PURGE LOSSES IN FRESH BEEF *BICEPS FEMORIS* ROASTS INJECTED WITH PLANT BASED PROTEOLYTIC ENZYMES

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Abstract – The injection of meat is a common method used to increase its perceived quality and enzymes can be injected to increase the tenderness of meat. To investigate the impact of enzymes on purge loss, enzymes from four plant sources (kiwi, papaya, ginger, kachri) as well as water (positive control) were injected into beef *biceps femoris* muscle. Injected muscles as well as dry controls were tumbled for 30 min. Muscles were stored at 4 ºC and purge losses were calculated after 24 hrs. Purge loss was found to increase with injection of the meat, as all injected samples had higher mean purge loss than the dry controls (P > 0.0001). The use of enzymes did not cause greater purge than the injection of pure water, but there were indications that some enzymes caused more purge than others. Papain and kachri both had lower purge losses than pure water (P=0.02 and P=0.03 respectively), while actinidin had greater purge losses than both kachri and papain (P=0.015 and P=0.01 respectively), although it was not significantly different from pure water. It was concluded that the injection of brine containing proteolytic enzymes did not cause increased purge losses compared to water injections.

Key Words – water retention, enzymatic tenderization, meat quality.

I. INTRODUCTION

Meat quality is the sum of several factors. Two such factors frequently repeated by consumers is the perceived tenderness of the meat and the juiciness [1]. Tender beef commonly comprises approximately 20 to 25% of a beef carcass [2], and methods enhancing tenderness and juiciness of the remainder of the meat in the beef carcass are therefore a common focus in the meat industry. One such method of post-mortem meat enhancement is the injection of meat with brines containing ingredients such as lactate, salt, phosphate and enzymes [3,4]. These treatments are used to increase the perceived quality of the meat by increasing the juiciness and tenderness of the product. Phosphates, salt and lactate all add to the ability of meat to hold water [3]. Enzymes however have the potential to decrease the water-holding capacity of an injected meat product because of their proteolytic effect that, although it increases meat tenderness [4], may also contribute to the denaturation of muscle proteins and the release of immobilized water.

When injecting enzyme brines, the ability of the muscles to retain the brine is of interest as the brine cannot be effective if it is not retained. Also, any added moisture will add to the weight of the meat, and so brine retention is of economic importance to the meat industry. To explore the effect of injected proteolytic enzymes on meat water-holding capacity, an experiment was performed to investigate the effects of injecting tough beef muscle with enzymes without the added water binding assistance of salts, phosphates or lactate. This paper will discuss the ability of injected beef *biceps femoris* muscle to retain plant enzyme brine during the first 24 hours of fresh storage.

II. MATERIALS AND METHODS

Thirty-six bovine *biceps femoris* muscles that were aged between 6 to 7 days post mortem were obtained from a local butcher. The meat was trimmed of any visible connective tissue prior to processing. Intramuscular pH was measured once at each end of the muscle, an average calculated and that average was considered the initial pH value of that muscle. Any muscles with mean pH values outside the pH range of 5.3 to 5.8 were excluded.

Three 7.6 cm wide roasts were cut from each muscle with muscle fibre direction of each roast parallel to the length of the roast. Roasts were randomly assigned to treatment with roast position within the muscle balanced within

treatment. Each roast position was assigned to each treatment 3 times each processing day, for a total of 9 roasts per processing day within each treatment. Processing occurred over two days, resulting in 18 replicates for each treatment.

All enzymes selected for study were from plant sources. Enzymes were obtained either previously isolated or were isolated using produce obtained at local supermarkets (Table 1). Papain and actinidin were commercially produced powders, while ginger and kachri enzyme mixtures were extracted from ginger root and kachri purchased at a local grocery. Crude enzyme mixtures from ginger and kachri were made by blending the plant material with water and solids were removed from the mixtures by filtering the plant homogenates through 1 mm^2 steel mesh and fine cotton. The ginger root was mixed with water in a 2 to 1 (w/v) ratio, while kachri was mixed in a 1 to 15 (w/v) ratio. The ginger and kachri extracts were then frozen, packaged under vacuum and stored at -20°C for 1-2 weeks until used.

Table 1: Brine used for injection

Treatment	Plant Source	Supplier	Brine Conc[1]. In Meat
Papain	Papaya	Enzyme Development Corp. (USA)	9
Actinidin	Kiwi	Ingredient Resources (Australia)	5
Kachri slurry	Kachri	From local grocery	440[2]
Ginger slurry	Ginger	From local grocery	825[2]
Water		Municipal water	
Control		None	No Injection

[1] Concentration in parts per million (ppm)
[2] Approximate values

A Fomaco Reiser injector was used (Reiser Canada, Ontario). For each of the five injection treatment (papain, actinidin, kachri, ginger, water), the beef roasts were injected to a green weight of 110% before being packaged under vacuum and gently tumbled for 30 min. Injection levels were based on literature values [4,5]. Dry controls were tumbled for 30 min as well and all roasts were then stored at 4 ºC overnight. After storage the meat was again weighed and the intramuscular pH of each roast measured.

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) using a one-way analysis of variance with enzyme treatment as the sole source of variation. The effect of roast position within muscles was included in the initial analysis of variance but was removed when it was found not to be significant. Sources of variation were considered significant at $P \leq 0.05$. Where sources of variation were significant, differences between treatment means were determined using least square means differences, with significance also at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

In general, muscle fiber structure and composition tends to vary greatly in a muscle as large as the beef *biceps femoris*. However, there was no effect of roast location within each muscle on purge losses nor was there an interaction between roast location and enzyme treatment.

All meat samples displayed some purge losses after 24 hours. As expected, the beef roasts injected with papain, actinidin, ginger, kachri, or water had incurred greater purge losses than the dry control roasts ($P<0.0001$). This increase in purge may have arisen not only from the added water but may have also resulted from the physical action of the needle injection, which can damage the meat fiber structure [6]. Overall, enzyme type was not associated with purge losses different from that of the injection of pure water used in the wet control. Papain had lower purge loss than other injection treatments including pure water, while actinidin had the highest purge losses (Table 2). These results suggested that the use of papain to tenderize beef may be advantageous as the reduced loss of moisture may mean a possibility of increased perceived juiciness.

The water holding capacity of muscle can be closely related to muscle pH [6], but in this study purge losses observed for enzyme treatment were not driven by pH because there

were no differences in mean pH between the enzyme treatments (Table 3). Also, there did not appear to be an effect of enzyme injection on the pH of the muscles as the mean pH value for whole muscle before injection was 5.53, while the mean values of the roasts after injection was 5.54 (Table 4).

Table 2 Mean purge loss by treatment (n=18)

Treatment	Mean (%)
Actinidin	2.79[a]
Papain	2.26[c]
Kachri	2.23[c]
Ginger	3.01[ab]
Water	2.74[ab]
Dry control	1.04[d]
S.E.M.[1]	0.146

[a,b,c,d] Means with different superscripts in columns are significantly different at $P < 0.05$ according to least square mean differences. [1] Standard error of the mean.

Table 3 pH values and detected change separated by treatment (n=18 per treatment)

Treatment	Mean pH after injection
Actinidin	5.54
Papain	5.54
Kachri	5.55
Ginger	5.54
Water	5.53
Control	5.57
S.E.M[1]	0.003

[1] Standard error of the mean.

Table 4 pH values for whole muscle (n=36) and overall roasts (n=108).

	Mean	Std. deviation
Whole muscle pH	5.53	0.086
Overall roast pH	5.54	0.032

Overall, the results of this preliminary study indicated that injection of enzymes did not affect the ability of meat to retain brine in the 24 hours immediately following injection. In fact, the loss of brine was on average less than 5 % of the injected green weight of the injected brine remained in the meat, thus ensuring that the enzymes had an opportunity to act. Further work is needed to examine the amount of drip associated with enzyme injection and prolonged storage of *m. biceps femoris* roasts.

IV. CONCLUSION

The injection of enzymes does not actively increase the purge losses of brine during the first 24 hours. To further increase water retention, salt or phosphate may be added to the brines provided these ingredients do not hamper enzymatic activity. Further study will examine protein heat solubility and stability of the samples, as well as shear force, drip and sensory analysis associated with the injection of each enzyme.

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