



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

Magdalena Montowska^{a,*}, Edward Pospiech^{a,b}

^a Institute of Meat Technology, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland

^b Institute of Agricultural and Food Biotechnology, Division of Meat and Fat Technology in Poznań, Głogowska 211, 60-111 Poznań, Poland

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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-

* Corresponding author. Current address: Marie Curie Research Fellow, School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, United Kingdom. Tel.: +44 115 95 66272; fax: +44 115 95 15102.

E-mail address: magdalena.montowska@gmail.com (M. Montowska).

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 frank-

furters 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 Brilliant 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

USA) and mean and standard deviation were calculated for selected spots. It was checked if the expression of the selected regulatory and enzymatic proteins differed significantly between raw meat and meat products consisted of the same meat species. For meat products, it was also checked if the expression of selected proteins differed significantly between pairs of samples B:A, B:C and J:D composed of the same meat species. Fisher test for small and independent samples was used. Differences in protein spot volume between the compared samples were not significant, and therefore the computation results are not presented in the paper.

2.5. Sequence alignment

The NCBI database (<http://www.ncbi.nlm.nih.gov>) was searched for amino acid sequences of selected proteins. The alignment of known sequences was constructed using the ClustalW2 program (Thompson, Higgins, & Gibson, 1994). The program calculates the best match for the selected sequences and lines them up so that identities, similarities and differences can be seen. A pairwise score for each pair of sequences to be aligned is calculated as the number of identities in the best alignment divided by the number of residues compared and gap positions are excluded (<http://www.ebi.ac.uk/Tools/msa/clustalw2>).

2.6. Statistical analysis

Statistical analysis was performed according to our previous work (Montowska & Pospiech, 2011b, 2012b). The protein spot volume data were imported into Statistica® Version 9 (StatSoft Inc.,

3. Results and discussion

3.1. Comparison of the proteomes of raw meat

The separations of skeletal muscle proteins extracted from samples collected 45 min *post mortem* revealed differences between the examined species in molecular weights (MW) and isoelectric points (pI) of numerous proteins, whose electrophoretic mobility was species-specific. However, the aim of our investigation was to find out if the species-specific proteins were degraded in processed meat products. Since meat products generally contain meat after several days of aging, in the next stage of our study the progress in proteolysis and *post mortem* protein degradation related with the meat aging was examined. For this purpose, the samples of the chicken meat were electrophoretically analysed after 48 h of

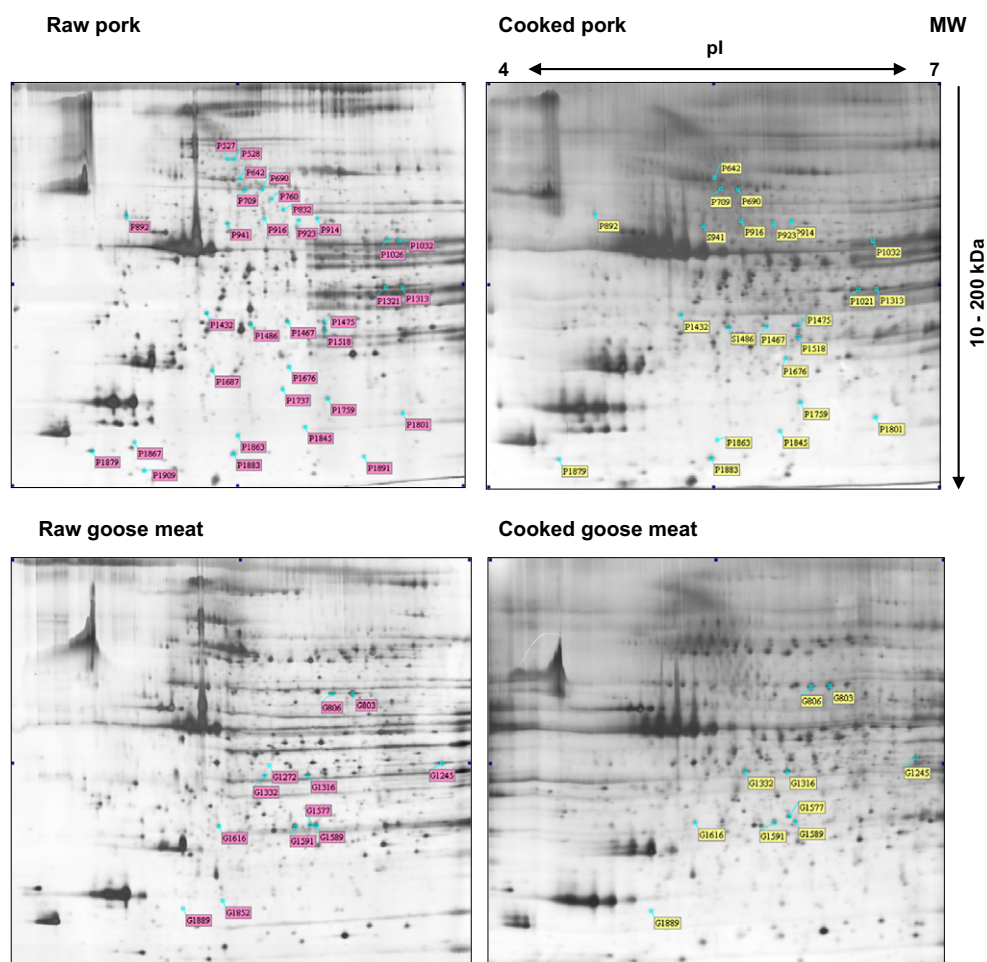


Fig. 1. Representative 2-DE gels of the skeletal muscle proteins extracted from raw and cooked meat after aging 144 h *post mortem*, pH range 4–7. Spots of species-specific electrophoretic mobility are numbered.

meat aging, other poultry species and pork were analysed after 144 h and the beef samples – after 336 h, i.e. when the meat was tender. In order to ensure the appropriate course of the aging process at 48 and 144 h, and for the cattle – at 336 h, the samples were thermally processed and the shear force was measured (data not shown).

Comparative analyses of the meat protein profiles extracted 45 min *post mortem* and after aging (48 h – chicken; 144 – pork, turkey, duck, goose; 336 h – cattle) were carried out. Low stability proteins were found among the spots of previously selected. During the aging their volume decreased considerably and some of them became totally degraded. As a result of the comparison those proteins were eliminated from further considerations and 130 spots were selected for further analysis of the influence of processing (from 12 to 33 spots from the analysed species), which were both relatively stable and species-specific after the period of meat aging. Examples of 2-DE gels of proteins extracted from raw pork and goose meat after aging as well as their comparison with the cooked meat are presented in Fig. 1. Magnified 2-DE gels of proteins extracted from tissue of six analysed species after aging period are presented in Fig. S1 (Supplementary Material). The results concerning myosin light chain isoforms were discussed previously (Montowska & Pospiech, 2011b, 2012b).

3.2. Comparison of the proteomes of cooked meat

After thermal processing 83 protein spots of all the six species retained their electrophoretic properties. Further study involved the analysis of only thermostable proteins, which were simultaneously characterised by the species-specific electrophoretic mobility, of which 16 came from the beef, 22 from pork, 12 from chicken, 10 from turkey, 13 from duck and 10 from goose. The pork and goose proteins are marked in Fig. 1.

We observed that the heating, which is the most destructive of the all technological processes applied in meat processing, deteriorated the quality of distribution of high molecular weight proteins more than that of low molecular weight proteins. Since the proteins retained their characteristic pIs and MWs, the heating did not change the position of proteins on the gel. The observed influence of heating on 2-DE meat protein separations was compatible with Hofmann's (1977) observations made on the basis of the SDS-PAGE technique. He reported that the thermal processing did not influence the protein migration or size of the molecules. However, the heating reduced the staining intensity, especially in high molecular weight proteins, such as myosin heavy chains. The changes in the staining of muscle proteins may be related with the processes of their degradation and/or aggregation (Hofmann, 1977).

3.3. Meat products

In the next step the influence of technological processes applied in meat processing on the degradation of previously selected species-specific spots was investigated. For this purpose proteins were extracted from meat products made in our own pilot plant (4 products) and from products purchased at supermarket (11 products). Meat products with diversified species composition (sausages, frankfurters) were analysed. Their production involved such technological processes as curing, smoking, cooking, roasting and semi-drying. High quality electropherograms we obtained from cold smoked as well as from cooked products. Protein profiles from 4 different processed meat products are shown in Fig. S2 (Supplementary Material).

2-DE protein profiles from meat products were compared with those extracted from the raw and cooked meat. Some of the proteins subjected to various technological processes were found to

be relatively resistant to processing and were characterised by almost identical electrophoretic mobility. In spite of denaturation these proteins were not significantly degraded and the 2-DE patterns were still characterised by species specificity. Therefore, the presence of most of the spots selected from the raw and cooked meat was confirmed in the meat products. When in doubt, we always assumed the negative result. This observation points to the fact that the further search for protein markers of meat authenticity is justified.

The main difficulties in the identification of individual spots we encountered in salami sausages made with the additive of starter cultures, which has positive influence on the acceleration of the ripening. As a result of high microbiological activity, muscle proteins are strongly degraded, even to short peptides, which give the product a specific flavour and taste (Hughes et al., 2002). Progressive protein degradation in the form of numerous additional spots was observed in our samples of salami (sample N is shown in Fig. S2). Relatively high fat content observed in commercial products did not affect the quality of our 2-DE separations.

The relatively small degradation changes in the thermally processed meat products may be influenced by the range of temperatures applied during the production which can affect the proteases. In the meat industry thermal processing resulting in higher temperatures than 72 °C in the centre of the product is rarely applied in the production of cold cuts. However, most proteolytic enzymes usually become inactivated at this temperature. Apart from heating, the presence of salts and pH changes also reduce the activity of proteases by their denaturation (Klement, Cassens, & Fennema, 1973; Toldra, Rico, & Flores, 1992). The main component of curing mixtures is sodium chloride and sodium nitrate (III). Additionally, a combination with phosphates and potassium nitrate (V) is often applied. It has been proved that the additive of salt influences the intensity of stained bands of meat proteins in raw and heated samples. Hofmann (1977) applied the SDS-PAGE technique and observed that sodium chloride and potassium nitrate (V) moderately reduced the staining of raw proteins, whereas sodium nitrate (III) reduced it very strongly, but disodium diphosphate increased the staining of myosin and myoglobin. Partial binding of the ions of those salts with proteins may be the reason. By contrast, when heating meat over 100 °C with the addition of each of those salts low molecular weight proteins were found to be more stable but large deterioration of the intensity of staining of high molecular weight proteins, especially myosin, was observed. This proves that smaller proteins are more resistant to damage caused by thermal processing and curing (Hofmann, 1977). Our results are consistent with the above observations. The intensity of high molecular weight proteins extracted from the meat products which underwent various technological treatments (including curing and heating) was worse than of low molecular weight proteins. The aforementioned facts lead to the conclusion that the possibility of quantitative analysis of individual proteins in meat products is limited to semi-quantitative detection due to influence of the type of salt, the applied temperature and heating time on the staining of proteins.

3.4. Identified proteins with species-specific expression

The species-specific spots observed in the largest number of products as well as well stained on preparative gels were selected for identification with the MS method. Among the spots analysed with the MS method 21 proteins were correctly identified. They had different functions in the cell, i.e. myofibrillar and blood plasma proteins, regulatory proteins and metabolic enzymes. Table 1 presents the identified proteins with the electrophoretic mobility

Table 1

Identified proteins from beef and pork with different species-specific electrophoretic mobility selected after meat aging and processing.

Spot number	NCBI accession number	Identified protein	Matched peptides ^a	Sequence coverage (%) ^b	Theoretical pI/MW (Da) ^c	Experimental pI/MW (Da) ^d	Probability (%) ^e
B476	P02769	Bovine serum albumin (<i>Bos taurus</i>)	16	25.9	5.76/69248	5.88/72200	100.0 ^e
B603	Q5R7D3	Heat shock 70.1 kDa protein (<i>Pongo abelii</i>)	16	27.9	5.32/70009	5.90/62230	25.0 ^e
B1369	Q3T149	Heat shock 27 kDa protein (<i>Bos taurus</i>)	7	38.3	5.96/22379	5.24/28045	100.0 ^e
B1360	Q3T149	Heat shock 27 kDa protein (<i>Bos taurus</i>)	10	57.7	5.96/22379	5.64/28381	100.0 ^e
B1396	Q5E946	Parkinson disease protein 7 (Protein DJ-1) (<i>Bos taurus</i>)	8	48.1	7.17/20022	6.38/26885	100.0 ^e
B1462	Q148H2	Similar to myosin light chain 1 slow-twitch muscle (MLC1sa) (<i>Bos taurus</i>)	12	49.3	5.24/23388	5.68/22523	100.0 ^e
B1557	Q32PA4	14 kDa phosphohistidine phosphatase (<i>Bos taurus</i>)	3	31.2	5.27/13990	5.19/16401	100.0 ^e
B1580	P11116	Galectin-1 (beta-galactose-binding lectin) (<i>Bos taurus</i>)	4	31.1	5.16/14734	4.97/14925	100.0 ^e
B1482	P13620	ATP synthase subunit d (<i>Bos taurus</i>)	3	15.5	5.91/18680	6.02/21100	100.0 ^e
P642	Q5R511	Heat shock 70.9 kDa protein (<i>Pongo abelii</i>)	7	12.1	5.70/73644	5.40/64313	50.0 ^e
P892	Q0VC48	Tropomodulin 4 skeletal muscle (<i>Bos taurus</i>)	3	8.41	4.51/39159	4.74/52813	100.0 ^e
P941	P31800	Cytochrome b-c1 subunit 1 (<i>Bos taurus</i>)	3	9.17	5.92/52702	5.31/50100	100.0 ^e
P1032	Q9XSJ4	Alpha-enolase 1 (<i>Bos taurus</i>)	6	13.4	6.38/47296	6.47/44902	100.0 ^e
P1313	Q5EA88	Glycerol-3-phosphate dehydrogenase, cytosolic (GPDH-C) (<i>Bos taurus</i>)	5	17.5	6.44/37623	6.49/36122	100.0 ^e
P1475	Q5S1U1	Heat shock 27 kDa protein (<i>Sus scrofa</i>)	5	22.2	6.23/22927	5.97/30934	100.0 ^e
P1486	Q5S1U1	Heat shock 27 kDa protein (<i>Sus scrofa</i>)	6	29.0	6.23/22927	5.47/30691	100.0 ^e
P1467	Q9TSX9	Peroxiredoxin-6 (<i>Sus scrofa</i>)	8	33.0	5.63/25021	5.72/31124	100.0 ^e

^a Number of matched peptides in the database search.^b Percent of coverage of the entire amino acid sequence.^c pI and MW recorded in NCBI database.^d pI and MW calculated from the spot position on the gel.^e Spot identified by ESI-MS.

specific to the cattle and pig, whereas Table 2 presents the proteins specific to the poultry species.

In the last stage we checked if there were differences in the primary structure of the identified proteins and if there were fragments specific only to a particular species. For this purpose the NCBI database was searched for homological sequences of proteins from the other species. As it turned out, the sequences of not all of the proteins in the species under investigation were known. In the NCBI database the most numerous representation comes from the sequenced proteins from three species, namely the cattle, pig and chicken. For the time being sequences of some turkey proteins and almost all of the duck and goose proteins are not known. The sequences available in the NCBI database were compared using CLUSTAL W2 software (Thompson et al., 1994). The score calculated for each pair of the sequences from the analysed species and the compared sequences of selected proteins are presented in the Supplementary Material (Tables S1–S3, Figs. S3–S6). As can be seen in the comparisons, the sequences differ depending on the species. Depending on the identified proteins the differences ranged from less than 10 to several dozen per cent. In this paper, only certain proteins are discussed in detail.

Of the selected species-specific spots two myofibrillar proteins were identified: troponin T and tropomodulin 4 and two blood plasma proteins: albumin and apolipoprotein B. Enlarged examples of some identified proteins extracted from differently processed products are presented in Figs. 2–4 (for more examples see Supplementary Material: Fig. S7). These 2-DE images confirm the

fact that amount of the proteins in the raw meat and the examined meat products did not change considerably. Poorer staining in the cooked meat samples may result from the reduced capacity to bind the stain as a result of thermal processing. On the other hand, better image in the thermally processed products, may have been caused by the additive of salt. This has been mentioned in the previous section of the paper.

Spot P892 selected from pork was identified as tropomodulin 4 (Tmod4; Table 1). Although the pI and MW (4.74/52.8) of the protein identified in this study are similar to those of the protein identified in the *semitendinosus* muscle in the cattle (4.69/48.0) (Bouley et al., 2004), in our 2-DE separations both from raw meat and processed products the spot was only specific to the pig. However, it was observed in raw smoked and cooked products, but it was not found in raw fermented products. This may indicate that the protein became degraded as a result of the activity of proteolytic enzymes. These results may suggest that technological treatments may lead to protein 'conservation' through their denaturation.

Two spots, one from the cattle (B476) and the other from the turkey (T587) were identified as serum albumin. Those two spots were specific to the investigated species, i.e. their molecular weights were similar but they differed in pI. The turkey spot was moved towards more acidic pH (pI 5.56), as compared with the cattle (pI 5.88). The cattle albumin is presented in Fig. 2. In Fig. 2d, which presents a product consisting not only of beef but also of pork is visible that the pork protein, which is most likely albumin

Table 2

Identified proteins from poultry species with different species-specific electrophoretic mobility selected after meat aging and processing.

Spot number	NCBI Accession number	Identified protein	Matched peptides ^a	Sequence coverage (%) ^b	Theoretical pI/ MW (Da) ^c	Experimental pI/ MW (Da) ^d	Probability (%) ^e or Mascot score ^f
C815	NP_001006686	Heat shock 70.1 kDa protein (<i>Gallus gallus</i>)	8	–	5.56/69620	5.37/75700	70 ^f
C956	P29616	Myosin heavy chain, cardiac muscle isoform (<i>Gallus gallus</i>)	10	7.90	4.99/127930	5.27/72532	100.0 ^e
C1061	P00548	Pyruvate kinase muscle isozyme (<i>Gallus gallus</i>)	19	41.89	7.35/57977	5.04/63251	100.0 ^e
C1348	P07333	Beta-enolase (<i>Gallus gallus</i>)	8	–	7.27/47196	6.63/51619	72 ^f
C1843	Q5F4B1	Phosphoglycolate phosphatase (PGPase) (<i>Gallus gallus</i>)	3	11.22	5.38/32974	5.51/37214	100.0 ^e
C2538	P80565	Fatty acid-binding protein, smooth muscle (SM-FABP) (<i>Gallus gallus</i>)	4	60.57	4.99/7969	5.90/15045	100.0 ^e
C2540	O13008	Fatty acid-binding protein, heart-type (H-FABP) (<i>Oncorhynchus mykiss</i>)	1	5.26	5.29/14520	5.71/14929	62.0 ^e
T587	P19121	Serum albumin precursor (Allergen Gal d 5) (<i>Gallus gallus</i>)	9	14.8	5.39/69871	5.56/65230	100.0 ^e
T1056	P51913	Alpha-enolase (<i>Gallus gallus</i>)	15	38.5	6.14/47275	6.19/41919	100.0 ^e
T1071	NP_990253	Troponin T, fast skeletal muscle isoform (<i>Gallus gallus</i>)	8	–	5.74/33668	6.76/41772	115 ^f
T1368	XP_418038	Apolipoprotein B (<i>Gallus gallus</i>)	6	–	6.57/25824	4.97/33569	80 ^f
T1482	P00548	Pyruvate kinase muscle isozyme (<i>Gallus gallus</i>) – fgm	8	19.4	7.35/57977	6.33/31127	100.0 ^e
T1861	O13008	Fatty acid-binding protein, heart-type (H-FABP) (<i>Oncorhynchus mykiss</i>)	2	12.0	5.29/14520	6.01/14568	100.0 ^e
D545	NP_990061	Annexin A6 (<i>Gallus gallus</i>)	8	–	5.42/75088	5.74/62660	81 ^f
D1385	P13804	Electron transfer flavoprotein subunit alpha (alpha-ETF) (<i>Homo sapiens</i>)	4	12.3	8.48/35057	6.16/33960	100.0 ^e
G1316	P13804	Electron transfer flavoprotein subunit alpha (alpha-ETF) (<i>Homo sapiens</i>)	3	10.2	8.48/35057	5.79/35720	100.0 ^e
G1577	Q00649	Heat shock 27 kDa protein (<i>Gallus gallus</i>)	6	27.5	5.71/21657	5.81/28917	100.0 ^e

^a Number of matched peptides in the database search.^b Percent of coverage of the entire amino acid sequence.^c pI and MW recorded in NCBI database.

also, was overlaid on the neighbouring Spot No. 348. These images confirm the species-specific expression of the spot B476.

Remains of the blood which was left in the muscles after bleeding are the source of serum albumin in meat samples. Albumin is a soluble protein. Therefore, it was identified in the fraction of sarcoplasmic proteins extracted from the muscles of cattle (Bouley et al., 2004), pig (Hwang, Park, Kim, Cho, & Lee, 2005) and chicken (Doherty et al., 2004). The intensity of the protein observed in the LM in Korean Native Black pigs was higher than in the Landrace breed and the intensity in Large White pigs was higher than in Meishan pigs (Park et al., 2007; Xu et al., 2009). Vallejo-Cordoba, Rodríguez-Ramírez, & González-Córdova (2010) compared the extracts of water-soluble proteins with the CE method and found a smaller amount of the protein in the cattle than in ostrich meat. Albumin was identified on 2-DE gels in semi-dry Bayonne hams after 9 and 15 months of ripening (Šklérp et al., 2011). The amount of albumin in the biceps femoris muscle was slightly higher than in the *semimebranosus* muscle (Théron et al., 2011).

In our study both spots, from the cattle and turkey, were observed in all of the products containing those types of meat, both raw smoked and cooked ones. Spot B476 was also present in salami samples, which confirms the results obtained from ripening hams and may indicate that the protein is slowly degraded by proteolytic enzymes. As results from the comparison of the amino acid sequences, they differ considerably depending on the species (Table S1). The differences between the cattle and pig amounted to 21%, between the chicken and turkey – 18% and 54–57% between different pairs of birds and mammals. Therefore, the identified peptides from the serum albumin are mostly characterised by species specificity (Fig. S3).

Among the identified proteins there were also those responsible for various regulatory functions in the cell as well as metabolic enzymes. Spot B1396, which was specific to cattle meat, was

identified as Parkinson disease protein 7 (DJ-1). It protects against oxidative stress and cell death. Chelhi, Picard, Hocquette, and Cas-sar-Malek (2011) observed up-regulation of DJ-1 associated with myostatin inactivation in double-musced cattle. DJ-1 is also listed among potential markers of beef tenderisation (Guillemin et al., 2011). In our study the protein was identified in all the meat products containing beef, namely roast sausage (sample K), cooked and semi-dry “Krakow” sausage (sample L) and in salami sausages (samples N and P). Images of DJ-1 protein are shown in Fig. 3. As results from the comparison of amino acid sequences, the protein is characterised by relatively small species diversity. The differences between the chicken and turkey amounted to 1% only (Table S2). The sequences differ only in one amino acid residue in position 124. In this position isoleucine (Ile124) can be found in the chicken, whereas threonine (Thr124) is found in the turkey (Fig. S4). This example clearly shows that the differences in the electrophoretic mobility of all proteins discussed in this study cannot be attributed only to differences in the primary structure. We can hypothesise that the observed differences in the electrophoretic mobility may be caused by differences in the secondary structure. Protein secondary structures, namely alpha helices, beta sheets and beta strands, are stabilised by hydrogen bonds in a given chain or between the neighbouring chains. It is likely that the denaturing conditions during 2-DE and denaturation caused by technological processes (curing, smoking, cooking) do not damage the protein secondary structure completely. Certain secondary structure elements which have been retained may be reflected in the electrophoretic mobility of individual proteins.

Spot D545, specific to duck meat, was identified as annexin A6. Annexins were relatively rarely identified in studies mapping skeletal muscle proteins. The following were identified: annexin V in the chicken PM (Doherty et al., 2004), annexin VII in the semiten-dinosus muscle of cattle (Bouley et al., 2004) and a fragment of

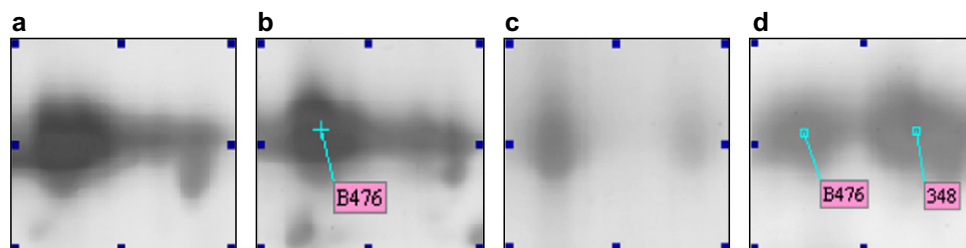


Fig. 2. Images of B476 spot extracted from differently processed products identified as bovine serum albumin: a – raw meat 45 min; b – raw meat 336 h; c – cooked meat; d – “Krakow” sausage (sample L).

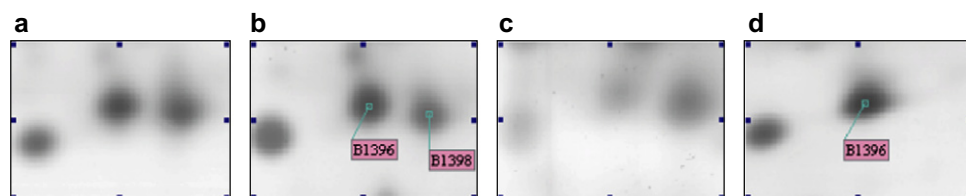


Fig. 3. Images of B1396 spot extracted from differently processed products identified as Parkinson disease protein 7 (DJ-1 protein): a – raw meat 45 min; b – raw meat 336 h; c – cooked meat; d – roasted sausage (sample K).

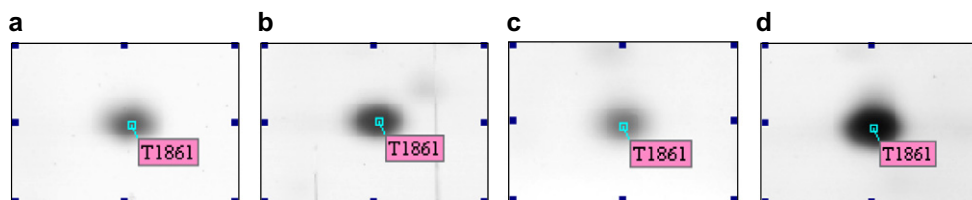


Fig. 4. Images of T1861 spot extracted from differently processed products identified as fatty acid-binding protein (H-FABP): a – raw meat 45 min; b – raw meat 144 h; c – cooked meat; d – poultry frankfurters (sample E).

annexin A6 in the LM of cattle (Laville et al., 2009). In our studies spot D545 was not degraded when thermally processed. Spots identified as H-FABP (C2538, C2540, T1861) were also relatively stable during the aging and processing. It is interesting observation higher intensity of spot T1861 in poultry frankfurters (sample E; Fig. 4). The product was made with the additive of phosphate salts, which might have positively influenced the staining capacity of the protein (Hofmann, 1977). The differences in the sequences proved to be relatively large in comparison with the other examined proteins: 8% between the pig and cattle, 16% between the chicken and turkey, 13–31% between the other pairs of species (Table S2, Fig. S5).

Among the investigated spots we identified 10 proteins functioning mainly as enzymes participating in various cellular metabolic processes. One of the smallest proteins specific to beef proved to be phosphohistidine phosphatase (PHP). The protein with the weight of 14 kDa and located in the cytosol was described in the pig liver in 2002 (Ek et al., 2002). PHP was previously identified in the cattle *semitendinosus* muscle (Bouley et al., 2004). Changes in the intensity were observed within 48 h *post mortem* in the LM of pigs with a different feeding regime. Higher intensity was found in the animals which had initially been on a restrictive diet (60%) and then had unlimited access to feed (Lametsch et al., 2006). In our research the presence of PHP was found only in one processed meat product, i.e. salami pepperoni (sample P). In the

other products, i.e. samples K, L and N, the presence of the protein was not observed. PHP may be more susceptible to degradation. However, this problem would require separate studies. There were very large differences in the primary structure between the analysed species (Table S3, Fig. S6).

In our studies probably for the first time phosphoglycolate phosphatase (PGPase; spot C1843) was identified in the chicken *pectoral* muscle using the ESI-MS technique. We observed this spot in processed meat products containing chicken meat (samples F and G). As in the case of albumin, the sources of PGPase are the remains of blood. However, while albumin was commonly identified in the muscles, PGPase was not. No other publications reporting the identification of the protein in the skeletal muscles of birds and mammals we found. Little is known about the regulation of the enzyme and about the molecular structure of eukaryotic PGPases. The enzyme is a homodimer composed of two subunits with the molecular weight of about 32 kDa. PGPase has chiefly been investigated in human red blood cells, where it has a significant function as phosphoglycolate is an effective activator of the hydrolysis of 2,3-bisphosphoglycerate (BGP) – the main modifier of haemoglobin affinity for oxygen (Mamedov, Suzuki, Miura, Kucho, & Fukuzawa, 2001; Rose, Grove, & Seal, 1986). At present there are only sequences of cattle and chicken available in the NCBI database. The chicken sequence was obtained from lymphocytes (Caldwell et al., 2004) and the cattle sequence was obtained from the liver

amounts, at the expense of thousands of proteins which are present in trace amounts. Owing to this fact those proteins may be considered as potential markers, which may in the future be applied to test the authenticity of meat products made from the six farm species. However, further research is necessary in order to determine whether species identification in complex products will be possible on the basis of the analysis of individual peptides without the electrophoresis stage.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2012.09.072>.

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Supplementary material to manuscript:

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

Magdalena Montowska ^{a,1,*} and Edward Pospiech ^{a,b}

^aInstitute of Meat Technology, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland

^bInstitute of Agricultural and Food Biotechnology, Division of Meat and Fat Technology in Poznań, Głogowska 211, 60-111 Poznań, Poland

Corresponding author. Tel.: +48 61 8487251; fax: +48 61 8487254.

E-mail address: magdalena.montowska@gmail.com (M. Montowska).

¹ Current address: School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

Table S1. CLUSTAL W2 pairwise scores calculated for pairs myofibrillar and blood plasma protein sequences from given species

Identified protein	No spot	NCBI Accession number	Compared species	Score
Myofibrillar proteins				
Tropomodulin-4	P892	Q0VC48.1 XP_001929690.1, NP_990105.1, XP_003214218.1	Cattle - Pig	97
			Cattle - Chicken	78
			Cattle – Turkey	79
			Pig - Chicken	78
			Pig - Turkey	79
			Chicken - Turkey	95
Troponin T (TnTf)	T1071	NP_990253.1, AAG43511.1, BAB92975.1, BAD15377.1	Cattle - Pig	97
			Cattle – Chicken	76
			Cattle – Turkey	73
			Pig – Chicken	75
			Pig - Turkey	73
			Chicken - Turkey	96
Blood plasma proteins				
Serum albumin	B476 T587	P02769.4 P08835.2 P19121.2 XP_003205725.1	Cattle – Pig	79
			Cattle - Chicken	44
			Cattle- Indyk	46
			Pig - Chicken	43
			Pig – Turkey	44
			Chicken - Turkey	88
Apolipoprotein B	T1368	NP_001038098.1, XP_003204582.1, XP_002691557.1, AAA74655.1	Cattle - Pig	76
			Cattle – Chicken	47
			Cattle - Turkey	45
			Pig – Chicken	46
			Pig - Turkey	45
			Chicken - Turkey	94
Pairwise scores are calculated as the number of identities in the alignment divided by the number of residues compared (gap positions are excluded)				

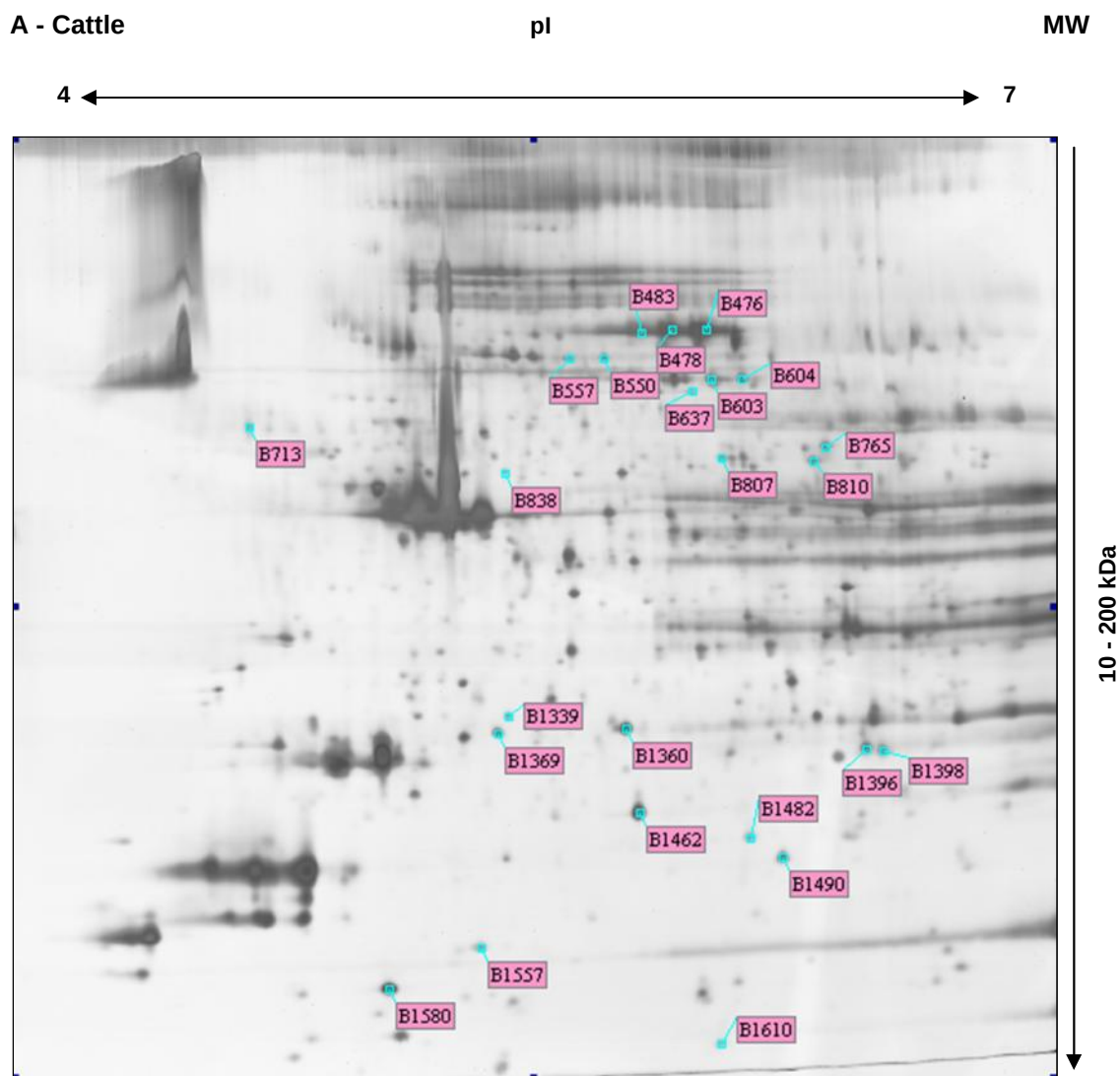
Table S2. CLUSTAL W2 pairwise scores calculated for pairs regulatory protein sequences from given species

Identified protein	No spot	NCBI Accession number	Compared species	Score
Parkinson disease protein 7 (PARK7/DJ-1 Protein)	B1396	Q5E946.1 NP_001072131.1 NP_989916.1 XP_003212226.1	Cattle – Pig	97
			Cattle – Chicken	88
			Cattle - Turkey	89
			Pig – Chicken	89
			Pig - Turkey	90
			Chicken - Turkey	99
Peroxiredoxin-6	P1467	Q9TSX9.3 O77834.3 NP_001034418.1, XP_003208644.1	Cattle - Pig	97
			Cattle – Chicken	86
			Cattle - Turkey	86
			Pig – Chicken	85
			Pig - Turkey	84
			Chicken - Turkey	97
Galectin-1 (Gal-1)	B1580	P11116.2 Q49I35.3 NP_990826.1, XP_003202288.1	Cattle - Pig	89
			Cattle – Chicken	54
			Cattle - Turkey	54
			Pig – Chicken	55
			Pig - Turkey	54
			Chicken - Turkey	99
Heat shock 27 kDa protein (HSP27)	P1475 P1486 B1360 B1369 G1577	Q5S1U1.1 Q3T149.1 Q00649.1 XP_003211746.1	Cattle - Pig	92
			Cattle – Chicken	66
			Cattle - Turkey	68
			Pig – Chicken	68
			Pig - Turkey	70
			Chicken - Turkey	98
Heat shock 70.1 kDa protein (HSP70.1)	B603 C815	NP_001006686.1, XP_003206814.1, ACD47154.2, ACD36583.2, Q27975.2 NP_001116600.1	Cattle – Pig	89
			Cattle – Chicken	84
			Cattle - Turkey	85
			Cattle – Duck	85
			Cattle - Goose	85
			Pig – Chicken	81
			Pig - Turkey	82
			Pig – Duck	82
			Pig - Goose	82
			Chicken – Turkey	98
			Chicken - Duck	97
			Chicken – Goose	98
			Turkey - Duck	99
			Turkey – Goose	99
Duck - Goose	99			
Heat shock 70.9 protein (HSP70.9)	P642	XP_003124024.1, Q3ZCH0.1 Q5ZM98.1	Cattle – Pig	98
			Cattle - Chicken	89
			Pig – Chicken	89
Annexin A6	D545	NP_990061.1, XP_003210442.1, DAA27272.1	Cattle – Chicken	78
			Cattle - Turkey	80
			Chicken - Turkey	96
Fatty acid-binding protein (H-FABP)	T1861 C2538 C2540	NP_001026060 XP_003212530.1 ABH10617.1 P10790.2 NP_001093401.1	Chicken – Turkey	84
			Chicken -Duck	87
			Chicken – Cattle	77
			Chicken - Pig	75
			Turkey - Duck	77
			Turkey – Cattle	70
			Turkey – Pig	69
			Cattle - Duck	75
			Pig – Duck	72
			Cattle - Pig	92
Pairwise scores are calculated as the number of identities in the alignment divided by the number of residues compared (gap positions are excluded)				

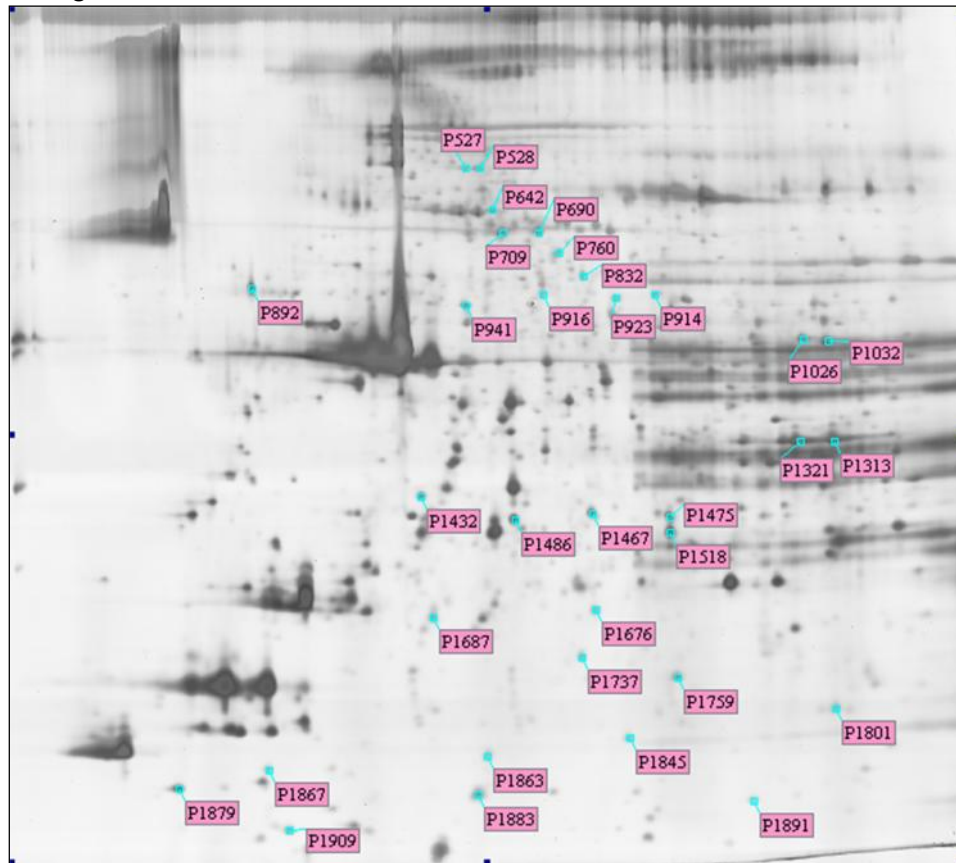
Table S3. CLUSTAL W2 pairwise scores calculated for pairs metabolic enzymes sequences from given species

Identified protein	No spot	NCBI Accession number	Compared species	Score
ATP syntase subunit d	B1482	P13620.2 XP_003133974.1, XP_001232599.1, XP_003211658.1	Cattle – Pig	83
			Cattle - Chicken	59
			Cattle – Turkey	60
			Pig - Chicken	54
			Pig – Turkey	52
			Chicken - Turkey	92
14 kDa phosphohistidine phosphatase (PHP)	B1557	Q32PA4.1 XP_003122371.1 XP_0012340073.1 XP_003208622.1	Cattle – Pig	88
			Cattle - Chicken	64
			Cattle – Turkey	51
			Pig - Chicken	64
			Pig – Turkey	51
			Chicken - Turkey	49
Cytochrome b-c1 subunit 1	P941	XP_001926664.2, P31800.2 XP_414356.1, XP_003210143.1	Cattle – Pig	92
			Cattle - Chicken	71
			Cattle – Turkey	68
			Pig - Chicken	72
			Pig – Turkey	69
			Chicken - Turkey	92
Alfa-enolase	P1032 T1056	Q9XSJ4.4 XP_003127599.1, XP_003127599.1, P51913.2 XP_003212299.1	Cattle - Pig	88
			Cattle – Duck	91
			Cattle - Chicken	91
			Cattle – Turkey	91
			Pig - Duck	81
			Pig – Chicken	82
			Pig - Turkey	82
			Chicken – Duck	97
			Turkey - Duck	97
			Chicken - Turkey	99
Beta-enolase	C1348	P07322.3 Q3ZC09.1 Q1KYT0.1	Cattle – Pig	98
			Cattle - Chicken	85
			Pig - Chicken	85
Glycerol-3-phosphate dehydrogenase (GPDH- C)	P1313	Q5EA88.3 NP_001177169.1, XP_003207253.1	Cattle – Pig	93
			Cattle - Turkey	69
			Pig - Turkey	69
Pyruvate kinase muscle isozyme	C1061 T1482	P00548.2 XP_003209367.1 DAA25831.1	Chicken – Turkey	99
			Chicken - Cattle	86
			Turkey - Cattle	87
Phosphoglycolate phosphatase (PGPase)	C1843	Q5F4B1.1 Q2T9S4.1	Chicken - Cattle	65
Electron transfer flavoprotein subunit alpha (Alpha-ETF)	G1316 D1385	NP_001069290.1, XP_001928767.1, NP_001025714.1, XP_003209417.1	Cattle – Pig	95
			Cattle - Chicken	79
			Cattle – Turkey	80
			Pig - Chicken	82
			Pig – Turkey	81
Chicken - Turkey	86			
Pairwise scores are calculated as the number of identities in the alignment divided by the number of residues compared (gap positions are excluded)				

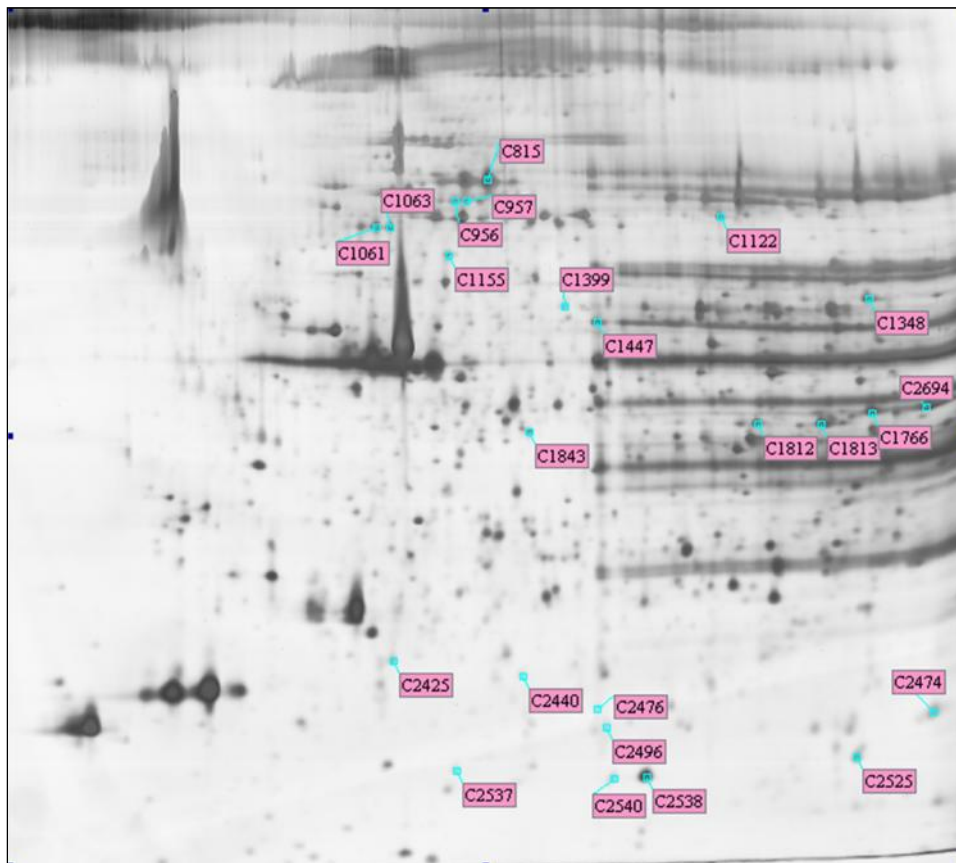
Figure S1. Representative 2-DE gels of proteins extracted from raw meat tissue *m. longissimus* (cattle, pig) and *m. pectoralis* (poultry) after meat aging, pH range 4-7, stained with silver nitrate. A – cattle 336 h; B - pig 144 h; C – chicken 48 h; D – turkey 144 h; E – duck 144 h; F – goose 144. Spots of species-specific electrophoretic mobility are numbered.



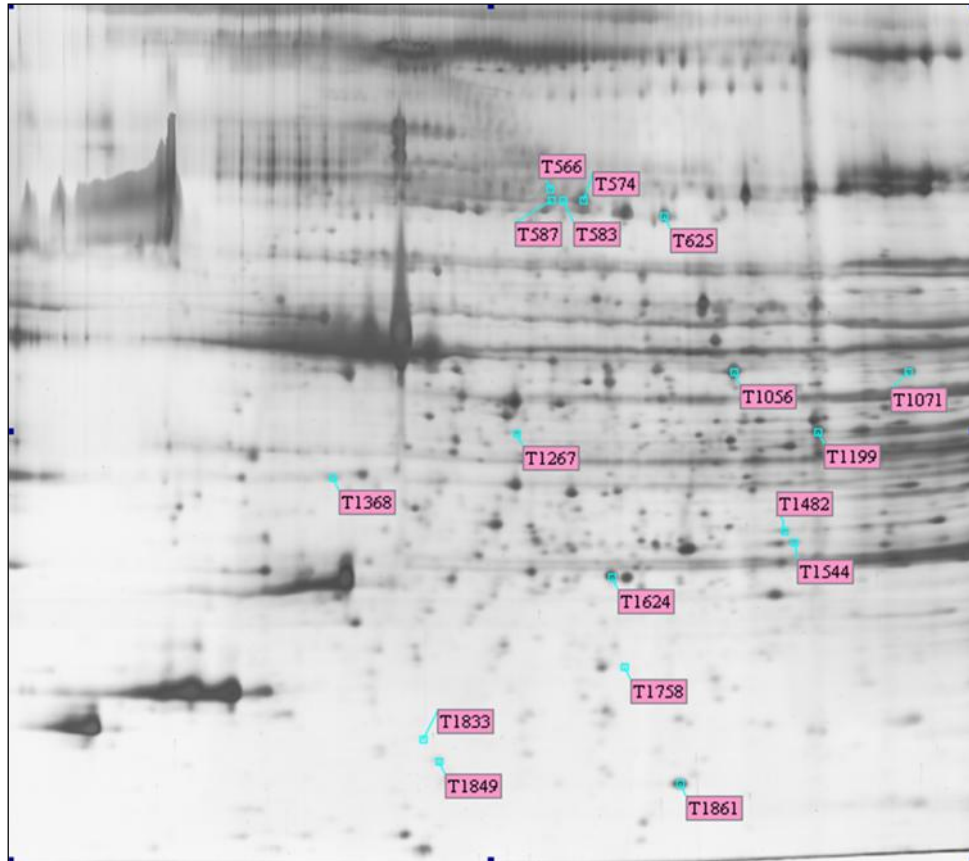
B - Pig



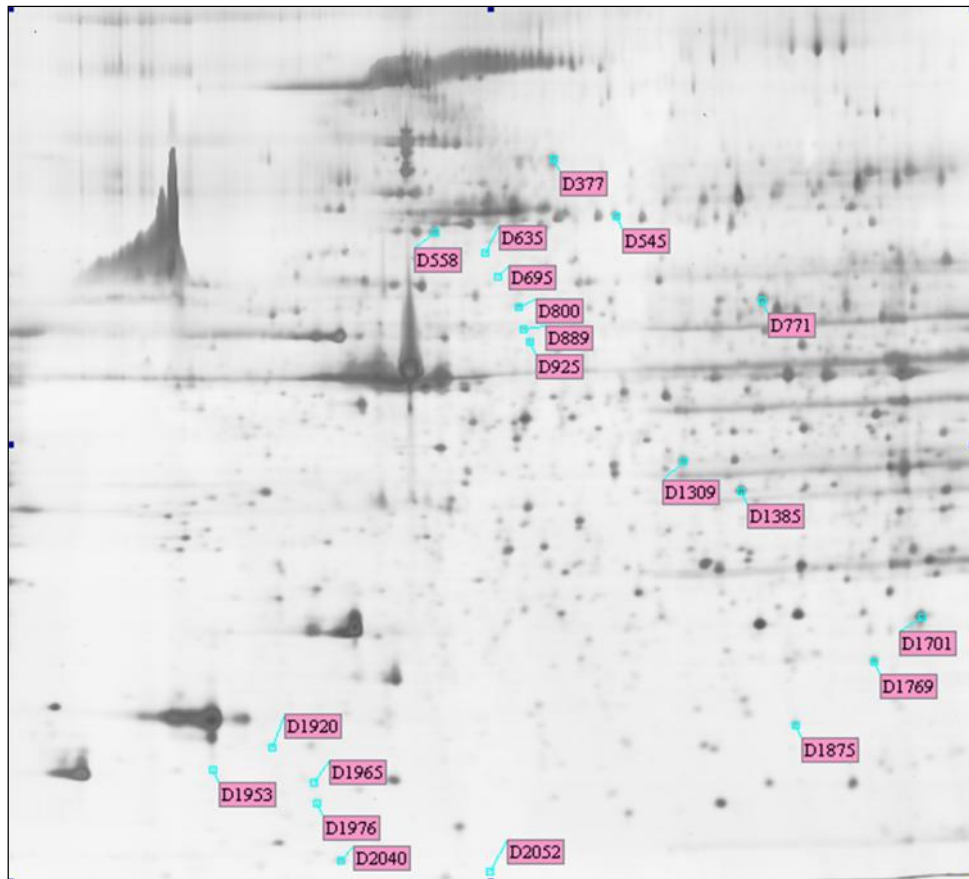
C - Chicken



D - Turkey



E - Duck



F - Goose

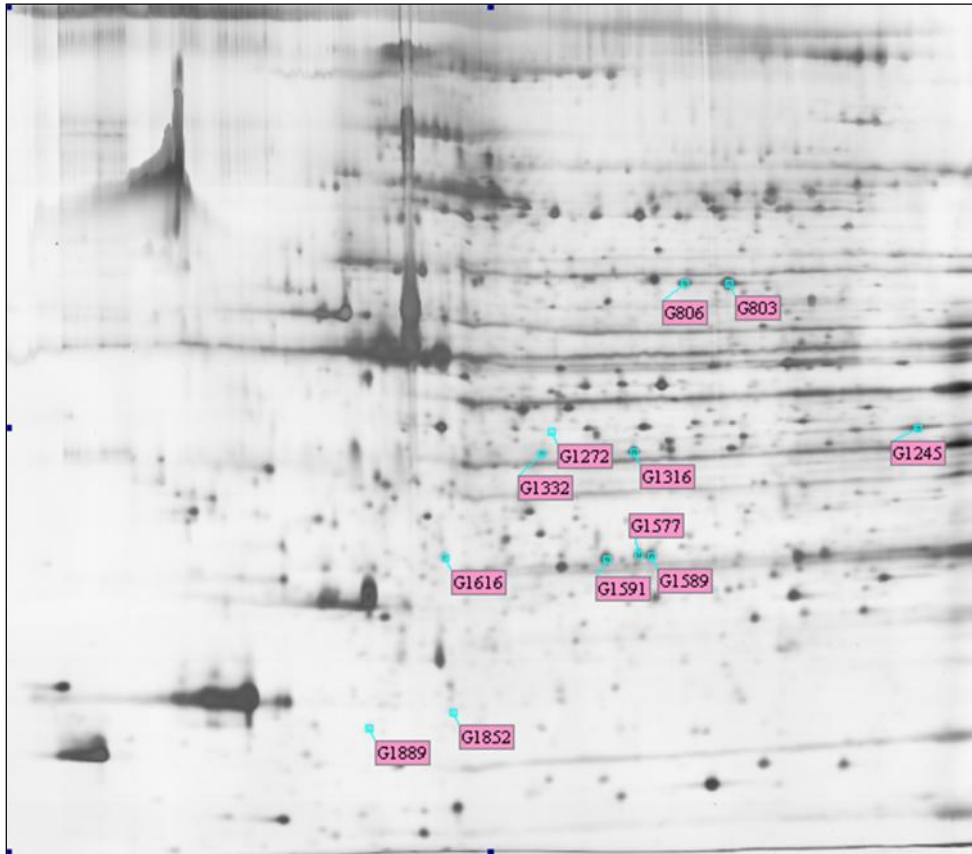
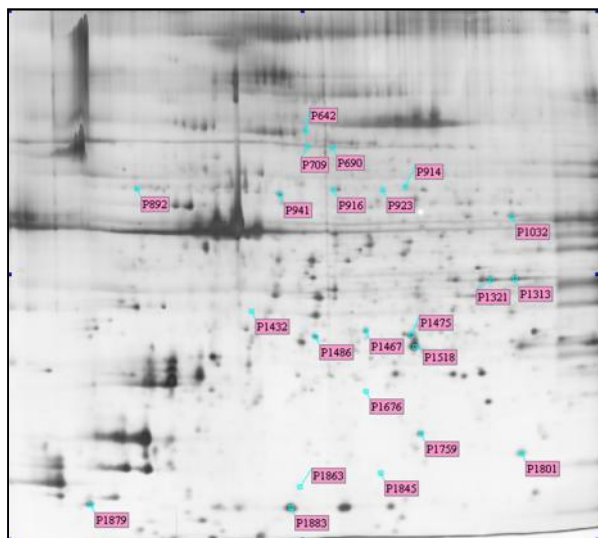
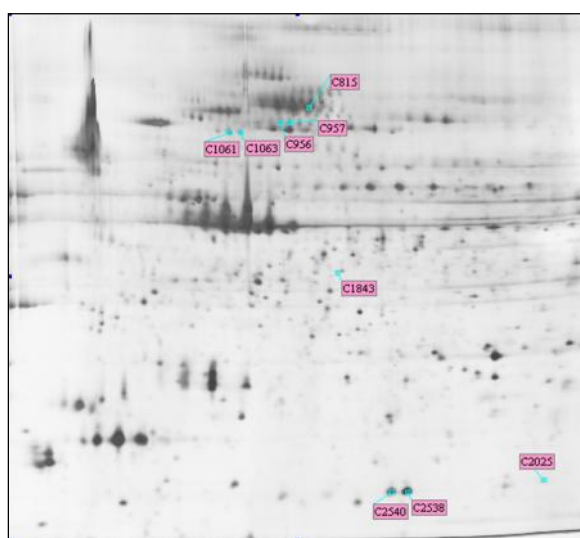


Figure S2. 2-DE patterns of proteins extracted from various meat products, pH range 4-7. Gels were produced in three technical replicates and stained with silver nitrate. A – Polish raw smoked sausage; F – chicken frankfurters; G – pork and poultry frankfurters; N – salami sausage. Capital letters before the numbers indicate the species of origin: B – cattle, P – pig, C – chicken, T – turkey.

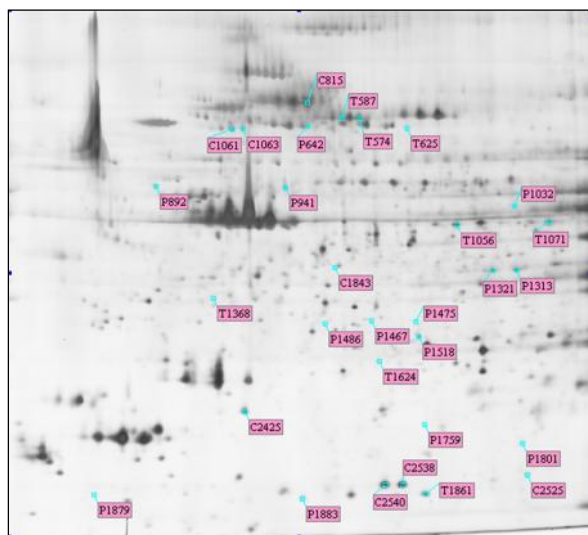
Sample A



Sample F



Sample G



Sample N

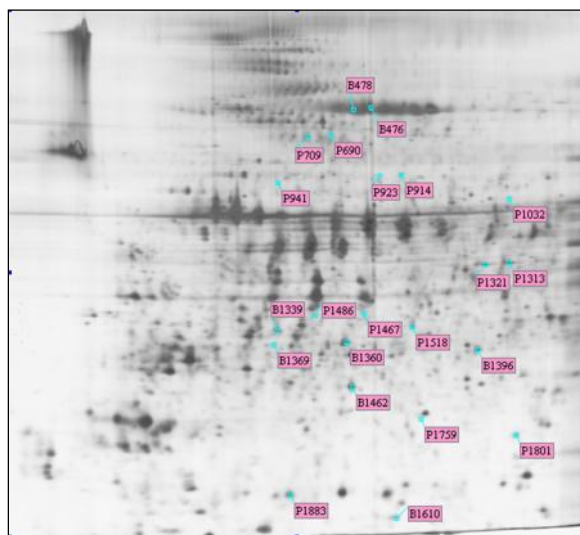


Figure S3. Amino acid sequences of known serum albumin. NCBI accession numbers: cattle (*Bos taurus*) P02769.4; pig (*Sus scrofa*) P08835.2; chicken (*Gallus gallus*) P19121.2; turkey (*Meleagris gallopavo*) XP_003205725.1. The alignment was constructed using CLUSTAL W2. Identified peptides by ESI-MS are highlighted.

G. gallus	MKWVTLISFIFLFSSATSRNLQRFARDAEHKSEIAHRYNDLKEETFKAVAMITFAQYLQR	60
M. gallopavo	MKWVTLISFIFLFSSATSRNLQRFARDAEHKSEIAHRYNDLKEETFKAVAMITFAQYLQR	60
B. taurus	MKWVTFISLLLLFSSAYSR---GVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQ	57
S. scrofa	MKWVTFISLLFLFSSAYSR---GVFRRDTYKSEIAHRFKDLGEQYFKGLVLIAFSQHLQQ	57
	*****:::***** ** . * :*****::** * : *:::*****:	
G. gallus	CSYEGLSKLVKDVVDLAQKCVANEDAPECSKPLPSIILDEICQVEKLRDSYGAMADCCSK	120
M. gallopavo	CSYDGLSKLVKDVVDLAQKCVANEDAPECSKSLPSIILDEICQVEKLRDSYGAMADCCSK	120
B. taurus	CPFDEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYGDMADCCCK	117
S. scrofa	CPYEEHVKLVREVTEFAKTCVADESAENCDSIHTLFGDKLCAIPSLREHYGDLADCCCK	117
	*::: ***:::*****:***: * *::: *::: *::: *::: *::: *::: *::: *	
G. gallus	ADPERNECFLSFKVSPDFVQPYQRPASDVICQEYQDNRVSLFGHFIYSVARRHPFLYAP	180
M. gallopavo	ADPERNECFLSFKVSPDFIQPYQRPASDVICKEYQDNRVSLFGHFIYSVARRNPYMYAP	180
B. taurus	QEPERNECFLSHKDDSPD--LPKLKPDNPTLCDEFKADEKKFWGKLYEIAARRHPYFYAP	175
S. scrofa	EEPERNECFLQHKNDNPD--IPKLKPDVALCADFQEDEQKFWGKLYEIAARRHPYFYAP	175
	:*****:* * ..** * : * . : * : : : : : : : : : : : : : : : : : : : *	
G. gallus	AILSFAVDFEHALQSCCKESDVGACLDTKIEVMREKAKGVSVKQYFCGILKQFGDRVFQ	240
M. gallopavo	TILSFAADYEHALQSCQESDVGACLDKETAMREKAKKISVKQYSCGILKKFGDRVFQ	240
B. taurus	ELLYYANKYNGVFQECQAEDKGACLLPKIETMREKVLASSARQRLRCASIQKFGERALK	235
S. scrofa	ELLYYAIYKDVFECCQAADKAACLLPKIEHLREKVLTSAAKQRLKCASIQKFGERAFAK	235
	: * : * : : : : : * : * * * * : * * * : : : : : * : : : : * : : *	
G. gallus	ARQLIYLSQKYPKAPFSEVSKFVHDSIGVHKECCGDMVECMDDMARMMSNLCSQQDVFS	300
M. gallopavo	AEKLVRLSQKYPKAPFSEVAKLVRESKDVHKECCGDMVECMDDMAEIIINMCSKQDVFS	300
B. taurus	AWSVARLSQKFPKAEFVEVTKLVTDLTKVHKECCGHDLLCADDRADLAKYICDNQDTIS	295
S. scrofa	AWSLARLSQRFKADFTEISKIVTDLAKVHKECCGHDLLCADDRADLAKYICENQDTIS	295
	* : : : * * : * * * : * : : * * * * : : : * : : : * : : *	
G. gallus	GKIKDCCEKPIVERSQCIMEAEFDEKPADLPSLVEKYIEDKEVCKSFEGHDAFMAEFVY	360
M. gallopavo	SKIKGCCEKPIMERSQCIMEAEFDEKPADLPSLVEKYIQDKEVCKSFEGHDAFMSEFVY	360
B. taurus	SKLKECCDKPLLEKSHCIAEVEKDAIPENLPLTADFAEDKDVCNKYQEAQDAFLGSFLY	355
S. scrofa	TKLKECCDKPLLEKSHCIAEAKRDELPAIDLPLEHDFVEDKEVCKNYKEAKHVFLGTFLY	355
	* : * * : * : : : * * : * * : * * : : : * : * * : : : : : * : * *	
G. gallus	EYSRRHPEFSIQLIMRIAKGYESLLEKCKTDNPAECYANAQEQNLNHIKETQDVVKTNC	420
M. gallopavo	EYSRRHPEFSTQLILRIAKGYESLLEKCKTDNPAECYANAQEQNLNHIKETQDVVKTNC	420
B. taurus	EYSRRHPEYAVSVLLRLAKEYEATLECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNC	415
S. scrofa	EYSRRHPDYSVSLLLRIAKIYEATLEDCCAKEDPPACYATVFDKFQPLVDEPKNLIKQNC	415
	*****::: : : : * * * : * * * : : * * : : : : : : : : : * * *	
G. gallus	DLLHDHGEADFLKSILIRYTKMPQVPTDLLLETGKKMTTIGTKCCQLGEDRRMACSEGY	480
M. gallopavo	DLLSAHGEPDFLKSILIRYTKMPQVSTETLLEIGKKMTAIGTKCCQLSEDRRMACSEGY	480
B. taurus	DQFEKLGEGYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDY	475
S. scrofa	ELFEKLGEGYGFQNALIVRYTKKVPQVSTPTLVEVARKLGLVGSRCCKRPEEERLSAEDY	475
	: : * * * : : : : * : * * * * * * * : : : : : * : * : * : * *	
G. gallus	LSIVIHDTCKRQETTPINDNVSQCCSGLYANRRPCFTAMGVDTKYVPPFPNPMFSFDEK	540
M. gallopavo	LSIVIHDMCRRQETTPINDNVSHCCSDSYAHRPCFTAMGVDTKYVPPFPNPMFSFDEK	540
B. taurus	LSLILNRLCVLHEKTPVSEKVTKCCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHAD	535
S. scrofa	LSLVLNRLCVLHEKTPVSEKVTKCCTESLVNRRPCFSALTPDETYKPKFVEGTFTFHAD	535
	* : : : : * : * * : : : : : : : : : * : * * * * * : * : *	
G. gallus	LCSAPAEEREVGQMKLINLIKRPQMTEEQIKTIADGFTAMVDKCKQSDINTCFGEEG	600
M. gallopavo	LCSAPAEEREVGQMKLINLIKRPQMTEEQIKTIADGFTAMVDKCKQSDINTCFGEEG	600
B. taurus	ICTLPDEKQIKKQATALVELLKHKPKATEEQLKTMENFVAFVDKCCAADDKEACFAVEG	595
S. scrofa	LCTLPEDEKQIKKQATALVELLKHKPHATEEQLRVTLGNFAAFVQKCCAAPDHEACFAVEG	595
	: * : * : : : . * : : * : * : * : * : * : * : * : * : * : * : *	
G. gallus	ANLIVQSRATLGIGA	615

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M. gallopavo    SNVVIIPGSAQDTWR 615
B. taurus      PKLVVSTQTALA--- 607
S. scrofa      PKFVIEIRGILA--- 607
               .:.:.:

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Figure S4. Amino acid sequences of known Parkinson disease protein 7 (DJ-1). NCBI accession numbers: cattle (*Bos taurus*) Q5E946.1; pig (*Sus scrofa*) NP_001072131.1; chicken (*Gallus gallus*) NP_989916.1; turkey (*Meleagris gallopavo*) XP_003212226.1. The alignment was constructed using CLUSTAL W2. Identified peptides by ESI-MS are highlighted.

```

G. gallus      MASKRALVILAKGAEEMETVIPTDVMRRAGIKVTVAGLTGKEPVQCSRDLICPDASLED 60
M. gallopavo   MASKRALVILAKGAEEMETVIPTDVMRRAGIKVTVAGLTGKEPVQCSRDLICPDASLED 60
B. taurus      MASKRALVILAKGAEEMETVIPVDVMRRAGIKVTVAGLAGKDPVQCSRDVVICPDASLED 60
S. scrofa      MASKRALVILAKGAEEMETVIPVDVMRRAGIKVTVAGLAGKDPVQCSRDVVICPDASLED 60
               *****.*****.***.*****.*****

G. gallus      ARKEGPYDIVLPGGNLGAQNLSA AVKDILKDQESRKGLIAAICAGPTALLAHGIGFG 120
M. gallopavo   ARKEGPYDIVLPGGNLGAQNLSA AVKDILKDQESRKGLIAAICAGPTALLAHGIGFG 120
B. taurus      AKKEGPYDVVVLPGGNLGAQNLSA AVKEILKEQEKRKGLIAAICAGPTALLAHEIGFG 120
S. scrofa      AKKEGPYDVVVLPGGNLGAQNLSA AVKDILKEQEKRKGLIAAICAGPTALLAHEIGFG 120
               *.*****.*****.***.***.*****

G. gallus      SKVITHPLAKDKMMNGAHYCYSES RVEKDGNILTSRGPGTSFEFG LAIVEALMGKEVAEQ 180
M. gallopavo   SKVTTHPLAKDKMMNGAHYCYSES RVEKDGNILTSRGPGTSFEFG LAIVEALMGKEVAEQ 180
B. taurus      SKVTTHPLAKDKMMNGSHYSYSEN RVEKDGLILTSRGPGTSFEFALKIVEVLVGKEVADQ 180
S. scrofa      SKVTTHPLAKDKMMNGSHYSYSEN RVEKDGLILTSRGPGTSFEFALAIVEALAGKEVADQ 180
               *** *****.***.***.***** *****.***.***.*****

G. gallus      VKAPLILKD 189
M. gallopavo   VKAPLILKD 189
B. taurus      VKAPLVLKD 189
S. scrofa      VKAPLVLRD 189
               *****.***.***

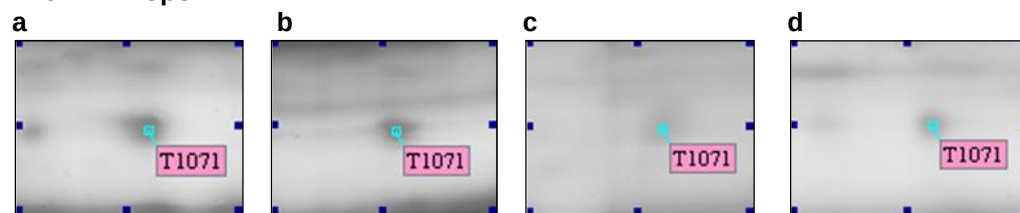
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Figure S5. Amino acid sequences of known heart-type fatty acid-binding protein (H-FABP). NCBI accession numbers: cattle (*Bos taurus*) P10790.2; pig (*Sus scrofa*) NP_001093401.1; chicken (*Gallus gallus*) NP_001026060.1; turkey (*Meleagris gallopavo*) XP_003212530.1; duck (*Anas platyrhynchos*) ABH10617.1. The alignment was constructed using CLUSTAL W2. Identified peptides by ESI-MS are highlighted.

Figure S6. Amino acid sequences of known 14 kDa phosphohistidine phosphatase (PHP). NCBI accession numbers: cattle (*Bos taurus*) Q32PA4.1; pig (*Sus scrofa*) XP_003122371.1; chicken (*Gallus gallus*) XP_001234073.1; turkey (*Meleagris gallopavo*) XP_003208622.1. The alignment was constructed using CLUSTAL W2. Identified peptides by ESI-MS are highlighted.

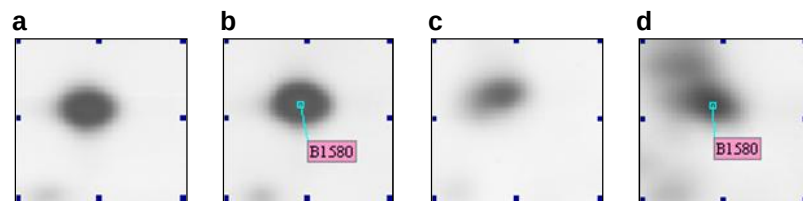
Figure S7. Images of selected spots extracted from differently processed meat products.

T1071 – TroponinT



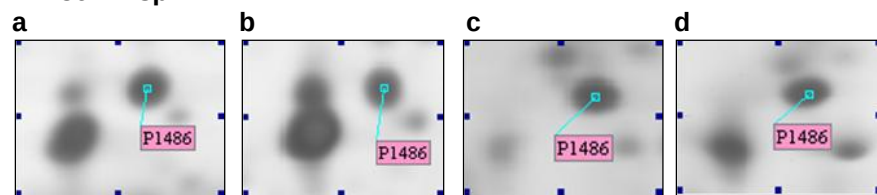
a – raw meat 45 min; b – raw meat 144 h; c – cooked meat; d – turkey and pork frankfurters (sample E)

B1580 – Galectin-1



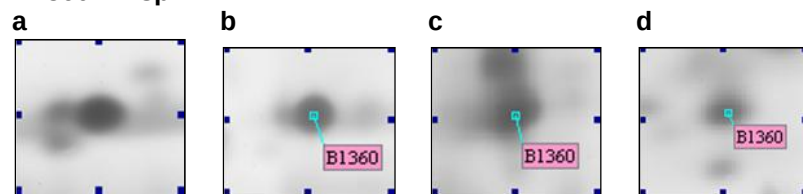
a – raw meat 45 min; b – raw meat 336 h; c – cooked meat; d – salami pepperoni (sample P)

P1486 – Hsp27



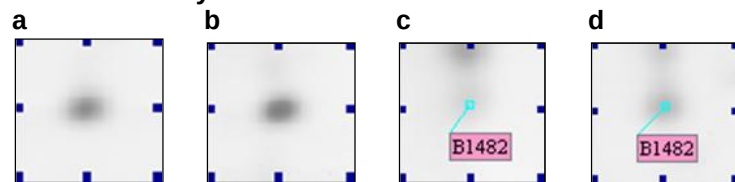
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B1360 – Hsp27



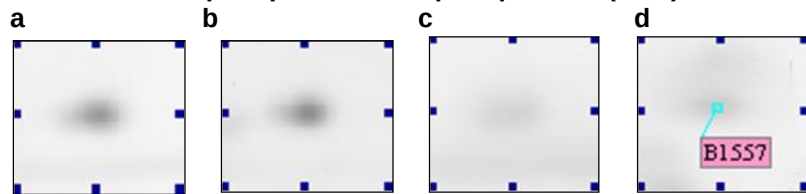
a – raw meat 45 min; b – raw meat 336 h; c – cooked meat; d – roasted sausage (sample K)

B1482 – ATP synthase



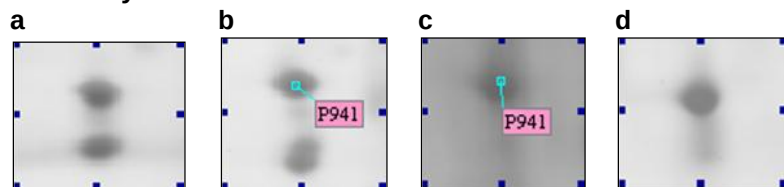
a – raw meat 45 min; b – raw meat 336 h; c – cooked meat; d – roasted sausage (sample K)

B1557 - 14 kDa phosphohistidine phosphatase (PHP)



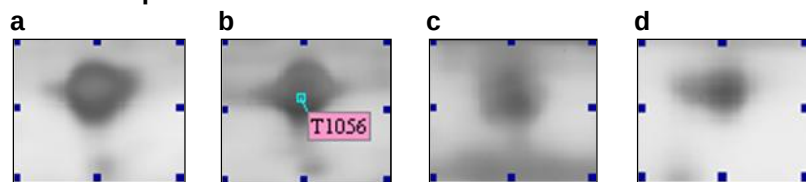
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P941 – Cytochrome b-c1



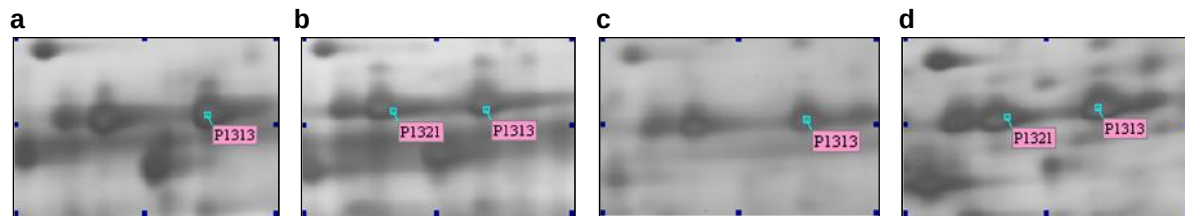
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T1056 – Alpha-enolase



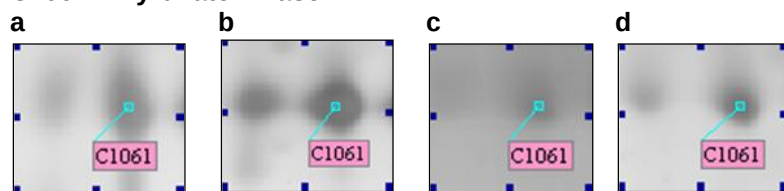
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P1313 – GPDH-C



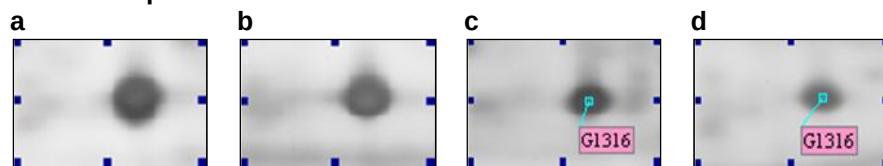
a – raw meat 45 min; b – raw meat 144 h; c – cooked meat; d – „Krakov” sausage (sample L)

C1061 – Pyruvate kinase



a – raw meat 45 min; b – raw meat 48 h; c – cooked meat; d – chicken frankfurters (sample E)

G1316 – Alpha-ETF



a – raw meat 45 min; b – raw meat 144 h; c – cooked meat; d – „Kabanos” sausage (sample M)