Journal of Agriculture and Agricultural Technology 8(1): 45-51, 2017

EFFECT OF COOKING TIME AND PAPAIN ON THE QUALITY OF BEEF

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ABSTRACT
The effect cooking time of level of papain enzyme and on the physico-chemical properties of beef was investigated. Beef cut from the shank of an aged cow was infused with varying quantities of 5% papain solution (15ml) and cooked for 30 and 60 minutes at 75^{0}C. Untreated beef sample served as the control. The effect of the treatments were determined by measuring the cooking yield, cooking loss, Water Holding Capacity (WHC), pH, soluble proteins, amino acid, minerals and sensory properties. The results show that increasing the level of papain enzyme significantly ($p<0.05$) reduced the WHC and cooking yield but significantly ($p<0.05$) increased the cooking loss, soluble protein, amino acid and mineral content of the beef samples. The samples cooked for 60 minutes and 15ml of 5% papain infusion was the most preferred by the sensory panelists.

Keywords: Beef meat, shank, aged, infused, physico-chemical, sensory

INTRODUCTION
Palatability of meat is affected by many factors principal among which is tenderness. Maiti *et al.* (2008) stated that meat tenderness is one of the most important attributes of meat eating quality. Meat tenderness is affected by pre and post slaughter factors. Pre-slaughter factors include breed, age, feeding, management and genetics while post slaughter factors include shortening, glycolysis, processing and cooking methods. The traditional animal husbandry system which is characterized by moving the animals from one place to another is widely practiced in developing countries such as Nigeria. Jiya (2001) reported that the physical activity of continuously moving from one place to another in search of pasture is a major cause of meat toughness. Consequently, meat from such animals will require a longer cooking or processing time before it can be tender or palatable for consumption. This is a major economic concern for meat processors.

Different methods are adopted, antemortem and postmortem, to improve meat tenderness. Antemortem methods of improving tenderness include oral supplementation of vitamin (Foote *et al*., 2004) and feeding of electrolytes (Maiti *et al.,* 2008) while postmortem methods include, aging, marination, electrical stimulation, cooking and treatment with enzymes such as papain (from papaya fruit), bromelain (from pineapple) and ficin (from fig tree) (Ionescu and Pascaru, 2008 and Istrati *et al*., 2012). Enzymes tenderizers usually break down peptide bonds between amino acids in collagen, producing a much tender cut of meat for cooking. The use of papain as a proteolytic enzyme and the mechanism by which it adds to the tenderness is well established: Pawar *et al*. (2003) reported that papain acts on the connective tissue of meat by breaking up the muco-polysaccharide of the substance matrix and progressively decreasing the connective tissue fibres to an amorphous mass; Ashie *et al*. (2002) reported that papain can degrade myofibrillar and collagen proteins yielding various sizes of protein fragments; Istrati *et al*. (2012) reported that beef muscle treated with papain had a softer texture and a lower resistance to applied pressure than untreated muscle; and Naveena and Mendiratta (2001) stated that the various ways by which meat is treated to improve tenderness have negative effects on the overall quality of the meat. However, information on the effect of papain infusion on the physico-chemical properties of muscle from the shank of spent cow is limited. In this paper, the effect of different quantities of papain infused into the shank of spent cow and cooking time on physical and chemical properties was studied.

MATERIALS AND METHODS
The experiment was carried out at Animal Production Department Laboratory, School of Agriculture and Agricultural Technology, Federal University of Technology, Gidan Kwano, Minna, Nigeria. The beef used for this study was obtained from Minna abattoir while the commercial papain powder (Starwest Botanicals: #210127-51) was ordered from India.

Samples preparation: The experimental beef was obtained from the shank of an aged cow of about ten years old (identified by dental set) that had finished gestation. The beef was cut into rectangular pieces of 5cm long, 3cm wide and 2cm thick. They were divided into 4 groups and infused with 5, 10 and 15mls of 5% papain solution and held at refrigerated temperature of 4^0C (model HRI 70T) for 24 hours in order for the enzyme to get into the muscle tissue. Untreated sample served as the control. The infused meats were cooked for 30 and 60 minutes at 75^0C in a water bath (model HH 60).

Analysis of physical properties: The Water Holding Capacity (WHC), Cooking Loss (CL) and Cooking Yield (CY) of the meat samples were determined using the procedures reported by Kauffman *et al.* (1992). For WHC, 10g (w_1) of the sample was wrapped in polyethylene and placed between a screw jack and pressed until all the free water was expelled. The meat sample was then removed, unwrapped and re-weighed (w_2). WHC was calculated using the relationship: (w_1-w_2/w_2) X 100. CL was calculated as the difference in weights before (w_1) and after (w_2) cooking while the cooking yield was calculated as a ratio of the weight of the cooked sample and the weight of the uncooked sample multiplied by 100.

Determination of chemical properties: Total protein (crude) and soluble protein of the meat samples were determined as described by AOAC (2000). The amino acid of meat samples were determined using the method reported by Bidlingmeyer *et al.* (1984). Mineral compositions of beef samples were determined using the method of AOAC (2000). The pH of the meat was measured based on the procedure that was outlined by AOAC (2000). 10 g of the cut samples were homogenised for 2 minutes with 90mL of distilled water using a laboratory blender (Nakal, Japan: Model 242). The meat suspension was filtered and the pH of the filtrate was measured with a digital pH meter.

Sensory evaluation of enzyme processed meat: Sensory evaluations of the meat samples were carried out using the method outlined by Ihekoronye and Ngoddy (1985). The meat samples were cut into small, bite sizes of 5g and juiciness, flavour and overall acceptability were scored using a 9 point hedonic scale (where 1 = dislike extremely and 9 = like extremely). The order of presentation of the samples to the panelist was randomized. Cold water was also served to the panelist to gargle and rinse their mouth after each evaluation.

Data analysis: The data collected from this study were subjected to a 2X4 factor analysis of variance (ANOVA) in a completely randomized design using Statistical Analysis System (1998). The variations in means were separated using Student-Newman-Keuls (SNK) test at 5% level of probability

RESULTS AND DISCUSSION

The effect of cooking time and papain addition on the physical properties of beef shank is shown in Table 1. Cooking for 60 minutes resulted in significantly ($p<0.05$) higher water holding capacity, cooking yield and pH and lower cooking loss. Conversely, lower cooking time (30 minutes) resulted in significantly ($p<0.05$) higher cooking loss. On the other hand, increasing the enzyme level significantly ($p<0.05$) decreased the water holding capacity and cooking yield but increased the cooking loss significantly ($p<0.05$). The meat sample treated with 5ml papain enzyme had the least cooking yield of 44% while the sample treated with 15ml had the highest cooking loss of 52.31% and the lowest pH of 5.33. The WHC of foods is the ability to hold its own and added water during the application of external force or heat. The higher WHC and CL observed in the sample cooked for 60 minutes may be due to the higher pH observed in the samples cooked for 60 minutes. Huff-Lonergan and Lonergan (2005) reported that as meat pH approaches the isoelectric point, there is increased attraction between the proteins causing a reduction in the ability of the proteins to hold water. In this study, there is a strong correlation between pH and WHC. For both cooking time and level of enzyme inclusion, samples with pH closer to the isoelectric point of protein have lower WHC. The changes in pH may be due to the type of amino acid (acidic or basic) released as a result of the protein degradation. WHC also affects CL. According to Jama *et al*. (2008), CL decreases with improving WHC. Honikiel (2004) also reported that CL is lower at higher pH. The trend observed in this study is in agreement with the report of these workers as samples with higher WHC recorded lower cooking losses.

Table 2 shows that crude protein was higher at 60 minutes cooking time while soluble proteins were higher at 30 minutes cooking time. As for effect of enzyme, soluble protein increased with increasing papain level. The lower soluble protein recorded at 60 minutes cooking time may be due to denaturation (Murphy and Marks, 2000) as heat is applied for a longer time compared to cooking for 30 minutes. The increase in soluble protein with increasing papain level may be due to the action of papain on myofibrils and collagen yielding more soluble proteins. Ashe *et al*. (2002) reported that papain degrades myofibrillar and collagen proteins into smaller fragments leading to proteins of different sizes with higher solubility. This result is in agreement with (Ionescu and Pascaru, 2008) who reported increasing levels of protein solubility as proteolytic enzymes concentrations increased.

The amino acid and mineral content of the samples are shown in Tables 3 and 4. Cooking for a longer period and increased level of enzyme treatment both resulted in higher levels of minerals and accumulation of free amino acids. Hence, cooking for 60 minutes yielded more minerals and free amino acids than cooking for 30 minutes while the samples treated with the enzymes had higher amino acids than the untreated sample. Furthermore, the sample treated with 15mL papain solution recorded the highest accumulation of free fatty acids and mineral followed by 10mL and 5mL. The level of free amino acid accumulation is a reflection of the level of protein degradation by the enzyme as reported by Ionescu and Pascaru (2008). As the enzyme levels increase, the level of free amino acids also increase. Hence, it is necessary to exercise caution in order to avoid over tenderization.

The sensory scores of the meat samples are shown in Table 5. The results show that cooking time and enzyme level had no significant effect on the appearance of the meat samples. However, the sample cooked for 60 minutes was rated higher than the one cooked for 30 minutes on the basis of tenderness, juiciness, flavour and overall acceptability. Similarly, increasing level of enzyme resulted in higher sensory scores for tenderness, juiciness, flavour and overall acceptability. This may be due to more intense degradation and the release of higher levels of flavour and aroma compounds as cooking time and enzyme level increase causing the meat to be more tender, juicier and more acceptable.

CONCLUSION

Beef cut from the shank of an aged cow cooked for 60 minutes exhibited better quality as evidenced in higher pH, better water holding capacity and cooking yield, more free amino acid and mineral and higher sensory scores compared with cooking for 30 minutes. Furthermore, use of papain enzyme enhanced the quality of beef. Increasing levels of papain lower the pH of beef resulting in lower water holding capacity and increased cooking loss. However, increasing the papain level resulted in higher protein solubility, free amino acids and minerals.

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Table 1: Effect of cooking time and papain infusion on the quality characteristics of beef meat

Treatment	WHC (g/g)	CY (%)	CL (%0	pH
		Cooking time (mins)		
30	0.26[b]	47.55[b]	52.39[a]	5.42[b]
60	0.34[a]	51.27[a]	48.08[b]	5.53[a]
SE±	0.002	0.31	0.13	0.01
		Enzyme (mls)		
0	0.34[a]	56.59[a]	43.25[c]	5.52[b]
5	0.32[b]	53.09[d]	46.12[b]	5.48[c]
10	0.29[c]	49.42[b]	49.67[b]	5.44[c]
15	0.27[d]	47.52[c]	52.31[a]	5.41[a]
SE±	0.003	0.44	0.19	0.01
TxE	p<0.004	p<001	p<001	p<001

[abcd] Means with different superscripts in the same column are significantly ($p<0.05$) different.
WHC: Water Holding Capacity, CY: Cooking Yield, CL: Cooking Loss

Table 2: The effect of cooking time, papain enzyme inclusion levels and their interaction on proximate and soluble protein of beef from shank of aged cow

Treatment	CP (%)	SP (% total protein)
Cooking time (mins)		
30	60.79[b]	0.64[a]
60	65.57[a]	0.58[b]
SE±	0.22	0.004
Enzyme (mls)		
0	53.64[d]	0.58[c]
5	63.81[c]	0.59[c]
10	65.85[b]	0.62[b]
15	69.43[a]	0.64[a]
SE±	0.31	0.01
TxE	p<001	P<001

[abcd]Means with different superscripts in the same column are significantly ($p<0.05$) different.
CP: Crude Protein, SP: Soluble Protein.

Table 3: Effect of cooking time and papain enzyme inclusion of amino acid content on beef from shank of aged cow (g/100g)

Parameter	Cooking time (mins)			Enzyme (mls)					
	30	60	SE±	0	5	10	15	SE±	TxE
Lys	4.82^{b}	4.91^{a}	0.01	5.06^{b}	3.96^{d}	4.51^{c}	5.97^{a}	0.01	p<.0001
Hist	3.29^{a}	2.98^{b}	0.01	1.97^{d}	3.63^{b}	4.38^{a}	4.57^{c}	0.01	p<.0001
Arg	7.11^{b}	7.37^{a}	0.01	6.67^{c}	8.13^{a}	7.68^{b}	8.48^{d}	0.01	p<.0001
Aspar	8.02^{b}	8.28^{a}	0.01	7.25^{d}	7.95^{c}	9.08^{a}	9.29^{b}	0.01	p<.0001
Threo	2.80^{b}	4.08^{a}	0.01	1.99^{d}	5.58^{a}	4.08^{b}	4.11^{c}	0.01	p<.0001
Ser	3.79^{b}	6.53^{a}	0.01	3.34^{d}	13.19^{a}	4.11^{b}	4.79^{c}	0.01	p<.0001
Glut	9.92^{b}	13.70^{a}	0.06	12.62^{b}	13.19^{a}	12.17^{c}	13.27^{d}	0.06	p<.0001
Prol	5.16^{b}	6.53^{a}	0	3.79^{d}	5.00^{b}	10.06^{a}	10.53^{c}	0.01	p<.0001
Glyc	5.58^{b}	5.89^{a}	0.03	4.48^{d}	6.07^{b}	7.37^{a}	7.02^{c}	0.04	p<.0001
Alan	5.62^{b}	6.22^{a}	0.03	5.52^{c}	6.38^{b}	7.99^{a}	8.79^{d}	0.04	p<.0001
Cyst	4.99^{a}	3.19^{b}	0.01	1.15^{d}	4.37^{b}	8.18^{a}	8.68^{c}	0.01	p<.0001
Val	4.98^{b}	6.38^{a}	0.01	3.77^{d}	6.51^{b}	7.33^{a}	5.09^{c}	0.01	p<.0001
Meth	3.43^{b}	4.19^{a}	0.01	2.86^{d}	4.29^{b}	4.58^{a}	4.52^{c}	0.01	p<.0001
Iso	4.21^{a}	3.87^{b}	0.01	3.23^{d}	3.69^{b}	5.93^{a}	6.32^{c}	0.01	p<.0001
leu	5.97^{b}	6.57^{a}	0.01	6.35^{b}	6.15^{c}	7.81^{a}	8.78^{d}	0.01	p<.0001
Tyr	3.91^{a}	3.87^{b}	0.01	2.34^{d}	3.28^{c}	5.88^{a}	5.07^{b}	0.01	p<.0001
Phen	3.71^{b}	4.50^{a}	0.01	3.54^{d}	4.53^{a}	4.29^{b}	5.07^{c}	0.01	p<.0001

^{abcd}Means with different superscripts in the same column are significantly ($p<0.05$) different. SE: Standard error, T =Time of cooking, E = Enzyme, Lys= Lysine Hyst= HystidineArg= ArginineAspar= Aspartic acid Threo= ThreonineGluto= Glutamic acid Prol= ProlineGlyc= Glycine Val = Valine Alan= Alanine Meth= MethionineIsoleu= IsoleusineLeus= Leusine Tyr= Tyrosine Phenyl= Phenylalanine

Table 4: Effect of cooking time and papain enzyme level on Mineral content of beef (g/100g)

Parameter	**Na**	**K**	**P**	**Fe**	**Ca**
Cooking time (mins)					
30	30.90^{b}	43.50^{b}	209.23^{b}	1.99^{b}	3.03^{b}
60	36.27^{a}	47.06^{a}	238.08^{a}	2.12^{a}	3.22^{a}
SE±	0.01	0.01	0.01	0.01	0.01
Enzyme (mls)					
0	30.74^{d}	36.94^{c}	241.69^{a}	1.81^{d}	2.68^{d}
5	31.03^{c}	35.32^{d}	211.41^{d}	2.09^{b}	3.20^{b}
10	31.65^{b}	44.19^{b}	217.79^{c}	1.97^{c}	3.16^{c}
15	40.92^{a}	64.68^{a}	223.72^{b}	2.38^{a}	3.46^{a}
SE±	0.01	0.01	0.01	0.01	0.01
TxE	p<.0001	p<.0001	p<.0001	p<.0001	p<.0001

^{abcd}Means with different superscripts in the same column are significantly ($p<0.05$) different, Na: Sodium, K: Potassium, P: Phosphorus, Fe: Iron, Ca: Calcium

Table 5: Effect of cooking time and papain enzyme level on sensory parameters of beef

Treatment	Appearance	Tenderness	Juiciness	Flavour	O/Acceptability
Cooking time (min)					
30	4.36^{a}	4.34^{b}	5.23^{b}	5.36^{b}	4.59^{b}
60	4.59^{a}	6.01^{a}	5.86^{a}	5.96^{a}	5.95^{a}
SE±	0.12	0.10	0.17	0.17	0.10
Enzyme (mls)					
0	4.35^{a}	2.53^{d}	4.85^{b}	5.00^{b}	2.78^{d}
5	4.43^{a}	4.78^{c}	5.28^{b}	5.48^{ab}	5.08^{c}
10	4.58^{a}	6.13^{b}	5.98^{a}	5.98^{ab}	6.20^{b}
15	4.55^{a}	7.28^{a}	6.08^{a}	6.20^{a}	7.03^{a}
SE±	0.17	0.15	0.24	0.24	0.15
TxE	$p<0.714$	$p<0.002$	$p<0.132$	$p<0.179$	$p<0.050$

^{abcd}Means with different superscripts in the same column are significantly ($p<0.05$) different. O: Overall