TENDERISING EFFECT OF BRINE-INJECTED PROTEOLYTIC ENZYMES ON AGED AND NON-AGED BEEF *SEMIMEMBRANOSUS*

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Abstract— Ageing is a cost-consuming procedure for the meat industry. Proteolytic enzymes (papain, actinidin or NS) were injected into aged (8-10 days) and non-aged beef (M. semimembranosus) to investigate whether or not ageing is necessary to achieve optimal eating quality of marinated meat products. The effects of ageing and proteolytic enzymes on eating quality were evaluated by a trained panel using a 15-point unstructured line scale. Furthermore, weight gain and cooking loss were measured. Injections of a marinade containing papain increased the tenderness, crumbliness and liver-like flavour of cooked semimembranosus but reduced the juiciness and meat flavour. Marinating with actinidin and NS had no significant effect on eating quality of marinated semimenbranosus. Furthermore, no effect of ageing before injection was observed on the textural attributes, although ageing was found to decrease juiciness and increase bitter taste, bouillon flavour and metallic flavour.

Index Terms— **ageing, beef, enzymes, injection, tenderness, marination**

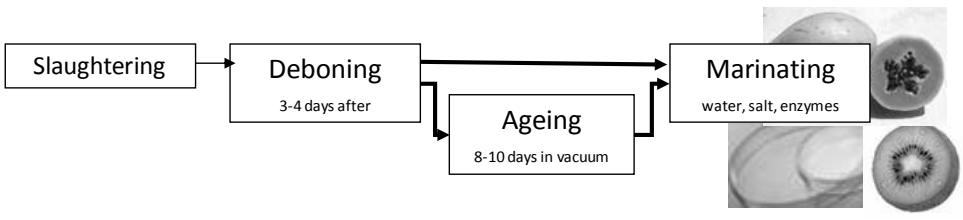
I. INTRODUCTION

The application of proteolytic enzymes from, for example, plants (Fogle et al., 1982; Lewis and Luh, 1988; Christensen et al., 2009; Andreasen et al., 2010) or microorganisms (Foegeding and Larick, 1986; Ashie et al., 2002) has been used to improve meat tenderness. Typical plant-derived proteases used as meat tenderisers include papain from papaya, bromelain from pineapple and ficin from fig. Some plant proteases, such as papain, may lead to over-tenderisation and a "mushy" texture, which may not be the case when marinating with actinidin (Kim and Taub, 1991; Ashie et al., 2002), because of differences in the physicochemical properties of the enzymes. Lewis and Luh (1988) found that a higher activity of actinidin was needed to obtain tenderness equivalent to bovine semitendinosus treated with papain. Furthermore, the actinidin-treated meat did not over-tenderise and had no off-flavours. NS is an aspartic protease from the fungal Aspergillus oryzae. Gerelt et al., 2000 compared the effect of papain and NS and found that NS had a lower and more specific degradation of myofibrils and intramuscular connective tissue. Injection with NS might reduce the risk of over-tenderisation of enzyme-marinated beef products.

The objective of the present work was to investigate the tenderising effects of marinade-injected proteases on beef M. semimembranosus and to investigate whether ageing is necessary if beef is injected directly after deboning.

II. MATERIALS AND METHODS

Twelve dairy cows were slaughtered in accordance with standard slaughtering procedures at a commercial Danish slaughterhouse. Carcasses were selected on the basis of age (48-63 months), carcass weight (267-311 kg) and EUROP conformation (O-P), EUROP fat class (2-3). The carcasses were deboned 3-4 days post mortem. The semimembranosus were removed from the right and left sides of the carcasses and transported to DMRI. The ultimate pH was measured, and the muscles were divided longitudinally into two pieces and the weight of each piece was recorded. Semimembranosus from six randomly selected animals were packed in vacuum bags and aged for 8-10 days at 2ºC before injection with a marinade. Semimembranosus from the six remaining animals were injected directly after deboning without further storage (non-aged).



Injection

Muscle samples were injected with marinades using a multi-needle injector (FGM 48 SC M3, Food Machinery Company (Fomaco), Køge, Denmark) with the following settings: 0.8 bar, 66 strokes/min and 3 bar up-pressure. Four brines were prepared, and the four muscle samples from each animal were randomly injected with one of the four marinades (papain, actinidin, NS or control). The four beef products were injected to obtain a desired weight gain of 8% and to contain the following concentrations of additives: 1) "Control": 0.46% maize starch (C*polar Tex, Danisco, Denmark), 0.3% maltodextrin and 0.6% NaCl; 2) "Papain": 50 ppm papain (extracted from papaya (Carica papaya), Danisco, Denmark), 0.46% maize starch (C*polar Tex, Danisco, Denmark), 0.3% maltodextrin and 0.6% NaCl; 3) "Actinidin": 0.05% actinidin (extracted from kiwi fruit, Actinidia chinensis), Creapharm, Denmark), 0.46% maize starch (C*polar Tex, Danisco, Denmark), 0.3% maltodextrin and 0.6% NaCl, and 4) "NS": 0.033% NS (extracted from Aspergillus oryzae, Novozymes, Denmark), 0.46% maize starch (C*polar Tex, Danisco, Denmark), 0.3% maltodextrin and 0.6% NaCl. After injection, the weight was recorded to calculate the weight gain, and the muscles were vacuum-packed and stored at 5 °C for 6 days.

Weight gain

Weight gain (WG) was measured by weighing the samples before and after injection using the relationship WG = 100(ma-mb)/mb, where *mb* is the weight of the sample before injection and *ma* is the weight of the sample after injection.

Sensory evaluation

The marinated beef products were cut into 2-cm-thick steaks and were equilibrated at room temperature (approximately 22 ºC) to an internal temperature of 10-15 ºC. The steaks were cooked on a pre-heated frying pan (160 ºC) to a core temperature of 75 ºC. The steaks were turned approximately every 2 minutes. Steaks were cut into 2.5 cm wide samples and served on a pre-heated plate to nine trained assessors. A descriptive analysis was performed in four sessions. The samples were evaluated using a 15-cm intensity scale (0 = slight and 15 = intense). The evaluated textural attributes were: hardness at first bite (force required to bite through the sample), crumbliness (amount of meat dust during chewing), tenderness (how easy it is to divide the meat during chewing) and juiciness (amount of juice after five to six times of chewing). The evaluated flavour and basic taste attributes were: meat (intensity of beef flavour), metal (intensity of coin-like metallic flavour), salt (intensity of salty flavour), sweet (intensity of sweet flavour), bitter (intensity of bitter flavour) and sour (intensity of sour flavour).

Cooking loss

Cooking loss (CL) was measured by weighing the samples before and after heat treatments using the relationship CL = 100(mb-ma)/ma, where *mb* is the weight of the sample before the thermal treatment and *ma* is the weight of the sample after the thermal treatment.

Statistical analysis

Data were analysed using a mixed model (SAS 8.2, 1999-2001). The model included "marinade" and "ageing" as fixed effects, and "animal" and "assessors" as random effects. Non-significant interactions were deleted from the model. Least squares (LSmeans) were calculated and separated using probability of difference. Levels of significance: $p > 0.05$ = non-significant (ns), $0.05 > p > 0.01$ = *, $0.01 > p > 0.001$ = **, $p < 0.0001$ = ***.

III. RESULTS AND DISCUSSION

Weight gain and cooking loss

The average weight gain after injection with marinade was 9.4±1.3% for non-aged beef and 10.0 ± 0.3% for aged beef. Cooking of the marinated beef steaks to a core temperature resulted in a cooking loss of 27.5±1.4% for non-aged beef and 29.8±0.3% for aged beef.

Sensory evaluation

Table 1 shows levels of significance for the two treatments and the interaction between them. The ageing process had a significant effect on four sensory attributes: juiciness, metallic flavour, bouillon flavour and bitter taste. No effect was observed on textural attributes related to tenderness of marinated beef samples.

Injection with proteolytic enzymes had a significant effect on all textural attributes and also meat flavour, liver-like flavour and salt taste. No significant effect of enzyme, ageing or the interaction between them was observed for acid taste or sweet taste.

Table 1. Levels of significance. Effect of enzyme injection and ageing 8-10 days (n=6)

	Attributes	Enzyme	Ageing	Enzyme*Ageing
Taste	Salt	0.0262	ns	ns
	Acid	ns	ns	ns
	Bitter	ns	0.0140	ns
	Sweet	ns	ns	ns
Flavour	Meat flavour	0.0010	ns	ns
	Liver	0.0002	ns	ns
	Metallic	ns	0.0068	ns
	Bouillon	ns	0.0437	ns
Texture	Hardness	< 0.0001	ns	ns
	Meat structure	< 0.0001	ns	ns
	Crumbliness	0.0329	ns	ns
	Tenderness	< 0.0001	ns	ns
Juiciness	Juiciness	0.0090	0.0168	ns

Figure 1 shows the effect of ageing. Bouillon flavour, metallic flavour and bitter taste were intensified during ageing compared with non-aged beef. Furthermore, ageing resulted in less juicy steaks.

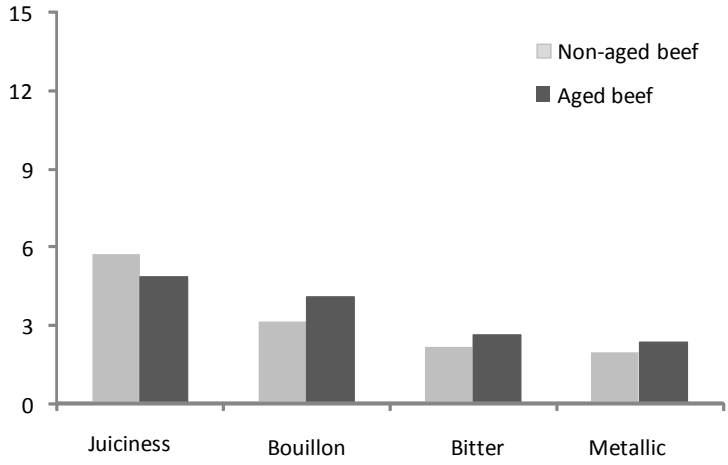


Figure 1. Effect of ageing on the sensory attributes juiciness, bouillon, bitter and metallic. The samples were evaluated using a 15-cm intensity scale (0 = slight and 15 = intense) (n= 6).

Figure 2 shows the effect of proteolytic enzymes. Only injection with papain differs from the other treatments, and therefore no significant effect on texture or flavour attributes was observed when beef was injected with actinidin or NS compared with the control.

Papain treatment of beef had a major effect on textural attributes and resulted in a lower intensity of hardness and meat structure and an increased intensity of tenderness and crumbliness (Figure 2). These textural changes could be explained by the degradation of myofibrillar proteins and an increase in the percentage of soluble collagen (Andreasen et al., 2010).

Furthermore, juiciness, salt taste (1.1 sensory units) and meat flavour (1.3 sensory units) were reduced compared with the control, and liver-like flavour was increased by 1.9 sensory units compared with the control (data not shown).

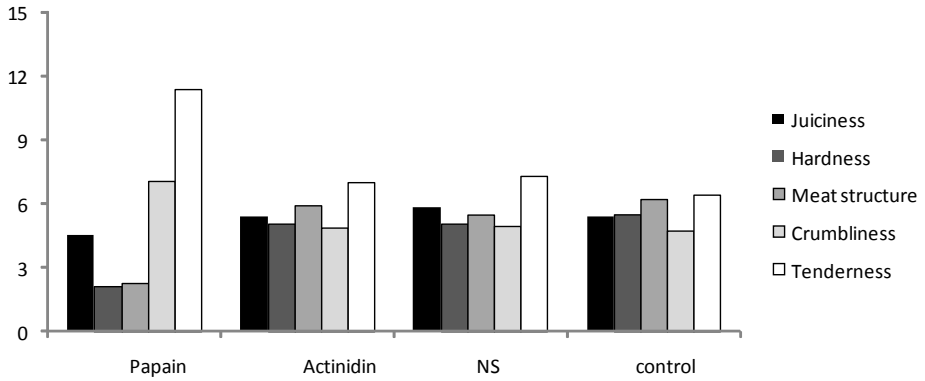


Figure 2. Effect of proteolytic enzymes on the sensory attributes juiciness, hardness, meat structure, crumbliness and tenderness. The samples were evaluated using a 15-cm intensity scale (0 = slight and 15 = intense) (n= 6).

IV. CONCLUSION

Ageing before injection with marinades had no effect on textural attributes. However, it decreased the juiciness of the meat and changed the flavour profile by increasing the bouillon flavour, metallic flavour and bitter taste.

Injection of a marinade containing papain increased the tenderness, crumbliness and liver-like flavour of cooked bovine semimembranosus, whereas it reduced the juiciness, meat flavour and salt taste. No effect of actinidin and NS was observed.

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