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Possibilities for developing texture-modified beef steaks suitable for older consumers using fruit-derived proteolytic enzymes

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Abstract

Meat intakes in the older population are commonly reduced because the relatively tough texture of meat can impair mastication. Fruit-derived proteolytic enzymes have been reported to have beneficial effects on tenderness, by causing significant degradation of myofibrillar proteins and collagen. Three treatments including: papain, bromelain and a 50:50 mixture of papain/bromelain, alongside one control were applied to beef M. *semitendinosus* steaks. Effects on Warner - Bratzler shear force, texture parameters, color, and cook loss were determined. Both enzymatic treatments that included papain significantly reduced WBSF values (P<0.05) and increased cook loss. Beef steaks tenderized with papain and papain/bromelain offer potential for inclusion in older consumers' diets, but improvement in tenderization may be associated with a reduction in processing yield.

KEYWORDS

tenderization, papain, bromelain, impaired mastication, meat

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Practical applications

Meat processors have a role to play in enhancing the availability of appropriate foodstuffs for older people, through developing targeted products that will meet the specialized nutritional and chemosensory needs of this cohort. Meat intakes in the older population are commonly reduced because the relatively tough texture of meat can impair mastication. In this study, beef steaks tenderized with papain and papain: bromelain (50:50) were demonstrated to produce more tender meat products, with a lower cook loss compared with tenderization with bromelain alone, which has relevance to the development of texture-optimized meat products that appeal to older adults with difficulty in mastication. This information could help meat processors to develop strategies for optimization of texture-modified beef products within their own businesses.

INTRODUCTION

According to the European Commission, by 2025 more than 20% of Europeans will be 65 years or over, a demographic change that is predominantly driven by an increase in the population aged over 80 years (European Commission, 2017). However, the specific needs of this cohort, comprising ~100 million citizens, are frequently disregarded by food processors, including companies focused on the development of novel, value-added and convenient meat products.

Rios et al. (2014) stated that older consumers do not identify with products currently available on the market, and the package labels neither address their needs nor coincide with the perception they have about their own health. There is thus a need for the meat industry to more fully consider the older demographic and to develop specific products to meet the wide variety of needs in this cohort (Botinestean et al., 2016; Goldman et al., 2014). As stated by Chen (2016), one of the most important aspect of the quality of life and the wellbeing of older consumers is having access to enjoyable and safe food. Although beef is a valuable source of

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protein, vitamins and minerals for older consumers, because the relatively tough texture of meat can impair mastication, meat intakes in the older population, who may have an increased prevalence of chewing difficulties, are commonly reduced and they miss out on an important source of high quality nutrients.

Fruit-derived proteolytic enzymes have softening effects on the integrity and fibre structure of meat, which may be of relevance in developing texture-modified targeted meat products for those with mastication difficulties. Five exogenous enzymes: bromelain, papain, ficin, *Bacillus Protease*, and *Aspartic Protease,* are currently classified as GRAS (Generally Recognized as Safe) by the USDA Food Safety Inspection Service (Calkins & Sullivan, 2007). Of these, the cysteine proteases papain and bromelain are the most well -studied in relation to meat tenderization (Bekhit et al., 2014). Papain and bromelain function by producing significant degradation of both myofibrillar and collagen proteins (Ashie et al., 2002; Kang & Rice, 1970).

Several authors (Zainal et al., 2013; Calkins & Sullivan, 2007) have reported a beneficial effect of fruit-derived proteolytic enzymes on tenderness, when used individually. However, to our knowledge, no study has examined the effects of a 50:50 combination of papain and bromelain on meat quality. Here, the aim was to evaluate if the combination of the two enzymes (papain and bromelain) has a more beneficial effect on beef tenderness, than treatment with either enzyme alone. Synergistic effects are postulated, since bromelain has been found to be very active against collagen proteins, while papain, a highly aggressive enzyme, causes significant degradation to both myofibrillar and collagen proteins, associated with severe disruption of the Z discs (Calkins & Sullivan, 2007).

The overall aim of this study was to investigate the potential tenderizing effects of papain and bromelain separately, and in combination, on tenderness, texture parameters, cooking loss, and color of beef M. *semitendinosus* steaks in order to understand their relevance to the development of products for older consumers or those with masticatory impairments. In

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parallel, application of a microstructure approach could contribute to a deeper understanding of the nature of the biochemical disruptions and alterations in muscle fibre structure and integrity that are likely to be occurring at the tissue scale, during tenderization with fruit-derived proteolytic enzymes.

1. MATERIAL AND METHODS

2.1. Raw material preparation

A total of 32 beef muscles [M. *semitendinosus* (*ST*)] from Holstein-Friesian steers were purchased from a local commercial processor on day 1 *post mortem* and aged for 7-10 days at 3°C. After aging, the muscles were trimmed of surface fat. The head and the tail of each muscle were discarded to minimize the variability within the muscle (Da Silva, 2015; Senaratne, 2011). The experiment was carried out in four runs with all four treatments analyzed in each run. Each muscle was cut into 8 steaks of uniform 2.54 cm thickness. Each *ST* steak was treated with either papain (P) (0.3g/100g meat), bromelain (B) (0.3g/100g meat) or papain/bromelain (PB) (0.15g:0.15g/100g meat) in a vacuum package, sealed, continuously tumbled for 20 minutes without vacuum, and cooked in a circulating water bath at 68°C for 20 minutes. Enzyme inactivation was achieved by heating at 82°C, for 5 minutes. Steaks were refrigerated overnight at 4°C. For the controls (C) the same technological treatments were applied, but without enzyme inclusion. Enzyme activity, associated with the effectiveness in meat tenderizing, varies among manufacturers. The enzymes used in this study were purchased from Enzybel, Belgium, with activity of 100 tyrosine units/mg (TU/mg) for papain, and 1200 gelatin digestion units/g (GDU/g) for bromelain. Manufacturers' recommended protocols were followed.

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2.2. Texture analysis

Warner-Bratzler shear force measurements (WBSF) analyses were carried out on cooked samples according to AMSA guidelines (AMSA, 2015) and Wheeler et al. (1996). Samples were sheared perpendicular to the fibre direction using the Instron Universal testing machine, Model 4464 (Instron Ltd., UK), with a load cell of 500 N, cross head speed 250 mm/min and data was analyzed in Bluehill®2 Software. Seven cores per steak were analyzed and following exclusion of the highest and lowest values, the average of five measurements was recorded for each sample.

For texture profile analysis (TPA), cooked samples were analyzed according to the method described by Bourne (1978) and Keenan et al. (2015). Three cores per treatment were obtained (diam. 25 × ht. 20 mm) and axially compressed to 90% of their original height in a two-cycle compression test using an Instron Universal Testing Machine Model 4464 (Instron Ltd., High Wycombe, UK). Force time deformation curves were obtained using a 2kN load cell applied at a cross speed of 500 mm/min. TPA recorded the following attributes: hardness (N), chewiness (N x mm), cohesion force (-), gumminess (N) and springiness (mm), as described by Keenan et al. (2015).

2.3. Color analysis and cooking loss

Measurements were taken using the CIE L*a*b* system with a dual beam xenon flash spectrophotometer (Hunter Lab Ultra Scan Pro, Inc., Reston, VA). The illuminant (D65, 10°) consisted of an 8° viewing angle and a 9.9 mm port size. Before each series of measurements, the instrument was calibrated using black glass and a white tile. Means of readings at three locations on each side of the thermal treated sample were assessed.

For cook loss, steaks were cooked at 68°C for 20 minutes in water bath, and the enzyme inactivation was achieved by increasing the temperature to 82°C, for 5 minutes. The weight of

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each sample was recorded before and after cooking. Cooking loss was expressed as the percentage of the weight difference.

2.4. Microstructure analysis

Treated beef samples (2 cm^3) were snap frozen in iso-pentane and liquid nitrogen (according to Meng et al. 2014) and stored at -80 °C. Sections were cut (20 μm thickness) using a Leica CM1950 cryostat (Leica Biosystems, Nussloch, Germany) after equilibration to specimen chamber temperature (-25 °C). Confocal scanning laser microscopy (CSLM) was used in conjunction with differential staining to visualize the impact of the enzymatic treatments on the meat fibre structure and integrity. Sections were stained with Nile Blue (0.1%) and examined under a Leica SP5 confocal microscope (Leica Microsystems GmBH, Mannheim, Germany).

2.5. Statistical analysis of data

Data were analyzed using one-way analysis of variance (ANOVA) in Genstat 14.1 (Rothamsted Experimental Station, Hertfordshire, UK) with animal included as a random effect. The potential tenderizing enzymatic treatment was considered as a fixed effect and significant differences among treatments were assessed using Fisher's LSD test, with the level of significance set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Texture analysis

Tenderness is the most important texture attribute to the consumer, and it reflects the commercial value of a meat cut (Chambers & Bowers, 1993) and Warner-Bratzler shear force (WBSF) is an objective measure of tenderness. In the present study, the treatments with papain and papain/bromelain significantly reduced WBSF values (P<0.05) from 46.24 N (C) to 37.95

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N for papain alone and 41.60 N for papain in combination with bromelain (Table 1). No effect on shear force was observed for bromelain alone compared with the control.

Texture Profile Analysis, which includes the evaluation of five textural parameters, is intended as a deformation test that permits mimicking of mastication. While most studies concerned with meat tenderness focus on WBSF values, here, TPA parameters (hardness, chewiness, cohesion force, gumminess and springiness), offered additional information about the textural characteristics of the meat products, since chewing difficulty is an important barrier to meat consumption in older people.

With regard to texture parameters, despite substantial replication, there was considerable variation within treatment, though a numerical reduction for hardness, chewiness, gumminess, and cohesion force values for enzyme-marinated steaks was observed (Table 1). As described by Bourne (2002) and Xiang et al. (2015), springiness is the ability of meat to resist external force, being a measure of the distance that the sample recovers its height during the time that elapses between the end of the first bite and the start of the second bite, therefore springiness is a measure of the product's elasticity. As well as the reduction in toughness observed through shear force analysis, springiness was also affected by the enzymatic treatments. All enzyme marinated samples had significantly reduced springiness compared with controls ($P<0.05$) (Table 1). Springiness effects were consistent across treatments, with no significant differences observed between different enzymatic treatments (Table 1).

Taken together, the WBSF and TPA springiness values suggest that treatment with papain and papain/bromelain could be considered as promising options for developing softer meat products. Optimization of various tenderizing treatments has potential to enhance the appeal of less tender cuts to older consumers and broaden the prospects for those with mastication impairment (Botinestean et al., 2016; Takei et al., 2015).

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3.2. Color analysis & cooking loss

Color was measured on both raw and cooked samples, and the effect of enzymatic treatments on the color parameters of beef *ST* steaks is presented in Table 2. For raw samples no effect was observed, which might be explained by the fact that most of the enzyme activity took place during the cooking process and these enzymes have reduced activity at low temperatures (4-10°C) (Calkins et al., 2007).

In this study, an effect on yellowness was observed for cooked samples. The values were significantly lower ($P<0.05$) when compared with control. This contrasts with Islam et al. (2013) who studied the possibilities of developing a meat tenderizer from papaya peel, and reported that there was no effect on the color of cooked samples. Additional denaturation of myofibrillar proteins and decreased production of metmyoglobin due to reduced myoglobin content associated with enzyme treatment could have played a role. The color opacity of cooked samples has been reported to be linked to both the denaturation of myoglobin as well as of actin and myosin (Pathare & Roskilly, 2016).

All the enzymatic treatments significantly ($P<0.001$) increased cooking loss from 30.61% (C) to a maximum of 38.62% (PB) (Table 2). An increased in cooking loss might be explained by the fibre destruction caused by enzymes inclusion during cooking, resulting in decreased water (juices) holding capacity. Extra myofibrillar shrinkage as well as the water movement from the myofilaments (actin and myosin) into the extracellular region of the muscle causes a reduction in water holding capacity, hence increased cooking loss. Therefore, the ability to retain water was considerably reduced, when enzymatic treatments were applied. Istrati et al. (2012) have studied the impact of beef tenderization with papain and bromelain on free and bound water, and also reported a negative effect on the water holding capacity of enzymatically treated samples. The reduction of bound water for the samples tenderized with papain and bromelain was likely due to the fragmentation of myofibrillar proteins.

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3.3. Microstructure analysis

CSLM is an innovative technique compared with the more traditional fluorescence microscopy, and it allows 3D imaging and clear visualization of the fibre structure of muscle tissues (Damez & Clerjon, 2008). Straadt et al. (2007) applied CSLM to investigate the changes in myofibres and myofilaments related to ageing and cooking muscle, respectively and concluded CSLM is a promising technique to investigate meat quality at microstructural level (Straadt et al., 2007). A key characteristic of confocal microscopy is three-dimensional imaging of food structures. In the present study, imaging analysis by CSLM proved to be an efficient tool for observing the textural changes in meat, permitting visualization of the disruption of the structure of meat fibres as a result of the tenderizing effect of fruit-derived proteolytic enzymes.

The microstructure images of treated and untreated meat samples are presented in Figure 1 (a-d) at lower resolution (20x) and in Figure 1 (e-h) at higher resolution (63x).

The lower resolution images (Figure 1 a-d) show differences between the control and enzyme-treated samples at the microstructural scale. In the control (Figure 1a), well organized bundles of myofibres with an intact structure can be observed. Fibre rupture, caused by the action of the enzymes, can be seen in the samples treated with fruit derived proteolytic enzymes [Figure 1 (b, c, d)]. The breaking down of myofibril tissue structure, observed for the treated samples, confirms the meat quality observations at macro-structural level. As mentioned by Ashie et al. (2002), enzymatic hydrolysis of meat myofibrils results in loss of fibre integrity and reduced shear forces. While papain is reportedly a more aggressive enzyme, compared with bromelain (Calkins & Sullivan, 2007), major differences in effect between the two enzymes were not evident from the images. However, the fibre disruption for the samples treated with 50:50 papain/bromelain (Figure 1 d) appeared to be greater, compared to either enzyme alone (Figure 1 b & c), suggesting possible synergistic effects due to different modes of action of each enzyme.

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The observed disruption of the muscle fibres through enzyme action may also be associated with the observed higher moisture losses incurred during the cooking process for all the enzymatic treatments compared to control and is consistent with observed reduction in processing yields and altered yellowness values of the cooked samples.

It is noteworthy that, when samples were visualized at a higher resolution (Figure 1 e-h), the images showed well organized bundles of myofibrils (23 - 65 µm) and intact sarcomere structure, in treated as well as control samples. We can speculate that the enzymatic activity may have taken place mainly at the surface of the fibre bundles through, for example, degradation of the perimysium, with minimal degradation of myofibrils and Z bands observed prior to enzymatic denaturation, which is consistent with the proposed modes of action of papain and bromelain (Calkins & Sullivan, 2007).

Few studies have applied microscopy to examine the microstructural effects of tenderizing fruit enzymes, but Chaurasiya et al. (2015) studied the effect of commercial bromelain and reverse micellar extracted and purified bromelain on meat tenderization using Cryo-SEM (visualized at 1000x). That study also reported ruptured tissues for enzymatic treated beef samples when the samples were analyzed at the microstructural level. Overall, the microstructural analysis of beef treated samples with papain, bromelain or a combination of papain and bromelain presented here are consistent with the instrumental or technological parameters reported in the present study and in the literature.

Chen (2016), Nyberg et al. (2015) and Takei et al. (2015) described the importance of developing and enhancing new food products adapted to older consumers, in order to promote health and prevent malnutrition. It is important to assess the technological performance of such products. In this screening study, fruit-derived proteolytic enzymes have been assessed in relation to developing texture modified meat products (beef steaks) targeted at older

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consumers. Providing a basis for optimizing beef steaks that require reduced mastication effort might lead to increased consumption by older consumers.

CONCLUSIONS

Both enzymatic treatments that included papain significantly reduced WBSF values. While bromelain did not significantly reduce the shear force values of the treated samples, all the other quality traits examined were affected similarly to papain and the combination of both enzymes. The effects on tenderness, color and cooking loss were similar for the samples treated with papain and for those treated with 50:50 papain and bromelain. The product tenderized with papain and papain/bromelain will have potential for developing texture-optimized meat products requiring a reduced mastication effort that could be beneficial for those with chewing difficulty, such as older consumers. CSLM proved to be an effective tool to characterize meat microstructure and understand the texture, color and cooking loss responses in proteolytic enzyme treated formulations, and also to visualize the differences between the tenderizing treatments. Images showed ruptured muscle fibres in enzymatic treated beef samples but an intact sarcomere structure. The microstructure analysis might contribute to a deeper understanding of the nature of the muscle fibre changes that take place at tissue scale during tenderization with fruit derived proteolytic enzymes. Future work could focus on sensory evaluation of the steaks prepared under the proposed enzymatic procedures to determine their acceptance among elderly consumers.

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ETHICAL STATEMENTS

Conflict of Interest: This is to state that the corresponding author and all of the authors have read and approved the final submitted manuscript, have no conflict of interest.

Ethical Review: This is to state that this study does not involve any human or animal testing.

Informed Consent: This is to state that the written informed consent was obtained from all study participants.

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Table 1. Effect of enzymatic treatments on Warner Bratzler Shear Force (WBSF) and texture profile parameters of cooked *ST* beef steaks

Enzymatic treatment[#]	**WBSF (N)**	**Hardness (N)**	**Chewiness (N x mm)**	**Cohesion force (-)**	**Gumminess (N)**	**Springiness (mm)**[*]
C	46.24±2.24[a]	316.04±93.90	784.81±267.93	0.85±0.01	123.28±31.30	6.10±0.71[a]
P	37.95±4.33[c]	264.93±79.78	524.46±107.03	0.83±0.01	97.92±20.23	5.29±0.36[b]
B	42.76±1.44[ab]	238.29±66.78	474.09±108.09	0.83±0.02	91.46±23.33	5.14±0.15[b]
PB	41.60±2.82[bc]	266.01±98.38	555.44±208.59	0.83±0.01	101.98±32.60	5.31±0.41[b]
P value	0.013	0.642	0.142	0.129	0.421	0.046
SEM	2.05	60.50	131.50	0.008	19.40	0.32

[a, b, c] – means within column that do not share a common superscript are significantly different ($P<0.05$)

[#] - the acronyms of the enzymatic treatments are described in Materials and methods, section 2.1.

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Table 2. Effect of enzymatic treatments on color parameters: lightness (L*), redness (a*), yellowness (b*) and cooking loss (%) of *ST* beef steaks

Enzymatic treatment[#]	**Color parameters**						**Cooking loss (%)** [B]
	Lightness (L*)		**Redness (a*)**		**Yellowness (b*)**		
	raw	*cooked*	*raw*	*cooked*	*raw*	*cooked* [A]	
C	40.51±2.49	45.00±4.78	8.25±1.12	6.40±0.50	9.64±1.44	13.12±0.37[a]	30.6±2.62[a]
P	38.26±3.24	48.30±3.08	8.84±0.84	6.13±0.77	9.47±1.85	12.29±0.33[b]	37.1±2.84[b]
B	37.82±3.27	49.33±1.98	8.51±0.57	6.30±0.67	8.54±1.33	12.32±0.62[b]	38.6±2.20[b]
PB	36.56±2.39	46.87±3.77	8.65±0.58	6.47±0.50	8.32±1.73	12.11±0.51[b]	38.6±0.90[b]
P value	0.315	0.382	0.768	0.880	0.584	0.046	<0.001
SEM	1.44	1.78	0.40	0.31	0.80	0.24	1.60

a, b – means within column that do not share a common letter are significantly different ($P<0.05$)[A], ($P<0.001$)[B]

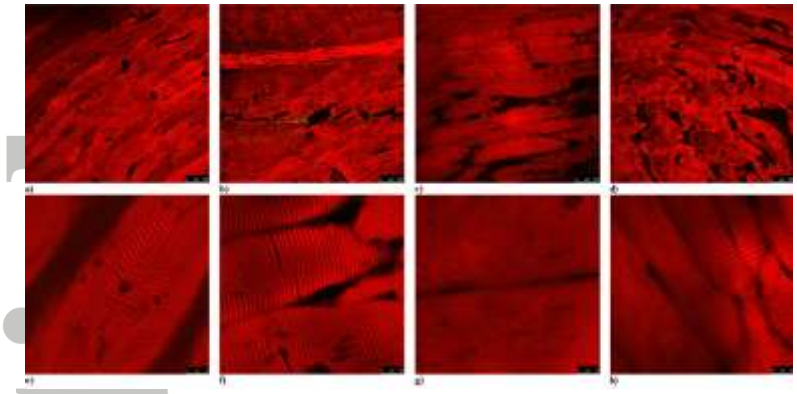
[#] - the acronyms of the enzymatic treatments are described in Materials and methods, section 2.1.

Figure 1. Confocal scanning laser microscopy (CSLM) images of cryostat sections:

- a-d (20x)
- e-h (63x)

of: control (a & e), papain (b & f), bromelain (c & g) and papain/bromelain (d & h) treated beef samples.

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