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EFFICIENCY OF DIFFERENT TYPE OF TENDERIZATION FOR IMPROVING TECHNOLOGICAL PROPERTIES OF BOVINE *BICEPS FEMORIS* MUSCLE

Daniela ISTRATI, Camelia VIZIREANU, Felicia DIMA

Food Science, Food Engineering, Food Biotechnology Department, Faculty of Food Science and Engineering, "Dunarea de Jos" University of Galati, 111 Domneasca Street, 800008, Romania,phone: 0040 336 130 180, fax: 0040 236 460 165.

Corresponding author email: daniela.istrati@ugal.ro

Abstract
Meat quality is defined as a combination of sensory characteristics and technological aspects, such as color, water-holding capacity, cooking losses, and texture. Tenderness and juiciness, has been considered as the most critical characteristics because it influences repeat purchases by consumers. Since natural aging is a long-term process, artificial aging is recommended to be widely used in the meat industry and catering. Thus, in the present study was investigated the effect of different type of tenderization including chemical methods (injection with 0.4 M $CaCl_2$), enzymatic methods (injection with papain extracted from papaya) and marinating in wine marinade on technological properties of bovine Biceps femoris muscle. The control samples were represented by raw meat stored in the same conditions as analyzed samples. After injecting with $CaCl_2$, papain and marinating, meat pieces were vacuum packed and stored at refrigeration temperature 4°C for 7 days. In this experiment, the influence of tenderization applied to beef meats was evaluated by monitoring pH evolution, storage losses, cooking losses, changes in meat texture. During storage and artificial tenderization of the samples vacuum packed, were noted for all indicators followed variations indicating the proteolysis process development, which signifies an improvement in meat tenderness.

Keywords: *beef tenderization, calcium chloride, papain, marinating*

INTRODUCTION

Consumer acceptance of meat is strongly influenced by the eating quality. 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 (Walsh et al.; 2010; Kargiotou et al., 2011). 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. Consumers have identified tenderness as the most important beef sensory attribute (Miller et al., 1995). Red meat industry needs to produce high quality meat of consistent tenderness to increase consumer confidence and encourage further purchase of meat products (Boleman et al., 1997; Han et al., 2009; Jayasooriya et al., 2007). 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 (Von Seggern et al., 2005). In general, muscles from the forequarter are less tender than those from the loin and these are classified as low value cuts. Therefore, there is considerable interest in developing strategies to improve palatability, in order to add value to these muscles (Molina et al., 2005). Papain (Schenkova et al., 2007) and calcium chloride (Ilian et al., 2004; Koohmaraie et al., 1998) have been the most studied and are probably the most effective tenderizing agents. However, papain has a tendency to over-tenderize the meat surface, leading to undesirable ''mushy" meat (Ashie et al., 2002), leading to a limited use as a commercial meat tenderizer. Although the infusion of $CaCl_2$ solution can improve meat tenderness (Koohmaraie et al., 1998), calcium ions reduce the color stability of fresh meat and decrease the product shelf life (Bekhit et al., 2005). 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 (Bjorkroth, 2005; Fernandez-Lopez et al., 2005; Pathania et al., 2010).

The objective of the present study was to investigate the influence of different methods of tenderization (enzymatic, chemical and marination) on technological properties of bovine *Biceps femoris* muscle vacuum packed and stored at refrigerated temperature.

MATERIALS AND METHODS

Materials

The raw material, utilized in research program, was represented by the beef thigh from adult cows. The meat was purchased in refrigerated state from a local slaughterhouse at maximum 24 hours 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). Garlic (*Allium sativum)* have been purchased from Quatre épices Company (Bucharest, Romania), lime-tree honey was purchased from S.C. Apisalecom S.R.L. (Bacau, Romania) and dry red wine, minimum 12% vol. alcohol content, from S.C. Viovin Prodserv S.R.L. (Odobesti, Romania).

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 g, cut along the muscular fibers. The meat pieces were then divided into four groups and were used for a certain treatment. For each treatment series were constituted, consisting of:

- Sample 1 – pieces of meat injected with 10% brine with papain addition to a concentration of 1,5 mg/100 g;
- Sample 2 – pieces of meat injected with 10% $CaCl_2$ to a concentration of 0.4 M;
- Sample 3 - pieces of meat marinated in dry red wine (300 ml/kg), honey (40 g/kg), garlic (9 g/kg), pepper (2 g/kg) and salt (5%);
- Sample 4 – control samples, pieces of meat without applying any treatment.

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. For marination treatment, meat slices were placed into polypropylene boxes. A 300 ml volume of the marinade per one kg of meat was then added to cover all the meat pieces, followed by agitation by hand to ensure an even distribution of the solid components of the marinades. All boxes were over-wrapped with a polyethylene cover and held at 4^0C for 48 hours. After approximately 24 hours the meat pieces and were turned over, to ensure uniform marination. Following marination, the meat samples were removed from the trays and the excess liquid was allowed to drain off. After tenderization treatments samples were vacuum packaged and stored at 4^0C for 7 days. Vacuum packaging was performed using a vacuum packaging machines, VACSY System, produced by the company Zepter International using a package type, with the following characteristics: permeability to O_2 (at 23^0C and 0% RH) <30 cm^3/m^2•24h•atm and CO_2 permeability (at 23^0C and 0% RH) = 150-200 cm^3/m^2•24h•atm.

Chemical analysis

Tenderness degree was determined according to the method described by Ionescu et all, 1992.

Cooking losses were calculated with the formula:

$$P= [Mi-Mf] x100/ Mi$$

where Mi= initial weight of the sample (raw meat);

Mf= final weight of the sample (after thermal treatment).

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 Thomson and col., 1997.

RESULTS AND DISCUSSIONS

pH evolution depending ontype of artificial tenderization and storage time at 4^0C

The pH of the meat has a special importance in its processing, directly influencing shelf life, color and quality of the meat (Simela, 2005). The tenderization of *Biceps femoris* muscle by injecting with $CaCl_2$, exogenous proteolytic enzyme (papain extracted from papaya) or marinating in wine marinade has changed the pH values. pH values were dependent on the type of treatment applied and time of storage at refrigeration temperature of 4^0C (Figure 1).

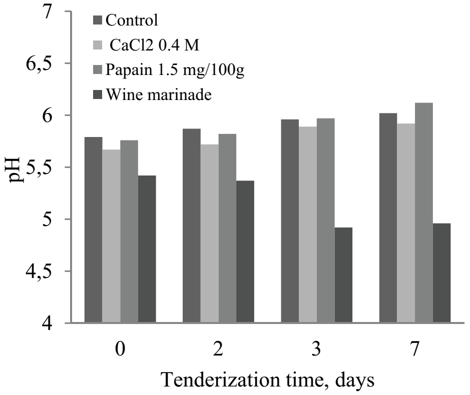

Figure 1. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on pH values

The experimental data showed the increasing of pH values with the increasing of ageing time in control samples and experimental samples tenderized with papain and $CaCl_2$ 4M. At the samples marinated in wine marinade had recorded a decrease of pH values between 0 and 3 days of storage with a slight increase after 7 days of storage. The highest value of pH was achieved at the samples injected with papain for maximum ageing time of 7 days. The pH at the samples marinated in dry red wine was maintained at low values because of the marinade composition (dry red wine contributes to the low pH of the marinade).

The decrease of pH values in samples marinated in wine marinade may be explained by organic acids from wine absorption by meat and lactic acid production by lactic acid bacteria. The honey from marinade was the nutritive substrate for lactic acid bacteria. Lactic acid accumulation in time, led to a decrease in pH values in marinated samples.

According to Koohmaraie et al., 1990 and Morgan et al., 1991 increased values of pH may play an important role in the activation of calpain system calcium-dependent and in improvement of meat tenderness. pH value of meat products is highly important because it has a major influence on water holding capacity (WHC), tenderness and juiciness (Goli et al., 2007).

Influence of artificial tenderization of *Biceps femoris* muscle on water holding capacity (WHC)

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. At the initial moment the best Water holding capacity had samples marinated in wine marinade which were followed by the control samples and the samples injected with $CaCl_2$ solution and papain (Figure 2).

Experimental data, showed in Figure 2, indicate the negative effect of exogenous proteolytic enzyme treatment and injection with $CaCl_2$ on water holding capacity of samples. Water holding capacity decreases with the increasing of aging time at refrigeration temperature of 4^0C. Thus, the best water holding capacity was recorded at initial time by the samples marinated in wine marinade, and the lowest value of water holding capacity was observed at the samples injected with papain for 7 days of aging. Reducing the amount of water bound of 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 (Figure 2).

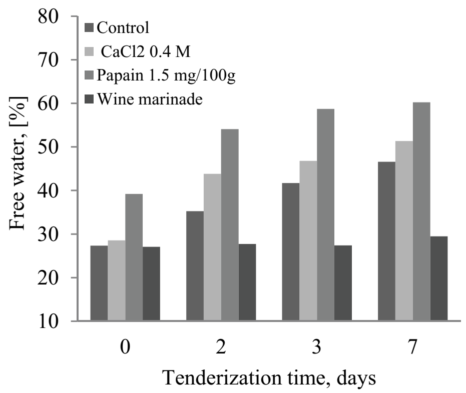

Figure 2. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on water holding capacity (WHC)

Decreased water holding capacity for the samples treated with $CaCl_2$ may be due to reduction of water absorption in the presence of Ca^{2+} ions (Gerelt et al., 2002). Wheeler et al., (1993) indicated that the addition of exogenous calcium activates calpain enzymes meat, which reduces WHC by myofibrillar proteolysis. Koohmararie et al., 1990 reported that calcium ions modify the native conformation of proteins myofibrillar promoting their denaturation. Also, water-holding capacity of fresh beef increases rapidly as pH values decrease below the isoelectric point of muscle (5.1), which increases the relative number of positive-charged protein groups (Hamm & Deatherage, 1960). Furthermore, the number of these reactive groups available to bind water is dramatically increased when muscle pH values were greater than 6.0 or below 4.0 (Gault, 1985). Additionally, Rao and Gault (1989) pointed out that acidification of meat below its isoelectric point favored the swelling and moisture retention by stromal proteins, whereas greater meat acidification, to pH levels of 4.0 and below, resulted in the swelling and retention of added moisture by the myofibrillar proteins.

The influence of artificial tenderization of *Biceps femoris* muscle on cooking losses

During the thermal treatment applied to beef take place a series of more or less intense chemical and physical changes. The meat texture is altered during the thermal treatment as a result of protein denaturation, meat dehydration, collagen hydrolysis, fat expulsion from fat cells and their dispersion in to the meat mass.

Figure 3 shows the evolution of cooking losses depending on the type of treatment applied to beef samples and the storage time at 4°C (storage time = 0 – 7 days). Artificial tenderization of *Biceps femoris* muscle led to significant changes in the cooking losses as compared to the control samples.

Experimental data indicate the negative effect of injection with $CaCl_2$ on cooking losses. The highest value of cooking losses was achieved at the samples injected with $CaCl_2$ at 2 days of ageing at refrigerated temperature. Koohmaraie et al. (1990) reported that injection of beef loins with $CaCl_2$ within 1 h postmortem resulted in increased cooking losses.

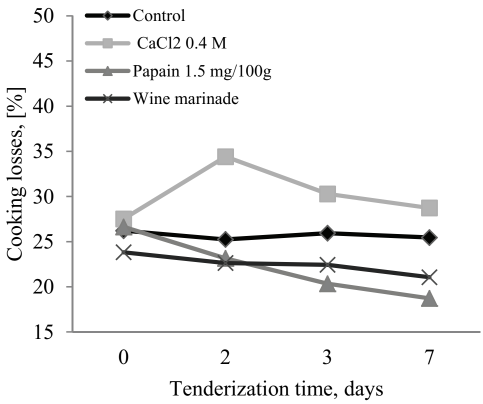

Figure 3. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on cooking losses

The result obtained in this study agreed with the work of Omojola (2007) who reported an increase in cooking loss as time post mortem increased. In a similar way, Wheeler et al. (1992) reported higher cooking loss values in beef injected with $CaCl_2$24 h post mortem compared with 0 h post mortem.

Experimental data indicate a decrease of cooking losses both in the samples tenderized with papain and the samples marinated in wine marinade, the lowest values being recorded at the samples marinated in wine marinade for the maximum time of ageing 7 days. Evolution of cooking losses of the samples injected with papain and marinated in wine marinade was closely related to the evolution of pH. The results from several studies have shown that lowering the pH of beef also reduced moisture losses during cooking contributing to an overall improvement in the juiciness of the cooked product (Aktas et al., 2003; Gault, 1985; Onenc et al., 2004).

The influence of artificial tenderization and storage time at 4^0C on rigidity index of beef cuts thermal treated by boiling

Rigidity index represents the resistance opposed by meat to compression (in the present study rigidity index was determined at boiled beef cuts). Meat samples injected with solutions containing papain and $CaCl_2$ and marinated in wine marinade, stored at 4^0C, showed rigidity index values higher than control samples, so indicating an improvement of meat tenderness (Figure 4).

The increase of the ageing time at refrigerated temperature resulted in significant increase of the rigidity index in the samples injected with papain. The highest values of rigidity index were recorded at the samples injected with papain for the maximum time of tenderization 7 days. Also, the lowest values of rigidity index were recorded at the control samples at initial time. Calcium chloride improves meat tenderness by increasing intracellular calcium ion concentration leading to activation of calpain enzymes and increasing fragmentation of muscle fibers. The mechanism of the tenderizing action of acidic marinades is believed to involve several factors including weakening of structures due to swelling of the meat, increased proteolysis by cathepsins and increased conversion of collagen to gelatin at low pH during cooking (Berge et al., 2001).

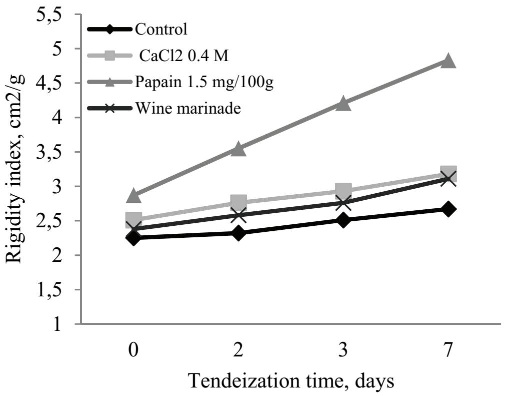

Figure 4. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on rigidity index

Insignificant increases of rigidity index of the samples injected with $CaCl_2$ and marinated in wine marinade can be explained by the high content of collagen in adult beef meat. The meat with a high content of collagen is generally tough and requires a prolonged heat treatment (Perez-Chabela et al., 2005).

CONCLUSIONS

Injection of beef cuts with papain cause an important improvement of functional properties of *Biceps femoris* muscle as compared with the injection with $CaCl_2$ and marination in wine marinade. 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.

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