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Influence of post - mortem treatment with proteolytic enzymes on tenderness of beef muscle

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Abstract

The treatment with proteolytic enzymes is one of the popular methods to increase meat tenderization. The aim of this study was to evaluate the enzymatic tenderization with some tropical plant enzymes derived from papaya and pineapple: papain and bromelain. Researches had been conducted on adult beef meat after 24 hours of slaughter. Proteolytic enzymes were added in different concentrations in the injection brine and then beef cuts were injected with a specific percentage of brine (10% v/w). Effects generated by injecting samples of adult beef with papain (2 mg/100g meat), bromelain (2 mg/100g meat) and a mixture of papain and bromelain (mixture ratio 1:1, enzyme added 2 mg enzyme/ 100g meat) were revealed after 24 - 48 hours of storage at 4^0C. Experimental data indicate that proteolytic enzymes weaken beef meat structure producing improvement of functional properties of adult beef. Papain and bromelain attacked connective tissue and myofibril proteins producing 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 samples treated with papain as compared with the samples tenderized with bromelain, papain in association with bromelain and the control.

Keywords: beef tenderization, brine injection, proteolytic enzymes, hydroxiproline, rigidity index

1. Introduction

Consumer acceptance of meat is strongly influenced by the eating quality including tenderness, juiciness and flavour [1]. Surveys have shown that tenderness is the most important palatability trait for consumers [2]. The red meat industry needs to produce high quality meat of consistent tenderness to increase consumer confidence and encourage further purchase of meat products [3-5].

Inconsistency in beef tenderness has been rated as one of the major problems faced by the meat industry [6]. Tenderness differs among bovine muscles from various anatomical locations largely because of differences in the structural components which influence tenderness namely the myofibrillar and connective tissue proteins [7,8].

In general, muscles from the forequarter are less tender than those from the loin and hence are classified as low value cuts. Therefore, there is considerable interest in developing strategies to improve palatability and hence add value to these muscles [9, 10].

While intervention strategies can be effective for tenderness enhancement in muscles such as the *Longissimus*, the *Semitendinosus* (ST) is resistant to tenderization due to high elastin content [11,12] and as a consequence is retailed as a relatively low value steak [13,14].

Conventional methods for tenderization such as modified chilling [15], extended ageing [15] altered carcass suspension [16] and prerigor skeletal

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separations [17] are not sufficient to guarantee tenderness of the eye of round. Calcium chloride injection [18] was also ineffective at tenderizing the ST muscle from mature cow. Exogenous enzymes originating from plants, bacteria and fungal sources have been used for centuries to improve tenderness by proteolytic activity. United States federal agencies recognize five exogenous enzymes – papain, ficin, brimelain, *Aspergillus oryzae* protease and *Bacillus subtilis* protease – as Generally Recognized as Safe (GRAS) to improve meat tenderness [19]. Each of these has been shown to have varying degrees of activity against myofibrillar and collagenous proteins [20,21].

This study investigated the application of tow GRAS enzymes – Papain and Bromelain – to adult beef muscle to determine improvement in tenderness and the mode of action [22].

2. Materials and Method

The raw material, utilized in research program, was represented by the beef thigh from adult cows (more than 9 years old). The meat was purchased in hot state from a local slaughterhouse at maximum two hour post-slaughter. Salt was of food-suitable purity, being a largely used additive in meat industry, papain and bromelain were purchased from Lay Condiments, Bucharest (Papain Chilko P, Bromelin EC 3.4.4.24) [23].

***Chemical analysis*:**Hydrolysis proteins degree was estimated by the determination of non-protein nitrogen according to the AOAC method, 1990, and aminic nitrogen according to the method described by Vâţă et al, 2000 [24].

Hydrolysis collagen degree was estimated by the determination of hydroxyproline from the liquid express at thermal treatment by boiling according to the colorimetric method indicated by the ISO 3496/1984 standard, with some modifications.

Soluble proteins from the liquid express at thermal treatment by boiling were determined by Lowry colorimetric method described by Ionescu, A. et al., 1992 [25].

Tenderness degree was determining according to the method described by Ionescu et al, 1992 [25].

Sample preparation:The adult beef meat separated from conjunctive tissue and fat was cut into pieces of the same size in length and thickness (1, 5 – 2, 0 cm) weighing approximately 150 – 200 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 proteolytic enzymes were added. For each treatment series were constituted, consisting of:

- *Control sample (C),* pieces of meat injected with 10% brine without papain addition;
- *Sample* P_1 – pieces of meat injected with 10% brine with papain addition to a concentration of 2 mg/100g meat;
- *Sample* P_2 – pieces of meat injected with 10% brine with a mixture of papain and bromelain (mixture ratio 1:1, enzyme added 2 mg enzyme/ 100g meat)
- *Sample* P_3 – pieces of meat injected with 10% brine with bromelain addition to a concentration of 2 mg/100g meat;

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 4^0C ± 1^0C for 0 - 48 hours aiming to achieving a uniform that diffusion of brine in the muscular tissue with or without enzymes addition and to deploying the activity of exogenous proteolytic enzymes.

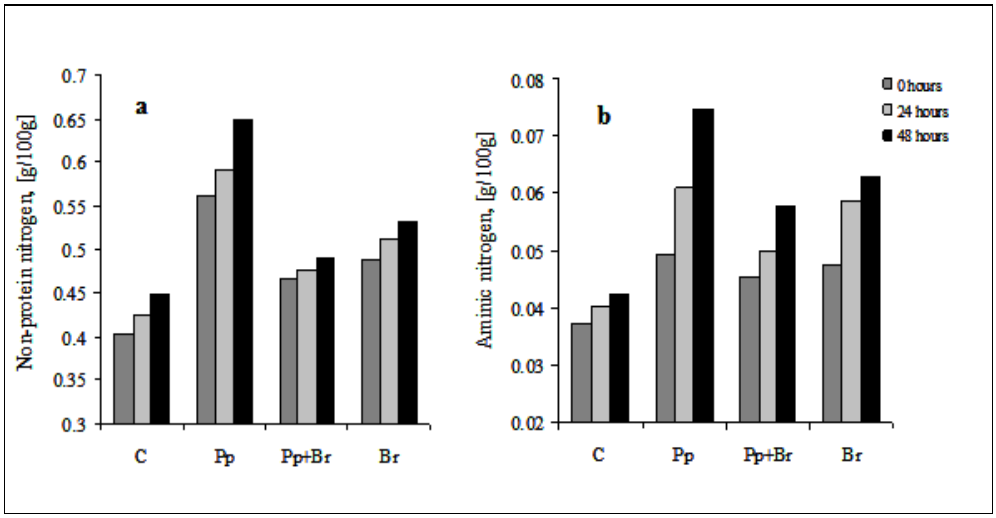

Figura 1. Influence of the enzyme treatment and duration of storage on the accumulation of non-protein nitrogen (a) and aminic nitrogen (b)
C – control sample; Pp– papain tenderization; Pp+Br –papain in association with bromelain; Br – bromelain tenderization.

Non-protein nitrogen and aminic nitrogen levels had an increasing trend throughout the enzymatic hydrolysis of beef, regardless of the presence or absence of exogenous proteolytic enzymes. Hydrolysis process intensity was higher in the sample injected with papain, in comparison with other variants of tenderization, bromelain injection and injection with papain in association with bromelain.

Samples tenderized with a mixture of exogenous proteolytic enzymes have been recorded accumulation of non-protein nitrogen and free amino acids higher than the control samples, but no evidence to the samples tenderized only with bromelain or papain. This finding allows us to assume that the two enzymes are hampering in their work when are used together for tenderizing meat.

At the meat tenderized with exogenous proteolytic enzymes, during aging has been accumulated amino acids from myosin fraction, represented, mainly by glycine, alanine, leucine and dicarboxylic amino acids [20].

At the molecular level, by breaking the peptide bonds and the accumulation of N-terminal groups from myosin fraction increase tenderization degree of meat, structural protein solubility, meat pH and improve water holding capacity.

Increased non-protein nitrogen level under the action of exogenous proteolytic enzymes leads to enhancing the assimilation of the meat. Meat treated with exogenous proteolytic enzyme has a capacity of assimilation by 16-20% higher compared to meat aged under normal conditions, without addition of enzymes [25].

The influence of *proteolytic enzymes* treatment on protein solubility

Losses of proteins in juice expressed from thermal treatment were significantly higher for meat treated with exogenous enzymes, compared with control sample (Fig. 2). By increasing the time of enzymes action, losses of soluble protein from the liquid express at thermal treatment by boiling increased significantly. Fragmentation of polypeptide chains, weakening the collagen network and thermal denaturation of the proteins are, in our view, the main factors that contributed to the elimination of water from meat and of some soluble substances in water.

Exogenous proteolytic enzymes, by breaking of the protein chains of muscle and collagen fibers and by their structural damage resulted to adult beef tenderization and in a better solubilization of proteins.

Our results showed more pronounced effect of papain on protein solubility compared with bromelain and the mixture of exogenous proteolytic enzyme (papain + bromelain).

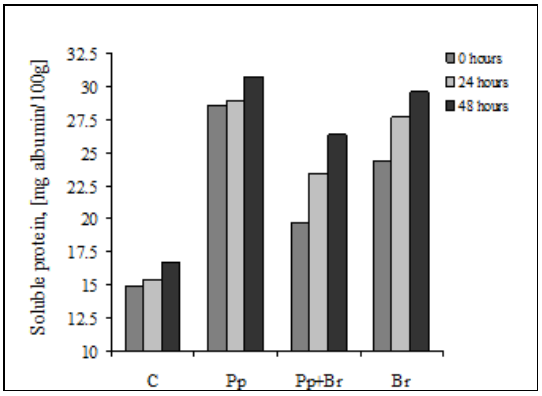

Figure 2. Influence of proteolytic enzymes treatment and storage time on loss of soluble protein from the liquid express at thermal treatment by boiling
C – control sample; Pp– papain tenderization; Pp+Br – papain in association with bromelain; Br – bromelain tenderization.

The influence of proteolytic enzymes treatment on conjunctive tissue proteins

Connective tissue content of meat is the main factor responsible for variability in beef tenderness. Exogenous proteolytic enzymes are effective both because of their action on muscle proteins and the collagen and elastin. The evolution of hydroxyproline accumulation depending on enzymatic treatment and ageing time at $4^0C \pm 1^0C$ is shown in figure 3.

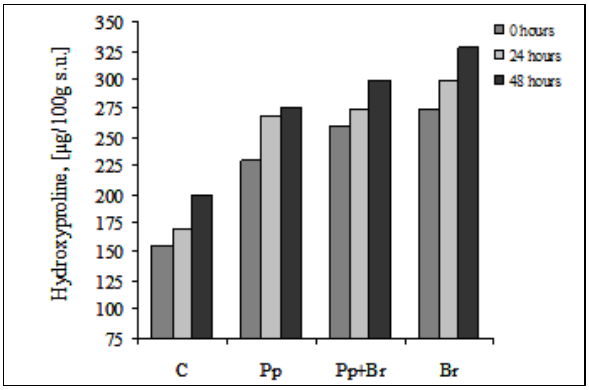

Figure 3. Influence of proteolytic enzymes treatment and storage time on the accumulation of hydroxiproline from the liquid express at thermal treatment by boiling
C – control sample; Pp– papain tenderization; Pp+Br – papain in association with bromelain; Br – bromelain tenderization.

Lowest accumulation of hydroxyproline were recorded at control sample, being represented by the free hydroxyproline in meat, released due to the action of collagenosis enzymes, own to muscle tissue, and hydroxyproline generated during thermal treatment applied to adult beef.

The mixture of bromelain with papain acted more intensely on the connective tissue in comparison with papain and control sample. Most pronounced degree of hydrolysis of the connective tissue was observed in the sample injected with bromelain, at 48 hours of ageing at $4^0C \pm 1^0C$. Increased levels of hydroxyproline accumulated in the liquid expressed at boiling of meat samples tenderized with papain in association with bromelain, evidenced both the collagenase action of papain and bromelain and the synergism between them. Bromelain is a vegetable endopeptidase with a strong affinity for collagen and elastin in muscle tissue.

Evolution of rigidity index

Dynamic of rigidity index based on enzymatic treatment applied and the time of storage at $4^0C \pm 1^0C$, is shown in figure 4.

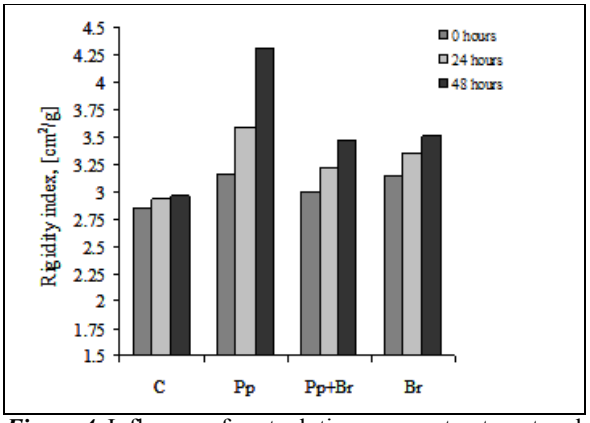

Figure 4. Influence of proteolytic enzymes treatment and storage time on rigidity index evolution

C – control sample; Pp– papain tenderization; Pp+Br – papain in association with bromelain; Br – bromelain tenderization

Both papain and bromelain and the mixture of these enzymes have resulted in increased rigidity index. Increasing the period of action of enzymes resulted in increased structural changes in muscle, rigidity index values increased throughout the ageing period. The highest values of rigidity index were recorded at 48 hours of ageing at $4^0C \pm 1^0C$ in the samples injected with papain, followed by the samples injected with bromelain and the samples

injected with papain and bromelain. Lowest values of rigidity index were recorded at the control sample.

4. Conclusion

Injection of beef cuts with proteolytic enzymes cause an important improvement of tenderness 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. Papain also has collagenase action, collagen being a major component of the conjunctive tissue. The small accumulations of amino acids and non protein nitrogen during maturation of bromelain treated samples, as compared with the samples treated with papain, are accounted for by the fact that bromelain is an endopeptidase which split proteins to form polypeptides of high molecular weight, peptides and small amounts of free amino acids and low affinity of the enzyme preparation to the actomyosin. Samples tenderized with a mixture of exogenous proteolytic enzymes have been recorded accumulation of non-protein nitrogen and free amino acids higher than the control samples, but no evidence to the samples tenderized only with bromelain or papain. This finding allows us to assume that the two enzymes are hampering in their work when are used together for tenderizing meat.Application of this technology could assist beef producers and processors to obtain meat products that can satisfy consumer's expectation.

Acknowledgements

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