IDENTIFICATION OF SPECIES IN RAW PROCESSED FISHERY PRODUCTS BY MEANS OF CELLULOSE POLYACETATE STRIP ELECTROPHORESIS

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ABSTRACT

A rapid, simple, and relatively inexpensive method--polyacetate strip electrophoresis--has been adapted for identifying species in raw processed fishery products. The method was used to separate the water-soluble proteins in a variety of samples, including drip exuded by products that had been frozen and thawed. The separated protein bands were then stained to establish a band pattern characteristic of each species of fish.

INTRODUCTION

In frozen processed fishery products such as fish blocks, portions, and sticks, it is extremely difficult and, in some cases, impossible to identify, by sensory means, the various species that may be present in the product. The need for identification arises because there often is a cost differential among the species, and a less costly species may be substituted for a more expensive one.

A method used to identify different species is based on electrophoresis. In the electrophoretic technique, an electrical attraction is used to differentially separate muscle proteins. The separation occurs because each type of protein differs in properties such as size and shape of the molecule but primarily because the molecules differ in net electrical charge. The differences in properties cause the protein molecules to migrate towards the electrodes at different rates.

When this technique was applied to the water-soluble proteins of fish, it was found that they gave reproducible characteristic patterns. The separated proteins were then fixed and stained in a supporting medium, such as starch gel, to give a "fingerprint" that is unique for each species.

The separation into band patterns depends on the properties of proteins in their natural state. This means that the sample being identified must not be denatured, as would be the case if the product has been completely cooked. A fully cooked sample cannot be used, but if the central sections of precooked portions are used, the amount of heat received at this point during normal commercial precooking is generally not great enough to denature the protein, so a satisfactory separation can be obtained.

The U. S. Food and Drug Administration was one of the first to apply the electrophoretic technique as an objective method of identifying fish. In 1960, Robert Thompson of that agency reported on a reproducible method of identification by starch gel zone electrophoresis of fish-protein extracts. This method is now employed routinely by the Food and Drug Administration to determine the species involved when substitution is suspected. Since 1962 when this technique was adopted, about 60,000 pounds of fishery products have been seized because of species mislabeling.

Although the Thompson method is reproducible and selective for different species, nonetheless it has several shortcomings that make it difficult for routine use in the field. It is time-consuming, taking 5 hours for electrophoresis alone, and it requires trained laboratory personnel to carry out the procedure.

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Recognizing these shortcomings, this laboratory has been investigating alternate procedures in an attempt to find or develop a method that is rapid, reproducible, inexpensive, and can be performed by untrained persons with a minimum of instruction. This paper reports on an adaptation of a method that fulfills those requirements.

METHOD

GENERAL: A sample of fish protein is applied to a cellulose acetate strip that serves as a supporting medium through which the proteins can travel. A fixed voltage is applied for a definite length of time. At the end of this period, the strips are stained, washed, and dried to fix the patterns.

PREPARATION OF FISH SAMPLES: Fresh: Approximately 3 grams of meaty portion of fish is ground in a mortar with 3 ml. of water and squeezed through several thicknesses of cheesecloth. If a centrifuge is available, pieces of fish may be placed in centrifuge tubes, and the fluid may be expressed by centrifugation. In this case no added water is required.

Frozen: The sample is thawed, and the drip that forms is used undiluted.

Freeze-Dried: Samples are reconstituted with water and treated in the same manner as the fresh samples.

Breaded Raw Sticks and Portions: The breading is removed by soaking the sticks or portions for a few seconds in water and scraping the breading off with a spatula. The samples are then handled as are the fresh or frozen samples.

Precooked Sticks and Portions: The breading and all surface meat is trimmed until the internal center section remains. This section is then treated as is a fresh sample.

EQUIPMENT: The cellulose polyacetate strips and the electrophoresis cabinet described below are products of the Gelman Instrument Company 1/; similar equipment may be obtained from other sources. The equipment (fig. 1) consists of (1) an electrophoresis cabinet divided into 4 compartments and (2) a constant-voltage power supply with an output potential of 300 volts.

Gelman Applicator
Cellulose acetate strips ($6\frac{3}{4}$" x 1")
Capillary tubes
Filter paper Whatman #1 sheets cut to convenient size

CHEMICALS:

Veronal buffer (pH 8.6 ionic strength 0.05)
- Sodium diethylbarbiturate 10.8 grams
- Diethylbarbituric acid 1.5 grams
- Distilled water to 1000 ml.

Stain
- 200 mg. Ponceau S stain dissolved in 100 ml. 5-percent trichloracetic acid

Wash
- 5-percent acetic acid

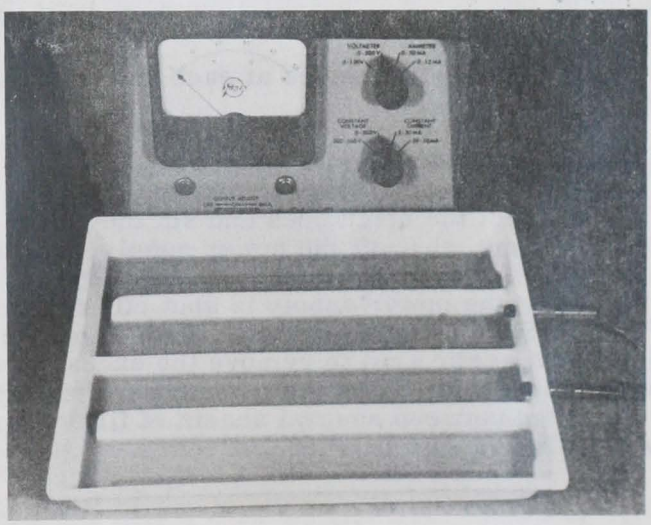

Fig. 1 - Electrophoresis cabinet and power supply.

PROCEDURE: 1. Soak the cellulose polyacetate strips for 30 minutes in the buffer (soaking is required to bring the strips back to their original gel structure). Use a new buffer supply each time. Since 6 strips can be run simultaneously, each with a different sample, identify each strip with a pencil notation before soaking it.

1/Trade and company names referred to in this publication do not imply endorsement of commercial products.

2. Add chilled (34° F.) buffer to each chamber of the cabinet and level to a point slightly below the compartment dividers.

3. After 30 minutes, remove the strips from the buffer, and gently blot them between sheets of Whatman #1 filter paper.

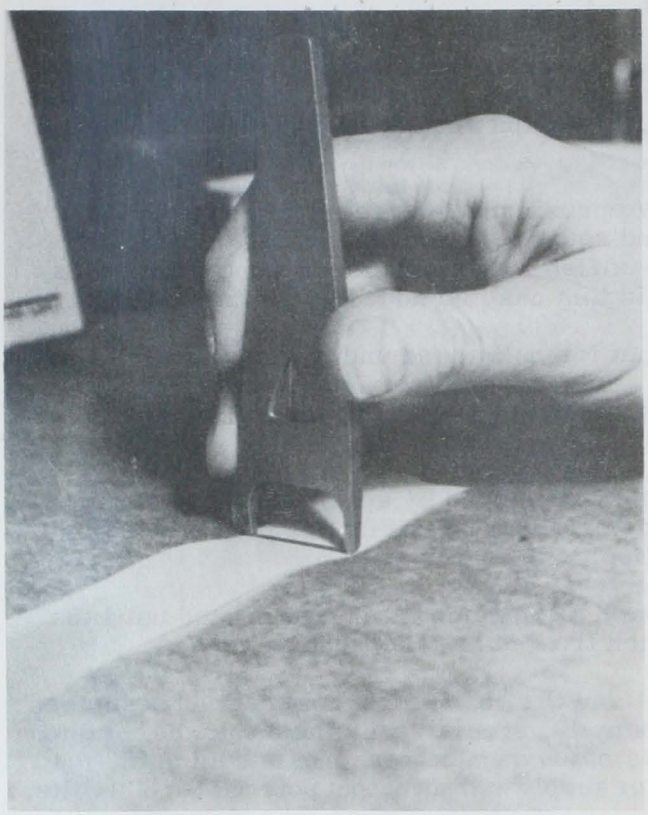

Fig. 2 - Application of sample to acetate strip.

4. Take up a sample of tissue fluid into a capillary tube, and transfer the sample to a special applicator. Draw the capillary tube along the applicator to within $\frac{1}{4}$ inch of both ends. Then press the applicator firmly against the strip about 2 inches from one end (fig. 2). Place the strip containing the sample across the cabinet dividers so that the sample is on the cathode side and both ends are immersed in the buffer in the 2 outer chambers.

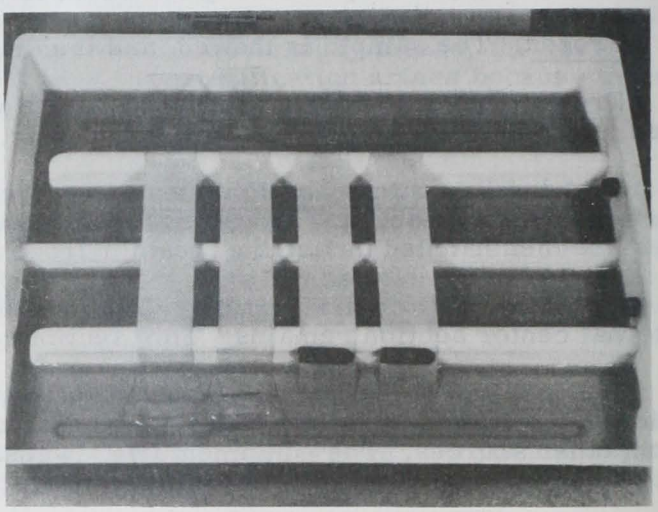

Fig. 3 - Electrophoresis cabinet showing strips secured in place.

Secure the acetate strips at each end with magnets or glass wedges to prevent slippage. Keep them taut (fig. 3).

5. Put the cabinet cover in place.

6. Connect the electrodes and set the power supply at 300 volts for 30 minutes. At the end of this time, shut off the power supply.

7. After the power supply is shut off, remove the strips from the cabinet, and place them in Ponceau S stain for 5 minutes. Then immerse the strips in a series of 3 rinsing solutions of 5-percent acetic acid to remove the excess dye. Rinse the strips until only the protein bands are left stained and the remainder of the strip is free from dye. Finally, blot the strips and dry them between several sheets of filter paper. Once the strips are completely dry, they may be retained as a permanent record.

RESULTS

Three or more samples each of ocean catfish, cod, cusk, haddock, ocean perch, pollock, and whiting were analyzed at separate times with consistent results. This method was also used for identifying (a) 122 samples of drip, (b) 26 samples in the dehydrated and precooked states, and (c) approximately 30 samples submitted by the inspection service. The results were verified by running known samples along with the unknown samples and comparing the patterns. Also, the accuracy of this method was confirmed by running duplicate samples, using other techniques.

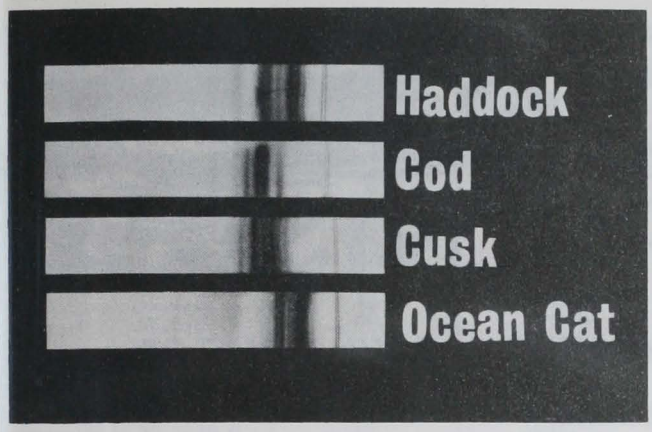

Fig. 4 - Typical acetate patterns from four different species.

Figure 4 illustrates the different patterns obtained from 4 common local species of fish.

CONCLUSIONS

This method of fish-species identification has several advantages over other electrophoretic methods in use:

1. It is more rapid, requiring only 45 minutes to complete after the strips have been soaked.

2. The reagents for the buffer, rinse solutions, and dye can be obtained premeasured from most chemical supply houses and require only dilution to volume.

3. The techniques are relatively simple; any person who is willing to exercise care can be readily trained to perform this analysis.

The cost of the basic equipment, including the power supply, ranges from about $150-$250, depending on the source. At the present time, the Bureau of Commercial Fisheries Technological Laboratory at Gloucester is investigating the possibility of reducing the cost of the cabinet equipment by using readily available materials to fabricate a homemade unit.

In the future, the possibility of species substitution in processed fishery products will undoubtedly be scrutinized more closely by regulatory agencies. It will, therefore, be to the advantage of a producer working with raw materials that have already been processed to some degree to have at his disposal a rapid, reliable method for determining the species in such products. First of all, such a method will ensure that he is receiving the species for which he is paying; secondly, it will preclude the possibility of conflict with regulatory agencies over the question of species when the finished product bearing the processor's name reaches the market place.

The use of the polyacetate strip electrophoresis technique provides a rapid and reliable method for doing this. It has the further advantage of being simple enough to permit application by those untrained in laboratory procedures.

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