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Effect of Different Marinades on the Tenderness of Beef

K.H.R. Wijayawardana, H.W. Cyril[1] and T.S.G Fonseka[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT. Tenderness was found to be the most important factor in determining the eating quality of beef. Based on a preliminary survey, information about tenderization techniques adopted in both hotels and households was collected. Thirteen marinades namely; tamarind, drumstick barks; papaya, pineapple, cannabis, tomato, cumin seed, lime, red wine, goraka, soya oil, curd and vinegar were among these tested and screened for their effectiveness. Six marinades namely; papaya, pineapple, vinegar, curd, red wine and cannabis were selected to investigate the effect on the shear values of beef at different marination conditions. All the beef samples subjected to surface marination at 4°C for 6 h showed a reduction in shear values than the control (reference). Reduction of shear values in curd, vinegar, papaya and cannabis treated meat was outstanding and the respective shear values were 4.3, 4.6, 5.6 and 5.5 kg. As the marination time increased to 14 h, in all the samples tenderness increased. Increase in time reduced the shear values in curd, vinegar and red wine remarkably. Injected marination for 14 h greatly reduced the shear values in all the treatments. Vinegar reduced the shear value by 42%. Others were in order of effectiveness, papaya, pineapple, cannabis, curd and red wine. Papaya developed more tenderness than pineapple at the same concentration. Solubility of myofibrillar protein in red wine and curd were 18.9% and 18.2% respectively. Acidity in the red wine and curd affected the solubilization of M-line and Z-line proteins in the myofibrils in addition to the myosin. Solubility of myofibrillar protein with papaya, and pineapple was not significantly different and they were next to curd and red wine. The cannabis has produced the third most solubility but the substance responsible is unknown. Vinegar has only solublized 9.86% of myofibrillar proteins. Collagen solubility was greater in vinegar treated beef and was 64.9%. This may be due to the presence of acetic acid resulting in low pH leading to acceleration of cathepsin activity. The others were in order of curd, papaya, pineapple, cannabis and red wine. In the same way lactic acid in the curd could affect the acceleration of cathepsin. Solubility of native collagen in papaya and pineapple treated meat were respectively 38.9% and 37.5%. This is presumably due to the action of papain and bromelin. Slight acidity in red wine has resulted 16.6% of solubility of collagen and part of myofibrillar proteins and it was not significantly different with cannabis treated meat at $p<0.05$, but in cannabis treatment the responsible chemical is unknown.

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1 Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

2 Faculty of Nutrition, Livestock and Fisheries, University of Wayamba, Makandura.

INTRODUCTION

Investigations of Kroft and Graf (1959) concluded that the tenderness is probably the single most important factor affecting consumer evaluation of meat quality. The major connective tissue that affects tenderness from the consumer point of view is the intra muscular perimycium and their susceptibility to damage. The quantity of collagen, fiber size and the number of cross-links between collagen molecule all contribute to meat toughness (Bailey and Light, 1989). However, the majority of the explanation for differences in tenderness of young animals is due to myofibril component. Ageing is the process in which meat toughened by rigour mortis is naturally tenderized (Lawrie, 1985). Although ageing improves beef quality, the cost of holding carcass for an extended chill temperature can be very high. According to Lawrie (1985), Buck and his team reported in 1959 that pre-slaughter injection of proteolytic enzymes such as papain, ficin and bromelin leads to develop tenderness in beef up to a satisfactory level. Use of bacterial enzymes to break down the collagen was found to be effective even at cooking temperature of 70-90°C (Wilson and Young, 1991). The use of marinating agents such as oil, organic acids, salts and even sugars have been able to reduce the connective tissue toughness in beef (Oreskovich and Bechtel, 1992).

The majority of beef available in Sri Lanka is produced from Indian breeds, which are of tough nature. Therefore, both tourist hotels and households use a wide range of tenderizers. However, their tenderizing practices have not been reported. Therefore, this work focussed on the investigation of the effectiveness of popular tenderizers used in Sri Lanka. Investigation included a preliminary screening for effective marinades. The six most popular marinades were used to evaluate their tenderizing ability on beef. The tenderness developed during surface and injected marination, and during prolonged marination was determined and was correlated to the solubility of myofibrilla protein and collagen.

MATERIALS AND METHODS

Preparation of meat samples

Longissimum dorsi muscle was used in the study and a cut of beef was taken 5 h after slaughtering. The size of the lean meat cuts used were, 1.5 × 4.5× 7 cm and two such cuts of meat were taken as a sample.

Preparation of marinades

Fifty grams of flesh from, ripened pineapple, half ripened tomato, fully matured papaya, goraka, tamarind, drumstick bark, lime and dill seeds were separately extracted in 200 ml water and filtered. For cannabis 1 g, boiled in 200 ml water and filtered. Vinegar, red wine, curd and soya oil were taken in 80 ml quantities. The pH of the marinades was measured soon after preparation using a pH metre (TOA, HM 20 S).

Surface and injected marination

In surface marination; each meat sample was placed in a plastic box, covered with a particular marinating solution and kept at 4°C for 6 h and 14 h. Injected marination was done by injecting the marinating solutions in to the interior of the cuts of meat at a spacing 1.5 cm using a syringe. Nearly 50 ml of solutions were injected to each of the meat cut. These cuts of meat were also placed in the plastic boxes and kept at 4°C for 14 h.

Measurement of tenderness

Tenderness was measured using the Warner Brazler shear force method (Braztler, 1949). Marinated meat cuts were cut into pieces of 1.5 × 1.5 × 7 cm with the fiber direction parallel to the longitudinal axis. These pieces were then immersed in a water bath at 80°C for 90 min in the sealed polythene bags and cooled for 20 min. After blotting with filter papers samples of meat were cut in to pieces of 1 × 1 × 7 cm as described by Bouton and Howard (1960). Each piece was then placed on the cutting edge of the knife of the Warner Brazler instrument and the tenderness was measured in term of kg pull.

Determination of myofibrilla protein

The fractionation of proteins was done following the method described by Lawrie (1985). The samples of 5 g minced marinated meat samples were put into 250 ml centrifuge tubes separately and 50 ml cold 0.03 M potassium phosphate buffer solution (pH 7.4) was added. Using the homogenizer (type B M 4, C 20) each of the samples in the tube was subjected to homogenization at medium speed for 2 min at temperature 0-4°C. These were then centrifuged for 20 min at 35000×g (at 4°C) using the model C F 15 D 2 Hitachi centrifuge. The supernatant was kept and the residue was re-suspended in another 50 ml of the same potassium phosphate buffer, homogenized and centrifuged as before. The residues remaining was extracted once with 50 ml of cold 1.1 M potassium iodide in 0.1 M potassium phosphate buffer solution (pH 7.40) using the same procedure as for the extraction of sarcoplasmic proteins. The supernatant was filtered through glass wool and the filtrate was used for myofibrilla protein determination by Kjeldhal method.

Determination of total collagen content

Each of 4 g, minced meat samples were digested with 100 ml of 6 N HCl, and reflexed for 16 h. The hot hydrolysate was then filtered through a diatomaceous earth filter. Fifty ml of each of the hydrolysate was then prepared for hydroxyproline determination. The absorption was measured at 558 nm in an optical glass cell in a spectrophotometer model S 104, WPA. Blank determination was also done and absorption measurement of the blank was subtracted from that of the sample and the hydroxyproline concentration of the latter was read from the calibration curve. A calibration curve, was developed using standard solutions. Standard solutions were prepared by diluting 10, 20 and 40 ml of this solution to 100 ml with water to obtain hydroxyproline concentrations of 1, 2 and 4 μg/ml respectively.

Determination of soluble collagen

Minced meat sample weighing about 4 g was placed in to 50 ml centrifuge tube and 12 ml of 1/4 strength Ringer's solution was added. The tubes were heated in a water bath at 77°C for 70 min and centrifuged (Hitachi model, CF 15 D 2) at 3000 g for 30 min. The supernatant were decanted and the residues were re-suspended in 8 ml of ¼ strength Ringer's solution at room temperature and centrifuged as above. The soluble collagen fraction added to the digestion flasks and hydrolysis and the hydroxyproline content was analysed. Finally soluble collagen contents were determined with respect to each treatment.

Statistical analysis

All experiments were conducted under the Complete Randomized Design with three replicates. Mean comparison was performed using the DMRT.

RESULTS AND DISCUSSION

Shear values of 6 h marinated beef, indicated that marination developed the tenderness in beef (Table 1). Out of the 13 marinades tested in the study, only eight marinades showed significant reduction in shear values of the meat, namely; tamarind, soya oil, drumstick bark, curd, papaya, pineapple, cannabis and vinegar. The highest tenderness resulted with curd and vinegar and their effects were the same. Next in order, tamarind, soya oil, drumstick bark, pineapple, papaya and cannabis have given lower shear

Table 1. Shear values of screen test under the surface marination.

Tenderizers	pH	Average shear value (kg)
Curd	3.5	3.10^{a}
Vinegar	3	4.30^{b}
Papaya	5.5	4.60^{b}
Tamarind	3.1	5.06^{b}
Cannabis/Ganja	7.5	5.10^{b}
Soya Oil	7	5.16^{b}
Pineapple	3.5	5.30^{cb}
Drumsticks	4.0	5.60^{c}
Cumin seed	5.1	6.66^{cd}
Red wine	4.5	7.00^{d}
Tomato	3.1	7.00^{d}
Lime	2.5	7.30^{d}
Goraka	1.9	9.06
Control	5.1	9.09

Means with the same superscripts are not significantly different at the 5% probability level.

values. Lime, tomato, red wine and cumin seed were found to be the marinades to reduce the shear values to lesser extent in comparison to the other marinades. Control had the average shear value of about 9.09 kg. The meat marinated with goraka extract had a value closer to that of control (9.06 kg).

Curd gave a closer shear value of 3.1 kg, proving it's highest tenderization effect. This would be due to the presence of lactic acid. Further data in Table 2, indicates that curd has the potential of increasing myofibrilla protein solubilization by 18.2% through marination. Saunders (1993) has shown that beef myofibrilla protein degradation was apparent below pH 4.5.

Table 2. Effect of marinades on solubility of myofibrilla proteins and collagens.

Marinade	Mean solubility of myofibrilla protein g/g of meat	Percent solubility of myofibrilla protein	Mean soluble collagen content µg/g	Percent solubility of collagen
Control	0.07	7.18^{f}	0.041	11.3
Vinegar	0.1	9.86	0.235	64.9^{a}
Cannabis	0.12	12.10^{d}	0.064	16.9^{d}
Pineapple	0.17	16.80^{b}	0.166	37.5^{c}
Papaya	0.18	17.54^{bc}	0.141	38.9^{c}
Curd	0.18	18.20^{c}	0.159	43.9^{b}
Red wine	0.19	18.90^{c}	0.045	16.6^{d}

Means with same superscripts in the same column are not significantly different at 5% probability.

Shear values of the vinegar treated beef were found to be second lowest values, 4.3 kg (Table 1). The greatest solubility of collagen has been resulted in the vinegar treated beef (64%). But the effect on myofibrilla protein solubility (Table 2) in the vinegar treated beef was lower (9.86 kg). In correlating the results of the shear values (Table 1), collagen solubility and myofibrilla protein solubility (Table 2), it would be appropriate to believe that, collagen solubility in the vinegar marinated beef would be the most important factor in improving the tenderness of beef. Rapid decline in pH of meat by acidic treatment has lead to speedup the activation of muscle Cathepsin and enhance collagen degradation (Stanston and Light, 1989). Saunders (1993) has reported that myofibrilla protein degradation in beef was apparent below pH 4.5, upon the acid proteinase enzymes. Meat marinated with papaya extractions showed third highest tenderness. As indicated in Table 2, 39% of the collagen was solubilized. The myofibrilla protein solubility (Table 2) of this sample was also found to be considerably high (17.54%). Therefore, both collagen solubility and myofibrilla protein solubility could have imparted desirable tenderness in papaya treated meat. Shear values, collagen solubility and myofibrilla protein solubility of papaya and pineapple was similar, indicating that action of both papaya and pineapple on meat tenderness is seemed to be same. Kang and Rice (1970) have shown that thiol proteases in the papain and bromelin cleave the myofibrilla protein and papain specially is more active on these proteins than on collagen. Tamarind had a pH value of 3.1 which was more close to the pH of vinegar. Curd was also able to reduce the shear value to about 5.06

kg. As in the other acidic marinades, drop in the ultimate pH of the meat could have caused to activate the enzymes on collagen and myofibrilla proteins. Comparatively low tenderness developed in the red wine treated meat could be attributed to its moderately high pH (Table 1). Protein analysis of the cannabis treated meat in Table 2 provides an evidence for the development of tenderness by slight solubility of collagen and myofibrilla protein after marination. Therefore it could be believed to contain natural enzymes or any other effective substance in cannabis, which may act on proteins, and it is non-investigated.

Marinating with soya oil resulted in a shear value of 5.1 and tenderness was not significantly different with the values obtained for pineapple, drumsticks, tamarind and cannabis. The popular tenderizing agent used by many Muslims for the meat preparation, cumin seed, has reduced the shear value to 6.6, which is little lower than the value for meat marinated with red wine. Tomato and drumsticks bark extractions had pH value of about 4.5 and their shear values were 7.0 and 5.6 kg respectively. The shear values of meat marinated with lemon and goraka obtained were little higher than others and 7.3 and 9.6 kg respectively. These findings imply that lowering the pH would not increase the tenderness proportionately. The most probable reason would be that at extremely low pH might have denatured the natural enzymes.

Table 2 further indicated that solubility of collagen in samples of meat marinated with vinegar, curd, papaya and pineapple is considerably higher than red wine and cannabis treatments. However, red wine found to be the marinade, which yielded highest myofibrilla protein solubility among all other marinades. The effect of myofibrilla protein solubility of the curd was also not significantly different to red wine. Of the marinades, papaya and pineapple were found to have satisfactory level of solubility of both collagen and myofibrilla proteins to give desired tenderness.

When marination at 4°C, all the meat samples have shown low shear values than the control. A part from the marination effect, low temperature treatment also might have contributed to develop tenderness in all the marinated meat samples.

Levie (1977) reported that relaxation of actomyosin complexes and partial degradation of collagens take place at low temperature storage. Increase in the marinating time from 6-14 h resulted in remarkable decrease in the shear values of the meat samples treated with curd and vinegar. The probable reason could be that the conditioning with acids might have increased the solubility of collagen in meat. Table 3 indicated that 14 h marination with pineapple, papaya and cannabis had not resulted significantly different shear values and showed a slight decrease when the duration of marination was increased.

This proves that acidic marinades give best result on prolonged marination than with plant enzymes. Red wine treated meat also shown to have comparatively high shear values at 6 h marination and showed 20% decrease over the 14 h marination (Table 3). Injected marination has a remarkable effect on improving the tenderness than the surface beef samples marination (Table 4). Regardless of the marinades, shear values of all the treatments subjected to injected marination appeared to have lower shear values than the respective shear values of surface marination. Vinegar, papaya and pineapple injected meat has shown remarkably higher percent reduction in shear values and they are, 42, 35 and 20% respectively. The reason could be that the uniform and deeper penetration of the marinades into the tissue and physical injury upon injection of the liquid extracts might have reduced the shear values. After injection, the shear values of the papaya, curd,

pineapple and cannabis was found to be not significantly different. The water injected control also resulted in 10% reductions in shear value. This may be due to enhance water retention and physical injury upon injection.

Table 3. Shear values of surface marinated beef.

Marinades	Mean shear values in kg		Percent decrease in the shear values
	6 h marination	14 h marination	
Curd	4.30[a]	3.09[a]	28
Vinegar	4.60[a]	3.50[a]	24
Ganja/ Cannabis	5.50[b]	5.02[b]	9
Papaya	5.60[b]	5.01[b]	11.0
Pineapple	6.00[b]	5.34[b]	11
Red wine	9.20[c]	7.4[c]	20
Control	10.00[d]	9.61[d]	4

Means with same superscripts in the same column are not significantly different at 5% probability level.

Table 4. Effect of surface and injected marination on tenderness of beef.

Marinades	Shear value averages (kg)		
	14 h surface marination	14 h injected marination	Percent decrease
Vinegar	4.10[a]	2.37[a]	42
Papaya	6.81[c]	4.42[b]	35
Curd	5.90[b]	4.90[b]	17
Pineapple	6.81[c]	5.35[b]	20
Cannabis	6.82[c]	5.47[cb]	19
Red wine	7.40[d]	6.62[c]	11
Control	10.61	9.58[d]	10

Means with same superscripts are not significantly different at 5% probability level.

CONCLUSIONS

Vinegar, curd, papaya, pineapple and cannabis found to be effective tenderizers on beef on surface marination at 4°C for 6 h. Injected marination tenders the beef much

effectively than surface marination. Injected marination with vinegar, papaya, pineapple and cannabis reduced the percent shear values by 42, 35, 20 and 19% respectively. However, injected marination with curd was not effective. Therefore, depending on the toughness of the cut, the marination time could be changed from 6-14 h Acidic marinades such as vinegar, curd and red wine gave best results on 14 h marination, than enzymic marinades. The desired level should be 75 ml of 2.5% commercial vinegar per kg beef for 14 h marination. Addition of about 100 ml of fresh curd per kg of beef and marination at 4°C for 14 h would give optimum tenderness in beef. Long term marination with cannabis before cooking has no significant effect on tenderness as it slightly degrades native collagen with its unknown substances. Addition of 200 ml red wine to 1 kg of beef and marination for 14 h would result satisfactory tenderness along with attractive colour. Using 100 ml of papaya milk, prepared by blending 80 g of fully matured peeled papaya fruits in 200 ml water, could tenderize 1 kg of beef satisfactorily under 14 h marination. Action of pineapple was very similar to the action of papaya and primarily affects on collagen at the concentration of 80 g in 200 ml extraction. However, at the same concentration papaya is more effective than pineapple in relevance to the development of tenderness.

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