The tenderization of bovine Biceps femoris muscle using marinades on the basis of wine

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Abstract

Fresh beef slices were marinated by immersion in marinades based on dry red wine, lime-tree honey, salt, spices and seasoning plants as thyme (Thymus vulgaris), marjoram (Majorana hortensis), garlic (Allium sativum) and horseradish (Armoracia rusticana). Control samples were represented by raw meat without marination treatment but packed and stored in the same conditions as marinated samples. After marination, meat pieces were packed under vacuum and stored at refrigeration temperature 4°C for 12 days. The combined effects of spices and marination on beef tenderness were evaluated by monitoring pH evolution, hydrolysis proteins degree, hydrolysis collagen degree and tenderness degree. Experimental data indicated that marination weaken beef meat structure producing improvement of functional properties of adult beef. Marinades attacked connective tissue and myofibril proteins producing increase of protein nitrogen, free amino acids and hydroxyproline contents in boiled beef cuts. A significant increase in tenderness by hardness measurement was observed in the samples marinated and boiled as compared with the control.

Keywords: Biceps femoris muscle, tenderness, spices, marination and texture.

Introduction

Among the organoleptic characteristics that contribute to meat quality, tenderness is recognized as the most important factor (KOOHMARAIE [1]), and consumers are willing to pay a premium for beef that is guaranteed to be tender (SHACKELFORD & al. [2]). Meat tenderness should be related mainly to the connective tissue and myofibrillar protein components of muscle while the relative contribution to tenderness of these components depends on factors such as the carcass location of the muscle, the degree of contraction of the myofibrils, and the cooking procedure applied (LAWRIE, SAWDY & al. [3, 4]).

Tenderness largely differs among bovine muscles from various anatomical locations because of differences in the structural components which influence tenderness namely the myofibrillar and connective tissue proteins (BELEW & al., VON SEGGERN & al. [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 to add value to these muscles (MOLINA & al., ROBBINS & al. [7, 8]). During the refrigeration period, a series of biochemical and physic-chemical modifications occur in meat. Tissue enzymes are the first which act, followed by bacterial enzymes activity. The assembly of modifications which take place during meat ageing, under the action of tissue proteolytic enzymes, improves the sensory characteristics of meat, defining meat as a foodstuff. These modifications, taking place in the post mortem period, are leading towards meat ageing (KOOHMARAIE, KOOHMARAIE & al. [1, 36]).
Papain (ASHIE & al., SCHENKOVA & al. [9, 10]), and calcium chloride (ILIAN & al., KOOMHARAIE & al. [11, 12]), 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 & al., LEWIS & al., IONESCU & al. [9, 13, 14]), which has limited its use as a commercial meat tenderizer. Although the infusion of CaCl₂ solution can improve meat tenderness (KOOMHARAIE & al., ISTRATI & al. [15, 16]), calcium ions reduce the colour stability of fresh meat and decrease the product shelf life (BEKHIT & al., HUNT & al. [17, 18]).

Marinating is a process which can be used also by consumers to improve tenderness, add taste and variety to the meat component of meals. Marination 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 for flavoring and tenderize meat products (BJORKROTH, PATHANIA & al. [19, 20]). Moreover, the shelf life of the meat may be positively affected by this process due to the pH of the solution, and due to the antimicrobial and antioxidant activity of some marinade ingredients (TOMPKIN & al., KARGIOTOU & al. [21, 22]). Marinades with a tenderizing capacity are particularly important in applications involving muscles rich in connective tissue. These muscles often lead to the cheaper carcass cuts and the tenderizing effect of marinating offers a commercially important tool of upgrading them (GAULT, LEWIS & al. [23, 24]). 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 & AL., OFFER & al. [25, 26]).

Therefore, the purpose of our study was to investigate the combined effects of spices and wine base marinades on tenderization process of bovine Biceps femoris muscle.

Materials and Methods

Materials

Beef samples (biceps femoris muscle; breed: Holstein Friesian; sex: female; age: 5 years) have been acquired from Meat Technology Centre of Galicia, Technology Park of Galicia, Ourense, Spain, after 24 hours postmortem and transported to the Food Technology Laboratory of the Faculty of Food Science of Ourense, University of Vigo, Spain. Marjoram (Majorana hortensis) and garlic (Allium sativum) have been purchased from Quatre épices Company (Bucharest, Romania), thyme (Thymus vulgaris) was acquired from Research Institute Plantavorel (Piatra Neamt, Romania), horseradish (Armoracia rusticana) from a local supermarket, lime-tree honey, 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).

Marinades

The marinades were: marinade 1 consists of dry red wine (300 ml/kg), honey (40 g/kg), garlic (9 g/kg), pepper (2 g/kg) and salt (5%); marinade 2 consists of dry red wine (300 ml/kg), honey (40 g/kg), garlic (9 g/kg), thyme (4 g/kg), pepper (2 g/kg) and salt (5%); marinade 3 consists of dry red wine (300 ml/kg), honey (40 g/kg), garlic (9 g/kg), marjoram (4 g/kg), pepper (2 g/kg) and salt (5%); marinade 4 consists of dry red wine (300 ml/kg), honey (40 g/kg), garlic (9 g/kg), horseradish (4 g/kg), pepper (2 g/kg) and salt (5%) and marinade 5 consists of dry red wine (300 ml/kg), honey (40 g/kg), garlic (9 g/kg), thyme (4 g/kg), marjoram (4 g/kg), horseradish (4 g/kg), pepper (2 g/kg) and salt (5%). The marinades were left at temperature of 18°C with intermittent agitation for at least one hour, to
allow the dry ingredients to hydrate. Control samples were represented by raw meat without marination treatment but packed and stored in the same conditions as marinated samples.

**Marination and storage of samples**

The beef *biceps femoris* muscle of right size of the carcass was collected. After removing the fat, ligaments and tendons from the muscle as much as possible, it was cut along the muscular fibers into total 31 parts with the same size (10 x 6 x 2 cm) and shape, weighing approximately 100 g. For each marination treatment, five 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 equal distribution of the solid components of the marinades. All boxes were over-wrapped with a polyethylene cover and held at 4°C for 48 h. After approximately 24h the meat pieces were turned over, to ensure uniform marination. Following marination, the meat samples were removed from the trays and the exceeding liquid was allowed to drain off for 5 minutes at 4°C and then they were vacuum packed in polypropylene bags type Side seal bags PA/PE, allfo Vakuumverpackungen, Frankfurt, Germany (thickness: 90μm; gas permeability: water vapors – 2,6 g/m²d; O₂ – 50 cm³/m²d; CO₂ - 150 cm³/m²d; N₂ – 10 cm³/m²d; mechanical strength: tensile strength MD – 40-50 N/15 mm; tensile strength TD – 30-40 N/15 mm; sealing temperature: 100-180°C; temperature consistency: - 50/+ 90°C) and were stored at 4°C for 12 days in a storage chamber.

**Analytical methods**

The chemical composition of adult beef utilized for analyses was determining by following methods: the water content according to the AOAC - 1995 method ; the total nitrogen content according to the SR ISO 9037:2007 standard; the fat content according to the AOAC - 1984 method and the pH using a micro pH 2002 pH-meter (CRISON Instruments S.A., Barcelona, Spain) according to the AOAC – 1984 method [27 - 29].

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 [30, 31].

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 3496/1994 standard, with some modifications [32].

Meat tenderness as hardness was measured by textural tests in TA.XT. Plus Texture Analyzer (Stable Micro Systems, Surrey, United Kingdom); for this test cooked and cooled samples were used. The samples were cooked placing vacuum package bags in a water bath with automatic temperature control (JP Selecta, Precisdg, Barcelona, Spain) until it reached an internal temperature of 70°C, controlled by thermocouples type K (Comark, PK23M, UK), connected to a data logger (Comark Dilligence EVG, N3014, UK).

After cooking, the samples were cooled to temperature of 18°C, placing vacuum package bags in a circulatory water bath set at 18°C during a period of 30 min and the percentage of cooking loss was recorded. Samples for hardness determination were obtained by cutting cubes of 1×1×1 cm (height×width×length) approximately perpendicular to the muscle fiber direction ant then compressing to 80% with a compression probe of 19.85 cm² of surface contact at a crosshead speed of 0.33 mm/s. There was an interval of 2 s between the first and second compression.

Statistical analysis was performed using the ANOVA Programme for Windows, in order to obtain medium values and standard deviations.
Results and Discussions

Our studies were realized at a laboratory level, in model systems, using as raw material adult beef muscle (*biceps femoris*) purchased at 24 hours after slaughtering. Experimental data, showing the chemical composition of adult beef used in our studies are presented in table 1. Chemical composition indicated a relatively lean meat, with 5.82% of fat.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chemical components*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture (g%)</td>
</tr>
<tr>
<td><em>Biceps femoris</em> muscle</td>
<td>76.8 ± 1.78</td>
</tr>
</tbody>
</table>

* Medium values and standard deviations

Analyzed beef presented a dark red colour, a good texture, gross muscular fibres, well highlighted, dry surface and full-grown connective tissue.

### Spices and marinades influence on adult beef pH values

In the present study, adult beef tenderization by marination (with different spices and seasonings) influenced the pH of beef (marinades pH was 4.57). pH values depended on the type of treatment and the storage time at 4°C (Table 2).

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Control</th>
<th>Marinade 1</th>
<th>Marinade 2</th>
<th>Marinade 3</th>
<th>Marinade 4</th>
<th>Marinade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.75± 0.61</td>
<td>5.75± 0.61</td>
<td>5.75± 0.61</td>
<td>5.75± 0.61</td>
<td>5.75± 0.61</td>
<td>5.75± 0.61</td>
</tr>
<tr>
<td>2</td>
<td>5.85± 0.88</td>
<td>5.06± 1.18</td>
<td>5.07± 0.60</td>
<td>5.07± 0.36</td>
<td>5.06± 0.14</td>
<td>5.06± 0.44</td>
</tr>
<tr>
<td>5</td>
<td>5.96± 0.37</td>
<td>4.92± 0.42</td>
<td>4.9± 0.53</td>
<td>4.93± 0.67</td>
<td>4.94± 0.72</td>
<td>4.94± 0.82</td>
</tr>
<tr>
<td>8</td>
<td>6.13± 0.35</td>
<td>4.96± 0.94</td>
<td>4.95± 0.22</td>
<td>4.97± 0.11</td>
<td>4.96± 0.15</td>
<td>4.96± 0.78</td>
</tr>
<tr>
<td>11</td>
<td>6.28± 0.59</td>
<td>5.07± 0.51</td>
<td>5.05± 0.73</td>
<td>5.08± 0.59</td>
<td>5.11± 0.60</td>
<td>5.14± 0.84</td>
</tr>
<tr>
<td>14</td>
<td>6.36± 0.10</td>
<td>5.18± 0.66</td>
<td>5.15± 0.81</td>
<td>5.17± 0.67</td>
<td>5.22± 0.41</td>
<td>5.24± 0.17</td>
</tr>
</tbody>
</table>

* Medium values and standard deviations

From data presented in table 2 it can be seen a decrease in pH values, in the first days of storage, in the experimental samples. The smallest pH value was determined in samples marinated in the marinade 2 which composition was red dry wine, tile honey, garlic, pepper and salt with thyme addition stored at 4°C for 5 days (2 days of marination and 3 days of vacuum packed storage). The pH values of experimental samples had an increasing trend after 5 days of storage and the same trend was followed by control samples after 2 days of storage at 4°C.

pH it is of great importance in meat processing having a direct influence on water holding capacity (WHC), tenderness and juiciness (BENDALL & al., GOLI & al. [33, 34]). pH values modifications are the result of the post mortem metabolism and the action of different substances added to meat during the marination and the technological process (GAULT [35]).
Spices and marination influence on muscle tissue proteins hydrolysis

In Figures 1 and 2 it can be followed the dynamics of different nitrogen fractions formation and accumulation, as result of the proteolytic activity under refrigeration conditions during the ageing period.

**Figure 1.** Dynamics of formation and accumulation of non-protein nitrogen in beef during marination (0-2 days) and subsequent storage at 4°C

**Figure 2.** Dynamics of formation and accumulation of aminic nitrogen in beef during marination (0-2 days) and subsequent storage at 4°C

Non-protein nitrogen and free amino acids had an increasing evolution during the entire ageing period. The non-protein nitrogen, respectively free amino acids were influenced by the treatment applied to meat samples and ageing time. The accumulation of non-protein nitrogen and of free amino acids in marinated samples was higher than in control samples, where the ageing is realized under the action of the muscular tissue enzymes. Most probably in the ageing process the of control samples were also involved the endogenous proteolytic enzymes such as proteinases activated by Ca\(^{2+}\) ions (calpaines), lysosomal proteinases...
The tenderization of bovine *Biceps femoris* muscle using marinades on the basis of wine

(cathepsines B, D, L, H). So the participation of proteolytic enzymes produced by the microbial flora of meat cannot be excluded.

Non-protein nitrogen and free amino acids levels, increased considerably in the first 48 hours of sample marination, followed by a slower increase period, which coincided with meat samples packaging and refrigeration at 4°C. Non-protein nitrogen and free amino acids accumulation in control samples increased during the whole storage time. The increase in non-protein nitrogen levels improves beef tenderness and also the degree of assimilation of nitrogen compounds from marinated beef.

The highest values in non-protein nitrogen and free amino acids levels (aminic nitrogen) were registered after 14 days of ageing at 4°C, in marinade 5 – with thyme, marjoram and horseradish (0,1457 aminic nitrogen, g/100g, respectively 0,647g/100g) while the smallest levels were registered in control samples.

**Spices and marination influence on connective tissue proteins hydrolysis**

In present study, the collagenic action of marination mixes was determined by determination of the hydroxyproline accumulation from the expressed liquid at marinated meats boiling and refrigeration storage at 4°C, under anaerobic conditions (Table 3). The expressed liquids were collected, clarified and used for hydroxyproline dosage. The values of free hydroxyproline and retrievable collagen contents, presented in table 3, are pointing out the hydrolytic action of marinades on beef collagen. The solubilization degree of collagen is depending on marinade types and ageing period. Significant differences between marinades used in this study couldn’t be noticed.

**Table 3. Influence of spices and marination of adult beef on the accumulation of hydroxyproline***

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Control</th>
<th>Marinade 1</th>
<th>Marinade 2</th>
<th>Marinade 3</th>
<th>Marinade 4</th>
<th>Marinade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>92.34±0.26</td>
<td>92.34±0.26</td>
<td>92.34±0.26</td>
<td>92.34±0.26</td>
<td>92.34±0.26</td>
<td>92.34±0.26</td>
</tr>
<tr>
<td>2</td>
<td>109.95±0.34</td>
<td>112.95±0.34</td>
<td>126.44±0.30</td>
<td>118.35±0.54</td>
<td>124.67±0.45</td>
<td>125.67±1.18</td>
</tr>
<tr>
<td>5</td>
<td>109.76±0.98</td>
<td>124.55±0.89</td>
<td>132.81±0.72</td>
<td>120.32±0.42</td>
<td>125.03±0.23</td>
<td>125.95±0.38</td>
</tr>
<tr>
<td>8</td>
<td>111.54±0.32</td>
<td>133.27±0.40</td>
<td>140.66±1.07</td>
<td>130.56±0.03</td>
<td>135.37±1.57</td>
<td>149.75±1.00</td>
</tr>
<tr>
<td>11</td>
<td>115.13±1.45</td>
<td>134.83±1.16</td>
<td>140.85±0.35</td>
<td>134.33±0.38</td>
<td>131.78±0.99</td>
<td>155.25±0.77</td>
</tr>
<tr>
<td>14</td>
<td>126.86±0.86</td>
<td>168.08±0.74</td>
<td>167.52±0.33</td>
<td>170.96±0.22</td>
<td>173.48±0.53</td>
<td>182.3±0.46</td>
</tr>
</tbody>
</table>

* Medium values and standard deviations

The increase of the ageing period lead to increasing of the hydroxyproline levels, maximum level being reached at the end of the largest period of marination (namely 14 days at 4°C). After this period it could be seen an increase of 1.82 times in free hydroxyproline in marinated samples of marinade 1 – red dry wine, tile honey, garlic, pepper; 1.81 times in marinated samples of marinade 2 – with thyme addition; 1.85 times in marinated samples of marinade 3 – with added marjoram; 1.87 times in marinated samples of marinade 4 – horseradish and 1.97 times in marinated samples of marinade 5 – with added thyme, marjoram and horseradish compared to control. The hydroxyproline level in control sample was lower, including the liberated hydroxyproline, as a result of endogenous collagenases and liberated hydroxyproline during the thermal treatment of beef.

Studies about meat tenderness showed that this feature is conditioned by myofibrillar proteins and also by the connective tissue proteins, especially collagen. Collagen is the predominant perimisial and endomisial connective tissue protein, representing 1.6 – 14.1 g/100 g of dry matter in meat. The connective tissue is one of the most important factors involved in meat tenderness, having 10% weight factor from the total number of influence factors. The perimisium connective tissue, representing about 90% of muscular connective tissue, is believed to have a major contribution in meat hardness (SIMELA [37]).
The increase of collagen content may favor the meat hardness, the highest level being observed in very young or very old animals. The tenderness decrease in meat is mainly related to the nature and number of cross-links between collagen fibers. The cross-links number increases with animal age, having an influence in collagen solubility. The proteoglycans presence, known as extracellular matrix producers, represents a supplementary factor influencing meat tenderness (NISHIMURA & al. [38]). It is considered that proteolytic enzymes act on the proteoglycans which are binding the fibrillar collagen and the collagens that are acting as interfibrilar bonds. After proteoglycans degradation, especially decorine, which protects the collagen fibres from proteolysis, the collagen may be susceptible of degradation.

**Spices and marination influence on adult beef tenderness**

Tenderness is the most important feature in meat texture and has the greatest influence on consumer’s perception. It is well known the fact that ageing process improves the beef tenderness because of proteolytic degradation of the myofibrillar fractions (KOOHMARAIE & al. [39]). In this study a progressive decrease in meat hardness along with the ageing period increasing in adult marinated beef samples and stored under anaerobic conditions could be observed (Table 4).

**Table 4.** Effects of spices and marination on textural properties of adult beef stored in anaerobic conditions at 4°C

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Control</th>
<th>Marinade 1</th>
<th>Marinade 2</th>
<th>Marinade 3</th>
<th>Marinade 4</th>
<th>Marinade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.11 ± 0.80</td>
<td>8.11 ± 0.80</td>
<td>8.11 ± 0.80</td>
<td>8.11 ± 0.80</td>
<td>8.11 ± 0.80</td>
<td>8.11 ± 0.80</td>
</tr>
<tr>
<td>2</td>
<td>7.96 ± 0.36</td>
<td>7.41 ± 0.36</td>
<td>7.59 ± 0.87</td>
<td>7.83 ± 1.12</td>
<td>6.51 ± 0.79</td>
<td>6.27 ± 1.32</td>
</tr>
<tr>
<td>5</td>
<td>7.84 ± 1.12</td>
<td>7.38 ± 0.97</td>
<td>7.12 ± 0.96</td>
<td>7.95 ± 0.84</td>
<td>6.38 ± 0.78</td>
<td>5.77 ± 0.47</td>
</tr>
<tr>
<td>8</td>
<td>7.87 ± 0.40</td>
<td>6.51 ± 0.47</td>
<td>6.24 ± 1.56</td>
<td>6.05 ± 0.68</td>
<td>5.24 ± 0.79</td>
<td>4.52 ± 0.87</td>
</tr>
<tr>
<td>11</td>
<td>7.48 ± 1.87</td>
<td>5.67 ± 1.42</td>
<td>5.32 ± 0.59</td>
<td>5.84 ± 0.95</td>
<td>5.82 ± 1.35</td>
<td>4.72 ± 1.26</td>
</tr>
<tr>
<td>14</td>
<td>6.34 ± 1.36</td>
<td>4.51 ± 0.93</td>
<td>4.39 ± 0.43</td>
<td>4.53 ± 0.24</td>
<td>4.34 ± 0.39</td>
<td>3.80 ± 0.72</td>
</tr>
</tbody>
</table>

* Medium values and standard deviations

Generally, by marination, adult beef tenderness was improved, but the modifications at the myofibrillar system level weren’t significantly different between marinated samples. The softening of the muscular tissue, even after 14 days of storage at 4°C, being similar in samples from 1 to 4 and slightly higher in sample 5 (table 4). The control sample presented a final higher hardness compared with the marinated samples in red dry wine with spices and seasonings.

The ingredients used in the preparations of acid marinades are, generally, organic acid solutions (acetic acid, lactic acid, citric acid, etc.), different types of vinegar, wines and fruit juice (BURKE & al. [40]). The meat tenderization mechanism with acid marinades isn’t completely known. It is believed that organic acids are involved in the muscle structure decay because the water absorption; improvement of the cathepsines activity and increase of collagen conversion to gelatin at low pH during cooking (BERGE & al. [25]). The connective tissue has an important role in beef tenderization. The acid breaks the transversal bounds of collagen, leading to the unstable structure loss of this connective tissue protein. Many studies pointed out that the low meat pH after the marination has positive effects on the texture, increasing the water holding capacity and the moisture content and also decreasing the thermal treatment losses.
Conclusions

The marination of adult beef *biceps femoris* muscle in marinades consisting of wine, honey, garlic with different spices and seasonings addition, lead to a decrease in pH values, an increase in free amino acids, non-protein nitrogen, hydroxyproline accumulation and a decrease in meat hardness. These results suggest that the marinades action, on myofibrillar proteins and on the connective tissue, had a positive effect on the tenderness of the adult beef *biceps femoris* muscle.

Optimal version of marinating of adult beef *biceps femoris* muscle was marinade 5 consisting of dry red wine, honey, garlic, thyme, marjoram, horseradish, pepper and salt.

Acknowledgements

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