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## FOOD SCIENCE & TECHNOLOGY | REVIEW ARTICLE

# Plant and bacterial proteases: A key towards improving meat tenderization, a mini review

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**Abstract:** Meat consumers are very concerned about the quality and tenderness of meat. Meat tenderness generally depends upon connective tissue, sarcomere length, and the proteolytic potential of muscle. Different physical and chemical methods are used to assess the tenderness of meat. Protease treatment is an efficient method used for meat tenderization. In the food industry, different proteases such as bromelain, papain, ficin, actinidin, and calpain are widely used for proteolytic degradation, to improve meat tenderness. Two structural components determine the toughness of meat, connective tissues composed of structural proteins and post-mortem changes in the sarcomere. Proteases play an important role in degrading the structural proteins in the connective tissues, thus reducing toughness of meat. Bacterial proteases are also used in meat tenderization. Bacterial proteases show effective proteolytic degradation of elastin and collagen, but have negligible or no effect in degrading myofibrillar proteins. The present review highlights the importance of plant and bacterial enzymes with special reference to meat tenderization.

**Subjects:** Environment & Agriculture; Food Science & Technology; Health and Social Care

**Keywords:** plant proteases; bacterial proteases; meat tenderization; meat quality

### 1. Introduction

The palatable quality of meat is influenced by several factors, among which meat tenderness is considered the most important determinant of consumer preferences. Meat tenderness generally depends upon connective tissues, sarcomere length, and the extent of proteolytic degradation of muscles (Kemp & Parr, 2012). In contrast, meat toughness is an undesirable attribute of palatable meat quality for consumers (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010). Toughness in meat primarily occurs because of the actomyosin effect (changes in myofibrillar proteins) or the background effect (because of the amounts of connective tissues or stromal proteins) (Chen, He, Jiao, & Ni, 2006). The quality and tenderness of meat and meat products can be improved in several ways using

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### PUBLIC INTEREST STATEMENT

The use of plant and bacterial enzymes are the useful method for the meat tenderization. There are many methods of tenderizing meat, chemically or physically but the popular method is the use of proteolytic enzymes. This review has innovation for the researchers and scientists in the field of enzymes with reference to meat tenderization. The combined use of plant and bacterial proteases was first time present together in a paper.

physical and chemical treatments. However, all treatments commonly emphasize on disrupting or degrading the connective tissues and myofibrillar proteins.

Myofibrillar toughness is caused by the onset of rigor mortis in slaughtered meat, and enzymatic breakdown of contractile proteins in post-slaughtered meat muscles causes tenderization of the meat (Naveena et al., 2011). In older animals, the formation of stronger and complex collagen cross-links in connective tissue increases; proteolytic enzymes can degrade connective tissue composed of 80% collagen, to tenderize meat (Gelse, Pöschl, & Aigner, 2003).

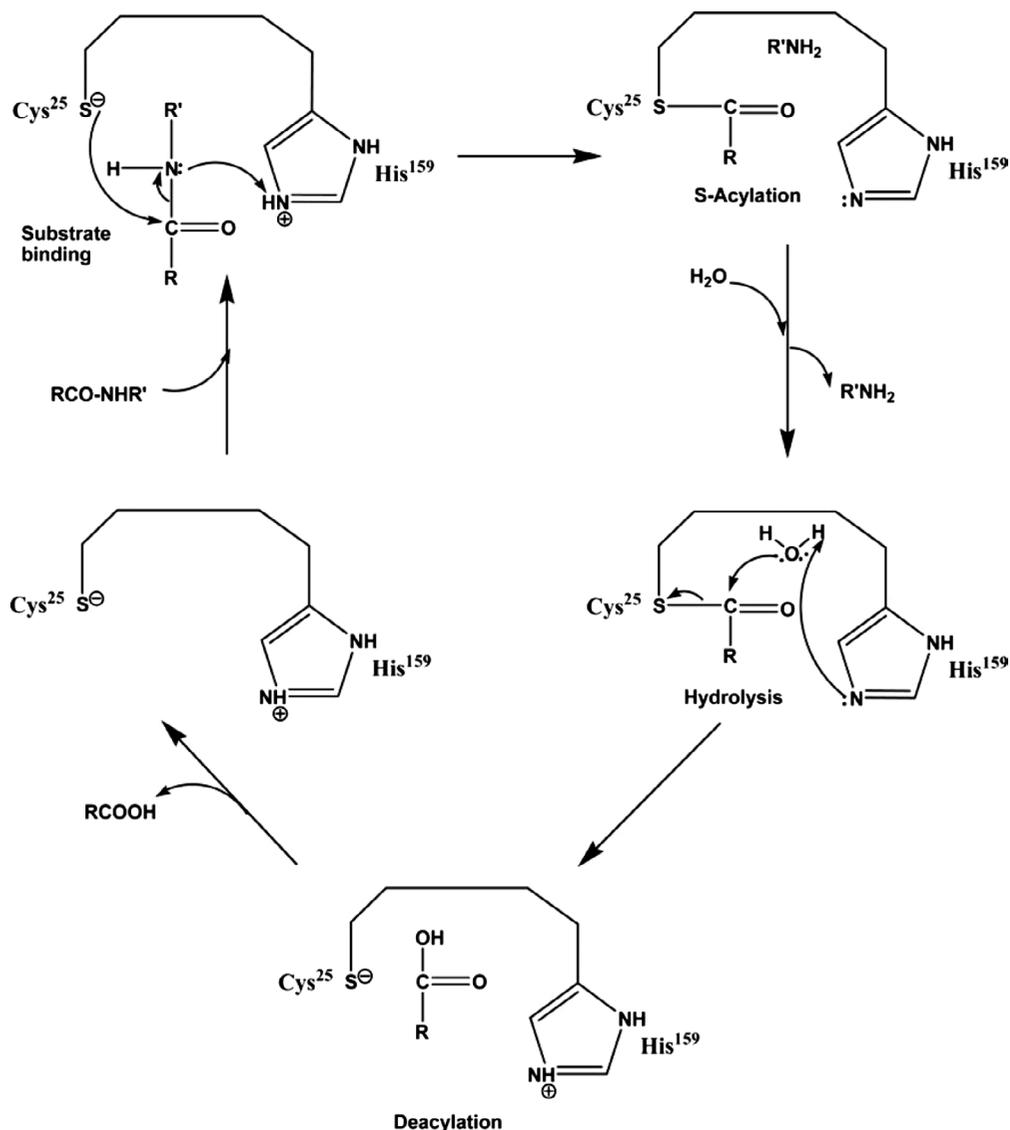
Meat tenderness is the most important attribute that governs consumer acceptability, consumer satisfaction, and recurrent purchasing trends and market value for meat and meat products (Grunert, Bredahl, & Brunsø, 2004; Mennecke, Townsend, Hayes, & Lonergan, 2007). The factors that influence the toughness of meat also contribute to the tenderness of meat. Meat tenderness depends on the type of muscle, pre- and post-slaughter factors, and postmortem pH and temperature (Anderson et al., 2012). The chemical composition, structure and amount of connective tissue, generally depends on the age of the animal and the specific muscle types, also affect meat tenderness (Bolumar, Enneking, Toepfl, & Heinz, 2013).

The use of exogenous proteases for meat tenderization is a relatively progressive method to improve meat quality. There are five exogenous proteolytic enzymes, plant proteases (papain, bromelain, and ficin), and proteases from *Aspergillus oryzae* and *Bacillus subtilis*, which have been approved as generally regarded as safe (GRAS) for use in the meat industry by the US Department of Agriculture (Ha, Bekhit, Carne, & Hopkins, 2012; Ketnawa & Rawdkuen, 2011). These proteolytic enzymes are mixed with the meat to breakdown the proteins in muscle and hydrolyze collagen and elastin, which helps in meat tenderization (Rawdkuen, Jaimakreu, & Benjakul, 2013). The use of enzymes reduces the amount of connective tissues and does not breakdown myofibrillar proteins. Papain and bromelain are the most commonly used plant enzymes for meat tenderization (Liu, Liao, Qi, & Tang, 2008). As meat tenderizers, proteolytic enzymes are best suited for degradation of collagen and elastin in connective tissue at relatively low pH and low temperature (Ryder, Ha, Bekhit, & Carne, 2015). The objective of this review was to gather the latest findings regarding the plant and bacterial enzymes which are used for the meat tenderization. Although the concept for the use of proteolytic enzymes is not new but the synergism between plants and bacterial enzymes added the value to this review.

The structure of myosin and actin filaments is affected by the plant proteases, papain, bromelain, and ficin (Wada, Suzuki, Yaguti, & Hasegawa, 2002). Ketnawa, Rawdkuen, and Chaiwut (2010) depicted that collagen from beef and giant catfish skin is degraded by bromelain obtained from pineapple peels. The plant proteases are better than bacterial enzymes, because of safety, standard problems; high concentrations of plant proteases can cause meat deformation (Chen et al., 2006). Moreover, the tenderness of meat is also assessed by activity of enzymes estimation, myofibrillar fragmentation index, hydroxyproline measurement, and scanning electron microscopic. Plant proteases also used to improve tenderization of meat by biochemical changes as well as micro structural changes (Maiti, Ahlawat, Sharma, & Khanna, 2008).

Additionally, tenderization can be achieved by application of electric current, dissipation of energy at the beginning of muscle contraction, and by changing the process of rigor mortis, such that rigor mortis sets in while the muscles are still warm. It shows that tenderization of meat has been started at a definite rate. Thus, temperature conditions and electric stimulation can be used to reduce the cold shortening. The methods employed for meat tenderization play a role in providing good efficiency and high quality of meat and meat products (Breidenstein & Carpenter, 1983). The enzymatic mechanism of protein hydrolysis by cysteine proteases is shown in Figure 1.

**Figure 1. Enzymatic mechanism of protein hydrolysis by cysteine proteases (Bekhit et al., 2014; Grzonka et al., 2007).**



## 2. Meat tenderization with plant proteases

The process of meat tenderization is essentially an enzymatic degradation, and proteolytic enzymes in meat are responsible for tenderization during aging. However, plant or microbial enzymes can be exogenously added when additional tenderization is necessary (Lantto et al., 2009). Commercial meat tenderization is usually achieved using exogenously added proteolytic enzymes from plants, such as papain, bromelain, and ficin, as well as bacterial collagenase (Abdel-Naeem & Mohamed, 2016). Garg and Mendiratta (2006) reported that the proteases derived from ginger rhizome and fruits of *Cucumis trigonus* Roxb plant were also effective in tenderization of meat. The connective tissues and muscle proteins are easily digested by the exogenously added proteases (Abdel-Naeem & Mohamed, 2016; Grzonka, Kasprzykowski, & Wicz, 2007).

The myofibrillar breakdown starts after activation of the enzymatic system and includes the proteins troponin-1, troponin-t, desmin, vinculin, meta-vincilin, dystrophin, nebulin, and titin (Koochmariaie, 1996). When meat tenderization occurs from z-to z-line attachment-and M-line attachments in sarcolemma with the help of costameric proteins and the elastic protein titin, degradation of three important cytoskeleton structures occurs (Taylor, Geesink, Thompson, Koochmariaie, & Goll, 1995). In muscles, these proteolytic enzymes have a significant role in post-mortem proteolysis

and meat tenderization (Koochmaria & Geesink, 2006). Cathepsins were the first enzymes used in meat tenderization, after which, calpain was introduced owing to its efficiency in changing the Z-line density seen in post-mortem, although it was not initially related to meat tenderization (Taylor et al., 1995).

The three familiar tenderizing enzymes from plants, i.e. papain, bromelain, and ficin are obtained from papaya, pineapple, and fig, respectively. The pH, temperature, and strength of hydrolysis of various enzymes in proteolytic degradation of myofibrillar proteins and collagen are summarized in Table 1. The activity and application of different plant and bacterial proteases is shown in Table 2.

**Table 1. pH, Temperature (°C) and strength of hydrolysis of myofibrillar proteins and collagen by various enzymes<sup>1</sup>**

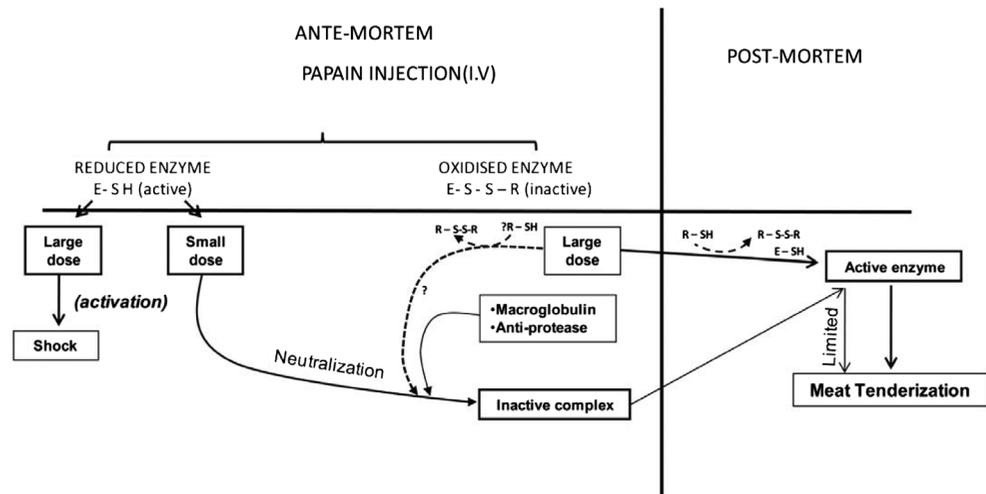
Protease	Active pH	Optimal pH	Active range	Optimal temperature	Hydrolysis of myofibrillar proteins	Hydrolysis of collagen
Papain	4.0–9.0	4.0–6.0	50–80	65–75	Excellent	Moderate
Bromelain	4.0–7.0	5.0–6.0	50–80	65–75	Moderate	Excellent
Ficin	5.0–9.0	7.0	45–75	60–70	Moderate	Excellent
Aspergillus	5.0–9.0	7.0	50–65	55–60	Moderate	Poor
Bacillus	2.5–7.0	<6.5	40–60	55–60	Poor	Excellent

<sup>1</sup>Derived from Calkin and Sullivan (2007).

**Table 2. The activity and application of different plant proteases**

Enzymes	Activity and application as meat tenderization	References
Papain	The recommended dose of papain for meat tenderization is 0.6% with better texture and quality of meat. The dose exceeding the limit may affect the quality and texture	Abdel-Naeem and Mohamed (2016)
Papain	The addition of ginger and papain powder improved the physico-chemical and sensory properties of camel burger patties	Akpan and Omojola (2015)
Bromelain	The purified bromelain completely ruptures the myofibril tissues in meat which shows higher tenderization of meat by using scanning electron microscopy analysis	Chaurasiya et al. (2015)
Ficin	This enzyme can be inhibited by oxidizing agents and divalent metals with small strengths and this inhibition can be changed	Ramezani et al. (2003)
Actinidin	The actinidin has less tenderization property as compared to other traditional plant proteases and still not approved as GRAS by FDA	Toohey, Kerr, van de Ven, and Hopkins (2011)
Calpain	Both m-calpain and $\mu$ -calpain are cysteine proteases, and their proteolytic activity is affected by oxidation, which can influence the quality of fresh meat	Zhang et al. (2013)
Bacterial enzymes	The bacterial enzymes hydrolyses the myofibril and collagen proteins more efficiently than the papain	Ha et al. (2013)

**Figure 2. Schematic diagram for the effect of papain in either active or inactive form in ante-mortem and post-mortem conditions (Bekhit et al., 2014; Dransfield & Etherington, 1981).**



### 3. Papain

Papain is an important plant protease derived from the latex of the papaya fruit. Latex is obtained by scoring, and then allowing the latex to dry on the fruit, to give crude material. Papain is purified by reducing contaminating agents and further extraction. This enzyme is stable at high temperatures and pressure and is inactivated in extreme conditions at 900 mpa, 80°C degree for 22 min. Papain is used in the meat industry as a tenderizer, owing to its proteolytic effect, as well as in beer making as an additive in flour (Starley, Mohammed, Schneider, & Bickler, 1999). It has the ability to hydrolyze larger protein molecules into smaller peptides and amino acids. For many years, papain has been used to breakdown tough fibers (Eshamah, Han, Naas, Acton, & Dawson, 2014). Moreover, papain can tenderize the meat surface and develop characteristic “mushiness” (Islam & Molinar-Toribio, 2013). The physiological role of papain in plants is to protect them from insects (Konno et al., 2004).

The three dimensional structure for papain has been determined (Kamphuis, Kalk, Swarte, & Drenth, 1984). Broad-spectrum enzymatic activity has been shown by papain in the pH range 5–8 and at 65°C temperature (Smith & Hong-Shum, 2003). Berger and Schechter (1970) demonstrated that papain has a specificity for amino acids with aromatic side chains such as Phe and Tyr at the P2 position. The synthetic peptides and inhibitors in mapping are the active sites of papain; within the active site, Cys25 and His159 are two of the essential residues for the protease activity (Bekhit, Hopkins, Geesink, Bekhit, & Franks, 2014). The schematic diagram for the effect of papain in either active or inactive form during ante-mortem and post-mortem conditions is shown in Figure 2.

### 4. Bromelain

Bromelain is a proteolytic enzyme and is obtained from the root of the pineapple plant after harvesting (Fileti, Fischer, & Tambourgi, 2010). The juice contains bromelain enzyme in soluble form. The processing involves precipitation of enzymes for further purification. Bromelain breaks down myofibrillar proteins and collagen and causes over-tenderization of meat. The use of bromelain in processing adult beef showed the best results at 10 mg/100 g meat, for 24 h at 4°C, followed by increasing the temperature at the rate of 1°C/min, until it reached 70°C. Bromelain is important for tenderization of meat in industries with controlled environment, and is useful for assurance of the microbiological quality and purity. Bromelain is commercially available in powdered form. It is estimated that 95% of the enzymes used in the United States are obtained from plant proteases like papain and bromelain, whereas microbially derived tenderizers are not used widely (Ionescu, Fillit, Jaffrezic-Renault, & Cosnier, 2008).

Reverse micellar extraction is used for separation and purification of bromelain from pineapple. The commercially obtained stem bromelain was also compared with the purified bromelain in meat tenderization. There was high bromelain recovery and purification in the reverse micellar extraction technique. In reverse micellar extraction, the toughness of meat was also reduced as compared to control (Chaurasiya, Sakhare, Bhaskar, & Hebbar, 2015).

### 5. Ficin and actinidin

Ficin is a well-known plant protease used in meat tenderization (Maróstica & Pastore, 2010). Ficin is a sulfhydryl or cysteine protease commonly obtained from *Ficus carica* (Fig. tree) that enhances the solubility of muscle proteins (Ramezani, Aminlari, & Fallahi, 2003). Ficin is an endoproteolytic enzyme present in the latex of Fig. trees (*F. carica* and *F. glabrata*). In 2008, ficin obtained from *F. racemosa* has a molecular weight of protein has 44.5 kDa, and shows maximum activity in the optimal pH range of 4.5–6.5 at 60°C. These properties make ficins a beneficial class of plant proteases for use in meat tenderization.

Actinidin is also a novel sulfhydryl protease extracted from gooseberry or the kiwi fruit. It has a molecular weight of 32 kDa. It is used commercially in meat industry to tenderize meat (Varughese, Su, Cromwell, Hasnain, & Nguyen Huu Xuong, 1992) and enhance the chemical processes related to degradation of the myofibrillar proteins into peptides. It is also involved in the activation of m-calpain throughout postmortem ageing (Ha et al., 2012). Actinidin has many applications in the food industry, because of its advantages over other plant proteases such as papain and ficin. Actinidin shows mild tenderizing activity even at high concentrations, preventing surface mushiness. It has a relatively low inactivation temperature (60°C), which makes it easier to control the tenderization process without overcooking (Eshamah et al., 2014; Tarté, 2009).

### 6. Calpain

Calpain is an important enzyme that is chiefly used for degradation of myofibrillar proteins. It also aids in meat tenderizing and improves water holding capacity during postmortem aging (Huff-Lonergan & Lonergan, 2005; Lonergan, Huff-Lonergan, Wiegand, & Kriese-Anderson, 2001). During postmortem refrigerated storage, calpain oxidation may adversely affect its proteolytic activity and negatively influence the quality of fresh meat (Zhang, Xiao, & Ahn, 2013).

The calpain system consists of three members—*m*-calpain,  $\mu$ -calpain, and calpastatin, which is the calpain-specific endogenous inhibitor (Goll, Thompson, Li, Wei, & Cong, 2003; Wendt, Thompson, & Goll, 2004). In the presence of calcium, calpains autolyze, and this autolysis is indication of their proteolytic activation during postmortem changes in muscles (Geesink & Koohmaraie, 1999). Both *m*-calpain and  $\mu$ -calpain are cysteine proteases, and their proteolytic activity is affected by oxidation, which can influence the quality of fresh meat (Zhang et al., 2013).

Meat tenderness undergoes changes after slaughtering due to the activity of the endogenous calpains and calpastatin. These calcium-dependent proteases degrade the myofibrillar proteins troponin, troponin T, troponin I, C-protein, connectin, titin, vinculin, and desmin. Calpains are inactivated by calpastatin and decreases myofibrillar breakdown and decreases tenderness of meat (Cheret, Delbarreladrat, Lamballerieanton, & Verrezbagnis, 2007; Gerelt, Rusman, Nishiumi, & Suzuki, 2005).

The endogenous concentration of these enzymes in meat influences meat quality (Koohmaraie, Shackelford, Muggli-Cockett, & Stone, 1991). Calpain is involved in postmortem proteolysis and meat tenderization in domesticated animals (Huff-Lonergan & Lonergan, 2005; Koohmaraie, 1992).

Although calpains improve the tenderization of meat, increased expression of the CAST gene decreases proteolysis and increases toughness of meat. In contrast, the calpain genes CAPN1 and CAPN2 are involved in the breakdown of myofibrillar proteins (Huff-Lonergan et al., 1996). CAPN1 plays a significant role in postmortem muscle proteolysis and in tenderization of meat (Kemp et al., 2010).

In bovine and ovine muscles, activity of CAPN2 is low (Camou et al., 2007; Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001). The most important muscle-specific calcium-dependent cysteine protease is the CAPN3 which has two structural domains called ISI and IS2 and the muscle specific proteins bind at N2 line region where proteolysis occurs (Geesink, Taylor, & Koohmaraie, 2005; Kemp et al., 2010).

## 7. Bacterial enzymes

Bacterial strains with proteolytic activity play an important role in the degradation of proteins in meat and meat products (Bekhit, 2010). *B. subtilis* contains two major proteases, subtilisin and neutral protease. The US Food and Drug Administration (FDA) has approved the GRAS status of these bacterial strains (FDA, 2001). The relative specific activity and low inactivation temperature of the bacterial proteases make them suitable for meat tenderization. Naveena, Mendiratta, and Anjaneyulu (2004) reported that the enzyme alkaline elastase, obtained from the alkalophilic *Bacillus* sp., breaks down the collagen, elastin, and myofibrillar proteins in tenderized meat. Alkaline elastase showed optimal activity at pH range 5.5–6.0 and temperature range 10–50°C. The hydrolytic activity of bacterial proteases in myofibrillar proteins is low as compared to that of the plant proteases; however, the hydrolytic activity of bacterial proteases in mediating collagen degradation was found to be intermediate to the hydrolytic activities of papain and bromelain (Yeh, Yang, & Tsai, 2002).

The commercially available bacterial proteases are obtained from *A. oryzae*. Payne (2009) reported that these proteases are stable over a wide range of pH and below 70°C. Aspartic proteases produced by *A. oryzae* show optimal activity at pH range 2.5–6.0, and at 75°C, its activity is reduced by 20% (Ashie, Sorensen, & Nielsen, 2002). The fungal proteases are usually effective in the proteolytic activity against elastin and collagen, but show negligible or no effect on myofibrillar proteins (Payne, 2009). Similarly, aspartic proteases from *A. oryzae* show negligible or no effect in the degradation of myofibrillar proteins in meat (Ha, Bekhit, Carne, & Hopkins, 2013).

## 8. Conclusions

Meat tenderness is the most important factor associated with meat palatability and consumer satisfaction. Different plant proteases like papain, bromelain, actinidin, and ficin have been used for tenderization of meat and meat products. Meat proteins can also be hydrolyzed by bacterial enzymes, such as proteases from *Aspergillus oryzae* and *Bacillus subtilis*. The bacterial proteases exhibit lower hydrolytic activity in myofibrillar proteins as compared to plant proteases. However, hydrolytic activity of bacterial enzymes in the collagen was found to be intermediate to the activities of papain and bromelain. Further, it is recommended that the combined use of plant and bacterial proteases may have synergistic effect on meat tenderization.

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### Competing Interest

The authors declare no competing interest.

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