EFFECT OF ASPARTIC PROTEASE FROM *ASPERGILLUS ORYZAE* ON THE TENDERNESS OF BEEF

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Introduction
Tenderness, the single most important palatability factor affecting consumer satisfaction of beef, is mainly associated with the structural integrity of myofibrillar and connective tissue proteins. One approach to increasing beef tenderness is to significantly reduce the amount of detectable connective tissues without causing extensive degradation of muscle fibres. This purpose may be achieved by controlled proteolysis of targeted proteins by addition of exogenous enzymes. Although enzyme tenderisation of meat has been used for many years, most commonly using enzymes of plant origin such as papain, bromelain, and ficin, previous reports on the effects of enzymatic tenderisation on tenderness of cooked meat have been unsatisfactory. Due to their broad substrate specificity, these enzymes tend to indiscriminately break down the major muscle proteins which often results in an extensive degradation of the meat structure and undesirable flavour and texture (McKeith *et al.*, 1994; Stefanek *et al.*, 2002).
Current technology in the area of enzyme engineering has revealed other novel sources of proteolytic enzymes, mainly of fungal origin. There has been however, little research on using novel proteases obtained from alternative sources (Ashie *et al.*, 2002; Stefanek *et al.*, 2002). The application of enzyme technology may provide a useful means of meeting consumer expectations for product quality and consistency.
The current study was conducted to determine the range of acceptable aqueous concentrations of proteinase from *Aspergillus oryzae* injected at 105% of raw weight and to determine if the enzyme preparation was active at refrigeration temperature following injection. In the second study we examined effectiveness of refined levels of the protease during moist and dry cooking.

Materials and Methods
Semimembranosus (SM) muscles from young Canada Grade A carcasses were used for this study. The major variables investigated in experiment one were level of proteinase from *Aspergillus oryzae* (Amano Enzyme USA Co., Ltd., Elgin, IL) (to deliver 0.001%, 0.0025%, 0.005% in injected product) and post-injection storage time (1, 7, 14 days). Each SM muscle was cut into four roasts (700g). The roasts designated for enzyme treatment were injected to 105% over the raw meat weight with enzyme solution and then each section was cut into three 2.5 cm steaks which were individually vacuum packaged and refrigerated (4°C) for 1, 7 and 14 days. Steaks were cooked in a conventional oven at 163°C to a final temperature of 71°C.
In experiment two, paired SM muscles were divided into two equal portions to yield four sections. One section was kept as a non-injected control, while the other three were injected to 105% over the raw meat weight with enzyme solution to give 0.0005%, 0.001% and 0.0015% of proteinase from *Aspergillus oryzae* in the final product. After injection, the muscle samples were vacuum packaged and stored overnight at 4°C. The following day each section was cut into four equal-sized roasts that were used for texture measurements after dry- and moist-heat cooking to 71 or 79°C. Roasts for moist-heat cooking were wrapped in aluminium foil before cooking.
Warner Bratzler shear force (WBSF) of 1.27x1.27x2.54cm core samples sheared perpendicular to the fibre direction was determined in experiment one. The variables measured on each roast in experiment two included: cook yield (% of green weight), expressible moisture (EM), WBSF, and instrumental texture profile analysis (TPA).

Results and Discussion
Experiment 1: Enzyme-treated meat showed a gradual reduction in shear force with an increase of enzyme concentration. The lowest concentration (0.001%) of enzyme reduced WBSF by about 25% as compared to the control while maintaining acceptable appearance and texture. However, steaks injected with the highest (0.005%) concentration of proteinase had localized spots with unacceptable mushy and creamy texture. Our results indicated no obvious difference amongst shear values following the different lengths of refrigerated storage (1, 7, 14 days) after injection. This suggested that this enzyme was relatively inactive at refrigerated temperatures and the tenderising effect occurs mainly during cooking. It appeared that the protease functioned quite effectively once cooking commenced and substrate temperature increased.
Experiment 2: Regardless of the cooking method, roasts cooked to a higher internal temperature appeared very dry/grainy and had lower cook yield compared to those cooked to 71°C (Table 1). This observation was well supported by the values obtained for EM with roasts cooked to 79°C having significantly ($p<0.001$) lower EM, thereby indicating lower amounts of free moisture due to loss during cooking. Enzyme-injected muscles had lower cook yield than non-

injected control samples. Enzyme-treated roasts had also significantly lower EM than roast beef made from non-injected meat indicating that there was less free moisture in the product as a result of larger cook loss.
Roasts cooked to 71°C exhibited lower TPA hardness, chewiness and springiness, but were more cohesive than those processed to 79°C. Roasts processed by the moist cooking regime had lower shear force values than those cooked by the dry method. However, the moist cook regime appeared to be more effective in the enzyme treated roasts. Enzyme injected roasts cooked by moist method were more tender (lower shear values) in comparison to non-injected control while with the exception of the highest (0.0015%) enzyme treatment, there were no differences in shear force among samples baked in the dry environment. In addition, a significant reduction of WBSF values of moist cooked roasts had already been achieved at 0.0005% enzyme level; whereas dry cooked roasts required 0.0015% enzyme. Regardless of the endpoint temperature, increased level of protease resulted in a gradual decrease in hardness of moist cooked roasts, but its effect was insignificant for roasts cooked by the dry regimen.
The significant decrease of hardness and shear force values due to enzyme treatment in moist cooked roasts compared to roasts processed by the dry method might have been due to the initially slower heating rate of the moist method and approx. 15 min longer period of time in the temperature range for increased activity of injected proteinase (20°C to 50°C).

Table 1: Processing and textural characteristics of cooked beef *semimembranosus* roasts.

		Cook yield (%)	Expressible moisture (%)	WBSF (N)	Hardness (N)	Cohesiveness (-)	Springiness (cm)	Chewiness (N*cm)
Endpoint temperature								
71		66.45a	10.46a	67.8	202b	0.401a	0.24b	20.8b
79		59.77b	6.89	70.7	226a	0.386b	0.27a	25.9a
Cooking method x Enzyme level								
Dry	0	65.9a	9.15a	86.4a	238.8ab	0.405a	0.27ab	26.1ab
	0.0005	62.6b	9.84a	83.3a	216.4abc	0.407a	0.26abc	22.7bc
	0.0010	62.0b	7.71b	78.8a	217.9abc	0.404a	0.25bc	22.5bc
	0.0015	61.1b	7.46b	59.3c	208.6bc	0.375c	0.25bc	19.9c
Moist	0	65.2a	10.14a	82.6a	250.9a	0.407a	0.28a	28.4a
	0.0005	63.6ab	9.55a	69.5b	229.6ab	0.399ab	0.28a	25.9ab
	0.0010	62.8b	7.88b	59.3c	188.7cd	0.386bc	0.25bc	18.3cd
	0.0015	61.8b	7.65b	53.8c	162.5d	0.379c	0.24c	15.0d

a-c, Means with different letters in the same column are significantly different ($P<0.05$).

Conclusions

Exploratory testing indicated that a negligible amount of proteolysis occurred at refrigeration temperature, suggesting that enzyme-treated meat could therefore be stored without any adverse enzymatic changes in product characteristics. This result shows the superiority of the proteinase from *Aspergillus oryzae* to currently used enzymes such as papain which retains its activity at refrigerated temperature even after cooking thus increasing the risk of both texture deterioration and flavour defects.
Generally, the positive effect of increased enzyme concentration on tenderness of beef was more pronounced in roasts cooked by a moist heat method. Owing to a longer time in the temperature range from 20°C to 50°C, the moist cooking regime yielded products with lower shear force values than those cooked by the dry method.

References

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