

Changes of proteins and biogenic amines in meat and meat products

Theses of Ph.D Dissertation

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I. INTRODUCTION

As a result of their high biological value, meat proteins play an important role in providing the required supply of essential amino acid intake, and meats also contain numerous important vitamins and micro-elements in an easily utilizable form. One goal of food sciences is to examine this system with such a complicated composition and structure, and well defined biological function, in respect to producing food products after the slaughter of an animal in which the utilization of the nutritional content as well as the physical and organoleptic properties are the most favorable. It is fundamentally characteristic of meat to go through continuous and significant changes following slaughter. The method of animal keeping and slaughter, the conditions of cooling, chopping and storage, as well as the different processing technologies decisively influence the quality of the product. The question of meat quality has become increasingly significant nowadays.

In the field of food analysis electrophoretic methods played a great role in isolating and cleaning proteins, as well as the description of animal and vegetable proteins during the last decades. Food industry procedures can modify the structure of food proteins to different degrees. The electrophoretic and chromatographic examination of meat protein structure - beyond the identification of different species and revealing foreign proteins - can also be applied to trace the different factors effecting the protein pattern (ex: species, keeping method, conditioning, freezing, heat processing, etc.). It is favorable that in the course of examining the changes in the meat proteins, after the electrophoretic isolation it is possible to use not only the generally applied protein stains, but also other - more sensitive and of a different specificity - detection methods can be used. The isolation of the proteins can serve as a basis for further, ex. immunological or enzymic investigations as well.

The question of food protein allergies is important in the case of meat products as well. In case of allergic disorders evoked by food it is important to omit the allergenic component (protein) from the diet. This is made difficult by foreign proteins existing in meat products in a „concealed” form, ex. as additives. The possibility of cross allergies further signifies the research of potential allergenic activity of meat products.

The level of biogenic amines is relatively low in fresh meats, but their concentration can rise significantly during storage or through protein degradation and microbial activity resulting from certain food industry technologies. Beyond health concerns resulting from their physiological activity, the identification of biologically active amines is important because their quantity and concentration suitably characterizes the freshness and quality of meats.

II. GOALS

I set the following tasks as goals for my research:

1. Determination of species specific protein patterns of the meat samples
 - according to the distribution of molecule mass of protein fractions
 - in case of pork meat using the immunoblot technique
 - using the isoelectric point based separation, with special attention to protein fractions containing iron

2. Study the effect of animal keeping methods and food-technology factors on muscle proteins
 - the study of protein structure from meat samples originating from pigs raised in mass-production and organic circumstances
 - determining the meat' conditioning process through the changes in myofibrillar protein structure
 - the effect of heat treatment and γ -irradiation to the protein structure of meats

3. Examination of potential allergenic characteristics of meat and meat-product protein components

4. Determination of biogenic amines in raw meat samples, and tracking the changes in quantity through storage and conditioning

III. RESEARCH METHODS

- ◆ SDS-polyacrylamide gelelectrophoresis
- ◆ electrophoretic blotting technique and immunostaining
- ◆ isoelectric point based separation of proteins in agarose or polyacrylamide gel
- ◆ pseudoperoxidase staining of heme proteins
- ◆ detection of non-heme iron bound to protein by ferriin reaction
- ◆ videodensitometric evaluation of electrophoretograms
- ◆ medium pressure liquid-chromathographic (FPLC) separation of proteins by gel filtration
- ◆ determination of biogenic amines with amino acid analyzer or HPLC technique (reverse phased ion pair chromatography, fluorescent detection)

IV. NEW SCIENTIFIC ACHIEVEMENTS

- Following the isoelectric focusing I determined the myoglobin pattern of meat samples from animal species frequently consumed in Hungary using pseudoperoxidase staining (Hofmann and Blüchel, 1986). I found that although the pattern is very similar in case of species closely related taxonomically ex. duck and goose, or pig and wild boar, while the main myoglobin patterns may coincide, the difference in distribution of secondary fractions makes identification possible.
- In case of pork, wild boar meat and beef I found that conditioning in a special ageing container on 2°C for three weeks does not considerably influence the myoglobin pattern.
- In accordance with the statements of literature, I found that the level of heat processing limits the applicability of determining the origin through the myoglobin pattern. Sausages reveal intensively staining streaks, in cooked sausages the electrophoretogram can not always be accurately evaluated, and in tinned meats the pattern of proteins has altered to such a degree that the origin of the meat can not be determined through the myoglobin pattern.
- Comparing the myoglobin pattern of samples from pork kept under mass-production and organic circumstances, I found a difference in the ratio of the two main myoglobin fractions: the ratio of 6.5 and 6.0 isoelectric point fractions in the examined organic meat samples was 47:53 on average, while in the control samples 66:34.
- In irradiated (0-25 kGy) pork leg samples I found the intensity of the myoglobin fractions decrease less compared to heat treated samples (50-121 °C), and in the fields above the 6.5 isoelectric point and below 6.0 isoelectric point I revealed the appearance of further fractions with peroxidase activity.
- Following the isoelectric focusing, on the meat proteins I used the „iron staining” technique based on ferriin reaction, which was formerly used to show the iron content of certain foods, like water or wine, as well as to mark proteins containing non-heme iron separated in native polyacrylamide gel. I found that with this method it is possible to specifically detect protein fractions containing non-heme iron in meats or other food samples.

- I saw, in accordance with statements in literature (Penny, 1980, Di Lisa et al., 1995) troponine-T fraction decomposition in pork, beef and wild boar meat samples through the three week conditioning process. I verified the existence of degraded products by immunoelectrophoretic analysis using rabbit serum containing antibodies for troponine.
- In certain Hungarian meat products I found the presence of soy or milk protein additives using the immunoblot technique. In some cases I detected cross-reaction in the meat of milk-giving animals - similarly to former observations in literature (Pastorello et al., 1995, Polgár et al., 1998) - using milk protein human serum.
- In accordance with literature data (Tschabrun et al., 1990, Hernandez-Jover et al., 1997) I found a relatively low biogenic amine level in fresh meat samples. I observed that from the fresh pork, beef and wild boar samples wild boar meat is characterized by higher tyramine and cadaverine values. I showed that in the examined PSE-type pork chop and round samples the concentration of biogenic amines is lower (in case of round samples, significantly lower) than in normal meat samples.
- My findings verified the continuous rise in tyramine, putrescine and cadaverine levels in meat samples (pork, beef and wild boar) through conditioning in a special container on 2°C. In the wild boar rib meat the putrescine and cadaverine concentration rose above 100 mg/kg as soon as the second week of conditioning, while the amount of histamine remained below 10 mg/kg in all three species through the four weeks of conditioning.

V. PUBLICATIONS AND LECTURES ON THE SUBJECT OF THE DISSERTATION

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