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Determining the authenticity of raw reformed breaded scampi (*Nephrops norvegicus*) by electrophoretic techniques

A. Craig, A. H. Ritchie & I. M. Mackie

Torry Research Station, 135 Abbey Road, Aberdeen AB9 8DG, UK

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A comparison was made between isoelectric focusing of aqueous extracts and sodium dodecyl sulphate (SDS) electrophoresis of SDS extracts of the 'meat' of raw reformed breaded scampi (*Nephrops norvegicus*) as a possible means of detecting adulteration with Pacific scampi (*Metanephrops andamanicus*) or tropical shrimp (*Penaeus indicus*). Only SDS electrophoresis gave profiles from mixed species products in which the characteristic protein zones of the component species could be identified.

INTRODUCTION

The identification of species of fish or shellfish by electrophoresis or isoelectric focusing of the flesh proteins is now a well-established procedure (Mackie, 1980, 1990). It is used when absolute identification is required; for example, in the enforcement of legislation on the correct naming of fish and fish products when only a portion of flesh is available for examination (Laird *et al.*, 1982; The Food Labelling Regulations, 1984).

The methods used depend upon the separation of the flesh proteins into species-specific profiles which, when compared with those of authentic species obtained under the same electrophoretic conditions, enable the species to be established unequivocally. The identity of raw fish or shellfish is determined from the profiles of the water-soluble or sarcoplasmic proteins obtained by isoelectric focusing (Mackie, 1990), while cooked fish, because of heat-denaturation of the proteins, is analysed by sodium dodecyl sulphate (SDS) electrophoresis of SDS extracts (Ann *et al.*, 1988; Scobbie & Mackie, 1988). Profiles of SDS extracts show greater overall similarity between species, as the main constituent proteins or sub-units are those of the myofibrils and connective tissue proteins which are of the same molecular mass for all animal species. None the less, there are sufficient differences in the profiles, arising possibly from the sarcoplasmic proteins, to enable differentiation of even closely related species such as cod (*Gadus morhua*) and haddock (*Melannogrammus aeglefinus*) to be made (Scobbie & Mackie, 1988).

When such procedures are applied to detect adulteration rather than substitution of one species by another (i.e. mixed species products) their success will depend upon the characteristic zones of the component species being identifiable in the profile of the mixture. For most species of fish, isoelectric focusing (IEF) of the sarcoplasmic proteins is the preferred analytical system as the profiles generally have more species-specific components, with differences between species being much greater than with SDS electrophoresis.

The IEF method, however, is not suitable for identifying mixtures of crustacean species, as the profiles have few zones and most of them are focused within the same narrow range of pH.

This paper demonstrates the successful application of SDS electrophoresis to the identification of scampi (*Nephrops norvegicus*) and other crustacean species such as tropical shrimp (*Penaeus indicus*) and Pacific scampi (*Metanephrops andamanicus*) when present in reformed scampi products.

MATERIALS AND METHODS

Samples of scampi (*Nephrops norvegicus*) were obtained from Aberdeen fish market, Pacific scampi and tropical shrimp from commercial sources, and breaded scampi, from retail outlets. All samples were held frozen at −30°C until required. Prior to extraction of proteins, the samples were allowed to thaw, the flesh separated from the shell and, in the case of the breaded scampi, from the coating.

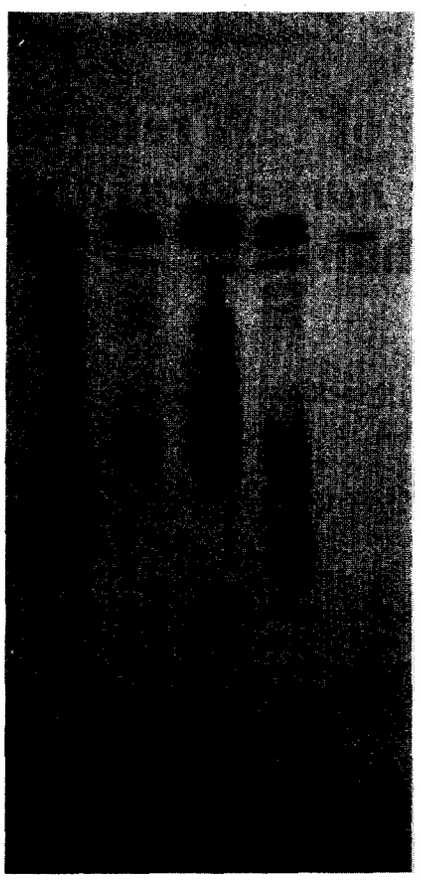

Fig. 1. Isoelectric-focused profiles of aqueous extracts: 1, scampi (*Nephrops norvegicus*); 2, sample A; 3, Pacific scampi (*Metanephrops andamanicus*); 4, sample B; 5, tropical shrimp (*Penaeus indicus*).

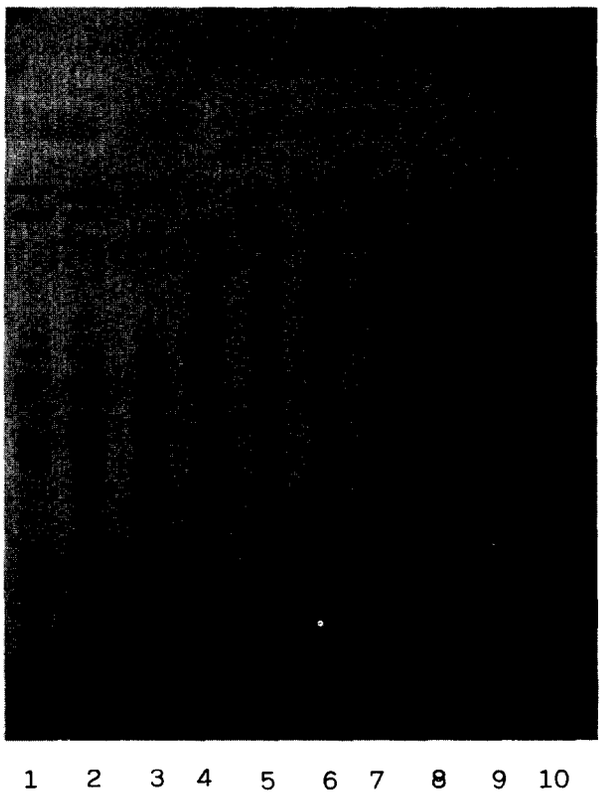

Fig. 2. Isoelectric-focused profiles of aqueous extracts: 1, cod (*Gadus morhua*); 2, haddock (*Melanogrammus aeglefinus*); 3, whiting (*Merlangius merlangus*); 4, scampi (*Nephrops norvegicus*); 5, sample; 6, scampi; 7, sample; 8, scampi; 9, sample; 10, scampi.

Preparation of protein extracts

Water-soluble protein extracts

Portions (5·0 g) of the minced flesh of each thawed sample were homogenised with 10 ml ice-cold distilled water using an Ultra-Turrax homogeniser. The homogenates were centrifuged for 15 min at (3000 *g*) and the supernatant solutions removed, spun in Eppendorf tubes for a further 10 min at 12000 *g* and stored at +1°C until required for isoelectric focusing.

SDS extracts

Portions (0·5 g) of each sample were homogenised in 2 ml of 2% (w/v) aqueous SDS solution and heated for 30 min at 60°C. The extracts were clarified by centrifugation at 3000 *g* for 10 min and the supernatant solutions diluted × 20 with a sample buffer (Laemmli, 1970) to which 2-mercaptoethanol had been added just prior to use. The samples were then heated in a boiling water bath for 2 min, cooled and clarified by centrifugation at 1200 *g* for a further 3 min. The supernatant solutions were stored at −18°C until required.

Isoelectric focusing (IEF)

Preparation of IEF gels

The IEF gels were cast in a gel mould 240 × 110 × 0·5 mm assembled with Gel Bond PAG film attached to the bottom plate. The acrylamide solution (5% acrylamide: bis, 29:1) containing the ampholytes (6%) was degassed using a rotary oil pump, and ammonium persulphate and tetramethylethylenediamine (TEMED) were added. This solution was injected between the glass plates by means of a large syringe fitted with a polythene canula and allowed to polymerise. A total volume of 30 ml solution was sufficient to prepare two IEF plates.

Conditions of isoelectric focusing

The IEF gel was placed on a ceramic cooling plate of a Bio-Phoresis cell, thermostatically controlled at +10°C. The electrode strips were evenly soaked in the electrolyte solution (0·5 M acetic acid at the anode and 0·5 M sodium hydroxide at the cathode) and applied to each end of the gel. Dry sample application papers were placed on the gel surface 20 mm in front of the cathode and 12·5 μl of each sample was loaded in all runs. Isoelectric focusing was then allowed to take place under limiting conditions of 2000 V, 25 mA and 25 W. The application strips were removed after 30 min and isoelectric focusing continued for a further 30 min.

After completion of the run the gel was fixed, stained with Coomassie Brilliant Blue R250, and destained according to the LKB Electrophoresis Laboratory Manual.

SDS electrophoresis

Preparation of SDS–PAGE gel

The SDS-PAGE separating gel was cast in a slab 140 × 140 × 1·5 mm from a 12·0% acrylamide total concentration (2·67% C—crosslinker). Gel and running buffers were 0·375 M Tris (hydroxymethyl) aminomethane (TRIS) titrated to pH 8·8 with HCl. A stacking gel of 4·0% total acrylamide concentration (2·67%C) containing 13 sample slots was prepared as described by Laemmli (1970).

Conditions of SDS–PAGE

Samples (10 μl) of the SD extracts were transferred to the slots and the gel cassette assembly, then mounted in a Bio-Rad Protean cell. Electrophoresis was carried out at a constant current of 30 mA per gel with the electrode buffer reservoir being kept at a constant +10°C by means of a circulating water bath. The run was considered to be completed when the marker dye reached the bottom of the gel (4–5 h).

The gels were then removed from the plates, fixed and silver-stained according to the method of Blum *et al.* (1989).

RESULTS AND DISCUSSION

In contrast with IEF profiles of fish, those of crustacea have few protein zones with the majority of them being found over the same narrow range of pH. This is shown in Fig. 1, where the IEF profiles of Atlantic scampi, Pacific scampi and tropical shrimp are compared. These profiles are not suitable for identifying the components of mixed crustacea products such as scampi/Pacific scampi or scampi/tropical shrimp. They are, however, suitable for detecting the presence of white fish species such as cod, haddock or whiting (*Merlangius merlangus*) (Fig. 2) which have species-characteristic zones well separated from those of scampi. The protein profiles of each crustacean species are, none the less, distinguishable from one another and can be used for the detection of substitution but not adulteration of scampi with one or both of the two species.

As SDS profiles of either raw or cooked fish are also known to be characteristic of the species of fish or shellfish, the three species of shellfish were compared by this method. The profiles obtained show, as expected, that although there are few species-specific zones, they are sufficiently well resolved from those of the other species to enable differentiation of even the closely related scampi species (Fig. 3). In this case, differences in mobility of the major fast-moving zones can

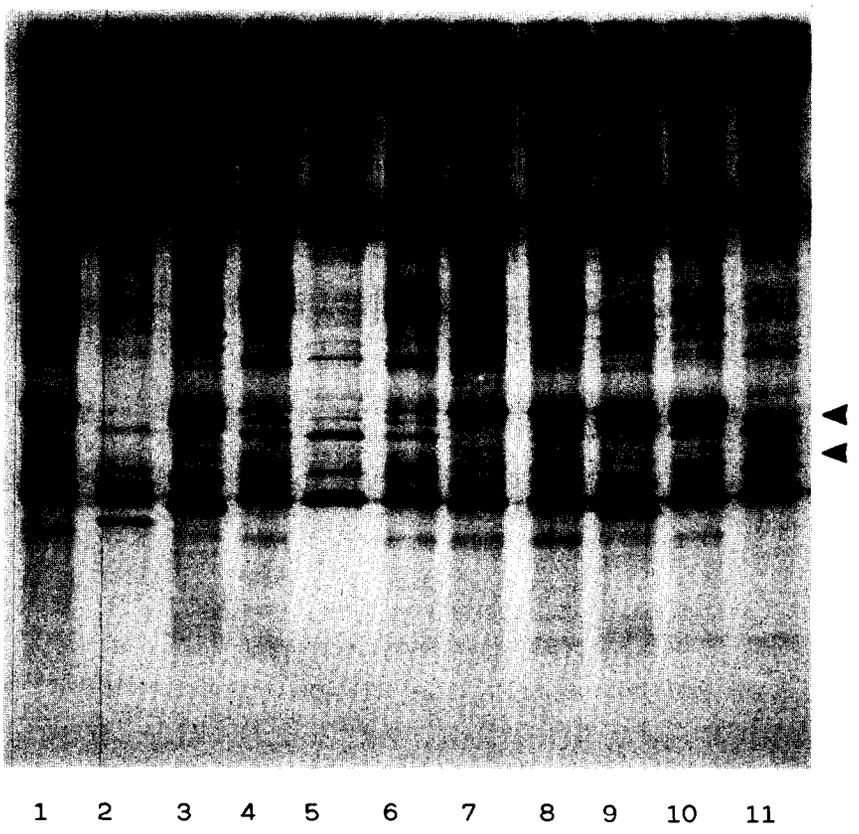

Fig. 3. SDS electrophoretic separation profiles: 1, scampi (*Nephrops norvegicus*); 2, sample C; 3, Pacific scampi (*Metanephrops andamanicus*); 4, sample D; 5, tropical shrimp (*Penaeus indicus*); 6, sample E; 7, scampi; 8, sample F; 9, Pacific scampi; 10, sample G; 11, tropical shrimp.

be reliably used to differentiate those two species. When their profiles are compared with those of tropical shrimp it is evident that there are major zones (arrowed) which are characteristic of this species and which can be used not only to identify it but also to detect its presence as an adulterant in scampi. Similarly, mixed-species products of Atlantic scampi and Pacific scampi could be identified.

Profiles of commercial samples of reputed scampi products are included in Fig. 3 where the main non-scampi bands are indicated by arrows. These protein sub-units which have molecular weights of less than 25 kDa are specific only for tropical shrimp. They represent low molecular weight fragments of myofibrillar or connective tissue proteins which are solubilised by SDS. These proteins do not appear on IEF which uses only water-soluble proteins for pattern recognition.

In conclusion, it can be stated that SDS electrophoresis of SDS extracts rather than IEF of water-soluble proteins is an effective means of detecting tropical shrimp or Pacific scampi in reformed scampi products. As the SDS profiles are not significantly affected by heat-denaturation of proteins on cooking, the SDS procedure has the additional advantage over IEF of being suitable for identifying species both in the raw and cooked states.

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