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ELECTROPHORETIC ISOFOCUSING IN STUDIES ON MEAT PROTEIN FROM VARIOUS ANIMAL SPECIES AND PORK STORED UNDER DIFFERENT CONDITIONS

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The aim of investigations was to evaluate the IEF separation patterns of proteins from meat of various animal species using direct contact electrophoresis (DCE) on agarose gel. These separation patterns were compared with the results of electrophoresis of meat centrifugal drip after deionization. All separations were accompanied by immunoblotting against titin. The comparison of IEF separations of proteins from meat of various animal species by DCE revealed that their patterns were similar to those of proteins from the centrifugal drip, although they were slightly more corrugated and of a more varied width. Changes in proteins from various fractions of the centrifugal drip of pork stored for 168 h were also analysed. The comparison of samples from measurements performed 48 and 168 h after slaughter revealed that, during meat storage, not only did the number of proteins in the drip increase but their IP also changed. As a rule, titin degradation products showed higher IP values.

INTRODUCTION

The functional and physicochemical properties of muscle tissue after slaughter depend mainly on the state of its proteins. These properties can be modified by the pH value. Professional literature provides scant information on protein changes, taking into account the values of the isoelectric point (IP) typical of both proteins and products of their breakdown [Lametsch *et al.*, 2001]. Most frequently, the IP is presented for major meat proteins, which are either extracted or washed from the tissue. This method is often combined with polyacrylamide gel electrophoresis with SDS when, following the separation against the IP in the so--called first direction, proteins are further characterised with regard to their molecular weights [Pernelle *et al.* 1986, Hirabayashi, 2000, Lametsch & Bendixen, 2001]. However, the progress in the area of protein assessment methodology allows IP determination without protein extraction from meat. This is possible due to the method of direct contact electrophoresis (DCE). In addition, the use of antibodies for protein detection enables ascribing a specified IP value to a given protein or a product of its degradation. Among many proteins responsible for meat tenderness, titin appears to deserve special attention [Greaser *et al.,* 2000].

The objective of the study was to compare the electrophoretic separations of meat as well as its centrifugal drip with regard to the IP for various types of raw material, using the method of direct contact electrophoresis of meat samples placed directly on gel. In the case of pork, the experiments were to show if different storage time and conditions influenced not only the quantities but also the IP values of proteins from different drip fractions. The protein applied for immuno-electrophoresis was anti-titin antibody mouse monoclonal IgM 9D10.

MATERIALS AND METHODS

The investigations were carried out on triplicate meat samples taken from pigs, cattle, calves, sheep, goats, chickens and turkeys. With the exception of pork, the remaining meat raw materials were purchased at a butchery shop, where they were sold on small trays wrapped in plastic foil restricting free access of air. In the case of large farm animals, meat was cut out from the *longissimus thoracis* muscle, and in the case of poultry – from breast muscles.

Samples of pork were obtained from controlled slaughter, 24 h *postmortem*. In this case, three types of samples were analysed. The first type was the drip loss collected from meat slices placed in plastic bags directly after slaughter and stored for 4 h in a water bath at 40°C, and then in a refrigerated room for 24 h. After sampling, it was centrifuged at 15 000 g for 20 min at 2°C. In this way, two fractions of drip were obtained: the supernatant, corresponding to the soluble fraction of proteins and the sediment, which constituted its solid part after centrifugation. The latter fraction was analysed by direct contact electrophoresis (DCE). The other two samples comprised centrifugal drips from meat. They were obtained as a result of meat centrifugation, after 48 and 168 h of cold storage. The conditions of meat centrifugation were the same as in the case of the sed-

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iment from the drip loss. Before placing the supernatant of the drip loss and the centrifugal drip on the gel, they were desalted using Sephadex – G10 columns.

The electrophoretic separation was conducted on 1.5% agarose gel. 12 g of sorbitol was added to 1.5 g agarose (neutral Agarose for IEF from Serva) and 100 mL water was used to dissolve this mixture. For each gel, 6 mL of agarose and 300 μL of Pharmalyte of 3–10 pH were used. The so-called gel bonds were employed to facilitate their manipulation [Pospiech *et al.*, 2003].

Protein separation was conducted using direct contact electrophoresis (DCE). Meat samples were placed on the gel for 3 min with no electricity and for 12 min after initiating the electrophoresis. Similar procedures were applied in the case of the sediment from the drip loss. Then the samples were taken off the gel, and proteins absorbed by the gel were separated. The electrophoresis was performed using the Pharmacia Flat Bed Apparatus FBE-3000 and constant power supply ECPS 3000/15. The initial settings were: power – 2 W, voltage – 1200 V, and current – 15 mA. The power was later increased (to 4 W) and so was the voltage (usually to 2300 V). The electrophoresis was terminated after 60 to 80 min. A similar procedure was used in the case of the examination of the sediment from the drip loss.

Protein fixation was performed using two solutions: sulfosalicylic acid (5%) (Merck) and TCA (10%) (Fluka) (5 min), and glacial acetic acid (30%) and ethyl alcohol (10%) dissolved in water (5 min). Later, the gel was dried using Whatman No. 1 filter paper and, during the final stage of drying, a fan with a fairly strong air blow. Then the gel was stained using the Coomasie Brilliant Blue R250 (Sigma) (5%) dissolved in a water solution containing ethyl alcohol (45%) and acetic acid (45%). After 5 min, the pigment solution was discarded and the gel was de-stained using a water solution containing 30% of glacial acetic acid and 10% ethyl alcohol. This process lasted *ca*. 30 min and then the gel was dried.

Immunoblotting was performed employing a nitrocellulose membrane, which was placed on the gel after the termination of the separation, and rinsed with a few drops of water. Before placing the membrane on the gel, it was wetted in a small amount of water and then, after placing it on the gel, it was covered with a few layers of filter-paper and pressed with a 1.5 kg weight for 10 s. After gentle drying, the membrane was first incubated in a TBST solution containing 1% BSA for 0.5 h and later with a primary antibody. Anti-titin mouse monoclonal antibody IgM 9D10 was used for this purpose [Fritz & Greaser, 1991]. Horseradish peroxidase conjugated with rabbit immunoglobulins to mouse immunoglobulins was used as the secondary antibody. The colored reaction was achieved using DAB (Sigma Fast™) in a solution with urea and H_2O_2 (Sigma Fast™).

In order to identify the IP of proteins, the Serva Mixture 9 Protein Test containing protein markers was used. This set comprised: cytochrome C (IP 10.65), ribonuclease A (IP 9.45), myoglobin from horse (IP 6.9–7.35), carboanhydrase from bovine erythrocytes (IP 6.0), β-lactoglobulin (IP 5.15–5.3), trypsin inhibitor from soybean (IP 4.5), glucoseoxidase (IP 4.2), and amyloglucosidase (IP 3.5).

RESULTS AND DISCUSSION

Figure 1 shows the IEF separations of meat proteins from various animal species, which were performed using direct contact electrophoresis and corresponding immunoblotting against the 9D10 titin antibody. Similar separations are shown in Figure 2, although in this case the centrifugal drip from muscle tissue was evaluated. When comparing these two separations, it is evident that the bands corresponding to the IP of proteins from the centrifugal drip were, with some exceptions, straight or slightly corrugated. Also the width of bands was more consistent along the entire length of the separation than in the case of analysis of proteins directly from muscle tissue. The above-described phenomenon can probably be attributed to the higher concentration of salts in the meat samples subjected to separation by their direct placement on the gel. Despite these differences, separations obtained by placing meat samples directly on the gel right before electrophoresis were similar to those obtained by the evaluation of the centrifugal drip. This may indicate that, as a result of direct contact of the meat sample with the gel, mainly proteins from the meat juice (relatively easily separable by centrifugation) are caught by the gel and then separated by electrophoresis. Therefore, direct contact electrophoresis can yield similar results to those obtained by analysis of the centrifugal drip. However, the conditions of pre-electrophoresis associated with the placement of the meat sample on the gel do not allow migration and then separation of proteins fixed in the myofibrillar structure of the muscle.

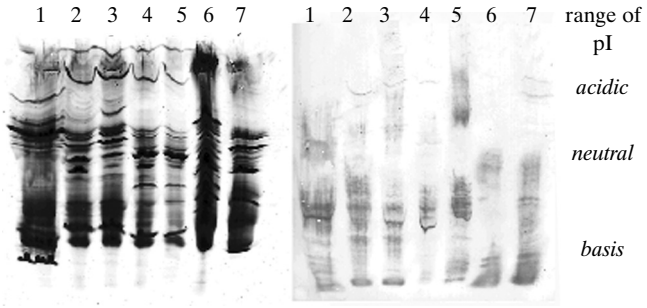

FIGURE 1. Isoelectric focusing of meat samples placed directly on gel (direct contact electrophoresis) and corresponding immunoblotting for titin 9D10 of various types of meat (1 – pork, 2 – beef, 3 – veal, 4 – lamb, 5 – goat, 6 – chicken, 7 – turkey).

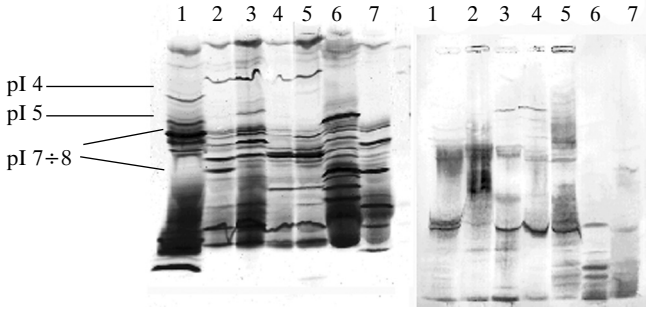

FIGURE 2. IEF of centrifugal drip from various types of meat and corresponding immunoblotting for titin 9D10 (1 – pork, 2 – beef, 3 – veal, 4 – lamb, 5 – goat, 6 – chicken, 7 – turkey).

The separations of proteins from meat of various animal species differed significantly in terms of the number of electrophoretic bands, their distribution on the gel, and proteins typical of a given animal group. In the case of ruminants (cattle, sheep and goats), special attention was drawn to the band whose IP was below 4. In the *longissimus thoracis* muscle of the pig, a similar band was characterized by the IP close to the value of 5. In the case of poultry (chickens and turkeys), despite a number of protein bands in this range, this particular band was not found. However, these muscle samples were characterised by a slightly greater number of bands with high intensity of staining, with the IP ranging from 7 to 8.

Immunoblotting performed against 9D10 titin usually showed a large number of protein bands both in muscles and in their drips, which responded positively to this antibody, irrespective of the origin of the sample. The greatest numbers of bands were found in meat samples from ruminants and pigs, fewer – in breast muscles from poultry. Attention should be paid to a fairly large number of proteins with a high IP which, due to the reaction with the Coomasie Brilliant Blue, was not identified on electrophoretic separations stained traditionally. Particularly large quantities of these proteins were observed in poultry meat, and slightly smaller - in meat from pigs and goats. The storage process favored protein degradation, including the breakdown of titin. Our observations from experiments, which employed SDS-PAGE with urea [Pospiech *et al.*, 2001] and without it [Pospiech *et al.*, 1996] indicated the presence of this protein both in the drip loss and the centrifugal drip. The obtained results showed that the proteins derived from titin degradation were characterised by higher IP values (above 6) (Figure 3). Their quantities increased with the time of storage. Definitely smaller quantities of its degradation products were found in the drip loss from a conditioned muscle, both in the sediment and supernatant. Fewer bands were found in the latter fraction. They were characterised by the IP ranging from 6 to 8 (Figure 3).

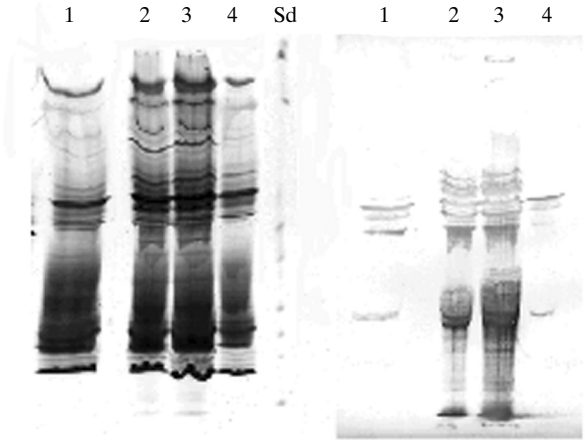

FIGURE 3. IEF of drip from pork stored at various conditions and corresponding immunoblotting for titin 9D10 (1 – sediment for drip loss of meat stored for 4 h at 40°C directly after the slaughter and then in cooled room, 2 – centrifugal drip (cd) from pork stored in cooled room 48 h after the slaughter, 3 – cd from pork stored in cooled room 168 h after the slaughter, 4 – supernatant from drip loss of meat stored for 4 h at 40°C directly after the slaughter and then in cooled room, Sd – standard proteins).

CONCLUSIONS

1. IEF separations of proteins from meat (direct contact electrophoresis) and from its centrifugal drip were similar and typical of a given species.

2. The electrofocusing of samples obtained from pork stored for 168 h revealed that with the prolonged time of storage not only did the amount of proteins in the drip increase but their IP character was also altered.

3. Titin degradation products were characterised by high IP values.

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OGNISKOWANIE ELEKTROFORETYCZNE W BADANIACH BIAŁEK MIĘSA RÓŻNYCH GATUNKÓW ZWIERZĄT I WIEPRZOWINY PRZECHOWYWANEJ W RÓŻNYCH WARUNKACH

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Celem badań była ocena rozdziałów IEF białek z mięsa różnych gatunków zwierząt (świń, bydła, cieląt, owiec, kóz, drobiu i indyków) przy wykorzystaniu elektroforezy bezpośredniego kontaktu (DCE) na żelu agarozowym a następnie porównanie ich z rozdziałami wycieku swobodnego i wirówkowego z tego surowca. Rozdziałom towarzyszył immunoblotting wobec titiny. Porównanie rozdziałów białek mięsa przy wykorzystaniu metody bezpośredniego kontaktu wykazało, że są one podobne do rozdziałów białek wycieku wirówkowego z tkanki aczkolwiek jakość rozdziałów była gorsza (mniej regularne prążki). Warunki elektroforezy wstępnej związanej z położeniem próbki mięsa na żelu nie pozwalały natomiast na migrację a następnie rozdział białek umocowanych w strukturze miofibrylarnej tkanki. Porównanie próbek wycieku wirówkowego z mięśni świń przechowywanych przez 168 godzin po uboju wskazuje, że podczas składowania mięsa zmienia się nie tylko ilość białek w wycieku lecz także ich IP. Produkty degradacji titiny miały zwykle wyższe wartości IP.