

Effects of Enzymes on Beef Tenderness and Palatability Traits

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SUMMARY

Established enzymes used for tenderization of beef (Bromelin and Ficin) were compared to a novel enzyme, NCT, in three phases of an experiment using beef inside round roasts. Phase A determined the optimal dosage levels of the enzymes to be used in the concurrent phases. Phase B compared Ficin and NCT tenderness and palatability traits of roasts cooked and then stored for 0, 1, 4, 8 or 12 weeks. Phase C compared Ficin and NCT effects on tenderness and palatability traits of roasts stored for 0, 1, 2, or 4 weeks and then cooked. Injection of beef roasts with the novel enzyme NCT improved tenderness (as quantified by myofibrillar tenderness, connective tissue amount, overall tenderness) numerically or statistically in Phases B and C with mild but significant ($P < 0.05$) depression of juiciness (Phases B and C) and cooked beef flavor intensity (Phase B). Application of this technology could assist U.S. beef producers and processors in their efforts to meet consumer expectations for product quality and consistency.

Key Words: Enzyme Tenderization, Bromelin, Ficin.

INTRODUCTION

Meeting consumer expectations for product quality and consistency (particularly for tenderness) has been identified as high priority by the U.S. beef industry (NCBA, 1998). According to results of a recent consumer survey (Moeller and Courington, 1998), one of the three primary factors that would motivate consumers to purchase more beef at retail markets is "improved product quality and consistency." When asked about the use of certain tenderization technologies to enhance beef's quality

and consistency, most consumers indicated that they would consider buying beef that has been marinated or injected with a solution if the addition of the solution improved product performance (Moeller and Courington, 1998).

Injection technology has only recently been adopted for widespread commercial use by the beef industry, and with the addition of tenderizing agents, could effectively enhance beef tenderness. Proteolytic enzymes derived from plants, such as bromelin (pineapple) and ficin (fig), have been used as meat tenderizers in America and Europe (Prusa et al., 1981). However, these enzymes often degrade the texture of the meat, due to their broad substrate specificity, thus leading to unfavorable taste due to overtenderization (Kang and Rice, 1970; Cronlund and Woychik, 1986). Therefore, the ideal meat tenderizer would be a proteolytic enzyme with specific affinity for collagen and elastin in connective tissues at the relatively low pH of meat, which would act either at the low temperature at which meat is stored or at the higher temperature achieved during cooking (Cronlund and Woychik, 1987).

With this in mind, Novozymes NA, Inc., has developed a protease derived from a *Rhizomucor* species with the commercial name of NCT. This study was developed to validate NCT's usefulness as a meat-tenderizing agent against competing proteases.

This study was conducted in three phases. The objective of Phase A was to compare the effects on tenderness of NCT as compared to Ficin or Bromelin at two dosage levels (high or low) and to a negative control when product was cooked to each of three final cooking temperatures (140°F, 155°F or 170°F). From Phase A, the ideal dosages of NCT and Ficin were determined for use in Phases B and C. The objective of Phase B was to study the effects of NCT and Ficin on cooked roast palatability after chilled storage for 0, 1, 4, 8 or 12 weeks. The objective of Phase C was to study the effects of NCT and Ficin on roast palatability

after chilled storage for 0, 1, 2 or 4 weeks prior to cooking.

MATERIALS AND METHODS

Phase A

Forty-five beef inside rounds (NAMP 168) were obtained at a commercial beef processing facility from carcasses of market cows and bulls. Rounds ($n=5$) were randomly assigned to one of three endpoint-cooking temperatures (140°F, 155°F or 170°F) for each enzyme evaluated (Bromelin, Ficin, or NCT). Each round was then divided into three roasts (2.5 to 4 lb each). The most anterior portion of the round served as the untreated control, the middle portion was injected with the low dosage level of the enzyme (20 ppm for Ficin and Bromelin, 0.003 AU/ 100g meat for NCT) and the posterior portion was injected with the high dosage level (40 ppm for Ficin and Bromelin, 0.01 AU/100g meat for NCT) of the treatment enzyme. Each roast was weighed (raw product weight) and then injected with the assigned enzyme and dosage level using a single-needle, hand-held injector. Each roast was injected at approximately 5 ± 2 different sites. Injection treatments were formulated so that the roast would retain 3 percent of the raw weight. All roasts were then vacuum packaged and stored under refrigeration (4°C) for 36 hours.

Roasts were wrapped in foil and then cooked in an electric convection oven (model series DN97, Hobart, Troy, OH) at 250°F. Internal roast temperatures were monitored continuously using copper constantan thermocouples attached to a digital readout thermometer (model 31380-KF, Atkins Technical, Gainesville, FL). Roasts were cooked in batches of 27 due to capacity limitations. Each batch contained one full replicate of the experimental design to evenly distribute extraneous sources of variation across all enzyme treatments, dose levels and endpoint temperatures. Roasts were randomly positioned in the oven and cooked to the specified endpoint temperature, removed from the foil wrap and allowed to equilibrate to room temperature (26°C).

Shear Force Determinations. Two to four slices (2.5 cm thick) were removed from the center of each roast. Six to nine cores (1.27 cm in diameter) were removed from the roast slices, parallel to the muscle fiber orientation and sheared once, perpendicular to the orientation of the muscle fibers, using a Warner-Bratzler shear machine. Peak shear measurements were recorded and averaged to obtain a single shear force value for each roast.

Statistical Methods. Data were analyzed using mixed models procedures (SAS, 1996). The statistical model included the fixed effects of enzyme (Bromelin, Ficin or NCT), endpoint temperature (140°F, 155°F or 170°F), dosage (A, B or C referring to control, low dose and high dose, respectively), and all of the two-, three-, and four-way interactions. In addition, the statistical model included the random effects of replication (1 through 5).

Phase B

It was determined from Phase A that the high dosage levels of Ficin (40 ppm) and NCT (.01 AU/100g meat) were ideal for further investigation. Thus, for Phase B, twenty-five inside rounds were obtained from the same commercial beef processing facility and randomly assigned to five different storage treatments (0, 1, 4, 8 or 12 weeks). Each round was then divided into three roasts (2.5 to 4 lb. each). The most anterior portion served as the untreated control, the middle portion was injected with the high dosage level of Ficin (40 ppm) and the posterior portion was injected with the high dosage level of NCT (.01 AU/100g meat). Each of the treated roasts were weighed (raw product weight) and then injected with the assigned enzyme and dosage level using a single-needle, hand-held injector. Each roast was injected at approximately 5 ± 2 different sites. Injection treatments were all formulated so that each roast would retain 3 percent of its raw weight. All roasts were then vacuum packaged and stored under refrigeration (4°C) for 36 hours. All roasts were then wrapped in foil and cooked in an

electric convection oven (model series DN97, Hobart, Troy, OH) at 250°F. Internal roast temperatures were monitored continuously using copper constantine thermocouples attached to a digital readout thermometer (model 31380-KF, Atkins Technical, Gainesville, FL). Roasts were cooked in batches of 27 due to capacity limitations. Roasts were removed when their internal temperature reached 155°F (approximate cooking time 2.5 hr.). Roasts designated for 0 week storage were then evaluated by members of a trained sensory panel and subjected to Warner-Bratzler shear force assessment. The remaining roasts were allowed to chill until they could be handled physically (approximately <1 hr), and then vacuum packaged and immediately stored under refrigeration (4°C). When stored roasts had reached their designated storage time, each was reheated in the same convection oven, using the same settings and methods, until reaching an internal temperature of 155°F. Samples were then removed for sensory evaluation and shear force evaluation as was previously described for Phase A.

Sensory Evaluation. One to three of the slices removed from the center of the roasts used for Phases B and C were used for sensory evaluation. Warm samples (1.3 x 1.3 x 2.5 cm) from each roast were evaluated by an eight-member, trained sensory panel. Panelists were trained for 2 weeks according to procedures outlined by Meilgaard et al. (1991) and AMSA (1995). Panelists assigned scores to each roast for tenderness and juiciness using 8-point, structured rating scales (AMSA, 1995). Additionally, panelists rated cooked beef flavor (0 = none detectable, 1 = slightly detectable, 2 = very strong) (AMSA, 1995). Panelists were also allowed to comment on any additional palatability characteristics they identified while eating.

Statistical Methods. Data were analyzed using mixed models procedures (SAS, 1996). The statistical model included the fixed effects of treatment (Control, Ficin or NCT), storage time (0, 1 or 4 weeks), and the two-way interaction. In addition, the

statistical model included the random effects of replication (1 through 5).

Phase C

Twenty inside rounds were obtained from the same commercial beef processing facility. Five rounds each were assigned to four storage periods (0, 1, 2 or 4 weeks). Each round was separated into 3 roasts and control or enzyme treatments were applied as outlined for Phase B. The roasts were vacuum packaged and stored under refrigeration (4°C) for 36 hours. At that time, roasts designated for 0 week storage were cooked in the manner described for Phases A and B, and sampled for trained sensory and shear force evaluation as was described previously.

Statistical Methods. Data were analyzed using mixed models procedures (SAS, 1996). The statistical model included the fixed effects of treatment (Control, Ficin or NCT), storage time (0, 1 or 4 weeks), and the two-way interaction. In addition, the statistical model included the random effects of replication (1 through 5).

RESULTS AND DISCUSSION

Phase A

Average cooking loss percentages were calculated and reviewed (results not presented). As expected, cooking loss increased with increased internal degree-of-doneness (cooking temperature) in all treatment combinations. Overall, it was observed that use of NCT resulted in lower cooking losses for roasts at the majority of dose and cooking temperature combinations than for roasts treated with Bromelin or Ficin (the exceptions were for dose 0 at 170°F, dose .01 AU at 155°F and dose 0.01 AU at 170°F). Some objectionable characteristics were observed for roasts treated with Bromelin or Ficin. Roasts treated with Bromelin or Ficin had localized "spots" with "mushy" texture due to uneven distribution of the injection solution. Mushiness was most evident in roasts treated with Ficin and cooked to the 140°F and 155°F. Roasts treated with Bromelin or Ficin that were cooked to 170°F displayed problems with uneven distribution of enzyme solution and,

due to moisture loss, the meat crumbled (in a fashion similar to that of sawdust) when cut for sampling. Mean differences in Warner-Bratzler shear force measurements between control and treated roasts are presented in Table 1. Because only roasts treated with Bromelin differed in tenderness ($P < 0.05$) from those treated with the other enzymes, it was decided by the sponsors of the study to conduct Phases B and C of the study using the high dosage levels of Ficin (40 ppm) and NCT (0.01 AU/g meat) as treatment levels.

Phase B

Cooking loss percentages again were calculated and reviewed (results not presented). As was the case in Phase A, roasts treated with NCT had numerically lower cooking losses than did roasts injected with Ficin, (42.24% versus 43.36%) but roasts in both treatments exhibited higher cooking losses than did control roasts (37.93%). The results for cooking loss were related to the endpoint cooking temperature to which roasts were cooked (155°F). Mean values for WBS force and differences from control in WBS force for Phase B roasts for 0, 1, 4, 8 and 12 weeks storage are presented in Table 2. There were no significant differences in WBS force values ($P > 0.05$) among roasts in treatment and control groups or among storage periods.

After completion of the mixed model procedure, no significant storage time effect was found ($P > 0.05$), but a significant treatment effect was observed ($P < 0.05$). Least squares means for sensory characteristics for Phase B roasts over all storage periods are presented in Table 3. Significant differences were found ($P < 0.05$) among roasts in treatment and control groups for all characteristics. Panelists rated the roasts treated with Ficin or NCT dryer than control roasts while Ficin-treated roasts were rated as having less connective tissue than control and NCT-treated roasts. Also, panelists determined that the control roasts and roasts treated with Ficin had more cooked beef flavor than roasts

treated with NCT. Again uneven distribution of the injection brine affected palatability of samples from roasts treated with Ficin; sensory evaluation panelists commented that those samples “crumbled” or had the texture of “sawdust” and a slight “liver” flavor. In addition, samples from roasts treated with Ficin differed widely in tenderness with samples from the same roast being rated “very tender” (scores of 6 or 7) to “very tough” (scores of 1 or 2). No unusual flavors or extreme differences in tenderness were observed for samples of control roasts or of roasts treated with NCT.

Phase C

Cooking loss percentages for Phase C roasts for storage periods of 0, 1, 2, or 4 weeks are presented in Table 4. Mean values for WBS force and differences from control in WBS force for Phase C roasts for 0, 1, 2, or 4 weeks storage are presented in Table 6. There were no significant differences among control and treatment groups or among storage treatments in cooking losses or WBS force values (Tables 4 and 5).

After completion of the mixed model procedure, no significant storage time effect was found ($P > 0.05$), but a significant treatment effect was observed ($P < 0.05$). Least squares means for sensory characteristics for Phase C roasts over all storage periods are presented in Table 6. Compared to control roasts, roasts treated with Ficin had less desirable ratings ($P < 0.05$) for juiciness and less intense ratings ($P < 0.05$) for cooked beef flavor but were not ($P > 0.05$) more tender (myofibrillar, connective tissue or overall ratings). Compared to control roasts, roasts treated with NCT had less desirable ratings ($P < 0.05$) for juiciness, did not differ in cooked beef flavor but were ($P < 0.05$) more tender (myofibrillar, connective tissue and overall ratings). Roasts treated with NCT were ($P < 0.05$) juicier and more tender (myofibrillar, connective tissue and overall ratings) but not different ($P > 0.05$) in cooked beef flavor from roasts treated with Ficin. Sensory evaluation panelists again encountered

irregular texture and flavor characteristics among samples of roasts treated with Ficin but no unusual texture or flavor characteristics were noted for samples of control or NCT-treated roasts.

CONCLUSIONS

Injection of beef roasts with the proteolytic enzyme NCT improved tenderness (as quantified by myofibrillar tenderness, connective tissue amount, overall tenderness) numerically or statistically in Phases B and C of the present study with mild but significant ($P < 0.05$) depression of juiciness (Phases B and C) and cooked beef flavor intensity (Phase B). Application of this technology could assist U. S. beef producers and processors in their efforts to meet consumer expectations for product quality and consistency. Further research should be conducted to identify optimal application of NCT to beef roasts (e.g. method of injection in conjunction with tumbling) to solve the enzyme distribution problem. It could also be advantageous to study the effects of use in combination with a salt and phosphate brine solution, to increase tenderness while preserving flavor and juiciness of the cooked product.

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Table 1. Least squares means and standard errors for WBS force values for roasts treated with NCT, Ficin or Bromelin, averaged over dosage and final cooking temperature.

Treatment	WBS force (kg)
NCT	4.98 ^b ± .22
Ficin	5.05 ^b ± .22
Bromelin	6.33 ^a ± .22

^{a,b} Means in the same column lacking a common superscript letter differ (P < .05).

Table 2. Mean values for WBS force and difference^a from control (± standard deviations) in WBS force for Phase B roasts for 0, 1, 4, 8 or 12 weeks storage.

	WBS force (kg)	Difference From Control (kg)
Stored 0 Weeks		
Control	8.4	
Ficin	6.7	1.7 ± 1.2
NCT	7.2	1.2 ± 2.0
Stored 1 Week		
Control	6.9	
Ficin	5.8	1.1 ± 0.8
NCT	8.1	-1.2 ± 4.9
Stored 4 Weeks		
Control	6.8	
Ficin	6.2	0.6 ± 1.8
NCT	6.7	0.2 ± 1.0
Stored 8 Weeks		
Control	7.0	
Ficin	5.2	2.2 ± 0.7
NCT	5.6	1.5 ± 1.5
Stored 12 Weeks		
Control	5.6	
Ficin	4.0	1.5 ± 1.7
NCT	6.1	-0.5 ± 1.3

^aDifference = Control - Treatment

Table 3. Least squares means (\pm standard errors) for sensory characteristics for Phase B roasts, over all storage periods.

	Control	Ficin	NCT
Juiciness ^v	4.03 ^a \pm .11	2.70 ^c \pm .12	3.15 ^b \pm .11
Myofibrillar tenderness ^w	3.82 ^a \pm .16	4.51 ^b \pm .16	4.28 ^b \pm .16
Connective tissue amount ^x	3.47 ^a \pm .16	4.34 ^b \pm .17	4.25 ^b \pm .16
Overall tenderness ^y	3.61 ^a \pm .16	4.26 ^b \pm .16	4.16 ^b \pm .16
Cooked beef flavor ^z	1.25 ^a \pm .04	1.13 ^b \pm .05	1.06 ^b \pm .04

^vJuiciness: 8 (extremely juicy) to 1 (extremely dry)

^wMyofibrillar Tenderness: 8 (extremely tender) to 1 (extremely tough)

^xConnective Tissue Amount: 8 (none) to 1 (abundant)

^yOverall Tenderness: 8 (extremely tender) to 1 (extremely tough)

^zCooked Beef Flavor: 0 (none detectable) to 2 (very strong)

^{ab}Means in the same row lacking a common superscript letter differ ($P < .05$)

Table 4. Mean cooking loss percentages for Phase C roasts for 0, 1, 2 or 4 weeks storage.

Treatment	Stored 0 Weeks	Stored 1 Week	Stored 2 Weeks	Stored 4 Weeks
Control	38.68	39.66	40.20	48.72
Ficin	38.65	45.63	47.31	43.98
NCT	31.81	45.55	43.02	37.46

Table 5. Mean values for WBS force and difference^a from control (\pm standard errors) in WBS force for Phase C roasts for 0, 1, 2 or 4 weeks storage.

	WBS force (kg)	Difference From Control (kg)
Stored 0 Weeks		
Control	5.6	
Ficin	5.3	0.3 \pm 1.8
NCT	5.0	0.6 \pm 0.9
Stored 1 Week		
Control	6.0	
Ficin	5.4	0.6 \pm 1.1
NCT	6.0	-0.02 \pm 1.7
Stored 2 Weeks		
Control	5.0	
Ficin	4.9	0.04 \pm 1.9
NCT	4.9	0.05 \pm 1.2
Stored 4 Weeks		
Control	5.3	
Ficin	5.1	0.2 \pm 1.8
NCT	4.7	0.6 \pm 1.5

^aDifference = Control - Treatment

Table 6. Least squares means (\pm standard errors) for sensory characteristics for Phase C roasts, over all storage periods.

	Control	Ficin	NCT
Juiciness ^v	5.08 ^a \pm .19	3.23 ^c \pm .19	4.35 ^b \pm .19
Myofibrillar tenderness ^w	4.20 ^b \pm .18	4.40 ^b \pm .18	4.99 ^a \pm .18
Connective tissue amount ^x	3.75 ^b \pm .18	4.12 ^b \pm .18	4.77 ^a \pm .18
Overall tenderness ^y	3.97 ^b \pm .19	4.23 ^b \pm .19	4.84 ^a \pm .19
Cooked beef flavor ^z	1.33 ^a \pm .06	1.14 ^b \pm .06	1.22 ^{ab} \pm .06

^vJuiciness: 8 (extremely juicy) to 1 (extremely dry)

^wMyofibrillar Tenderness: 8 (extremely tender) to 1 (extremely tough)

^xConnective Tissue Amount: 8 (none) to 1 (abundant)

^yOverall Tenderness: 8 (extremely tender) to 1 (extremely tough)

^zCooked Beef Flavor: 0 (none detectable) to 2 (very strong)

^{ab}Means in the same row lacking a common superscript letter differ ($P < .05$)