Volume 24

D 42804 F

Fleischwirtschaft International

Journal for meat production, processing and research

2 / 2009

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Application of a new micro-enzymatic preparation in processing of canned beef meat products

Keywords
- Enzymatic preparation
- *Streptomyces* species 82
- Beef meat

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The production of canned beef meat, rich in connective tissue is widely spread in the Republic of Macedonia. These products are very tough and rigid, with lower organoleptic features. The aim of this investigation was to improve the structure and succulence using characteristic methods. For that reason proteolysis enzymes were applied, which had been extracted from *Streptomycin* species 82, bacteria with characteristic collagen degrading activity. The examination affirmed that the production of canned meat with added collagen degrading enzyme showed higher hydrolytic activity resulting in better succulence and structure of the products. The final products were scored higher in sensory traits because of higher spiciness and better structure.

One of the most important meat processing technologies is the maturation process, i.e. preparing of meat products to be ready for consumption. Due to this process the meat becomes more appealing, with characteristic taste and flavour because of the changes in protein structure through enzyme activity. The proteolysis of the meat results in many changes and modifications of its muscular structure. During the maturation process changes of the muscular components give the meat a better structure.

Meat maturation is a very expensive process because of long cold storage, decreasing mass and increasing evaporation (weight loss). A lot of different methods were used for meat maturation and softening: needle picking, pressuring, ultrasound treatment, meat beating with hammer, chemical methods, electro stimulation, but the most useful method was the treatment with enzyme reagents (5, 7, 8, 9, 15, 17).

Many enzyme applications have been used in the food industry, which contribute to the traditional and the new food quality, for increasing stuff utilisation and intensifying technological processes.

During the last few years authorities from the meat industry have paid special attention to enzyme applications using them for improving meat quality performances. Meat manufacturers used many herbal, animal and microbiological enzymes for quality improvement (3).

Within the last few years, microbiological enzymes have won the first place in meat processing because of some specific technological and financial preferences. Furthermore, these enzymes are more specific in hydrolysing peptide bonds compared to proteolytic enzymes from herbal and animal origin. The first detailed researches are closely connected to the isolation and the study of collagenase induced by *Clostridium histoliticum*, which is described as cloistered peptidase (EC 3.4.24.3) similar to tissue collagenase. The isolated enzyme is able to hydrolyse the native collagen at physiological pH and temperature conditions. It attacks the same peptide bonds (X-Gly) as the tissue collagenase sequence (Pro-X-Gly-Pro-).

The proteolytic preparation Pronase – a commercially available mixture of proteinases isolated from the extracellular fluid of *Streptomyces griseus* – is isolated from bacteria that can decompose diagonal bonds of the tropo-collagen molecules by releasing monomeric chains from the spiral chain. Its activity extends to both denatured and native proteins leading to complete or nearly complete digestion into individual amino acids. The investigations of hydrolytic activity of *Pseudomonas* confirm that it produces enzymes that attack the native collagen and elastin as well as their hydrolysed forms of gelatine and peptide. The microbial collagenases are usually synthesised in a complex of other enzymes thus thoroughly impacting the various components of connective tissues, membrane structures and nutritional compounds. That explains the presence of elastins, specific peptides, hyaluronidases and phospholipases in the various enzyme preparations with collagenous influence. All enzyme reagents do not have essential proteolysis activity and do not achieve the effect necessary for meat processing. Some of them did catalyse muscle proteins; however, they did not have any effect on connective tissue proteins, responsible for meat toughness. For optimal activity different enzymes require an acidic or alkaline pH value, which is far from the normal pH of meat.

The canned meat producing industry tended to exploit meat rich in connective tissue. The most usual method to accelerate meat softening is by adding collagen degrading proteolytic enzymes (10, 11, 14).

The main target of this research project was to investigate special enzymes of microbial origin and to determine their activity concerning collagen degradation in order to apply them with canned meat processing technology. The experiments were especially related to proteolytic changes within this connective tissue containing meat in order to improve the quality of the final products.

Material and methods

With this study a new micro-enzymatic preparation of *Streptomyces* species 82 was used that has a very high collagen degrading activity. By using this enzymatic preparation the hydrolytic process was accelerated. The processing was carried out according to the principles of good manufacturing practice (GMP). The meat used for research was derived from cattle of East Frisian breed, which were 15 months old and weighted 280 to 300 kg. All primary processing had been carried out under best sanitary and hygienic conditions (GHP). After slaughtering and dressing the meat was stored in cold chambers at +4 °C. The processing meat was classified due to the technological specification for beef meat corresponding to the Macedonia legislation. In this case raw beef meat of category 2 was used.

The proteolytic enzyme applied during this study was added with 75, 110 and 230 phytase units (PU)/kg. The control sample was produced without added enzyme. The recipes for the control and treatments 1 to 3 of the canned meat products – beef meat in stew and beef luncheon meat – are shown in Table 1.

Beef luncheon meat was produced according to the following procedure: Meat dough was produced from chilled beef meat finely minced and mixed in a cutter. During the cutting process ingredients, additives and ice were added. Beef hearts and fat were minced to an aver-

Received: 10 March 2006 | reviewed: 17 March 2006 | revised: 20 July 2006 | accepted: 8 December 2008

age particle size of 18 mm using a meat grinder.

Thereafter all the components were blended while adding the enzyme preparation. The mixture was then stored for maturation in a chilling room at 0 °C to +4 °C for a period of 48 h. After these two days the meat batter was filled into appropriate cans. The filled, sealed and washed cans were sterilised and then transported to the department for labelling and packing.

The technological procedure for production of beef meat in stew was as follows. The second grade beef meat was minced with a meat grinder to an average granulation of 24 mm. Subsequently, salt, gelatine, spices and the enzyme preparation were added. After blending all components the product was stored in a chilling room at 0 °C to +4 °C for a period of 48 h. Afterwards, it was filled into the cans. The filled cans were sealed and sterilised and then transported to the department for labelling and packing.

Sterilisation formula:

$$\frac{T_h + T_s + T_c}{S} = \frac{15\,min + 45\,min + 15\,min}{120°C}$$

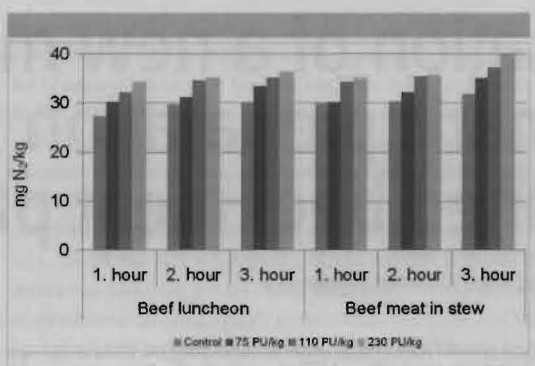
T_h – Time for warming up of the autoclave, T_s – Time for sterilising of the product, T_c – Time of cooling and S – Temperature of sterilisation

Characteristics of proteolysis preparation:

- pH optimum 6 to 7.5
- Proteolytic activity 300 PU/g enzyme
- Temperature optimum 45 to 50 °C
- Inhibiting conditions: pH 4.5 and pH 9

To determine alpha-amine nitrogen and the hydrolysis level of the proteins 500 g of the sample were homogenised and 1000 cm³ of distilled water were added. The mixture was stirred periodically over a period of 30 min. The quantity of free α-amine nitrogen in the diluted sample was determined by inhydrin method. The hydrolysis level of proteins was obtained after adding the quantity of free amine nitrogen to the overall quantity of nitrogen in the reference sample determined by the Kjeldahl method.

The dilution of proteins was determined by mixing 2 g of meat emulsion from the stuffing with 8 cm³ of 0.6 M NaCl, 0.005 M potassium phosphate, pH=0.7. 10 cm³ of the protein suspension were centrifuged at 10,000 spins per minute for 15 min with the laboratory centrifuge T23. The Kjeldahl method was used to determine the dilution of proteins in the supernatant and the result was added to the overall content of proteins in the meat batter. The extra cellular enzyme with elastin and collagen hydrolysing features was extracted from *Streptomyces* species 82 at the Microbiological laboratory of the Bulgarian Academy of Science.



Source: Kuzelov and Kirovska Cigulevska Fleischwirtschaft International 2/2009

Fig.: Increase of soluble proteins in Beef luncheon meat and Beef meat in stew

For the evaluation of the sensory characteristics of the products the indicators surface appearance, colour, consistency, aroma, taste, succulence and general estimate were used. The sensory characteristics of the samples were described by 9-point-scale developed by VNIIMP, Moscow (All-Union Research Institute for the Meat Industry).

The results were mathematically and statistically elaborated by MS Excel programme in accordance to established statistical methods. Average values, standard deviation and varying coefficient of the experiment, reliable intervals and presence of statistically important differences were determined by the method of Duncan and Newman-Keuls.

Results and discussion

One of the opportunities to evaluate the possible time of impact of the enzyme preparation over the meat raw materials is to determine the level of hydrolysis. That is of essential importance since there is a limited chance of keeping the filled cans for too long before their thermal treatment. That's why measurements had been conducted the first, the second and the third hour after the filling of the cans before sterilisation. The results are presented in Tables 2 and 3. The results show increase of the hydrolysis level after 2 hours of implementing the enzyme preparation, and even more dramatic increase was obtained after the third hour with both types of cans.

The results that were derived from investigating the influence of the enzyme preparation over the solubility of myofibrillar proteins in the stuffing of cans filled with beef meat luncheon, and beef meat in stew are shown in the Figure. It shows that the level of solubility of the myofibrillar proteins increased significantly as a result of their partial hydrolysis under the influence of the enzyme preparation with an activity of 230 PU/kg and prolonged impact of 2 to 3 hours. The sampled cans with beef luncheon meat showed an increased share of soluble proteins with 10.97%, 13.94% and 16.02% after 1, 2 and 3

Tab. 1: Recipes for the manufacture of canned products

Raw materials and additives (kg)	Control	Treatment 1	Treatment 2	Treatment 3
Beef meat in stew				
Beef meat, category II	100	100	100	100
Gelatine	2.0	2.0	2.0	2.0
Nitrite curing salt	1.1	1.1	1.1	1.1
Onion	0.3	0.3	0.3	0.3
Red pepper	0.1	0.1	0.1	0.1
Enzymatic preparation (PU/kg)	–	75	110	230
Beef luncheon meat				
Beef meat, category II	45	45	45	45
Beef hearts	20	20	20	20
Beef fat	26	26	26	26
Starch	4	4	4	4
Nitrite curing salt	2.2	2.2	2.2	2.2
Emulsifier	2	2	2	2
Enzyme reagent	–	75	100	230
Phosphate	0.3	0.3	0.3	0.3

Source: Kuzelov and Kirovska Cigulevska Fleischwirtschaft International 2/2009

Tab. 2: α-amine nitrogen values and hydrolysis rate in canned beef luncheon meat

Sample	1 h after filling	2 h after filling	3 h after filling
	α-amine nitrogen in mg/kg		
Control	142.53 ± 1.97	155 ± 2.03	176.14± 2.10
75 PU/kg	283.16 ± 3.81	411.22 ± 3.06	624.79 ± 2.95
110 PU/kg	355.18 ± 2.45	516.73 ± 2.90	784.40 ± 4.98
230 PU/kg	715.23 ± 4.50	971.1 ± 6.12	1320.50 ± 8.02
	Hydrolysis rate in %		
Control	0.72 ± 0.09	0.80 ± 0.11	0.94 ± 0.12
75 PU/kg	1.31 ± 0.28	2.26 ± 0.51	3.35 ± 0.26
110 PU/kg	1.64 ± 0.12	2.83 ± 0.34	4.15 ± 0.49
230 PU/kg	3.90 ± 0.40	4.70 ± 0.45	6.11 ± 0.51

Source: Kuzelov and Kirovska Cigulevska Fleischwirtschaft International 2/2009

Tab. 3: Quantity of α-amine nitrogen and hydrolysis rate of proteins in canned beef meat in stew in dependence on enzymatic activity

Sample	α-amine nitrogen in mg/kg		
	1 h after filling	2 h after filling	3 h after filling
Control	156.15 ± 1.80	170.23 ± 2	182.4 ± 2.09
75 PU/kg	310.18 ± 3.11	456.23 ± 3.72	643.25 ± 3.08
110 PU/kg	389.5 ± 2.4	571.15 ± 2.89	810.26 ± 4.9
230 PU/kg	763.2 ± 4.4	988.36 ± 6.2	1450.0 ± 8.12
	Hydrolysis rate in %		
Control	0.82 ± 0.1	0.91 ± 0.11	0.98 ± 0.12
75 PU/kg	1.39 ± 0.32	2.3 ± 0.31	3.42 ± 0.37
110 PU/kg	1.74 ± 0.12	2.9 ± 0.33	4.28 ± 0.5
230 PU/kg	3.95 ± 0.41	4.68 ± 0.44	6.22 ± 0.61

Source: Kuzelov and Kirovska Cigulevska — Fleischwirtschaft International 2/2009

Tab. 4: Sensory evaluation of canned beef

Indicators (scores 1 to 9)	Control	Treatment 1	Treatment 2	Treatment 3
Luncheon meat				
Consistency	6.24 ± 0.38	7.11 ± 0.26	8.45 ± 0.39	6.92 ± 0.41
Succulence	6.82 ± 0.41	7.20 ± 0.27	8.26 ± 0.41	6.63 ± 0.35
Aroma	7.10 ± 0.36	7.23 ± 0.27	7.87 ± 0.29	6.92 ± 0.29
Taste	6.73 ± 0.29	6.92 ± 0.31	7.29 ± 0.32	6.82 ± 0.31
Colour	7.65 ± 0.32	7.72 ± 0.23	7.78 ± 0.28	7.53 ± 0.27
General estimate	7.85 ± 0.30	7.93 ± 0.27	8.10 ± 0.37	7.65 ± 0.28
Meat in stew				
Consistency	6.75 ± 0.37	7.64 ± 0.31	8.62 ± 0.39	7.34 ± 0.35
Softness	6.08 ± 0.36	7.56 ± 0.37	8.47 ± 0.38	7.28 ± 0.37
Aroma	7.49 ± 0.39	7.51 ± 0.28	7.65 ± 0.34	7.80 ± 0.38
Taste	7.15 ± 0.35	7.32 ± 0.33	7.38 ± 0.30	7.40 ± 0.32
Colour	7.83 ± 0.40	7.92 ± 0.37	7.96 ± 0.37	7.76 ± 0.34
General estimate	7.94 ± 0.36	7.98 ± 0.29	8.30 ± 0.35	7.70 ± 0.30

Source: Kuzelov and Kirovska Cigulevska — Fleischwirtschaft International 2/2009

hours due the usage of an enzyme activity of 110 PU/kg compared to the control samples without enzyme. Increasing the proteolytic activity of the enzyme preparation to about 230 PU/kg the results were 4.97%, 16.67% and 21.94%, respectively. The quantity of soluble proteins in the cans with beef stew measured 1, 2 and 3 hours after adding the enzyme of 110 PU/kg increased to 12.22%, 15.41%, 16.39%, while after adding the enzyme preparation with an activity of 230 PU/kg the increase measured 17.73%, 16.67% and 21.68% compared to the control.

The sensory evaluation is very important for food products in general. The results of the sensory tests (Tab. 4) show statistically significant differences between the test and control samples regarding the indicators of consistency, succulence, aroma, taste and general estimate. The indicator of coexistence that is a summarised characteristic of all mechanical traits (structure, plasticity, tenderness etc.) also shows differences between the test and the control samples.

Significantly higher scores were given to the samples produced with the enzyme preparation of 110 PU/kg compared to the control samples and those with an enzyme activity of 230 PU/kg. The samples with a higher activity of enzymes showed unacceptably soft coexistence, while those without enzyme showed explicit hardness. The succulence of the final product is higher with canned meat products treated with an enzyme preparation of 110 PU/kg. Enzyme preparations with higher activity made the product significantly softer, while their absence resulted in a final product with dry consistency. The test and control samples also differed with minor degree in taste and aroma. Taste and aroma were evaluated better for products treated with the enzyme preparation of 110 PU/kg. Only the indicator of the cut-off surface colour showed no significant differences between the test and the control samples.

The complex sensory assessment showed that coexistence, aroma and taste were the most important for the quality level; in minor degree was the succulence.

The results of the sensory analysis show that the used enzyme preparation significantly improved the quality of the canned meat produced with meat from large ruminant animals rich in connective tissue (with low functional characteristics). Some articles show that meat samples treated with proteolysis enzyme have had more soluble myofibrillar proteins and more fractions with higher electrophoresis activity. The main reason for that are changes in protein structure, solubility in concentrated solutions (for dissociation of actomyosin in actin and myosin: 3, 11, 17).

Conclusions

- Collagen enzymatic preparation increased and improved the proteolytic hydrolysis in canned beef meat.
- Added enzyme resulted in increased hydrolysis and better solubility of proteins thus inducing better succulence of the final product.
- Better complex index and better effects were shown with beef meat products treated with 110 PU/kg enzyme.
- Enzyme treated samples showed better sensory characteristics than control samples (softness, aroma and taste).

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