



International 58th Meat Industry Conference “Meat Safety and Quality: Where it goes?”

Identification of biomarkers of horse muscle tissue using proteomic strategy

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Abstract

Proteomic analysis of horsemeat detected protein fractions, which were located on two-dimensional electrophoregram over a wide range of molecular masses and isoelectric points. The majority of the revealed proteins possessed molecular masses from 10 to 95 kDa. More than 130 protein fractions were detected, for which the proteomic profiles were similar to those of beef. Mass-spectrometric characteristics of seven horse proteins, in particular, the myosin light chains (MLC) and the tropomyosin proteins were obtained. Several proteins of MLC family in combination with tropomyosin proteins can be considered to be biomarkers, useful in analyzing raw meat and meat products containing horsemeat.

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Peer-review under responsibility of scientific committee of The 58th International Meat Industry Conference (MeatCon2015)

Keywords: muscular proteins; beef; horsemeat; biomarkers; proteomics

1. Introduction

Since the beginning of the 21st century, the development of biological sciences received a powerful impetus as a direct consequence of the decryption of genomes of a number of eukaryotic organisms and, above all, the human genome¹. In parallel, effective techniques to study biopolymers and various metabolites were invented. This led to the beginning of the new biological disciplines which are generally called “omics”². One of them is proteomics,

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which systematically studies protein products of gene expression³. These circumstances became the reason to consider the 21st century as the beginning of the era of post-genomic biology⁴.

With the beginning of the post-genomic era, proteomic technologies are considered a powerful tool to study raw meat and meat products, in particular specific biomarkers⁵, and determination of non-muscle proteins in meat and meat products⁶.

The aim of this research was to study horse muscle proteins and compare the obtained data to find potential meat biomarkers for identification horsemeat components in comminuted and cooked meat products.

Nomenclature

C	Coverage (% of covering the entire amino acid sequence by identified peptides)
Da	Dalton
kDa	kilo Dalton
M	Match peptides (number of matched peptides)
Mm	molecular weight
nm	nanometer
pI	isoelectric point
S	Score (measure of compliance or “scorecard”)

2. Materials and methods

Horse muscle samples (*m. Longissimus dorsi*) were obtained from a local meat-processing factory in Moscow region.

O’Farrell two-dimensional electrophoresis with isoelectric focusing in the pH gradient formed by carrier ampholytes was used with subsequent protein detection by staining with Coomassie R-250 was used to separate proteins.

Identification of protein fractions was performed after trypsin digestion techniques on MALDI-TOF MS and MS/MS mass spectrometer Ultraflex (Bruker, Germany) with UV laser (336 nm) using the positive ion mode in the mass range of 500-8000 Da with calibration of the known trypsin autolysis peaks. Analysis of the mass spectra of tryptic peptides was performed using the Mascot software, option Peptide Fingerprint (Matrix Science, USA), followed by a database search in the National Center for Biotechnology Information (NCBI).

Comparative analysis of proteomic profiles of beef and horsemeat was conducted using information modules “Proteins skeletal muscle of cows (*Bos taurus*)” from the database “Proteomics muscular bodies” (<http://mp.inbi.ras.ru>).

3. Results and discussion

Up to 130 protein fractions were obtained (Fig. 1).

The protein fractions identified ranged in molecular mass from 10 to 95 kDa. In general, the horsemeat proteomic profile was similar to the beef one (Fig. 1 a and b, respectively). The results of MS identification of seven protein fractions (Fig. 1a, fractions 1 – 7) confirmed the above claims.

Table 1 summarizes the data regarding these fractions, indicating that five of them are similar to the corresponding beef proteins (Fig. 1b).

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