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EXPLORING POSSIBILITIES FOR QUALITY IMPROVEMENT OF MEAT RAW MATERIALS FROM CATTLE RUMINANT ANIMALS BY ENZYMATIC TREATMENT

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Abstract: The production of beef meat is a widely spread in Republic of Macedonia, and the beef is very abundant with binding tissue that influences certain hardness and tenacity both to the meat and the final meat product, thus diminishing its organoleptic quality. That demands for seeking adequate methods to improve the consistency and succulence of meat. For that purpose, were used a proteolytic enzyme preparations derived from the bacteria *Streptomyces species 82* which have a high proteolytic activity. In this study was determined increased solubility of myofibril proteins at the meat (*Muscles supraspinatus*) and beef hearts when treated with enzyme preparations. Both *Muscles supraspinatus* and beef hearts showed a general tendency to increase the quantity of free amino acids when an enzyme preparation with activity of 110 PU/kg was added.

Key words: enzyme preparation, *Streptomyces species 82*, *Muscles supraspinatus*, beef heart, free aminoacids, organoleptic quality

Introduction

The big interest in beef meat and beef meat products (very rich in binding tissue) by consumers from some regions in R. Macedonia imposes the necessity of introducing methods for improving organoleptic features (succulence, consistency etc.) in their production technology. Endeavors to improve the organoleptic features of the meat occur far in the ancient times. Indians used some herb leaves to wrap up the meat of wild animals in order to soften it. By that, the proteinase of the herb leaves triggered processes that are analogue to the processes of the meat enzymes *(Kuzelov et al., 2002)*. The speeding up of the meat ripening process in industry is one of the most actual problems of contemporary meat science

(Setandrey and Toldra, 2001; Hughes et al., 2000). That was determined by the fact that processes of meat ripening are the ones that contribute the most to the structural, mechanical, aromatic and nutritional features of meat. Also it increase the market acceptance of the final product from one side, and the emerging necessity for energy optimization of the production on the other side *(Kuzelov et. al., 2007)*

A variety of methods for improving the softness and the organoleptic features of the meat is used today – like tumbling, pressing, high pressure treatment, use of ultrasound, chemical methods, electric stimulation etc. *(Kuzelov et. al, 2002)* The use of enzyme preparation proved to be one of the best methods for treatment of the meat *(Kuzelov et.al. 2009)*. Enzyme preparations from herbal origin (*Papain, Bromelin, Fycin*) and from animal origin (*Trypsin and Chymotrypsin, Pepsin, Pancreatin*) are being used for that purpose (*Ionescy et. al., 2008; Wang, 2001).*Lately, because of their technological and economical advantages, the enzyme preparations from microbial origin (*Rozim A-4, Rozim P-11, P-15, Hydrolasys D, Mezenterin, Oryzine, Terrozine, Flavozine etc.*) are more frequently practiced than the enzyme preparations from herbal and animal origin *(Mandl, 2000).*

Under the influence of the proteolytic enzyme preparations, the binding tissue becomes feebler. The collagen fibers loosen up and loose their ability to be dyed, loose their fibril structure and turn into an amorphous mass. The elastic fibers are also torn to segments thus loosing the ability to get dued. After a while, the segmentation of the fibers reduces and they are thoroughly dissolved remaining in the mass as foreign particles *(Morgan et al., 1993).*

In this research was studied the influence of the microbiological enzyme preparation derived from the bacteria *Streptomyces species 82* over the quantity of free amino acids in the meat raw material from the large ruminant animals.

Materials and Methods

The research was conducted on *Musculus supraspinatus*[1] and beef hearts collected from 10 cattle of East Frisian breed, 15 months old, weighing 280 to 300 kg each. From the collected raw materials we prepared control and test samples. The test samples were injected with 2% Sodium Chloride and three different

[1] Anatomic characteristics of the muscle – an upper back, scapular muscle hatched on *fosse supraspinata* at the scapula. At the beginning it is wide starting from the scapular cartilage and the scapular rim, continues distally towards the scapular neck thickening and splits into two branches embracing the *tuberositos supraglenoidalis* and ends at the big and the small hole of the shoulder's bone. Its function is to stretch out and fixate the shoulder's joint. The muscle is widely used in the meat processing industry for production of various products.

quantities of enzyme preparation - 75, 110 and 230 PU/kg. The control samples were not treated with any enzyme preparation. All samples were packed in polyethylene foil and kept at a temperature between +2ºC and +4ºC.

Proteolysis enzyme was extracted from *Streptomyces species 82*, a bacterium with the following characteristics: pH optimum of 6.0-7.5, proteolytic activity of 300 PU/kg, temperature optimum of 45-50 °C, inactivation temperature of 70 °C. Its inhibition occurs at pH 4.5 if in acid environment, or at pH 9.0, if in alkaline environment. Enzyme preparation is obtained from Microbiological laboratory of the Bulgarian academy of sciences Sofija. .

The meat samples (*Muscles supraspinatus*) had been cut into 50-80 g slices and grouped in three test samples each injected with 75 PU/kg, 110 PU/kg and 230 PU/kg respectively. Apart from that, one sample was left enzyme free (not treated) as a control sample. Both test and control samples were packed in PET foil and kept at temperature of +2°C to +4°C. . The influence of the enzyme preparation on the solubility of the proteins in *Musculus supraspinatus* and beef hearts was measured on the 2^{-nd}, 48^{-th}, 72^{-nd} and 96^{-th} hour.

The amino acid constitution of the meat was determined by ion-exchange chromatographic analysis. Prior to determining the amino acid constitution the sample must be hydrolyzed by acid hydrolysis. In the course of our research we used acid hydrolysis with 6n HCl. The mixture of amino acids was examined by amino analyzer (AAA) produced in Czechoslovakia by the SPN company, type Hd 1200E. The type of appliances determined Theanalytical conditions by the method of external standard *(Specman et al., 1985; Bos et al., 1983).*

The protein solubility was determined by mixing 2 gram of meat emulsion with 8 cm^3 of 0.6 M Sodium Chloride, 0.05 M Potassium Phosphate at pH of 7.0 (23a, 94, 95). 10 cm^3 of the protein suspension were centrifuged at 10,000 spins per minute for 15 minutes with the laboratory centrifuge T-23. To determine the solubility of proteins in the supernatant the Kjeldahl method was used *(Kjeldahl, 1983; Ksenz, 2002)*. Results were statistically elaborated by ANOVA MS Excel program 1997-2003 in accordance to established statistical methods. Determined average values, standard deviation and x- average of the experiment, reliable intervals and presence of statistically important differences using Duncan test *(Statistica v.6 Stat Soft 2003).*

Results and Discussion

The technological and organoleptic characteristics of the meat are dependent of state of the myofibril albumens. That was reason, it was tried to determine the influence of the used microbial proteolytic enzyme preparation on the structural (myofibril) albumens in the muscle tissue. The experiment's results

of solubility variations of myofibril albumens in the enzyme treated and non-treated meat samples are shown on Table 1.

Table 1. Solubility variations of myofibril albumens in Muscles Supraspinatus from large ruminant animals treated with enzyme preparation

Sample type	mgN/kg values after							
	2 hours		48 hours		72 hours		96 hours	
	x	Sd	x	Sd	x	Sd	x	Sd
Control sample	37.20	± 1.08	32.15	± 1.17	28.74	± 1.07	30.18	± 1.14
75 PU/kg of	37.20	± 1.08	35.77	± 1.36	32.10	± 1.15	33.34	± 1.20
110 PU/kg of	37.20	± 1.08	39.72	± 1.36	37.61	± 1.40	37.72	± 1.56
230 PU/kg of	37.20	± 1.08	42.58	± 1.62	38.47	± 1.50	40.59	± 1.87

The results of this study show that the enzyme preparation influences on the solubility of myofibril albumens and it decreases 48 hours post mortem for 13.58% compared to the initial values. The further storage at a temperature of +2+4°C slowly increases the solubility of myofibril albumens.

Unlike the control sample, when the muscle tissue is enzyme treated, the solubility of the myofibril albumins results in a significant raise for the same period of 48 hours *post mortem* compared to the solubility of the control sample values, i.e. 11.26% when treated with 75 PU/kg of enzyme, 23.55% when treated with 110 PU/kg of enzyme and 32.44% when treated with 230 PU/kg of enzyme. 72 hours *post mortem* the values still show significant raise compared to the solubility of the control sample values, i.e. 11.69% when treated with 75 PU/kg of enzyme, 30.86% when treated with 110 PU/kg of enzyme and 33.86% when treated with 230 PU/kg of enzyme, while 96 hours *post mortem* the raise is more moderate, i.e. 10.47% when treated with 75 PU/kg of enzyme, 24.98% when treated with 110 PU/kg of enzyme and 34.49% when treated with 230 PU/kg of enzyme. The enzyme preparation that was used causes structural changes in the albumin complex (*actomyosine*) and showing increase of extracted myofibril albumens compared to the control sample.

Table 2 shows the impact of enzyme treatment on albumens of the heart tissue.

The results of this study show a significant decrease of the solubility of myofibril albumens in the beef heart 48 hours *post mortem* and minor increase of solubility after 72 and 96 hours *post mortem* while the sample is kept on 0°C.

Table 2. Solubility variations of myofibril albumens in heart tissue from large ruminant animals treated with enzyme preparation

Sample type	mgN/kg values after							
	2 hours		48 hours		72 hours		96 hours	
	x	Sd	x	Sd	x	Sd	x	Sd
Control sample	28.31	± 1.12	22.73	± 1.05	24.12	± 1.08	25.87	± 1.04
75 PU/kg of enzyme	28.31	± 1.12	25.85	± 1.08	27.82	± 1.08	30.15	± 1.16
110 PU/kg of enzyme	28.31	± 1.12	31.01	± 1.15	35.64	± 1.26	36.75	± 1.30
230 PU/kg of enzyme	28.31	± 1.12	33.26	± 1.18	35.08	± 1.32	37.42	± 1.38

When an enzyme preparation with lowest activity is used (75 PU/kg) 48 hours *post mortem* the increase of albumen solubility in comparison to the control sample is not so dramatic (13.73%), but it significantly raises when treated with enzyme preparation with higher activity (36.43% for 110 PU/kg and 46.33% for 230 PU/kg). The results evidence the active influence of the enzyme preparation on the albumen complex of the heart muscle tissue of large ruminant animals.
The quantity of free amino acids in Muscles supraspinatus and the beef heart muscle is shown in tables 3 and 4 respectively.

Table 3. Change in quantities of free amino acids in Muscles supraspinatus from large ruminant animals treated with enzyme preparation of 110 PU/kg

Amino-acids	gr./100 gr. of *M. Supraspinatus*			
	without enzyme		with enzyme	
Alanine	1.40	± 0.12	1.54	± 0.14
Arginine	1.27	± 0.40	1.33	± 0.19
Aspartic acid	1.98	± 0.22	2.01	± 0.16
Cysteine	0.24	± 0.04	0.31	± 0.03
Glutamic acid	3.46	± 0.25	3.63	± 0.25
Glycine	1.30	± 0.11	1.44	± 0.14
Histidine	0.70	± 0.15	0.72	± 0.15
Isoleucine	1.05	± 0.13	1.15	± 0.14
Leucine	1.72	± 0.24	1.73	± 0.21
Lysine	1.78	± 0.22	1.86	± 0.19
Methionine	0.53	± 0.16	0.58	± 0.18
Phenylalanine	0.87	± 0.12	0.98	± 0.12
Proline	1.02	± 0.12	1.22	± 0.18
Serine	0.89	± 0.16	1.03	± 0.17
Threonine	0.96	± 0.12	0.98	± 0.17
Tryptophan	0.23	± 0.06	0.32	± 0.12
Tyrosine	0.70	± 0.15	0.81	± 0.10
Valine	1.15	± 0.18	1.28	± 0.12
Total	21.25	± 0.23	22.92	± 0.27

Table 4. Change in quantities of free amino acids in beef heart muscle from large ruminant animals treated with enzyme preparation of 110 PU/kg

Amino-acids	gr./100 gr. of *Heart*			
	without enzyme		with enzyme	
Alanine	1.28	± 0.15	1.43	± 0.13
Arginine	1.20	± 0.17	1.52	± 0.16
Aspartic acid	1.76	± 0.20	1.88	± 0.15
Cysteine	0.25	± 0.03	0.32	± 0.06
Glutamic acid	2.45	± 0.12	2.83	± 0.15
Glycine	2.45	± 0.12	2.83	± 0.15
Histidine	1.21	± 0.17	1.38	± 0.19
Isoleucine	0.72	± 0.10	0.76	± 0.17
Leucine	1.16	± 0.15	1.25	± 0.18
Lysine	1.83	± 0.16	2.14	± 0.20
Methionine	0.51	± 0.10	0.63	± 0.12
Phenylalanine	0.88	± 0.14	0.95	± 0.11
Proline	0.81	± 0.13	1.02	± 0.10
Serine	1.00	± 0.18	1.07	± 0.12
Threonine	0.92	± 0.09	1.13	± 0.16
Tryptophan	0.22	± 0.02	0.31	± 0.08
Tyrosine	0.65	± 0.19	0.93	± 0.13
Valine	1.11	± 0.14	1.22	± 0.12
Total	20.41	± 0.20	23.60	± 0.28

The quantity of free amino acids in the final product is of utmost importance for determining the basic nutritional ingredients of the meat and it is particularly meaningful when meat raw materials with low hydrolyzing characteristics are being used for production of meat goods, which do not thoroughly digest during human consumption. The results of forming and accumulation of free amino acids during the treatment of the beef meat and heart muscle tissue show general tendency to increasing the quantities of the free amino acids when treated with enzyme preparation of 110 PU/kg – an activity that we determined to be the best based on our research.. The exploration of influence of the enzyme derived from bacteria *Streptomyces species 82* over the myofibril proteins solubility are in correlation with the similar explorations of other authors, who conducted the examination of the impact of enzyme preparation of different origin on the properties of meat. *Mandl (2000)* had isolated and studied the collagenase extracted from *Clostridium hystoliticum* described as clostridio-peptase with the same EK 3.4.24.3. as tissue collagenase. The isolated enzyme is capable to hydrolyze the native collagen with physiological pH and temperature by activating the same peptide bonds (X-gly) as the tissue collagenase with the sequence (-Pro-X-Gly-Pro-). *Rabirs et. al. (1986)* had found that the enzyme *Tereto*, derived from *Trihoderma resei* of MC680 type had proteolytic characteristics like the animal proteinase *catepsyn D* and produced solubility of myofibril proteins and softness of

the meat. *Zapelena et.al. (1999)* state that the enzyme proteinase K produced from *Tritirachium album* mushroom hydrolyzes the muscle tissue faster and deeper than the enzymes from animal origin (trypsin and chimotrypsin), thus softening the meat. *Ionescu et al. (2008)* had studied the effect of papaine on myofibril proteins and had determined that the solubility of meat's myofibrillar proteins treated with papaine is 25-30% bigger than the one not treated with papaine. *Ashei et al. (2006)* were explore the effect of papain of beef meat on tenderness of meat and determined that the beef meat treated with papain tenderness is increased by 25-30% compared with beef meat treated with papain.

Table 5. Analysis of variance of the levels of amino acids obtained from the Muscles supraspinatus and beef heart treated with enzyme preparation with activity 110 PU / kg

Source of Variation	*SS*	df	*V*	F	P-value
Between Groups	0.018356	1	0.018356	0.031492	0.860265 *ns
Within Groups	18.65172	32	0.582866		
Total	18.67007	33			

Legends:
SS- Sum of squares
df-Degree of freedom
V- Varianc
***ns-Non significant**

The Table 5 shows no statistical significant difference between the two groups in the amount of amino acids obtained ($p>0.05$).

Conclusion

The above said facts imply the conclusion that under the influence of the enzyme preparation from the *Streptomices species 82* bacteria, the solubility of myofibril albumins in Muscles supraspinatus, as well as in the beef hearts, increases significantly. The enzyme preparation improves the albumen solubility of the heart muscle tissue from large ruminant animals.

The results of these tests are very important because the use of the enzyme preparation obtained from the bacterium *Streptomyces species 82* improves tenderness the meat because of the solubility of proteins and thereby reduce the process of maturing. That will lead to a good economic effects (shorter time of maturation of the meat and lower costs in the process of maturation) in meat industry. Enzyme preparation is gift received from Microbiological laboratory of the Bulgarian academy of sciences Sofia.That would help solve the problem of lack of protein from meat and to improve the quality of meat and meat products.

Ispitivanje mogućnosti za poboljšanje kvaliteta sirovine – mesa goveda tretmanom enzimima

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Rezime

Proizvodnja goveđeg mesa je široko rasprostranjena u Republici Makedoniji, a goveđe meso sadrži puno vezivnog tkiva što utiče na čvrstoću i jačinu sirovine, kao i finalnih proizvoda od mesa, i na taj način smanjuje njegov senzorni/organoleptički kvalitet. To zahteva traženje adekvatnih metoda za poboljšanje konzistencije i sočnosti mesa. U tu svrhu korišćeni su proteolitički enzimatski preparati dobijeni od bakterije *Streptomyces species 82* koje imaju izrazitu proteolitičku aktivnost. U ovom istraživanju je određivana povećana rastvorljivost miofibrilnih proteina u mesu (*Muscles supraspinatus*) i goveđim srcima nakon tretmana enzimataksim preparatima. I mišić *Muscles supraspinatus* i goveđa srca su pokazala generalnu tendenciju povećanja količine slobodnih amino kiselina pri dodavanju enzimskog preparata koji ima kativnost od 110 PU/ kg.

References

ASHEI I., SORENSEN T.L., NILSEN P.M. (2006): Effects of Papain and a Microbial Enzyme on Meat Proteins and Beef Tenderness. Journal of Food Science, 2138-2142

BOS K.D., WERBBEK C., SLUMP P. (1983): Gas-liquid chromatographic method for the analysis of metionine and methionine sulfide in proteins, J. Agric Food Chem., 31, 1, 63-66.

HUGHES M.C., HEALY A., Mc.SVENEY P.L.H. (2000): - EE. onelli proteolytic specificity of cathepsin D on bovine F- aktin, Meat Science 56, 2, 165-172.

IONESCU A., APRODU J., PASCARU G. (2008): Food Technology, New Series Year II (XXXI), 9-16.

KJELDAHL J.C.C.T. (1983): En my Methode til Kvaelstofbestemmelse i organiske Stoffer, Medd. Carsberg lab., 2, ;p. 10-27, Compt. Rend. Traw. Lab. Carsberg, 2, p.1-12, Anal. Chem., 22.

KSENZ M. V. (2002): Izvestija vuzov, Piscevaja tehnologija Nomer 1, 52.

KUZELOV A, KIROVSKA CIGULEVSKA O. (2009): Aplication of a new micro-emnzymatic preparation in processing of canned beef meat products Fleischwirtshaft international, 2, 63-65.

KUZELOV A. TRAJCOVA D., MARKOVA N., BALABANOVA B. (2007): Colagenase Enzyme Influence Upon Structural mechanical properties of meat cans, Univerzity Goce Delcev Stip, Faculty of Agriculture Year Book, 49-56
KUZELOV A., VASILEV K. VELKOVA JORGOVA K. (2002): Influence of microbial enzyme preparation upon structural and mechanical properties of meat raw materials from big ruminants, Food Tasting Industry, 4, 13-14.
MANDL I. (2000): Collagenases and Elastases. Advances in Enzymology, Interscience Publishers, London, p. 123-125.
MORGAN, J.B. T.L. WHEELER, M. KOHMARAIE, J.W. SAVEL J.D. CROUSE,(1993): Meat tenderness and the Calpain Proteolytic System in Longissimus Muscle of Young Bulls and Steers, Journal of Animal Science 71, 6, 1471-1476.
RABIRS F., ALLEU A., WALCEN E.J., COHEN K.S. (1986): Meat tenderization with a proteolytic enzyme from *Trihoderma resei* Patent.
SPECMAN A., STEIN D.W., MORE S. (1985): Automatic residing aparature for use in the chromatography of aino acids, Anal. Chem., 30, 1119-1205.
SETANDREY M.A., TOLDRA F. (2001): Dipeptidyl peptidase IV from porcine skeletal muscle: purification and biochemical properties, Food Chemistry 75.
2, 159-168.
ZAPELENA M.J., DSIATSARAN I., BELLO J. (1999): Dry Fermented sausages made with a protease from *Aspergillus orizae* and/or a starter culture, Meat science, 52, 403.
WANG, F.S. (2001): Lipolyticand proteolytic properties of dry-cured boneless hams ripened in modified atmospheres, Meat Science, 59, 1, 15-22.

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