MORPHOLOGICAL CHANGES IN STRUCTURELESS SAUSAGES PREPARED FROM ENZYMATICALLY MODIFIED BEEF HEARTS KATYA VULKOVA¹, ALEXI KOSTOV², MILOSLAV ZHIKOV³ and STEFAN DANCHEV¹

Higher Institute of Food and Flavour Technologies, 26 Lenin Blvd., Plovdiv 4002, Bulgaria ²Meat Packing House, 1 Malashevska St., Sofia, Bulgaria ³Institute of Meat Technology, 65 Cherni Vruh Blvd., Sofia, Bulgaria.

SUMMARY: The morphological changes in structureless sausages prepared from enzymatically modified beef hearts were established. The studies were carried out using the microbial protease "Mezenterin 11-11" and beef hearts modified with different amounts of the same enzyme. The modified beef hearts were added to pork, and sausages were produced in the traditional method. The electron microscopical studies indicated that "Mezenterin 11-11" had caused destruction of the endomysium, sarcolemma, myofibrils and the sarcoplasm of the muscle fibres. A better emulsifying ability of the meat mix was established as well as better quality of the finished product. The organoleptic analysis of frankfurters pointed out that samples with enzyme levels of 0.01% and 0.02% received the highest grades: 0.87 ± 0.28 and 8.27 ± 0.32 , respectively.

INTRODUCTION: One of the methods currently used to improve meat quality is modification by appropriate proteolytic enzymes.

After an extensive research work a number of different technologies of protease application in the meat industry were developed. The subject of protease employment in the meat industry in Bulgaria is described on a larger scale by Velinov (1971), Sinha (1981), Velinov (1987) and Danchev (1988).

During the past decade some researchers directed their investigations towards the enzymatic modification of lowfunctional meat like beef hearts aiming at their effective utilization in the production of frankfurters (Brekke et al., 1981) and (Smith et al., 1984). Heart muscles have low waterholding capacity (Wiley et al., 1979) and low emulsifying ability (Forrest et al., 1975). The most common method of enzymatic modification is the partial protein hydrolysis using proteases (Brekke et al., 1981). According to Phillips et al. (1981) enzymatic modification does not violate protein nutritive value; it is non-toxic, specific and is carried out with enzymes that are authorized for food applications.

Other authors (Smith et al., 1984) found out that 30% enzymatically modified beef hearts plus 70% beef skeletal musculature, at low salt concentration, significantly increase frankfurter yields in comparison to control non-modified frankfurters prepared with 2.3% of salt. The same authors (Smith et al., 1985) pointed out that protein solubility and emulsifying capacity of mechanically deboned poultry were improved by partial hydrolysis of myofibrillar proteins using the sour protease "Milezyme" AFP 2000. The purpose of the present study was to establish the effect of the bacterial proteolytic enzyme preparation "Mezenterin 11-11" on the texture and organoleptics of cooked sausages.

MATERIALS AND METHODS: The studies were carried out with the microbial protease "Mexenterin 11-11" whose proteolytic activity was 210 PU per g of enzyme preparation, temperature optimum 55-60°C, and pH 6.5.

The beef hearts were ground through a 2 mm plate and were treated with the enzyme in the following concentrations: Experiment 1 - 0.01%; Experiment 2 - 0.02%; Experiment 3 - 0.03%; Experiment 4 - 0.05%. Thirty percent of the beef hearts thus prepared were added to 70% pork. Each meat batch was finely ground for three minutes. Each experiment received additions of 25% ice and 2.2% salt. Control samples were prepared in the very same way without enzyme treatment of the beef hearts. Sausages weré produced in the traditional way. Samples from the experimental and control specimens were treated in the established way and cut on Ultramicrotom LKB 8800. Meat slices were stained according to Reynolds (1963) and were analysed on an electron microscope "Tesla" BS-613 at 80 kV. The finished product was subjected to organoleptic evaluation by twelve-member taste panel.

RESULTS AND DISCUSSION: The results from the electron mic^r roscopical studies of the control samples are given in Fig.1. The different degree of destruction of myofibrils results fro⁰ the combined action of the grinding process and sodium chlori de.

The electron microscopical picture of frankfurters produced with enzymatically modified beef hearts (Experiment 1) differs significantly (Fig.2).



Fig.1. Electron microscopical picture of longitudinally cut control frankfurter. Magnified 10 000 x.

It can be noticed that due to the action of the microbial protease the myofibrils of the beef hearts have been hydrolyzed to a moderately acceptable degree. The significant disruption of the protofibrils contributes to the increase of th' number of the hydrophilic centres capable of holding water which is organoleptically expressed by the better texture and juiciness of the frankfurters. The partial enzyme hydrolization of the myofibrillar components exerts favourable influence on the formation of fine protein membranes round the fatty globules (Fig.3). Thus, the emulsifying ability of the meat mix is improved, hence the quality of the finished product. Similar is the electron microscopical picture of Experiment 2 frankfurters (Fig.4).



Fig.2. Electron microscopical picture of 0.01% enzymatically modified frankfurter. Magnified 14 000 x.



Fig.3. Formation of protein membranes round the fatty globules. Mafnification 14 000 x.

It can be noticed a very fine net in the lighter areas (Fig.5). The hydrolysis of the myofibrillar components is more expressed that is why they are more finely disintegrated while at certain spots they have dissolved and completely lost their structure. The result is an increased amount of dissolved myo-

fibrillar proteins as well as better emulsifying capacity of the meat emulsion. The higher level of the microbial protease in Experiment 3 increases the amount of the dissolved myofibrillar proteins (Fig.6). Around the smaller fatty globules there can be seen well shaped protein membranes that serve as an indicator for a good emulsifying capacity. It is noticeable that proteins in the zones of connection of myofibrils i.e. on the borderline between the cells that is typical of the heart musculature are relatively not so much affected by the enzymatic action. The further increase of the level of the microbial protease in Experiment 4 contributes to the further stronger dissolution of the myofibrillar proteins resulting in a more spready texture that is uncharacteristic of frankfurters (Fig.7).

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Fig.4. Electron microscopical pictute of a piece of frank furter modified with 0.02% of enzyme. Magnified 14 000 x.

The organoleptic evaluation of frankfurters prepared with different levels of "Mezenterin 11-11" is given in Table 1.

The result obtained indicate that samples from Experiments 1 and 2 received the highest grades. This is due mostly to the better texture and juiciness of the finished products, and partially to their taste and flavour.

Experimental frankfurters NoNo 3 and 4 have soft, spready

texture that is uncharacteristic of this type of sausages and that is due to the more extensive destruction of muscle proteins.



Fig.5. Electron microscopic picture of a piece of frankfurter modified with 0.02% of enzyme. Magnified 19 000 x.



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Fig.6. Electron microscopic picture of a piece of frankfurter modified with 0.03% of enzyme. Modified 10 000 x.



Fig.7. Electron microscopical picture of a piece of frankfurter modified with 0.05% of enzyme. Magnified 14 000 x.

Table 1. Organoleptic evaluation of frankfurters prepared with different levels of the enzyme preparation "Mezenterin 11-11".

Parameter		Туре	of Sample		
	Control	Exp.1	Exp.2	Exp.3	Exp.4
Texture Juicines Flavour Taste Outer	5.92±0.42 s6.21±0.47 6.83±0.45 6.91±0.52	9.27 ⁺ 0.31 8.94 ⁺ 0.29 7.86 ⁺ 0.41 8.42 ⁺ 0.29	8.27 ⁺ 0.42 8.45 ⁺ 0.39 7.75 ⁺ 0.32 7.54 ⁺ 0.29	6.84 [±] 0.34 6.05 [±] 0.28 6.00 [±] 0.41 6.72 [±] 0.31	4.35 ⁺ 0.26 5.26 ⁺ 0.31 6.04 ⁺ 0.27 5.80 ⁺ 0.30
appear. Colour Overall evaluat.	8.75 [±] 0.41 8.25 [±] 0.51 7.15 [±] 0.34	8.95 ⁺ 0.29 9.43 ⁺ 0.32 8.87 ⁺ 0.28	8.35 ⁺ 0.40 8.75 ⁺ 0.35 8.27 ⁺ 0.32	7.40 ⁺ 0.38 8.68 ⁺ 0.40 7.02 ⁺ 0.37	7.60 ⁺ 0.42 7.80 ⁺ 0.24 6.12 ⁺ 0.28

CONCLUSIONS: 1. The enzyme preparation causes destruction of the endomysium, the sarcolemma, the myofibrils, and the sarcoplasm of the muscle fibres.

2. The microstructural changes show that "Mezenterin 11-11" provokes hydrolysis of the muscle components so it can be used

to tenderize tougher meat.

3. The enzymatic modification of beef hearts with "Mezenterin 11-11" is an effective method of improving the technological characteristics of the heart musculature.

4. The most appropriate enzyme levels determined by our studies are 0.01% and 0.02%.

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