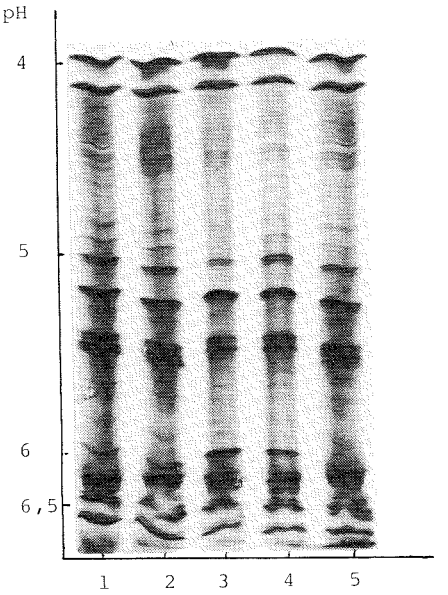

Fig. 4. The electrofocusing protein patterns of cod caught in the Barents Sea, stated pH range 4–6.5.

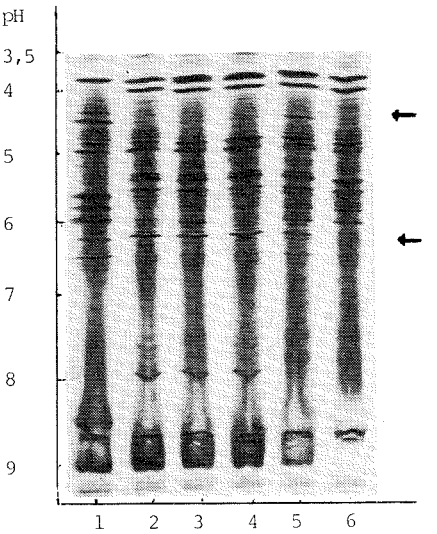

Fig. 5. The electrofocusing protein patterns of saithe and cod and mixtures of the two species, stated pH range 3.5–9.

No 1 Saithe
No 2 Cod with 1% saithe
No 3 Cod with 3% saithe
No 4 Cod with 5% saithe
No 5 Cod with 10% saithe