B/5

ON SOME CHANGES IN MUSCLE PROTEINS OCCURRING UNDER THE ACTION OF THE BACTERIAL PROTEASE E-30

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РЕЗЮМЕ

Были проведены исследования для определения некоторых изменений в мышечных белках, наступающих под действием энзимного препарата Е-30. Говядину и телятину, рубленые на мясорубке, обрабатывали 0,5, 1 и 2%-ными водными растворами Е-30. Исследовали воздействие энзимного препарата на мышечные белки посредством определения количества свободных аминокислот и разваримости коллагена. Описаны наступившие под действием Е-30 изменения в экстрагируемости водорастворимых, солерастворимых и нерастворимых мышечных фракций.

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On a effectué des recherches pour l'établissement de certaines modifications ayant lieu dans les protéines musculaires sous l'influence du produit d'enzymes E-30. On traite de la viande de bovins et de veau, moulues dans un cutter, avec des solutions aqueuses de l'E-30 aux concentrations de 0.5%, 1% et 2%. On examine l'influence du produit d'enzymes sur les protéines musculaires par la détermination de la quantité des acides aminés libres et de la solubilité du collagène. On décrit les modifications, obtenues sous l'influence de l'E-30, dans l'extractivité des fractions musculaires insolubles et de celles-ci qui sont solubles dans l'eau et dans les solutions salines.

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Studies were carried out to determine some changes in muscle proteins, occurring under the action of the enzymatic preparation E-30. Beef and veal, ground in a meat grinder, were treated with 0.5%, 1% and 2% water solutions of E-30.

The action of the enzymatic preparation on muscle proteins was studied by determining the quantity of free amino acids and collagen solubility on heating. The changes set in under the action of E-30 in the extractability of water-soluble, salt-soluble and insoluble muscle fractions are described.

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Es wurden Untersuchungen durchgeführt zur Feststellung einieger Veränderungen in dem Muskeleiweiss, die unter der Einwirkung des Enzymepräparates E-30 eintreten. Gewolftes Rind- und Kalbfleisch wurde mit 0.5, 1 und 2%-igen wässrigen Lösungen von E-30 behandelt. Durch die Bestimmung der Menge der freien Aminosäuren und der Kolagenlöslichkeit wurde die Einwirkung des Enzympräparates auf das Muskeleiweiss untersucht. Es werden die unter der Einwirkung des E-30 eintretenden Veränderungen im Extrahierungsvermögen der wasser- und salzlöslichen, sowie auch der unlöslichen Muskelfraktionen beschrieben.

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During the recent years in the literature appeared much informations for intensifying the process of meat curing by way of proteolitic enzymatic preparations. Many proteases have been investigated from vegetable, animal and microbial origin. Lately, in the Institute of Microbiology with the Bulgarian Academy of Science, was produced a bacterial protease E-30. Its characteristic peculiarities as pH optimum, temperature optimum, gelatinase and elastasae activities are described in detail by Nachev et al. (4, 5, 6). Karadjov et al. established that E-30 is composed of four fractions, the first of which is amilase, and the rest proteolytic enzymes.

The investigations of Velinov (1972) on the hystological changes in the muscle tissue following the action of the E-30 enzyme, demonstrate that tha latter affects the endomisial membranes of the sarcoleme, the myofibriles, the elastic fibres and under specific conditions,also the perimisial connective tissue (1, 3, 3).

Muscle innoculations by way of a multi-needle device of veal and ox meat with water solutions of E-30 significantly increases the tenderness of the meat (Nestorov et al., 1972).

The present investigations were made to elucidate the action of bacterial protease E-30 on the quantity of hydrolized products, which do not precipitate by threechloracetic acid, on the boiling of collagen and the extractibility of the water soluble, salt soluble and not soluble protein fractions of the meat. This will further elucidate the action of the preparation.

Material and Methodics

The investigations were made with bacterial protease E-30, produced in the Institute for Microbiology with the Bulgarian Academy of Science. Comminuted veal and ox meat was treated with 0,5%, 1% and 2% water solutions of the enzymatic preparation (activity 100000 PUN) for four hours at 37°C and 4°C. The enzymatic treatment was made, while to 100 gm comminuted meat was added 10 ml from the enzymatic solutions, and to the controls, correspondingly 10 ml of destilled water. The quantity of the hydrolyzed protein products which do not precipitate by threechloracetic acid were established by spectrophotometry at 280 nm. Solubility of colagen was established after the method of Solovyov (1966). The quantity of oxyprolin after the method of Neuman and Logan (1950).

The action of E-30 on the extractibility of the different fractions of muscle proteins was investigated by treatment of 5 g comminuted meat from M. long. dorsi with 1 ml water solution from E-30 (containing 1500 p.p.m./ml) for two hours at 20°C. The extraction of the muscle proteins was made after the modified by Kang & Rice method of Hagarty et al. (1963). Total nitrogen from the separate muscle fractions was established after the Kjeldal method. Controls were similarly treated, but the enzymatic solution was changed with 1 ml destilled water.

Results and Discussion

The results from our investigations on the quantity of the hydrolyzed protein products,which do not precipitate with

threechloracetic acid, are given on fig. 1 and 2.

Data from fig. show, that even with low plus temperatures, under the action of the enzyme, the quantity of the products from the protein hydrolyses, which do not precipitate with threechloracetic acid is augmented with 41.5% in comparison with the controls. The action of the enzyme upon the comminuted meat is significantly stronger at 37°C (fig. 2), while the quantity of the hydrolized protein products which do not precipitate with threechloracetic acid is augmented with 108%.

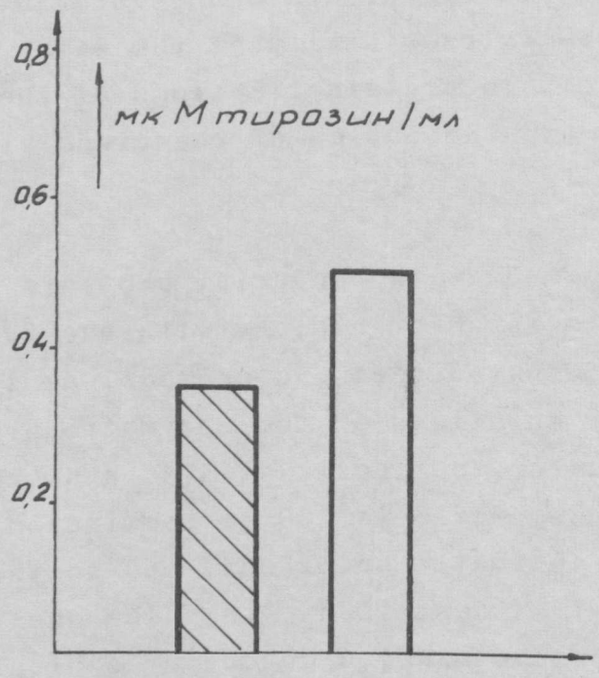

Fig.1. Hydrolycs products soluble in TClAA; conditions - 1% sol. E-30 at 4°C

The changes in boiling the colagen, appearing under the action of 1% solution of E-30 et 4°C and 37°C for four hours are given on fig. 3 and 4. It could be seen, that under both temperature regimes the degree of colagen boiling is higher in comparison with the controls. Evident is a significantly higher activity of the enzyme at 37°C.

The results from the investigations of samples from comminuted meat treated with 0,5%, 1%, and 2% water solutions of E-30 at 37°C are reflected on fig. 5 and 6.

Data on fig. 5 and 6 show that with the increase of the enzyme concentration, the hydrolic processes of muscle and connective tissue proteins are accelerated withiut having any linear dependance. From the investigated different concentrations of enzyme solutions, best suited for the production

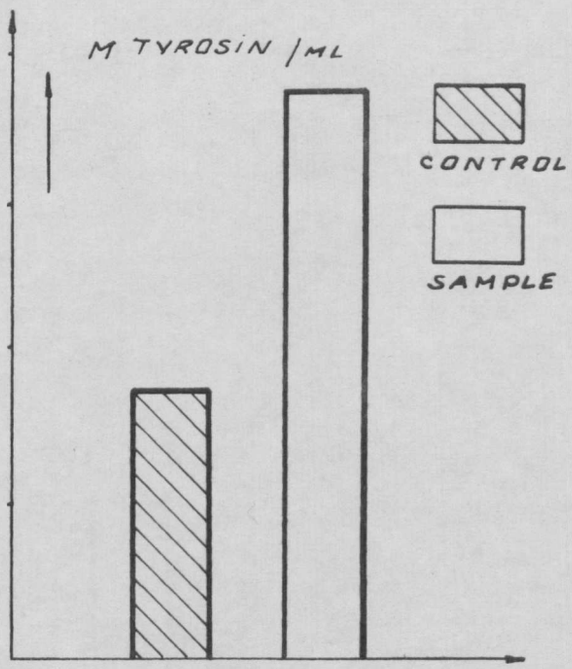

Fig. 2. Hydrolycs products soluble in TClAA; conditions - 1% sol.E-30 at 37°C

of paste sausages was the 0,5% water solution of E-30.

On fig. 7 are given the changes in the extractibility of the water soluble, salt soluble, and insoluble fractions of muscle proteins in percent towards the total nitrogen.

From data on fig. 7 it is evident that the fraction of the insoluble proteins decreases significantly while the fractions of the water soluble and salt soluble muscle proteins increase. It is evident, that proteins from the connective tissue, under the action of E-30 undergo changes leading to their extractability.

Conclusions

Our investigations on some changes exibited in muscle proteins, under the action of the bacterial protease E-30 permit the following conclusions:

1. The quantity of the hydrolytic protein products which do not precipitate with threechloracetic acid increase. This process is better evidenced at 37°C than at 4°C.

2. Under the action of the bacterial protease E-30 the colagen boiling is increased.

3. The bacterial protease E-30 hydrolizes mainly the insoluble fraction and comparatively to a lesser degree soluble and salt soluble fractions of muscle proteins.

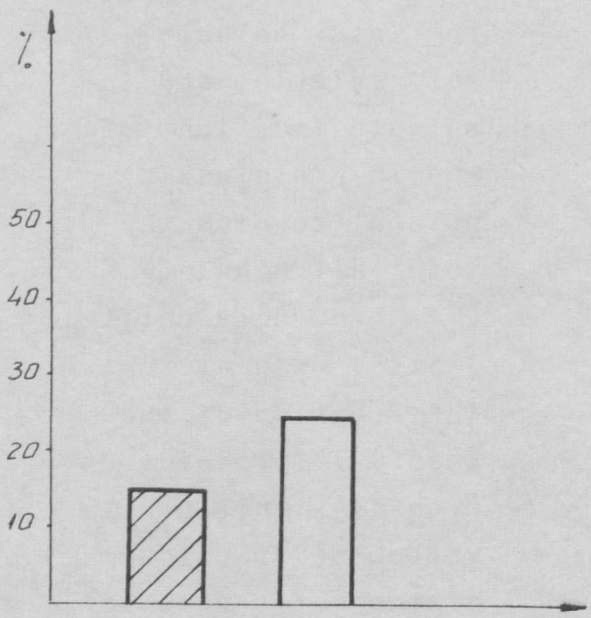

Fig. 3

Boilability of colagen:
conditions - 1% sol. E-30
at 4°C

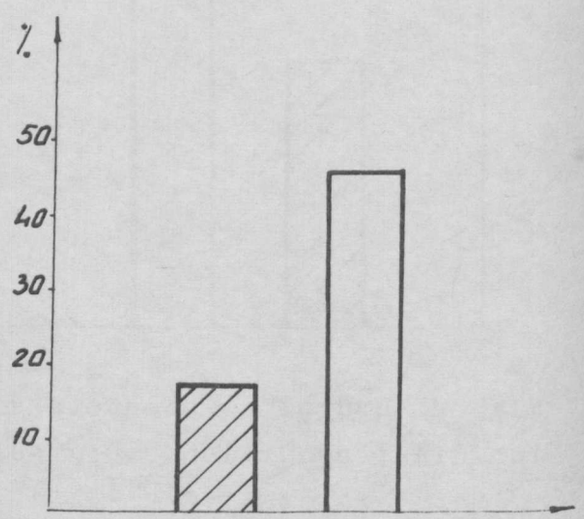

Fig. 4

Boilability of colagen:
conditions - 1% sol. E-30
at 37°C

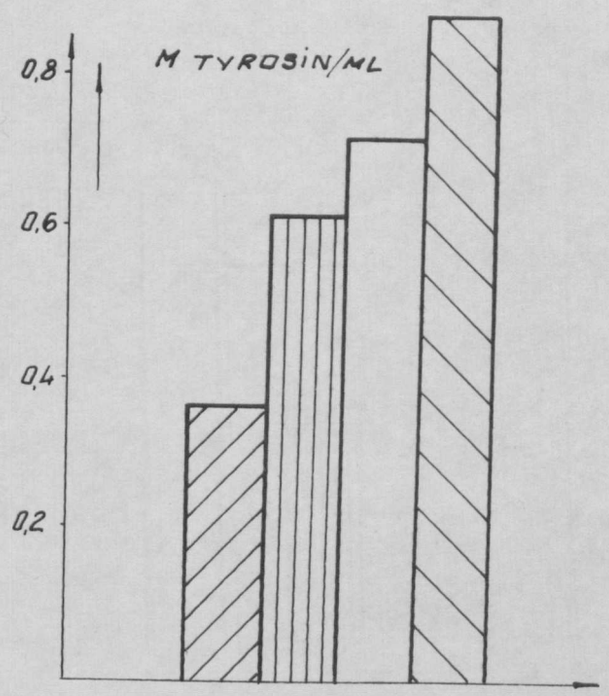

Fig. 5

Hydrolyc products soluble
in TClAA at 37°C

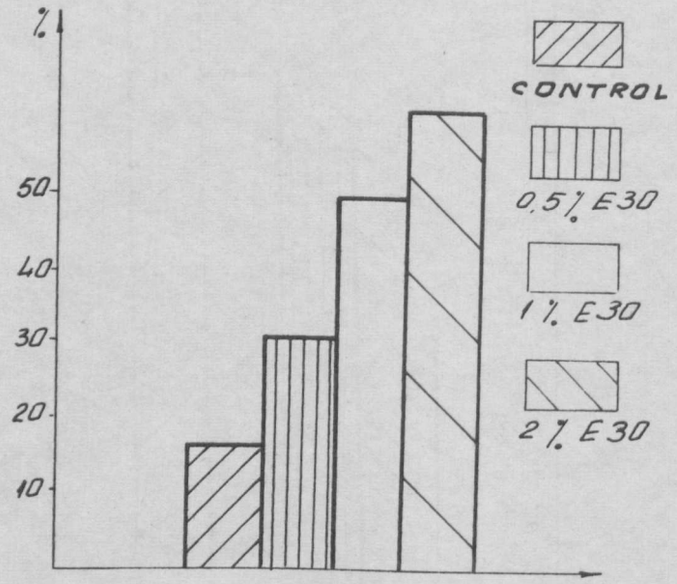

Fig. 6

Boilability of colagen
at 37°C

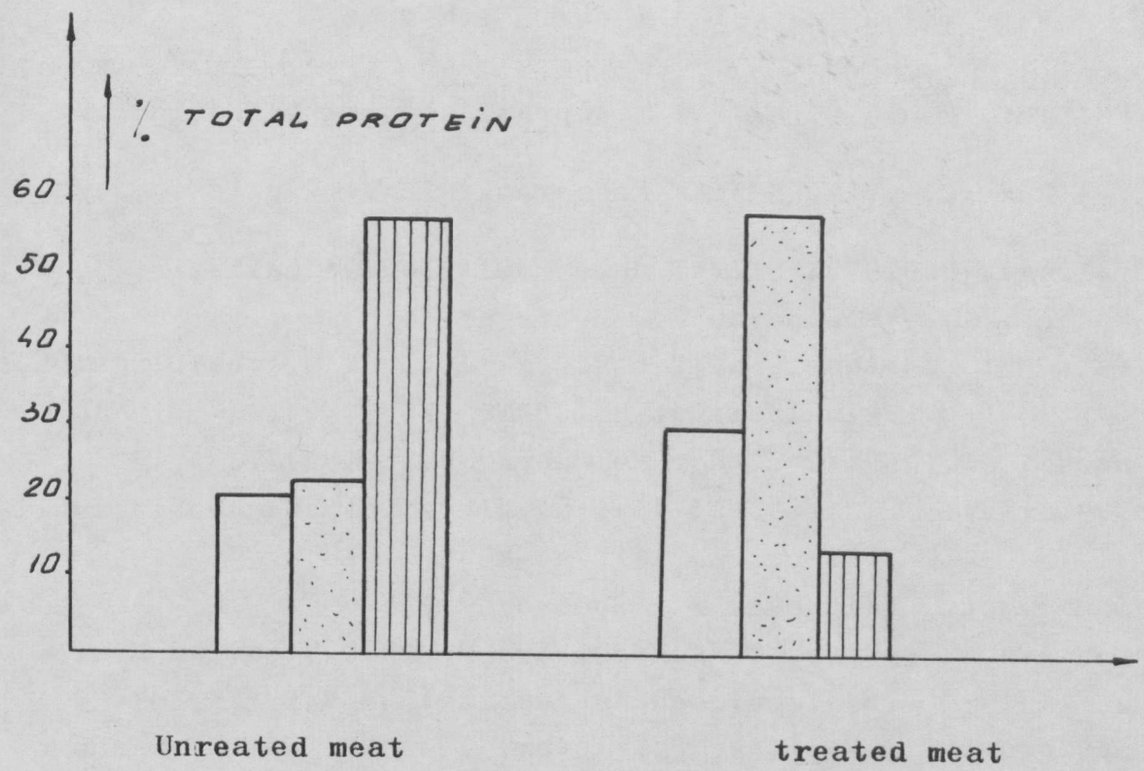

Fig. 7

Solubility of muscle proteins

- Fraction of the water soluble muscle proteins
- Fraction of the salt soluble muscle proteins
- Fraction of the insoluble muscle proteins

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