



Enhancing Steak Taste, Juiciness, Tenderness, and Acceptability through Bromelain-Enzyme Marination: A Sensory-Focused Approach

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Abstract

This study aimed to evaluate the effects of bromelain and papain proteolytic enzymes on the physicochemical and sensory properties of beef steak. Treatments included a control (P0, without proteases, papain marination for 20 minutes (P1) and 30 minutes (P2), and bromelain marination for 10 minutes (P3) and 15 minutes (P4). Parameters measured were moisture content, pH, water activity (a_w), water holding capacity (WHC), color (L^* , a^* , b^*), and sensory attributes (aroma, taste, aftertaste, juiciness, tenderness, and overall acceptability). The results showed no significant differences ($p > 0.05$) in moisture, pH, a_w , WHC, or color across treatments. However, significant improvements were observed in sensory attributes except for the aroma. Bromelain treatment, particularly at 15 minutes, resulted in higher scores for tenderness, juiciness, and overall acceptability. Its selective proteolytic action on collagen and elastin enhanced meat texture and moisture retention without excessive protein degradation. Conversely, papain treatment led to some off-flavor development and a softer texture due to its broader proteolytic activity. In conclusion, bromelain proved more effective than papain in improving the sensory quality of beef steak. Marination with bromelain for 10–15 minutes is recommended as a natural tenderization method to enhance consumer acceptance and meat quality.

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Introduction

Meat is an essential source of animal protein in human diets due to its high content of essential amino acids, vitamins, and minerals. However, one major challenge in meat consumption, especially local beef, is its tough texture, particularly in muscle parts rich in connective tissue. Enhancing meat tenderness is a key focus in processing meat-based products like steak. One approach to improve tenderness is through the application of proteolytic enzymes, which can degrade structural proteins such as collagen and myofibrils, resulting in a softer texture. Bromelain (extracted from pineapple) and papain (extracted from papaya leaves) are among the most commonly and effectively used proteases.

Bromelain demonstrates strong proteolytic activity against collagen and myosin, significantly improving meat tenderness without adversely affecting color or flavor when used at appropriate concentrations (Abril et al., 2023). In contrast, papain is a more aggressive enzyme that can extensively degrade connective tissues, but may cause over-tenderization or mushy texture if not properly controlled (Razali et al., 2023).

Previous Studies have shown that both enzymes, at 100 ppm concentration, effectively disrupt meat protein structure, significantly reducing shear force in steaks (Maqsood et al., 2018). Papain-treated meat exhibited superior tenderness compared to the control. Similarly, bromelain was effective in enhancing meat tenderness by hydrolyzing difficult peptide bonds.

Previous research has indicated that bromelain and papain can positively affect the texture, flavor, and overall organoleptic properties of steak. Bromelain, in particular, has demonstrated the potential to reduce meat hardness and enhance flavor, making it more appealing to consumers (Utami et al., 2017). This enzyme also improves the water-binding capacity of meat, crucial for maintaining moisture and juiciness post-cooking (Sakhr & Khatib, 2019).

Both enzymes act specifically on peptide bonds in proteins, altering the structure of muscle and connective tissues (Ullah et al., 2022). In the food industry, the use of proteases in meat processing has gained attention for their role in improving meat texture and flavor. Furthermore, proteases enhance the nutritional value of meat by increasing amino acid bioavailability and generating bioactive peptides

beneficial to health (Sakhr & Khatib, 2019).

The effectiveness of these enzymes depends on concentration, treatment time, and application temperature. However, results may vary depending on meat type, application method, and processing parameters. Further studies are necessary to explore the impact of bromelain and papain on steak, especially in terms of texture, cooking loss, and sensory properties.

To date, research on the application of bromelain and papain on Indonesian local beef varieties such as Bali, Ongole, or PO (Peranakan Ongole) cattle is limited. These breeds generally produce tougher meat due to genetic factors, feed, and slaughter age, differing significantly from imported beef. This poses a challenge in developing processed products like steak, which require high tenderness as a key sensory attribute (Razali et al., 2023). This research fills a crucial gap by evaluating the practical applicability of natural proteases specifically on Indonesian local beef, thereby contributing new insights into enzyme-marination strategies for improving the sensory quality of underutilized meat resources. The findings offer context-specific recommendations relevant to tropical and developing meat industries that differ from those in Western contexts to commonly studied.

This study provides a comparative and contextual approach by using protease extracts derived from fresh, locally grown pineapples and papayas in Indonesia, better reflecting realistic applications in the local meat industry. Therefore, this research aims to evaluate and compare the effects of bromelain and papain on the physical and sensory characteristics of beef steak, providing applicable recommendations for producing high-quality meat products tailored to modern consumer preferences and enhancing competitiveness in both domestic and global markets.

Materials and Methods

Materials

The materials used in this study included beef (topside cut) from PO cattle, ripe pineapple, papaya leaves, distilled water (aquadest), black pepper, and salt. The equipment utilized consisted of an analytical balance (Ohaus, USA), a blender (Philips, Indonesia), PTFE-coated cookware (Maxim, Indonesia), and a stove.

Methods

Beef Preparation

Topside cuts of beef were selected and standardized in size for all treatments, with each sample having a thickness of 1 cm and a weight of 200 grams.

Preparation of Pineapple Protease Extract

The pineapple extract was prepared through several steps, including selection, peeling, washing, cutting, blending, and filtering. Pineapples were selected based on maturity—ripe but not overly soft. The fruits were peeled, and the eyes were removed before washing. The cleaned pineapples were then cut into small pieces and blended until smooth. The resulting pulp was filtered to separate the juice from the residue. The filtrate (juice) was collected for use in the subsequent stages.

Preparation of Papaya Leaf Protease Extract

Fresh papaya leaves (*Carica papaya*), in a mature-green stage, were harvested from local plants near the research site (Semarang, Indonesia). The leaves were thoroughly washed under running water to remove dirt and debris, then drained until no water remained. A total of 100 grams of papaya leaves were weighed and chopped into small pieces to facilitate extraction. The chopped leaves were blended with 200 mL of sterile distilled water (ratio 1:2 w/v) for 2–3 minutes until a homogeneous slurry was obtained. The crude extract was filtered through a double layer of muslin cloth to separate the pulp from the liquid. The filtrate, containing the proteolytic enzyme papain, was used for further experimentation.

Marination and Enzyme Application

Beef samples (200 g) were immersed in either pineapple protease or papaya leaf protease until fully submerged (200 mL). The marination durations were as follows: P1 = 20 minutes and P2 = 30 minutes for papaya protease, and P3 = 10 minutes and P4 = 15 minutes for pineapple protease. A control sample without enzyme treatment (P0) was also included.

Grilling Process

After marination, the beef samples were rinsed with mineral water (Aqua, Indonesia) to remove excess enzyme, then seasoned with salt and pepper. Grilling was performed on PTFE-coated cookware by cooking each side of the meat 175-181°C for 3 minutes.

Moisture Content

A 3-gram sample was placed in a hot air oven at 102°C for 24 hours until a constant weight was obtained. The moisture content was calculated by subtracting the final dry weight from the initial weight (AOAC, 2005)

$$\text{Moisture Content (\%)} = \frac{(W1 - W2)}{W1} \times 100\%$$

W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying

pH determination

Three grams of the sample were measured and blended with 20 mL of distilled water to form a homogeneous mixture. The pH was then determined using a pH meter (Bakhsh et al., 2021).

Water Holding Capacity (WHC)

A 5 gram portion of the sample was centrifuged at 4000 rcf for 30 minutes at 4°C. Water holding capacity (WHC) was calculated as the percentage of retained water using the following equation

$$\text{WHC (\%)} = \frac{W_a}{W_b} \times 100\%$$

W_a = sample weight after centrifugation

W_b = sample weight before centrifugation

Water Activity (a_w) determination

The water activity (a_w) was determined using an a_w-meter after the sample was ground to a homogeneous consistency using a mortar

Color determination

Color analysis was conducted using a digital colorimeter. The values for lightness (L^*), redness (a^*), and yellowness (b^*) were recorded. Three independent measurements were taken at random locations on the surface of each sample to ensure accuracy and representativeness.

Sensory Test

A sensory evaluation was conducted to assess aroma, taste, aftertaste, tenderness, juiciness, and overall acceptability of the samples. The evaluation involved five treatments: P0 (control), P1 (20 min papain), P2 (30 min papain), P3 (10 min bromelain), and P4 (15 min bromelain). There were 66 panelists who participated in the test, and they were untrained panelists. Panelists will receive samples with a random 3-digit code. Panelists evaluated each attribute using a 4-point intensity scale. Taste was evaluated on a four-point scale ranging from 1 (not savory) to 4 (very savory). Aroma was assessed from 1 (no meat-like aroma) to 4 (intense meat-like aroma). Texture was rated based on tenderness, with scores ranging from 1 (not tender) to 4 (very tender). In addition, a hedonic test was conducted for overall acceptance using a 4-point scale, where 1 indicated 'dislike very much' and 4 indicated 'like very much'.

Data Analysis

The data were first tested for normality and homogeneity of variance using the Shapiro–Wilk test and Levene's test, respectively. If the data were normally distributed and exhibited homogeneity of variances ($p > 0.05$), a one-way Analysis of Variance (ANOVA) was conducted, followed by Duncan's multiple range test for post hoc comparisons. Conversely, if the data did not meet the assumptions of normality or homogeneity ($p < 0.05$), the non-parametric Kruskal–Wallis test was applied. When significant differences were found ($p < 0.05$), Dunn's test was used for pairwise comparisons. All statistical analyses were performed using IBM SPSS Statistics version 2

Results and Discussion

Physicochemical quality of beef steak

The analysis indicated that there were no significant differences ($p > 0.05$) in moisture content among all treatments, including control and those treated with papain or bromelain (Table 1). Proteolytic enzymes such as papain and bromelain have been reported to enhance meat tenderness by breaking down structural proteins, which may consequently lead to moisture loss during cooking (Radiati & E., 2010; Rawdkuen et al., 2012). Furthermore, bromelain activity is known to cause higher levels of protein denaturation, contributing to the release of water from muscle tissues (Sunarsih & E.,

2008).

Similarly, water activity (a_w) did not differ significantly across treatments. Water activity is an important factor in determining the microbiological stability and sensory quality of meat products. Proteolytic enzymes such as bromelain and papain have been shown to denature proteins and influence the distribution of water within muscle tissue (Gokoglu et al., 2017; Xu et al., 2020). When applied in excessive concentrations or over extended periods, these enzymes may impair the meat's ability to retain water, thereby reducing a_w values (Truc et al., 2023). Lower a_w may contribute to improved shelf life by inhibiting microbial growth and maintaining meat texture and juiciness (Mohan et al., 2016).

The pH values of all treatments ranged from 6.63 to 6.75, with no significant differences observed. Previous studies have indicated that proteolytic enzyme activity can influence pH by promoting the release of amino acids and acidic groups from muscle proteins (Gerelt et al., 2000; Smith-Marshall et al., 2012). Marination using papaya leaves, which contain papain, has also been reported to lower meat pH due to the formation of free acids (Wahyuni & L., 2018).

Water holding capacity (WHC) values also showed no significant differences between treatments. WHC is commonly affected by the degree of protein structure modification, which influences the ability of meat to retain water (Ionescu et al., 2008; Mohan et al., 2016). Overexposure to proteolytic enzymes may result in excessive protein degradation, potentially decreasing WHC (Choi et al., 2012; Lima et al., 2012). In some cases, extended marination using bromelain has been shown to excessively soften tissue structure, thereby reducing its capacity to retain water (Choi et al., 2016).

The absence of statistically significant differences in moisture content, water activity, WHC, pH, and color parameters following papain and bromelain marination may be attributed to several interrelated factors. The efficacy of proteolytic enzymes like papain and bromelain is highly dependent on variables such as concentration, marination time, temperature, and pH. If these parameters are not optimized or sufficiently varied, enzymatic activity may be inadequate to induce measurable changes in meat quality attributes (Kumar et al., 2015; Lasekan et al., 2016; Mcfeeters, 2004). Additionally, interactions with other marinade components or unfavorable physicochemical conditions may inhibit enzymatic action, reducing its impact on structural modifications within the meat matrix (Inguglia et al., 2019).

Furthermore, variability in the intrinsic properties of the meat, such as muscle type, water content, and protein-fat composition, could overshadow the effects of enzymatic treatment, particularly when measurement

Table 1. Physicochemical of beef steak with the marination of bromelain and papain

Sample	Moisture ^{ns}	a_w ^{ns}	WHC ^{ns}	pH ^{ns}
P0	65.58 ± 3.61	0.82 ± 0.04	83.04 ± 6.02	6.72 ± 0.45
P1	63.06 ± 2.49	0.81 ± 0.04	85.94 ± 2.05	6.67 ± 0.45
P2	61.76 ± 1.69	0.80 ± 0.04	85.38 ± 4.94	6.63 ± 0.45
P3	60.08 ± 4.08	0.79 ± 0.05	82.36 ± 5.45	6.72 ± 0.41
P4	60.33 ± 6.44	0.80 ± 0.04	83.70 ± 6.61	6.75 ± 0.39

*P0: control, P1: 20 min papain, P2: 30 min papain, P3: 10 min bromelain, P4: 15 min bromelain

tools lack the sensitivity to detect subtle changes (Ehsanur Rahman et al., 2023; McFeeters, 2004; McFeeters et al., 2023). External inconsistencies during marination, including temperature fluctuation or non-uniform marinade application, may also contribute to high variability and diminished statistical power. Therefore, a more refined experimental approach that carefully controls these factors may yield clearer distinctions in future studies (Karageorgou et al., 2023).

Table 2 showed the color values of beef steak with the marination of bromelain and papain. The analysis showed that there were no significant differences ($p > 0.05$) in L^* values among all treatments, including control, papain, and bromelain applications. The L^* value indicates the lightness of meat, with higher values representing a brighter appearance. Previous studies have demonstrated that bromelain and papain can improve meat lightness through protein structure modification (Gokoglu et al., 2017). Furthermore, marination with non-recombinant bromelain has also been shown to increase L^* values in beef (Santos et al., 2020).

Table 2. Color values (L^* , a^* , b^*) of beef steak with the marination of bromelain and papain

Sample	L^* ns	a^* ns	b^* ns
P0	13.64 ± 3.67	1.95 ± 1.26	2.94 ± 1.96
P1	13.71 ± 3.60	0.87 ± 1.02	1.71 ± 1.82
P2	13.88 ± 3.56	1.19 ± 0.46	2.63 ± 1.02
P3	12.37 ± 2.62	1.02 ± 0.95	1.49 ± 1.55
P4	13.36 ± 3.96	1.86 ± 1.18	2.31 ± 1.76

* P0: control, P1: 20 min papain, P2: 30 min papain, P3: 10 min bromelain, P4: 15 min bromelain

Similarly, no significant differences ($p > 0.05$) were found in a^* values, which represent the redness intensity of meat. The a^* value is commonly affected by myoglobin content and its oxidation state. It has been reported that proteolytic enzyme activity can lead to the degradation of myoglobin pigments, thereby reducing red color intensity (Ye et al., 2021). In line with this, (Razali et al., 2023) observed that recombinant bromelain derived from MD2 pineapple reduced a^* values in goat meat.

The b^* values, indicating the yellowness of the meat, also showed no significant differences ($p > 0.05$) between treatments. Changes in b^* values are typically associated with modifications in protein and lipid structures due to enzymatic activity, which alter light reflection in the yellow wavelength range. (Nadzirah et al., 2016) found that bromelain treatment increased b^* values in beef as a result of such structural changes. The appropriate selection of enzyme type and marination duration is considered crucial for optimizing meat color attributes to meet consumer preferences.

Sensory characteristics of beef steak with the marination of bromelain and papain

In this study, the sensory characteristics of beef steak marinated with bromelain and papain were evaluated to determine the effects of enzymatic treatment (Table 3). The P2 treatment using papain received a lower panel score for aroma intensity compared to the control (P0), while the highest aroma score was recorded in the P4 treatment with bromelain.

This outcome may be attributed to the proteolytic activity of bromelain, which produces peptides and free amino acids that contribute to the formation of volatile aroma compounds (Bhattarai et al., 2021). However, prolonged application of papain may lead to the development of undesirable aroma compounds (Ketnawa et al., 2011).

Panelists assigned the highest taste scores to the P2 (papain for 30 minutes) and P3 (bromelain for 15 minutes) treatments, indicating that the optimal duration of enzyme application can enhance meat flavor. Both papain and bromelain hydrolyze proteins into peptides and free amino acids, which are associated with the enhancement of umami taste/ savory (Ketnawa et al., 2011).

Treatments with papain and bromelain showed variation in off-flavor and aftertaste characteristics. Extended marination times with papain (P2) lead to the formation of bitter compounds. Additionally, the type of protease used significantly influences off-flavor formation. Protease derived from papaya leaves was associated with higher off-flavor intensity compared to bromelain. Papain, due to its broad and less selective proteolytic activity, hydrolyzes various meat proteins, including myofibrillar and connective tissue proteins. Excessive protein degradation may result in the accumulation of peptides and amino acids, which, during cooking, can undergo Maillard reactions or lipid oxidation, leading to the formation of volatile aldehydes and ketones that contribute to off-flavors and bitter flavour (Wang et al., 2020).

Both papain and bromelain treatments improved juiciness scores compared to the control. This improvement is likely due to enhanced water-holding capacity (WHC) resulting from myofibrillar protein degradation, which allows better moisture retention during cooking (Bhattarai et al., 2021). Juiciness was higher in the bromelain-treated samples than in those treated with papain. This can be explained by the more selective action of bromelain on connective tissue proteins such as collagen and elastin (Ketnawa et al., 2011). Unlike papain, bromelain does not excessively degrade myofibrillar proteins, thereby preserving the meat's structure and water retention capacity. Conversely, the more aggressive proteolytic activity of papain can result in over-degradation, damaging muscle structure, and causing increased moisture loss during cooking (Bhattarai et al., 2021; Gokoglu et al., 2017). The perceived juiciness of beef, when marinated with bromelain or papain, can differ even if the moisture content remains similar. Furthermore, the structural changes induced by marination and enzyme activity influence how water is held within the meat. Even if the overall moisture content is unchanged, the distribution and release dynamics of that moisture can vary. For example, tenderization can cause a more immediate release of liquids, which contributes to a juicier taste perception during early stages of chewing (Zhang et al., 2023). Additionally, other components like fat content play a critical role in juiciness perception. Fat can act as a lubricant, influencing the sensory characteristics of the meat. A higher fat content in meat can lead to a richer perception of juiciness, independent of the moisture content (Behrends et al., 2005). Cooking methods also affect how moisture and fats are retained or released in

Table 3. Sensory characteristics of beef steak with the marination of bromelain and papain

Sample	Aroma	Taste	Off-flavor	Juiciness	Tenderness	Overall Acceptance
P0	2.21 ± 0.89 ^{ab}	1.85 ± 0.85 ^a	1.14 ± 0.43 ^a	1.38 ± 0.52 ^a	1.27 ± 0.45 ^a	1.38 ± 0.63 ^a
P1	2.02 ± 0.64 ^{ab}	1.94 ± 0.68 ^{ab}	2.21 ± 0.8 ^c	1.68 ± 0.59 ^{ab}	1.91 ± 0.72 ^b	1.58 ± 0.56 ^{ab}
P2	1.88 ± 0.81 ^a	2.33 ± 0.92 ^{bc}	2.36 ± 0.94 ^c	2.08 ± 0.77 ^{bc}	2.59 ± 0.91 ^c	1.83 ± 0.87 ^b
P3	2.03 ± 0.80 ^{ab}	2.38 ± 0.78 ^c	1.26 ± 0.56 ^{ab}	2.23 ± 0.72 ^c	2.56 ± 0.84 ^c	2.41 ± 0.76 ^c
P4	2.35 ± 0.89 ^b	2.27 ± 0.83 ^{bc}	1.23 ± 0.55 ^b	2.30 ± 0.84 ^c	2.98 ± 0.89 ^c	2.61 ± 1.07 ^c

* P0: control, P1: 20 min papain, P2: 30 min papain, P3: 10 min bromelain, P4: 15 min bromelain

a-c significant differences ($P < 0.05$)

the meat, impacting juiciness (Xu & Falsafi, 2023). Lastly, the subjective nature of sensory evaluation can account for perceived differences in juiciness. Panelists may base their perception on a combination of factors such as texture, fat release, and immediate moisture release rather than on objective moisture content measurements alone (Demir et al., 2021). These complex interactions illustrate why similar moisture levels do not directly translate to similar juiciness perceptions.

The highest tenderness scores were observed in the P4 (bromelain 15 minutes) and P3 (bromelain 10 minutes) treatments, confirming the effectiveness of bromelain in improving meat tenderness. These proteases break down collagen and myofibrillar proteins, reducing muscle rigidity (Bhattarai et al., 2021). Tenderness is also influenced by marination time, as longer exposure allows deeper enzyme penetration into muscle tissues, resulting in uniform and thorough tenderization not only on the surface but also within deeper layers (Manohar et al., 2016). Moreover, the tenderness of meat treated with bromelain (P3) was higher than that treated with papain (P1). According to (Chaurasiya et al., 2015), bromelain leads to faster and more uniform degradation of connective tissue, particularly in collagen-rich muscle, which accounts for the greater tenderness observed in bromelain-treated samples (Gokoglu et al., 2017).

The application of either bromelain or papain improved the overall acceptability of the steak. However, bromelain-treated samples received higher acceptability scores than those treated with papain. This is consistent with sensory evaluations by (Bhattarai et al., 2021), who reported that meat marinated with bromelain was rated higher than papain in overall acceptability. Other studies also show the same indication that Bromelain, derived from pineapple, has been used to improve the texture and overall quality of beef (Santos et al., 2020). Panelists described the bromelain-treated meat as more tender, juicy, easy to chew, and free from undesirable flavors or odors. In contrast, meat treated with papain was sometimes perceived as overly soft or bitter by some panelists. Treatment P4 (bromelain 15 minutes) achieved the highest score for overall acceptance, suggesting that bromelain, when applied at an optimal duration, can significantly enhance the sensory quality of beef steak.

Conclusion

The application of proteolytic enzymes, bromelain and papain, did not significantly alter the physicochemical parameters of beef steak, including moisture content, water activity, pH, water-holding capacity (WHC), and color values (L^* , a^* , b^*). However,

both enzymes demonstrated notable effects on sensory characteristics. Bromelain-treated samples, particularly at 15 minutes of marination, consistently showed superior outcomes in aroma, juiciness, tenderness, and overall acceptability compared to papain and control treatments. Overall, bromelain was more effective than papain in improving the sensory quality, taste, juiciness, tenderness, and overall acceptance of beef steak. An optimal marination duration of 10–15 minutes using bromelain is recommended to achieve better texture, flavor, and consumer acceptability in steak products.

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