Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2012, **13** (1), pp. 081 – 089

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

EFFECT OF MARINATION WITH PROTEOLYTIC ENZYMES ON QUALITY OF BEEF MUSCLE♦

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Received: June 16, 2011
Accepted: November 25, 2011

Abstract: During storage and thermal treatment meat suffers a number of biochemical and physical-chemical changes in the substrate protein, changes that take place with varying intensity depending on the method of preservation utilized and temperature of thermal treatment applied. Application of different treatments aimed to influence the proteolytic activity as is the case of enzymatic tenderization of beef.

Improving the meat tenderness with proteolytic enzymes is promising, but current legislation restricting the use of proteolytic enzymes from bacterial origin and recommended tenderizers salts containing papain, ficin and bromelain. Recent research revealed that meat marinating before grilling results in a reduction of heterocyclic amine content after thermal treatment. Also, the addition of fruit pulp, garlic or other spices contributes to decreased production of heterocyclic amines because of their antioxidant activity.

In the present study was aimed influence of exogenous proteolytic enzymes on adult beef tenderness. To increase the tenderness of adult beef were used exogenous enzymes preparations (papain and bromelain) and natural sources of enzymes using pineapple and papaya fruit. It was intended to establish the correlation between enzymatic activity of enzymes used in the study, the processing technology and changes in the physical-chemical and biochemical characteristics that occur during storage in refrigerated conditions (evolution of the rigidity index and water holding capacity, cooking losses and cooking yield of the samples injected/marinated with enzymes).

Keywords: *beef tenderization, injection, marination, proteolytic enzymes, rigidity index*

♦ Paper presented at the *International Conference of Applied Sciences, Chemistry and Chemical Engineering* – **CISA 2011**, 28-30 April 2011, Bacău, Romania

INTRODUCTION

Meat quality can be defined as a combination of diverse properties of fresh and processed meat. These properties contain both sensory characteristics and technological aspects, such as color, water-holding capacity, cooking losses, and texture [1]. Of the sensory characteristics, eating quality, which consists of flavor, tenderness and juiciness, has been regarded as the most critical characteristics because it influences repeat purchases by consumers. 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].

Inconsistency in beef tenderness has been rated as one of the major problems faced by the meat industry [4]. 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 [5, 6]. 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 [7, 8].

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 and as a consequence is retailed as a relatively low value steak [9, 10]. Conventional methods for tenderization such as modified chilling [11], extended ageing altered carcass suspension [12] and prerigor skeletal separations [13] are not sufficient to guarantee tenderness of the eye of round. Calcium chloride injection [14] 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, bromelain, *Aspergillus oryzae* protease and *Bacillus subtilis* protease – as Generally Recognized as Safe (GRAS) to improve meat tenderness. Each of these has been shown to have varying degrees of activity against myofibrillar and collagenous proteins [15, 16]. Marinating is an effective means of enhancing the quality of meats. Marinating is the process of soaking or injecting meat with a solution containing ingredients such as vinegar, lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids to flavor and tenderize meat products [17].

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), papaya and pineapple fruits were purchased from a local supermarket.

Chemical analysis

Rigidity index was determined according to the method described by Ionescu *et al.* [18]. Cooking losses (*CL*) were calculated with the formula:

$$CL = \frac{m_i - m_f}{m_i} \times 100$$

where: m_i - initial mass of the sample (raw meat); m_f - final mass of the sample (after thermal treatment).
Thermal processing yield (Y_{TP}) was calculated according to equation:

$$Y_{TP} = \frac{m_f}{m_i} \times 100$$

where: m_f - final mass of the sample (after thermal treatment); m_i - initial mass of the sample (raw meat).
pH was determined according to A.O.A.C. method (1984), with a Hanna digital pH-meter.
Water holding capacity was determined according to the method described by Fujimaki and Tsuda, cited by [19].

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 five groups and were used for a certain treatment. For each treatment series were constituted, consisting of:

- *Sample P_1* – pieces of meat injected with 10% brine with papain addition to a concentration of 2 mg/100 g meat;
- *Sample P_2* – pieces of meat injected with 10% brine with bromelain addition to a concentration of 2 mg/100 g meat;
- *Sample P_3* – pieces of meat marinated in a composition consisting of 15 g of papaya pulp, 200 mL dry red wine, 2 tablespoons honey, 2 tablespoons grated horseradish, 2 teaspoons garlic, 1 tablespoon thyme, 1 tablespoon marjoram, salt and pepper.
- *Sample P_4* – pieces of meat marinated in a composition consisting of 15 g of pineapple pulp, 200 mL dry red wine, 2 tablespoons honey, 2 tablespoons grated horseradish, 2 teaspoons garlic, 1 tablespoon thyme, 1 tablespoon marjoram, salt and pepper.
- *Sample P_5* – (control sample) - pieces of meat without enzyme addition.

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±1°C for 0 - 48 h aiming to achieve a uniform diffusion of brine with enzymes addition in the muscular tissue and to deploy the activity of exogenous proteolytic enzymes. In the case of marinated samples, all the components of marinade were mixed in plastic vessel and then the meats, initially weighed, were immersed in the marinade so the meat mass to be fully covered by it. Samples thus prepared were coated with polyethylene film and stored at 4±1°C for 0 - 48 h.

Thermal treatment applied to beef cuts consisted in: the samples were boiled in hermetically sealed test tubes on water bath by gradual heating (about 1°C/minute) up to reaching the thermal centre of the temperature of 70°C, which was maintained for 10 minutes. 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 expressed at thermal treatment was collected and weighed.

RESULTS AND DISCUSSION

Influence of marination with proteolytic enzymes on pH values

The pH of the meat has a special importance in its processing, directly influencing shelf life, color and quality of the meat [20]. The tenderization of the adult beef by injecting with exogenous proteolytic enzymes (papain, bromelain) and the marinating in the marinade with the addition of natural sources of exogenous proteolytic enzymes (papaya and pineapple) changed the pH values of beef meat. pH values were dependent on the type of treatment applied and time of storage at refrigeration temperature of 4±1°C (Table 1).

From the data in Table 1, it can be seen increasing pH of meat with increasing ageing duration in both control samples and the experimental samples tenderized with exogenous proteolytic enzymes by injection or marinating. The highest value of pH was achieved at the samples injected with papain for maximum ageing time of 48 h. The pH at the samples marinated with papaya and pineapple was maintained at low valuesclose to those of control samples, probably because of the marinade composition (dry red wine contributes to the low pH of the marinade).

Table 1. *Influence of marination with proteolytic enzymes on pH values*

Time of ageing [hours]	pH				
	Control sample	Papain[1]	Bromelain[2]	Papaya[3]	Pineapple[4]
0	5.61	6.29	6.18	5.89	5.77
24	5.63	6.14	6.33	5.93	5.85
48	5.68	6.42	6.35	5.95	5.87

[1] samples injected with 2 mg papain /100 g, percent of brine injection 10%;
[2] samples injected with 2 mg bromelain /100 g, percent of brine injection 10%;
[3] samples marinated with papaya fruit (15 g papaya pulp);
[4] samples marinated with pineapple fruit (15 g pineapple pulp).

Increasing the pH of meat has led to improvement of water retention by displacing the pH meat isoelectrical point of myofibrillar protein (5.2 ÷ 5.3 in red meat). It also takes place an increase in the efficiency of heat treatment and the percentage of bonded water and both taste and tenderness of meat have improved. According to the data reported by Takahashi [21], tenderness of meat increases proportionately with increasing pH.

Influence of marination with proteolytic enzymes on cooking losses

Cooked meats have suffered significant changes, one of the most important being weight loss, due to juice and fat losses recorded. In Table 2 are recorded the analytical data of weight loss at thermal treatment depending on the type of treatment applied to beef samples and the storage duration at 4±1°C (storage time = 0 - 48 h).

Table 2. *Influence of marination with proteolytic enzymes on cooking losses*

Time of ageing [hours]	Cooking losses, [%]				
	Control sample	Papain[1]	Bromelain[2]	Papaya[3]	Pineapple[4]
0	30.96	11.61	18.73	25.46	26.81
24	29.75	10.45	17.83	24.37	25.35
48	27.09	8.18	16.52	22.35	23.61

[1] samples injected with 2 mg papain /100 g, percent of brine injection 10%;
[2] samples injected with 2 mg bromelain /100 g, percent of brine injection 10%;
[3] samples marinated with papaya fruit (15 g papaya pulp);
[4] samples marinated with pineapple fruit (15 g pineapple pulp).

The experimental data showed a decrease in losses at thermal treatment on both enzymatic tenderized meat and control samples with the increasing of storage duration. The evolution of thermal losses was closely related to pH, its growth has resulted in increased water retention capacity with positive implications on losses at thermal treatment.
Correlating the tenderizer effect of enzymes used in the study with the losses at thermal treatment was noted a decrease of losses which have been minimal at the samples injected with papain for an ageing period of 48 h, followed by samples injected with bromelain and the samples marinated with papaya and pineapple. The biggest losses at thermal treatment were recorded at the initial time in control samples.
Thus, after 48 h of aging thermal losses for the samples injected with papain were 18.91% lower, for the samples injected with bromelain were 10.57% lower, for the samples marinated with papaya were 4.74% smaller and for the samples marinated with pineapple meats were 4.74% lower compared with the control sample.

Influence of marination with proteolytic enzymes on thermal processing yield

In Table 3 are recorded the analytical data of thermal processing yield depending on the type of treatment applied to beef samples and the storage time at 4±1°C (storage time = 0 - 48 h).

Table 3. *Influence of marination with proteolytic enzymes on thermal processing yield*

Time of ageing [hours]	Thermal processing yield, [%]				
	Control sample	Papain[1]	Bromelain[2]	Papaya[3]	Pineapple[4]
0	59.21	87.35	79.44	71.26	70.01
24	61.28	89.21	80.15	72.83	71.28
48	73.17	90.05	81.25	75.31	74.21

[1] samples injected with 2 mg papain /100 g, percent of brine injection 10%;
[2] samples injected with 2 mg bromelain /100 g, percent of brine injection 10%;
[3] samples marinated with papaya fruit (15 g papaya pulp);
[4] samples marinated with pineapple fruit (15 g pineapple pulp).

The experimental data showed an increase of thermal processing yield with the increasing of storage period for all the experimental variants. The biggest values of thermal processing yield were recorded at the samples injected with papain fellow by the samples injected with bromelain, samples marinated with the addition of papaya, samples marinated with the addition of pineapple and the control samples for 48 h of ageing at refrigeration temperature of 4±1°C.

Influence of marination with proteolytic enzymes on water holding capacity

One of the characteristics that define the quality of meat is its ability to retain its own water and water added. Other attributes of meat, such as juiciness, flavor and color are related to water holding capacity. Also, a close correlation is between water holding capacity and weight losses, which take place in meat during storage or thermal treatment. Experimental data, showed in Table 4, indicate the negative effect of exogenous proteolytic enzyme treatment on water holding capacity of thermal treated samples.

Water holding capacity decreases with the increasing of aging time at refrigeration temperature of 4±1°C. Thus, the best water holding capacity was recorded at initial time by the control samples, and the lowest value of water holding capacity was observed at the samples injected with papain for 48 h of aging. The samples marinated with natural sources of papain and bromelain (papaya and pineapple) have recorded values of water holding capacity higher compared to the samples injected with pure enzymes but lower compared with control samples.

Table 4. *Influence of marination with proteolytic enzymes on water holding capacity*

Time of ageing (hours)	Water holding capacity, [%]									
	Control sample		Papain[1]		Bromelain[2]		Papaya[3]		Pineapple[4]	
	Free water	Bound water	Free water	Bound water	Free water	Bound water	Free water	Bound water	Free water	Bound water
0	35.61	64.38	44.10	55.80	41.68	58.32	40.17	59.83	39.97	60.03
24	38.25	61.74	53.70	46.20	45.71	54.29	42.75	57.25	41.75	58.25
48	47.07	52.92	54.10	45.80	50.75	49.25	48.98	51.02	45.62	54.38

[1] samples injected with 2 mg papain /100 g, percent of brine injection 10%;
[2] samples injected with 2 mg bromelain /100 g, percent of brine injection 10%;
[3] samples marinated with papaya fruit (15 g papaya pulp);
[4] samples marinated with pineapple fruit (15 g pineapple pulp).

Reducing the amount of water bound to the enzymatic tenderized meats can be explained by the changes of myofibrillar protein structure as a result of exogenous proteolytic enzyme action. The degree of fragmentation of structural proteins was higher, the water holding capacity of meat was lower, and that is the case of samples treated with papain and those injected with bromelain (Table 4).

Influence of marination with proteolytic enzymes on rigidity index of beef cuts thermal treated by boiling

Rigidity index value reflects the degree of meat tenderness. At the samples tenderized with enzymes rigidity index has a specific evolution, conditioned by the physical and chemical changes (pH and water holding capacity evolution) and biochemical changes (degree of proteolysis), which occurred during storage.

The evolution of rigidity index depending of enzymatic treatment applied to beef samples and the storage time at 4±1°C (storage time = 0 - 48 h) is shown in Figure 1.

Increasing the action time of exogenous proteolytic enzymes led to a significant increase in rigidity index values of samples thermal treated by boiling. Thus, after 48 hours of aging have recorded an increase in the rigidity index 2.22 times for the samples injected with papain, 1.93 times for the samples injected with bromelain, 1.5 times for the samples marinated with papaya and 1.34 times for the samples marinated with pineapple while for the control samples the increase was only 1.11 times.

Tenderization of beef meat by injection with papain resulted in meats with soft texture. The resistance to pressure of the samples injected with papain is much lower compared with the samples injected with bromelain, samples marinated with papaya addition, samples marinated with pineapple addition and control samples.

The decrease of the resistance to compression of the samples enzymatic treated is explained by the action of the enzyme by weakening muscle structure, the loss of physical integrity of muscle fibers and profound changes in the structure of cover membrane consisting mainly from collagen.

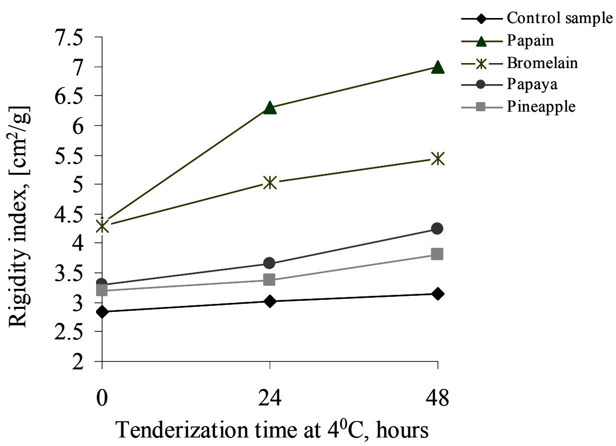

Figure 1. *Influence of marination with proteolytic enzymes on rigidity index of beef cuts thermal treated by boiling*

CONCLUSION

Beef tenderization with papain and bromelain determined a significant improvement of the adult beef quality. In the case of beef meat tenderized by marinating, samples weight increase due to incorporation of the juice from marinade; these weight increases are dependent on marinade recipe and size of samples. As a result of thermal treatment were recorded losses of juice, depending on the nature of enzyme used in the study, samples size, duration and temperature of thermal treatment. Although rigidity index of the samples injected with papain solution is higher compared to other samples, the percentage of bound water is lower, because papain has a slightly destructive action of muscle tissue and therefore water holding capacity is low. The juiciness of the samples enzymatic tenderized is closely related to the pH of the samples, tenderization method used in the study and, not least, the size of the samples. Samples tenderized using marinades had a high percentage of juiciness (moisture of the meat).

ACKNOWLEDGEMENTS

This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project, *Organizing the National Interest Postdoctoral School of Applied Biotechnologies with Impact on Romanian Bioeconomy*, project co-financed by the European Social Fund through the Sectoral Operational Program Human Resources Development 2007-2013.

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