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Application of exogenous enzymes to beef muscle of high and low-connective tissue

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ABSTRACT

Exogenous enzymes tenderize meat through proteolysis. *Triceps brachii* and *Supraspinatus* were randomly assigned to the seven enzyme treatments, papain, ficin, bromelain, homogenized fresh ginger, *Bacillus subtilis* protease, and two *Aspergillus oryzae* proteases or control to determine the extent of tenderization (Warner–Bratzler shear and sensory evaluation) and mode of action (myofibrillar or collagen degradation). Sensory evaluation showed improvement ($P < 0.0009$) for tenderness and connective tissue component and all except ginger had a lower shear force than the control ($P < 0.003$). Ginger produced more off-flavor than all other treatments ($P < 0.0001$). Only papain increased soluble collagen ($P < 0.0001$). Control samples were only significantly less than ficin for water soluble ($P = 0.0002$) and *A. oryzae* concentrate for salt soluble proteins ($P = 0.0148$). All enzyme treatments can increase tenderness via myofibrillar and collagenous protein degradation with no difference among high and low-connective tissue muscles.

1. Introduction

Consumers' perception of overall palatability is affected by many traits but tenderness is often cited as the most important (Huffman et al., 1996; Savell et al., 1987, 1989). Just as importantly, consumers are willing to pay a premium for increased tenderness (Miller, Carr, Ramsey, Crockett, & Hoover, 2001).

Tenderness is a complex, multi-factorial trait that has been described in three primary components: actomyosin effect of myofibrillar proteins, the background effect of connective tissue and the bulk density or lubricating effect of fat (Savell & Cross, 1988). Collagen, total and soluble, has been shown to impact palatability (Cover, Hostetler, & Ritchey, 1962; Herring, Cassens, & Briskey, 1967; Rhee, Wheeler, Shackelford, & Koohmaraie, 2004). The physical state of myofibrillar proteins also plays an important role in tenderness (Cover et al., 1962; Locker, 1960; Marsh & Leet, 1966). Most methods of improving tenderness will impact on one or more of these traits. Exogenous enzymes originating from plants, bacteria, and fungal sources have been used for centuries to improve tenderness by proteolytic activity. United States federal agencies (CFR, 2009, chap. 424; 1999, chap. 184) recognize five exogenous enzymes – papain, ficin, bromelain, *Aspergillus oryzae* protease, and *Bacillus subtilis* protease – as Generally Recognized as Safe (GRAS) to improve meat tenderness. Each of these has been shown to have varying degrees of activity against myofibrillar and collagenous proteins (Ashie, Sorensen, & Nielsen, 2002; El-Gharbawi & Whitaker, 1963; Gerelt, Ikeuchi, & Suzuki, 2000; Kang & Rice, 1970; Kim & Taub, 1991; Miyada & Tappel, 1956; Takagi et al., 1992; Tsai, Yamasaki, & Tamura, 1984; Whitaker, 1994). In addition to these GRAS enzymes, many others have been suggested. Among the most promising, zingibain, from ginger (*Zingiber officinale Rosocoe*), has been shown to degrade both myofibrillar and collagenous proteins (Lee, Sehnert, & Ashmore, 1986; Naveena, Mendiratta, & Anjaneyulu, 2004; Thompson, Wolf, & Allen, 1973).

This study investigated the application of the five GRAS enzymes (papain, ficin, bromelain, *B. subtilis* protease, two variations of *A. oryzae* proteases) and ginger to high and low-connective tissue beef muscles to determine improvement in tenderness and the mode of action.

2. Materials and methods

2.1. Experimental design

An 8×2 factorial design (enzyme treatment and muscle, respectively) was used to determine tenderization and mode of action of enzyme treatments on beef muscles of high and low collagen content. Two muscles, *Triceps brachii* (TRB) and *Supraspinatus* (SUP), were purchased from Greater Omaha Packing Company (Omaha, Nebraska). Muscles were randomly assigned to one of eight treatments of water and papain (PAP), bromelain (BRO), ficin (FIC), *B. subtilis* protease (BAC), *A. oryzae* concentrate (ACONC), *A. oryzae* 400 (A400), or ginger (GIN). Whole muscles were treated 7 days post-mortem. A randomized complete factorial design of two muscles by eight treatments and 12 samples per muscle/treatment combination resulted in a total of 192 samples. Five steaks were cut from each muscle and analyzed for collagen content

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and solubility, protein solubility, Warner–Bratzler shear force, and sensory evaluations were performed by a trained panel.

2.2. Sample preparation

Ninety-six of each beef USDA select grade *Triceps brachii* (IMPS 114E) and *Supraspinatus* (IMPS 116B) were delivered to the University of Nebraska Meat Laboratory on day four post-mortem. All muscles were trimmed of visible fat and connective tissue and the *Triceps brachii* (long head) were isolated at day six post-mortem. Muscles were randomly assigned to one of eight treatments. Concentrations for each enzyme were determined during preliminary investigations to maximize tenderizing effects while minimizing negative effects on eating quality (Table 1). Enzymes from the Enzyme Development Corp. (New York, NY) were stored in a walk-in cooler at 4 ± 1 °C until use. Ginger was prepared by peeling fresh ginger and homogenizing, using liquid nitrogen and a Waring blender (Waring Products Division, New Hartford, CT) and stored at −80 °C until use. Enzyme solutions, using a five needle stitch pump injector, were injected into muscles to 105% of green weight; control samples were not injected. All treatments were vacuum tumbled (Model TU-120; Roshermatic, Osanabruck, W. Germany) for 10 min and rested for 30 min. Steaks, 2.54 cm thick, were cut from the anterior end of each muscle and vacuum packaged. Steaks 1 and 2, used as an extra sample and for laboratory analysis, respectively, were stored at 4 ± 1 °C for 7 days and then frozen. Steaks 3–5 were frozen at 4 h post-injection and used for Warner–Bratzler shear force, sensory panel evaluation, and an extra steak, respectively. Frozen samples were stored at −20 ± 5 °C until analyses.

2.3. Warner–Bratzler shear force

Steaks were thawed for 24 h at 4 °C and cooked on a flat top grill sprayed with Vegalene pan coating (Par-Way/Tryson Companies, St. Clair, MO) (GE Model HGA, General Electric, Chicago Heights IL) set to 176–190 °C. Steaks, turned once at 35 °C, were cooked to an internal temperature of 70 °C. Temperature was monitored with an OMEGA 450ATT thermometer with a type T thermocouple (OMEGA Engineering, Inc., Stamford, CT). Immediately following cooking, samples were placed in a plastic zipper bag and submerged in an ice-water bath for at least 15 min to minimize residual proteolytic activity. Steaks were placed at 4 °C for 4 h before coring. At least six, 1.27 cm diameter cores were taken from each steak parallel to the muscle fiber orientation. Warner–Bratzler shear force was determined from the average peak force of six cores using an Instron Universal Testing Machine (Model 55R1123, Instron Corp., Canton, MA) equipped with a 500 kg load cell.

2.4. Trained sensory panel evaluation

The sensory panel, composed of community members, UNL staff and graduate students, was trained according to AMSA (1995) guidelines and then sensitized using TRB that had been untreated or treated with varying enzymes at different concentrations. Members were trained to identify varying degrees of tenderness, connective tissue, juiciness and off-flavors. Eight samples were served per panel session representing each of the treatments from one muscle. Steaks were thawed for 24 h at 4 °C before being cooked to 70 °C on a flat top grill set to 176–190 °C and held for up to 15 min in a 55 °C warming table until serving. Samples, 1.27 × 1.27 × 2.54 cm, were served immediately after cutting to panelists who sat in individual booths under red incandescent lighting. Panelists evaluated the samples using 8-point scales, anchored with eight representing extremely tender, no connective tissue, extremely juicy, and extreme off-flavor and one representing extremely tough, abundant amount, extremely dry and no off-flavor for tenderness, connective tissue, juiciness and off-flavor intensity.

2.5. Soluble and insoluble collagen

Samples were powdered using liquid nitrogen and a Waring blender (Waring Products Division, New Hartford, CT) and then stored at −80 °C. Soluble and insoluble collagen was determined as described by Hill (1966) and Cross, Carpenter, and Smith (1973). The procedure was modified by including 10 min in a 40 °C water bath prior to 80 min in a 70 °C water bath.

Filtrates were analyzed for hydroxyproline content by the procedures modified from Bergman and Loxley (1963) and Kolar (1990). Conversion factors of 7.52 and 7.25 were used for heat soluble and heat insoluble collagen, respectively (Goll, Hoekstra, & Bray, 1963). Each sample was prepared in duplicate and read in triplicate.

2.6. Water and salt soluble protein

Samples were powdered using liquid nitrogen and a Waring blender (Waring Products Division, New Hartford, CT) and then stored at −80 °C. Salt and water soluble proteins were extracted to determine the extent of proteolysis of muscle proteins, as an indicator of overall enzyme activity. Water soluble and total soluble proteins were determined as described by Joo, Kauffman, Kim, and Park (1999).

2.7. Statistical analyses

Data were analyzed using a factorial design, two muscles × eight enzyme treatments, by analysis of variance (ANOVA) using Proc GLIMMIX of SAS (Version 9.1, Cary, NC). Separation of means was conducted using LSMEANS and PDIFF when the level of significance indicated by ANOVA was $P \leqslant 0.05$. In the case of significant muscle by treatment interactions, SLICE and SLICEDIFF functions were used to separate differences.

Table 1
Enzymes, abbreviations, commercial name, supplier's stated activity, parts per million of enzyme used in the application and enzyme activity per kg of meat.

Protease	Abbreviation	Commercial name	Supplier stated activity (per mg)[a]	Application per kg	
				PPM	Enzyme activity[a]
Papain	PAP	PANOL® 300 PURIFIED PAPAIN	>300 MCU	9.0	3300 MCU
Bromelain	BRO	ENZECO® BROMELAIN 240	228–276 MCU	14.5	3080 MCU
Ficin	FIC	ENZECO® FICIN 260	250–300 MCU	9.0	2728 MCU
Aspergillus oryzae	ACONC	ENZECO® FUNGAL PROTEASE CONCENTRATE	400 HUT	21.1	8448 HUT
Aspergillus oryzae	A400	ENZECO® FUNGAL PROTEASE 400	400 HUT	17.8	7128 HUT
Bacillus subtilis	BAC	ENZECO® NEUTRAL BACTERIAL PROTEASE 160 K	152–184 PC	8.8	1478 PC
Zingibain	GIN	Ginger Rhizome	–	2021.0	142 PC

[a] Measures used to state enzyme activity MCU – milk clot unit, HUT – hemoglobin unit of tyrosine, and PC – proteolysis activity of casein.