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Full Length Research Paper

Influence of spice and wine based marinades on bovine *Biceps femoris* muscle tenderness

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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 prepared without marinating treatment but packed and stored in the same conditions as marinated samples. After marination, meat pieces were packed under vacuum and stored at 4°C for 12 days. The combined effects of spices and marination on beef tenderness were evaluated by monitoring pH evolution, the protein and collagen degree of hydrolysis and tenderness. Experimental data indicated that marination weaken beef meat structure, producing improvement of functional properties of adult beef. Marinades use lead for an increase in the protein nitrogen fraction, 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.

Key words: Acidic marination, beef meat, marinating time, meat hardness, pH.

INTRODUCTION

Of all the organoleptic characteristics that contribute to meat quality, tenderness is recognized as the most important factor (Koohmaraie, 1992), and consumers are willing to pay more for beef that is guaranteed to be tender (Shackelford et al., 2001). Meat tenderness can be related principally 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 (Sawdy et al., 2004).

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 (Belew et al., 2003). 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; Ionescu et al., 2008), 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; Istrati et al., 2008), calcium ions reduce the colour stability of fresh meat and decrease the product shelf life (Bekhit et al., 2005). Marinating is a way by which consumers can improve tenderness, add taste and variety to the meat component of meals. Marination is the process of soaking or injecting

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meat with a solution containing ingredients such as vinegar, lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids (Pathania et al., 2010). Moreover, the shelf life of the meat may be positively affected by this process due to the acidic or alkaline nature of the solution and also the antimicrobial and antioxidant activity of some marinade ingredients (Kargiotou et al., 2011). Marinades with a tenderizing capacity are particularly important in applications involving muscles rich in connective tissue. These muscles are the cheaper carcass cuts, and the tenderizing effect of marinating offers a commercially important solution to improve them (Gault, 1991). Therefore, the purpose of our study was to investigate the combined effects of spice and wine base marinades on bovine *Biceps femoris* muscle tenderization.

MATERIALS AND METHODS

The raw material utilized in this research program was represented by the beef thigh from adult animals (*Biceps femoris* muscle; breed: Holstein Friesian; sex: female; age: 5 years). The meat was purchased in refrigerated state from a local slaughterhouse at maximum of 24 h post-slaughter. Salt was of food-suitable purity, being a largely used additive in meat industry. Marjoram (*Majorana hortensis)* and garlic (*Allium sativum)* was 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

Marinades composition is presented in Table 1. Marinades were left at room temperature with intermittent agitation for at least one hour, to allow the dry ingredients to hydrate (marinades pH was 5.2). Control samples were represented by raw meat without marinating treatment but packed and stored in the same conditions as marinated samples.

Marinating treatment 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 fibres into 31 parts with the same size (10 × 6 × 2 cm) and shape, weighing approximately 100 g. For each marinating 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 even 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 24 h, the meat pieces 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 for 5 min at 4°C, and then they were vacuum packaged in polypropylene bags, type Side seal bags PA/PE, allfo Vakuumverpackungen, Frankfurt, Germany (thickness: 90 μm; gas permeability: water vapours: 2.6 g/m^2d; O_2: 50 cm^3/m^2d; CO_2: 150 cm^3/m^2d; N^2: 10 cm^3/m^2d; mechanical strength: tensile strength MD: 40 to 50 N/15 mm; tensile strength TD: 30 to 40 N/15 mm; sealing temperature: 100 to 180°C; temperature consistency: -50/+90°C) and were stored at 4°C for 12 days in a storage chamber.

Analytical methods

The following determinations were carried out in order to characterise the beef meat used: water content according to the AOAC (1995) method, total nitrogen content according to SR ISO 9037 (2007) standard (for samples digestion and distillation was utilized by Kjeldahl Velp Scientifica UDK 127 System), fat content according to the AOAC (1984) method utilizing Fat Extractor SER 148 and pH with a micro pH 2002 pH-meter (CRISON Instruments S.A., Barcelona, Spain) according to AOAC (1984) method. Protein degree of hydrolysis was estimated by the determination of non-protein nitrogen according to AOAC (1990) method and aminic nitrogen according to the method described by Vata et al. (2000). Collagen degree of hydrolysis was estimated by the determination of hydroxyproline (HP) content of the meat sample based on the procedure of Nueman and Logan (1950) with few modifications as suggested by Naveena and Mendiratta (2001). Two gram meat sample was hydrolysed with 40 mL of 6 N HCl for 18 h at 108°C. The hydrolysate was filtered, and the volume adjusted to 50 ml with distilled water. Then, 25 ml of hydrolysate was taken and pH was adjusted to 7.0, using 40% NaOH and the volume was adjusted to 50 ml again with distilled water. One millilitre of aliquot from this solution was used for hydroxyproline estimation. Absorbance was measured at 540 nm, using UV VIS Double Beam PC and Scanning auto cell spectrophotometer, model UVD-3200 (Labomed, Inc., U.S.A) and the hydroxyproline content was determined by referring to a standard graph.

Meat tenderness was measured by textural tests in TA.XT Plus texture analyzer (Stable Micro Systems, Surrey, United Kingdom); hardness: 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 room temperature, placing vacuum package bags in a circulatory water bath set at 18°C during a period of 30 min, and the percentage 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 fibre direction and then compressing to 80% with a compression probe of 19.85 cm^2 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 Statistica 5.1. Programme for Windows-Microsoft excel statistics. Means and standard deviations were calculated among samples.

RESULTS AND DISCUSSION

The present study was realized at a laboratory level in model systems, using raw material as adult beef muscle (*biceps femoris)* purchased at 24 h after slaughtering. Experimental data, showing the chemical composition of adult beef used in the present study are presented in

Table 1. Marinades composition.

Marinade ingredient	U.M	Sample					
		Control	Marinade 1	Marinade 2	Marinade 3	Marinade 4	Marinade 5
Dry red wine	ml/kg	-	300	300	300	300	300
Lime-tree honey	g/kg	-	40	40	40	40	40
Allium sativum	g/kg	-	9	9	9	9	9
Thymus vulgaris	g/kg	-	-	4	-	-	4
Majorana hortensis	g/kg	-	-	-	4	-	4
Armoracia rusticana	g/ kg	-	-	-	-	4	4
Pepper	g/kg	-	2	2	2	2	2
Salt	%	-	5	5	5	5	5

Table 2. Chemical composition of beef.

Sample	Chemical component					
	Moisture (g/100 g)	Dry substance (g/100 g)	Total nitrogen (g/100 g)	Fat (g/100 g)	Non-protein nitrogen (g/100 g)	Aminic nitrogen (g/100 g)
Biceps femoris muscle	76.8±1.78	23.2±1.21	2.72±0.13	5.82±0.29	0.379±0.01	0.112±0.03

Values are given as mean ± standard deviations.

Table 3. Changes in pH values of beef meat during marination (0 to 2 days) and subsequent storage at 4°C.

Storage time (days)	pH					
	Control	Marinade 1	Marinade 2	Marinade 3	Marinade 4	Marinade 5
0	5.75 ± 0.04^{a}	5.75 ± 0.04^{a}	5.75 ± 0.04^{a}	5.75 ± 0.04^{a}	5.75 ± 0.04^{a}	5.75 ± 0.04^{a}
2	5.85 ± 0.06^{a}	5.06 ± 0.28^{c}	5.07 ± 0.07^{c}	5.07 ± 0.28^{cd}	5.06 ± 0.27^{cd}	5.06 ± 0.33^{cd}
5	5.96 ± 0.03^{ab}	4.92 ± 0.28^{cd}	4.9 ± 0.20^{cd}	4.93 ± 0.21^{d}	4.94 ± 0.14^{d}	4.94 ± 0.18^{d}
8	6.13 ± 0.11^{ab}	4.96 ± 0.71^{d}	4.95 ± 0.20^{d}	4.97 ± 0.11^{d}	4.96 ± 0.13^{d}	4.96 ± 0.21^{d}
11	6.28 ± 0.19^{b}	5.07 ± 0.28^{d}	5.05 ± 0.27^{d}	5.08 ± 0.07^{d}	5.11 ± 0.11^{d}	5.14 ± 0.07^{d}
14	6.36 ± 0.14^{b}	5.18 ± 0.14^{d}	5.15 ± 0.04^{d}	5.17 ± 0.07^{d}	5.22 ± 0.07^{d}	5.24 ± 0.03^{d}

Values are given as mean ± standard deviations; values in the same row and column followed by different superscripts are significantly different ($p < 0.05$).

Table 2. Analyzed beef presented a dark red colour, a good texture, gross muscular fibres, well highlighted, dry surface and full-grown connective tissue.

Marination influence on adult beef pH values

Beef meat marination and storage at 4°C had a great influence in pH values (Table 3). Values in Table 3 show significant differences ($p < 0.05$) between control and marinated samples. During the whole storage period, pH values in the control sample was higher, increasing continuously, from 5.75 to 6.36, while in marinated samples a decrease and an increase in pH values was observed. The decrease in pH values of the experimental samples was observed after the marination and in the first days of storage when we found the lowest pH values (4.90 to 4.94). There were no significant differences between marinated samples; this fact allowed us to conclude that spices addition had no effect on pH evolution. The pH decrease in experimental samples during marination and refrigeration storage may be explained by organic acids from wine absorbtion by meat and lactic acid production by lactic acid bacteria. Honey was the nutritive substrate for lactic acid bacteria. Lactic acid acumulation in time, led to a decrease in pH values in marinated samples. The increase in the pH values was observed in marinated samples after the 5th day of storage, and in the control samples, may be determined by the processes involved in meat maturation at

Table 4. Evolution of non protein nitrogen in beef during marination (0 to 2 days) and subsequent storage at 4°C.

Storage time (days)	Non-protein nitrogen (g/100 g)					
	Control	Marinade 1	Marinade 2	Marinade 3	Marinade 4	Marinade 5
0	0.379±0.11^{a}	0.379±0.11^{a}	0.379±0.11^{a}	0.379±0.11^{a}	0.379±0.11^{a}	0.379±0.11^{a}
2	0.395±0.13^{ab}	0.445±0.09^{ab}	0.445±0.06^{ab}	0.447±0.10^{ab}	0.453±0.03^{ab}	0.457±0.14^{ab}
5	0.433±0.10^{b}	0.471±0.10^{b}	0.475±0.10^{b}	0.477±0.08^{b}	0.484±0.06^{b}	0.493±0.12^{b}
8	0.459±0.20^{b}	0.500±0.12^{bc}	0.503±0.10^{bc}	0.506±0.11^{bc}	0.513±0.10^{bc}	0.523±0.10^{bc}
11	0.467±0.12^{b}	0.541±0.07^{bc}	0.545±0.11^{bc}	0.547±0.10^{bc}	0.555±0.12^{bc}	0.566±0.17^{bc}
14	0.471±0.15^{b}	0.623±0.11^{c}	0.623±0.13^{c}	0.621±0.15^{c}	0.635±0.15^{c}	0.647±0.17^{c}

Values are given as mean ± standard deviations; values in the same row and column followed by different superscripts are significantly different ($p < 0.05$).

Table 5. Evolution of aminic nitrogen in beef during marination (0 to 2 days) and subsequent storage at 4°C.

Storage time (days)	Aminic nitrogen (g/100 g)					
	Control	Marinade 1	Marinade 2	Marinade 3	Marinade 4	Marinade 5
0	0.112±0.003^{a}	0.112±0.003^{a}	0.112±0.003^{a}	0.112±0.003^{a}	0.112±0.003^{a}	0.112±0.109^{a}
2	0.112±0.001^{a}	0.130±0.007^{ab}	0.135±0.01^{ab}	0.136±0.018^{ab}	0.134±0.014^{ab}	0.137±0.127^{ab}
5	0.118±0.004^{ab}	0.137±0.001^{b}	0.136±0.011^{b}	0.137±0.008^{b}	0.139±0.005^{b}	0.138±0.128^{b}
8	0.119±0.001^{ab}	0.137±0.002^{b}	0.138±0.011^{b}	0.137±0.016^{b}	0.139±0.011^{b}	0.139±0.136^{b}
11	0.121±0.004^{ab}	0.138±0.002^{b}	0.137±0.015^{b}	0.137±0.016^{b}	0.140±0.013^{b}	0.142±0.128^{b}
14	0.121±0.005^{ab}	0.139±0.003^{b}	0.138±0.018^{b}	0.138±0.017^{b}	0.144±0.010^{b}	0.145±0.134^{b}

Values are given as mean ± standard deviations; values in the same row and column followed by different superscripts are significantly different ($p < 0.05$).

refrigeration storage temperatures. These results are in agreement with that reported by Burke et al. (2003) and Kargiotou et al. (2011). 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).

Marination influence on muscle tissue proteins hydrolysis

Non-protein nitrogen and free amino acids had an increasing evolution during the entire ageing period (Tables 4 and 5). The non-protein nitrogen, respectively free amino acids, was influenced by the treatment applied to meat samples and ageing time. We found significant differences ($p < 0.05$) between the marinated samples and control, starting from 8th day till the 14th day of ripening. The accumulation of non-protein nitrogen and of free amino acids in marinated samples was higher than in the control samples, where the ageing is realized under the action of muscular tissue enzymes. We consider that the ageing of control samples were also involved in the endogenous proteolytic enzymes such as proteinases activated by Ca^{2+} ions (calpaines), and lysosomal proteinases (cathepsines B, D, L, H). We cannot exclude the participation of proteolytic enzymes produced by the microbial flora of meat. Non-protein nitrogen and free amino acids levels increased considerably in the first 48 h 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 nitrogenous compounds from marinated beef.

The highest values in non-protein nitrogen and free amino acids levels (aminic nitrogen) were registered after 14 days of ripening at 4°C, in marinade 5: with thyme, marjoram and horseradish (0.146 aminic nitrogen, g/100 g, respectively, 0.647 g/100 g) while the lowest levels were registered in the control samples. We found no significant differences ($p < 0.05$) between aminic nitrogen levels in marinated samples compared to the control samples. These results in non protein nitrogen and aminic nitrogen levels show that a weak proteolysis was present in the myofibrillar and the sarcoplasmic proteins of the meat system. The limited proteolytic processes led to polypeptides, small peptides and amino acids (components of the non protein nitrogen) accumulation which have an important role in defining the desired meat

Table 6. Influence of spices and marination of adult beef on the accumulation of hydroxyproline.

Storage time (days)	Hydroxyproline (μg/100 g s.u)					
	Control	Marinade 1	Marinade 2	Marinade 3	Marinade 4	Marinade 5
0	92.34±0.11^{a}	92.34±0.11^{a}	92.34±0.11^{a}	92.34±0.11^{a}	92.34±0.11^{a}	92.34±0.11^{a}
2	109.95±1.97^{b}	112.95±1.02^{b}	126.44±0.75^{b}	118.35±0.47^{b}	124.67±0.68^{b}	125.67±0.51^{b}
5	109.76±0.82^{b}	124.55±1.30^{c}	132.81±0.98^{c}	120.32 ±0.95^{c}	125.03±0.88^{b}	125.95±0.72^{b}
8	111.54±0.47^{c}	133.27±0.72^{d}	140.66±0.17^{d}	130.56±0.31^{d}	135.37±0.25^{c}	149.75±0.82^{c}
11	115.13±0.58^{d}	134.83±0.27^{e}	140.85±0.57^{d}	134.33±0.62^{e}	131.78±0.81^{d}	155.25±0.82^{d}
14	126.86±0.76^{e}	168.08±0.83^{f}	167.52±0.31^{e}	170.96±0.66^{f}	173.48±0.52^{e}	182.3±0.24^{e}

Values are given as mean ± standard deviations; values in the same row and column followed by different superscripts are significantly different ($p < 0.05$).

flavour. During the refrigeration period, a series of biochemical and physicochemical modifications occur in meat. Tissue enzymes are the first to act, followed by bacterial enzymes. 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 an aliment. These modifications, taking place in the post mortem period are leading towards meat ageing (Koohmaraie, 1996).

Marination influence on connective tissue proteins hydrolysis

The role of collagen is of particular interest, as it has been proposed that collagen is actually the determining factor in the textural differences among various muscles (Bailey, 1989). The values of free hydroxyproline content presented in Table 6, are pointing out the hydrolytic action of wine base marinades on adult beef *Biceps femoris* muscle. The solubilization degree of collagen was influenced by marinade type and ageing period. In the present study, significant differences in hydroxyproline content were observed between control and wine based marinades ($p < 0.05$). The increase of the ageing period led to an increase in the hydroxyproline levels, maximum level being reached at the end of the 14th day. After this period, we could see 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 the control. The hydroxyproline level in the 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 to 14.1 g/100 g of dry matter from meat. The connective tissue is one of the most important factors involved in meat tenderness, having a 10% weight factor from the total number of factors. The perimisium connective tissue representing about 90% of muscular connective tissue is believed to have a major contribution in meat hardness (Simela, 2005). The increase of collagen content may favour 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 fibres. The cross-links number increases with animal age, having an influence in collagen solubility (Nishimura et al., 2003). The mechanism by which collagen molecules in a fibre are rendered soluble is unclear. The two processes that could be responsible are (1) peptide bond hydrolysis; and (2) slow breakage of covalent cross-links. The rate of aspartyl peptide bond hydrolysis increases markedly as the pH falls from 6 to 4 (Offer and Knight, 1988), and this could explain why acid marinades help to tenderize meat. The alternative mechanism by which collagen may be solubilised is through breakdown of the cross-links. Some of these bonds are easily ruptured by pH changes, heat or denaturing agents, and other links, while relatively stable, may still breakdown slowly particularly at acid pH (Offer and Knight, 1988). A second possibility is that the optimal pH for activity of cathepsins is in the range of 3.5 to 5.0 and hence the lowering of meat pH in an acid marinade may well enhance proteolytic attack by these enzymes.

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 a well known fact that ageing process improves beef tenderness because of proteolytic degradation of the

Table 7. Effects of spices and marination on textural properties of adult beef stored in anaerobic conditions at 4°C.

Storage time (days)	Hardness (kg)					
	Control	Marinade 1	Marinade 2	Marinade 3	Marinade 4	Marinade 5
0	8.11±0.62^{a}	8.11±0.62^{a}	8.11±0.62^{a}	8.11±0.62^{a}	8.11±0.62^{a}	8.11±0.62^{a}
2	7.96±0.06^{a}	7.41±0.17^{a}	7.59±0.14^{a}	7.83±0.17^{a}	6.51±0.41^{b}	6.27±0.57^{b}
5	7.84±0.71^{a}	7.38±0.18^{a}	7.12±0.06^{a}	7.95±0.57^{a}	6.38±0.24^{b}	5.77±0.17^{b}
8	7.87±0.59^{a}	6.51±0.08^{b}	6.24±0.21^{b}	6.05±0.13^{b}	5.24±0.75^{c}	4.52±0.37^{c}
11	7.48±0.06^{a}	5.67±0.31^{b}	5.32±0.27^{c}	5.84±0.55^{b}	5.82±0.71^{c}	4.72±0.28^{c}
14	6.34±0.14^{b}	4.51±0.31^{c}	4.39±0.38^{d}	4.53±0.21^{c}	4.34±0.33^{d}	3.80±0.38^{d}

Values are given as mean ± standard deviations; values in the same row and column followed by different superscripts are significantly different ($p < 0.05$).

myofibrillar fractions (Koohmaraie et al., 2002). In this study we could see a progressive decrease in meat hardness, along with the ageing period increase in adult marinated beef samples stored under anaerobic conditions (Table 7). Significantly decreases in meat hardness were observed in wine base marinades samples, compared to the control ($p < 0.05$). Generally, by marination, adult beef tenderness was improved, modifications at the myofibrillar system level being significantly different between marinated samples ($p < 0.05$) and at different sampling points. Marination in base marinades consisting of wine, honey, garlic, pepper and salt with the addition of horseradish decrease meat toughness compared to the other treatments. Horseradish is a seasoning plant commonly used in traditional marinades for red meats. It is a powerful meat tenderizer and the ancients used it to tenderize dried meats (Schar, 2010). However, further studies are required in order to evaluate the tenderization mechanism of the horseradish on meat cuts.

The control sample presented a final higher hardness compared with the marinated samples in red dry wine with the addition of spices and seasoning plants. Similar observations were reported by Burke et al. (2003) and Kargiotou et al. (2011). 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 et al., 2003). The meat tenderization mechanism with acid marinades is not completely known. It is believed that organic acids are involved in the muscle structure decay because of the water absorption; improvement of the cathepsines activity and increase of collagen conversion to gelatine at low pH during cooking (Berge et al., 2001). 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. Gault (1991) and Offer and Knight, (1988) found 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.

Conclusion

This study showed that beef marinating process in marinades consisting of wine, honey, garlic with different spices and seasoning plants led to beef muscle (*Biceps femoris*) tenderization. The mechanism of tenderization appears to involve a decrease in pH values, an increase in protein hydrolysis and solubilization of connective tissue collagen. Due to the richness of beef meat in connective tissue and tendency to be of low economic value, these results suggest that marinating may by way add value to these cuts and prevent economic losses. Therefore, 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*) might be used as a marinating agent for improving the tenderness of tough beef meat.

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