

SPECIES IDENTIFICATION OF MEAT BY ELECTROPHORETIC METHODS

Magdalena Montowska, Edward Pospiech

Agricultural University of Poznań

Abstract. Electrophoretic methods can be used to identify meat of various animal species. The protein electrophoresis, especially the IEF of the sarcoplasmic proteins, is a well-established technique for species identification of raw fish and is used in the control of seafood authenticity. However, in the case of the analysis of heat-processed fish, the method is applicable only to those species which possess characteristic patterns of the heat-stable parvalbumins. Heat-denatured fish muscle proteins may be solubilised by urea or sodium dodecylsulfate (SDS) and separated by urea-IEF or SDS-PAGE, respectively. The comparison of these two methods allowed to conclude that, basically, each of them can be used for species identification of heated fishery products. However, extensively washed products may be preferentially analysed by the SDS-PAGE, because most of the parvalbumins are washed out leaving mainly myosins. On the other hand, the IEF method may be preferred for the differentiation of closely related species rich in parvalbumins isoforms. It is evident from the literature data that species-specific protein separations yield proteins of low molecular weight made up of three light chains of myosin (14-23 kDa), troponin (19-30 kDa) and parvalbumin (about 12 kDa). Investigations showed that the SDS-PAGE method can be used to identify meats of: cattle, sheep, lambs, goats, red deer and rabbits. The technique allowed researchers to identify the following myofibrillar and sarcoplasmic muscle proteins: myosin and actin, α -actinin, tropomyosin, troponin. SDS-PAGE allowed the identification of myofibrillar proteins taking into account their molecular weights which was not possible with the assistance of the PAGIF because too many protein bands were obtained. It was possible to obtain differences in the separation of proteins characteristic for certain species, e.g. beef, resulting from the presence of single myofibrillar proteins.

Key words: IEF, SDS-PAGE, species identification

INTRODUCTION

In recent years, species identification of meat has been gaining in practical importance. This has been caused, on the one hand, by a lack of confidence of consumers

Corresponding author – Adres do korespondencji: Mgr Magdalena Montowska, Institute of Meat Technology of Agricultural University of Poznań, Wojska Polskiego 31, 60-624 Poznań, Poland, e-mail: magmont@au.poznan.pl

regarding the origin of marketed products and, on the other hand, by the concern of producers to ensure the safety of the consumers' products. Unfortunately, without an effective system of control of food articles available on the market, consumers cannot be certain of their appropriate quality as well as the absence of the harmful effect of these articles on the human organism. The need for meat species identification derives from the lack of confidence regarding the statement of the manufacturer concerning the meat species used in a given product, occurrence of allergies to the protein of certain animal species as well as some religious considerations (Hindus do not eat beef, followers of Islam and Judaism – pork) as well as the prohibition of trading of meat derived from animal species under protection or threatened with extinction.

It is necessary to control meat products which were subjected during their manufacture to various technological processes causing significant property changes of the applied tissues and raw materials. It is not uncommon to add to such products various plant raw materials which may, additionally, contribute to difficulties in the process of their identification. It may also be necessary to determine the percentage composition of an article manufactured using different meat species.

The optimal identification method should allow the identification of meat species, both in raw materials and in processed meat products containing animal - and plant derived components. It is also important that the applied method should make it possible to identify meat species in products subjected to thermal processes. Furthermore, the employed method should be sensitive and simple.

Methods applied currently for the identification of muscle tissues of unidentified species are frequently based on different indices and, therefore, the results obtained with the assistance of these methods cannot be compared in practice. The essence of these methods is to show the presence of typical constituents or their proportions characteristic for the muscle tissue of a given species.

The following methods can be employed to determine the species of meat, its composition and origin:

- electrophoretical,
- immunological,
- chromatographic,
- genetic.

The presented article discusses examples of the species identification of products of animal origin with the assistance of electrophoretic methods.

THE ESSENCE OF ELECTROPHORETIC METHODS

The electrophoretic methods are based on the separation of proteins in the electric field following their extraction from the muscle tissue and later placed on special media. Initially, starch gels were employed and later polyacrylamide and agarose ones. At the present time, the electrophoretic separation can be conducted on polyacrylamide gels (PAGE), on polyacrylamide gels containing a denaturing agent (sodium dodecyl sulfate) (SDS-PAGE) or by isoelectric focusing (IEF) on the agar or polyacrylamide gel (PAGIF).

The IEF method consists in the separation on a gel which is characterised by the pH gradient resulting from the addition of ampholyte. Individual proteins move towards the

pH value which is in agreement with their isoelectric point (Ip). The PAGE electrophoresis is also referred to as native electrophoresis due to the absence of a denaturing agent. In this method, the protein separation depends on the electrical charge and sizes of protein molecules. Individual proteins move towards the anode or cathode depending on their charge. In the case of the SDS-PAGE, protein molecules with a negative charge imparted onto them by the SDS move only towards anode with the speed depending, with few exceptions, primarily on their molecular weight. There is a linear correlation between the distance travelled by the proteins and the value of the decimal logarithm of their molecular weight which makes it possible to determine the molecular weight of proteins. With the assistance of two-dimensional electrophoresis (2-DE), it is possible to identify meat of various related species of fish, birds and mammals. The obtained protein separation is visible with naked eye (in the case of proteins containing colour component) or following appropriate staining. In the latter case, the most common dyes include: Coomassie blue, silver salts or enzymatic staining [Hofmann 1997].

APPLICATION OF THE IEF METHOD

Protein separation with the assistance of the IEF method is influenced by many factors, among others, by: meat pH and meat defects, age and gender of animals, way of nutrition, rearing conditions, meat storage conditions, activity of native or microbiological proteases as well as the occurrence of polymorphism. It is possible to carry out separation focusing on a single constituent, e.g. myoglobin which is a species specific muscle dye independent of the above-mentioned factors and whose chains are relatively short. Employing myoglobin as an indicator of species, the same separation can be obtained by examining different muscles of the same animal but also by different animals of the same species. It was demonstrated that in the case of meats of such animal species as: cattle, horses, pigs, sheep, roe deer, kangaroos, camels, brown bears, rabbits, hares, chickens, ducks and ostriches the set of myoglobin bands is characteristic and they can be identified on the basis of myoglobin patterns [Hofmann 1997]. In the case of closely related animals, e.g. pig and wild boar, roe deer and fallow deer, the myoglobin patterns are so similar that they rule out possibilities of their identification. The IEF technique can be employed for the identification of related animals with low myoglobin content, for example, a chicken and turkey, using pseudo-peroxidase staining with the aid of a mixture composed of odianizidine and hydrogen peroxide. Some to several minutes after the treatment, red-brown bands appear on the gel. The IEF method based on the myoglobin analysis is also effective in the situation of mixtures composed of different species. Effective electrophoretic separation is possible when fresh meat, muscle fluid as well as lyophilised muscle extracts derived from animals of the same species are used. The band distribution is always identical and the conditions of storage, freezing and de-freezing do not influence the results [Hofmann 1997].

When the IEF method is used for the species identification of meats subjected to thermal treatment, the obtained results are influenced by the height of the temperature during the technological process. Although high temperature does not affect the relocation of the isoelectric points, the bands are not sharp and this may make the assessment more difficult. Therefore, it is recommended to conduct the analysis on one gel onto which the examined samples as well as the reference sample should be placed. In the

case when the tissue was heated to less than 100°C, pseudo-peroxidase staining is sufficient. However, when the applied temperature exceeded 100°C, one of the possibilities is staining with silver compounds. Staining with silver compounds turned out effective in the case of the identification of: cattle, swine, sheep, horse and roe deer semi-processed and processed meats as well as in the case of processed articles consisting of muscle tissues derived from different animal species, provided the proportion of a given tissue is sufficient, e.g. pork and beef can be identified in mixtures subjected to thermal treatment if their proportions exceed 10% [Hofmann 1997].

APPLICATION OF THE PAGE AND SDS-PAGE METHODS

PAGE electrophoresis can be used for the protein identification of meats derived from: pigs, cattle, horses, sheep, fish, reindeer, moose, goats and bears. The method can be employed for the identification of meats of related animal species, including wild animals, provided that the examined material was not subjected to thermal treatments. The identification of meat additives of different animal species in processed products heated to the temperature of 70°C is possible on condition that the examined proteins are dissolved in a 6 M solution of guanidine chloride and employment of isoelectric focusing followed by enzymatic staining [Pyz-Łukasik 1998].

The SDS-PAGE (Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis) method makes it possible to analyse proteins which dissolve poorly in solvents other than SDS solutions. This method can be applied to analyse proteins which do not dissolve in urea-containing solutions. The method can be utilized for protein quality analyses, for example, proteins of various species of fish or for the quantitative analysis which takes into account different degrees of binding of the applied dye by individual proteins. This method is not very convenient because the obtained results can be influenced by many factors, among others, by: age, way of animal nutrition, stress, meat quality deviations as well as the lack of standards since the lyophilized meat extract which might be used as the reference can yield different results than the extract from fresh meat [Minkiewicz et al. 2004].

EXAMPLES OF SPECIES IDENTIFICATION OF MAMMALIAN MEAT

Identification techniques of meats derived from different animal species are based on the examination of muscle extracts. The employed techniques use either muscle drip or squeezed juice which contain most of the soluble meat proteins and even some structural proteins released into them either as a result of the applied technological processes or factors affecting the animals during the short period before slaughter. Among the electrophoretic methods, the most suitable for the comparison of proteins from different animal species are: PAGE, SDS-PAGE and PAGIF [Parisi and Aguiari 1985].

Investigations carried out by Parisi and Aguiari [1985] showed that the SDS-PAGE method can be used to identify meats of: cattle, sheep, lambs, goats, red deer and rabbits. The technique allowed researchers to identify the following myofibrillar and sarcoplasmic muscle proteins: myosin and actin, α -actinin, tropomyosin and troponin.

SDS-PAGE allowed the identification of myofibrillar proteins taking into account their molecular weights which was not possible with the assistance of the PAGIF because too many protein bands were obtained. It was possible to obtain differences in the separation of proteins characteristic for certain species, e.g. beef, resulting from the presence of single myofibrillar proteins.

Hofmann [1985] obtained somewhat different results using the SDS-PAGE method. The aim of his experiments, in which muscles and their water extracts were used, was to identify and compare several muscles derived from cattle, pigs and horses. Separations of the muscle proteins of these three animal species showed identical bands. Distinct differences were observed in the intensity of the stained bands. These differences were also observed between the protein separations of different muscles derived from the same species. A considerable variability in the intensity of the myoglobin bands derived from different muscles of the same species confirmed their wide diversification with regard to the myoglobin content. On the basis of his experiments Hofmann [1985] concluded that the SDS-PAGE method cannot be employed for the species identification of muscles derived from cattle, pigs and horses. However, simultaneously, he conducted a number of studies with the aim to check possibilities of the identification of different animal species, i.e. cattle, pigs, horses, red deer, roe deer, fallow deer and wild boar using the isoelectric focusing on the polyacrylamide gel (PAGIF). Protein bands in the swine and wild boar separations were situated almost at the same positions but differed with regard to their intensity. No apparent differences were found between separations of the roe deer and fallow deer, while those of the red deer and roe deer differed with regard to the intensity of bands which were less intensively dyed in the upper range of the separation. Bearing in mind the results of the above experiments, it is not at all certain if the IFE is capable of differentiating closely related species. It was further found that different muscles derived from the same species can yield different separations. In experiments conducted on other species which were not closely related (rabbit, duck, chicken), the obtained separations were more diversified and there were no problems with their species identification [Hofmann 1985].

In further investigations, the IEF of soluble proteins of: cattle, pigs, wild boar, horse, roe deer, fallow deer, rabbit and chicken was employed for the species identification on the basis of the determination and comparison of the bands of myoglobin which is a species specific muscle dye and whose chains are relatively small with regard to their molecular weight. The separations of myoglobin bands can be observed without gel staining. In order to achieve greater diversity in the distribution of myoglobin bands, an ampholyte with a narrow pH 5-9 was applied. Moreover, attempts were made to achieve better separations by the application of higher protein concentrations using squeezed juice instead of the muscle extract and employing 1 mm thick gels. The determined species yielded characteristic separations of two or more bands of myoglobin. Different muscles gave identical separations. Wild boar separations were poorly visible because the employed extracts derived from frozen meat stored for three years. The remaining samples were either fresh or frozen for a short period of time. The obtained myoglobin separations were distinctly characteristic for the determined species [Hofmann 1985].

EXAMPLES OF SPECIES IDENTIFICATION OF FISH MEAT

Species identification of fish products subjected to thermal treatment processes

Protein electrophoresis, in particular the IEF of sarcoplasmic proteins, is a well known technique used for the species identification of fresh fish and is applied to control the authenticity of marine products [Mackie 1996]. On the other hand, in the case of fish products subjected to processes of thermal treatment, this method can be applied only for the determination of species which give characteristic separations of the parvalbumins stable after heating [Plowman and Herbert 1992, Rehbein 1992]. Proteins of fish muscles denatured at high temperatures can be dissolved either by urea or SDS and separated, respectively, either by IEF or SDS-PAGE [Scobbie and Mackie 1988].

The above methods were employed to carry out experiments on the species identification of cooked fish [Rehbein et al. 1999]. Fish was filleted, placed in plastic bags and heated in a water bath to the temperature of 85°C for 2 min from the moment the above temperature was achieved by the sample. Next, the samples were cooled down to room temperature and stored at -20°C.

Samples were divided into three groups. The first group consisted of 10 reference samples of raw muscles derived from 10 fish species. The second group included 5 cooked fish species determined with the aid of the IEF with urea, whereas the third one comprised 5 cooked fish species determined with the assistance of the SDS-PAGE.

The commercially available gels were employed for the identification of raw and cooked filleted fish:

- CleanGels for the IEF,
- Immobiline Dry Plates for the IEF,
- ExcelGels for the SDS-PAGE.

Two methods were applied to determine the concentration of proteins in the examined extracts: the spectrophotometric method which assesses optic density at 280 nm and Coomassie staining (Bio-Rad Protein Assay). Cooking reduced by about 50% the amount of soluble proteins when the extraction was conducted using a urea-containing buffer. On the other hand, when the solution contained SDS, the same quantities of protein were extracted from raw and cooked tissues.

The IEF technique used two different gel types which separated proteins in the form of bands in the acid part of the gel. These bands can represent parvalbumins or light myosin chains as well as troponin C. Cooking did not change significantly the separation of proteins from the fish raw muscles which may indicate that the raw muscle can be used as reference material for the identification of cooked fish. The interspecific differences concerning the pI values were sufficient to identify all the tested fish species. Both types of gels yielded similar results. The IEF technique with urea turned out to be an appropriate method for the identification of cooked fish as only one of 35 identifications was incorrect [Rehbein et al. 1999].

In the case of the SDS-PAGE method where staining with silver compounds was employed, small quantities of protein were used (about 3 µg per path). Small quantities of proteins (74 ng BSA and lysozyme per path) were used for the standardization of staining with silver. The region with a high molecular weight extending from actin (42 kDa) to heavy myosin chains (200 kDa) was characterized by some strong bands and was situated in the same place for all the examined species, while the number of less

stained bands revealed a certain diversity between species. However, this diversity was too small to allow identification of species [Rehbein et al. 1999].

The researchers succeeded to identify all the examined species despite the fact that some of them were genetically closely related and belonged to the same family (*Gadidae*), e.g. cod (*Gadus morhua*), whiting (*Merlangius merlangus*), haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*). Only one of the total of 40 samples was identified incorrectly.

The comparison of the methods indicated that, generally speaking, both of them are suitable for the species identification of heated fish products, although the SDS-PAGE method appears to be better suited for the analysis of well-washed raw materials because the majority of parvalbumins are washed out and what remains is mainly myosin. On the other hand, IEF with urea is better suited for the identification of closely related species rich in parvalbumin isoforms.

It is evident from literature data [Civera and Parisi 1991, Scobbie and Mackie 1988, Sotelo et al. 1992] that species-specific protein separations yield proteins of low molecular weight made up of three light chains of myosin (14-23 kDa), troponin (19-30 kDa) and parvalbumin (about 12 kDa). Species identification of fish can be carried out using the SDS-PAGE method taking into consideration protein characterized by proteins of molecular weight less than 30 kDa.

Species identification of smoked and marinated fish products

The above methods were also employed for species identification of smoked and marinated fish products derived from the salmonid family and smoked eels [Mackie et al. 2000]. The procedures employed to extract proteins in the SDS solution and the electrophoretic analysis with the SDS-PAGE method were described by Pineiro et al. [1999], whereas those used for the protein extraction with the assistance of urea and isoelectric focusing – by Etienne et al. [1999]. In standard samples (raw fish) water-soluble proteins were assayed employing IEF using ready-made Ampholine PAG commercial gels; 10 µl of sample was placed on gels.

The SDS-PAGE method was not sufficiently appropriate for the unequivocal identification of smoked and marinated fish derived from the salmonid family. Identification between the smoked sea trout (*Salmo trutta*) and the Atlantic salmon (*Salmo salar*) was not possible because their separations were very similar. Somewhat greater differences were observed in the case of the rainbow trout (*Oncorhynchus mykiss*) but they were not sufficiently large to carry out the identification with complete certainty. Smoked arctic char (*Salvelinus alpinus*) was easily identified from among other processed species. On the other hand, all raw reference species were identified correctly. The SDS-PAGE method was less suitable for the identification of processed samples due to distinctly smaller changes in the separation of protein bands.

Smoking and marinating processes appear to cause relatively smaller differences in the separation of proteins obtained using the SDS-PAGE and IEF with urea methods which makes the identification of closely related species a little more difficult. These changes concern, in particular, the SDS-PAGE analyses. Nevertheless, despite these problems, all the three analytic methods, namely: native electrophoretic, SDS-PAGE and IEF can be employed for the identification of trout and salmon species. Although in the case of processed products the SDS-PAGE method yields more protein bands than

the IEF method, nevertheless in the case of the former method, too few differentiating bands were obtained to carry out a correct identification. Therefore, the IEF method is commonly considered to be better for the identification of trout and salmon species. On the other hand, for such species as *arctic char* and rainbow trout, the SDS-PAGE method turned out to be more suitable. It is clear from the above investigations that the native IEF is not suitable for the identification of the smoked sea trout and the Atlantic salmon as well as the marinated Atlantic salmon, although the method can be applied as a supplementary analysis [Mackie et al. 2000].

The above investigations showed that species belonging to the eel family can be identified with the assistance of both SDS-PAGE and IEF with urea methods. However, the IEF method with urea is better suited for the identification of the two Atlantic species: the European (*Anguilla anguilla*) and American (*Anguilla rostrata*) eels. On the other hand, the native IEF method can be employed to distinguish the short-finned eel (*Anguilla australis*) from the Atlantic species. However, since the industrial smoking conditions frequently differ from one another, this method provides only supplementary information [Mackie et al. 2000].

Martinez et al. [2001] used the SDS-PAGE method for the identification of the following fishes: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*), minke whale (*Balanoptera acutorostrata*) and harp seal (*Phoca groenlandica*). The investigations were carried out on both raw as well as processed (cooked, cold and hot smoked and marinated) samples. Experimental samples were analysed on the following four gels: ExcelGel, on which 3 µg of proteins were placed stained with silver, Tris-Tricine gels – 7 to 10 µg proteins stained with Coomassie Brilliant Blue, NuPAGE Bis-Tris gel – 12 µg proteins stained with colloidal blue and Anderson gel – 1.5-2 µg proteins stained with silver. The first three gels were ready-made commercial gels.

The Anderson gel distinguished itself with the strongest differentiating strength showing six diagnostic bands between the Atlantic salmon and the rainbow trout. The ExcelGel showed three bands, Tris-Tricine gel – two bands and the NuPAGE Bis-Tris gel – only one differentiating band. Raw and cooked samples of the same species yielded the same profiles on all experimental gels with the exception of the ExcelGel. The separations of the smoked and marinated samples differed quite significantly from the raw samples, which made the identification more difficult. The obtained separations were not sufficient to distinguish between the smoked and marinated salmon and rainbow trout. In addition, the fastest migrating bands were found blurred and pathways distorted on the majority of gels [Martinez et al. 2001].

Identification can also be performed by estimating band molecular weights and their comparison with the published values. However, when carrying out the estimation, it is essential to remember that it is possible to obtain slightly different values for the same bands changing times of migration, temperature, pH values of the buffer etc. In order to assess the authenticity of a product, samples should be analysed on the same gel and in identical conditions. The performed investigations revealed that the Anderson gel turned out to be the best of all the applied gels. It allowed obtaining proteins in the range appropriate for the most changeable myofibrillar proteins – troponin and light chains of myosin. The weakening of the faster migrating bands was less visible on this gel than on the ExcelGel indicating that fewer interactions with polymers occur in it [Chrambach and Radko 1998]. It was further found that commercial gels are usually characterized by

the composition satisfactory for many users and are suitable for a wider range of samples. It is also evident from the performed investigations that the intraspecific polymorphism of myofibrillar proteins emphasizes the need for extreme caution in the case when the identification is based on the presence or absence of only one or a few bands. That is why it is necessary to apply gels which produce a greater number of diagnostic bands [Martinez et al. 2001].

Species identification of fresh fish

Native IEF and 2-DE electrophoresis were utilized to identify freshwater fish often referred to as “perch”, i.e. the European perch (*Perca fluviatilis*), the Nile perch (*Lates niloticus*), the European pikeperch (*Stizostedion lucioperca*) and a breeding hybrid of sunshine bass (*Morone chrysops x saxatilis*) [Berrini et al. 2006]. The above-mentioned species are usually sold as fillets because whole fish contain excessive quantities of fishbone. The most valuable species is the European perch which is also the most expensive one. On the other hand, general classification of the products as “perch” without specification of the fish species may be misleading for consumers and result in the falsification of products.

From each of the investigated species, 6 samples of sarcoplasmatic proteins extracted from the muscles of white fish were analysed. The native IEF was conducted on polyacrylamide gels employing the Ampholine PAGplates placing on them 40 µg of the sample. Gels were stained using Coomassie Brilliant Blue R250. In the case when the 2-DE was applied IEF was carried out on the Immobiline DryStrip and the SDS-PAGE was carried out on polyacrylamide gels stained with silver nitrate.

Using native IEF, protein separations were obtained and compared. Each analysed species yielded a characteristic band separation thanks to which it was easy to carry out identifications. The performed IEF showed that there were no bands common for the four tested species. There was only one European pikeperch sample in which a distinct band in pI 4.26 appeared which was not present in the remaining five samples but it did not prevent correct identification of this species. Also other studies [Tepedino et al. 2001] reported poor intraspecific polymorphism of muscle proteins.

Majority of bands appeared in the pH range of 4.0-7.0. According to Rehbein [1990], proteins of white muscles which give distinct bands in the acid part of the gel comprise, primarily, parvalbumins. In native conditions, their pIs are found in the range between 3.8 and 5.3 and they are species specific. Two larger bands visible on the protein separations of the European perch (pI 4.30 and 4.83), Nile perch (pI 3.91 and 4.08) and European pikeperch (pI 4.07 and 4.52) can be included in this group of proteins. The hybrid of the sunshine bass showed four bands which can be classified as parvalbumins. The second difference concerned the band in pI 7.05 present in the samples of the European and Nile perch and European pikeperch and missing in the samples of the sunshine bass. These differences could be attributed to the fact that the sunshine bass is a hybrid which developed from the crossing of the white bass (*Morone chrysops*) female and striped bass (*Morone saxatilis*) male in the process of breeding [Berrini et al. 2006].

Considerably greater possibilities of species identification are predicted when two-dimensional (2-DE) electrophoresis is employed. Species investigation of fish sold as “perch” carried out by Berrini et al. [2006] demonstrated that all 2-DE patterns were

species-specific. Numerous spots corresponding to particular proteins and species occurred, primarily, in the acid part of the pH gradient. The Sunshine bass was easily recognised due to the presence of five spots of proteins which were absent in the remaining species. Samples of the European perch contained many small spots spread over the acid pH gradient. Apart from the distinct differences, seven spots of proteins were common for all the four examined species.

CONCLUSION

Electrophoretic methods are widely employed in investigations of food products. Protein electrophoresis is a particularly well-known technique used for the species identification of raw fish and is applied to control the authenticity of marine products. The electrophoretic methods, apart from their application for the species identification, may also be useful for the proteomic investigations connected with the assessment of the freshness of products, falsification of food articles of both plant and animal origin or processing conditions.

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IDENTYFIKACJA GATUNKOWA MIĘSA METODAMI ELEKTROFORETYCZNYMI

Streszczenie. Metodami elektroforetycznymi można identyfikować mięso różnych gatunków ssaków i ptaków. Elektroforeza białek, a zwłaszcza IEF białek sarkoplazmy jest dobrze poznaną techniką do identyfikacji gatunków surowych ryb i jest stosowana do kon-

troli autentyczności produktów morza. Natomiast w wypadku produktów rybnych poddanych procesom obróbki cieplnej metoda ma zastosowanie tylko do oznaczania gatunków dających charakterystyczne rozdziały stabilnych po ogrzaniu parwalbumin. Zdenaturowane w wysokiej temperaturze białka mięśni ryb mogą być rozpuszczone przez mocznik lub SDS i rozdzielone przez zastosowanie odpowiednio IEF lub SDS-PAGE. Porównując obie metody stwierdzono, że w zasadzie są one odpowiednie do identyfikacji gatunkowej ogrzewanych produktów rybnych. Jednak surowce intensywnie myte lepiej analizować metodą SDS-PAGE, ponieważ większość parwalbumin jest wymywana, a zostaje głównie miozyna. Jednocześnie IEF z mocznikiem jest lepszą metodą do różnicowania blisko spokrewnionych gatunków bogatych w izoformy parwalbumin. Z danych literaturowych wynika, że specyficzne gatunkowo rozdziały białek dają białka o małej masie cząsteczkowej złożone z trzech lekkich łańcuchów miozyny (14-23 kDa), troponiny (19-30 kDa) i parwalbuminy (około 12 kDa). Badania wykazały, że metodą SDS-PAGE można różnicować mięso bydła, owiec, jagniąt, kóz, jeleni i królików. Technika tą identyfikowano szczególnie miofibrylarne białka mięśni: miozynę i aktynę, α -aktyninę, tropomiozynę i troponinę. SDS-PAGE pozwoliła na rozpoznanie tych białek z uwzględnieniem ich mas cząsteczkowych, co nie było możliwe z zastosowaniem PAGIF, ponieważ uzyskano zbyt dużą liczbę pasm białek. Stało się możliwe uzyskać różnice w rozdziałach białek charakterystyczne dla pewnych gatunków, np. wołowiny, wynikające z obecności pojedynczych wyróżniających się białek.

Słowa kluczowe: IEF, SDS-PAGE, identyfikacja gatunkowa

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