

*Original Research Article*

**ENHANCEMENT OF SHELF LIFE OF BEEF USING *RICINUS COMMUNIS* LEAVES**

**ABSTRACT**

**Aims:** *Ricinus communis* leaves are used indigenously by some villagers in Nigeria to preserve fresh beef due to lack of electricity. The goal of this study is to scientifically determine the efficacy of fresh leaves of *R. communis* in beef preservation.

**Study design:** This study was designed using three **method** of preservation at three different storage times.

**Comment [DZ1]:** methods

**Place and duration of Study:** Beef sample was purchased from Animal Science department Abattoir, University of Ibadan, and analysis carried out at Nutrition Research Laboratories, Department of Biochemistry, Ibadan, Oyo State.

**Methods:** The effect of storage time (12h, 24h and 36h) on the nutritional qualities of beef preserved with *R. communis* leaves compared with fresh samples (control), refrigerated and beef preserved under ambient temperature was evaluated. At the end of each of the three storage periods, the physicochemical and sensory properties of the beef samples were determined using standard techniques. The data were analysed using a one-way analysis of variance while comparisons were made among the preservation methods against control using Independent T-Test (XLSTAT version 20).

**Results:** It was found that there are significant differences ( $P < 0.05$ ) between the treatment modalities used (ambient, *R. communis* and refrigeration) in comparison with control across various time intervals (12, 24 and 36h) in most of the investigated parameters except in Ash (24h, 36h).

**Conclusion:** *Ricinus communis* leaves help extend the shelf life of fresh beef, as well as regulate the pH of beef samples.

*Keywords: Beef, Nutritional quality, Preservation, Ricinus communis, Storage time.*

## INTRODUCTION

Beef is known as the flesh of meat derived from cows, bulls, or other bovines. Apart from pork and poultry, beef is the most consumed meat worldwide [1]. Water, protein, carbohydrates, and lipids are the major constituents of beef, while the minor components include vitamins, enzymes, pigments, and flavour compounds [2]. The combination of all these constituents gives beef its unique structure, texture, flavour, colour and nutritive value. Beef and its products are good sources of high-quality proteins and their amino acid constituent usually compensate for the deficiencies in other staple foods. Iron in beef is easily absorbed. It also enhances the absorption of iron and zinc from other foods. Beef is a rich source of vitamins in the B group [3]. Due to the nutritive component of beef, microorganisms of various types invade rapidly and cause spoilage. Therefore, adequate and long-lasting preservation methods are applied to maintain its safety and quality [4].

The processing and preservation methods of beef products in Nigeria are incomparable with those in advanced countries. Most natives in villages and less developed cities in Nigeria use traditional methods for preserving beef and beef products. Refrigeration at a temperature of about 4°C is the best way to preserve meat slaughter in good hygienic condition [4] but in Nigeria and most African countries, meat is predisposed to conditions that increase its contamination from the growth of pathogens and microorganisms due to a lack of electricity in the slaughterhouse [4]. There are regulations governing meat preservation by refrigeration; however, in many parts of Africa, this does not happen or is expensive to maintain, presumably because of the unstable power supply [4]. Several preservation methods, such as salting, drying, wet curing, and the use of chemicals, have been described [5]. Studies have shown that the high level of salt in beef is associated with the incidence of high blood pressure and hypertensive diseases [6, 7]. The use of chemicals such as Nitrite in retaining the reddish colour in meat and its bacteriostatic effect on *Clostridium botulinum* make it widely acceptable [8], but the reaction of the amines in meat with nitrite has been implicated in the formation of nitroso-compound, a potential carcinogen [8]. Hence, there is a need for an alternative method which will be devoid of dangerous side effects.

**Comment [DZ2]:** why you not used bacterial indicators in your study

*Ricinus communis* (*R. communis*), commonly known as the Castor plant (leaves), is traditionally used for beef preservation in many Nigerian villages due to poor or no electricity supply. *R. communis* belongs to the family *Euphorbiaceae*, a soft, wooden small tree widely spread all through the tropics and temperate regions of the world [9]. *R. communis* grows well along stream banks, river beds, bottomlands, and just above many hot areas where the soil is well drained with sufficient moisture and nutrients to encourage growth.

The natural product of *R. communis* Leaves have showed the presence of different phytochemicals such as alkaloids, saponins, flavonoids, terpenoids, and cardiac glycosides [10]. The leaves also has antibacterial activity against gram negative bacteria such as *E. Coli* as well as gram positive (staphylococcus)[11]. The objective of this study is to scientifically evaluate the traditional claims of use of *R. communis* in beef preservation and the effect of storage time on the physico-chemical qualities of the beef preserved with *R. communis*.

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## MATERIALS AND METHODS

**2.1 Sample collection:** The flank part of the Cow was purchased from the Animal Science Department Abattoir, University of Ibadan, Oyo State, Nigeria immediately after slaughter, while the leaves for preservation were harvested fresh from Ojoo, Ibadan, and identified before use at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria (Forest Herbarium No. FHI 1136578), where a voucher specimen was deposited.

**2.1.1 Sample preparation and groupings:** A weighing balance was used to weigh 750g of fresh beef in triplicate. The 750g of fresh beef were then divided into three equal parts, weighing 250g each, to represent the three different storage periods (12, 24, 36 hours). Every analysis were done at the end of each storage period using different method of preservation. The last portion of the beef served as the control group, while the other three portions of the beef underwent three different preservation methods at different storage times. There are four groups as shown below, one serves as the control while the other three depict the three method of preservation.

Group I: preserved under ambient temperature (room temperature);

Group II: Beef preserved with *R. communis* leaves;

Group III: Beef preserved by refrigeration and

Group IV: Control.

In group 1, each 250grams of the beef were placed on a clean aluminum foil and left under ambient temperature for 12, 24 and 26 hours whereas in the second group (beef preserved with *R. communis* leaves), each 250g of fresh beef were individually wrapped with *R. communis* leaves and stored at different storage time. In the third group, the same portion of the beef were preserved by placing it in a small plate and refrigerated, at the end of each storage time, samples were brought out for analyses. In control group, all analyses were carried out immediately after purchase at zero hour.

## 2.2 Physico-chemical analysis

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**2.2.1 Determination of pH:** The degree of alkalinity of the beef was determined by using a digital pH meter (PHS- 25 Microfield instrument England) according to the method described by Lampra Debbarma et al. [12]. pH value of the beef was determined by weighing 3 grams of sample into a blender with 27mL of distilled water and homogenized until smooth slurry was formed. The digital pH meter was placed in a buffer solution to allow equilibrium for two minutes before placing it into the prepared slurry.

**2.2.2 Proximate analysis:** Proximate analysis was carried out on each of the samples. The micro-Kjeldahl method described by AOAC [13] was used to determine the crude nitrogen content and the value multiplied by 6.25 to obtain crude protein. Fat, carbohydrate, ash and moisture contents were also determined by AOAC (13) .

**2.2.3 Water-holding capacity:** The water-holding capacity of the sample was determined by the method of Adewunmi and Oluyode [14]. A small sample was pressed between 2 filter papers with a plexiglass for over 1 minute using a table device. The quantity of juice released from the sample was measured indirectly by measuring the area of the filter paper wetted, relative to the area of the pressed sample. The compressed area of beef sample and the area covered by the water let out onto the piece of tracing paper was traced out and then transferred onto a graph sheet. The calculated area was then used to calculate the water holding capacity.

$$\text{WHC} = \frac{(\text{Meat film area})}{\text{Area of spread juice}} \times 100$$

**2.2.4 Cooking loss:** Cooking loss was determined according to the method described by Adewunmi and Oluyode [14]. Sausage samples from each time post-mortem were taken and weighed before cooking for 20 minutes. Cooked samples were allowed to cool and re-weighed. Cooking loss was calculated in percentage using:

Cooking Loss (%) =

$$\frac{\text{Weight of the sample before cooking} - \text{the weight of the sample after cooking}}{\text{Weight of sample before cooking}} \times 100$$

Weight of sample before cooking

**2.2.5 Determination of extract release volume (ERV):** The extract release volume was determined following the method of Lampra Debbarma et al. [12]. Twenty-five grams of meat samples were blended in a laboratory blender with 100 ml distilled water for 2 minutes and filtered by Whatman no.1 filter paper. The volume of the aqueous portion of the filtrate collected in a graduated cylinder in the first 15 minutes was taken as the ERV of the sample.

**2.2.6 Drip loss:** Drip loss (DL) was measured as following the method described by Bao et al. [15]. Two hundred and fifty grams (250g) of meat sample was used as initial throughout the storage period. After each storage period, the sample was wiped and dried with filter paper and weighed.

% Drip Loss = 
$$\frac{\text{Actual weight} - \text{Final weight}}{\text{Actual weight}} \times 100$$

Actual weight

**2.2.7 Determination of Lipid peroxidation:** Malondialdehyde [MDA] was estimated as a thiobarbituric acid reacting substance (TBARS) as described by Ashok et al. [16]. A 20g of beef was homogenised with 100 ml of distilled water for 2 minutes. The mixture was filtered through Whatman filter paper, to 2.5 ml of the filtrate was added to 2.5 ml of 20 % Trichloroacetic acid (TCA) and 2.5 ml of 0.02M thiobarbituric acid (TBA). The solution was incubated at 100°C for 35 minutes and cooled afterwards under a running tap absorbance read at 532 nm and the result was expressed as TBARS (mg MDA/kg beef) with values calculated as follows:

TBARS = O.D x 7.8

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where O.D = Absorbance of the sample at 532nm.

**2.2.8 Organoleptic attributes:** The sensory evaluation of the beef samples was conducted at the Nutritional and Industrial Biochemistry Research Laboratory, Department of Biochemistry as well as the Animal Science Department of the University of Ibadan. A total of ten (10) trained individuals aged between 20 and 30 years were used to access two replicates of the prepared sausage. The samples were evaluated using a 9-point hedonic scale for flavour, colour, juiciness, tenderness, texture, aroma and overall acceptability Fadare and Arogbo [17]. The samples were rated on a nine-point hedonic scale with a maximum score of extremely high condition while the lowest score of 1 was assigned to the poorest condition Oyedepo and Oshibanjo [18]. Equal bite size from the four treatments was coded and served. Each sample was evaluated independently of the other.

**2.3 Statistical analysis:** The data were analysed using one-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA) while comparisons were made between the *R. communis* treated beef, ambient and refrigerated beef samples against control using Independent T-Test (XLSTAT version 20). The level of significance was taken to be  $p < 0.05$  and values were expressed as Mean  $\pm$  SD.

### 3. RESULTS AND DISCUSSION

The physio-chemical and proximate analysis are shown in Tables 1 and 2 respectively. It was observed that, except in a few instances, there are significant differences between the treatment modalities (ambient, *R. communis* and refrigeration) used as compared with the control across various time intervals (12, 24 and 36h).

At the 12h interval, significant differences exist in all parameters except in the EE (refrigeration), TOTAL (refrigeration), NFE(refrigeration), ERV (ambient), pH (*R. communis*), Drip loss (ambient, *R. communis*, refrigeration) and WHC (ambient & *R. communis*).

At the 24h interval, significant differences exist in all parameters except in the ASH (ambient, *R. communis* and refrigeration), TOTAL (ambient), NFE (ambient & *R. communis*), pH (refrigeration), Drip loss (ambient, *R. communis* and refrigeration) and WHC (ambient and refrigeration). At the 36h interval, a significant difference ( $P < .05$ ) exists in all parameters except in the ASH content (*R. communis* and

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refrigeration), pH (refrigeration), Drip loss (ambient, *R. communis* and refrigeration) and WHC (refrigeration).

Beef preserved with *R. communis* leaves shows no significant difference in the pH at 12h duration, but at 24 and 36h intervals there is a significant difference ( $P < .05$ ) in the pH, whereas the refrigerated beef samples showed no significant difference in pH at both the 24 and 36h intervals. The gradual but consistent rise in the degree of meat's alkalinity in *R. communis* preserved and ambient temperature beef at 24 and 36h storage period may be due to production and accumulation of odoriferous nitrogenous compounds, as well as degradation of amino acid [19, 20].

At zero hour, the control group had no DL, when compared with the other three groups at different storage periods. Group 1 (meat under ambient temperature) shows the highest percentage of drip loss. There was no significant difference ( $P < .05$ ) in drip loss in all groups when compared with the fresh meat samples. The increase in drip loss may be due to the degeneration of protein, sarcomere shortening [21] and myosin degeneration [22] resulting in myosin shrinkage, which draws the thick and thin filaments more closely together [23].

Extract release volume is another index for determining beef of good quality. There was a significant difference among all three groups as compared with control except at 12h interval ambient. There was a significant increase ( $P < .05$ ) in the extract release volume of beef preserved with *R. communis* at 12h as well as refrigerated beef samples at 12 and 24h when compared with control and ambient temperature meat groups. This work showed that at 12h in all groups, there is beef of good quality which corroborates [12] Lampra Debbarma et al., 2021) findings. The significant decrease in ERV in 24h *R. communis*, ambient and 36h refrigerated beef may be due to microbial spoilage.

The degree of oxidative rancidity in fats may also be determined by its thiobarbituric acid (TBA) number. The thiobarbituric acid measures the breakdown of products of unsaturated fatty acid oxidation. The TBA number of a sample increases as it becomes more rancid, but a certain amount of variation is observed in TBA numbers obtained for fresh samples. As with peroxide value, a low TBA value is not an absolute indicator of fat quality, aldehydes may have not been formed or volatile aldehydes may have been lost during processing and storage [23]. In this study, there was a

significant increase ( $P < .05$ ) in TBARS value in all the groups as compared with control. The breakdown of valuable contents (fat, protein and carbohydrates) in meat leads to the development of flavours, off-odours and often slime formation which makes the meat product undesirable for human consumption.

**Table 1: The physico-chemical analysis of beef samples at various storage period**

Beef Preservation methods	Storage time (HOURS)	pH	ERV(ml)	TBARS (mgMDA/kgbeef)	DL(%)	WHC(%)
Ambient Temperature	12	5.33±0.01*	33.00±1.41	0.20±0.01*	240.76±13.07	37.65±2.33
<i>R. communis</i> leaves	12	5.70±0.03	46.00±1.41*	0.23±0.04*	244.07±8.39	40.97±3.54
Refrigerated	12	5.58±0.02*	47.00±1.41*	0.20±0.05*	249.15±1.20	41.88±1.38*
Ambient temperature	24	5.49±0.01*	27.00±0.00*	0.22±0.05*	233.45±23.41	37.65±3.33
<i>R. communis</i> leaves	24	5.62±0.03*	21.00±0.00*	0.18±0.01*	244.82±7.33	42.69±1.85*
Refrigerated	24	5.74±0.01	45.00±0.00*	0.21±0.04*	248.19±2.57	36.61±1.87
Ambient temperature	36	6.29±0.02*	7.00±0.00*	0.28±0.04*	233.11±23.89	40.84±1.18*
<i>R. communis</i> leaves	36	6.80±0.03*	8.00±0.00*	0.20±0.03*	238.79±15.86	31.51±0.11*
Refrigerated	36	5.81±0.01	28.00±0.00*	0.28±0.03*	246.75±4.60	31.65±1.25
Control	0	5.78±0.03	35.00±0.00	0.02±0.00	250.00±0.00	35.00±0.00

Mean±SD, n = 3, \* means significant difference,  $P < .05$

**Table 2: Proximate Analysis of beef at different storage period**

Beef Preservation Methods	Storage time (HRS)	ASH	EE	MC	NFE	Crude protein	TOTAL
Ambient Temperature	12	1.45±0.07*	0.77± 0.078*	76.54±0.09*	6.13± 0.04*	15.60±0.06*	93.95±0.07*
<i>R. communis</i> leaves	12	1.25 ±0.06*	1.49± 0.72*	75.71±0.13*	4.36± 0.05*	17.92±0.11*	95.68± 0.04*
Refrigerated	12	1.50 ±0.04*	2.07± 1.32	75.12±0.11*	4.84± 0.05	17.75±0.08*	95.26± 0.08
Ambient temperature	24	1.10 ±0.01	1.77± 0.15*	72.37±0.05*	4.66± 0.04	20.36±0.09*	95.39± 0.02
<i>R. communis</i> leaves	24	1.06± 0.03	0.60± 0.11*	73.97 ±0.05*	5.95± 5.59	14.80±0.28*	90.13± 0.05*
Refrigerated	24	1.99±1.43	1.31± 0.10*	75.25± 0.08*	10.42±0.04*	12.25±0.07*	89.66± 0.06*
Ambient temperature	36	1.37± 0.04*	0.61± 0.09*	72.88± 0.03*	9.86± 0.05*	15.52±0.11*	90.20± 0.03*
<i>R. communis</i> leaves	36	1.08± 0.04	2.17± 0.06*	73.46± 0.20*	3.79± 0.05*	19.83±0.11*	96.29± 0.05*
Refrigerated	36	1.01± 0.02	1.54± 0.09*	75.34± 0.09*	7.59± 0.05*	14.80±0.13*	92.49± 0.06*
Control	0	1.05± 0.021	3.71± 0.13	74.18± 0.04	4.76± 0.05	16.54±0.09	95.32± 0.05

Mean±SD, n = 3, \* means significant difference, P<.05

Data in Table 3 showed that there was a significant difference in the variables investigated in the different treatment modalities (ambient, *R. communis* and refrigeration) across the time intervals (12, 24 and 36h) in comparison with control (fresh beef), except in some instances. At the 12h interval, significant differences (P < 0.05) do not exist in all sensory parameters except in the Texture (Ambient). At the 24-hour interval, significant differences do not exist in all sensory parameters except in the Juiciness (Ambient and Refrigeration), Texture (Ambient and Refrigeration) and Overall (Ambient). At the 36h

interval, a significant difference does not exist in all parameters except in the Aroma (Ambient), Juiciness (Ambient and Refrigeration), Overall (Ambient) and Flavour (Ambient).

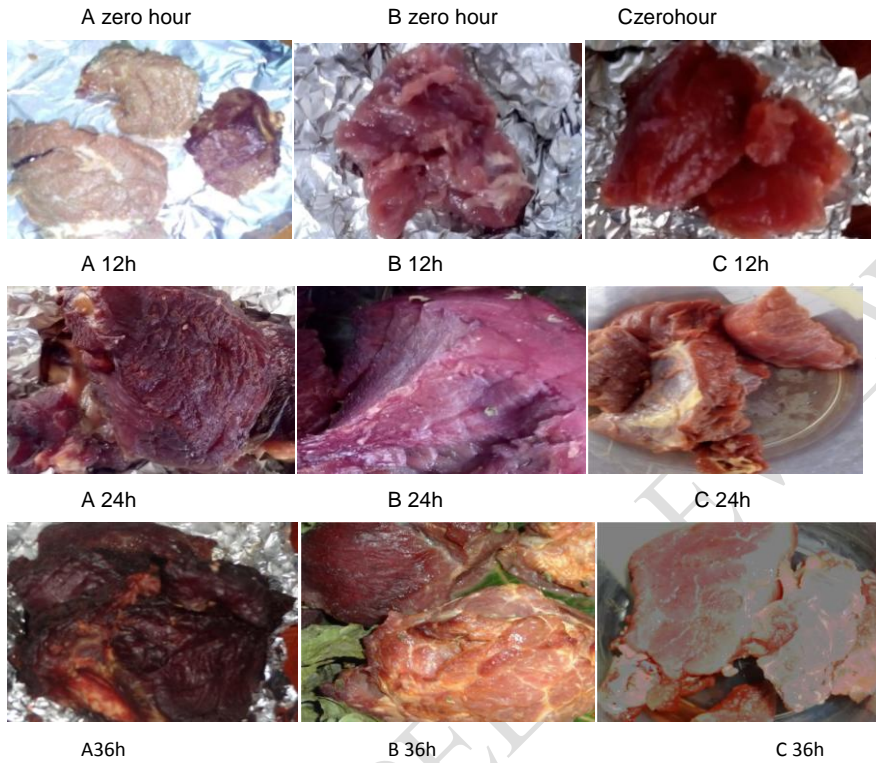
**Table 3: Sensory analysis of beef at various storage time**

Preservation Methods	Storage time	Aroma	Colour	Tenderness	Juiciness	Texture	Flavor	Overall
Ambient temperature	12h	6.00±2.35	5.60±2.07	5.20±1.79	4.00±2.12	4.00±0.71*	5.80±2.05	6.60±1.95
<i>R. communis</i>		7.20±0.84	6.40±1.52	5.20±1.10	4.40±1.52	5.80±1.30	7.40±0.89	7.00±1.41
Refrigerated		6.00±1.87	6.40±0.55	5.60±2.51	6.20±1.92	5.20±1.30	6.60±1.52	7.00±1.87
Ambient temperature	24h	5.20±2.49	5.00±2.35	6.20±1.10	3.00±0.71*	4.00±1.41*	5.00±2.35	5.20±1.30*
<i>R. communis</i>		6.80±1.10	6.00±1.41	4.40±1.67	5.40±1.34	5.60±1.52	6.80±0.45	7.00±0.00
Refrigerated		7.00±1.73	8.00±0.71	6.00±1.41	4.00±1.73*	5.20±1.10*	6.60±1.52	7.00±1.00
Ambient temperature	36h	3.60±2.30*	4.60±2.51	5.80±2.17	2.60±0.55*	5.20±2.17	4.40±1.82*	4.20±1.79*
<i>R. communis</i>		5.00±2.35	5.60±1.67	5.80±2.17	4.60±1.82*	5.80±1.10	6.00±1.00	6.20±0.84
Refrigerated		6.20±1.79	6.60±0.55	6.40±1.52	4.20±1.30*	5.80±1.30	5.40±1.52	7.00±1.00
Control		7.40±1.52	7.60±1.14	6.60±1.67	6.60±1.67	7.00±1.23	7.40±1.52	7.80±1.30

Mean±SD, n = 3, \*means significant difference, P<.05

Figure 1. Beef samples undergoing different methods of preservation at different storage times





A: Beef at Ambient temperature

B: Beef preserved with *Ricinus communis* leaves

C: Refrigerated beef

## CONCLUSION

From the study, we deduced that *Ricinus communis* leaves help in extending the shelf life of fresh beef for 24h as well as regulating the pH of beef samples. Hence, further study is needed to know the desirability of the practice of preserving beef with *Ricinus communis*.

Comment [DZ9]: Not accurate

## ETHICAL APPROVAL

Not applicable to this work. It is not a human or animal study.

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Comment [DZ12]: Name of journal

UNDER PEER REVIEW

