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Effects of papain enzyme treatment on the tenderness of beef from the chuck cut of spent cow

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Abstract

An experiment was conducted to investigate the effects of papain enzyme concentration and cooking time on beef tenderness from the chuck meat of spent cow. The papain enzyme was obtained from precipitate that settled out from a two-carbon organic co-solvent system which comprised 77% equal volume of ethanol and acetone and 23% pawpaw fruit liquid extract (crude protein solution). Uniformed-sized of beef from the chuck portion of spent cow was marinated with different concentrations of papain extract (0 ml, 10 ml, 15 ml), aged in the freezer and cooked at 75°C for 30 and 60 minutes in a water bath. Data was collected on physico-chemical properties of the meat as well as the sensory evaluation. Cooking time and levels of inclusion of papain enzyme significantly ($P<0.05$) affected the meat quality characteristics measured. There were interactions between cooking time and levels of enzyme inclusion in the results of water holding capacity and pH. The amino acid content, mineral and the sensory evaluation of the chuck meat were all significantly ($P<0.05$) affected by the cooking time, levels of inclusion as well as interactions between cooking time and enzyme inclusion levels. The result showed that the papain extract derived from the two-carbon organic co-solvent extraction of pawpaw fruit could be used as an effective meat tenderizer within a short cooking time. It was concluded that papain enzyme treated beef from the chuck of spent cow can be cooked for 30 minutes with up to 15mls level of inclusion for better meat tenderization.

Keywords: Papain enzyme, cooking time, chuck beef, Spent cow quality
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Introduction

The traditional animal husbandry system is characterized by moving the animals from one place to another, as such a major cause of meat toughness (Jiya, 2001). Due to this toughness, meat would need long cooking or processing time before it can be tender or palatable for consumption. Furthermore, the time it consume (or the time used) to tenderize a piece of tough meat is of high economic concern to the processor. Children, people with tooth problems and the aged find eating such meat a very big challenge, if not softened or tenderized. It is therefore necessary to identify simple and economical means by which tough meat can be tenderized. Tenderness been a vital factor in the consumer's perception of meat quality, In order to improve beef tenderness, different physical and enzymatic methods have been adopted (Savell and Shackelford, 1992; Tyszkiewicz *et* al., 1997). Tenderness is affected by breed, age, feeding, suspension of carcass during slaughter, electrical stimulation, chilling rate and aging, mechanical marination, freezing, thawing and cooking (Crouse *et* al., 1991).Tough cuts of papain as a proteolytic enzyme and the mechanism by which it add to the tenderness is well established. Chuck beef consists of the neck, shoulder and upper arm. Beef chuck yields tough but very flavourful cut of meat with a good deal of connective tissue, it is considered best when cooked slowly in a liquid to tenderize it (Lori Alden, 2005). Papain comes from *carica papaya* (pawpaw) and bromelain comes from pineapple. There limited study on the extract of pawpaw on

meat tenderisation, nor is there any effect of cooking time on chuck of aged meat from cow. Thus, this study focused on papain enzyme which is extracted from papaya (pawpaw) is focused on its effects on the tenderness of beef from the chuck cut of spent cow

MATERIALS AND METHODS

Location of the experiment

The experiment was carried out at the Animal Production Department Laboratory, School of Agriculture and Agricultural Technology, Federal University of Technology Minna, Niger State. Minna lies between Latitude 9^0 30 North and Longitude 6^0 33 East of the equator. Its annual rainfall is between 1200 mm and 1300mm. Minna experiences a mean temperature of between 38^0C and 47^0C, with lowest in the month of August and highest in the month of March. The characteristic vegetation is mainly of Guinea savanna (Post Graduate School Prospectus, FUT Minna, 2012).

Source of experimental materials

The beef used for this study was obtained from Minna Abattoir located at Tayi village. The enzyme was obtained from unripe pawpaw fruit sourced from Department of Crop Production Research Farm, Federal University of Technology Minna.

Enzyme extraction

Papain enzyme was extracted from pawpaw using the method described by Rex and Daumantas (1997) with slight modifications. Unripe papaya fruits, weighing 3900g were harvested early in the morning. They were washed with potable water to remove all adhering extraneous matter. They were then cut into small sizes and pounded manually by means of mortar and pestle. The pounded papaya fruit was soaked in cold distilled water for one hour after which it was filtered using a muslin cloth. The residue was discarded and 500 ml of the filtrate was added to 1 litre of an equal mixture of acetone and ethanol (acetone 1:1 ethanol) and allowed to stand for another one hour, during which a precipitate evolved. The precipitate was decanted and washed with cold distilled water. This was repeated until the enzyme was observed at the base of the beaker in the form of smaller, white crystals. The enzyme yield (total amount of enzyme extracted from pawpaw fruit) was calculated as: -

Enzyme yield (%) = weight of extracted crude enzyme/weight of fruit used

Experimental design and application of enzyme and cooking of beef

A 2x4 factorial completely randomized design (CRD) was used. The experimental meat was obtained from an aged cow of about ten (10) years old that had finished its reproductive cycle. The age of the animal was identified using dentition method. The beef sample was taken from the chuck and cut into pieces of 5cm length and 3cm width. The pieces of meat were divided into eight (8) groups. They were treated with 0 (control), 5, 10 and 15 millilitres of papain enzyme concentration respectively. The infused meats were cooked for 30 and 60 minutes at 75^0C (Ionescu *et al.*, 2008). Thus there were 4 levels of papain enzyme concentration and 2 levels of cooking time. The infused meat was cooked for 30 and 60 meat or meat from aged animal can also be made soft by forcing little but very sharp blades in the cut of meat towards impeding or reduced protein level. Likewise, soaked meat that contain external proteolytic enzymes like papain, bromelain or ficin can be tenderize using injection to supplement the enzymes within the meat (Michael 2007). The use of minutes at 75^0 C in a water bath after the addition of distilled water to the beaker that was used for cooking.

Data Collection

The water holding capacity of the meat sample was determined using the procedures reported by Kauffman *et al.* (1992). A portion of the meat from the chuck were cut, weighed and kept in a container. Water holding capacity was determined by cutting a section of meat weighing 10g. The sample was pressed using a screw jack until all the free water was expelled. The meat sample was then removed and re-weighed. The difference in weight of meat sample represents the weight of removed fluid. This was calculated and expressed as a

percentage of the initial sample weight and recorded as water holding capacity of the meat.

The cooking yield and cooking loss were determined using the procedures of Kauffman *et al.* (1992). A piece of the chuck was selected for broiling. Broiling was done on a hot plate (Thermostatically control). The racks were covered with perforated aluminium foils for ease of drainage; the hot plate was preheated for five minutes before loading in the samples, which was broiled to a temperature of 72^{0}C as measured with a skewer meat thermometer. The sample was allowed to cool to room temperature. Excess fluid was cleaned up with paper serviette and their weights were taken and recorded. The difference between the pre-cooked weight and post cooked weight was the cooking loss. While the cooking yield was calculated as cooked weight/thawed weight x100.

The pH of the meat was measured based on the procedure outlined by AOAC (1990). 10g of the cut samples were mixed with 90ml of distilled water for 2 minutes using a blender; the meat suspension was filtered and the meat's pH was measured with a digital pH meter.

Proximate composition (Moisture, Ether extract, Crude protein and Soluble Protein) of the samples were determined using the method of AOAC (1990). The amino acid profile of meat samples were determined reported by Spackman *et al.* (1958) by means of Technicon Sequential Multi sample amino acid analyzer, (TSM).

Mineral composition of beef samples were determined using atomic absorption spectrophotometer and Perkin, Elma 500 instrument, after wet ashing mixed with nitric, perchloric and sulphuric acid, while phosphorus was determined by the phospsho-vanamolybolate method of AOAC (1990). Sodium and potassium were determined using flame photometer model – EEL, Calcium was determined using method of AOAC (1990).

Sensory evaluations of the meat were carried out using a 9-point hedonic scale (where 1=dislike extremely and 9=like extremely) as described by Iwe (2002). The treated meat samples were cut into smaller sizes and presented in plates to an untrained panel composed of twenty members who scored the meat samples on the basis of tenderness, colour, overall acceptability, juiciness and flavour. The order of presentation of the samples to the panellist was randomized. Cold water was also served to the panellist to gurgle and rinse their mouth after evaluation of each of the sample.

Data analyses

The data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 1998.Version 9.0). The variations in means were separated using Student-Newman-Keuls (SNK) test at 5% level of probability

Results

Meat quality characteristics of chuck meat as affected by papain enzyme, cooking time and their interaction showed (Table 1) that water holding capacity, cooking yield, cooking loss and pH of chuck meat cooked for 60 minutes were significantly ($P<0.05$) higher than those cooked for 30 minutes, while enzyme inclusion levels showed that water holding capacity and pH were higher ($P<0.05$) in the control and reduces as the inclusion levels increases, but it was observe that 5 ml papain enzyme inclusion had the least pH value.

Water holding capacity (WHC) decreased ($P<0.05$) as the enzyme treatment levels increased with the chuck meat treated with 15 ml having the least (0.27%) WHC. Cooking yield showed that the control was significantly ($P>0.05$) higher when compared to those included at 5, 10 and 15 ml inclusion levels which are similar. Cooking loss increased significantly ($p<0.05$) as the level of the enzyme increased. pH value was statistically ($P<0.05$) highest in the control samples (5.84) followed by those treated with 15 ml (5.48), then10 ml (5.45) and the least in 5 ml (5.32)papain treated chuck. There were significant ($P<0.01$) interaction between cooking time and enzyme inclusion levels in water holding capacity and pH. Water holding capacity was significantly higher with 60 minutes cooking time at 0 ml inclusion of papain enzyme and least at 30 minutes cooking time with 15 ml enzyme inclusion. Similarly, 60 minutes cooking time was highest with 0 ml enzyme inclusion and lowest at 30 minutes cooking time with 5 ml enzyme inclusion level.

Table 1. Meat quality characteristics of chuck meat as affected by papain enzyme, cooking time and their interaction

Treatment	WHC	CY	CL	pH
Cooking time (mins)				
30	0.31^{b}	43.61^{b}	8.85^{b}	5.41^{b}
60	0.41^{a}	44.02^{a}	9.23^{a}	5.61^{a}
SE±	0.00	0.66	0.13	0.01
Enzyme (ml)				
0	0.49^{a}	49.51^{a}	6.79^{d}	5.84^{a}
5	0.39^{b}	40.90^{b}	8.09^{c}	5.32^{d}
10	0.29^{c}	41.64^{b}	9.84^{b}	5.39^{c}
15	0.27^{d}	43.20^{b}	11.44^{a}	5.48^{b}
SE±	0.00	0.94	0.18	0.01
TxE	p<0.001	p>0.6989	p>0.2898	p<0.001

[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 TxE = Interraction between Time and enzyme inclusion

Effect of cooking time, papain enzyme inclusion levels and their interaction on proximate composition and soluble protein of chuck meat is presented on Table 2. Cooking time significantly (p<0.05) affect moisture content, crude protein, fat and soluble protein. The CP content was significantly highest when chuck meat was cooked for 60 minute, however, fat and soluble protein were higher at 30 minutes cooking time. Moisture content was not influenced by cooking time. Enzyme treatment levels showed significant (p<0.05) difference in all the parameters measured. The moisture content of meat treated with 5 ml of papain enzyme was significantly (p<0.05) higher than all the other treatments which were similar. Crude protein and soluble protein had similar trend, their values increase as the papain enzyme level increased. Fat content of chuck treated with 0 and 5 ml of enzyme levels were higher numerically but not statistically than that of the chuck treated with 10 and 15 ml. The interaction between cooking time and enzyme inclusion levels were significant on moisture content (p<0.01), crude protein (p<0.01), fat (p<0.03), and soluble protein (p<0.01). Moisture content was significantly (p<0.05) higher at 60 minutes cooking time and with 15 ml papain enzyme inclusion level and least moisture content was recorded at 30 minutes cooking time with 10 ml enzyme inclusion level. Similarly, crude protein was highest when chuck meat was cooked for 60 minutes with 15 ml enzyme inclusion level, the least crude protein content was recorded at 30 minutes cooking time with 0 ml enzyme inclusion. Fat content was highest at 30 minutes cooking time with 0 and 5 ml inclusion of papain enzyme; the least was recorded at 60 minutes cooking time with 10 ml papain enzyme inclusion. Soluble protein content was significantly (p<0.05) higher with 30 minutes cooking time when 15 ml papain enzyme was added and lowest at 0 ml enzyme inclusion and 30 minutes cooking time.

Effect of time of cooking, papain enzyme inclusion levels and their interaction on amino acid content of beef from chuck of aged cow is presented in Table 3. The result showed that all the amino acids measured had higher (p<0.05) values when the beef samples were cooked for 60 minutes with the exception of threonine and cystine that were significantly (p<0.05) higher at 30 minutes cooking time.

Enzyme levels showed that lysine, cystine, isoleucine and tyrosine were significantly (p<0.05) higher when beef samples were treated with 15 ml enzyme and lowest at 0 ml when treated with 0 ml enzyme.

Histidine, arginine, aspartic and valine acid were significantly (p<0.05) higher when samples were treated with 10 ml enzyme inclusion. Threonine, glycine, leucine and phenylalanine was significantly (p<0.05) higher in the control samples.

Serine, alanine and methionine were significantly (p<0.05) higher at 5 ml enzyme level and lowest at

15 ml enzyme levels. Glutamic acid and proline were significantly ($p<0.05$) higher at 5 ml and lowest at 10 ml. Glycine, leucine were higher with 0 ml and lowest with 15 ml enzyme inclusion, while phenylalanine was highest with 0 ml samples and lowest with 10 ml enzyme inclusion. There were interaction effect ($p<0.05$) between cooking time and enzyme inclusion level in all the amino acid evaluated. The result showed that all the amino acid content were significantly ($p<0.05$) higher in the papain enzyme treated meat with higher values at 60 minutes cooking time with 10 ml enzyme inclusion with the exception of threonine and cysteine that were higher at 30 minutes cooking time.