PAPER

EFFECTS OF HIGH PRESSURE AND MARINATION TREATMENT ON TEXTURE, MYOFIBRILLAR PROTEIN STRUCTURE, COLOR AND SENSORY PROPERTIES OF BEEF LOIN STEAKS

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ABSTRACT

The influence of high pressure/marination treatment on the texture, myofibrillar protein structure, color and sensory properties of beef loin steaks was studied. Combined high pressure and marination treatment at 550 MPa significantly increased beef tenderness, but had a "whitening/brightening" effect on the color of the samples ($P<0.05$). High-pressure processing caused protein degradation, leading to texture development. Furthermore, the panelists gave the highest overall impression score to the 150 MPa pressurized samples. These results show that combined high pressure and marination treatment at 550 MPa can potentially improve the textural properties of beef loin steaks, although it is less favored than pressurization treatment.

Keywords: beef, high pressure, marination, protein degradation, texture

1. INTRODUCTION

High pressure processing has been a hot topic of study among scientists because of its positive effects on the safety, quality and sensory properties of food products (EVRENDİLEK *et al.*, 2008). Addressing the increasing demand for minimally processed foods with high nutritional and sensory quality, the food industry has employed high pressure processing to develop high quality, fresh, additive-free food products with an extended shelf life. In the European Community, high pressurized foods are classified as ''novel foods'' (CAMPUS, 2010). This novel technology also offers an alternative to pasteurization treatment and could have great potential for heat-sensitive foods (DURANTON *et al.*, 2011).

There is particular interest in researching the effects of high pressure on the food matrix (SUN and HOLLEY, 2010). It has been discovered very recently that short-time high pressure application at lower temperatures develops tenderness in meat and meat products while minimally changing the natural characteristics of the product (BAJOVIC *et al.*, 2012). It has been hypothesized that applying pressure to meat in the postmortem period could cause changes to the enzymes and proteins, especially to the gelatin characteristics of myofibrillar proteins, color, microbial load, ultrastructure and textural properties of meat (SCHENKOVA *et al.*, 2007). In research studies, pressure levels applied to post-rigor meat generally ranged from 100 to 600 MPa with short processing times (5-20 min.) at 15-60ºC, according to the purpose of the study (CHAN *et al.*, 2011; KRUK *et al.*, 2011; MCARDLE *et al.*, 2013; GROSSI *et al.*, 2014; SIKES and TUME, 2014; GIMENEZ *et al.* 2015). Researchers have also reported that high pressure technology is a physical additive-free process for meat tenderizing and softening due to its effects on the gel-forming ability of proteins and on texture (BUCKOW *et al.*, 2013). The effective pressure levels have varied from 150 to ≥500 MPa (5 min, 20ºC) for meat tenderization (SUN and HOLLEY, 2010). Furthermore, post-rigor meat tenderization without bleaching of color can be achieved with pressure levels up to 300 MPa for a few minutes at room temperature (CAMPUS, 2010).

Marination treatment using plant additives is another natural way to preserve meat and meat products. In recent years, natural plant extracts with high phenolic contents have been used in meats, due to their safety characteristics and beneficial effects on health, synthetic chemical preservatives. There are numerous studies about plant extracts (such as grape seed, green tea, pomegranate, peanut skin, garlic, rosemary, olive leaf, moringa leaf, nettle, myrtle, and mint leaf extracts) used in meat and meat products (AKARPAT *et al.*, 2008; ALP and AKSU, 2010; YU *et al.*, 2010; DEVATKAL *et al.*, 2010; HAYES *et al.*, 2010; COLINDRES and BREWER, 2011; RABABAH *et al.*, 2011; BISWAS *et al.*, 2012; DAS *et al.*, 2012; ÖZVURAL and VURAL, 2012; CAO *et al.*, 2013). Among these natural extracts, oleoresin rosemary (Herbalox®) has been commonly included in food processing as a shelf life extender and flavor developer (AHN *et al.*, 2007). In addition, there are many studies in the literature about the antimicrobial and antioxidant activities of rosemary in different food materials (BOTSOGLOU *et al.*, 2007; SASSE *et al.*, 2009; NIETO *et al.*, 2010; PUANGSOMBAT and SMITH, 2010; COLINDRES and BREWER, 2011; WOJCIAK *et al.*, 2011; GIBIS and WEISS, 2012; MATHENJWA *et al.*, 2012; KIM *et al.*, 2013). The literature studies reported that the effective usage level of rosemary extract varied between 0.02-10% in a marinade solution for retarding lipid oxidation and improving sensorial characteristics in meat and meat products (AHN *et al.*, 2007; AKARPAT *et al.*, 2008; ROJAS and BREWER, 2008; WOJCIAK *et al.*, 2011).

The use of a combination of pressure and marination treatment can be an alternative preservation method for meat producers. The combined treatment of pressure and marination can be more efficient at improving meat quality attributes and increasing shelf life. It was reported that the combined treatment of high pressure and natural antioxidants as a multi-hurdle approach can be an alternative treatment in the meat industry (HYGREEVA and PANDEY, 2016). However, there are relatively few studies regarding the combined use of high pressure and natural extracts in meat and meat products, and generally chemical preservatives were used for marination in these studies (SCHENKOVA *et al.*, 2007; OHNUMA *et al.*, 2013; KIM *et al.*, 2014; GIMENEZ *et al.*, 2015; RODRIGUES *et al.*, 2016). In addition, oregano, rosemary, papain plants and carvacrol were used as natural antioxidants for meat and meat products in the studies evaluating a combination of high pressure and marination treatment (BRAGAGNOLO *et al.*, 2005; GOMEZ-ESTACA *et al.*, 2007; de OLIVEIRA *et al.*, 2015). Although very few studies have been published about the effects of rosemary extract and high pressure treatments on sardines and chicken breast meat, to the best of our knowledge, there has been a lack of information about the effects of combined high pressure and rosemary extract marination on beef quality (BRAGAGNOLO *et al.*, 2005; GOMEZ-ESTACA *et al.*, 2007). The literature studies about combined use of high pressure and natural antioxidants are still in an early stage, and more studies are needed to be conducted (HYGREEVA and PANDEY 2016). According to these informations, the main goal of this study was to research the combined effects of high pressure and marination treatment on the textural, color and sensory properties of beef loin steaks and to improve natural new textured meat products.

2. MATERIALS AND METHODS

2.1. Materials

Beef loin steaks were supplied by a local retail butcher and cut into 2×10×4 (height×width×length) cm uniform portions weighing an average of 50-70 g before high pressure and marination treatment. Oleoresin rosemary extract (Herbalox® Type W seasoning oil) was supplied by Kalsec Inc. (Kalamazoo, Michigan, USA) and used for marination. It is dispersible in water (polar carriers) and oil (nonpolar carriers) with agitation and has a brown, viscous, liquid appearance.
Eight groups of samples were used in the experiments based on high pressure treatment and high pressure/marination treatment. The samples were divided into (i) 0:control (non-pressurized samples), (ii) 150/0 (150 MPa HPP), (iii) 350/0 (350 MPa HPP), (iv) 550/0 (550 MPa HPP), (v) 1: marinated, non-pressurized sample (vi) 150/1 (150 MPa HPP/marination), (vii) 350/1 (350 MPa HPP/marination) and (viii) 550/1 (550 MPa HPP/marination) groups. All experiments were carried out in triplicate.

2.2. Marination treatment

Marinades were prepared with oleoresin rosemary extract. Preliminary experiments were performed to determine the appropriate marinade concentration for preserving and developing meat quality characteristics. Each sample was placed in a polyamide/polyethylene bag (Apack Ambalaj, İstanbul, Turkey) containing 10 ml of marination solution (including 5% oleoresin rosemary extract) and was kept overnight at 4ºC. On the following day, the marinades were removed from the packages, and all

samples were vacuum packaged in double pouches to prevent contamination of the samples by the pressurization medium from bags breaking due to pressurization.

2.3. High-pressure processing

High-pressure processing was applied to the non-marinated and marinated samples. As a result of adiabatic heating, pressure treatment increases the temperature of pressure-transmitting fluid and samples, depending on the product composition and initial temperature of the sample (KOCA *et al.*, 2011). For this reason, the initial temperatures of the samples were adjusted before the high pressure treatment, and the final temperature of the samples after pressurization was monitored with a computer program and found to be approximately 20±2ºC.
The high pressure process was carried out in a MSE-CIP-WB-5500 high pressure food processor (MSE Teknoloji Ltd., Gebze, Turkey) with a 0.7 L vessel volume. Propylene glycol (Kimetsan Co., Ltd., Ankara, Turkey) was used as the pressure-transmitting fluid. The pressure vessel was surrounded by coils connected to a cooling circulator (model RE1050S, Lauda Dr R. Wobser GmbH & Co. KG., Germany). The temperature of the pressure vessel and the pressure-transmitting fluid inside the pressure vessel were controlled with these coils. The inherent ramp rate was 5 MPa/s, and the pressure was increased to the test pressures of 150 MPa, 350 MPa and 550 MPa within approximately 30 s, 70 s and 110 s, respectively. The samples were held at test pressures for 5 min. After the pressurization, decompression was manually performed in approximately 20 s. During the pressure treatments, the temperature of the pressure-transmitting fluid was monitored with two K-type thermocouples mounted to the center of the top closure of the pressure chamber and positioned close to the sample. In addition, the treatment cycle was controlled by a computer program throughout the pressurization. After pressurization, all samples were stored at 4ºC prior to analysis within 24 h.

2.4. Texture profile analysis

Texture profile analysis (TPA) of samples was carried out with a TA-XT Plus Texture Analyzer (Stable Micro Systems, England). Beef loin steaks were cooked in a water bath at 80ºC until reaching an internal temperature of 72ºC and then cooled to room temperature for 45 min before texture analysis. A 5 kg load cell was used in the experiments. The cylindrical samples (1 cm diameter and 2 cm length) were compressed across the fiber direction in two consecutive cycles to 50% of their original height using a cylindrical probe, 38 mm in diameter. The sample was placed under the probe that moved downwards at a constant speed of 2.0 mm s^{-1} (pre-test), 2.0 mm s^{-1} (test), and 5.0 mm s^{-1} (post-test). A time of 5 s was allowed to elapse between the two compression cycles. The TPA parameters (hardness, springiness, cohesiveness, gumminess, chewiness and adhesiveness) were expressed as described by MOCHIZUKI (2001). The measurements of each sample were replicated at least six times. All textural analyses were conducted using Texture Exponent software version 4.0.9.0. (Stable Microsystems Ltd., Surrey, England).

2.5. Protein solubility

Myofibrillar proteins were extracted according to the method described by CLAEYS *et al.* (1995). Samples of 2.5 g of minced meat were homogenized with 25 mL of 0.05 M Tris, 0.25 M sucrose and 1 mM EDTA buffer, pH 7.6. Homogenate was centrifuged at 1000 × g for 10

min. After centrifugation, the supernatant was removed, and the pellet was suspended in 25 mL of 0.05 M Tris, 0.05 M EDTA buffer, pH 7.6 and sedimented at 1000 × g for 10 min. Then, the supernatant was removed again, and the pellet was resuspended in 25 mL of 0.15 M KCl and centrifuged at 1000 × g for 10 min. The same procedure was carried out three times. The myofibril solution was lyophilized and used for further analysis.
The lyophilized myofibril extracts were analyzed for protein concentration. The extracts were dissolved in sample buffer (2×Laemmli buffer, 2-mercaptoethanol, bromophenol blue, pH 6.8). Then, the dissolved extracts were placed in a water bath at 50°C overnight and filtered using Whatman no. 1 filter paper. After filtration, the protein concentration of the extracts was determined using the Bio-Rad Quick Start Bradford Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) based on the Bradford method (BRADFORD, 1976). Bovine serum albumin was used as the standard. The myofibrillar protein solubility of the samples was expressed as mg protein/mL extract solution.

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to the method of LAEMMLI (1970) using a 12% separation gel and 4.5% stacking gel (bisacrylamide: acrylamide 1:37 [w/w]). The protein concentration of the loaded sample was adjusted to 10 μg for each sample. A protein broad range marker (Bio-Rad Unstained SDS-PAGE standards, 161-0317) was used as the molecular weight standard (6.5-200 kDa). The electrophoresis run was carried out at 100 V in a Mini-PROTEAN Tetra Cell electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA). After the runs, the gels were stained with 0.01 Coomassie blue, 50% methanol and 10% acetic acid and then destained in 10% methanol and 7% acetic acid. The gels were visualized, and protein molecular weights were estimated using Bio-Rad Versadoc 4000 MP and Quantity One Software (Bio-Rad Laboratories, Hercules, CA, USA). Electrophoresis was carried out in duplicate.

2.7. Cooking loss

Beef loin steaks were placed in plastic bags and cooked in a preheated water bath until the internal temperature of the samples reached 72ºC. Then, the samples were taken from the water bath, and excess moisture on the surface of the samples was removed with filter paper. Subsequently, the samples were cooled to room temperature and reweighed. The cooking loss (CL) was expressed as a percentage of the weight difference before and after cooking using the following formula described by RODRIGUES *et al.* (2016):

$$CL = (\text{initial weight} - \text{final weight}) / \text{initial weight} \times 100$$

2.8. Color measurements

The color of the samples was measured using a colorimeter (Minolta Chromameter CR-300; Minolta Camera Co., Ltd., Osaka, Japan) with illuminant D65 (light source) and a 10º observation angle. The beef loin steak packages were opened and exposed to air for 10 min prior to analysis. A CIELAB system was used to determine the color attributes, and the results were expressed as L^* (lightness), a^* (redness and greenness), and b^* (yellowness and blueness). For each sample, five color readings were taken (one at the center and the others from different sides of the sample) at room temperature. The total differences in the color reading values were calculated as described by JUNG *et al.* (2003):

$$\Delta E = \sqrt{(L^* - L^*_{ref})^2 + (a^* - a^*_{ref})^2 + (b^* - b^*_{ref})^2}$$

The color values of the non-pressurized samples were used as a reference for the sample groups pressurized without marination in calculating ΔE, and the color values of marinated/non-pressurized samples were used as a reference for the marinated pressurized sample groups in calculating ΔE.

2.9. Sensory evaluation

Eight graduate students and lecturers in the Department of Food Engineering at Manisa Celal Bayar University participated in the sensory tests as panelists. The panelists were asked to evaluate the sensory parameters of appearance, color, texture, chewiness, juiciness, flavor and overall acceptability. A hedonic scale of 1-5 was used for each attribute. The 5-point hedonic scale was as follows: like very much (5), like much (4), like (3), like slightly (2) and dislike (1). Unsliced raw and cooked samples were presented to the panelists to rate their preferences in terms of appearance, color and texture attributes. In addition, cooked samples were sliced, and a sliced sample from each group was presented to the panelists to rate their preferences in terms of chewiness, juiciness and flavor attributes. The samples were served on plates that were randomly identified with three-digit codes, and a cup of water and bread were given to the panelists to eliminate the residual taste of the samples (DJENANE *et al.*, 2011).

2.10. Statistical analysis

All of the experiments were repeated on three separate occasions. The statistical analyses were performed using SPSS Version 25.0 (SPSS INC., 2017). The experimental data were expressed as the means ± standard deviations. A two-way analysis of variance was conducted to evaluate the effects of high pressure and marination treatment, and the significant differences between pairs of means were tested by Duncan's multiple range test at a confidence level of $P<0.05$. The results of the sensory analysis using a hedonic scale were evaluated by Friedman's (non-parametric) rank test and a Wilcoxon test was used to test for pair differences ($P<0.05$) (MEILGAARD *et al.*, 2015).

3. RESULTS AND DISCUSSION

3.1. Texture profile analysis

The textural properties of marinated and marinated pressurized samples are presented in Table 1. Both pressure and marination treatment had a significant effect on the hardness, gumminess and chewiness of the samples ($P<0.05$). High pressure treatment alone significantly affected all texture profile parameters, whereas marination treatment was only effective on cohesiveness and adhesiveness ($P<0.05$). These results suggest that pressure affects the normal texture, marination with rosemary extract partly affects the texture, while the pressure and marination interaction increase the effects on the textural properties of samples.

Table 1. Texture profile parameters of beef loin steaks marinated with rosemary extract and treated with high pressure.

A: Pressure level	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)	Adhesiveness
0	33.6±0.8^{c}	0.55±0.02^{a}	0.67±0.006^{a}	22.4±0.4^{c}	12.5±0.6^{c}	0.57±0.06^{c}
150	41.0±0.9^{b}	0.60±0.02^{a}	0.62±0.006^{b}	25.4±0.4^{b}	15.1±0.7^{b}	0.35±0.06^{ab}
350	57.9±0.8^{a}	0.55±0.02^{a}	0.59±0.006^{c}	34.2±0.4^{a}	18.7±0.8^{a}	0.47±0.06^{bc}
550	26.2±0.8^{d}	0.48±0.02^{b}	0.62±0.006^{b}	16.4±0.4^{d}	7.7±0.7^{d}	0.18±0.06^{a}
SL	0.0	0.04	0.0	0.0	0.0	0.01
B: Marination						
0	39.2±0.6	0.56±0.02	0.64±0.004^{a}	25.0±0.3	14.0±0.5	0.33±0.04^{a}
1	38.7±0.6	0.52±0.02	0.61±0.004^{b}	24.2±0.3	13.0±0.4	0.46±0.04^{b}
SL	NS	NS	0.0	NS	NS	0.04
A×B						
SL	0.0	NS	NS	0.0	0.0	NS
Samples						
150/0**	34.9±1.2^{d}	0.63±0.05	0.64±0.013	22.4±1.2^{d}	14.0±1.2^{b}	0.27±0.03
350/0	61.9±1.3^{a}	0.54±0.04	0.61±0.012	37.7±1.2^{a}	20.3±2.7^{ab}	0.47±0.22
550/0	28.3±3.7^{e}	0.51±0.07	0.65±0.002	18.4±0.9^{e}	9.0±2.1^{c}	0.19±0.12
150/1	47.0±2.0^{c}	0.57±0.03	0.60±0.008	28.3±0.8^{c}	16.2±0.4^{b}	0.43±0.05
350/1	53.9±2.9^{b}	0.55±0.04	0.57±0.004	30.7±0.8^{b}	17.0±1.5^{ab}	0.47±0.07
550/1	24.1±1.8^{f}	0.44±0.03	0.60±0.013	14.4±1.0^{f}	6.4±0.2^{c}	0.17±0.04

*The results are the mean values of three replicates (n=8) ± standard error. Means with alphabetical superscripts (a-f) in the same column (within each main effect) are significantly different (P<0.05).
**The first number refers to the pressure level, and the second refers to the rosemary extract added (5%). 0: no added extract,
1: added extract.
***L^*: lightness; a: redness and greenness; b: yellowness and blueness; ΔE: total color difference; SL: significance level;
NS: not significant.

The combination of marination and high pressure treatment led to an increase in hardness at up to 350 MPa and a slight decrease in hardness at higher pressure values (550 MPa) (P<0.05). High pressure treatment alone also showed a similar trend in the hardness values of the samples, whereas marination treatment alone had no significant effect on hardness (P>0.05). Our results were in agreement with those of MA and LEDWARD (2004), who reported that high pressure treatment at or above 200 MPa increased meat hardness. These results could be attributed to the aggregation of pressure-treated myofibrillar proteins at 100-300 MPa, causing increased hardness (MA and LEDWARD, 2004; SIMONIN *et al.*, 2012; RODRIGUES *et al.*, 2016). The hardness values of all of the samples were significantly decreased at 550 MPa high pressure treatment (P<0.05). In the literature, the decrease in hardness at high-pressure values was explained by the enzymatic hydrolysis of muscle proteins (MALINOWSKA-PANCZYK *et al.*, 2013). Furthermore, the lowest hardness values were observed in marinated pressurized (550

MPa) samples. These results showed that pressure treatment of previously marinated meat can be more effective for providing softer texture than pressure treatment alone.
The secondary parameters of gumminess and chewiness showed similar changes with hardness. The gumminess and chewiness values of the samples increased with a high-pressure treatment up to 350 MPa and decreased significantly at the higher pressure value of 550 MPa. There was also a significant interaction between pressure level and marination on gumminess and chewiness values ($P<0.05$).The results indicated that the marinated pressurized beef samples were more tender, less gummy and less chewy than the samples that were pressurized alone. It was reported that the loss of myosin structure induced a decrease in gumminess when the texture profile of pressure treated samples was examined with thermograms (ANGSUPANICH and LEDWARD, 1998).
No significant interaction was found between pressure level and marination for springiness, cohesiveness and adhesiveness of the samples ($P>0.05$). The springiness, cohesiveness and adhesiveness values of the samples changed variably at different pressure levels. Pressure treatment alone had a significant effect on these texture attributes of the samples, whereas marination treatment alone was only effective on cohesiveness and adhesiveness ($P<0.05$). At the 150 MPa high pressure treatment, the springiness values increased while the cohesiveness and adhesiveness values decreased. A similar relationship was found by ANGSUPANICH and LEDWARD (1998) and ASHIE *et al.* (1997). On the other hand, some opposing results were found by MALINOWSKA-PANCZYK *et al.*, (2013). At 350 MPa and 550 MPa, the springiness, cohesiveness and adhesiveness decreased. This could be explained by the protective effects of high pressure against heat denaturation of meat proteins (FERNANDEZ-MARTIN *et al.*, 1997).
In general, fresh meat tenderization depends on resolving two components: actomyosin toughness and background toughness. Actomyosin toughness is related to myofibrillar proteins, while background toughness is related to connective tissue and stromal proteins (SUN and HOLLEY, 2010). The effects of high pressure treatment on meat tenderization can be explained by the changes to myofibrillar protein structure. Two possible mechanisms cause myofibrillar protein dissociation and the subsequent decrement in toughness of the meat: thermal degradation of muscle proteins and the enzymatic hydrolysis of proteins (SIMONIN *et al.*, 2012). Pressure breaks up the myofibrillar structure and accelerates enzyme activation in meat as mentioned above. In the present study, rosemary extract also showed a positive effect on some textural properties of samples; however, the higher tenderization effect on beef loin steaks was achieved by the combination of high pressure and marination treatment.

3.2. Protein solubility

Protein solubility is one of the most important functional properties of meat proteins (VAN LAACK *et al.*, 2000). As a consequence of increased interactions between protein constituents and water, protein solubility can change and cause significant alterations to meat texture (CHEFTEL and CULIOLI, 1997).
The effects of high pressure and marination treatment on the solubilization of myofibrillar proteins are shown in Fig. 1. According to the results, the high pressure and marination treatment had no effect on myofibrillar protein solubility in the samples. However, the protein solubility of the samples generally decreased with increasing pressure when compared to the untreated group. Similar results have been found in previous studies. CHAPLEAU *et al.* (2003) found decreased myofibrillar protein solubility in beef samples subjected to pressure treatment (≤600 MPa) compared to control samples.

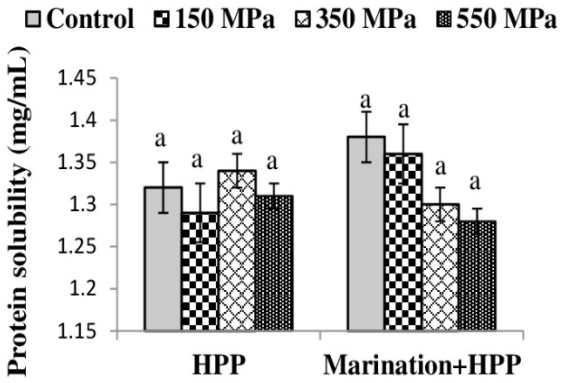

Figure 1. Effects of high pressure/marination treatment on the myofibrillar protein solubility of beef loin steaks.

Furthermore, MALINOWSKA-PANCZYK *et al.* (2013), SOUZA *et al.* (2011), GROSSI *et al.* (2012) and CHAN *et al.* (2011) also reported similar decreases in protein solubility for cod, salmon, pork and beef samples (>60 MPa pressure treatment), pork (215 and 600 MPa pressure treatment) and turkey meat (≤600 MPa pressure treatment), respectively.
The literature reports that protein solubility is a good indicator of protein denaturation (VAN LAACK *et al.*, 2000). Additionally, protein solubility decreases with increasing pressure due to the formation of insoluble protein aggregates that can no longer be extracted (MARCOS and MULLEN, 2014).

3.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Postmortem degradation of myofibrillar proteins has been reported to be an essential part of postmortem tenderization. The increase in protein degradation reflects lower mechanical tenderness and promotes the development of meat texture (SOUZA *et al.*, 2011). For this reason, it is of great importance to understand the effects of high pressure processing on myofibrillar proteins in considering textural changes in pressurized meat, as described above.
Fig. 2 shows the SDS-PAGE profile of myofibrillar proteins from each of the high-pressure and marination-treated samples. The volume intensity of each protein band is also presented in Fig. 3. The protein bands extracted from the samples for myosin heavy chain (MHC) (200 kDa), C-protein (135 kDa), α-actinin (95 kDa), desmin (53 kDa), actin (43 kDa), tropomyosin (36 kDa) and myosin light chain (MLC1, MLC2) (24 kDa, 14 kDa) were identified on a SDS-PAGE gel. Similar myofibrillar proteins were also identified by CHAPLEAU *et al.* (2003), CHAN *et al.* (2011), OMANA *et al.* (2011) and SPERONI *et al.* (2014) in beef, turkey, poultry and meatball samples, respectively.
In general, increasing the applied pressure reduced the band intensities of the myofibrillar proteins. On the other hand, the SDS-PAGE profile of the marinated pressurized samples was similar to that of the samples that were pressurized alone; therefore, we suggest that marination treatment had no effect on myofibrillar protein degradation. The pressure-treated samples had the lowest protein band intensities, and the molecular weights of

mainly degraded myofibrillar proteins ranged from 53 kDa to 200 kDa (MHC, C-protein, α-actinin and desmin).
The band intensities of MHC, C-protein, α-actinin and desmin extracted from the pressurized samples were noticeably decreased compared to those of the control samples. The decreased band intensities may have been caused by protein aggregation due to intermolecular disulfide bond formation at the higher pressure levels (ANGSUPANICH *et al.*, 1999). Myofibrillar proteins were partly degraded with high pressure treatment, and MHC protein was the most degraded protein in the SDS-PAGE profile. However, MLC2 protein was found to be unaffected or even decreased in intensity with applied pressure. This may be because myosin aggregation mechanisms involve the dissociation of heavy chains from light chains, so that only myosin heavy chains form aggregates under pressure (SPERONI *et al.*, 2014).

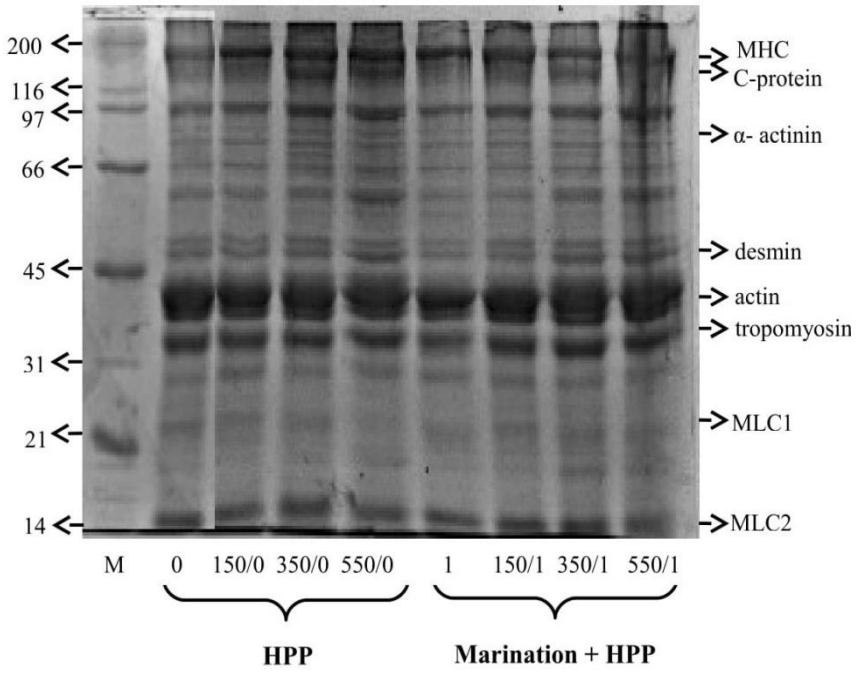

Figure 2. SDS-PAGE patterns of myofibrillar proteins isolated from beef loin steaks. M: Marker, 0: no added extract (control), 150/0, 350/0, 550/0: pressurized, 1: added extract, 150/1, 350/1, 550/1: marinated pressurized

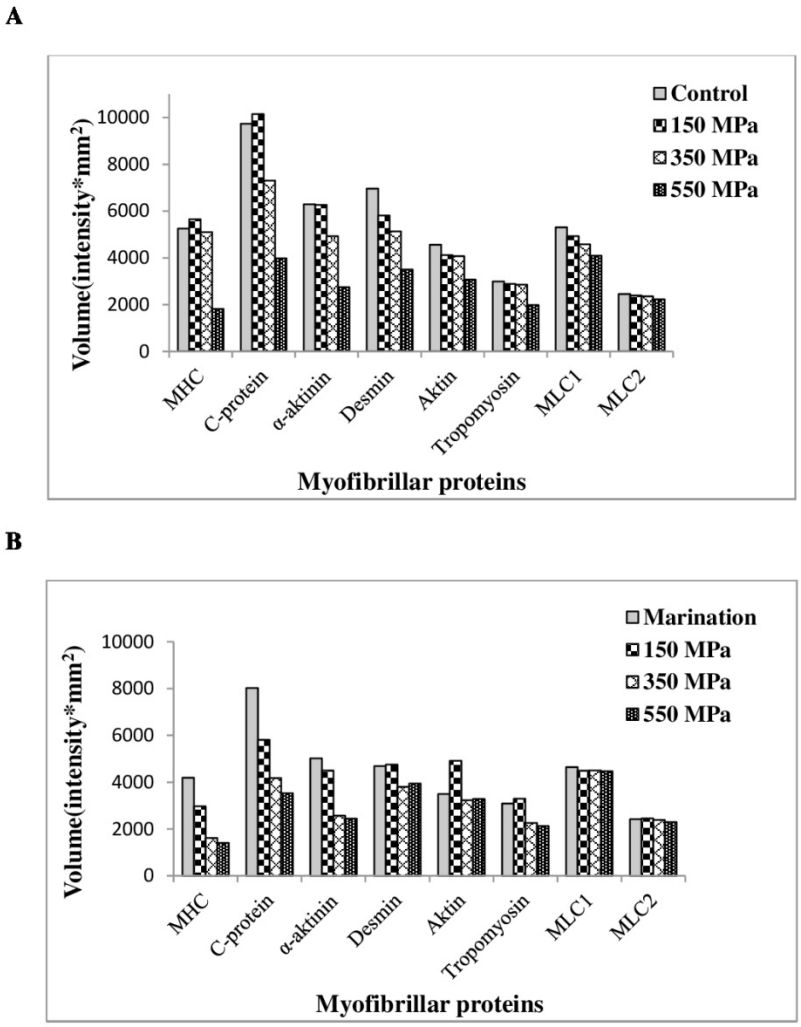

Figure 3. A: The volume intensity of the different protein bands from SDS-PAGE for the high-pressurized sample groups. B: The volume intensity of the different protein bands from SDS-PAGE for the marinated high-pressurized sample groups.

The changes in band intensities of myofibrillar proteins under pressure are attributed to conformational changes in proteins and thereby decreased solubility due to denaturation following covalent linking or increased solubility due to degradation into lower molecular weight compounds. Our results are in accordance with this explanation. The protein band intensities and solubilities decreased in parallel with increasing pressure.

3.4. Cooking loss

Table 2 shows the cooking loss values of the samples. The results indicated that there was no significant interaction between pressure level and marination for cooking loss values of the samples ($P>0.05$). However, it was found that pressure level and marination separately had a significant effect on cooking loss ($P<0.05$). A significant difference was observed in all pressure levels compared to the control group ($P<0.05$).

Table 2. Color and cooking loss values of beef loin steaks marinated with rosemary extract and treated with high pressure.

	L^*	a^*	b^*	ΔE	Cooking Loss (%)
A: Pressure level					
0	40.86±0.5^{c}	9.4±0.5^{b}	11.8±0.3^{d}		40.60±0.6^{a}
150	40.69±0.5^{c}	10.0±0.5^{ab}	12.8±0.3^{c}	2.8±0.4^{c}	38.03±0.6^{b}
350	54.41±0.5^{b}	11.3±0.5^{a}	18.6±0.3^{a}	15.4±0.4^{b}	37.55±0.6^{b}
550	57.20±0.5^{a}	8.7±0.5^{b}	17.6±0.3^{b}	17.5±0.4^{a}	39.22±0.6^{ab}
SL	0.0	0.01	0.0	0.0	0.0
B: Marination					
0	46.25±0.3^{a}	10.4±0.3^{a}	14.3±0.2^{a}	12.0±0.4	37.64±0.4^{a}
1	50.33±0.3^{b}	9.3±0.3^{b}	16.1±0.2^{b}	11.8±0.4	40.05±0.4^{b}
SL	0.0	0.02	0.0	NS	0.0
A×B					
SL	0.04	NS	NS	NS	NS
Samples					
150/0**	37.50±0.7^{e}	10.4±1.9	12.1±1.2	3.6±0.9	36.18±0.9
350/0	52.20±0.9^{c}	12.3±0.9	17.3±1.0	14.6±0.9	36.24±2.1
550/0	56.06±1.4^{b}	9.1±1.5	16.7±0.9	17.8±0.9	38.19±0.9
150/1	43.89±1.2^{d}	9.6±0.7	13.6±0.4	2.1±0.8	39.87±0.9
350/1	56.63±0.9^{ab}	10.2±0.3	20.0±0.6	16.2±0.6	38.85±0.4
550/1	58.34±1.8^{a}	8.3±1.2	18.4±0.5	17.2±1.9	40.24±0.7

*The results are the mean values of three replicates (n=8) ± standard error. Means with alphabetical superscripts (a-d) in the same column (within each main effect) are significantly different (P<0.05).
**The first number refers to the pressure level, and the second refers to the rosemary extract added (5%).
0: no added extract (control), 1: added extract.
***L^*: lightness; a^*: redness and greenness; b^*: yellowness and blueness; ΔE: total color difference;
SL: significance level; NS: not significant.

Both pressure and marination treatment generally resulted in an increase in cooking loss values except for the marinated pressurized (350 MPa) group, but the differences were not significant (P>0.05). The cooking loss values of the samples that were pressurized alone increased with increasing pressure. A similar trend was determined by KIM *et al.* (2014) who reported increased cooking loss values in beef samples pressurized at 300, 450 and 600 MPa compared to the control group. In addition, NETO *et al.* (2015) reported that 100, 200, 300 and 400 MPa high-pressure treatment led to increased cooking loss values in beef samples. These authors also reported that high pressure levels and changes in myofibrillar protein structure at these pressures had a negative effect on the water holding capacity of meat and consequently increased cooking loss. In addition, MARCOS *et al.* (2010) explained that sarcoplasmic proteins decreased high-pressure effects on cooking loss but that the increased denaturation of sarcoplasmic proteins induced by pressure had a negative effect on the cooking loss values of meat. The cooking loss values of the marinated pressurized samples decreased at 350 MPa and then increased with increasing pressure. Similar results were obtained by BARBANTI and PASQUINI (2005) in marinated meat. Increasing cooking loss values might be attributed to lower water binding capacity and moisture loss during cooking.

3.5. Color

The color measurements of beef loin steaks marinated with rosemary extract and subjected to high pressure are shown in Table 2. Statistical analysis showed a two-way interactive effect between pressure level and marination for L^* values of the samples ($P<0.05$). Pressure level and marination separately had a significant effect on the a^* and b^* values of the samples ($P<0.05$).

The L^* values showed an increasing trend, while the a^* and b^* values increased up to 350 MPa and then decreased. Marination with rosemary extract also caused a significant increase in L^* and b^* values and decrease in a^* values ($P<0.05$). Pressure level had a significant effect on L^* values at pressures above 150 MPa compared to the control group ($P<0.05$). It was also found that the a^* values significantly changed at 150 and 350 MPa pressure levels, whereas the b^* values significantly changed at all pressure levels ($P<0.05$). Similar results were also reported by KIM *et al.* (2014), MARCOS *et al.* (2010), MCARDLE *et al.* (2010), OHNUMA *et al.* (2013) and RODRIGUES *et al.* (2016) for beef *M. Longissimus dorsi*, beef supplemented with conjugated linoleic acid, beef *Longissimus lumborum*, beef *M. Pectoralis profundus* and beef treated with sodium hydrogen carbonate, respectively. The highest L^* values were determined in marinated pressurized (550 MPa) samples. These increases in L^* values caused discoloration of the beef samples, which was attributed to the whitening/brightening effect between the range of 200 to 350 MPa and ferrous (Fe^{2+}) myoglobin oxidation to ferric (Fe^{3+}) metmyoglobin at pressures above 400 MPa in the literature (SIMONIN *et al.*, 2012; BUCKOW *et al.*, 2013). The whitening/brightening effect occurred due to the following changes: (i) protein coagulation causing loss of sarcoplasmic and myofibrillar protein solubility, affecting their structure and surface properties and (ii) myoglobin denaturation and the displacement or release of the heme group (BUCKOW *et al.*, 2013). MUSSA (1999) also reported that the lighter color in pressurized meat could be related to alterations in the water content of samples due to drip loss. In the present study, visual color observations of the samples are also shown in Fig. 4.

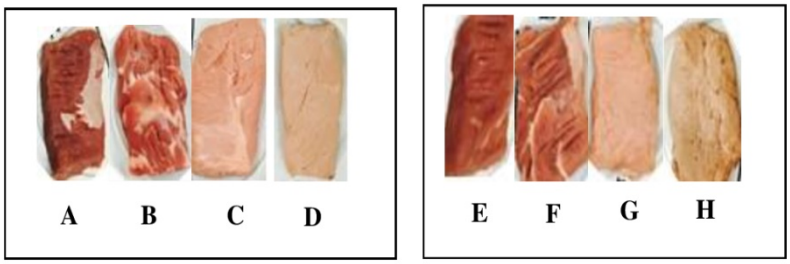

Figure 4. Visual color observation of beef loin steaks. A: control (unpressurized), B: 150 MPa, C: 350 MPa, D: 550 MPa, E: marinatedunpressurized, F: marinated/150 MPa, G: marinated/350 MPa, H: marinated/550 MPa.

Increasing pressure caused the increase in a^* values of samples up to 350 MPa; then, the a^* values of the samples decreased at pressures above 350 MPa. JUNG *et al.* (2003) found that pressure treatment up to 300 MPa decreased metmyoglobin content and higher pressures led to increased metmyoglobin content in the beef samples. These authors also explained

the increases in a^* values at pressures up to 300 MPa by the activation of enzymes causing metmyoglobin reduction. Our results are in agreement with previous reports for beef samples (JUNG *et al.*, 2003; MARCOS *et al.*, 2010; LOWDER *et al.*, 2014).
The b^* values represent the intensity of yellowness and blueness in the samples. In the present study, b^* values showed a similar trend with a^* values. Increasing pressure caused by the increase in b^* values of samples up to 350 MPa and then the b^* values of the samples decreased at pressures above 350 MPa. Similar results were found by MCARDLE *et al.* (2010), who reported higher b^* values in beef samples pressurized at 300 MPa compared to the samples pressurized at 200 MPa and lower b^* values in beef samples pressurized at 400 MPa compared to the samples pressurized at 300 MPa. On the other hand, GOUTEFONGEA *et al.* (1995) reported an increase in b^* values for minced meat samples pressurized at 600 MPa (20ºC for 30 min). These authors related the increase in b^* values to the change of the myoglobin chemical state. CARLEZ *et al.* (1995) also stated that the increase in b^* values was due to metmyoglobin formation.
The total color difference (ΔE) indicates the evaluation of color changes. The results revealed that there was no significant interaction between pressure level and marination for ΔE values of the samples ($P>0.05$). However, the pressure level had a significant effect on ΔE values ($P<0.05$). An increase of 10 units in ΔE is thought to significantly change the appearance of meat color (JUNG *et al.*, 2003). In the present study, an increase of 10 units in ΔE values was found in the samples pressurized at 350 and 550 MPa.

3.6. Sensory evaluation

The sensory evaluation results of the raw and cooked samples are shown in Table 3 and Table 4. In general, the addition of rosemary extract (5%) did not positively affect the sensory scores of the samples. It was observed that pressurized samples were evaluated as better than marinated pressurized samples. Increasing pressure caused a decrease in the sensory scores of the samples. The panelists showed slightly Friedman rank test significant preferences in appearance, color and texture attributes of raw samples and chewiness, juiciness and overall impression attributes of cooked samples ($P<0.05$). According to the results of the raw samples, the control samples received the highest score for appearance and texture, whereas 150 MPa pressurized samples were rated highest for color. The results of cooked samples also showed that 150 MPa pressurized samples received the highest score for appearance, color, texture, chewiness and overall impression, while 350 MPa pressurized samples were rated highest for juiciness.
The panelists did not notice the color difference between the pressurized and non-pressurized cooked samples. It has been reported that high pressure treatments caused visible color changes in raw meat, but the color difference decreased extremely after cooking. Our results are in agreement with those of MOR-MUR and YUSTE (2003) and SIMONIN *et al.* (2012). In addition, the panelists recognized the color differences in the raw samples. According to the pair comparisons, there was a significant difference between the control samples and pressurized as well as marinated pressurized samples ($P<0.05$) in color scores. Similarly, the appearance of the raw samples was also significantly influenced by the treatments ($P<0.05$). These results were also supported by the color measurements shown in Table 2.

Table 3. Sensory evaluation of pressurized and marinated pressurized raw samples

	Appearance	Color	Texture
0	4.80±0.4	4.70±0.6	4.30±0.7
1	4.10±0.6	4.15±0.7	4.05±0.7
150/0**	4.75±0.4	4.75±0.4	4.20±0.8
350/0	2.60±1.2	2.65±1.2	3.70±1.0
550/0	2.00±1.0	2.00±0.9	3.35±0.8
150/1	3.60±0.8	3.75±0.9	3.80±0.8
350/1	2.15±0.8	2.25±0.9	3.70±1.0
550/1	1.70±0.9	1.90±1.1	3.40±0.9
SL	0.0	0.0	0.0

*The results are the mean values of three replicates (n=8) ± standard error.
**The first number refers to the pressure level, and the second refers to the rosemary extract added (5%).
0: no added extract (control), 1: added extract, SL: significance level.

The panelists tended to give lower scores for the texture attributes of cooked and raw samples than for the control group. The pair comparisons of texture scores of the control group and the other sample groups were significant except for 150 MPa pressurized groups (150/0, 150/1) and marinated 350 MPa pressurized group ($P<0.05$). Surprisingly, no significant difference was found in the texture attributes of the cooked samples ($P>0.05$). These results were not in agreement with TPA measurements reported in the previous sections, which were significantly affected by pressure ($P<0.05$). The contrast between the results could be due to the different preferences reflected by the panelists regarding texture. On the other hand, the panelists gave higher chewiness scores to the pressurized samples (150/0, 350/0) and marinated pressurized samples (350/1) compared to the control group, and the pair significant differences were found between the control group and the 350 MPa pressurized group (350/0, 350/1) ($P<0.05$).

Table 4. Sensory evaluation of pressurized and marinated pressurized cooked samples

	Appearance	Color	Texture	Chewiness	Juiciness	Flavor	Overall Impression
0	3.80±1.0	4.20±0.8	3.95±0.8	3.45±1.2	3.50±1.1	3.80±1.0	3.55±1.1
1	4.00±0.9	4.20±0.7	4.00±0.8	3.55±1.1	3.35±1.1	3.65±1.0	3.55±0.9
150/0**	4.20±0.8	4.25±0.8	4.10±1.1	4.20±0.8	4.00±0.8	4.10±0.9	4.10±1.0
350/0	4.05±0.9	3.05±1.1	3.95±0.8	4.15±0.7	4.25±0.9	4.30±0.8	4.00±0.7
550/0	4.00±0.9	3.05±1.1	3.55±0.9	3.50±1.4	3.50±1.2	3.90±1.0	3.40±1.1
150/1	3.60±1.1	3.60±0.8	3.40±0.9	3.55±0.8	3.40±0.8	3.60±0.7	3.50±0.8
350/1	3.70±1.0	2.90±1.0	3.80±0.8	3.70±1.1	3.65±1.0	3.60±1.0	3.20±1.0
550/1	3.80±1.0	3.15±1.3	3.55±1.1	3.20±1.0	3.15±0.9	3.65±0.8	2.75±0.8
SL	NS	NS	NS	0.0	0.0	NS	0.0

*The results are the mean values of three replicates (n=8) ± standard error.
**The first number refers to the pressure level, and the second refers to the rosemary extract added (5%).
0: no added extract (control), 1: added extract, SL: significance level.

It was determined that the samples pressurized up to 350 MPa had higher juiciness scores than the control group, while the juiciness scores decreased at 550 MPa pressure levels. MACFARLANE (1973) stated that decrements in juiciness scores were attributed to increased moisture retention based on the defragmentation of structural proteins into hydrophilic peptides/free amino acids.
The panelists gave the highest overall impression scores to the 150 MPa pressurized samples; however, no significant differences were found between the overall impression scores of 150 MPa pressurized samples and the control group ($P>0.05$). The 350 MPa pressurized samples also received the highest scores for flavor in all sensory characteristics of the cooked samples. According to the sensory evaluation results, the panelists preferred the 150 MPa and 350 MPa pressurized samples most, which were more tender, chewier, juicier and tastier than the control group. In addition, the marinated pressurized samples had lower sensory scores than the samples that were pressurized alone. It was reported that the sensory acceptance among panelists of high pressurized meat products varied and that they generally reported good sensory scores, although with some alterations in aroma and taste components (SIMONIN *et al.*, 2012).

4. CONCLUSION

A pressure of 550 MPa improved the tenderness of beef loin steaks but caused a light color in the meat appearance. High pressure processing caused protein degradation, leading to a great change in the protein profile of samples and thereby the development of meat texture. The panelists preferred the samples pressurized at 150 MPa and 350 MPa in the sensory panel. The best overall beef quality was achieved with the combined application of high pressure and marination. High pressure treatment has important positive effects on beef quality due to limitations regarding meat discoloration. In this regard, high pressure and marination treatment present a good alternative strategy for developing reliable and healthier meat products with desirable texture. However, further studies are needed for process optimization.

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