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Effect of post-stunning bleeding time on physical, sensory and microbial status of rabbit meat

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Target Audience: *Scientists, Processors, Public health personnel*

Abstract

This study was carried out to investigate the effect of post-stunning bleeding time on the physical, sensory and microbial status of Rabbit meat. The study was carried out in a completely randomized design with fifteen (15) male Dutch breed of Rabbits with an average weight of 1.7 kg, which were randomly assigned to 5 treatment groups (3 rabbits/treatment). The rabbits were stunned and bled at different time intervals; 0, 10, 40 and 60 minutes (T2, T3, T4 and T5, respectively), Rabbit in the first treatment group (T1) were conventionally slaughtered using Halal method. The processed meat samples where grilled and evaluated for organoleptic, physical, chemical analysis and microbial properties. The results for organoleptic properties showed that there was significant difference (P>0.05) in mean scores for overall acceptability, with grilled rabbit meat from Rabbit bled after 60 minute (T5), having the lowest score (6.40) and highest score at T3 (7.60). The pH values were within acceptable limits (5.5-6.5). Percentage cooking loss, thermal shortening and Water holding capacity were highest in T5. Microbial load for both Bacteria and Fungi on raw and cooked samples were within satisfactory limit (i.e. <1/2 million/g). However, all samples were contaminated with Staphlococci and Bacillus spp. It was concluded that meat from rabbit bled up to 60minutes post stunning does not have adverse effect on the physical parameters, microbial load and overall acceptability of the processed meat as all values were within acceptable limits.

Key words*: Rabbit meat; Stunning; Bleeding; Organoleptic; Microbial.*

Description of Problem

In many parts of the world, meat makes up a considerable portion of a typical diet. Meat has been defined as the flesh of animals which is suitable as food. Meat makes a valuable contribution to diets because of its high biological value and an excellent source of amino acids, vitamins and minerals. Meat from rabbit is highly digestible, tasty, having low-calories and often recommended by nutritionists over other meat types (1). Meat quality consist of; nutritional properties such as appropriate proportions of bioactive compounds, proteins, lipid and their essential constituents; sensory characteristics such as appearance, texture and flavor; health which depends on fat and saturated fatty acid content; and technological factors; such as processing.

Bleeding, also known as sticking is the removal of blood from an animal as quickly as possible. It is done to kill the animal with minimal damage to the carcass and to remove the blood which provides an ideal medium for the growth of bacteria. Bleeding involves severing the carotid arteries and jugular veins, or the blood vessels from which they arise. The animal then dies from loss of blood. It is important that all major blood vessels are severed. If only one carotid artery is cut the animal may take over a minute to die. Proper bleeding can improve the quality of meat during storage (2). The amount of blood lost from meat after slaughtering varies according to the time the animal is bled after stunning. Stunning is the act of rendering an animal unconscious before bleeding. This is done to reduce the stress associated with bleeding the

animal directly. The presence of blood in meat encourages microbial proliferation in the meat. Hence, the longer the rabbit stay (post stunning) without bleeding, the higher the chances of meat being contaminated by microbes which may further lead to deterioration in the physical and sensory qualities of the meat due to blood loss. Most consumers' decision to purchase meat is guided by the perception of healthiness, safety and sensory traits such as colour, tenderness, juiciness and aroma or flavor. This study was conducted in order to reveal how we can obtain healthy and acceptable meat and to discover at what levels meat can be seen as unacceptable or harmful for consumption due to microbial contamination and deterioration in quality parameters.

The aim of this study is to determine the effect of post-stunning bleeding time on physical, sensory and microbial status of Rabbit meat.

Materials and Methods

Study location

The study was carried out in the Teaching and Research Farm, Faculty of Agriculture, Kaduna State University. The University is located in Kafanchan town of Jema'a Local Government Area of Kaduna State, located at latitude 9.58^0N, longitude 8.29^0E with an elevation of 733m above sea level (3).

Sample collection

Fifteen male Rabbits (Dutch breed), with an average weight of 1.7kg were obtained from the Teaching and Research Farm of Ahmadu Bello University Zaria.

Stunning and slaughter procedure

Mechanical stunning (direct blow) to the head was stunning method used for treatments 2, 3, 4, and 5. The blow was manually done with precision and force.

Rabbits were bled by severing the jugular vein and the carotid arteries, trachea and esophagus below the jaws as described by (4). Rabbits were stunned and bled horizontally at different time intervals with their heads pointing downwards as described by (5).

Sample preparation

The rabbits were randomly allotted into five different treatments with each treatment consisting of three (3) rabbits in a completely randomized design. The procedure is outlined below:

Treatment 1- Halal method of slaughtering (control)

Treatment 2 – stun rabbit and bleed animal immediately (0 minutes)

Treatment 3 - bleeding was done 20 minutes after stunning

Treatment 4 - bleeding was done 40 minutes after stunning

Treatment 5 – bleeding was done 60 minutes after stunning.

Determination of blood loss;

During exsanguination, blood was collected in a beaker and weighed. The amount of blood loss was measured using the formula given by (6):

Blood loss (%) = BW/ LW × 100

Where: BW (kg) = weight of blood lost by dead animal (based on ECG) and

LW (kg) = live body weight pre-slaughter.

Processing of rabbit carcass:

The carcasses were skinned using procedures described by (7) washed, weighed and chilled at 4^0C for 24 hours and fabricated into four quarters.

Grilling of rabbit meat:

Thirty grams (30g) of meat from the hind quarter of each rabbit was grilled using an electric barbecue machine. The ingredients used were purchased from Yakowa market, Kafanchan Jema'a LGA of Kaduna State. The spices used and their proportions are shown in Table I.

Table 1 Types and levels of ingredients used in grilling the rabbit meat.

Ingredient constituents	Proportion by weight(g)	Proportion in mixture (%)
Dried pepper	60	32.79
Maggi	55	30.06
Onga	30	16.39
Salt	38	20.77
Total	**183**	**100**

The samples to be cooked were marinated for 10 minutes and grilled on the grilling machine for 25minutes. Groundnut oil was added while grilling and meat samples turned intermittently to ensure even doneness. Weight before and after grilling was taken from samples and used in calculating the percentage cooking loss. Samples for the determination of the physical properties were taken to the food technology laboratory of Kaduna Federal Polytechnic, Kaduna state for analysis.

Physical Parameters Analysis:

Cooking Loss and Thermal Shortening;

Meat samples of known weight and length were boiled for 25min between 160 – 180^0 C to an internal temperature of 70^{0}C. They were removed and cooled to room temperature (25^{0}C) before they were reweighed and their weights and lengths retaken. The difference between the initial weight and length and the final weight and length was recorded as the cooking loss and thermal shortening of meat (7). Thus

$$\text{Cooking loss} = \frac{\text{Initial meat wt} - \text{Final meat wt}}{\text{Initial meat wt}} \times 100$$

$$\text{Thermal shortening} = \frac{\text{Initial meat length} - \text{Final meat length}}{\text{Initial meat length}} \times 100$$

Moisture Content;

Percentage moisture content was determined by the oven method, by heating 10 grams of meat samples at 80^{0}C to a constant weight. The difference in weight before and after grilling divided by weight before grilling multiplied by 100 was recorded (8).

$$MC = \frac{WFS - WSS}{WFS} \times 100$$

Where MC = Moisture Content (%), WSS = Weight of grilled Sample and WFS = Weight of Fresh Sample

Water Holding Capacity;

Water holding capacity was determined following a slightly modified method of (9). Intact samples (10 x 10 x 5 mm) were weighed individually from the different treatments on two filter papers and pressed for a minute, using a 10 kg weight. The amount of water released from the sample was measured indirectly by measuring the area of the filter paper wetted relative to the area of pressed sample.

$$WHC = \frac{100 - [Ar - Am] \times 9.47}{Wm \times Mc} \times 100$$

Where Ar = Area of water released from meat (cm^2), Am = Area of meat sample (cm^2), Wm = Weight of meat in mg, Mc = Moisture content of meat % 9.47 is a constant factor.

pH Measurement;

pH was measured with a portable pH-meter equipped with a glass electrode (3 mm diameter conic tip) suitable for meat penetration.

Microbial Analysis:

The following types of bacteria were enumerated

- Mesophilic Bacteria (Total Plate Count); using standard plate count method

- Yeast and Mould; Using standard plate count methods.
- Coliform bacteria; Using standard plate count method.

Microbial load guide in animal food product, according to (10) was used to interpret result.

Table 2 Standard Microbial Load Specification on animal food product.

Grade	TVC(total viable count)/g at 30^{0}C	Description
1	<1/2million	Satisfactory
2	1/2millionto<10million	Passable
3	10million and more	Unsatisfactory

Source: (10).

Sensory evaluation of meat samples

Grilled rabbit meat samples were cut into bites sample sizes and served in plates to a twenty member semi-trained panelist (11). The panelists received each sample separately, rinsing their mouth in-between samples, the meat samples were presented sequentially to the taste panelists on clean saucers (12). The organoleptic parameters that were evaluated include; appearance, taste, odor, texture and provision for a score on overall acceptability. A 9 point hedonic scale was used with a score of 9 indicating like extremely, and 1 indicating dislike extremely. A score below 5 was considered not acceptable (13). The meat products were coded with alphabets indicating no information about the samples to avoid bias in preferred treatments.

Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) using (14), while significant means were separated with Duncan multiple range test of the same software.

Results and Discussion

Physical Parameters of Rabbit Meat

Table 3: Physical Parameters of Rabbit meat bled at different Time Intervals.

Parameters	T1	T2	T3	T4	T5	SEM
Blood loss	1.54^a	1.35^a	0.60^b	0.42^b	0.27^b	0.11
pH fresh	6.20^a	6.00^b	5.58^c	5.54^c	5.05^d	0.02
pH grilled	5.60^a	5.40^b	5.38^b	5.34^b	4.50^c	0.03
Cooking loss	28.51^{ab}	28.64^{ab}	18.41^b	31.36^{ab}	34.33^a	2.57
Thermal Shortening	16.75^b	16.34^b	14.59^b	27.89^a	30.95^a	2.13
WHC	26.80^b	26.22^b	18.54^b	49.84^a	50.28^a	2.75

All values are means of triplicate determination. $T_{1=}$ halal method of slaughter, T_2= stunned and bled immediately, T_3=stunned and bled after 10mins, T_4= stunned and bled after 40mins, T_5= stunned and bled after 60mins. WHC = Water Holding Capacity.

Table 3 shows the mean values of the physical parameters of the Rabbit meat samples. Values obtained for blood loss showed the highest mean in T_1 (1.54) which is statistically same with T_2 (1.35). The lowest value was recorded in T_5 (0.27) which is statistically same with T_3 and T_4, (0.60 and 0.42 respectively). Bleeding is greatly affected by the time of slaughter 'post stunning'. The longer the time interval between stunning and bleeding the more blood will be trapped in the veins, capillaries, etc of the meat. Mean values obtained for the pH of the fresh meat samples recorded the highest pH value in T_1 (6.20) and the lowest value at T_5 (5.05). The mean values obtained for the pH of the grilled meat samples showed that T_1 (5.60) had the highest value while T_5 (4.50) had the lowest values and they are statistically different. The pH values of the fresh and grilled meat showed that the processing of the rabbit meat led to a notable change in the pH value of the meat. However, the grilled meat had a lower value compared to the fresh meat of same treatments. The pH values for fresh rabbit meat were similar to maximum accepted limit of 6.0 suggested by (15) and sited by (16) for fresh meat, suggesting that the products were produced from well-nourished and rested stock.

Values obtained for cooking loss showed highest mean in T_5 (34.33) which is statistically similar to T_1, T_2 and T_4 with values 28.51, 28.64 and 31.36 respectively. The lowest value was recorded in T_3 (18.41). (17) indicated that cooking loss is mostly water and could be caused by protein denaturation which allows less water to be entrapped within the protein structures held by capillary forces. (18) said that water or cooking loss is of economic concern because it affects weight loss along the distribution chain.

The mean values obtained for thermal shortening showed that T_5 (30.95) had the highest value which is statistically same with T_4 (27.89) and the lowest value was recorded in T_3 (14.58) which is statistically same with T_1 and T_2 (16.75 and 16.34). Thermal shortening also occurred as a result of structural changes caused by heating.

The mean values for Water holding capacity showed that T_5 (50.28) had the highest value which is statistically same with T_4 (49.84) and the lowest value was recorded in T_3 (18.55) which is statistically same with T_1 and T_2. Water holding capacity refers to the ability of meat to retain its water during application of external forces (19). This is important in meat processing as it influences the overall eating quality (13). There was an observed trend between cooking loss, thermal shortening and water holding capacity which agrees with (17) who reported that losses in meat is mostly water and could be caused by protein denaturation during processing which allows less water to be trapped within the protein structures held by capillary forces.

Microbial Counts of Rabbit Meat

Total Plate Count for Mesophilic Aerobic Bacteria;

The total plate count for Mesophilic Aerobic Bacteria is shown in Table 4. The result shows that all the meat samples were contaminated with microorganisms i.e. there was no correlation between time of bleeding and bacterial contamination. The Total Plate Count for all samples fell within the satisfactory limit with reference to standard microbial load specification (10). The total plate count for mesophilic aerobic bacteria for the fresh rabbit meat was highest in T_4 (1.25×10^2). While T_1, T_2 and T_5 had the lower values (1.0×10^2). The Processed (grilled) meat samples had Total Plate Count (TPC) highest in T_2 (1.35×10^2) and the lowest values were recorded at T_3 and T_4 (1.0×10^2). The presence of blood in meat encourages microbial proliferation in the meat. From table iii, T_1 had the highest value for blood loss and this can be a possible reason for the low value for TPC for the fresh sample of

same treatment. Possible sources of microorganisms in the meat could be inherent micro-flora in normal tissues of Animals; others are through air, environment, or contamination due to slaughtering, handling and processing conditions as suggested by (20).

Table 4: Total Plate Count for Mesophilic Aerobic Bacteria in rabbit meat bled at different time intervals.

Treatment	TPC fresh	TPC grilled	Description	Bacillus species	Staphylococci species
T_1	1.0 x 10^2	1.25 x 10^2	Satisfactory	+	+
T_2	1.0 x 10^2	1.35 x 10^2	satisfactory	+	+
T_3	1.1 x 10^2	1.0 x 10^2	satisfactory	+	+
T_4	1.25 x 10^2	1.0 x 10^2	satisfactory	+	+
T_5	1.0 x 10^2	1.2 x 10^2	satisfactory	+	+

All values are means of duplicate determination. $T_{1=}$ halal method of slaughter,T_2= stunned and bled immediately,T_3=stunned and bled after 10mins,T_4= stunned and bled after 40mins, T_5= stunned and bled after 60mins. TPC means Total Plate Count, + = Positive.

Total Fungi (Yeast and Mould) Count;

Table 5 shows the total Fungi Count from the different meat samples**.** The Total Fungi Count from the meat samples are shown in Table 5. Results from fresh meat samples showed that T_2 and T_5 did not have any value for fungi count, while T_1, T_3 and T_4 all had same values of 1.0 x 10^2. Processed rabbit meat samples all had same/uniform value of 1.0 x 10^2. The microscopic identification of the fungal isolates shows the presence of mould species with a black powdery colonies appearance. The absence of fungi in the fresh form of T_2 and T_5 could be as result of proper hygiene practices all through the handling of the meat. The low number and frequency of occurrence of isolate can be linked to the level of hygiene of the raw meat and the various handling and processing methods and the low moisture content of samples which agreed with report by (21). According to (22) microbial growth on meat is higher prior to processing and lower after drying processes. This may explain the reduction in the types and number of micro-organisms that were isolated from the samples.

Table 5: Total Fungi (yeasts and moulds) count for Rabbit meat bled at different time intervals

Treatment	FC fresh	FC grilled	Cultural characteristics	Microscopic identification
T_1	1.0x 10^2	x 10^2	Black powdery	Mould species
T_2	Nil	x 10^2	Black powdery	Mould species
T_3	1.0 x 10^2	x 10^2	Black powdery	Mould species
T_4	1.0 x 10^2	x 10^2	Black powdery	Mould species
T_5	Nil	1.0 x 10^2	Black powdery	Mould species

All values are means of duplicate determination. $T_{1=}$ halal method of slaughter,T_2= stunned and bled immediately,T_3=stunned and bled after 10mins,T_4= stunned and bled after 40mins, T_5= stunned and bled after 60mins. FC means Fungi Count

Organoleptic Parameters of Meat samples:

Table 6: Organoleptic Parameters of Rabbit meat samples bled at different time intervals.

Treatment	T_1	T_2	T_3	T_4	T_5	SEM
Aroma	7.30^{a}	7.53^{a}	7.15^{ab}	7.13^{ab}	6.50^{b}	0.72
Taste	7.48^{a}	7.23^{a}	7.43^{a}	7.15^{a}	6.03^{b}	0.75
Color	7.25	7.03	7.13	7.40	6.80	0.68
Tender	6.78	6.95	7.00	6.86	6.50	0.89
Juiciness	7.45^{a}	7.00^{ab}	7.03^{ab}	6.58^{bc}	6.20^{c}	0.82
OA	7.58^{a}	7.35^{a}	7.60^{a}	7.13^{a}	6.40^{b}	0.74

$T_{1=}$ halal method of slaughter, T_2= stunned and bled immediately,T_3=stunned and bled after 10mins,T_4= stunned and bled after 40mins, T_5= stunned and bled after 60mins. OA=Overall Acceptibility.

Table 6 shows the results for the different organoleptic parameters. Rabbit stunned and bled after 60 minutes (T_5) recorded the lowest values in all of the parameters. The highest value for aroma, was recorded in T_2 (7.53) Rabbit stunned and bled immediately, which is statistically the same with T_1(7.30) rabbit slaughtered by Halal method (7.30), and statistically similar to T_3 and T_4 with values (7.15) and (7.13) respectively. The lowest value for aroma was recorded in T_5 (6.50) rabbit stunned and bled after 60minutes, which is statistically similar to T_4 (7.13), rabbit stunned and bled after 40minutes, and T_3 (7.15), rabbit stunned and bled after 10minutes .The highest value for taste was recorded in T_1 (7.48) which is statistically the same (P>0.05) with T_2 , T_3, and T_4,with mean values of 7.23, 7.43 and 7.15 respectively, while the lowest value for taste was recorded in T_5(6.03). The highest value for juiciness was recorded in T_1 (7.45) which is statistically similar to T_2 and T_3 with values 7.00 and 7.03 respectively. The lowest value was recorded at T_5 (6.20) which is statistically similar to T_4 (6.58). The observed relationship between Juiciness, taste, cooking loss and percentage moisture content agrees with statement by (23) who reported that Juiciness is made up of two effects; the impression of moisture released during chewing and also the salivation produced by flavor factor. Meat juices play an important role in conveying the overall impression of palatability to the consumer. They contain many of the important flavor components and assist in the process of fragmenting and softening the meat during chewing (19).

The values obtained from color and tenderness of the meat is not significant. Cross *et al* (24) as sited by Omojola (25) reported that tenderness is considered as the most important trait in meat quality. It has also been identified as the most critical eating quality that determines whether consumers are repeat buyers. However, it is worthy of note that as a result of the heat treatment and the use of spices which have tenderizing effect, the degree of tenderness is affected when compared to fresh meat. (26) as sited by (27) reported that most citizens of developing countries like Nigeria prefer less tender meat or meat product probably for longer chewability.

The highest value for overall acceptability was recorded in T_3 (7.60) but statistically the same (P> 0.05) with T_1, T_2, and T_4 with values 7.58, 7.35 and 7.13 respectively. The lowest value for overall acceptability was recorded at T_5 (6.40). Using the 9-point hedonic scale, the meat samples all scored above 5, which is the limit of acceptability for the Organoleptic parameters considered.

Conclusion and Applications

1. The results from this study showed no specific difference between the physical

parameters of both fresh and processed rabbit meat with the time of bleeding (up to 60 minutes) post stunning.

2. The microbial status of meat from all samples was within safe limit; hence, safe for consumption.
3. The Overall acceptability score was lowest in grilled meat from rabbits bled 60 minutes post-stunning; although the mean score for overall acceptability was above 5 (which is the least acceptable value) implying that the product is still within acceptable range by man.
4. Meat from rabbit bled up to 60minutes post stunning does not have adverse effect on the physical parameters, microbial load and overall acceptability of both raw and processed meat.

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