INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 1, p. 102-112, 2021

REVIEWPAPER OPEN ACCESS

Meat Tenderization through Plant Proteases- A Mini Review

Ali Ikram, Saadia Ambreen, Amber Tahseen, Areeg Azhar, Khadija Tariq, Tayyba Liaqat, Muhammad Babar Bin Zahid, Muhammad Abdul Rahim, Waseem Khalid*, Naqash Nasir

Institute of Home and Food Sciences, Government College University Faisalabad, Pakistan

Key words: Meat Tenderization, Proteases, Papain, Bromelain, Ficin.

http://dx.doi.org/10.12692/ijb/18.1.102-112 Article published on January 20, 2021

Abstract

The tenderness and quality of meat is very concerning for meat consumers. Meat tenderness relies on connective tissue, and muscle proteolytic ability. The use of various chemical tenders is the subject of the majority of research studies on the meat tenderness. However, there are certain drawbacks of these chemical tenders on one or the other sensory characteristics of meat. Few natural tenderizers may be used to counteract these adverse effects of chemical goods. Natural tenderizers are certain vegetables and fruits containing proteolytic enzymes that are responsible for rough meat tenderization. The use of exogenous proteases to enhance the tenderness of meat received tremendous interest in order to consistently produce meat tenderness as well as add value to low-grade cuts. The overview elaborates the sources, characteristics, and uses of plant proteases for the tenderization of meat. Furthermore, it highlights the impact of plant protease on the meat quality and effect on the meat proteins. Plant enzymes (including papain, ficin and bromelain) have been thoroughly studied as tenderizers for meat. The efficient use of such enzymes in raw meat calls for the determination of their enzyme kinetics and features and an understanding of the effect of the meat surrounding ambient (pH, temperature) conditions upon enzyme function. This allows for the creation of optimum conditions for tendering fresh meat and the removal or mitigation of any harmful effects on other quality characteristics.

* **Corresponding Author:** Waseem Khalid ✉ waseemkhalid@gcuf.edu.pk

Introduction

Consumer's choice of meat is increasing day by day because of the awareness of the quality of the meat, especially red meat and purchasing mainly dependent on it (Thorslund, 2016; Mullen *et al.*, 2017). Tenderness, juiciness and flavor are the three main characteristics which affect the sensory attributes of the meat (Aaslyng and Meinert, 2017; Picard and Gagaoua, 2017). The consumer's choice of purchasing has been mainly dependent on the tenderness of the meat (Mullen *et al.*, 2017; Wang *et al.*, 2017; Bhat *et al.*, 2018). The high level of difference in sensory qualities represents the main cause of consumer irritation with beef with the concern of tenderness as the major anxiety (Ellies-Oury *et al.*, 2016; Picard and Gagaoua, 2017).

The tenderness of the meat is a result of several causes which can be considered consecutively. The "background toughness" is the appearances of the muscle primarily its connective tissue (collagen), both its amount and type (Veiseth, 2004). This depends on appearances like sex, animal age, muscle type, species and state of nutrition (Wheeler, 2000; Ahmad *et al.*, 2020). Various muscle fiber types vary in their content of collagen and therefore muscles with typical fiber type arrangement also vary in their tenderness (Maltin, 2003). More collagen has been observed in Slow-twitch muscles which are stated as less tender (Nanda *et al.*, 2020). Small fibers have a relationship with muscles and fiber diameters have been declared as more tender in cattle (Renand, 2001).

Tenderization of meat with exogenous proteases is one of the best approaches. USDA permitted the five exogenous protease enzymes (papain, bromelain, ficin, *Aspergillus oryzae* protease, and *Bacillus* subtilis protease) that are GRAS "generally recognized as safe". In which three of these enzymes (papain, ficin, and bromelain) are derived from plants (Sullivan and Calkins, 2010). The most famous plant proteases are bromelain (EC 3.4.22.32), papain (EC 3.4.22.2), and ficin (EC 3.4.22.3) from pineapple, papaya, and fig respectively are used in, pharmaceutical, industrial processes, and other food processing. 5% of the worldwide sales of proteases is represented by these three plant proteases enzymes (Barberis *et al.*, 2008).

Muscle protein can be digested by the use of these enzymes during mixing. Collagen and elastin can be hydrolyzed which support the meat tenderization (Wada, 2002), noted that myosin and actin filaments are affected by the use of plant thiol proteases (bromelain, papain, and ficin). Ketnawa and Rawdkuen (2011) also reported from the meat of beef collagen and giant catfish skin can be degraded by the use of bromelain from pineapple peels. The muscles of beef, quid and farmed giant catfish can also be damaged by the Protease from Calotropisprocera latex efficiently (Ketnawa and Rawdkuen, 2011). Plant proteases are superior to bacterial borrowed enzymes generally because of safety complication such as pathogenicity and many other harmful effects. A measured amount of these enzymes are used because an excessive amount of enzymes would affect the structure of the meat (Qihe *et al.*, 2006).

Papain in meat tenderization

Papaya naturally found in tropical and subtropical regions of the world but in Pakistan, province of Punjab and Sind are rich in lavish green plantations of papaya (Oad *et al.*, 2001). Papain (EC 3.4.22.2) is found in the fluid of pawpaw plant and it is a cysteine protease which protects the plants from the attack of the insects (Konno *et al.*, 2004). The structure of the papain is three dimensional (eddine Derardja *et al.*, 2019). Having a broad range of pH (4-8) as well as excessive optimal temperature (over 60 °C) papain showed a wide range of enzymatic activity. The uniqueness of papaya proteinase I is generally based on the site of substrate P-Two (the best site for the better affinity of obligatory the enzymes just for substrate union remaining n-end for the cleavage sites) (Hong-Shum and Smith, 2011).

Artificial inhibitors and polypeptides in plotting the active site of papaya proteinase proved that papaya proteinase includes the accuracy for aromatic side chains having amino acids, for example, the positions

of Tyr and Phe at the P2 position in the protease action two most important residues are Cys25 and His159 inside the active site of the papain. (Norazmi *et al.*, 2020) Crude extracts comprise other proteases comprises the crude extracts (papaya peptidase A and chymopapain). Pure papaya proteinase shows the optimum activity at the temperature 50-57 °C and pH range from 5.8-7.0 while phosphoprotein is used as a substrate (Grzonka *et al.*, 2007).

The process and stableness of papaya proteinase are conserved for several weeks during the storage at 4 °C. by oxidizing and reducing agent's papain can also be inactivated and activated by the use of oxidizing and reducing agents respectively. During the partly reversed of the thiol protein i.e sodium metabisulfite and cysteine thiol is oxidized which lead to inactivation (Grzonka *et al.*, 2007). Bacterial enzymes are lower than the Proteolytic enzymes that are linked with safety problems such as disease. The vitality of the meat can be reduced by dissolving the proteolytic enzymes in muscle protein i.e elastin and collagen. However, the accurate amount of enzyme should be used, because dissolution can be occurred by using the excessive amount (Rawdkuen *et al.*, 2013). It is recommended that to develop a flexible and effective property for female papain with a concentration of 0.025%/3% (w / w) can be used who paid for the use of effective ones (Khanna and Panda, 2007).

Thiol protease is also known as cysteine endopeptidase. The emerald contains enzymes such as endopeptidase, glycyl, chymo papain, caricain and papain having a total enzyme content of 80% (Azarkan *et al.*, 2003). The basic amino acid bonds are broken down by the use of these enzymes (Llerena-Suster *et al.*, 2011) that's a reason papain is used in the beverage industry, and in the process of confirming the skin reduction (Huet *et al.*, 2006) The dose of papain recommended by the researchers for meat tenderization is 0.6% which gives the better quality and texture of meat. Texture and quality of the meat may be affected by the use of high dose (Abdel-Naeem and Mohamed, 2016). Sensory and physicochemical properties of camel burger patties are improved by the use of papain and ginger powder (Akpan and Omojola, 2015).

The papaya extract also showed the anti-bacterial activity against *C. Albicans, E. coli, S. aureus, Listeria monocytogens, S.dysenteria, P. fluorescens, P. vulgaris, S. typhimurium, Pseudomonas aeruginosa and S. marcescens* (Ogunjobi and Ogunjobi, 2011; Eshamah *et al.*, 2014; Tewari *et al.*, 2014). It has been reported that 90% of deaths associated to estimate related to pathogens of *Listeria monocytogenes* (19%), of *Salmonella* (28%), *Norwalk virus* (11%), *Campylobacter* (6%) of Toxoplasma (24%) and *Escherichia coli* O157: H7 (3%). According to previous studies, the results of *Listeria monocytogenes* in chicken is varied from 20.6-24.1% and in processed meat 0 to 15%. During processing, many kinds of organisms and bacterial pathogens are inserted into the meat that affects the nutritional value of the food and cause of any damages in the human body. This is the main reason for the microbiological potential that improves lifespan (Gudbjörnsdóttir *et al.*, 2004).

The main reason of this study was to raise the life of Kabila to stop *Salmonella Typhimurium* and the characteristics of bags improved bromelain, papain, and Ficini usually are thiol proteases (Barrett and Chen, 2004), often called sulphydryl or thiol having a molecular weight is in between 21 to 30 units kilodaltons (kDa) and a studies of tenderization of the meat showed that these usually are low endoproteinase and moisture can be attracted by the certain substrates (Schwimmer, 1981).

Papainastic and Papainastic proteases are synthesized in inactive plants occasionally, which involve the first division as a part of the intramolecular polypeptide, two C- and n-terminal circuits (Wiederanders, 2003) papain enzyme model was used to study the mechanism of cysteine protease activity (Cstorer and Ménard, 1994) The activity of papain enzyme is responsible for the formation of ions between His159 and Cis 25 pair residues (Cstorer and Ménard, 1994; Grzonka *et al.*, 2007).

Cysteine proteins occur during the restoration of S-acyl enzymes when the water molecule reacts with the center and release of N-terminal and starts the new cycle (Cstorer and Ménard, 1994). Sullivan and Calkins (2010) also concluded that collagen is more readily soluble in papain-treated samples as compared to the other treatments (ficin and bromelain).

Bromelain in meat tenderization

Bromelain is a proteolytic enzyme which is obtained from the pineapple plant is used as a tenderization of meat. Bromelain was used to tenderize the beef, chicken meat and mutton (Bille and Taapopi, 2008; Ketnawa and Rawdkuen, 2011). Anatomy of myosin and actin filaments of myofibrillar proteins have been impacted by the thiol proteases. the proteolytic enzymes are used as a stimulated of fragmentation of myofibrils and for the disturbance of the shape of the intramuscular connective tissue in meat. Bromelain is used to spit the peptide bonds of tyrosine, lysine, glycine, and alanine at their carbonyl end. By computation, bromelain is used to enhance the meat tenderness and is recognized as (GRAS) generally recognized as safe by the United States federal agencies (Sullivan and Calkins, 2010).

The physicochemical properties of beef could be changed by the tenderization process and its study is necessary to coordinate with its nutritional values. The structural components of body cells are made up by the building block of proteins, amino acids, which are non-essential and essential amino acids. Dispensable or Non-essential amino acids are the amino acids that are produced in the body. Meanwhile, indispensable or essential amino acids are the amino acids that cannot be prepared by our body and we should get it from our diet (Muhammad-Lawal and Balogun, 2007). Peraza-Mercado *et al.* (2010) stated that harmful health effects occur in our body if essential amino acids are not available in an adequate form.

Zhao *et al.* (2012) concluded that when beef meat is treated with papain or bromelain for 1 hour at 37°C all of the myofibrillar proteins (including AC and MHC) are degraded into fragments whose molecular weights are less than 20 kDa. Additionally, they also said that bromelain and papain are thiol proteases, and meat structure and myofibrillar is extensively degraded by the strong activity towards the myofibrillar proteins. When buffalo meat is tenderized by the treatment with ammonium hydroxide both collagen tissues and myofibrillar proteins are fragmented (Naveena *et al.*, 2011). The firmness of meat is reduced when myofibrillar protein is breakdown into small peptides with low molecular weights. However, the final tenderization of meat depends on the weakening and degree of alteration of myofibrillar structures (Kemp *et al.*, 2010).

Ketnawa and Rawdkuen (2011) reported that samples treated with bromelain sowed high TCA-soluble peptides content because of high muscle protein hydrolysis. When meat is extracted with bromelain extract then collagen is hydrolyzed into small peptides. Ha, *et al.* (2012) showed that bromelain, papain and zingibian preparations appear to have a comparable hydrolysis profile to both topside myofibril extracts and beef connective tissues. According to Wada *et al.* (2002) the structure of AC and MHC filaments of myofibrillar proteins was affected by the thiol plant protease. Research proved that bromelain was obtained from pineapple wastes which contain peels, cores, and leaves. The bromelain is also present in the fruits and stems of pineapple in a large amount as compared with their waste material. Bromelain is commercially available in the market by the name of varieties such as McCormick and Knorr in compare with papain and ficin. Myofibril proteins in meat such as actomyosin and nebulin titin are hydrolyzed efficiently by the bromelain and papain, as expose by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Hage *et al.*, 2012).

Sullivan and Calkins, 2010 reported bromelain enhanced the tenderness and sensory properties of meat as compared to other exogenous enzymes. The tenderizing effect is formed on myofibrillar and

myosin proteins of course dried sausage by the addition of bromelain in it Aishima. Structural changes in the protein of meat which is dealing with pineapple juice with success relieve total reflection infrared spectroscopy (Gurumallesh *et al.*, 2019; Naveena *et al.*, 2004).

Ketnawa and Rawdkuen (2011) exhibit that extensive the breakdown of peptides or proteins has occurred on chicken and beef when bromelain is treated at up to 20 %(w/w). In oyster meat, bromelain can proceed as a high-quality hydrolysis agent for hydrochloric acid and at the same time, offered higher adequacy scores in the sensory assessment of oyster sauce formation (Chuapoehuk and Raksakulthai, 1991). Myofibril tissues are completely ruptured by the use of purified bromelain in meat which showed high meat tenderization by the use of scanning electron microscopy analysis (Chaurasiya *et al.*, 2015).

The fruit (ec 3.4.22.33, 25 units kilodaltons (kDa)) and stem (ec 3.4.22.32, 24.5 units kilodaltons (kDa)) in the Ananascomosus plant take out bromelain which is present in the mixture of cysteine proteases. Both of these enzymes (proteins) are hemoglobin A1c (HbA1c) polypeptides, however they're clinically non-identical. Proteolytic activity is low in bromelain of stem and showed not as much uniqueness for amide bond differentiate with Pineapple (Ananas comosus) (Chen and Barrett, 2004; Grzonka *et al.*, 2007).

Several studies widely investigated the catalytic activity of the majority of the proteases with the assist of different synthetic peptides. The bromelain has a biological catalysts action spectra which is lower as compared to papain with known protein breakdown action on artificial peptides at a pH range 5.0-7.5 as well as an optimal temp of 50°C (Grzonka *et al.*, 2007). The peptidase may have diverse uniqueness that was accredited with a little string variations (ALBEE *et al.*, 1997). Pure bromeliase is stable when stored at temperature -20°C (Buttle *et al.*, 1990), and cysteine is an important compound to activate the bromelain (Napper *et al.*, 1994). In the juice, bromelain enzyme is present in soluble form. Further purification is done by the precipitation of enzymes in processing. The main causes of meat over-tenderization are the breakdown of collagen and myofibrillar proteins by the action of bromelain. Best results are seen in the adult beef processing by the use of bromelain at 10 mg/100 g meat, at 4°C for 24 hours followed by raising the temperature at the speed of 1°C/min, until it reaches 70°C. At a controlled environment, most industries are using bromelain which is important for the tenderization of meat, assurance of the purity and microbiological quality of meat. Bromelain is commercially accessible in powdered form. It is estimated the United States used 95% enzymes that are attained from plant proteases i.e. bromelain and papain, while enzymes that are derived from the microbial source aren't widely used (Fillit *et al.*, 2009).

Effect of Concentration

Bromelain action (0.29 /121.9 miligrams of animal meat proteins) was observed to become more effective in enhancing the tenderness of meat and also observed that action was less active against the myofibrillar proteins and more active towards the collagen proteins (Ramanathan *et al.*, 2020).

Larick and Foegeding (1986) in comparison with the efficiency of commercially made, bromelain (.2 milligram), papain (1 mg), ficus protease (.06 ml), as well as collagenase (0.2 ml) on the hydrolysis of myofibrillar proteins and also collagen. Ramanathan *et al.*, (2020) observed that the efficiency of solubilization of collagen was in the subsequent order; papain < bromelain < ficin < collagenase, while myofibrillar proteins activity was in the following arrange; collagenase< papain < ficus protease< Ananas comosus.

Calkins and Sullivan (2007) mentioned that the collagen is hydrolyzed by the use of papain (9 ppm) and bromelain (14.5 ppm), while in the subsequent arrangement bromelain < ficus protease < papain. Ramanathan *et al.* (2020) also reported the bromelain is less effective tenderizer as compared to papain and that the concentrations of both enzymes

depend on the tenderizing effect. Clearly, there's not any type of harmony between the results as well as it's difficult to attain a common trend because levels of enzyme can change in between the research studies. It is essential to keep in mind that the usage of peptidase concentration may dependent upon the meat cut, storage temperature, and also using the other interventions.

Alarcon-Rojo *et al.* (2019) reported that the sensory properties and tenderness of semimembranosus (SM) and longissimus lumborum (LL) muscles from old cows are affected by the use of bromelain, papain mixture, aging combination, and blade tenderization. Additionally, they stated how the harmony had been superior in cold boned longissimus lumborum in comparison with hot boned longissimus lumborum. But, their study showed the severe cold shortening was caused during the freezing of hot-boned longissimus lumborum samples (-39°C) as indicated by the untreated control samples which showed the very low sensory score. In the semimembranosus muscle (SM) of young cattle kiwi-fruit juice-based solution was injected and then smart stretch TM is used for packing (M Taylor and L Hopkins, 2011).

It was observed that more significant tender meat is produced by using sheer force. However, on compressing the SM no effect was observed which suggests that there wasn't any type of effect on the connective tissues of the SM Investments Corporation (Toohey *et al.*, 2011).

Temperature Influences

By using an artificial substratum, (Larick and Foegeding, 1986) stated that vitro protease activity is minimal from 19-39°C for store-bought, Ananas comosus, papain ficus protease, as well as collagenase by using a synthetic substrate (Azocoll). The Temp range had been >35-40°C for the ficin and bromelain; 39-60°C for collagenase; and >50°C for papain. The activity of ficin, bromelain, along with papain is not affected by heating at 70°C, but the activity of collagenase has been removed due to the higher deactivation of temprature for ficin as well as bromelain (˜75°C), papain (90°C). Over tenderization can be happened after cooking at the medium or rare degree of doneness by the high residual activity. Just like all the reactions which include these digestive enzymes, it's always depends upon the enzyme substrate percentage, temperature, as well as time. Such factor must be measured throughout the optimization and using a peptidase meat tenderization process. The tenderness of bromelain and papain treated meat is achieved by the cooking methods.

Ramanathan *et al.* (2020) stated that the high actions of tenderization and proteinase were achieved in cooking food slowly rather than fast cooking on high flame, and thus in slower cooking lower concentration of bromelain and papain is used than in fast cooking to produce tenderness at the same level. This means that the concentration of enzymes which is injected in the meat sample should be customized according to the designed cooking method and the meat sample that will be used.

Ficin in meat tenderization

Ficin is a well-recognized plant protease which is used in tenderization of meat (Maróstica and Pastore, 2010). Ficin is a cysteine or sulfhydral protease usually obtained from Ficus carica (Fig tree) that enhanced the solubility of muscle proteins (Ramezani *et al.*, 2003). Ficin is an enzyme that breakdown the peptide bonds of non-terminal amino acids in proteins present in the latex of Fig. trees (F. glabrata and F. carica).

In 2008, the molecular weight of protein in ficin which is obtained from F. racemosa has 44.5 KDa, and maximum activity was shown at 60°C in the best pH range of 4.5-6.5 due to these properties, ficins are used in meat tenderization as a beneficial class of plant proteases.

Conclusion

Consumer satisfaction and meat palatability is associated with meat tenderness. Numerous proteases are derived from various sources i.e. plant,

animal and microbes but plant proteases are used for the best results to tenderize the meat. Different researches showed that Papain, ficin and bromelain are plant proteases which act as a tenderizer in meat and meat products. The effective use of protease concentration will dependent on the storage temperature, meat cut, and by using the other interventions. These factor needs to be measured during the optimization and application of a protease meat tenderizing procedure. The tenderness of bromelain and papain treated meat is achieved by the cooking methods. This means that the concentration of enzymes which is injected in the meat sample should be customized according to the designed cooking method and the meat sample that will be used.

References

Aaslyng MD, Meinert L. 2017. Meat flavour in pork and beef–From animal to meal. Meat science **132,** 112-117. https://doi.org/10.1016/j.meatsci.2017.04.012

Abdel-Naeem HH, Mohamed HM. 2016. Improving the physico-chemical and sensory characteristics of camel meat burger patties using ginger extract and papain. Meat science **118,** 52-60. https://doi.org/10.1016/j.meatsci.2016.03.021

Abe M, Arai S. 1991. Some properties of a cysteine proteinase inhibitor from corn endosperm. Agricultural and biological chemistry **55(9),** 2417-2418. https://doi.org/10.1080/00021369.1991.10870959

Ahmad MN, Hilmi NHN, Normaya E, Yarmo MA, Bulat KHK. 2020. Optimization of a protease extraction using a statistical approach for the production of an alternative meat tenderizer from Manihot esculenta roots. Journal of Food Science and Technology, 1-11. https://doi.org/10.1007/s13197-020-04317-5

Akpan I, Omojola A. 2015. Quality attributes of crude papain injected beef. Journal of Meat Science and Technology **3(04),** 42-46.

Alarcon-Rojo AD, Carrillo-Lopez LM, Reyes-Villagrana R, Huerta-Jiménez M, Garcia-Galicia IA. 2019. Ultrasound and meat quality: A review. Ultrasonics sonochemistry **55,** 369-382.

Albee KL, Bernasconi RJ, Edmunds T. 1997. Complete amino acid sequence of ananain and a comparison with stem bromelain and other plant cysteine proteases. Biochemical Journal **327(1),** 199-202. https://doi.org/10.1042/bj3270199

Azarkan M, El Moussaoui A, Van Wuytswinkel D, Dehon G, Looze Y. 2003. Fractionation and purification of the enzymes stored in the latex of Carica papaya. Journal of Chromatography B **790(1-2),** 229-238. https://doi.org/10.1016/S1570-0232(03)00084-9

Barberis S, Guzmán F, Illanes A, López-Santín J, Wilson L, Álvaro G, Clapés P. 2008. Study cases of enzymatic processes Enzyme Biocatalysis (p 253-378), Springer.

Barrett AJ, Chen JM. 2004. Thimet oligopeptidase Handbook of proteolytic enzymes (p 352-356), Elsevier. https://doi.org/10.1016/B978-0-12-079611-3.50093-8

Bhat ZF, Morton JD, Mason SL, Bekhit AEDA. 2018. Applied and emerging methods for meat tenderization: A comparative perspective. Comprehensive Reviews in Food Science and Food Safety **17(4),** 841-859. https://doi.org/10.1111/1541-4337.12356

Calkins CR, Sullivan G. 2007. Adding enzymes to improve beef tenderness. Beef Facts Product Enhancement, National Cattleman's Beef Associat-ion. Centennial Colorado: Cattlemen's Beef Board.

Cdc C. 2011. Estimates of foodborne illness in the

United States. Atlanta: Center for Disease Control and Prevention (CDC).

Chaurasiya RS, Sakhare P, Bhaskar N, Hebbar HU. 2015. Efficacy of reverse micellar extracted fruit bromelain in meat tenderization. Journal of food science and technology **52(6),** 3870-3880.

Chuapoehuk P, Raksakulthai N. 1991. Use of papain and bromelin in the production of oyster sauce. Witthayasan Kasetsart sakha Witthayasat.

Cstorer A, Ménard R. 1994. Catalytic mechanism in papain family of cysteine peptidases Methods in enzymology (Vol. 244, pp. 486-500): Elsevier. https://doi.org/10.1016/0076-6879(94)44035-2

eddine Derardja A, Pretzler M, Kampatsikas I, Barkat M, Rompel A. 2019. Inhibition of apricot polyphenol oxidase by combinations of plant proteases and ascorbic acid. Food chemistry: X, **4,** 100053. https://doi.org/10.1016/j.fochx.2019.100053

Ellies-Oury MP, Cantalapiedra-Hijar G, Durand D, Gruffat D, Listrat A, Micol D, Saracco J. 2016. An innovative approach combining animal performances, nutritional value and sensory quality of meat. Meat science **122,** 163-172. https://doi.org/10.1016/j.meatsci.2016.08.004

Eshamah H, Han I, Naas H, Acton J, Dawson P. 2014. Antibacterial effects of natural tenderizing enzymes on different strains of Escherichia coli O157: H7 and Listeria monocytogenes on beef. Meat science **96(4),** 1494-1500. https://doi.org/10.1016/j.meatsci.2013.12.010

Foegeding EA, Larick D. 1986. Tenderization of beef with bacterial collagenase. Meat science **18(3),** 201-214. https://doi.org/10.1016/0309-1740(86)90034-3

Grzonka Z, Kasprzykowski F, Wiczk W. 2007. Cysteine proteases Industrial enzymes (p 181-195): Springer.

Gudbjörnsdóttir B, Suihko ML, Gustavsson P, Thorkelsson G, Salo S, Sjöberg AM, Bredholt S. 2004. The incidence of Listeria monocytogenes in meat, poultry and seafood plants in the Nordic countries. Food Microbiology **21(2),** 217-225. https://doi.org/10.1016/S0740-0020(03)00012-1

Gurumallesh P, Alagu K, Ramakrishnan B, Muthusamy S. 2019. A systematic reconsideration on proteases. International journal of biological macromolecules **128,** 254-267. https://doi.org/10.1016/j.ijbiomac.2019.01.081

Hage DS, Anguizola JA, Bi C, Li R, Matsuda R, Papastavros E, Zheng X. 2012. Pharmaceutical and biomedical applications of affinity chromatography: recent trends and developments. Journal of pharmaceutical and biomedical analysis **69,** 93-105. https://doi.org/10.1016/j.jpba.2012.01.004

Huet J, Looze Y, Bartik K, Raussens V, Wintjens R, Boussard P. 2006. Structural characterization of the papaya cysteine proteinases at low pH. Biochemical and biophysical research communications **341(2),** 620-626. https://doi.org/10.1016/j.bbrc.2005.12.210

Ionescu RE, Fillit C, Jaffrezic-Renault N, Cosnier S. 2008. Urease–gelatin interdigitated microelectrodes for the conductometric determination of protease activity. Biosensors and Bioelectronics **24(3),** 489-492. https://doi.org/10.1016/j.bios.2008.06.021

Kang CK, Rice EE. 1970. Degradation of various meat fractions by tenderizing enzymes. Journal of food science **35(5),** 563-565. https://doi.org/10.1111/j.1365-2621.1970.tb04809.x

Kemp C M, Sensky PL, Bardsley RG, Buttery PJ, Parr T. 2010. Tenderness–An enzymatic view. Meat science **84(2),** 248-256.

https://doi.org/10.1016/j.meatsci.2009.06.008

Ketnawa S, Rawdkuen S. 2011. Application of bromelain extract for muscle foods tenderization. Food and Nutrition Sciences **2(05),** 393. https://doi.org/10.4236/fns.2011.25055

Konno K, Hirayama C, Nakamura M, Tateishi K, Tamura Y, Hattori M, Kohno K. 2004. Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. The Plant Journal **37(3),** 370-378. https://doi.org/10.1046/j.1365-313X.2003.01968.x

Llerena-Suster CR, Priolo NS, Morcelle SR. 2011. Sodium tetrathionate effect on papain purification from different Carica papaya latex crude extracts. Preparative biochemistry & biotechnology **41(2),** 107-121. https://doi.org/10.1080/10826068.2011.544230

Taylor MJ, Hopkins LD. 2011. Patents for stretching and shaping meats. Recent patents on food, nutrition & agriculture **3(2),** 91-101.

Maltin C, Balcerzak D, Tilley R, Delday M. 2003. Determinants of meat quality: tenderness. Proceedings of the Nutrition Society **62(2),** 337-347. https://doi.org/10.1079/PNS2003248 https://doi.org/10.1079/PNS2003248

Maróstica MR, Pastore GM. 2010. Some nutritional, technological and environmental advances in the use of enzymes in meat products. Enzyme research, 2010.

Mullen AM, Alvarez C, Zeugolis DI, Henchion M, O'Neill E, Drummond L. 2017. Alternative uses for co-products: Harnessing the potential of valuable compounds from meat processing chains. Meat science **132,** 90-98. https://doi.org/10.1016/j.meatsci.2017.04.24

Nanda RF, Rini B, Syukri D, Thu NNA, Kasim A. 2020. A Review: Application of Bromelain Enzymes in Animal Food Products. Andalasian International Journal of Agriculture and Natural Sciences (AIJANS) **1(01),** 33-44.

Napper A, Bennett S, Borowski M, Holdridge M, Leonard M, Rogers E, Shames S. 1994. Purification and characterization of multiple forms of the pineapple-stem-derived cysteine proteinases ananain and comosain. Biochemical Journal **301(3),** 727-735. https://doi.org/10.1042/bj3010727

Naveena B, Kiran M, Reddy KS, Ramakrishna C, Vaithiyanathan S, Devatkal SK. 2011. Effect of ammonium hydroxide on ultrastructure and tenderness of buffalo meat. Meat science **88(4),** 727-732. https://doi.org/10.1016/j.meatsci.2011.03.005

Naveena B, Mendiratta S, Anjaneyulu A. 2004. Tenderization of buffalo meat using plant proteases from Cucumis trigonus Roxb (Kachri) and Zingiber officinale roscoe (Ginger rhizome). Meat science **68(3),** 363-369. https://doi.org/10.1016/j.meatsci.2004.04.004

Norazmi M, Shahrain M, Normaya E, Nor M, Iqbal A, Halim K. 2020. The Applicability of Using a Protease Extracted from Cashew Fruits (Anacardium occidentale), as Possible Meat Tenderizer: An Experimental Design Approach. Journal of texture studies. https://doi.org/10.1111/jtxs.12529

Oad F, Lakho A, Khan A, Ansari A, Sheikh F, Khail M. 2001. Economics of papaya in Malir district, Karachi–Pakistan. International Journal of Agriculture and Biology **4,** 477-481.

Ogunjobi AA, Ogunjobi T. 2011. Comparative study of antibacterial activities of ethanol extracts of the bark and seeds of garcinia kola and caricapapaya. African Journal of Biomedical Research **14(2),** 147-152.

Picard B, Gagaoua M. 2017. Proteomic investigations of beef tenderness Proteomics in Food Science (p 177-197): Elsevier. https://doi.org/10.1016/B978-0-12-804007-2.00011-4

Qihe C, Guoqing H, Yingchun J, Hui N. 2006. Effects of elastase from a Bacillus strain on the tenderization of beef meat. Food chemistry **98(4),** 624-629. https://doi.org/10.1016/j.foodchem.2005.06.043

Ramanathan R, Mafi GG, Yoder L, Perry M, Pfeiffer M, VanOverbeke DL, Maheswarappa NB. 2020. Biochemical changes of postmortem meat during the aging process and strategies to improve the meat quality. In Meat Quality Analysis (p 67-80). Academic Press. https://doi.org/10.1016/B978-0-12-819233-7.00005-7

Ramezani R, Aminlari M, Fallahi H. 2003. Effect of chemically modified soy proteins and ficin-tenderized meat on the quality attributes of sausage. Journal of food science **68(1),** 85-88. https://doi.org/10.1111/j.1365-2621.2003.tb14119.x

Rawdkuen S, Jaimakreu M, Benjakul S. 2013. Physicochemical properties and tenderness of meat samples using proteolytic extract from Calotropis procera latex. Food chemistry **136(2),** 909-916. https://doi.org/10.1016/j.foodchem.2012.08.077

Renand G, Picard B, Touraille C, Berge P, Lepetit J. 2001. Relationships between muscle characteristics and meat quality traits of young Charolais bulls. Meat science **59(1),** 49-60. https://doi.org/10.1016/S0309-1740(01)00051-1

Schwimmer S. 1981. Source book of food enzymology: AVI Publishing Co. Inc.

Smith J, Hong-Shum L. 2011. Food additives data book: John Wiley & Sons.

Sullivan GA, Calkins C. 2010. Application of exogenous enzymes to beef muscle of high and low-connective tissue. Meat science **85(4),** 730-734. https://doi.org/10.1016/j.meatsci.2010.03.033

Tewari BB, Subramanian G, Gomathinayagm R. 2014. Antimicrobial properties of Carica papaya (Papaya) different Leaf Extract against E. coli, S. aureus and C. albicans. American Journal of Pharmacology and Pharmacotherapeutics **1(1),** 025-039.

Thorslund CA, Sandøe P, Aaslyng MD, Lassen J. 2016. A good taste in the meat, a good taste in the mouth–Animal welfare as an aspect of pork quality in three European countries. Livestock science **193,** 58-65. https://doi.org/10.1016/j.livsci.2016.09.007

Toohey E, Kerr M, Van de Ven R, Hopkins D. 2011. The effect of a kiwi fruit based solution on meat traits in beef m. semimembranosus (topside). Meat science **88(3),** 468-471. https://doi.org/10.1016/j.meatsci.2011.01.028

Veiseth E, Shackelford S, Wheeler T, Koohmaraie M. 2004. Factors regulating lamb longissimus tenderness are affected by age at slaughter. Meat science **68(4),** 635-640. https://doi.org/10.1016/j.meatsci.2004.05.015

Wada M, Suzuki T, Yaguti Y, Hasegawa T. 2002. The effects of pressure treatments with kiwi fruit protease on adult cattle semitendinosus muscle. Food chemistry **78(2),** 167-171. https://doi.org/10.1016/S0308-8146(01)00395-8

Wang LL, Han L, Ma XL, Yu QL, Zhao SN. 2017. Effect of mitochondrial apoptotic activation through the mitochondrial membrane permeability transition pore on yak meat tenderness during postmortem aging. Food chemistry **234,** 323-331. https://doi.org/10.1016/j.foodchem.2017.04.185

Wheeler T, Shackelford S, Koohmaraie M.

2000. Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Science **78(4),** 958-965. https://doi.org/10.2527/2000.784958x

Wiederanders B. 2003. Structure-function relationships in class CA1 cysteine peptidase propeptides. Acta Biochimica Polonica **50(3),** 691-713. https://doi.org/10.18388/abp.2003_3661

Zhao GY, Zhou MY, Zhao HL, Chen XL, Xie BB, Zhang XY, Zhang YZ. 2012. Tenderization effect of cold-adapted collagenolytic protease MCP-01 on beef meat at low temperature and its mechanism. Food chemistry **134(4),** 1738-1744. https://doi.org/10.1016/j.foodchem.2012.03.118