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Species-specific expression of various proteins in meat tissue: Proteomic analysis of raw and cooked meat and meat products made from beef, pork and selected poultry species

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ABSTRACT

The aim was to search for proteins differentiating the six species (cattle, pig, chicken, turkey, duck and goose) and relatively stable during the meat aging and only slightly degraded in ready-made products. The two-dimensional electrophoresis was used for analysis of the protein profiles from raw meat and frankfurters and sausages (15 products). The observed species-specific differences in protein expression in raw meat were retained in processed products after finishing the entire technological process. Regulatory proteins, metabolic enzymes, some myofibrillar and blood plasma proteins were identified, which were characterised by the electrophoretic mobility specific to the given species. Large differences in the primary structure were observed in serum albumin, apolipoprotein B, HSP27, H-FABP, ATP synthase, cytochrome bc-1 subunit 1 and alpha-ETF. Some of these proteins have potential to be used as markers in authentication of meat products.

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1. Introduction

In recent years there have been many studies where the skeletal muscle proteins were mapped, including the cattle (Bouley, Chambon, & Piccard, 2004; Chaze, Bouley, Chambon, Barboiron, & Picard, 2006), pig (Kim et al., 2004), chicken (Doherty et al., 2004) and sheep (Hamelin et al., 2007). Protein profiles between pure line breeds of pigs were compared, such as Norwegian Landrace vs Duroc (Hollung, Grove, Færgestad, Sidhu, & Berg, 2009), Meishan vs Large White (Xu et al., 2009). The influence of the type of fibres on proteolysis in the longissimus muscle of Landrace and Korean native black pigs was analysed (Park, Kim, Lee, & Hwang, 2007). Complex studies on the method of pig breeding and gender on the level of proteins in the longissimus muscle proved the influence of those factors on the expression of numerous proteins (Kwasiborski et al., 2008). Proteomic studies indicate differences in the proteomes of grass-fed and grain-fed Japanese Black Cattle (Shibata et al., 2009), differences in the expression of sarcoplasmic and myofibrillar proteins extracted from white and red skeletal muscles of pigs (Kim et al., 2004), sarcoplasmic proteins extracted from four muscles of sheep with the majority of fast fibres (Hamelin et al., 2007) and proteins extracted from the semimembranosus muscle and biceps femoris muscle from Bayonne ham (Théron et al., 2011). In the above examples only differences in the quantity of individual proteins in the analysed proteomes were found. No qualitative differences in the protein composition between the compared samples were observed.

To date, the literature provides a few publications with studies of processed meat products, analyses of protein composition and the degree of protein degradation at the end of the technological process. Fermented sausages (Díaz, Fernandez, De Fernando, de la Hoz, & Ordoñez, 1997; Hughes et al., 2002; Molly et al., 1997) and dry cured hams (Di Luccia et al., 2005; Larrea, Hernando, Quiles, Lluch, & Pérez-Munuera, 2006; Mora, Sentandreu, & Toldra, 2010; Šklerp et al., 2011) are the products which have been best investigated in this respect. However, there are no proteomic studies analysing thermally processed meat products, which are the largest segment on the market. Processed meat products consist of fat, spices, various salts, antioxidants, plant additives or milk proteins. Examining of protein changes is particularly difficult in such products due to their different composition, complexity and often heterogeneity.

The aim of our study was to search for differences in the protein expression between the six examined species (cattle, pig, chicken, turkey, duck and goose), and further to check whether the species-

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specific proteins were strongly degraded in processed meat products. Proteins with species-specific expression and simultaneously not significantly degraded during processing could be used in authenticity tests of meat products made from the analysed species. The methods based on the proteomic approach may be applied not only to species identification but also to other authenticity issues (for review see Montowska & Pospiech, 2011a, 2012a). The applied approach aimed at stable proteins during processing distinguishes this study from other publications on meat proteomics. The 2-DE method was used for analysis of the proteins extracted from raw meat and those from meat products. We checked if the inter-species differences in protein expression observed in raw meat were retained in meat products which underwent the whole technological process consisting of a sequence of treatments, i.e. curing, mincing, smoking, cooking and drying.

In our previous papers we described the inter-species differences in myosin light chain isoforms (MLC) in raw meat of six species, namely cattle, pig, chicken, turkey, duck and goose (Montowska & Pospiech, 2011b) as well as we confirmed that MLC isoforms retain their species-specific electrophoretic mobility after processing, including minced meat and various meat products (Montowska & Pospiech, 2012b). This study presents the results concerning other proteins, including those from the group of regulatory proteins and metabolic enzymes as well as two other myofibrillar proteins (troponin T and tropomodulin). Although the functions and structure of the proteins discussed in this study have been relatively well investigated, especially in various species of mammals and inferior vertebrates, which are the most common object of scientific experiments, there are no publications discussing the influence of technological processes on degradation of those proteins in ready to eat meat products.

2. Materials and methods

2.1. Sample preparation

Meat and meat products made of six farm species, namely cattle (*Bos taurus*), pig (*Sus scrofa*), chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), duck (*Anas platyrhynchos*) and goose (*Anser anser*), were examined in the present study. Samples were collected and prepared according to our previous work (Montowska & Pospiech, 2011b). Five samples of fresh meat from each species in two terms were collected ($n = 60$). The initial samples were excised within 45 min post mortem from the *longissimus* muscle (LM – cattle, pig) and the *pectoralis* muscle (PM – poultry). The latter samples were collected after meat aging. The aging times were determined as described previously (Montowska & Pospiech, 2012b). Samples were cut out at 48 h (chicken), 144 h (pig, turkey, duck and goose) or 336 h (cattle) *post mortem*. Verification of the degree of meat aging was carried out by shear force measures of cooked meat (data not shown).

Processed meat products were manufactured in our own pilot plant or purchased at supermarkets ($n = 15$) as reported previously (Montowska & Pospiech, 2012b). The Polish raw smoked sausage made from pork (sample B) and tree types of frankfurters prepared only from pork (J – control sample) and separately from pork with the addition of 15% milk protein preparation (sample H) and with 15% soy protein isolate (sample I) were processed in our pilot plant. All of these frankfurters were fine comminuted, smoked and cooked.

Meat products purchased at supermarkets included the following commodities: Polish raw smoked sausage made from pork (sample A), coarsely minced, raw and smoked frankfurters made from pork (sample C), fine comminuted, smoked and cooked frankfurters made from various species (sample D – pork; E – turkey and pork with the addition of cheese; F – chicken; G – pork and poultry), coarsely minced smoked and roasted sausage made from pork and beef (sample K), coarsely minced smoked, cooked and semi-dried "Krakov" sausage (sample L), "Kabanos" sausage made from goose, turkey and pork (sample M), raw fermented salami made from beef and pork (samples N and P).

For subsequent 2-DE analysis, a 0.1 g of ground sample was solubilised in 1 mL of lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 2% carrier ampholyte pH 4–7, 40 mM DTT) containing Protease Inhibitor Mix (GE Healthcare Bio-Sciences, Uppsala, Sweden). Protein concentration was determined using a 2-D Quant Kit (GE Healthcare Bio-Sciences). The gels were produced in triplicate.

2.2. Cooking conditions

The meat of the six analysed species is known for its diversified tenderness. For this reason different conditions of thermal processing were applied when each of the meat types was heated. Meat slices of about 25 mm in thickness were wrapped in aluminium foil, placed in a Rational Combi convection oven and heated to the temperature of 75 °C. The heating time fluctuated from 30 min (PM from chicken and duck), through 40–60 min for pork and other types of poultry, up to 90 min for the LM from cattle. Samples of about 2 g were cut from the cooked meat and stored at the temperature of −80 °C in order to carry out further 2-DE analyses.

2.3. 2-DE

2-DE analysis of protein profiles was carried out in triplicates as previously described (Montowska & Pospiech, 2011b, 2012b). Briefly, a sample volume equivalent to 90 μg (for analytical gels) or 1000 μg (for preparative gels) of protein extract was loaded onto IPG strips pH 4–7, 24 cm long (GE Healthcare Bio-Sciences). Following in-gel rehydration (7 M urea, 2 M thiourea, 2% w/v CHAPS, 0.5% carrier ampholyte, 0.001% bromophenol blue), samples were focused at 20 °C (the voltage was stepwise increased to 8000 V, reaching a total of 70,000 Vh) using an Ettan IPGphor 3 unit (GE Healthcare Bio-Sciences). IPG strips were then reduced and alkylated using buffers containing 6 M urea, 30% w/v glycerol, 2% w/v SDS, 50 mM Tris–HCl, pH 8.8 and 0.002% bromophenol blue, supplemented successively with 1% w/v DTT or 2.5% w/v iodoacetamide, for 15 min each. SDS–PAGE was performed on 15% polyacrylamide gels (200 × 260 × 1 mm) in an Ettan DALTsix Large Vertical System (GE Healthcare Bio-Sciences). The separation was run at 10 °C with 1 W per gel for 45 min followed by 9 W per gel. Analytical gels were stained with a silver nitrate according to procedure 4 with the addition of glutaraldehyde described by Sørensen et al. (2002), while the preparative gels for MS analysis were stained using colloidal Coomassie Brillant Blue (Sigma–Aldrich, Steinheim, Germany). The gels were scanned on an ImageMaster Scanner (GE Healthcare Bio-Sciences). Spot detection and quantification were performed using ImageMaster 2D Platinum 7.0 software.

2.4. Protein identification by MS analysis

Protein identification by mass spectrometry was performed as previously described (Montowska & Pospiech, 2012b). Selected spots from chicken and turkey were investigated using an Autoflex MALDI-TOF spectrometer (BrukerDaltonics, Bremen, Germany) and from all other species using a Premier Q-TOF spectrometer with nanoAcquity UPLC attachment (Waters, Milford, Massachusetts, USA). Proteins were identified by Peptide Mass Fingerprinting. The SwissProt and Trembl protein databases were searched

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