# EFFECTS OF BROMELAIN ENZYMATIC PROTEOLISIS ON SOME RHEOLOGICAL AND TECHNOLOGICAL PROPERTIES OF BEEF

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**Abstract**

*The research focused on the effect induced by two enzymatic proteolytic solutions based on bromelain on certain rheological and technological traits of beef. There were studied samples from Longissimus dorsi muscle, non-enzymatically treated (control, NET group) or treated with natural originated bromelain solution (ABE group, solution 50%, triturated pineapple and filtered liquid phase, mixed with distilled water 1:1) and with bromelain food additive (BFA group, Bromelain powder 2000 GDU/g, solution 1%). There have been run 120 analytical tests (3 experimental factors x 4 reasoning criteria x 10 repetitions). The results indicated decreased Adams consistency, compared to control group, by 72.7% (ABE) – 90.9% (BFA) and reduced shear force by 16.56% (ABE, 39.30N) – 23.31% (BFA, 36.12N), vs. NET samples (47,10N). Water holding capacity was 13.70-20.79% decreased while the cooking yield was 10.68-12.70%, consequently to the bromelain proteolysis. Although textural improvement of the meat has been noticed, assessed instrumentally through tenderness descriptors, the proteolytic treatment induced, however, loss of beef technological properties. It remains to study to what extent the usage of bromelain, as proteolysis agent, could efficiently improve meat tenderness, without affecting significantly its technological or sensorial properties, knowing that bromelain could impregnate to meat, in certain situations, a slightly bitter and astringent taste, not accepted by some consumers.*

**Key words**: beef, proteolysis, bromelain, Adams consistency, shear force, water holding capacity, cooking yield

## INTRODUCTION

Enzymes were empirically used in food manufacturing long time before they were discovered and before the subsequent biochemical phenomena were understood. In deep knowledge of enzymatic actions is essential for the future usage of agricultural resources in producing better and healthier food. There is a high amount of enzymes involved in relevant biochemical changes in food components, such as carbohydrates, proteins and lipids hydrolysis or bioconversion, with final impact on food sensorial properties: colour, flavour, texture[11]. The evaluation and the technological usage of such complex systems require knowing perfectly the biochemical pathways and especially those linked to proteolysis and lipolysis [2].

Meat tenderness is considered as one of its main qualitative trait. This is related to the connective intermuscular and interfibrilar connective tissue quantity, to the sarcomere length and to the muscle proteolytic potential [5]. It is known that a harder, firmer texture of the meat is lowering the acceptance of this food in consumers[4]. The treatment with exogenous proteases is one of the most modern ways to improve meat tenderness. Some proteases, such as the papain, bromelain, both derived from vegetal raw matters, are used on a large scale as meat tenderising agents [1].They could digest the muscle proteins, including the collagen and elastines. They are available either as extracts from the originating fruit (bromelain from pineapple and papain from papaya), either as stable, lyophilised food additives. It seems

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they also have disadvantages hence they hydrolyse excessively both myofibrilar proteins and the connective ones. When used inappropriately, in terms of concentration and timing, they could lead to poor quality meat, such as exudative, texture less and bitter meat [8].

Within such conjuncture, we aimed to investigate the action of two enzymatic proteolytic solutions based on bromelain (pineapple extract and food additive), on the textural and technological features of beef.

## MATERIAL AND METHOD

Biological material consisted in 120 beef samples, issued from adult cattle *Longissimus dorsi* carcasses. The samples have been prepared in prism shaping, sized 3 cm length x 3cm width x 1.5 cm thickness. They were randomly and symmetrically distributed in 3 groups, in accordance with the experimental factors:

- NET group (40 samples, control group, non enzymatically treated);
- ABE group (40 samples treated with pineapple extract containing natively bromelain: solution 50%, triturated pineapple and filtered liquid phase, mixed with distilled water) (ABE group);
- BFA group (40 samples treated with bromelain food additive: BROMELAIN 2000 GDU/g, powder reconstituted to 1% solution).

The experimental factors have been applied for 7 hours on the studied samples. Then, 10 repetitions have been run for each reasoning criterion (Adams consistency and shear force as textural descriptors, instrumentally measured to assess tenderness; water holding capacity and cooking yield, measured to assess technological quality).

Effect of bromelain proteolysis on the meat texture has been tested through its deformation degree, using an Adams consistometer and through its shear force level, using a Warner Bratzler instrument.

The Adams consistometer measures the deformation of any sample, induced by gravity or by a discoid probe of known weight positioned on the sample put on a metal or glass plane surface. This one has a series of concentrically engraved circles with the origin situated in its geometrical center, the spot where the sample is also put for analysis. The circles radiuses increase by 3 mm steps. The method we used was adapted after Mateescu [7]. The samples from the three groups have been placed on the center of the consistometer. A discoid metallic press of 1 kg has been put on the top of each sample. After 30 seconds of action, it was identified the outer circle to which the sample extended (was deformed). The number corresponding to this limit is known as Adams consistency. More tender is the meat, higher the Adams value will be, therefore the consistency or firmness will be lower, if it would be considered from the sensorial perspective

The instrumental methods used to assess the mechanical properties of the muscle and to predict the textural meat features are based on shear forces measuring, applied on the samples via an Allo-Kramer, Warner-Bratzler or single blade dynamometer [12]. An TA.XT Stable Micro Systems analyser has been used in our research, to measure the total force (Newtons-N and Kg force-kgf) required to cross-cut the sample, using a mounted Warner-Bratzler shear blade.

Water holding capacity has been tested in accordance with the method proposed by Rawduken et al. [10]. Meat samples have been grinded, homogenized, introduced in glass tubes (20g sample/tube + 30 ml NaCl 6%). Meat and saline solution have been stirred for 1 minute then cooled down to 4°C, for 15 minutes. The resulted volumes were measured for each tube then they were submitted to centrifugation, for 25 minutes, at 3000 rotations per minute. The volume of humid precipitate that remained in the tube after supernatant removal, expressed as percentage from the initial volume was considered as the individual water holding capacity for each sample.

Cooking yield was measured through the method proposed by Ketnawa and Rawduken [6]. The samples have been submitted to 170°C thermal treatment for 30 minutes, to simulate the conditions existing in a cooking oven. The initial and the final weight of the samples have been measured and the cooking yield represented the percentage value of the final weight out of the initial one.

The data issued form the analyses have been statistically processed to obtain the main statistical descriptors, according to the methods described by Petrie & Watson [10].

## RESULTS AND DISCUSSIONS

Adams consistency had higher value in the meat treated with bromelain food additive solution, 1% (4.20±0.13 Adams units), therefore a decrease of the firmness (consistency) by 90.9%, compared to the non-treated samples in the control group (2.20±0.13 Adams units) (Fig. 1). In the beef treated with 50% pineapple extract, the Adams consistency was 72.7% improved. This suggested the presence of intense proteolysis in both positive treatments, hence the prism shape muscle samples extended much more than the non-treated beef samples, from the origin point of the Adams consistometer, when the discoid metallic probe was applied as deforming agent on all samples.

The shear force (table 1) had the highest value 47.10±1.58N in the non-treated meat, while the ones recorded in proteolysis samples were of 39.30±0.75 (bromelain from pineapple extract) and of 36.12±0.92N (bromelain food additive) (16.56-23.31% less force needed to cross cut samples) (Fig. 2).

Table 1 Dynamics of beef textural traits, as induced by bromelain proteolysis

| Trait | Group | Mean ($\bar{X}$) | Std. error ($\pm s_{\bar{x}}$) | v% | ±% vs. Control |
|---|---|---|---|---|---|
| Adams consistency (units) | NET (control, non-treated meat) | 2.20 | 0.13 | 19.17 | - |
| | BFA (bromelain food additive, 1%) | 4.20 | 0.13 | 10.04 | +90.91% |
| | ABE (pineapple extract, 50%) | 3.80 | 0.13 | 11.10 | +72.73% |
| Shear force (N/ kgf) | NET (control, non-treated meat) | 47.10/ 4.803 | 1.58/ 0.161 | 10.58 | - |
| | BFA (bromelain food additive, 1%) | 36.12/ 3.683 | 0.92/ 0.093 | 8.02 | -23.31% |
| | ABE (pineapple extract, 50%) | 39.30/ 4.007 | 0.75/ 0.046 | 3.66 | -16.56% |

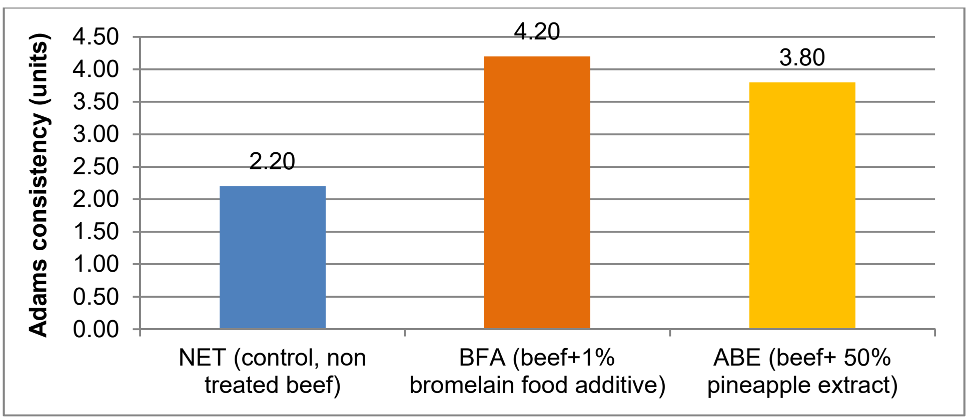


Fig. 1 Experimental factors influence on the *Longissimus dorsi* samples Adams consistency

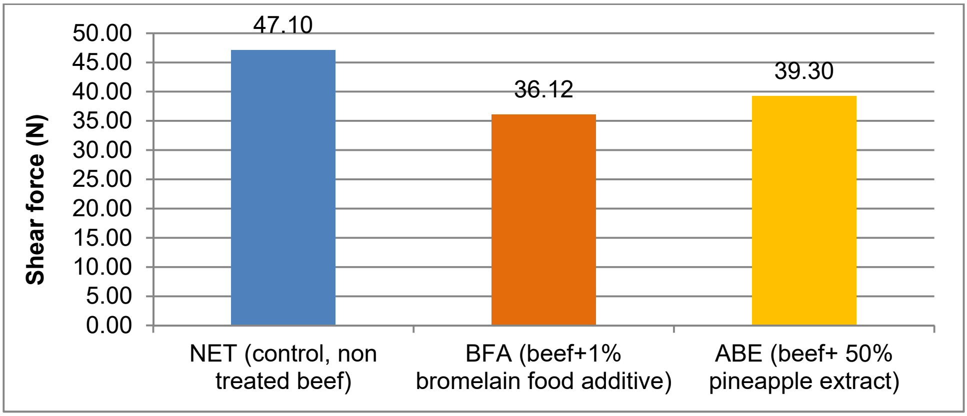


Fig. 2 Experimental factors influence on the *Longissimus dorsi* samples Shear force

According to the data presented in table 2, the best results related to the meat Water holding capacity were obtained in the non-treated beef (25.10±0.37%), suggesting a better structure than the beef samples submitted to proteolysis, where the proteins in connective tissue and structure were both affected by bromelain action.

In the BFA group (beef treated with bromelain food additive, 1%), the water holding capacity had an average value of 19.88±0.17%, therefore 20.79% lower than the control samples.

In the samples treated with pineapple extract (50% concentration), the same technological parameter presented an average value of 21.66±0.20 % (-13.70% compared to the non-treated meat) (Fig. 3).

The highest cooking yield was also noticed in the non-enzymatically treated meat (84.24±0.33%), while the lowest one (73.54±0.47%) was found in the samples treated with 1% solution of bromelain food additive. The beef submitted to preoteolysis with natural bromelaine solution (pineapple extract 50%) had an intermediate value for the cooking yield (75.24±0.30%) (Fig. 4). For this technological feature, the bromelain treatments induced decreases of 10.68-12.70%, compared to the non-treated samples, suggesting that the proteolysis induced higher exudation which favoured plasmatic water leakage and vaporising, upon the heating action of cooking.

Table 2 Dynamics of beef technological traits, as induced by bromelain proteolysis

| Trait | Group | Analytical value ($\bar{X}$) | Mean standard Error ($\pm s_{\bar{x}}$) | Variation coefficient v% | ±% vs. Control |
|---|---|---|---|---|---|
| Water holding capacity (%) | NET (control, non-treated meat) | 25.10 | 0.32 | 3.98 | - |
| | BFA (bromelain food additive, 1%) | 19.88 | 0.17 | 2.71 | -20.79% |
| | ABE (pineapple extract, 50%) | 21.66 | 0.20 | 2.93 | -13.70% |
| Cooking yield (%) | NET (control, non-treated meat) | 84.24 | 0.33 | 1.25 | - |
| | BFA (bromelain food additive, 1%) | 73.54 | 0.47 | 2.03 | -12.70% |
| | ABE (pineapple extract, 50%) | 75.24 | 0.30 | 1.26 | -10.68% |

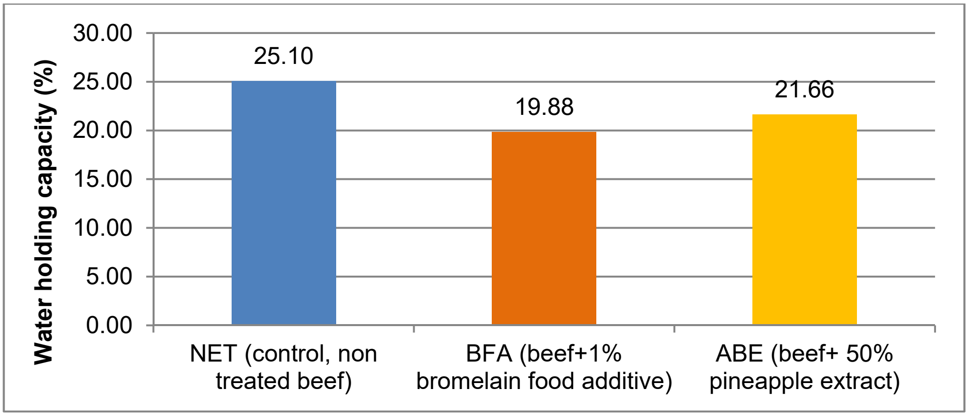


Fig. 3 Experimental factors influence on the *Longissimus dorsi* samples Water holding capacity

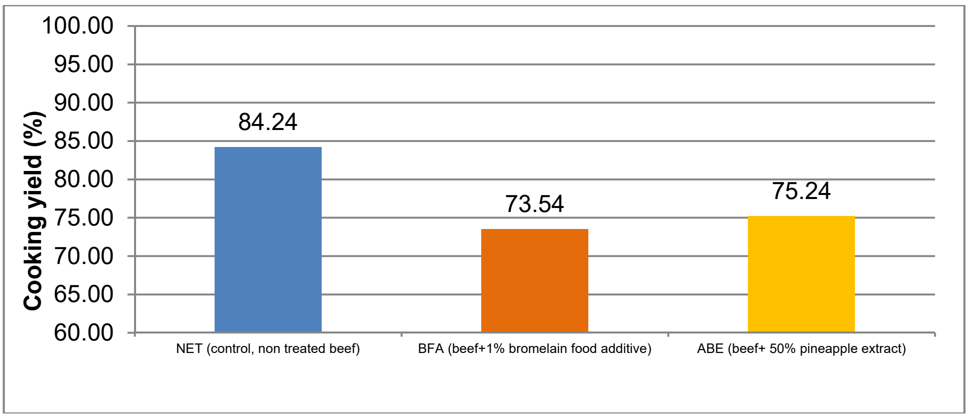


Fig. 4 Experimental factors influence on the *Longissimus dorsi* samples Cooking yield

Although both textural descriptors were improved, as data achieved via instrumental tests revealed, it remains to run sensorial testing, as follow-up of the research. It is known that vegetal originated proteases could enhance the texture, especially the tenderness of the meat, affecting, though, the taste and flavour, pushing them toward bitterness and astringency. Also, the decrease of the technological quality, despite the good results observed in texture profile suggests the non-specificity of the bromelain for meat proteins, meaning that such a protease hydrolyses both connective and myofibrilar proteins. It would be recommended to analyse comparatively vegetal and microorganism proteases, in order to find the more appropriate enzyme-substrate affinity, to achieve tenderness through mostly connective tissue hydrolysis. Such a more specific hydrolysis could be also instrumentally tested through NIRS total collagen analysis in the treated sample or through electrophoresis, as alternative follow-ups of the research.

## CONCLUSIONS

The experimental factors induced samples tenderness improvement, while the subsequent technological properties decreased.

Adams consistency was improved by 72.7 – 90.9 %, suggesting better proteolysis in bromelain 1% food additive;

Shear force (N) decreased by 16.56 – 23.31%, suggesting improved tenderness in the treated samples, while the untreated meat kept its raw texture.

Water holding capacity decreased by13.70-20.79% in bromelain treated samples.

The same trend was noticed for the cooking yield values, the calculated means indicating 10.68-12.70% less yield in the samples submitted to experimental factors.

The best textural results have been achieved when the bromelain food additive 1% was used as proteolysis agent. When it is considered to use such enzymes in the food processing industry, the loss of technological properties, as consequences of undifferentiated, nonspecific proteolysis must be considered. It remains the alternative of microbial originated proteases that could match better with the substrate, in order to decompose the connective intermyofibrilar tissue. However, for the common consumer in domestic gastronomy, bromelain is available as tenderizing agent through the preparation of a meat maturing marinade, in which the pineapple should be introduced up to 50%.

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