The influence of enzymatic tenderization with papain on functional properties of adult beef

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Abstract

From all types of treatments used to improve meat tenderness (mechanical, chemical, enzymatic tenderization), has been studied the enzymatic tenderization with papain a tropical plant enzyme derived from papaya. In this research has been evaluated papain influence on adult beef because pork, mutton and chicken meat are enough tender. Papain was added in injection brine and than the beef cuts was injected with a specific percent of brine. Experimental data indicate that papain weaken beef meat structure produced improvement of functional properties of adult beef. Papain attacks connective tissue and myofibril proteins determinate increase of hydroxiproline and free amino acids content in boiled beef cuts. A significant increase in tenderness by rigidity index measurement was observed in the meats treated with papain as compared with the control. The sensorial tests of beef treated with papain were bigger than control. This experiment confirms that papain is a proteolytic enzyme that causes improvement of meat quality.

Keywords: Tenderness, adult beef, papain, rigidity index

1. Introduction

The most important characteristics of meat quality are tenderness, juiciness and flavor. Consumers consider tenderness as the most important factor in determining eating satisfaction of beef (Issanchou, 1996, Boleman, Miller, Taylor, Cross, Wheeler, Koohmaraie, Johnson and Savell, 1997). Tenderness is defined as the ease of mastication, which involves the initial ease of penetration by the teeth, the ease with which the meat breaks into fragments and the amount of residue remaining after mastication (Lawrie, 1998).

Meat tenderness depends on specie, breed, age, sex and individual skeletal muscle tissue of animal. Tenderness originates in structural and biochemical properties of skeletal muscle fibres, especially myofibrils and intermediate filaments, and of the intramuscular connective tissue, the endomysium and perimysium, which are composed of collagen fibrils and fibres (Takahashi, 1996).

The mechanical stability of collagen fibrils increased markedly with chronological ageing.

Meat produced from old animal is tough and has a lower eating quality. Improvement of meat tenderness of aged cattle is necessary to increase functional properties and value of the meat (Shiba, 2004). Meat can be tenderized in the different ways: mechanical methods (mincing or hitting to crush the conjunctive and muscle tissue), chemical methods by injecting in the muscle solutions with chemical substances (salt, sodium chloride, sodium polyphosphate, potassium lactate, sodium diacetate all dissolved into water, R.K., 2003), enzymatic methods used proteolytic enzymes like papain, bromelin or ficin.

Enzymatic tenderization can be made in a humid way (by injecting in the muscle solutions with enzymatic preparates) or in a dry way (pressing tender mixtures on the meat surface).
The end of the tenderization process and the method employed for ageing and thermal processing should be established to get an optimum sensorial quality of the meat.

2. Materials and methods

The study employed adult beef thigh post rigor (24 hours after slaughter), purchased from the commercial distribution in refrigerated form. Salt was of food-suitable purity, being a largely used additive in meat industry and papain was purchased from Lay Condiments, Bucharest (Papain Chilko P).

**Chemical analysis:** The chemical composition of adult beef utilized for analyses was determining by the water content according to the AOAC- 1995 method; the total nitrogen content according to the SR ISO 9037:2007 method; the fat content according to the AOAC, 1984 method and the pH with a Hanna digital pH-meter. Hydrolisis proteins degree was determining by the non-protein nitrogen according to the AOAC method (1990) and the aminic nitrogen according to the method described by Vâtă and all, 2000. Tenderness degree was determining according to the method described by Ionescu and all, 1992.

The sensorial analysis of papain-tenderised meats was achieved by a team of samplers, made up of 5 specialists, using a 5-point scale (1-I dislike it; 2- I do not like or dislike it; 3- I like it; 4-I like it moderately; 5- I like it very much).

**Sample preparation:**

The adult beef thigh separated from the gross conjunctive tissue and fat was cut into pieces of the same size in length and thickness, weighing approximately 120 g, cut along the muscular fibers. The meat pieces were then divided into four groups and were used for a certain treatment. They were injected with brine made up of: salt 2 g and water 98 g to which various amounts of papain were added. For each treatment series were constituted, consisting of:

- **Control sample (M),** the pieces of meat were injected with 10% brine without papain addition;
- **Sample A** – the brine was completed with papain to a concentration of 0.003 mg/100g meat. The injection was 10%;
- **Sample B** – the brine was completed with papain to a concentration of 0.005 mg/100g meat. The injection was 10%;
- **Sample C** – the brine was completed with papain to a concentration of 0.007 mg/100g meat. The injection was 10%.
- **Sample D** – the brine was completed with papain to a concentration of 0.010 mg/100g meat. The injection was 10%.

The injection was performed manually with a syringe, so that the entire brine quantity could be uniformly pumped into the whole muscular mass. The eliminated brine was reinjected. The injected meats were wrapped with a polyethylene film and stored at 4°C for 24-48 hours aiming to achieving an uniform that diffusion of brine in the muscular tissue with or without papain addition and to deploying the activity of exogenous proteolytic enzymes.

After ageing, the beef cuts were boiled in hermetically sealed test tubes on a water bath by gradual heating (about 1°C/minute) up to reaching the thermal centre of the temperature of 83°C, which was maintained for 10 minute.

After boiling, the samples were immediately cooled on water bath cooled by means of ice, after which they were stored at refrigeration temperature over night. The boiled meats brought to room temperature were carefully removed from the test tubes and were weighed after being tapped with filter paper.

The juice percentage expressed at thermal treatment was calculated with the formula:

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\text{% expressed juice} = \frac{\text{Mass of meat after injection and maturation} - \text{Mass of boiled and cooled meat}}{\text{Mass of meat after injection and maturation}} \times 100
\]
3. Results and Discussions

The research used adult beef thigh post rigor at 24 hours after slaughter purchased from specialized stores in refrigerated state. The data resulted from chemical composition analyses of beef showed a relatively lean meat (5.94% ± 1.5% fat), with average protein content (17.5% ± 1.09%) and water content (75.6% ± 1.06), the average values and errors being calculated with the statistic program Sigma plot 2001 for five different lots of beef.

**Influence of papain on muscular proteins hydrolysis:**

The hydrolysis degree of muscular proteins was appreciated by determination of non-protein nitrogen content and free amino acids from boiled enzymatic tenderized beef and also by the content of soluble proteins from the juice released from thermal treatment.

Papain is a powerful proteolytic prepared obtained from papaya latex by purification which activate all delitescent potential. It has a large specific of substratum catalyzing segregation of – CO - NH - chemical bond. According to the internal or terminal position of the peptidic bonds, proteases are exopeptidase and endopeptidase. Papain belongs to exopeptidase group (it has cystein in center).

In present study the hydrolysis degree depended by intensity of applied enzymatic treatment, being bigger when utilized level of injected papain was higher, and ageing duration longer. The smallest hydrolysis degree was observed at the control where has been used for injection only brine without papain addition, accumulation of non-protein nitrogen and free amino acids are influenced by the enzyme action proper to muscular tissue.

The level of non-protein nitrogen and free amino acids increased in the same time with papain addition and time of ageing increasing. By increasing of non-protein nitrogen accumulation will obtain improvement of tenderness and assimilation degree of nitrogen compound from beef enzymatic tenderized (fig. 1, 2).

The accumulation o free amino acids increased proportional with the addition of papain. In contrast with other exogenous proteolytic enzymes, papain being an endopeptidase determined a smaller accumulation of free amino acids because of chains polypeptide bursting in fragments with large molecular weight.

Papain acts different to structure proteins of muscular tissue hydrolyzing less actomyosin, protein which is forming in time of muscular rigidity, than myosin.

<table>
<thead>
<tr>
<th>Chemical components</th>
<th>Content</th>
<th>g%</th>
<th>g% s.u.</th>
</tr>
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<tbody>
<tr>
<td>Moisture</td>
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<tr>
<td>Dry substance</td>
<td></td>
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<tr>
<td>Total nitrogen</td>
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<tr>
<td>Total proteins</td>
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<tr>
<td>Fats</td>
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<td>0.892</td>
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<tr>
<td>Aminic nitrogen</td>
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</tr>
<tr>
<td>Ammonia</td>
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<tr>
<td>pH</td>
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<td>6.03</td>
<td>-</td>
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Tenderness degree:
The degree of rigidity was used as a measure of determining tenderness degree of beef enzymatic tenderised with papain. The rigidity index represents the resistance opposed by meat to compression (in the present study rigidity index was determined at boiled beef cuts).

The increase of the level of papain added as well as the increase of the duration of the enzyme activity led to a considerable increase of the values of the rigidity index in thermally treated samples by boiling (fig. 3). The lowest values of the rigidity index were observed for the control (injected only with brine with no papain addition) as compared to samples tenderised with different quantities of papain.

The increase of the level of papain addition determined a significant increase of the rigidity index in all samples the highest values being observed to the samples injected with 10 mg papain/100 g carne (10% w/w).

No matter the thermal treatment applied to beef, there are a series of more or less intense chemical and physical modification.

The study on beef cuts tenderized with papain registered losses at thermal treatment, the highest values were observed to the control samples (fig. 4). The increase of the papain level added and the period of enzyme tenderization cause a significant decrease of the cooking losses.

Sensorial analysis:
Sensorial analysis consisted in assessment of palatability characteristics of enzymatic tenderised beef thermally treated by boiling. By the increasing of the level of papain added and by the increasing of ageing time from 24 to 48 hours was observed a considerable fragmentation of muscular fibers, part of the enzymatic beef cuts injected with papain became soft after boiling, with very low resistance to mastication. Also, some samples exhibited, after thermal treatment, areas with texture similar to a paste probably due to the inappropriate distribution of the injected solution or to an overtaking of necessary time of tenderization. The flavour of the thermally treated meat was not significantly affected. Due to the small losses at thermal treatment the samples tenderized with papain were juicier as compared to the control samples.

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**Figure 1. Influence of the papain level on the accumulation of non-protein nitrogen**

![Figure 1](image-url)
Figure 2. Influence of the papain level on the accumulation of free amino acids.

Figure 3. Influence of the papain level on the rigidity index of thermally treated beef by boiling.

Figure 4. Influence of papain enzymatic tenderisation on losses at thermal treatment.
4. Conclusions

Injection of beef cuts with papain cause an important improvement of functional properties of adult beef. Papain is a powerful proteases preparation, with great under-layer specificity, catalyzing the breaking of the peptidic bonds in the protein molecules and their degradation products to amino acids. The increase of the papain level, from injection brine, as well as the increase of the time of ageing determined a significant weakening of the meat structure.

Papain tenderization of the adult beef determined improvement of tenderness, flavour and juiciness. It is recommended to use papain doses as low as possible, in order to avoid advanced tenderization and obtaining meats with soft structure, with very low resistance to mastication and paste texture.

Application of this technology could assist beef producers and processors to obtain meat products that can satisfy consumer’s expectation.

Acknowledgement

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References


Barbonti D., Posguini M., Influence of cooking conditions on cooking loss and tenderness of raw and marinated chicken breast meat, Society of Food Science and Technology, Elsevier, 2005: 38 :895-901


