EFFECT OF A BACTERIAL ENZYME PREPARATION ON THE SOLUBILITY AND ELECTROPHORETIC PROPERTIES OF MUSCLE PROTEINS

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INTRODUCTION

One of the most important technological processes in meat production by means of which raw meat products receive desirable consumer properties is the process of ageing (1,3). The necessity of providing consumers with meat of high organoleptic quality (tenderness first of all) on one hand, and the pursuit by meat processors to reduce both duration of natural ageing, and the considerable energy costs, on the other, have stimulated the search for more efficient tenderizing technologies. There are numerous varieties of methods for improving meat tenderness mechanically that, however, present certain difficulties and disadvantages (2,4).

In the past years attention in meat industry has increasingly been paid to proteolytic enzyme preparations as an effective means for accelerating the ageing process and improving meat quality. There are experiments on partial proteolysis of mechanically deboned poultry meat using acid protease which increases the solubility of miofibrillar proteins as compared to control miofibrillar proteins from deboned meat (5). Particularly good results, in this respect, are observed when using enzymes produced by microorganisms - fungi and bacteria. The major advantage of this kind of enzymes is the possibility to obtain preparations with strictly definite properties and activity. The changes that occur when modifying meat with proteolytic enzymes can also be observed during its natural ageing. The difference lies in the fact that the former set in more rapidly and more intensively.

The objective of the present study was to examine the effect of a proteolytic enzyme preparation isolated from Bacillus mesentericus on skeletal and heart muscles.

METHODS AND MATERIALS

Animals from one flock of the "Black-motley" and "Merinofleisch" breeds were used to receive beef and lamb, respectively, for the purposes of the study.

M.Longissimus dorsi was excised bilaterally from the warm carcasses (up to 2 hrs after production), and from the cooled carcasses (48 hrs after production). The right-side specimens were tested, and the left side ones were used as controls 0.3 g enzyme solution corresponding to 137 PU per 1 kg of meat was injected together with 2% NaCl solution.

The enzyme preparation used was "Mezanterin 11-11" from Bacillus mesentericus having the following characteristics:

- Dry subtance, % 85
- Ash content, % against d.s. 28,12
- Total nitrogen, % against d.s. 5,32
- Total protein, % against d.s. 33,25
- Proteolytic activity, PU/g enzyme preparation 210
- Optimum temperature range 55-60°C
- Optimum pH 6,5

Both test and control samples were packed in PVC bags and stored at +2 - +4°C.
In order to establish protein solubility changes against Helander's chart (6), about 5 g of the product were homogenized in a porcelain mortar with quartz sand. This method, according to Lindenberg (7), gives the best results in extracting muscle proteins.

The total water-soluble nitrogen, containing the sarcoplasmic fraction and nonprotein nitrogen, was extracted by potassium phosphate buffer with pH=7,3 and ionic strength=0,03. The homogenized sample was deluged with 50 ml of the abovementioned buffer, and extracted for 2 hours. Then the sample was centrifigated for 15 min at 5000 rpm. The resultant centrifugate was filtered. Two more extractions were performed under the same conditions. The total filtrate volume was measured. A portion of 40 ml was taken out and the total nitrogen of the water-soluble proteins was determined by Kjeldahl's method. In 50 ml of the same extract proteins were precipitated with 10 ml of 60% trichloroacetic acid for 15 min; after filtering, the precipitate was washed with 20 ml of 10% trichloroacetic acid. The volume of the resultant filtrate was measured and the amount of nonprotein nitrogen was determined in a 40 ml portion by Kjeldahl's method.

To extract the miofibrillar proteins, the remaining sample, after separation of the water-soluble protein, was deluged with 50 ml of 1,1 M KJ dissolved in 0,1 M potassium phosphate buffer with pH=7,6. Triple extraction was performed, each lasting 2 hrs. Following each extraction, centrifugarion was performed for 15 min at 5000 rpm. The insoluble residue, after extracting the water-soluble and salt-soluble nitrogen, was washed with distilled water and the total amount was transferred into a Kjeldahl flask to determine the quantity of the insoluble in KJ proteins by Kjeldahl's method.

The nitrogen in the sarcoplasmic proteins was calculated by substracting the nonprotein N from the total protein extracted by 0,03 M potassium phosphate buffer. The miofibrillar protein was determined by substraction of the total protein extracted by 0,03 M potassium phosphate buffer plus the insoluble protein in KJ from the total nitrogen of the sample.

All analyses on protein fractioning and extraction were carried out in a cool chamber at 2-4°C because of the sarcoplasmic fraction's tendency towards denaturation.

The changes in the electrophoretic properties of proteins were determined in the following way: 10 g of the average sample were homogenized in a high-speed homogenizer with pending knives. One gram of the resultant homogenate was suspended in 10 ml lysing buffer with the following composition: 66 mM Tris, pH=6,8, 2% SDS, 28 M β-mercaptoethanol, 1% glycerol, o,05 bromphenol blue. After dissolving, the sample was boiled for 5 min in water bath. Portions of the resultant lysate were mixed with corresponding amounts of lysing buffer and were loaded on 12,5% polyacrylamide gel prepared according to Laemli (8). The protein content of the tested samples had been previously determined by Kjeldahl's method. The electrophoresis was performed at a constant voltage of 45-50V for 12 hrs on 16/16 cm gels. The movement of the bromphenol blue across the gel served as an indicator. The electrophoresis was precluded when the dye reached the bottom of the gel.

RESULTS AND DISCUSSION

The experimental results about the solubility of miofibrillar proteins in enzyme treated and non-treated meat are shown on Figures 1 and 2.

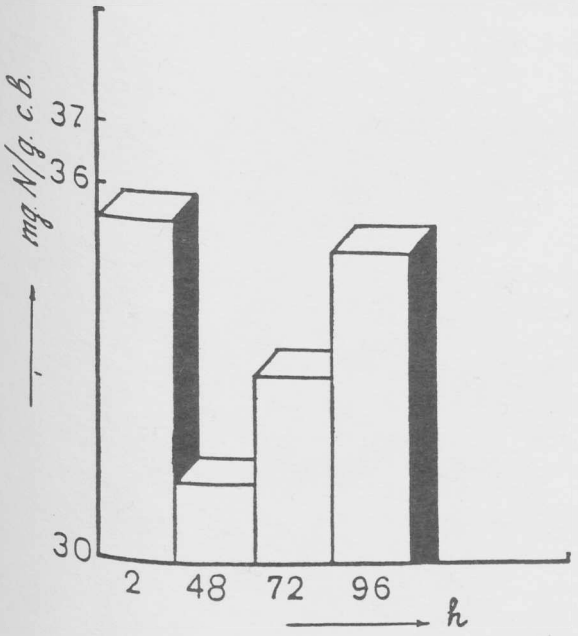

Fig.1 Changes of miofibrillar protein solubility (mg N/g dry substance . 10^{-3}) in non-treated meat.

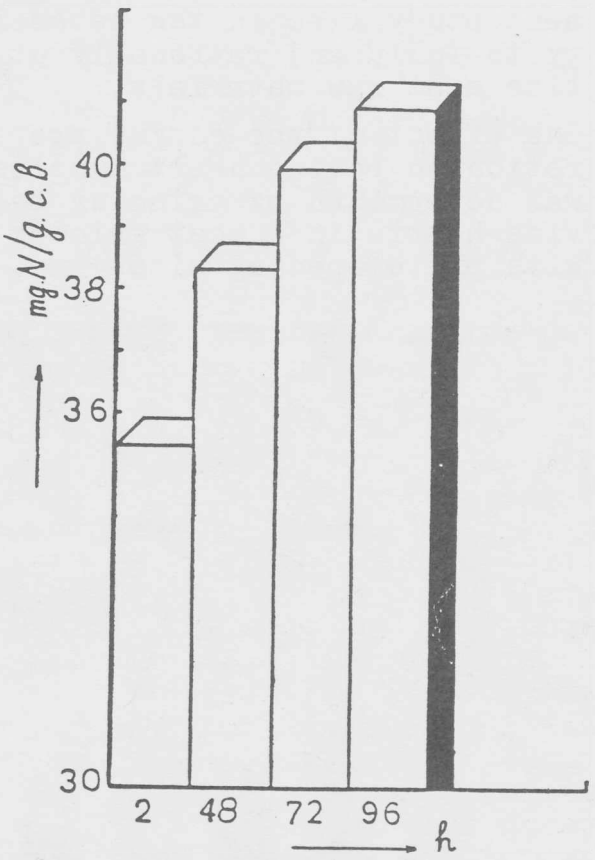

Fig.2 Changes of miofibrillar protein solubility (mg N/g dry substance.10^{-3}) in enzyme treated meat.

It is obvious that miofibrillar protein solubility in non-treated meat is reduced to 87,4% of its initial value at 48 hrs post mortem. With further storage of non-treated meat, miofibrillar protein solubility starts to rise slowly.

By contrast to non-treated meat there is considerable increase in miofibrillar protein solubility already 48 hrs post mortem (107,8% of the initial value), and continues to increase with further storage of enzyme treated meat. At 72 and 96 hrs post mortem miofibrillar solubility of enzyme treated meat is 112,6 and 115,2% of the initial value respectively.

The proteolytic enzyme preparation used by us caused considerable changes in muscle structure resulting in an increased amount of extracted miofibrillar proteins as compared to control samples. The increased solubility of the miofibrillar proteins in enzyme treated meat is also related to the increased meat tenderness as compared to non-trated meat.

The results about the tenderizing effect of the bacterial enzyme preparation "Mezenterin 11-11" on skeletal muscles suggested the idea to study its effect on tissues of lower tech

nological importance. Such tissues, for example, are the cardiac muscle, muscles of the subbrachial and subfemoral areas, etc. The expedience of the present study lies in the necessity to fully and rationally utilize meat raw materials.

The effect of the enzyme preparation on bovine heart muscles was determined by grinding bovine hearts in a meat grinder with plate opening size 4 mm. 0,02 g of 0,02% enzyme preparation solution were added to 1 kg of the sample, corresponding to 42 PU/kg of sample. The preparation was allowed to act on the sample for 30 and 60 min, respectively, at 30°C. The results about the changes of the electrophoretic properties of proteins are shown on Fig.3.

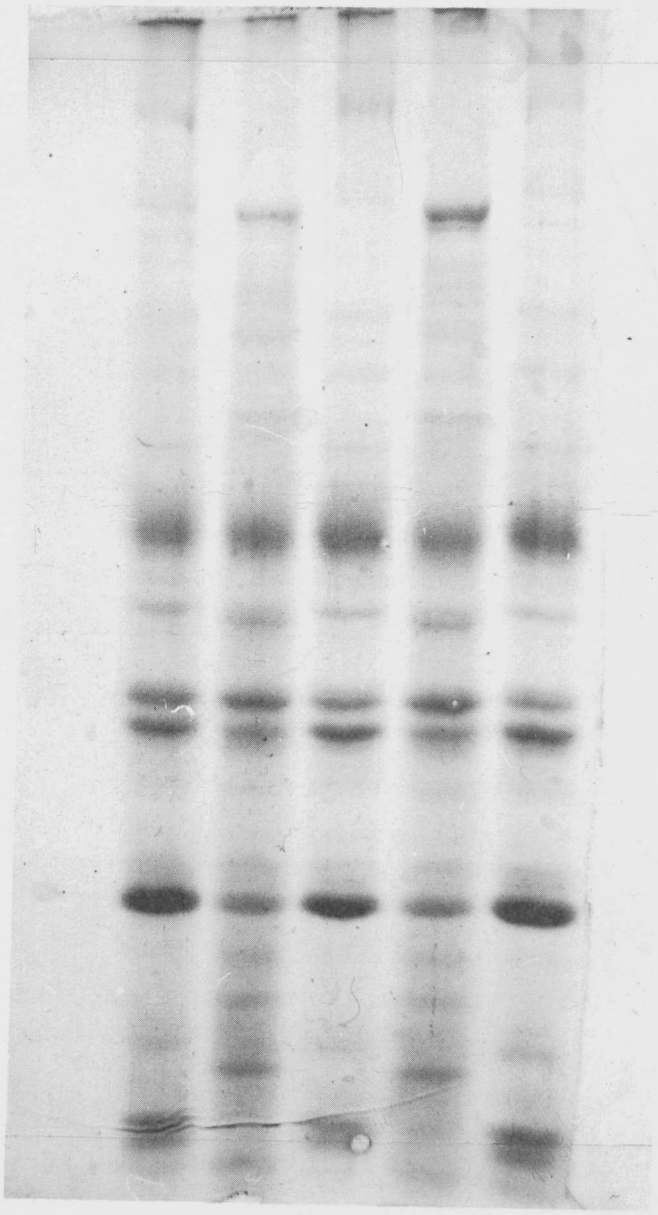

Fig.3 SDS-polyacrylamide gel electrophoretic profile of bovine heart muscle proteins: 1-initial sample; 2-enzyme treated-30 min; 3-non-treated-30min; 4-enzyme treated-60 min; 5-non-treated-60 min.

The results obtained indicate that considerable changes have occurred in the electrophoretic profile of enzyme treated samples. There is an obvious decrease of high molecular fractions and presence of low molecular ones which, apparently, are degraded products of myosin.

Consequently, the enzyme preparation used by us is an effective means to accelerate hydrolytic processes of muscle proteins related to changes in the structural, mechanical and hydrophilic properties of meat. It can be concluded that this method of treatment can considerably contribute for the intensification of technological processes and improvement the quality of low functional meat.

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