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Original scientific paper

ASSESSING THE EFFECT OF THERMAL TREATMENT ON MEAT PROTEINS USING PROTEOMIC METHODS

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Abstract
The results of studying the effect of various temperatures on the protein composition of minced meat from porcine m. longissimus dorsi by two-dimensional electrophoresis are presented. The most complete distribution of protein fractions was observed in fresh raw minced meat, and when it was exposed to negative temperature, there was a sharp decrease in protein components (carbonic anhydrase 3, αβ-crystallin), as well as a decrease in the staining intensity of protein spots of the main constitutive fractions (tropomyosin alpha 1, myosin light chain 1). In the case of heat treatment, structural muscle proteins were retained with some changes in high molecular weight fractions, namely, protein molecules degraded to compounds with a simpler structure. It was noted that fractions of tropomyosin β-chain, triosephosphate isomerase 1, myosin light chains 2 were not detected after minced meat was frozen, while tropomyosin alpha 1 was retained in all samples.

Introduction

It is known that introduction of processing technologies with different temperature variations is of crucial significance to meat technological characteristics [1,2]. The conditions of treatment, especially temperature, cause a cascade of chemical and physical changes that affect meat proteins. Occurring protein modifications include denaturation and aggregation [3,4], fiber shrinkage and collagen solubilization [5], the Maillard reaction [6] or protein oxidation [7]. Therefore, a range of used temperature conditions for meat processing and its following preparation leads to potentially different impacts on protein modification.

If meat products are exposed to the reactive oxygen species, the oxidative stress is induced by proteins, which cause chemical destruction, changes in protein functionality, loss of essential amino acids and possibly product digestibility [8]. With occurrence of oxidation, not only basic and aromatic amino acids are transformed into carbonyl, but also thiol groups are replaced with formation of disulfide bridges, which cause polymerization and subsequent aggregation of proteins [9]. Oxidation of aromatic amino acids can lead to denaturation, polymerization, aggregation, fragmentation, changes in hydrophobicity, solubility, gel formation and emulsification, which ultimately affect the physico-chemical condition of proteins [10].

Therefore, the main aim of the research was to understand how an impact of different temperatures (freezing and heat treatment) can cause biochemical changes in meat systems, which can affect food quality of meat proteins, using comparative analysis of two-dimensional maps.

Materials and methods

The object of the research was minced meat from porcine m. longissimus dorsi: raw, raw after freezing at a temperature of minus 40 °C and heat treatment, namely cooking until reaching 68 °C in the sample center. Minced meat was homogenized using a cutting mill Retsch GM 200 (Retsch GmbH, Germany) with a gradual increase of the knife revolution speed from 2000 rpm to 5000 rpm.

Two-dimensional electrophoresis (2-DE)

The samples described above were studied by the method of two-dimensional electrophoresis (2-DE). At the first stage, isoelectric focusing (IEF) was performed at 3650 V/h in tube gels (2.4 mm × 160 mm); 0.02 M orthophosphoric acid and 0.02 M sodium hydroxide were used as the anode and cathode buffers, respectively. After IEF, gels were incubated for 10 min. in 2.5 ml of the equilibration buffer I (6 M urea, 20% glycerol, 2% SDS and 1% DTT in 50 mM tris-HCl buffer, pH 8.8), then in equilibration buffer II (6 M urea, 20% glycerol, 2% SDS and 4% iodoacetamide in 375 mM tris-HCl buffer, pH 8.8).

After that, electrophoresis with sodium dodecyl sulfate was carried out. For this, the equilibrated gels were transferred to the 12.5% polyacrylamide gel (170 mm × 180 mm × 1.5 mm). Electrophoresis was performed using the buffer contained 25 mM tris -HCl, 192 mM glycine and 0.1% SDS at 30 mA on the gel until the dye front reached the end of the gel [11,12].

Visualization of the protein fractions and image analysis

Protein spots were visualized by staining with Coomassie Brilliant Blue G-250. Two-dimensional electrophoregram in the wet state were used for computer densitometry. Their digital images were obtained using a Bio-5000 plus scanner (Serva, Germany). Scanned images were analyzed with the software package ImageMaster ™ 2D Platinum based on Melanie 8.0 (GE Healthcare and Genebio, Switzerland), which automatically detected and quantified protein fractions. Then digitized 2 DE images were compared by the matching method.

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Results and discussion

Two-dimensional (2D) electrophoregram of investigated minced meat presented in Figure 1 showed similar distribution of fractions that corresponded to the multi-module database «Proteomics of Muscular Organs» [13] regarding *Sus scrofa*. The greatest quantity of protein fractions was found in native/raw minced meat, while a noticeable decrease in protein quantity was observed in the sample after freezing. Decomposition of the group of protein fractions with a molecular weight range of 65 kDa to 250 kDa was revealed in minced meat after heat treatment (Figure 2). It is interesting that tropomyosin beta chain (TPM2 33.5 kDa; pI 4.71), was not detected after freezing; with that, the fraction of tropomyosin alpha 1 (TPM1 33.5 kDa) was retained in all samples.

In addition, the degenerative effect on the myosin light chains 2 (MLC2 18.5 kDa; pI 4.89) was revealed both at the negative and positive temperatures. The similar picture was found for the fraction triosephosphate isomerase 1 (TPI 1 23.0 kDa; pI 6.80), which was intensively pronounced in raw minced meat, absent in the sample after freezing and found in small amounts in cooked minced meat. A uniform decrease in the staining intensity of protein spots of carbonic anhydrase 3 (30.5 kDa; pI 7.55), αβ-crystallin (20.0 kDa; pI 7.60) and myosin light chain 1 (MLC1 21.0 kDa; pI 4.90) from native minced meat to frozen was observed.

Conclusion

Various thermal treatments of meat systems differently affect the protein profile, in particular, the quantity of proteins and their staining intensity. Using the proteomic analysis by the method of two-dimensional electrophoresis, the greatest quantity of fractions with the pronounced intensity of the protein spots was found in native raw minced meat.

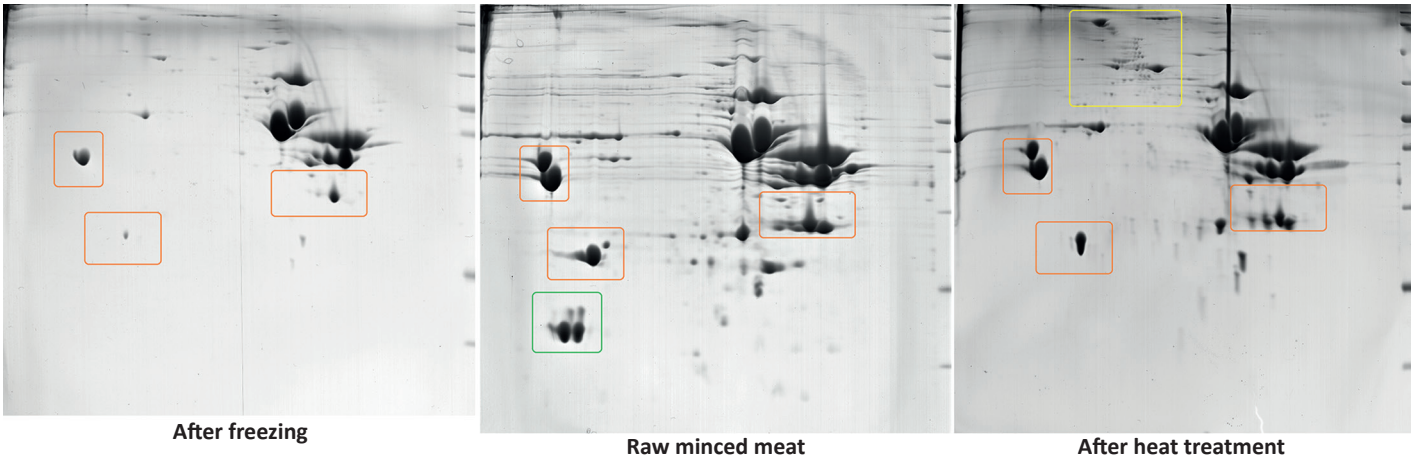

Figure 1. 2D electrophoregram of minced meat with different thermal treatment

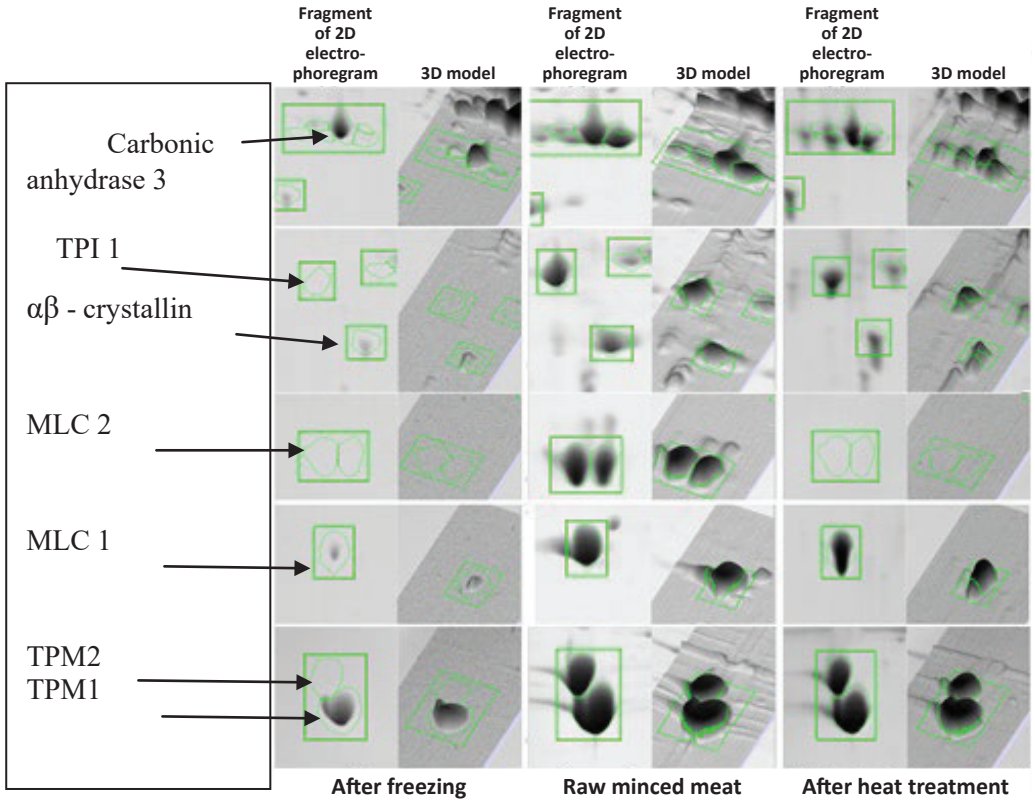

Figure 2. Enlarged fragments of 2D electrophoregram

In the case of sample freezing, part of protein compounds (triosephosphate isomerase 1, myosin light chains 2 and tropomyosin beta chain) was lost, which was caused by the mechanical impact of water crystals on the muscle fiber walls and protein denaturation due to water separation from the protein substance. It was found that in the sample after heat treatment, several proteins with the molecular weight higher than 65 kDa disintegrated into a set of spots of fractions of the same name that were present in the native minced meat, and part of proteins was not detected on the 2D electrophoregram.

These interpretations can ensure understanding of an effect of different food processing strategies and focus attention on biofunctional and nutritional properties of meat proteins.

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