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BROMELAIN TREATMENTS EFFECT ON PORK LOIN HISTOLOGICAL, TEXTURAL AND TECHNOLOGICAL PROPERTIES

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Abstract

Bromelain is a vegetal originating enzyme known for its effects on meat tenderization. The purpose of this study was to investigate to which extent the treatment with two sources of bromelain affects the histological properties of pork loin (myocytes integrity, proportion of connective and pure muscular tissues) as well the textural ones (shear force) and its technological parameters (drip loss and cooking yield). Forty-five slices of swine Longissimus dorsi muscles (10 mm thickness) were used as biological material, randomly assigned to a control group (CG-no bromelain hydrolytic treatment-15 slices), to B1 group (triturated pineapple, diluted with distilled water 1:1, resulting 50% aqueous extract - 15 slices kept in marinade 12 hours) and to B2 group (bromelain 1% aqueous solution prepared from commercial food additive, bromelain powder of 2000 GDU/g - 15 slices, kept in marinade 12 hours). Tissue square pieces were sampled from the center of the loins and submitted to paraffin impregnation technique, followed by a hematoxylin, eosin, methylene blue staining and studied via photonic microscopy for the histometric assessments. Cubic samples of were also cut from each slice and submitted to a shear force reading cell. Water holding capacity was then measured on the meat provided by half of the remained slice surface, using the grinding-centrifugation technique while cooking yield was calculated after samples weighing and cooking through deep frying at 250°C into sunflower oil, during 15 minutes. The results suggested that bromelain treatments affected the histological integrity of the samples, more than 17.5 % of endomysium being hydrolyzed in experimental groups, compared to control one. This resulted in apparent increase of the surface occupied by muscular tissue (+6.92% B1vs. CG and +13.18% B2 vs. CG) ($P<0.05$; $P<0.01$), versus the one occupied by the connective fibers, as observed and measured within the microscopic field. Bromelain treatments induced better tenderness, suggested by lower instrumentally measured shear force (52.13 N in CG, 48.52 N in B1 and 44.48 N in B2). However, better water holding capacity was measured in CG (WHC=16.78%) due to less tissue disintegration, compared to experimental groups B1 (WHC=14.21%) and B2 (WHC=13.47%) ($P<0.05$). The cooking yield was consequently better in CG (CY=74.35%), due to lower exudation than in enzymatically treated meat (B1, CY=71.82%; B2, CY=70.25%). Therefore, bromelain enzymatic treatment improves the histological and, subsequently, textural features of pork loin, while the technological properties were reduced by the enzymatic proteolysis. It still remains to investigate to which extent the textural improvement is justified by loss of technological properties and by certain taste and flavor expected alterations, due to the known bromelain bittering potential.

Key words: *pork loin, bromelain, connective tissue, shear force, water holding capacity, cooking yield.*

INTRODUCTION

Ultimate gastronomic quality of the meat, given by its nutritional, textural, sensory and technological traits are strongly influenced by pre-cooking treatments and cooking methods (Kharb and Ahlawat, 2010; Haskaraca et al., 2014). One of the ways to enhance sensory properties of meat is to marinate it a few hours prior to cooking, using a mixture of spices, sauces and beverages (Kim et al., 2018). Microbial or vegetal originated enzymes are known to improve meat tenderness, due to the proteolytic effects induced into cell membranes and on the connective stroma of muscles (Sullivan and Calkins, 2010; Chaudary et al., 2015). Many vegetal species are known as sources of such enzymes, that could be used *per-se* as hydro soluble enzymatic complexes or standalone after extraction and purification: pineapple (Nadzirah et al., 2016), *Calotropis procera* (Rawduken et al., 2013), mango (Dhital and Vangnai, 2019), kiwi (Koak et al., 2011), asparagus (Ha et al., 2013), ginger (Naveena and

Mendiratta, 2001), figs (Singh et al., 2019) papaya (Verma et al., 2018), *Averhoa bilimbi* (Ismail et al., 2018), Korean pear (Hao-Liet al., 2009). Besides the proteolytic effects, it is known that such enzymes also have bactericidal or bacteriostatic effect on most foodborne pathogens (Eshamah et al., 2014), therefore a double advantage by using them. Within this state of knowledge, this study aimed to investigate the effect of bromelain, a proteolytic enzyme originated in pineapple on the pork loins sensory, textural and technological traits, using two types of enzymatic treatment solutions (one issued directly from the fruit itself and prepared in dilution with distilled water and the other reconstituted from a commercially available bromelain powder, used as food additive in the meat industry.

MATERIALS AND METHODS

Biological material comprised 45 slices of *Longissimus dorsi* muscles, 10 mm average thickness, cut from adult pigs half carcasses. They were randomly allotted in three groups, in relation with the usage of the experimental factor, bromelain enzyme, as following:

* *CG control* - no bromelain treatment (15 slices)

* *B1 group* (15 slices kept in marinade 12 hours, marinade obtained from triturated pineapple pulp, diluted with distilled water 1:1, resulting 50% aqueous extract)

* *B2 group* (15 slices, kept in marinade 12 hours, marinade consisted in bromelain 1% aqueous solution prepared from commercial food additive, bromelain powder of 2000 GDU/g).

Square pieces of tissue were sampled from the center of the loins and submitted to paraffin impregnation technique, followed by a hematoxylin, eosin, methylene blue staining and studied via photonic microscopy for the histometric assessments. General protocol applied for parrafination and staining was adapted from the methods proposed by Mobini and Asaid Khoshoii, 2013, using a using a spin tissue impregnation processor - THERMOSCIENTIFIC STP-120-2, a rotary automatic microtome - histology line - THERMOSCIENTIFIC HM355S, an automatic tissue stainer - histology line - Varistain Gemini AS – THERMOSCIENTIFIC.

The smears were analyzed by microscopic measurements (Motic M230 with camera, endorsed with Motic Image 3+ software) to assess myocytes and 1st order muscular fascicles cross section areas (sqµm). By difference from the whole area of fascicles and the areas of cross-sectioned muscle cells, contained by each 1st order fascicle, it was found the part occupied by the connective stroma in muscle architecture. Knowing these dimensional and morphostructural details, myocytes density (number of muscle cells per sqmm of muscle) and proportion of main tissue categories (% pure muscular tissue and % connective tissue) were also calculated, following a method applied in similar investigations in the past, by some of the authors in the present paper (Radu-Rusu et al., 2019). One hundred readings on muscle cells and eight readings on 1st order musce fascicle were carried on per each smear (one microscopic blade elected from each slice of loin).

Perten Instruments TVT 7600 texture analyzer equipped with straight Warner Bratzler blade and with the appropriate rig - heavy duty stand – was used to test the cutting strength in a single cycle compression mode, following a methodology published by Bratcher et al., 2006. The maximum peak force (Newtons) was considered as the shear force necessary to cut the meat sample. Two samples were tested per slice; therefore 30 repetitions were run in order to acquire cutting strength data in each treatment group.

Water holding capacity has been tested in accordance with the method proposed by Rawduken et al., 2013: 20 g of grinded, homogenized samples, transferred into glass tubes with 30 ml NaCl 6%, stirred for 1 minute and cooled 4℃, for 15 minutes then were centrifuged 25 minutes, at 3000 rpm. After centrifugation, the moist precipitate remained in the tube after supernatant removal (% of the initial measured volume) was considered as the individual water holding capacity for each sample.

Cooking yield was measured through to 180ºC oil frying for 10 minutes, to simulate the conditions existing in a frying pan. Using gravimetric differences and relative ratios, the cooking yield was obtained (%) (Wyrwisz et al., 2012).

Acquired data were statistically processed using MsExcel Data Analysis toolpack, in order to obtain the descriptors (mean, standard deviation, coefficient of variation - CV%) and to run comparisons between treatments (single factor ANOVA) (Wu et al., 2016).

RESULTS AND DISCUSSIONS

Under the action of bromelain solutions, the pork loin slices modified certain of histological properties, thus the apparent proportion of pure muscular tissue increased and the one of connective tissue decreased (table 1). Of course, this phenomena, observed within the microscopic field is in fact due to the destruction of sarcolemma and to the certain level of liquefaction of connective tissue between the myocytes, leading to the increase occupied by the muscle cell content within the total area of a 1st order muscle fascicle. Thus, in control group (normal muscle) the diameter of myocytes was measured at 56.38±4.65µm and the action of proteolytic enzyme bromelain obtained straight from pineapple pulp triturate induced significant enlargement of cells apparent thickness, by 13.85% ($P<0.05$), till 64.19±6.30 µm.

Table 1. Histological and textural properties of pork loins, as influenced by bromelain proteolysis

Trait	Group	Mean	±StDev	CV%	± % vs. CG
Muscle cells thickness (µm)	CG	56.38^{a}	4.65	8.25	100%
	B1	64.19^{b}	6.30	9.81	+13.85
	B2	68.79^{c}	7.13	10.37	+22.01
% Pure muscular tissue (%)	CG	71.34^{a}	4.49	6.29	100%
	B1	76.28^{b}	6.83	8.95	+6.92
	B2	81.23^{c}	9.29	11.44	+13.86
Connective tissue in muscle (%)	CG	28.66^{c}	1.80	6.29	100%
	B1	23.72^{b}	2.12	8.95	-17.24
	B2	18.77^{a}	2.15	11.44	-34.51
Shear force (Newtons)	CG	52.13^{c}	5.35	10.27	100%
	B1	48.52^{b}	5.74	11.83	-6.93
	B2	44.48^{a}	5.64	12.69	-14.67

ANOVAS, on the means from the same trait:
$^{ab,\,bc}$ – significant differences for *P<0.05*
^{ac} – *distinguished significant differences for P<0.01*

Usage of the bromelain reconstituted solution of 1% concentration from commercially available food additive powder induced more severe degradation of connective tissue surrounding muscle cells, thus an increase of diameter by 22.01%, till an average measured value of 68.79±7.13µm ($P<0.01$).

Data in table 1 and figure 1 also reveal the consequence of apparent cell inflating on the tissue constituent categories of *L. dorsi* analyzed slices. Thus, pure muscular tissue increased from 71.34% in control group (not treated samples) to 76.28% in B1 group (+6.92%), respectively to 81.23% in B2 group (+13.86%), while the connective tissue conversely decreased from 28.66% till 23.72% and 18.77%. Each stage of treatment induced significant and distinguished significant differences.

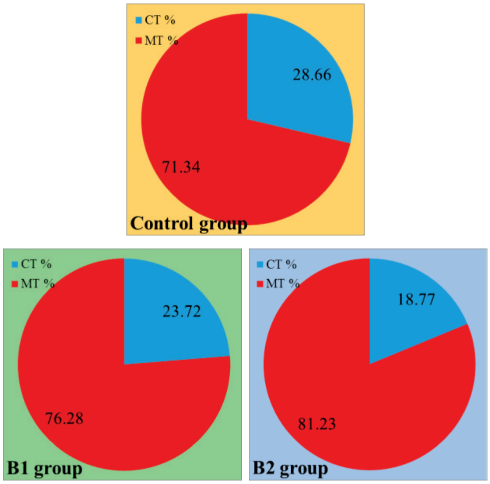

Figure 1. Transformation of tissue structure of pork loin muscles, under the influence of bromelain treatments

Of course, these findings observed in the microscopic field must not be interpreted like a literal change of muscle structure, in terms of hypertrophy of myocytes and atrophy of connective fibers, but more likely like a wiping of membrane limits and fiber delimitation, leading to such an apparent aspect and to the subsequent dynamics of the two categories of tissues, due to photonic microscopy observations.

As expected, due to reduction of connective like tissue proportions, the shear forces necessary to cross-cut the samples (in an imitative test for the consumers' teeth bite force) gradually decreased from 52.13 N in untreated samples to 48.52 N in B1 group (bromelaine from pineapple extract), respectively to 44.48N in B2 group (bromelaine food additive), resulting in an instrumentally measured tenderization of meat by 6.93 to 14.67%, due to proteolysis (figure 2).

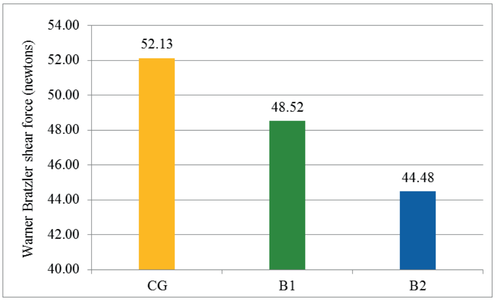

Figure 2 – Dynamics of Warner-Bratzler shear force (N) of pork loin muscles, under the influence of bromelain treatments

These alterations in meat ultrastructure, observed at the microscopic level also affected the technological properties of the meat, such as water holding capacity and cooking yield (table 2 and figure 3).

Table 2. Technological properties of pork loins, as influenced by bromelain proteolysis

Trait	Group	Mean	±StDev	CV%	± % vs. CG
Water Holding Capacity (%)	CG	16.78[a]	1.38	8.25	
	B1	14.21[b]	1.39	9.81	-15.32
	B2	13.47[b]	1.40	10.37	-19.73
Cooking yield (%)	CG	74.35[a]	4.68	6.29	
	B1	71.82[b]	6.43	8.95	-3.40
	B2	70.25[b]	8.04	11.44	-5.51

ANOVAS, on the means from the same trait:
[ab] – significant differences for *P<0.05*

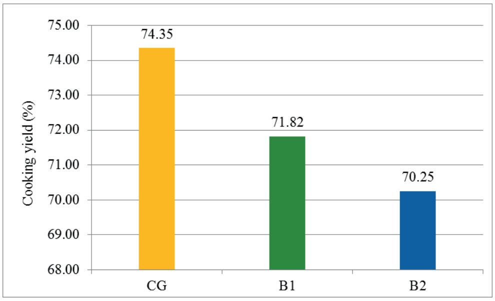

Figure 3. Dynamics of Cooking yield (%) of pork loin muscles, under the influence of bromelain treatments

Water holding capacity significantly decreased from 16.78 till 14.21% and 13.47% in B1 and B2 groups, compared to CG group, suggesting more water drip or loss due to degradation of the connective stroma and to the breakage of cell membranes.

This dynamics also affected cooking yield and, in the case of the raw fried muscle slices, the mass reduced to 74.35% after hot oil frying, while in the samples treated with pineapple extract, the decrease was more pronounced, with 3.41%, in comparison with the control group and reached 71.82% ($P<0.05$).

Bromelain solution reconstituted from food additive powder induced lower cooking yields, by 5.51%, compared to the raw pork loin.

CONCLUSIONS

Basing on the original findings in our study, it could be stated that the enzymatic treatment seems to beneficially affect the sensory quality of the meat, because the tenderization would attract more the consumers. However, looking at the technological features that decreased, bromelain usage should be better reasoned, because tenderness could increase at the cost of juiciness and at the cost of commercial efficiency, especially when the meat is used for processing or when is cooked for public food services, where gravimetric loss are straightly linked to economic effects.

However, enzymatic proteolysis treatments could be successfully used in order to improve the quality of spent meats, such as those issued from aged animals or of those meats with different unconformities, like pale-soft exudative and dry-firm-dark defects.

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