EFFECT OF AGEING UNDER TROPICAL CONDITIONS ON THE EATING QUALITIES OF BEEF

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ABSTRACT

Beef is a major source of animal protein in Ghana but most of it comes from old and poorly conditioned animals, which produce tough meat with poor eating qualities. The eating quality of tough beef can, however, be improved by methods of tenderizing such as ageing, electrical stimulation and application of enzymes. The purpose of this work was to study the effect of ageing under tropical conditions with high ambient/room temperatures (average 35°C) on the eating qualities of beef. Fresh beef longissimus dorsi muscle from a matured Sanga bull was used. The muscle was cut into four equal steaks measuring 10 cm long with an average weight of 373g in duplicates and subjected to ageing treatments for 5, 10 and 15 days at a temperature of 2°C ± 2°C. The control samples were frozen throughout the experiment. After each period of ageing, the samples were immediately frozen to halt further ageing process. Swabs were taken on the samples for microbiological analysis before and after each ageing period to determine the microbial quality of the steak after ageing. Fourteen untrained sensory panelists assessed the eating qualities: tenderness, beef flavour, juiciness and abnormal flavour of the samples. The ageing process resulted in highly significant improvements in tenderness and juiciness of the beef steak. Ageing for 10 to 15 days produced very tender steaks whilst the control steaks remained very tough (P< 0.001). The beef steaks became more juicy with increasing time of ageing and beef flavour intensity was significantly (P< 0.01) enhanced from day 5 to becoming strong by the day 15, whilst abnormal flavour intensity was not affected by the ageing process. On acceptability, majority of the panelists preferred the steaks aged for 10 or 15 days as the best meat. The ageing process did not have any detrimental effect on drip loss and microbial quality of the beef steaks. The problem of very tough beef from old animals in tropical countries can be minimized considerably through the practice of ageing.

Key words: Ageing, Flavour, Beef, Juiciness, Tenderness
INTRODUCTION

Meat production records for developing countries, particularly in sub-Saharan Africa show that beef cattle leads the chart on meat production and consumption [1]. Beef is a major source of animal protein in Ghanaian dishes [2] and the demand for it is constant throughout the year due to its relatively lower price [3]. Meat from cattle on the Ghanaian market mostly comes from older and poor body conditioned animals [4]. The meat from these animals tend to be tough (metallic) than meat from young cattle[1]. Old animals, especially draught animals, have a high content of tough connective tissues in the muscle which make them tough and therefore require prolonged cooking at higher cost in terms of fuel wood, gas or electricity. Young animals produce more tender meat. However, the proteolytic enzyme system decreases as the animal grows older resulting in tough meat. Intense stress on an animal prior to slaughter is known to have an influence on beef tenderness. The best cut from an animal can be made tough by pre-slaughter stress whilst an older animal can produce relatively tender meat if the animal is docile, humanely handled and slaughtered and the meat is properly aged. Unfortunately, in Ghana just as in most developing countries, animals are often subjected to stressful pre-slaughter conditions[4].

The most important aspect of meat quality is the eating quality which is the level of overall eating satisfaction and a function of the combined effects of tenderness, juiciness and flavour [5]. Meat tenderness is an important factor in consumer perception of meat quality [3] and most consumers consider tenderness to be the single most important component of meat quality. Meat tenderness is defined as the quality of the cooked meat which is associated with easy chewability and minimal loss of desirable texture [6]. It is also described as the softness or pressure exerted by the teeth to bite the meat and how easily it fragments [7]. Therefore, variations in tenderness at the consumer level must be controlled to improve consumer satisfaction. Meat tenderness is the most difficult trait to predict but important in determining quality and consumer acceptance. The real magnitude of the problem of tenderness is that most unsatisfied consumers rarely complained or return the products but simply avoid purchasing such tough products in the future.

Meat tenderness is influenced by pre-slaughter factors such as breed, age and feeding, and postmortem factors such as suspension of carcass during slaughter, electrical stimulation, chilling rate ageing, freezing, thawing and cooking [8, 9, 10, 11]. Quick freezing and ageing before rigor mortis sets in, pressure cooking, disruption of muscles by blades or hummer and muscle stretching are methods used to tenderize meat [12, 13]. The feeding regimes of animals can have significant effect on meat tenderness [14]. The amount of muscular exercise of the animal from which the cut is obtained also plays a role in tenderness [14,15]. Tenderness also depends on the amount of connective tissues present between the muscular fibres and to a lesser extent on the thickness of the muscle fibres themselves [5]. Meat tenderness may be judged by measurement of soluble collagen, sarcomere length, amount of
myofibril fragmentation or the shear force needed to cleave a standard sample of cooked meat[16].

Postmortem ageing is a natural process which improves the palatability attributes of meat, especially cut from the loin and rib. The tenderizing effect of ageing is more evident in carcasses from older animals than in the usually more tender lean meat from younger animals [17]. Ageing of meat also called ripening or conditioning is defined as the practice of holding carcasses or cuts under refrigerated condition at temperatures of 1 to 3 ºC (32 to 34ºF) [17]. Ageing, therefore, refers to holding of meat at refrigerated temperatures for an extended period to allow natural enzymatic reactions to take place to enhance tenderness and flavour, thus, allowing proteolytic enzymes to break down some of the complex proteins contained in the muscle [18,19]. Apart from ageing or conditioning of meat, other methods of meat tenderization are either too expensive or have a negative effect on sensory attributes of meat or have limitation for exploitation in restaurants and at household levels [20]. The ageing process is not needed if meat is to be ground, cured or made into sausage [21]. Improving the tenderness of beef through ageing or conditioning mainly depends on the optimum refrigeration temperature of about 2ºC which is easily applicable in the temperate countries where ambient temperatures are normally below 20ºC compared to the over 35 to 40ºC in the tropics [17].

The spoilage of meat, loss of weight and the development of off-odours and flavours during the ageing period can be attributed to one or more of the following possibilities [22]: (i) Off-odour in the chill room adsorbed by the meat. However, the most common off-odour comes from excess growth of bacteria, yeasts and moulds on the meat and chill room walls or floor. Also, storing any other product in the room that has an odour will contribute to the problem. (ii) Poor sanitation during slaughtering, chilling and packaging. Contamination with microorganisms causes off-odours, off-flavours and spoilage. (iii) Excessive ageing results in an accumulation of microorganisms which produced the off-odour. (iv) Shrinkage occurs during the ageing period; the longer the ageing period the greater the total loss in weight. Also, the longer the ageing period, the greater the need for trimming of lean and fat surfaces that have dried excessively or have detectable growth of microorganisms. Using a cost-effective method to improve the eating quality of tough tropical beef will contribute to increased meat consumption and improvement in nutritional status of most people in the developing countries.

The objective of this study was to determine the effect of ageing or conditioning at a temperature of 2ºC ± 2ºC on the eating quality of beef under tropical conditions of high ambient temperatures.
MATERIALS AND METHODS

The experiment was conducted at the University for Development Studies Meat and the Microbiology Laboratories in Tamale, Ghana. Fresh post-rigor beef, *longissmus dorsi* loin from a matured Sanga bull (about 10 years old), butchered 24 hr after slaughtering was used. All fats and connective tissues were trimmed off the muscle. The muscle was then cut into eight steaks of equal length each measuring 10 cm long with an average weight of 373g. The steaks were vacuum packed and labeled in duplicates as Control A and B, T1A and T1B, T2A and T2B, T3A and T3B. All samples, excluding the control were aged at a temperature of 2 ºC ± 2 ºC for varying time (days) in a Fagor refrigerator (Talleras Raman, Spain) as follows: Samples T2 were aged for 5days, T3 were aged for 10days and T4 were aged for 15days. The control (T1) samples were frozen throughout the experimental period to prevent any enzymatic action. At the end of each period of ageing, the aged samples were also frozen to halt further ageing All the samples were then thawed for 12 hours in a Fagor refrigerator (Talleras Raman, Spain) at a temperature of 2 ± 2ºC after the 15th day. The average room (ambient) temperature during the ageing period was 35 ± 2 ºC.

Microbiological analysis

Microbial identification and total viable count (TVC) was determined on each beef steak before and after ageing. The microbial load was obtained by swabbing the surface of the samples using sterile cotton wool. The swabbed microbes were inoculated unto separate dishes of blood agar and incubated at 37ºC for 24 hours in an incubator. Cultures were made on blood agar media from various diluents and incubated separately. Gram staining technique was used to identify the growth of microorganisms in the colonies. The microbial loads on the samples were determined and expressed in log10 colony forming units per cm² (cfu /cm²).

Weight loss of samples

The beef steaks were weighed before and after ageing to determine the differential weight loss as a result of shrinkage and drip loss from the steaks during the ageing periods.

Sensory analysis

The samples were thawed and oven-grilled (Turbofan, Blue Seal oven, UK) to a core temperature of 70ºC for sensory assessment. The grilled samples were trimmed of all burnt surfaces, sliced into cubes of (2 cm³) and wrapped with coded aluminum foil to keep them warm. Fourteen untrained sensory panelists were served with the coded beef samples. Pieces of bread were used as neutralizer between samples. With the aid of questionnaires, the eating qualities (tenderness, beef flavour, juiciness and abnormal flavour) of the various samples were determined by the sensory panelists using a five-point category scale as follows:

1. **Tenderness**: 1- Very tender, 2- Tender, 3- Intermediate, 4- Tough, 5- Very tough.
2. Beef flavour: 1-Very strong, 2- Strong, 3- Intermediate, 4- Weak, 5- Very weak.
4. Abnormal flavour: 1- Very weak, 2-Weak, 3- Intermediate, 4-Strong, 5- Very strong.
Overall acceptance was also determined by the percentage of the panelists.

Statistical analysis
The data generated were analysed using a general lineal model (GLM) of analysis of variance (ANOVA) of MINITAB version 13.0 [23].

RESULTS
The ageing process had significant effects on most of the organoleptic qualities such as tenderness, beef flavour, juiciness and overall acceptability of the beef steaks but did not have any impact on abnormal flavour intensity in the beef steaks (Table 1). The tenderness of the beef steaks improved significantly with increasing time (days) of ageing (P < 0.001). Thus ageing for 10 to 15 days produced fairly tender steaks whilst the control steaks remained tough. There was a significant effect of ageing on juiciness of the beef steaks (P<0.001) (Table 1). The results demonstrated that the beef steaks became more juicy with increasing time of ageing.

The ageing process had significant (P< 0.01) effect on the beef flavour of the samples, such that beef flavour intensity increased gradually from day 5 and became strong by the day 15. Incidentally, the ageing process did not have any negative effect on the flavour of the samples. Hence, the intensity of abnormal beef flavor remained very weak in the samples throughout the trial. On overall acceptability, the panelists rated the control sample as the least preferred because it was considered to be tough and dry. Majority (70%) of the panelists indicated the samples aged for 10 to 15 days to be the best meat (Table 1).

An average of 1.5 percent of the initial weights of the aged beef samples was recorded as drip and thaw loss after ageing. The control samples which were frozen throughout recorded the highest thaw/drip loss of 5 percent on thawing.

Microbiological quality
The microbes identified on all the beef steaks before ageing included Staphylococcus spp and Escherichia coli which were of identical quantities (4.7 × 10 cfu/cm²) (Table 2). The final microbial count showed marginal increases in their numbers with increasing ageing. Generally, the microbial loads recorded on the beef samples before ageing indicated that the level of contamination of the samples was very minimal.
DISCUSSION

Eating qualities
The purpose of ageing or conditioning or "ripening" or refrigerated storage above freezing point of beef is simply to allow "natural processes" to improve its palatability attributes, mainly flavour, juiciness and tenderness. Whilst a muscle is undergoing changes associated with tenderness, chemical breakdown of certain muscle and fat constituents occurs, resulting in a more intense flavour and aroma [22]. In general, these changes in flavour and aroma are desirable to most consumers. However, undesirable flavours and aroma can develop during ageing, mainly due to the effects of microbial growth, rancidity of the fat and adsorption of off-odours if they are present in the chill room, which was not the case in this study.

The ageing process significantly improved two most important organoleptic qualities of meat namely tenderness and juiciness. This observation agrees with earlier reports that ageing improves meat tenderness [7,17, 24]. The tenderness obtained in this study due to ageing process indicates that there was some degree of degradation of some structural proteins by proteolytic enzymes (18, 19). The result indicated that there were increasing rates of tenderizing activities in the beef up to the 10th day but these activities might have decreased from the 10th and perhaps ceased at the 15th day. It has been reported that most of the advantages of ageing beef are achieved by the end of seven to 10 days of ageing [22]. The trend of the current results conforms to that of earlier reports [21,25], which indicate that tenderization is relatively rapid during the early stages (3 to 7 days) of ageing and continues to increase but at a much slower rate thereafter. During ageing, naturally occurring enzymes, calpains and cathepsins (proteases) found in muscle, breakdown specific protein strands in the muscle fibre in a process called proteolysis [18,19]. As meat ages, proteolysis is enhanced. The calpain proteolytic enzyme system is responsible for the specific peptide bond cleavage which causes early postmortem tenderization [7]. The calpain system, the primary enzyme system responsible for the ageing process, is comprised of three primary components, µ-calpain, m-calpain and their inhibitor, calpastrin which are all calcium- dependent [25].The calpains undergo autolysis and as such they do not over- tenderize meat. The breaking or fragmentation of the myofibril protein strands by these natural enzymes result in improved tenderness [26]. Longer ageing periods, for instance beyond 28 days, result in little benefits to enhanced palatability and may be detrimental in terms of increased and unwanted microbial growth and abnormal flavour development[26].

The flavour intensity obtained in this study agrees with a previous report stating that true beef flavour is fully developed at about 11 days of ageing and that cooked, un-aged beef lacks a typical beef flavour [17]. The weak abnormal flavour intensity in the samples could also be attributed to the vacuum packaging effect that prevented the meat from absorbing any flavour from the chiller and also eliminated oxidative rancidity. This finding supports an earlier report that “in the bag” ageing produces fewer off odours and off flavours in meat [17].
The most important aspect of meat eating quality is the level of overall eating satisfaction. Eating satisfaction is a function of the combined effect of tenderness, juiciness and flavour [5] obtained during this ageing process thereby producing very acceptable or palatable meat from the 10th to 15th day of ageing. Among the palatability attributes of beef, tenderness is the attribute most sought after by consumers and thus the need for ageing of beef to improve this attribute.

During the ageing process, weight loss should be expected. The weight loss is caused by dehydration of the lean and fat and can be at high proportions depending on relative humidity, air flow and the temperature in the ageing chamber. However, an additional advantage of “in the bag” ageing is reduced weight loss since moisture is retained in the bag and partly re-absorbed. The high percentage of drip lost in the control samples can be attributed to the continuous formation of ice crystal in the muscle fibres which then melt on thawing resulting in higher exudate [27]. The plastic packaging does not allow loss of moisture; instead the meat absorbs this moisture resulting in an increased juiciness and tenderness[28].

**Microbiological quality**

When spoilage bacterial population reaches 10 million per gram of a food substance it will begin to produce chemical changes that are apparent to the senses [29]. The low levels of initial contamination of the samples used in this study facilitated the production of good quality meat by the end of the ageing process. The disappearance of the *Staphylococcus spp* was due to lack of favourable conditions (air and ideal temperature) during the ageing period. The *E. coli* survived because they are psychrophilic and could withstand the ageing temperatures. Meat is described as “spoiled” when bacterial count or load is more than $10^7$/cm$^2$ or $10^8$/g [30] and meat will, therefore, ‘spoil’ when the TVC value goes beyond $10^6$/cm$^2$ because of off-odours which start to develop at approximately $10^7$ - $10^8$ organisms cm$^{-2}$ [7]. The final microbial loads recorded on the samples in this study were within the marginal limits such that they will not cause spoilage of the products or harm the consumer [7, 30]. It also indicates that there was no loss of vacuum, whereby the air present in the bag, surrounding the meat, will enhance the growth of aerobic microorganisms and cause rapid spoilage [22].

**CONCLUSION**

The problem of toughness in beef from old animals in tropical countries can be reduced considerably through the practice of ageing or conditioning. Ageing will make tropical tough beef tender and juicy with enhanced beef flavour thereby improving its eating qualities. A high standard of meat hygiene is a prerequisite for ensuring that eating qualities of beef are not compromised during ageing. Packaging of the meat in polythene bags could be practiced at homes and restaurants in the tropics as this reduces or prevents contamination of the meat and weight loss during ageing. Beef can, therefore, be successfully aged in a vacuum bag under tropical conditions. At a temperature range of $2^\circ$C ± $2^\circ$C, beef should be aged for at least 10 days and not longer than 15 days for desirable eating qualities. Before ageing, all visible fat attached to the meat should be trimmed off to prevent rancidity.
Table 1: Effect of ageing on the eating quality of beef steaks (5 points category of scale)

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Control</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>SED</th>
<th>SIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenderness</td>
<td>4.8c</td>
<td>3.4b</td>
<td>2.4a</td>
<td>1.8a</td>
<td>0.30</td>
<td>***</td>
</tr>
<tr>
<td>Beef flavour</td>
<td>3.5b</td>
<td>3.0ab</td>
<td>2.5a</td>
<td>2.0a</td>
<td>0.49</td>
<td>**</td>
</tr>
<tr>
<td>Juiciness</td>
<td>4.0c</td>
<td>3.0b</td>
<td>2.4a</td>
<td>2.0a</td>
<td>0.34</td>
<td>***</td>
</tr>
<tr>
<td>Abnormal flavour</td>
<td>1.8</td>
<td>2.2</td>
<td>2.2</td>
<td>2.1</td>
<td>0.38</td>
<td>ns</td>
</tr>
<tr>
<td>Overall acceptability (%)</td>
<td>1</td>
<td>29</td>
<td>35</td>
<td>35</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a b c  means in a row with the same superscript are not significantly different.

ns: not significant; **: significant P< 0.01; ***: significant P < 0.001.

SED: Standard error of difference.
Table 2: Micro-organisms and Microbial load on beef steaks before and after ageing

<table>
<thead>
<tr>
<th>Ageing time (days)</th>
<th>Microbes before ageing</th>
<th>Microbial load before ageing (cfu/cm²)</th>
<th>Microbes identified after ageing</th>
<th>Microbial load after ageing (cfu/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><em>Staphylococcus</em> spp <em>and E. coli</em></td>
<td>4.7 × 10</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus</em> spp <em>and E. coli</em></td>
<td>4.7 × 10</td>
<td><em>Staphylococcus</em> spp <em>and E. coli</em></td>
<td>1.6 × 10²</td>
</tr>
<tr>
<td>10</td>
<td><em>Staphylococcus</em> spp <em>and E. coli</em></td>
<td>4.7 × 10</td>
<td><em>E. coli</em></td>
<td>1.5 × 10²</td>
</tr>
<tr>
<td>15</td>
<td><em>Staphylococcus</em> spp <em>and E. coli</em></td>
<td>4.7 × 10</td>
<td><em>E. coli</em></td>
<td>3.6 × 10²</td>
</tr>
</tbody>
</table>
REFERENCES


