# EFFECT OF PAPAIN AND BROMELIN ON MUSCLE AND COLLAGEN PROTEINS IN BEEF MEAT

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Received 28 May - Accepted 9 July

**Abstract**

*The effects of papain and bromelin on muscles and collagen proteins in beef meat were evaluated by injecting brine supplied with enzymes in different concentrations. The effects produced by papain and bromelin, together with the endogenous enzymes of meat, were established by the determination of nitrogenous compounds resulted through the degradation of meat proteins and of hydroxyproline produced from collagen during enzymatic tenderization and thermal treatment of the meat. Papain and bromelin led to a limited hydrolysis of beef meat proteins, to a loss of physical integrity of muscle and connective tissue, accompanied by a high solubility of structural proteins, and to an improvement of the beef meat tenderness. The tenderization effects of papain and bromelin were monitorized, both on the raw meat during ageing at 4°C for 24 and 48 hours and on the cooked meat.*

**Keywords**: papain, bromelin, myofibrillar proteins, collagen, rigidity index, tenderness

## 1. Introduction

Tenderness is one of most important meat texture attributes which affects the perception of beef meat, by the customers (Brooks et al., 2000; Morgan et al., 1991). Tenderness depends on the structural integrity of the myofibrils and of connective tissue which surrounds the muscle fibers and on their properties (Bailey, 1972; Koohmaraie et al., 1988; Koohmaraie, 1994; Nishimura et al., 1996a, 1996b; Penny, 1980). Collagen plays an important role in the meat texture. The contribution of connective tissue to the secondary toughness of meat is dependent on the quantity, type and intermolecular cross-links of collagen which is the main component of connective tissue (Bailey and Light, 1989; Bertran et al., 1992; Light et al., 1985). By formation of the cross-links between the collagen molecules, the meat of old animals becomes harder. In order to obtain tender meat at industrial level, it proceeds either to the slaughter of young animals, or to the storage of meats for ageing at low temperatures (0-4°C). During the ageing it takes place a limited process of proteolysis which leads to ultrastructural changes in skeletal muscle and to the improvement of meat tenderness (Koohmaraie, 1992a, 1992b). In the USA, beef meat is classified into groups, based on different levels of palatability. Between the marbling and the palatability of beef meat, there is a direct correlation: meats with a high level of marbling are juicier and tenderer. In the last years different tenderization techniques of sheep and beef meat were applied. These techniques include mechanical tenderization, storage at high temperatures, injection with calcium chloride, electrical stimulation, ultrasonation, high pressure treatment and enzymatic tenderization (Cheftel and Culioli, 1997; Koohmaraie et al., 1988; Koohmaraie, 1992a, 1992b). The enzymes of vegetable origin, such as papain, bromelin and ficin and bacterial collagenase (Foegeding and Larick, 1986; Kang and Rice, 1970; Stanton and Light, 1987) were often used for postmortem meat tenderization, without establishing exactly, which enzyme is more efficient. These enzymes have a large spectrum of action being involved in degradation of the main proteins of myofibrillar muscles (myofibrillar proteins, collagen), and sometimes lead to over – tenderization and to a product with a pasty texture (Miller, 1995). Nowadays there is not clear evidence which refers to collagen degradation during post-

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mortem ageing of beef meat, but only to the structural weakening of the endomysium and perimysium during the ageing for long periods of time (Nishimura et al., 1998; Takahashi, 1996). Phillips et al. (2000) tried to establish the way in which the disruption of connective tissue matrix contributes to the improvement of tenderness.

The present study aimed at the evaluating papain and bromelin effects on the beef meat proteins, at determining the optimal quantity and time of enzyme action and at estimating of the effect of thermal and enzymatic treatment on the beef meat tenderization.

## 2. Materials and Methods

### 2.1. Materials

The commercially farmed beef of approximately 24 months of age were taken from a single commercial producer and slaughtered at an EU approved abattoir using standard international procedures.

The salt, tripolyphosphate and sugar used for the study are food additives which are currently used in meat industry.

We tested the following enzymes: *bromelin* [EC 3.4.4.24] and *papain* [EC 3.4.4.10], purchased from Difco, N.F.VIII (Difco Laboratories, Detroit, Michigan).

### 2.2. Chemical analysis

*Moisture content* was determined by drying the sample for 24 hours at 100$^{o}$C, according to method AOAC, 1995.

*Total nitrogen content* was determined according to the method AOAC, 1995. The *content of global proteins* was established by multiplying the percentage of the total nitrogen content by 6.25 proteins.

*Fat content* was determined according to the method AOAC, 1984 which is based on the extraction of free fat substances from analyzed sample with ethylic ether.

*The measurement of the pH* was made according to the procedure AOAC, 1984; 10g of sample were homogenized for 2 minutes with 90 ml of distillated water using a laboratory blender; the meat suspension was filtrated and the pH was determined with a pH- meter digital Hanna.

### 2.3. Level of hydrolysis

The partial hydrolysis level of the beef muscle proteins by the exogenous enzymes was appreciated by determination of the following nitrogen fractions:

*Non-protein nitrogen* - The fraction of non-protein nitrogen was determined after protein precipitation with trichloroacetic acid and removing it by filtration, according to the method AOAC, 1990. The filtrate free of proteins was mineralized, with concentrated sulfuric acid in presence of catalysts; the content of nitrogen was determined from the mineralized sample after dilution and alkalization by the micro-Kjeldahl method.

*Aminic nitrogen* - The free amino acids, expressed as tyrosine, were dosed from the extract free of proteins and polypeptides insoluble in trichloroacetic acid solution, using the colorimetric method for the determination of the amino acids.

*Soluble proteins,* from the juice expressed during the boiling of the enzymatic treated meat, were determined through the colorimetric method of Lowry, modified by Miller. Soluble proteins were expressed as bovine serum albumin.

*Solubilization of collagen* - The solubilization of collagen was measured by determining the free hydroxyproline from the juice which was eliminated at the boiling of the enzymatic treated samples, according to the method Standard ISO 3496/1997.

*Tenderization level* was appreciated by determination of the rigidity index. Compression method is based on recording sample deformation when a force is applied perpendicular to muscle fibers (Voisey, 1976). Rigidity index (*Ir*) was estimated through the relation:

$$Ir=a/g \ [cm^2/g]$$

where: $a$ = the surface occupied by meat after pressing it with a weight of 1kg for 10 minutes, $cm^2$, while $g$ = the mass of meat to be pressed, g.

### 2.4. Preparation of samples

The beef ham (24 hours after slaughter) was chosen from fats and connective tissues and was divided in equal pieces in length and thickness, having the approximate weigh of 100 g. The cutting of pieces was made along the muscle fibers. The pieces of meat were divided into 4 groups; each group, having at least 5 pieces, was treated in a different way:

The blank sample (M, control) - the pieces of meat were injected with 10% brine, without adding enzymes;

Sample A – the brine used for the injection was supplied with enzyme. The meat was injected with 10% brine with 10 mg enzyme.

Sample B - the level of the injected enzyme was 15 mg/100g meat;

Sample C - the level of the injected enzyme was 20 mg/100g meat.

The brine used for injection was made up of: salt 10g, sugar 10g, tripolyphosphate 2.5 g and water 86.5 g.

The injection was made manually, through an injector with a single needle, so that the entire quantity of brine could be pumped in muscle mass. The eliminated brine was re-injected. The meat injected was packed and stored at the temperature of 4$^{o}$C, for 24, 48 and 72 hours, to allow the diffusion of the brine with or without added enzymes into muscle tissue, and the ageing of the meat by the action of exogenous and endogenous enzymes on their substrate.

After the ageing, each sample was placed in very well closed experimental tubes and was boiled in water bath by increasing the temperature with about 1$^{o}$C/min. until the sample achieved 70$^{o}$C on the thermal center; this temperature was maintained for 10 minutes. After boiling, the samples were made to become cold immediately in water bath with ice; afterwards they were kept in refrigeration conditions for 12 hours. The boiled samples were brought at room temperature, were taken out carefully from the experimental tubes, and after removing the excess of juice from the surface using filter paper, they were weighed. The juice resulted during the boiling was collected, weighed and used for different analysis. The percentage of expressed juice during thermal treatment was calculated with the relation:

*% Expressed juice = [Mass of the meat after ageing - Mass of the meat after boiling and cooling] ·100/Mass of meat after ageing*

### 2.5. Statistical analysis

Five experimental batches were realized for each kind of treatment. Statistical analysis, which consist in evaluating the mean values, standard error and standard deviation with the framing into the confidence interval of 95%, was performed using Sigma Plot 2001/Statistics Data software. Experimental data were fitted using Table Curve 2D software and the regression equations were established based on statistical criteria ($r^2$, Fit Standard Error or F Statistic).

## 3. Results and Discussion

### 3.1. The composition of meat

The experiments were made at the laboratory level using the ham of adult beef. In table l, are reported data referring to the chemical composition of adult beef meat. Analyzing these values it can be seen that the meat was well chosen according to fats (5.52%+1.24% fats) and has a mean content of water of 75.8+1.3% and protein of 17.81+1.02%.

***Table 1***. Chemical composition of the beef meat

| Chemical components | Content | |
|---|---|---|
| | g% | g% d.s. |
| Moisture | 75.8±1.3 | - |
| Dry substance | 24.0±1.3 | - |
| Total nitrogen | 2.85 | 11.875 |
| Total Proteins | 17.81 | 74.21 |
| Fats | 5.52 | 23.00 |
| Non-protein nitrogen | 0.202 | 0.842 |
| Aminic nitrogen | 0.068 | 0.283 |
| Ammonia | 0.018 | - |

### 3.2. The influence of exogenous enzymes on protein hydrolysis

The proteolytic activity of papain and bromelin on the adult beef meat proteins was estimated by monitoring the variation of non protein and aminic nitrogen levels during ageing.

Both papain and bromelin led to a limited hydrolysis of beef meat proteins. The level of hydrolysis was more accentuated when using higher levels of papain and bromelin and longer enzyme action times. The lowest level of hydrolysis was noticed at the samples that were injected with brine without enzymes, in which case the changes regarding the contents of non protein nitrogen and free amino acids were determined only by the endogenous proteolytic enzymes of the muscle and those synthesized by the psychrophilic microflora of the natural contamination of meat.

The evolution of non protein nitrogen was similar to all experimental lots for the entire process of ageing. The levels of non protein nitrogen increased

continuously, varying in accordance with the applied treatment (Figure (1) and Figure (2)).

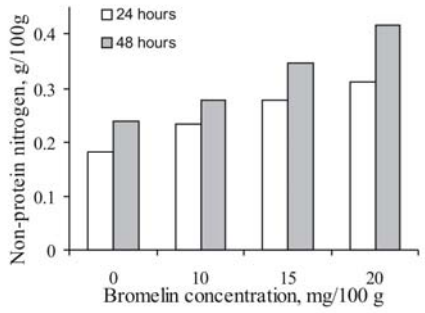


***Figure 1.*** The influence of bromelin on non protein nitrogen accumulations

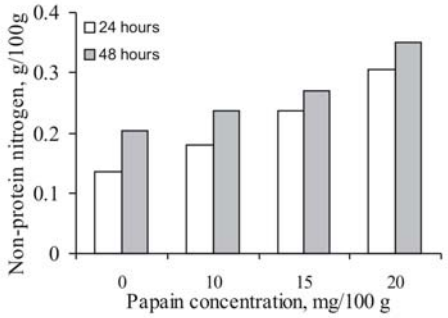


***Figure 2.*** The influence of papain on non protein nitrogen accumulations

The highest nitrogen accumulations were recorded for tenderized samples with bromelin, and we can appreciate the better specificity of bromelin for the proteins of beef meat.

The values presented in Figure (3) show that the levels of free amino acids, as a main component of non protein nitrogen, increased proportionally to the enzyme levels.

For both enzymes the increase of free amino acid levels was relatively small, due to the fact that the used enzymes are endopeptidases that cleave the polypeptides chains into fragments with high molecular mass. Papain, compared to bromelin, acted more intensively on the polypeptide fractions of the beef muscle and led to higher accumulations of free amino acids. Papain is a cystein proteinase that contains a thiol group Cys 27 in the catalytic site acting like a nucleophilic group. It acts upon the peptide bonds between Arg, Lys, and Gly residues, but not on the bonds between residues of amino acids with acid character, which are major in the myosin molecule (42%). Papain has a different action on the structural tissue proteins: it hydrolyses less the actomyosin, a protein found in muscular tissue after the installation of muscular rigidity, than myosin. Myofibrillar proteins are faster hydrolyzed by papain when they are in a denaturated state (Rattrie and Regenstein, 2000).

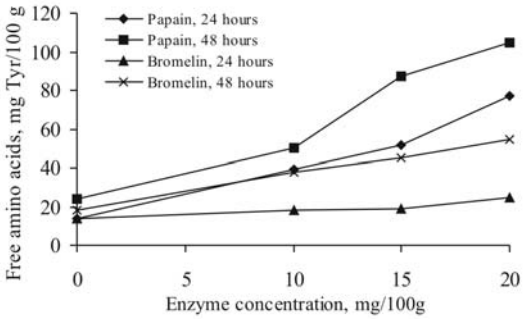


***Figure 3.*** The influence of papain and bromelin concentration on the free amino acid levels

The aminic nitrogen represents only one fraction of the non protein nitrogen, the rest being composed of peptides with smaller molecular mass. The increase of non protein fractions improves both the beef meat tenderization and the assimilation degree of nitrogenous compounds from the beef meat.

The regression curves showed that there is a non linear dependence between the level of enzyme added and the accumulation of low molecular weight nitrogenous compounds as result of hydrolysis activity of papain and bromelin, soluble in trichloroacetic acid (non protein nitrogen, free amino acids)

Generally in complex system of meat and particularly in our experimental conditions, it is not excluded the participation of other factors, such as the intensity and uniformity of the thermal treatment, meat piece structure and thickness, which can compete to the liberation of soluble low molecular weight nitrogen compounds. Values over 0.9 of the correlation coefficients indicate that the accumulations of non protein nitrogen and free amino acids are well correlated with the enzymes level that was added and with the time of action, in case of both bromelin and papain.

### 3.3. The influence of exogenous enzymes on the soluble proteins

The influence of the exogenous proteolytic enzymes on the beef meat was also evaluated by determining

the solubility of the protein in the expressible juice after the thermal treatment.

The results of our study showed that proteolysis altered the properties of beef meat proteins, by increasing its solubility. The losses of proteins in expressible juice at the thermal treatment were significantly higher in case of samples treated with enzymes, compared to control samples, without enzymes (Figure 4). The increase of enzyme level and of the enzyme action time caused a significant increase of the protein losses in the expressible juice at the boiling. The breaking of polypeptide chains, the weakening of collagen network and thermal denaturation of the proteins, are, in our opinion, the principal factors that contributed to the elimination of water and some soluble substance from the meat.

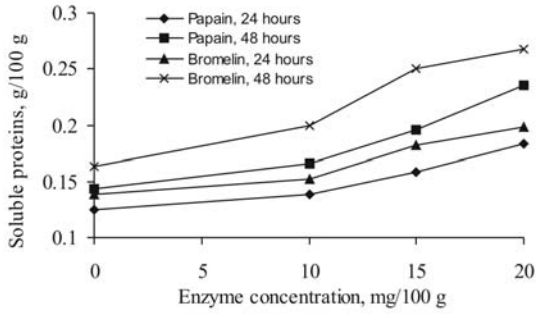


***Figure 4.*** The influence of the papain and bromelin levels on the soluble protein losses

The highest solubility of proteins, in the case of the treatment with both bromelin and papain, was recorded for the highest tested level of enzymes (20 mg/100g) and the longest time of ageing (48 hours). The solubility of enzymatically treated meat proteins, increased differentially after boiling, being 9 times and 5.5 times higher than the one of the blank sample, in case of bromelin and papain, respectively. For all studied cases the highest correlation coefficients ($r^2$>0.92) related to the relationship between the added enzyme level and the contents of soluble proteins in expressible juice after boiling was obtained by modelling the experimental data through a non linear mathematic equation.

### 3.4. The influence of exogenous enzymes on the protein connective tissue

Bromelin and papain also demonstrate collagenase activity by solubilisation of collagen, the principal constituent of connective tissue which enters muscle organization. The collagen solubilisation was estimated through the hydroxyproline accumulations that varied with the applied treatment, by varying the bromelin or papain levels and the tenderization time. Compared to the enzymatically treated meat, the level of hydroxyproline from the blank sample was significantly lower, and was due to the (i) free hydroxyproline normally present in meat, (ii) hydroxyproline obtained as a consequence of endogenous collagenase action and (iii) the one taken out during the thermal treatment applied to the meat.

The action of bromelin was more intensive upon the connective tissue, compared to papain, leading to a more advanced solubilisation of the collagen (Figure 5). It is believed that papain affects the mucoproteins and the collagen of the connective tissue of the meat, more likely than other structural proteins. Under papain action, the collagen suspensions are transformed into a compact gel. These observations suggested the fact that the tenderization effect of the papain may be mostly due to the degradation of connective tissue, which is one of the four principal factors involved in the tenderization of meat, together with the shortness of the muscle, the ageing of meat under the action of endogenous enzymes and the marbling degree of meat (Miller et al., 1995).

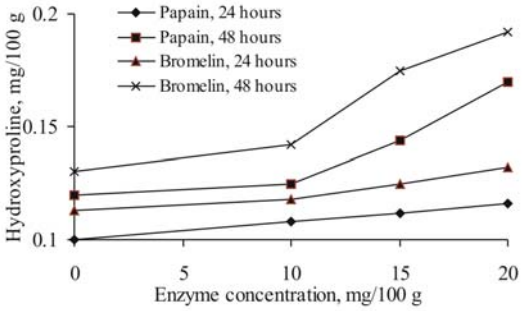


***Figure 5.*** The influence of bromelin and papain levels on hydroxyproline contents

It is supposed that proteases act on the proteoglycans which link the fibrillar collagen II and on the collagens which act as interfibrillar bridges. Nowadays experimental studies are focused on finding innovative methods to disrupt the connective tissue matrix, in order to improve meat tenderization (Phillips et al., 2000).

The collagenase activity of bromelin was about twice higher than papain activity. According to Ashie et al. (2002), the bromelin exhibits a more

accentuated hydrolytic action on collagen than on myofibrillar proteins.

The effect of bromelin or papain on the connective tissue, which covers muscle fibers and fiber bundles, was also noticed by performing a sensorial analysis on the boiled meat. Our results agree with the observations of other research groups who tendered the beef meat in marinade using level of proteolytic enzymes higher than 0.01 UA/100 g meats.

### 3.5. The tenderization degree and the losses at the thermal treatment

The influence of bromelin and papain on the tenderization degree of the beef meat was estimated by establishing the rigidity index, which is a measure of the resistance opposed by the meat to the compression test. This parameter was evaluated for both raw meat and thermal treated one and we could note a significant increase of the rigidity index value by increasing the enzyme levels and their action time on the substrate (Figure 6, 7 and 8), indicating continuous weakening of meat structure during the enzymatic ageing.

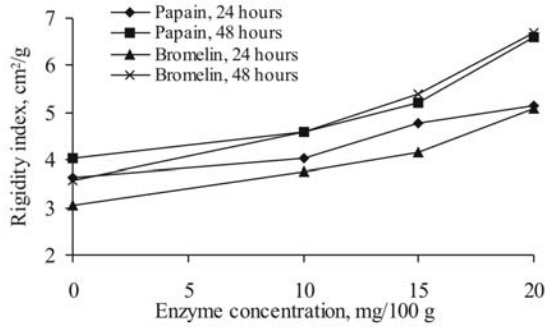


***Figure 6.*** The variation of the rigidity index depending on the added enzyme level and ageing time (boiled meat)

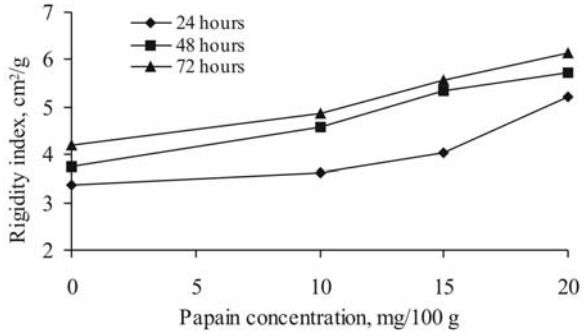


***Figure 7.*** The variation of the rigidity index depending on the added papain level and ageing time (raw meat)

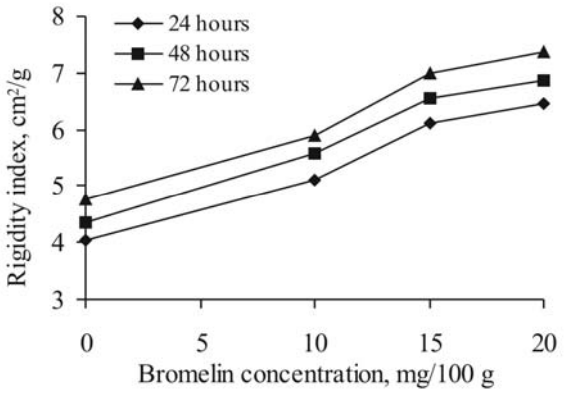


**Figure 8.** The variation of the rigidity index depending on the added bromelin level and ageing time (raw meat)

The rigidity index increased exponentially with the added enzyme levels and the correlation coefficients were higher than 0.9, for all studied cases.

The rigidity index increased exponentially with the added enzyme levels and the correlation coefficients were higher than 0.9, for all studied cases.

The enzymatic ageing of beef meat with bromelin or papain for 24 and 48 hours, led to the improvement of the hydrophilic characteristics of meat proteins and to the decrease of juice losses at thermal treatment compared to the samples without exogenous enzymes (table 2). By increasing the levels of added enzyme and of the time of ageing, the juice losses at thermal treatment significantly increased.

***Table 2.*** The losses at thermal treatment

| Enzyme, mg/100g | Juice losses, % | |
|---|---|---|
| | Meat treated with bromelin | |
| | Ageing period at 4°C, hours | |
| | 24 | 48 |
| 0 | 30.81±1.25 | 34.36±1.32 |
| 10 | 20.88±0.98 | 33.44±1.20 |
| 15 | 16.25±0.82 | 28.43±1.08 |
| 20 | 17.24±0.78 | 32.37±1.06 |
| | Meat treated with papain | |
| 0 | 32.71±1.16 | 36.77±1.08 |
| 10 | 30.53±1.22 | 34.45±1.21 |
| 15 | 28.77±0.98 | 32.69±1.10 |
| 20 | 30.30±1.12 | 34.22±1.20 |

A better tenderization was obtained for samples treated with bromelin compared to those treated with papain and to the blank sample. Due to the marked

structure weakening of the enzymatically treated meat, one should consider the substantial decrease of boiling time in order to avoid the pasty texture.

## 4. Conclusions

The development of adult beef meat texture is a complex process, which in our experiment, was based on the weakening of structural elements of the muscle, by using the exogenous proteolytic enzymes such as papain and bromelin.

The main effect of papain and bromelin on the beef meat consisted in limiting proteolysis of the proteins, which was emphasized through the dynamics of non protein nitrogen, free amino acid and soluble protein levels of the juice resulted at the boiling of enzymatically treated meat with different levels of enzymes and for different periods of time.

Papain and bromelin showed hydrolytic activity on the connective tissue, leading to a better tenderization of the adult beef meat. From the analytical point of view, this fact was counted through determination of the soluble collagen from the expressed juice after the thermal treatment of the enzymatically treated meat.

In order to avoid advanced structural degradation of the meat, the enzymatic treatment has to be carefully monitorized by limiting the levels of the papain and bromelin and the tenderization period.

In conclusion, we recommend the following parameters to be used for the enzymatic and thermal treatment of the beef meat: the enzyme doze-10mg/100g meat; the tenderization time-24 hours, at 4$^{o}$C, followed by a thermal treatment in a dynamic regime by increasing the temperature with 1$^{o}$C/min until achieving 70$^{o}$C in the thermal centre to completely inactivate papain and bromelin.

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