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# EFFECTS OF PAPAIN SPICES MIXTURE (SPIZYM) ON MEAT TENDERNESS

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## ABSTRACT

The purpose of this research was to know the effect of the long soaking with papaya extract (PE) with spices toward tenderness of Beef meat. Uniform-sized chunks of beef was marinated with concentration of (Spizym) (2.5% (w/w)).The research used experiment method based on Completely Randomomized Design, with 5 long soaking (without soaking, 15 minutes, 30 minutes, 45 minutes, and 60 minutes) at room temperature, each treatment was 3 replicated. The physico-chemical and quality characteristics (Tenderness, juiciness, color and flavor) of the marinated samples were determined. Result indicated that meat tenderness increase with the long of soaking with (Spizym).cooking yields decreased in all of the tested samples, especially when the concentration of mixture increased. In addition, a reduction of meat firmness and toughness were also observed in all of the samples when compared to the control. The best physico-chemical and quality characteristics of beef meat were 45, 15 minutes soaking at 30, 45 minutes cooking respectively.

**KEYWORDS**: Papain Extract, Proteolytic Enzyme, Beef Meat, Tenderness.

## 1. INTRODUCTION

Tenderness is one of most important meat texture attributes which affects the perception of beef meat, by the customers.[7,22] Tenderness is a complex trait. Generally, the two primary

structural features of muscle that influence tenderness are integrity of the myofibrils (termed the actomyosin effect) and the connective tissue contribution (termed a background effect).

Collagen plays an important role in the meat texture. The contribution of connective tissue to the secondary toughness of meat is dependent on the quantity, type and intermolecular cross-links of collagen which is the main component of connective tissue.[2] By formation of the cross-links between the collagen molecules, the meat of old animals becomes harder.

Identified six characteristics of perceived tenderness.[9] Three characteristics relate to the myofibrillar portion, two relate to product adhesion and the remaining one relates to connective tissue components. Myofibrillar proteins are located intracellularly while connective tissue proteins (primarily collagen) are located extracellularly. Stated simply, some muscles may lack palatability because of myofibrillar proteins while others may lack palatability because of the connective tissue proteins. A plausible tenderization strategy should target one or both of the main structural constraints to be successful.

In order to obtain tender meat at industrial level, it proceeds either to the slaughter of young animals, or to the storage of meats for ageing at low temperatures (0-40C°). During the ageing it takes place a limited process of proteolysis which leads to ultrastructural changes in skeletal muscle and to the improvement of meat tenderness.[3]

In the last years different tenderization techniques of beef meat were applied. These techniques include mechanical tenderization, storage at high temperatures, injection with calcium chloride, electrical stimulation, ultrasonation, high pressure treatment and enzymatic tenderization.[19] The enzymes of vegetable origin, such as papain, bromelin and ficin and bacterial collagenase[8] were often used for postmortem meat tenderization, without establishing exactly, which enzyme is more efficient. These enzymes have a large spectrum of action being involved in degradation of the main proteins of myofibrillar muscles (myofibrillar proteins, collagen), and sometimes lead to over – tenderization and to a product with a pasty texture.[11]

Papain (EC 3.4.22.2) is an endolytic plant cysteine protease enzyme which is isolated from papaya (Carica papaya L.) latex. Papain is obtained by cutting the skin of the unripe papaya and then collecting and drying the latex which flows from the cut. The greener the fruit, more active is the papain.[27] Papain shows extensive proteolytic activity towards proteins, short

chain peptides, amino acid esters and amide links and is applied extensively in the fields of food and medicine.[28]

Papain is used in meat tenderizers; the major meat proteins responsible for tenderness are the myofibrillar proteins and the connective tissue proteins.[17] United States federal agencies recognize five exogenous enzymes – papain, ficin, bromelain, Aspergillusoryzae protease and Bacillus subtilis protease – as Generally Recognized as Safe (GRAS) toimprove meat tenderness. enzyme distribution depends on the time, the temperature and the concentration of enzymes.[5] Also, the addition of fruit pulp, garlic or other spices contributes to decreased production of heterocyclic amines because of their antioxidant activity.

The red meat industry needs to produce high quality meat of consistent tenderness to increase consumer confidence and encourage further purchase of meat products.[6]

In the present study was aimed influence of Papain- spices mixture (Spizym) on beef tenderness and time of enzyme action and at estimating of the effect of thermal and enzymatic treatment on the beef meat tenderization. To increase the tenderness of beef were used enzyme preparations (papain and spices) from natural source of enzyme, using papaya fruit.

## 2. MATERIAL AND METHOD

### 2.1. Collect of Papain samples

Papain was collected from local papaya fruits before ripe (Taiz government). Papain juice collected manually from period July to August. Volume of papain juice was 200 ml, put it within dry and clean test tubs, then it were translated to laboratory rapidly and coldly.

### 2.2. Collect of Meat samples

The raw meat, utilized in research, was represented by the beef thigh from adult cows (more than 9 years old). The beef meat and was purchased in hot state from Thamar central slaughterhouse at maximum six hour post-slaughter. Then it put in cold box to laboratory rapidly and keep it in refrigerated.

### 2.3. Spices mixture samples

Spices mixture (Black pepper - cumin - white pepper - turmeric - laurel leaves) were obtained from Thamar central supermarket. put it in box and keep it at room temperature in dry place.

**2.4. papain-spices mixture samples Preparation**

Enzymatic solution was prepared by taken certain g from enzyme pulp as mentioned below and completed it to 100 ml. 1 g spices was added to enzymatic solution, a solution was shaked very well, then all meat samples were treated. That way every sample received equal amounts of spices and salt which are typically added to commercial meat tenderizers to develop desirable flavor.

**Table. (1): Papain- spices mixture (Spizym) concentrations.**

| Enzyme pulp g | Distilled water ml | Enzyme Percentage % |
|---|---|---|
| 2.5 | 97.5 | 2.5 |

**2.5. Meat Samples preparation**

The adult beef meat separated from conjunctive tissue and fat was cut into pieces of the same size in length and thickness weighing approximately 100 g, cut along the muscular fibers. The meat pieces were then divided into five slices and were used for a certain treatment. For each treatment series were constituted, consisting of

Sample P1 – slice of meat soaked for 15 minute with 2.5% papain- spices mixture.

Sample P2 – slice of meat soaked for 30 minute with 2.5% papain- spices mixture.

Sample P3 — slice of meat soaked for 45 minute with 2.5% papain- spices mixture.

Sample P4 – slice of meat soaked for 60 minute with 2.5% papain- spices mixture.

Sample P5 – (control sample) - pieces of meat without enzyme addition.

**Effects of cooking period on palatability of soaked meats in papain-spices mixture**

Samples soaked as mentioned above and the control meat were sealed in heat-resistant bags, and subjected to cooking in boiled water for different periods (15,30,45 and 60 minutes).

**Meat samples a soaked (15,30,45 and 60 minutes) with papain –spices mixture (2.5%)**

Samples were cooked for (15,30,45 and 60 minutes), then the Sensory were evaluated (tenderness, juiciness, flavor, color) after each period, compared with control.

**3. RESULTS AND DISCUSSIONS**

**1. Effect length of soaking withpapain –spices mixture (2.5%)**

**1. Effect long soaking (15 min) and long cooking (15-60minut) on meat palatability**

The meat samples were soaked for 30 min at room temperature. Samples were cooked for 15,30,45 and 60 min at a temperature of 150 C°. A fife-member panel judged the tenderness,

juiciness, color and flavor of the cooked samples using a 5-point hedonic scale where 1 = poor and 5 = best desirable attribute.

**Table. (2). Sensory evaluation of beef meat, soaking (15 min) and long cooking (15-60 minut.**

| Palatability characteristics | Cooking periods/ minutes | | | |
|---|---|---|---|---|
| | **15** | **30** | **45** | **60** |
| Juiciness | 1.5 | 3.4 | **4.2** | 2.6 |
| Tenderness | 3.4 | 4 | **4.6** | 2 |
| Color | 2.8 | 3 | **4.4** | 4.5 |
| Flavor | 3 | 3.9 | **4.1** | 1.5 |

Measurements of the effect of meat soaking with mixed enzyme toward meat palatability was on Table (2). Some physico- chemical characteristics occurrences at a meat was treated with papain and spices. Cooked meats have suffered significant changes, one of the most important being weight loss, due to juice and fat losses recorded with long cooking, but these changes specially loss of fat lead to good flavor and color.

In figure (1) are showed a increase in tenderness and the brown color desired in meat. One of the characteristics that define the quality of meat its ability to retain its own water and water added. Other attributes of meat, such as juiciness, flavor and color are related to water holding capacity. Also, a close correlation is between water holding capacity and weight losses, which take place in meat during thermal treatment. Experimental data, showed in Table (1), indicate the positive effect of mixed enzyme treatment on water holding capacity of thermal treated samples. Water holding capacity decreases with increasing cooking period. According to[10], the best water holding capacity was showed at initial time by the soaked and control samples.

Soaked samples

Control samples

**Figure. (1) Soaked beef meat samples (15 min), and long cooking (15-60 min) compared with Control samples.**

**1. Effect long soaking (30 min) and long cooking (15-60minut) on meat palatability**

This being a study on meat tenderization, special emphasis was placed on texture, where 1 represented the toughest and 5 represented the most tender as perceived by the teeth and palate.

Data on the sensory evaluation of meat soaked for 30 min are presented in table (3). Texture was the most important attribute to consider in this sensory evaluation. Mixed papain clearly had a tenderizing effect on the beef samples. A Cooking at 30 min had twice as much as in tenderness, juiciness, color and flavor.

Based on the comments of the sensory panel, the meat was somewhat mushy and grainy like chopped liver. Also, it had a slightly bitter taste, perhaps due to the breakdown of some of the protein fibers to amino acids like L-tryptophan & L-tyrosine.[4]

**Figure. (2): Soaked beef meat samples (30 min), and long cooking (15-60 min) compared with Control samples.**

**Table. 3: Effect of mixed papain enzyme on the sensory evaluation of beef based on a hedonic scale.**

| **Palatability characteristics** | **Cooking periods/ minutes** | | | |
|---|---|---|---|---|
| Juiciness | **15** | **30** | **45** | **60** |
| Tenderness | 3 | **3.8** | 3.5 | 2.4 |
| Color | 2.4 | **4.3** | 3.4 | 2 |
| Flavor | 3 | **4.5** | 3.4 | 2.5 |

Ageing and marinating, regardless of the method applying, resulted in a reduction of muscle hardness, springiness, cohesiveness, connective tissue perceptibility and stringiness as well as an increase of tenderness and juiciness. A similar effect of ageing on muscle texture was reported by.[7,14,19, 18,20,24,25]

**2. Effect long soaking (45 minut.) and long cooking (15-60minut) on meat palatability**

Experimental data showed that the beef has the best tenderness, juiciness, color and flavor in using the mixture of papain enzyme at soaking 45 min and cooking for 30 minutes as seen in table (4) and figure (3). Tenderization of beef meat was treated with papain resulted in meats with soft texture. The resistance to pressure of the samples with papain is much lower compared with the control samples. The decrease of the resistance to compression of the samples enzymatic treated is explained by the action of the enzyme by weakening muscle structure, the loss of physical integrity of muscle fibers and profound changes in the structure of cover membrane consisting mainly from collagen. The improved meat tenderization with vegetable cysteine proteases is due to the higher breakdown of myofibril proteins and the disruption of the muscular fibril structure in the experimental samples compared to the control ones.[15] showed higher activity for myofibrillar fraction with stronger solubilizing activity on connective tissue.[21] reported that papain and pressure treatment had synergistic effect on improving tenderness with higher collagen solubility.[16] suggested that papain infusion plus forking technology was more suitable for tenderizing spent hen meat cuts than injection method.[13] also showed the synergistic effect of papain and sodium tripolyphosphate in increasing the tenderness of chicken gizzard.

**Table. 4: Effect of mixed papain enzyme on the sensory evaluation of beef based on a hedonic scale.**

| Palatabilitycharacteristics | Cooking periods/ minutes | | | |
|---|---|---|---|---|
| | **15** | **30** | **45** | **60** |
| Juiciness | 1.5 | **4.7** | 3.2 | 2 |
| Tenderness | 3.1 | **4.9** | 3.8 | 2.3 |
| Color | 4.2 | **4.6** | 4 | 3.5 |
| Flavor | 4 | 4.5 | **4.7** | 4 |

**Figure. (3): Soaked beef meat samples (45 min), and long cooking (15-60 min) compared with Control samples.**

**3. Effect long soaking (60 min) and long cooking (15-60minut) on meat palatability**

Measurements of the effect of meat soaking with mixed enzyme toward meat palatability was on Table (5).

The lower the water retention rate is observed at soaking for long period (60 min). This is caused by the higher rate of hydrolysis of the meat proteins, respectively by the gelatinization of the samples and the release of terminal peptides and amino acids.[1] reported that, By increasing the levels of added enzyme and of the time of ageing, the juice losses at thermal treatment significantly increased.

However the level of tenderness was excessive leading to a relatively poor flavor score (1.3) at 60 C° and this similar to study by Smalling et al., had scored that flavor dry cured hams without treatment was prefer than treatment with the papain.[26]

**Table 5. Effect of mixed papain enzyme on the sensory evaluation of beef based on a hedonic scale.**

| Palatability characteristics | Cooking periods/ minutes | | | |
|---|---|---|---|---|
| | **15** | **30** | **45** | **60** |
| Juiciness | 1.8 | 3.4 | **4** | 1.3 |
| Tenderness | 2.4 | **4.1** | 3.5 | 1.5 |
| Color | 3.8 | **4** | 3.5 | 3 |
| Flavor | 3.5 | 4.2 | **4.5** | 1.3 |

In the table 5,it is clear that the meat samples treated with papain for 60 minutes, samples show muscle fiber deformation and breakdown, and the color fades. The dissociation of the muscle fibers at such high rate is to be avoided since the outward appearance of the meat is of major importance to the consumers. According to[23], higher concentrations of the papain preparations were able to hydrolyse the meat connective tissue proteins in a non-specific manner. whereas the papain samples, particularly the high soaked ones, undergo complete

breakdown of the connective tissue. Such intense hydrolysis leads to protein loss, deterioration of the organoleptic qualities of the meatand meat received high tenderness but high score for bitterness too.[12]

## CONCLUSIONS

Treatment with proteolytic enzymes results in disorganization and disintegration of the collagen structural elements, which in turn loosens and disrupts the intermolecule bonds.

Research result indicated that meat tenderness would increased with longer soaking in Papain spices mixture (Spizym. The optimal variant for hydrolysis with minimal protein loss and optimal preservation of the orgonoleptic qualities of the product is recorded in the soaking of the raw samples in papain solutions with preferred Palatability characteristics. The best physico-chemical and quality characteristics of beef meat were 45, 15 minutes soaking at 30, 45 minutes cooking respectively.

## REFERENCES

1. Aurelia I., Iuliana A., Gina P., Effect of papain and bromelin on muscle and collagen proteins in beef, The Annals of the University Dunarea de Jos of Galati , Fascicle VI – Food Technology, New Series, II (XXXI), 2008; 9-16.
2. Bailey, A. J., The basis of meat texture. Journal of the Science of Food and Agriculture, 1972; 23: 995–1007.
3. Bailey, A. J. And Light, N. D., Connective Tissue in Meat and Meat Products. 1989;(pp 1-355). Elsevier Applied Science, New York.
4. Belitz H.-D., W. Grosch& P. Schieberle. Food Chemistry, 4th ed. Springer-Verlag, Berlin Heidelberg, 2009; p34.
5. Bernholdt, H.F., Enzymes in Food Processing. Second Ed. Academic Press, New York, San Fransisco. London, 1975.
6. Boleman, S.J., Boleman, S.L., Miller, R.K., Taylor, J.F., Cross, H.R., Wheeler, T.L.:Consumer evaluation of beef of known categories of tenderness, Journal of Animal Science, 1997; 75: 1521-1524.
7. Brooks, J. C., Belew, J. B., Griffin, D. B., Gwartney, B. L., Hale, D. S., Henning, W. R., Johnson, D. D., Morgan, J. B., Parish, F. C., Reagan, Jr., J. O., Savell and J. W.,. National beef tenderness survey-1998. Journal of Animal Science, 2000; 78: 852–1860.
8. Cheftel, J.C. and Culioli, J.,. Effects of high pressure on meat: A review. Meat Science, 1997; 46(3): 211-236.

9. Cover et al. 1962.
10. Daniela Istrati, CameliaVizireanu, Felicia Dima, RodicaDinică. Effect of marination with proteolytic enzymes on quality of beef muscle, 13(1): 081-089, ISSN 1582-540X, Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry St. Cerc. St. CICBIA, 2012; 13(1).
11. Foegeding, E. A. and Larick, D. K., Tenderization of beef with bacterial collagenase. Meat Science, 1986; 18: 201–210.
12. Gereld, B., Ikeuchi, Y., Suzuki, A.:. Meat tenderization by proteolytic enzymes after osmotic dehydration, Meat Science, 2000; 56: 311-318.
13. Grover, R.K. et al.Indian J. Poult. Sci., 2005; 40(2): 202-205.
14. Islam M.N., & E. Molinar-Toribio.. Desarrollo de ProductosNovedosos de Papaya Panamena: Encurtidos en forma de Deditos y Rayados. Tecnociencia, 2013; 15(1): 43-56.
15. Jorgova V. K., Danchev S., Kostov A.: Effect of bacterial enzyme preparation on the solubility and electrophoretic properties of muscle proteins. Proceedings, International Congress of Meat Science and Technology, 1989; 35: 913-917.
16. Khanna, N. 1995. Ph.D. Dissertation, CCS HAD, Hisar.
17. Khanna, N. and P.C. Panda,. The effect of papain on tenderization and functional properties of spending hen meat cuts. Indian J. Anim. Res., 2007; 41: 55-58.
18. Kołczak T., Pospiech E., Palka K., Łącki J., Changes of myofibrillar and centrifugal drip proteins and shear force of psoas major and minor and semitendinosus muscles from calves, heifers and cows during post-mortem ageing, Meat Sci., 2003; 64: 69–75.
19. Koohmaraie, 1992a, 1992b.
20. Maher S.C., Mullen A.M., Moloney A.P., Drennan M.J., Buckley D.J., Kerry J.P., Colour, composition and eating quality of beef from the progeny of two Charolais sires, Meat Sci., 2004; 67: 73–80.
21. Mendiratta, S.K. 1992. Ph.D. thesis, CCS HAU, Hisar.
22. Miller et al., 2001.
23. Minh H.A., Bekhit A.E.D., Carne A., Hopkins D.L.: Characterisation of commercial papain, bromelain, actinidin and zingibain protease preparations and their activities toward meat proteins. Food Chemistry, 2012; 134 95–105.
24. Monson F., Sañudo C., Sierra I., Influence of cattle breed and ageing time on textural meat quality. Meat Sci., 2004; 68, 595–602.

25. Palka K., The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. Meat Sci., 2003; 64: 191–198.
26. Smalling, J.B., J.D. Kemp, J.D. Fox and W.G. Moody., Effect of antemortem injection of papain on the tenderness and quality of dry cured hams. J. of. Anim. Sci., 1971; 32(6): 1107-1112.
27. Tsuge, H., T. Nishimura, Y. Tada, T. Asao and D. Turk et al.,. Inhibition mechanism of cathepsin L-specific inhibitors based on the crystal structure of papain- CLIK148 complex. Biochem. Biophys. Res. Commun, 1999; 266: 411-416. DOI: 10.1006/bbrc.1999.1830
28. Uhlig, H.,. Industrial Enzymes and their Applications. 1st Edn., John Wiley and Sons, New York, 1998, ISBN-10: 0471196606: 454.