BEEF FACTS

Product Enhancement

Adding Enzymes to Improve Beef Tenderness

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Importance of Beef Tenderness

Beef palatability is affected by many factors and tenderness is cited as one of the most important. Consumers are willing to pay a premium for a guaranteed tender product with the potential to increase the value of the middle meats over $60 per carcass (Miller *et al*., 2001). Consequently, the meat industry is in a continual search for methods to improve the tenderness of the middle meats and upgrade other cuts and muscles to steak quality. This search for tender product not only adds value to the industry but can help to supply a more consistent and uniform product to consumers while increasing overall beef satisfaction.

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). Fat also plays a minor role. Cover *et al*. (1962) identified six characteristics of perceived tenderness. 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. This difference in distribution of key proteins within the muscle increases the challenge of improving tenderness. 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.

Inherent Proteolytic Enzymes

The primary mechanism of postmortem improvement in tenderness is through the disruption of the native structure of the muscle. Of the methods identified as having a positive effect on tenderness, the easiest and oldest is postmortem cooler aging. The calpain system, the primary enzyme system responsible for the aging process, is comprised of three primary components, μ-calpain, m-calpain and calpastatin. Calpains and their inhibitor calpastatin are calcium-dependent (Goll *et al*., 2003). Calpain proteolysis of myofibrillar proteins occurs through the degradation of the z-line, costameric proteins and titan (Taylor *et al*., 1995). Negligible degradation occurs for collagen primarily due to its extracellular nature. Postmortem proteolysis is self limiting because the calpains undergo autolysis (Goll, *et al*., 2003). As a result of this self-degradation, calpains do not over-tenderize meat.

Table 1: Enzymes classified as Generally Recognized As Safe (GRAS)

Enzyme	Type	Source	Protease class
Papain	Vegetable	Papaya	Cysteine
Bromelain	Vegetable	Pineapple	Cysteine
Ficin	Vegetable	Figs	Cysteine
Bacillus Protease	Bacterial	Bacillus subtilis	Serine
Aspartic Protease	Fungal	Aspergillus oryzae	Aspartic

Exogenous Enzymes

Research into the use of exogenous proteolytic enzymes has been conducted for over 60 years and has investigated countless enzymes from plant, bacteria, and fungal sources. Exogenous enzymes added to meat to enhance tenderness react differently to the myofibrillar and connective tissue portions of the meat. Currently, just five of the many exogenous enzymes that have been studied have been classified as 'Generally Recognized as Safe' (GRAS) by USDA's Food Safety Inspection Service (FSIS) and

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come from varying plant, bacterial, and fungal sources (Table 1). Consequently, only these five can be added to meat for the purposes of enhancing tenderness. Additional, promising sources of exogenous enzymes have been identified. In particular, enzymes have been isolated from kiwi fruit (actinidin) and ginger that show potential for future inclusion in meat systems.

Temperature is probably the most controllable factor influencing enzyme activity. Most of the exogenous enzymes used to tenderize meat have an optimal activity in the 50-70°C range (Table 2). As a result, most of the activity takes place during the cooking process and to a lesser degree during cooler storage. Product pH will also influence enzymatic activity. Most of the enzymes have an optimal pH in the normal range of meat, but some function best at a more acidic or alkaline pH for optimal protein degradation. The addition of salts can have positive or negative effects on enzymatic activity. Many of the enzymes do not penetrate meat, causing the method of application to play an important role in uniform distribution into the meat product. The proper identification of enzyme, time, temperature and pH will be dependant upon the processing system and desired product. An overview of optimal pH and range of activity is found in Table 3.

Enzymes are often sold on the basis of activity; however, the assay depends upon which enzyme is being tested. Papain, bromelain and ficin activity is often measured in milk clot units. Enzymes from *Aspergillus oryzae* are measured in hemoglobin units of tyrosine. Enzymes from *Bacillus subtilis* are measured by a proteolytic test on casein. Assay details are found in Table 4. The relationships among assays are not strong, making it difficult to directly compare the activities of enzymes. The same enzyme can show differing results in activity levels and optimal pH and temperature ranges depending upon the substrate. The milk clot assay relies on a subjective determinate of clotting time while the others provide results using a spectrophotometer. The assay used is partly based on which one conforms to the optimal activity level for each enzyme, depending on pH, temperature and substrate.

GRAS Enzymes

Papain

Papain, a mixture of enzymes from the papaya fruit, has been studied for its tenderizing effects since the 1940's. Papain is a highly aggressive, indiscriminate enzyme causing significant degradation to both myofibrillar and collagen proteins, yielding protein fragments of several sizes (Ashie *et al.*, 2002). Even though papain shows massive disruption of the Z disc, as

Table 2: Temperature effects on activity

Enzyme	Active range	Optimal temperature
Papain	50-80	65-75
Bromelain	50-80	65-75
Ficin	45-75	60-70
Bacillus	50-65	55-60
Aspergillus	40-60	55-60

Table 3: pH optimal and range of activity

Enzyme	Active pH	Optimal pH
Papain	4.0-9.0	4.0-6.0
Bromelain	4.0-7.0	5.0-6.0
Ficin	5.0-9.0	7.0
Bacillus	5.0-9.0	7.0
Aspergillus	2.5-7.0	<6.5

Table 4: Enzyme activity assays

Name	Abbr.	Substrate	pH	Assay Time	Temp	Measures	Common Uses
Milk Clot Unit[1]	MCU	Nonfat Dry Milk	4.5	2-3.5	40	Activity based on time to clot milk substrate in reference to a known standard's clotting time	Vegetative
Tyrosine Unit	TU	Casein	6.0	60	40	Amount of tyrosine release	Vegetative/ Bacterial
Plant Proteolytic (Papain Unit)	PU	Casein	6.0	60	40	Tyrosine released by papain standard	Vegetative
Casein Digestion	CDU	Casein	7.0	30	40	Amount of enzyme to degrade 1.5 µg of Tyrosine / minute	Vegetative/ Bacterial
Gelatin Digestion	GDU	Gelatin	4.5	20	45	Amount of enzyme to degrade 1 mg of Amino nitrogen	Bromelain
Hemoglobin Unit[2]	HUT	Denatured Hemoglobin	4.7	30	30	Amount of enzyme to degrade 1.1 µg of Tyrosine	Fungal
Proteolytical Unit[3]	PC	Casein	7.0	30	37	Amount of enzyme to degrade 1.5 µg of Tyrosine / minute	Vegetative/ Bacterial
Spectrophotometic Acid Protease Unit	SAP	Casein	3.0	30	37	Amount of enzyme to degrade 1 µmol of Tyrosine / minute	Fungal

[1]Assay used most commonly in Industry for vegetative enzymes.
[2]Assay used most commonly in Industry for fungal enzymes.
[3]Assay used most commonly in Industry for bacterial enzymes.
From: Balls and Hover, 1937; Food Chemical Codex, 1996; Dapeau, 1976; Enzyme Development Corporation.

observed in aged meat and collagen, it is much more effective when injected into the product due to its poor ability to penetrate surfaces (Gottschall and Kies, 1942). The optimal temperature range for papain is 65-80°C (Gottschall, and Kies, 1942) but Ashie *et al.* (2002) did find a significant increase in tenderness after one and two weeks of storage. Tappel *et al.* (1956) showed that a majority of the tenderizing activity occurs during the cooking process. Although active over a wide range of pH, the highest activity occurred in the pH range of 4.0-6.0 (Landmann, 1963). Studies disagree on the ability of papain to solubilize collagen with some showing little to no degradation and some a major increase in collagen solubility. Caution must be taken when using papain in order to prevent over-tenderization creating a mushy or mealy product. The proteins preferentially degraded by the GRAS enzymes are identified in Table 5.

Table 5. Strength of hydrolysis of myofibrillar proteins and collagen by various enzymes.

Protease	Hydrolysis of myofibrillar proteins	Hydrolysis of collagen
Papain	Excellent	Moderate
Bromelain	Moderate	Excellent
Ficin	Moderate	Excellent
Aspergillus	Moderate	Poor
Bacillus	Poor	Excellent

Bromelain

An enzyme mixture coming from pineapple, bromelain (a cystein protease), has been studied since the 1950's. Bromelain first degrades 40% of the collagen in the sarcolemma followed by degradation of myosin in the myofibrillar component (Wang *et al.*, 1958; Kang and Rice, 1970). This enzyme has low, but significant, activity at 0°C which dramatically increases at 50-70°C. The activity is sustained without a marked decrease up to 80°C (El-Gharbawi and Whitaker, 1963; Tappel *et al.*, 1956). Kim and Taub (1991) found that 5.0 is the optimal pH for bromelain activity. McKeith *et al.* (1994) found a significant increase in tenderness when an enzyme solution was injected into muscle versus dipping or tumbling in brine.

Ficin

Ficin, a vegetable-based enzyme, is derived from figs. The enzyme exhibits less activity against all types of proteins when compared to papain and bromelain. This thiol protease, although capable of minimal degradation of collagen and elastin, will preferentially degrade myofibrillar protein. Ficin is able to degrade elastin at temperatures as low as 20°C whereas it has little activity against collagen and myofibrillar proteins below 40°C. Optimal activity occurs between 60-70°C (El-Gharbawi and Whitaker, 1963; Foegeding and Larick, 1986). El-Gharbawi and Whitaker (1963) also showed the optimal pH for activity is near 7 for collagen and myofibrillar proteins and approximately 5.0-5.5 for elastin degradation. Sensory evaluations tend to show only a marginal increase in tenderness when compared to untreated samples (Wang *et al.*, 1958).

Bacillus subtilis proteases

A blend of proteolytic enzymes (alkaline elastase and neutral protease) derived from *Bacillus subtilis* were approved as GRAS in 1999 (FDA, 1999). Takagi *et al.* (1992) show alkaline elastase specifically degrades collagen and elastin with little degradation of myofibrillar protein resulting in less tenderizing effect but also decreasing the possibility of over-tenderizing. *Bacillus subtilis* proteases' optimal and activity ranges vary depending upon the organism from which they are produced. McConn *et al.* (1964) found neutral protease activity to rapidly increase at 50°C with a dramatic drop above 65°C. The same authors found a broad pH activity from 5.0-9.0 pH with an optimal pH found at 7.0. Enzymatic activity occurs during storage for one, two and three days at 4°C as measured by a decrease in relative hardness (Qihe, 2006). This bacterial protease was developed to be an inexpensive alternative for ficin.

Aspergillus oryzae proteases

Aspergillus oryzae produces an aspartic protease that shows a self-limiting proteolytic activity in meat systems (Ashie *et al.*, 2002). Proteolytic activity of *Aspergillus* has been known and studied since the 1950's (Underkofler *et al.*, 1958). Myofibrillar proteins are the main substrate for activity while little, if any, collagen breakdown occurs. This aspartic protease shows minimal activity during refrigerated storage through 14 days of storage but increases to an optimum at 55°C before dropping dramatically at 60°C. The enzyme from *Aspergillus* is active in acidic conditions and activity is maintained up to pH 7.0 before rapidly declining (Ashie *et al.*, 2002). As a self-limiting enzyme, some limited degradation can improve tenderness without the risk of a mushy or mealy texture.

Enzyme Application

An experiment was conducted at the University of Nebraska-Lincoln to compare all of the GRAS enzymes and evaluate the activity and tenderizing effect of each in muscles of different connective tissue contents. The study examined at the degradation of the myofibrillar and connective tissue components of muscle.

Materials and methods

Due to the difficulty of comparing the activity of enzymes based on different assays, a study was completed comparing the five GRAS enzymes and one control. Enzymatic activity and tenderizing effects were compared in the *supraspinatus* (high connective tissue) and *triceps brachii* (low connective tissue) muscles. Enzymes representing each of the GRAS enzymes (2 variations for *Aspergillus*, concentrate and 400) were obtained from Enzyme Development Corporation (Table 6) and activity was measured using four assays. Steaks were injected with varying levels of each enzyme to determine the highest level of enzyme that could be used without causing mealy or mushy texture from over-tenderization (Table 7). Additional *supraspinatus* and *triceps brachii* muscles were injected to 105% of green weight with a water and enzyme solution, tumbled for ten minutes and rested for one hour prior to cutting into steaks. Two steaks were frozen four hours post injection to be cooked to 70°C and evaluated using Warner-Bratzler shear force and trained

Table 6. Commercial names of enzymes used for data in tables 7-9

Protease	Commercial name	Stated activity units/mg
Papain	PANOL® 300 PURIFIED PAPAIN	>300 MCU
Bromelain	ENZECO® BROMELAIN 240	228-276 MCU
Ficin	ENZECO® FICIN 260	250-300 MCU
Aspergillus	ENZECO® FUNGAL PROTEASE CONCENTRATE	400 HUT
Aspergillus	ENZECO® FUNGAL PROTEASE 400	400 HUT
Bacillus	ENZECO® NEUTRAL BACTERIAL PROTEASE 160 K	152-184 PC

Table 7. Amount of enzyme added for results in tables 8-9

Protease	Enzyme target level (ppm)	Enzyme Activity Units added (per lbs)
Papain	9.0	1500 MCU
Bromelain	14.5	1400 MCU
Ficin	9.0	1240 MCU
Aspergillus Conc	21.1	3840 HUT
Aspergillus 400	17.8	3240 HUT
Bacillus	8.8	672 PC

Table 8. Shear force and sensory measures of tenderness

Protease	Shear force (kg)	Sensory tenderness rating	Sensory connective tissue rating
Papain	3.52^{a}	5.90^{a}	5.31^{a}
Bromelain	3.80^{ab}	5.44^{cde}	5.04^{bc}
Ficin	3.84^{ab}	5.46^{de}	4.83^{bcd}
Aspergillus Conc	3.86^{b}	5.82^{ab}	5.12^{ab}
Aspergillus 400	3.94^{b}	5.76^{ab}	5.04^{bc}
Bacillus	4.05^{b}	5.60^{bcd}	4.94^{bcd}
Control	4.39^{c}	5.00^{f}	4.34^{e}

$^{a-f}$Means within a given column with a common superscript do not differ significantly.
Lower readings for shear force reflect increased tenderness.
Higher readings for sensory ratings reflect better tenderness and lower amounts of connective tissue on 8-point hedonic scales.

sensory panel ratings. One steak was stored in a 4°C cooler to be used in laboratory assays comparing collagen and myofibrillar protein solubility as an indicator of protein degradation.

Results

The pretest to identify the highest level of enzyme for each treatment without detrimental effects on tenderness was successful. As a result, there were few differences in tenderness among the treatments (Figure 1). For shear force, the control was the least tender sample while papain had the lowest numerical value and was significantly more tender than all treatments except bromelain and ficin (Table 8). Sensory evaluation showed more differences but the results were similar - the control was the least tender while papain was the most tender, numerically, and significantly more tender than all but the *Aspergillus* treatments. The comparison for connective tissue in the samples also reflects the trend in the previous measures. The control (untreated) samples had the most connective tissue while samples treated with papain had the least, and were significantly lower than all but *Aspergillus*-treated samples.

Papain nearly doubled the soluble collagen content and percent solubility (Figure 2) compared to the other samples (Table 9). It should be noted that in the collagen extraction procedure, the assay temperature does not deactivate papain, allowing for residual activity. There were no statistical differences among the other treatments for soluble collagen. The control samples had the most insoluble collagen and were statistically higher than all but the *Bacillus* and *Aspergillus* 400 treatments. Comparing the enzyme treatments for percent collagen, the control had the second lowest numerical solubility and significantly less than all but and *Aspergillus* 400 treatments. These data reflect the higher insoluble content found in these three treatments.

All treatments prove to increase tenderness of the product in comparison to the control. Depending upon the system and degree of degradation desired, each has its purpose and place within the meats industry. Prior to deciding upon which enzyme to use, one must analyze the system in place. Factors such as raw material, holding time and temperature, other ingredients found within the brine, handling, and cooking procedures need to be evaluated to determine which enzyme will provide the desired outcome.

Table 9. Measures of collagen for enzyme-treated samples

Protease	Soluble	Insoluble	Total	Solubility %
Papain	0.92^{a}	6.76^{a}	7.65^{ab}	12.36^{a}
Bromelain	0.43^{b}	6.60^{a}	7.06^{a}	6.43^{bcd}
Ficin	0.54^{b}	7.31^{ab}	7.83^{ab}	7.66^{b}
Aspergillus Conc	0.53^{b}	7.56^{ab}	8.12^{abc}	7.27^{bc}
Aspergillus 400	0.42^{b}	8.42^{bc}	8.84^{bc}	4.89^{d}
Bacillus	0.42^{b}	8.37^{bc}	8.79^{bc}	5.03^{cd}
Control	0.46^{b}	8.93^{c}	9.29^{c}	4.98^{cd}

$^{a-d}$Means within a given column with a common superscript do not differ significantly.

Figure 1. Warner-Braztler shear force values for beef muscles treated with an enzyme solution

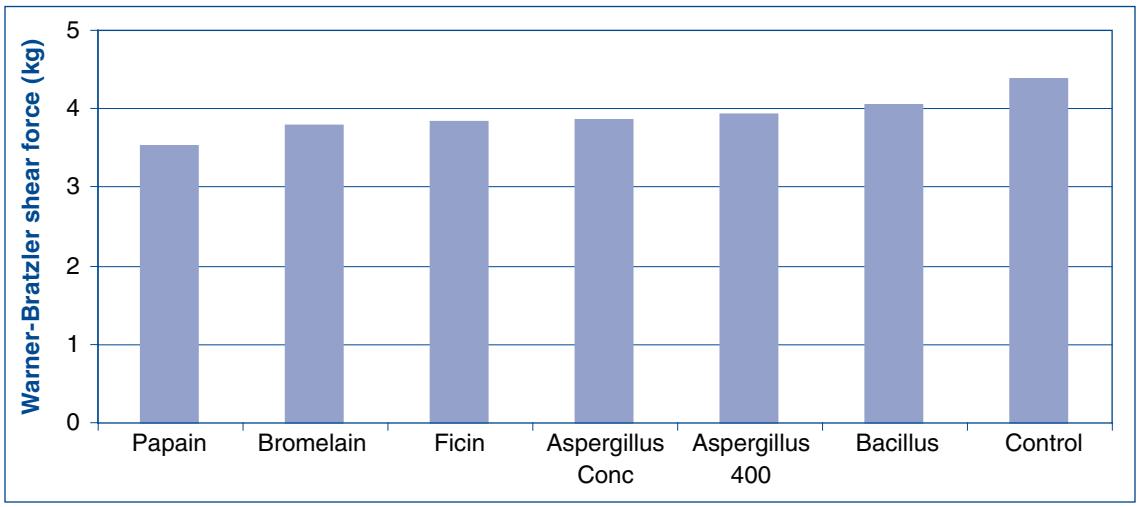
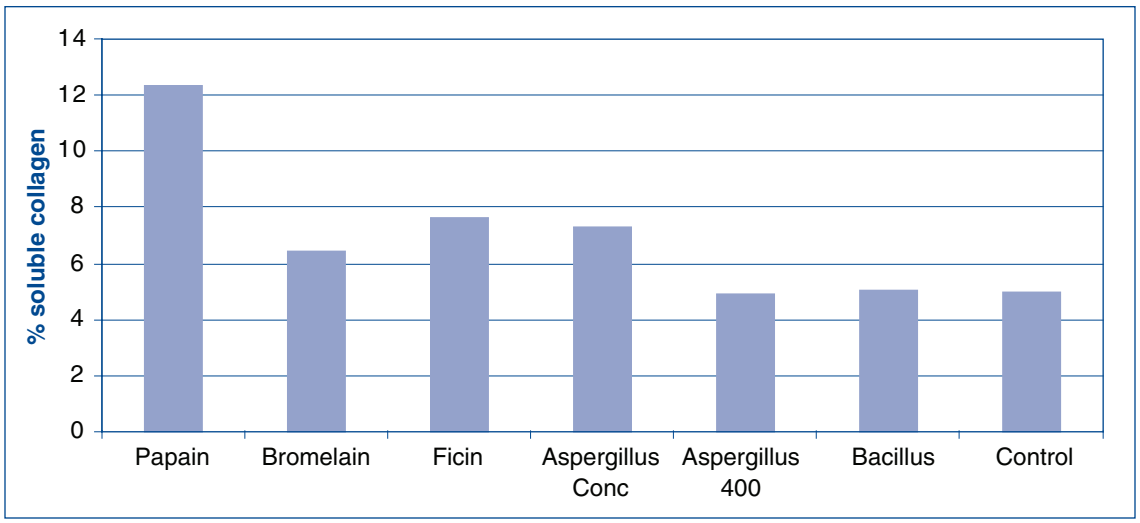


Figure 2. Percent solubility of total collagen for beef muscles treated with an enzyme solution



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