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Effect of Boiling, Oven Drying and Roasting On the Proximate Composition and Microbial Loads of Cane Rat

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ABSTRACT: The study examined the effect of proximate composition, mineral and microbial loads of cane rat (*Thryonomys swindarianus*) meat of different processing methods. Three (3) live adult cane rats were purchased from reputable cane rat domestication farm in Abeokuta and transported to the wildlife laboratory of the Federal University of Agriculture where the samples were slaughtered and subjected roasting, boiling and oven drying. Small piece of each processed cane rat meat was extracted and placed in sterile plastic containers for proximate, mineral and microbial loads for three (3) consecutive days. The standard procedure described by Holt *et al.,* (1994) method was used for the analysis of moisture content, crude protein, total available carbohydrate and ash. Data obtained were subjected to analysis of variance using least significant difference to separate the means and line graph was used for the microbial count. The results showed that from day 1 to day 3 oven dried meat had the highest ash, F. extract, C. protein and C. fat content compared with other processing methods. In the same vein, high potassium, iron and magnesium content was recorded in oven dry cane rat. Furthermore, there are presence of Staphylococcus aureus, Bacillus spp, Salmonella spp and Escherichia coli in the meat samples which can cause serious health problems. To encourage sustainable utilization of bush meat consumption in urban and rural areas, oven dried bush meat is the most appropriate method of processing, this should be used and encourage by the seller.

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Keywords: Bushmeat, Microbial, Animal protein, Mineral

Bushmeat represents a vital dietary which serves as alternative source of animal protein among greater majority of rural people in Africa (Oduntan *et al.,* 2012). Many poor rural dwellers depends on bush meat consumption and trade as a means of livelihood (Emelue and Idaewor, 2018). Cane rat is the most preferred among the wild animals (Byanet *et al.,* 2015). The acceptability of cane rat as food is as a result of the nutritional quality of the meat (Adu *et al.,* 2017). In Nigeria, the meat is regarded as the king of bush meat because of its exceptional tastes (Ibitoye, 2019). The meat is specially prepared and sold in special joints in cities and towns. Cane rat is high in iron, calcium and phosphorous and very low in cholesterol (Ogogo *et al.,* 2017) when compared to other conventional meat types such as rabbit, chicken, goat, sheep and cow. As reported by (Owen and Dike, 2013), for normal growth and development, 34g of animal protein must be consumed per person per day. However, in Nigeria, due to high ignorance and poverty levels, animal protein consumption stands averagely at 7-10 g/person/day (Ebenebe and Okpoko, 2015) and Livestock meat appears to be unavailable and unaffordable by many Nigerians (Meludu and Onoja, 2018). Increase in demand for cane rat as resulted in the use various capturing techniques such as baiting with poison which is cruel and poses a serious threat to human health (Ogada, 2014). Aside the high nutritional value to the consumers, generally, meat often are ideal culture media for microbial growth and spoilages bacteria because of their perishable nature (Afrin *et al.,* 2002). In several countries, a number of meat borne infections has been reported on fresh raw meat such as cane rat (Mukhopadhyay *et al.,* 2009). Despite the contribution to the daily protein intake and the role they play in the livelihood of most Africans, cane rat meat is a source food-borne illness resulting from improper handling, slaughtering, transporting and how this meat are sold in Nigerian markets. This study was carried out to provide information on the adequate processing method of bush meat in order to maintain bush meat

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quality and to minimize possible incidence of food-borne infections amongst consumers.

MATERIALS AND METHODS

Study area: The study was conducted in the Department of Forestry and Wildlife Management, Federal University of Agriculture Abeokuta (FUNAAB), Nigeria. FUNAAB is located along Alabata road, on 10,000 hectares of land, in North-East part of Abeokuta, Ogun State. It's located between Latitude 7^0 30'N and Longitude 3^0 54'E. The region enjoys mean annual rainfall of 1113.1 mm, mean monthly temperature varies between 22.9 ^{0}C-36.32 ^{0}C and relative humidity ranges between 75.52 ^{0}C and 88.15 ^{0}C (Aiboni, 2001).

Sample collection: Three (3) live adult cane rats were purchased from Department of Forestry and Wildlife Management Domestication uint, Federal University of Agriculture Abeokuta (FUNAAB) for this study. The samples were collected in a transport cage for easy transportation and to ease the stress of the specimen. The cane rats (*Thryonomys swindarianus*) were slaughtered, washed and then subjected to Smoking, boiling and oven drying methods. After processing, the samples were taken to the Microbiology Laboratory within 24 hours for further tests to determine the proximate composition and microbial loads of the cane rat carcass.

Microbiological analysis: The samples were processed for microbiological analyses by dissecting in order to isolate the enteric bacteria. One gram of the intestinal gut was homogenized in 9 ml of sterile normal saline after which the homogenized samples were serially diluted to 10-4 (Douglas and Amuzie 2017). For isolation of the bacteria from the skin, ten (10) grams of the whole cane rat was submerged into 90 ml of sterile normal saline and shaken vigorously in order to dislodge the bacteria associated with it. Further 10 - fold serial dilutions were carried out by adding 1 ml of the initial dilution to 9.0 ml of appropriate diluents. Finally, 0.1 ml of appropriate dilutions was inoculated on dried nutrient agar, Mannitol salt agar and MacConkey agar.

Isolation and enumeration of bacterial isolates: An aliquot (0.1 ml) of 10-3 to10-4 dilutions of each samples were inoculated on Nutrient agar (for total Heterotrophic bacteria), while an aliquot (0.1 ml) of 10-3 dilution on Mannitol and MacConkey agar for isolation and enumeration of Staphylococci species and enteric bacteria respectively, using the spread-plate technique as described by Prescott *et al.,* (2005). The plates were inoculated in duplicates and incubated under aerobic condition at 37°C for 24 hours except for the MacConkey plates to be used for the isolation of faecal coliform that was incubated at 45°C for 24 hours. The numbers of colonies in each plate was counted and mean values calculated for duplicate dilutions, which was expressed as colony forming unit per gram $(cfu\ /\ g)$ using the equation below;

$$cfu/g = \frac{NC}{Dilution} \times Volume\ plated\ (0.1\ ml)$$

Where NC = number of colonies

Maintenance of pure culture: Discrete bacterial colonies that grew on the respective media plates was sub cultured using streak plate method onto fresh medium and incubated for 24 hours at 37°C. The pure bacterial cultures was then be maintained according the method as adopted by Amadi *et al.,* (2014) using ten percent (v/v) glycerol suspension at -4°C.

Characterization and identification of isolates: The isolates were characterized based on their appearance on the culture media that is; shape, colour, wetness, dryness, etc, while identification of the characterized isolates were done via Biochemical tests such as Gram Reaction, Catalase, Oxidase, Motility, Citrate, Indole, MR/VP, Glucose, Lactose, Fructose, Manitol, Sucrose, Galactose according to Bergey's Manual of Determinative Bacteriology (Yalcin *et al.,* 1995).

Proximate Analysis: The proximate composition of the edible cane rat specimen were determined according to standard methods described by Holt *et al*., (1994) which was carried out in duplicates. The parameters to be analyzed includes: moisture content, crude protein, total available carbohydrate, ash, and averages taken.

Determination of moisture: The crucibles were cleaned and dried using the air oven for 10 minutes. They were kept in the desiccators to cool and weighed. The samples were thoroughly mixed and 5 g weighed into the crucibles. Crucibles plus content will be placed in the oven at 103 ± 2°C overnight. The crucibles were then be removed and reweighed after cooling. They were dried for another one hour to ensure constant weight. The moisture content was calculated using the formula below:

$$\%\ moisture = \frac{LWS\ (g)}{WS\ (g)} \times 100$$

Where LWS = loss of weight of sample; WS = weight of sample

Determination of crude protein: This wasill be done using the Micro-Kjehdhal method. Half gram (0.5 g) of the cane rat sample were weighed into one quarter size filter paper, one tablet of catalyst were added followed by 10 ml concentrated sulphuric acid in a digestion flask in duplicates. The flasks were then be placed in the heating unit inside the fuming cupboard and heated slowly until the sample is boiled. The temperature was then be increased until foaming ceases and the content of the flask completely liquefied. The digestion will be done by boiling vigorously while agitating the flask until the solution becomes completely clear. Digestion will be terminated, samples cooled and weighed into a 100 ml flask with distilled water. Five millilitres (5 ml) of boric acid mixed. Indicator solution was be transferred into a 100 ml conical flask placed at the end of the condenser of the micro kjehdhal distillation apparatus so that the adapter was be dipped into the liquid. Ten millilitres (10 ml) aliquot of sample was pipetted into a micro kjehdhal flask for distillation. Ten millilitres (10 ml) of 45% Sodium hydroxide (90 in 200 ml) was be poured carefully down the inclined neck of the solution. The flask was immediately attached to the splash head of the distillation apparatus. Steam was passed through alkaline liquid (i.e. NaOH + aliquot) slowly until it is boiled. The liquid was be trapped and distilled into 5 ml boric acid in the conical flask until 50 ml of the distillate is collected with a green colour and then titrated with 0.045 N Sulphuric acid. The bank was prepared in the same way. Crude protein was calculated as follows:

$$N_2\,\% = \frac{Titre - Blank\ \times N \times 1.4}{Weight\ of\ sample}$$

N2 = nitrogen; N = normality of acid

$$Crude\ protein\,\% = Total\ Nitrogen\ (\%)\ \times 6.25$$

Where 6.25 = Conversion factor.

Determination of fat: The Micro-Soxhlet extraction method was used. Two-grams (2 g) of the dried samples used for the determination of moisture content was used for fat extraction. This is to make the fat more available for extraction. The samples were wrapped in a filter paper and held with the clip in the extraction unit in which a weighed flask containing 50 ml of petroleum ether (60-90°C) will be attached while on the heating plate. The extractor was connected to a reflux condenser on a steam bath for 3 hours. The petroleum ether extract was evaporated to dryness at 100°C for 5 minutes. The flask will be cooled in the desiccator and weighed. Extractable fat was calculated using the equation;

$$\%\,Fat = \frac{\text{WFF} - WWF}{\text{WSBD}} \times 100$$

Where WFF = weight of empty flask (g); WWF = Weight of without fat; WSBD = weight of sample before drying

Determination of Ash (AOAC 1990): Six crucibles were washed and placed in the oven for 5 minutes. The crucibles were removed, cooled in the desiccators for one hour and weighed. 5 g of the sample was weighed into each crucible, placed on hot plate under a fume hood and temperature was increased slowly until smoking ceases and the samples becomes completely charred. The crucibles was placed inside the muffle furnace and ashed overnight at 550°C. The crucibles was removed from the furnace and placed in the desiccator for an hour. When cooled to room temperature, each crucibles plus ash was weighed and weight of ash calculated as follows;

$$\frac{C - A}{B - A} \times \frac{100}{1}$$

Where A= Weight of empty dish; B = Weight of dish + unashed sample; C = Weight of dish + ashed sample

$$ADStd\ \times WS\ (g) = 25\ \times ADS$$

ADStd = absorbance of dilute standard; WS = weight of sample; ADS = absorbance of dilute sample

Determination of Crude Fibre: Five gram of each of the sample were heated with 1.25% H2SO4 for 30 minutes and then filtered. The residue was washed with distilled water until it was acid free. 1.25% solution NaOH was used to boil the residue for 30 minutes and was filtered and washed several times with distilled water until it was alkaline free. The residue was put in a crucible and dried at 105^0C in an oven overnight. After cooling in a desciccater and weighed (W1). It was ignited in a nuffle furnace at 550^0C for 90 minutes to obtain the weight of the ash (W2).

$$\%\,Crude\ fibre = \frac{\text{W1} - W2}{\text{Weight of sample}} \times 100$$

Microbial Quantifications: 10(g) of the sample was aseptically removed and homogenized in a Stomacher 400 Circulator Lab Blender (Seward, Worthing, UK) with 90 mL of 0.1% peptone salt solution. Further serial dilutions was made in the same diluent and used for standard plate enumerations. Total aerobic mesophilic and psychrotrophic bacteria were determined on Plate Count Agar (pour plate method)

with incubation at 30^{0}C for 72 h, and 7^{0}C for 10 days, respectively; Enterobacteriaceae on Violet Red Bile Glucose Agar at 37^{0}C for 24 h; Escherichia coli on Tryptone Bile X-Glucuronide Medium (TBX) at 44^{0}C for 24 h, lactic acid bacteria on MRS Agar (pour plate method) in anaerobiosis (Anaerogen 2.5L) at 30^{0}C for 72 h; Brochothrix thermosphacta on Streptomycin Thallous Acetate (STA) agar with STA selective supplement at 25^{0}C for 48 h; Pseudomonas spp. On Pseudomonas Agar base with CFC supplement at 25^{0}C for 72 h; yeasts and moulds on Yeast Extract Glucose Chloramphenicol Agar (pour plates method) after incubation at 25^{0}C for 5 days. Where not specified, spread plate method was used. The bacterial counts was be expressed as log Colony-Forming Units (CFU) per gram of sample.

Data Analysis: Descriptive statistics such as line graph was used to describe the growth in microbial loads of the different processed meat samples and least significant different was used to separate the means. The rate of change in growth of microbial count was determined using this formula:

$$Gradient = \frac{Y}{X}$$

Where Y= total bacteria count; X = number of days

RESULTS AND DISCUSSION

Effect of processing methods on proximate content of cane rat: Table 1 shows the proximate composition of cane rat. In the first day, the result shows that oven dried meat had the highest ash, F. extract, C. protein, C. fat content by representation of 2.37%, 30.06%, 19.32%, 15.39% respectively while low moisture and carbohydrate were recorded. It was also shown in the result for the second day that, oven dried meat had the highest ash, F. extract, C. protein, C. fat content representing 1.89%, 26.38%, 20.92%, 12.10% respectively. Low moisture and carbohydrate was recorded in oven dried meat by representation of 34.16% and 3.99%. In the same vein, on the third day, oven dried meat had the highest ash, F. extract, C. protein, C. fat 2.03%, 27.02%, 19.93%,13.38 respectively. Low moisture content was recorded in oven dried meat representing 32.76% while lowest carbohydrate was recorded in boiled meat representing 2.17%.

Effect of processing methods on mineral content of cane rat: Mineral composition in table 2 shows that, in the first day, oven dry meat had the highest Magnesium (11.67 mg/l), Potassium (62.22 mg/l) and Iron (1.15 mg/l) while Calcium (6.88 mg/l) was found to be higher in boiled meat sample. The result from the second day shows that Magnesium (9.83 mg/l) and Potassium (57.67 mg/l) was found to be high in oven dried meat while boiled meat had the highest Calcium (7.62 mg/l) and Iron (1.78 mg/l). Furthermore, on the third day, boiled meat had the highest Calcium (8.06 mg/l) and Iron (1.45 mg/l) while oven dried meat had the highest in Magnesium (10.43 mg/l) and potassium (59.63 mg/l).

Identification of organisms in the processed meat: The result in Table 3 shows the identification of organisms in the processed meat. The positive (+) sign denotes there is presence of microorganisms while the negative (-) sign denotes there is absence of microorganisms. The letter A, B, C, D and E represents Streptococcus species, Bacillus species, Salmonella species, Staphylococcus aureus and Escherichia coli respectively. In boiled, A,C,D were present in day 1, A,B,C,E present in day 2, A,B,C,D,E present in day 3. In roasted, B,D,E were present in day 1, B,C,D present in day 2, and A,B,C,D,E present in day 3. In oven dried, A,D were present in day 1, A,B present in day 2, and A,B,D present in day 3. *Rate of change in growth of microbial count:* The result in Table 4 shows the rate of change in growth of microbial count. The mean values for the rate of change in growth of microbial count on boiled, roasted and oven dried processed meat are 0.7, 0.6 and 0.25. The higher value of rate of change was obtained in boiled meat (0.7), this implies that the longer the days of boiled processed meat the more the microbial count.

Table 1: Effect of processing method on proximate content of cane rat

Treatments		**proximate**					
Day	**Processing methods**	Ash %	F. extract %	C. protein %	Moisture %	C. Fat %	CHO %
1	Boiled meat	1.82	13.04	15.56	55.22	7.04	6.54
	Oven dry meat	2.37	30.06	19.32	29.57	15.39	2.51
	Roasted meat	0.89	10.79	17.77	59.21	5.06	5.68
2	Boiled meat	1.29	11.11	15.45	60.31	5.58	4.78
	Oven dry meat	1.89	26.38	20.92	34.16	12.10	3.99
	Roasted meat	0.41	9.23	15.65	64.11	3.73	6.35
3	Boiled meat	1.92	15.08	15.17	59.10	6.02	2.17
	Oven dry meat	2.03	27.02	19.93	32.76	13.38	3.62
	Roasted meat	0.92	11.42	15.77	61.32	4.16	5.81
	LSD	0.2017*	0.1969**	0.3791**	0.1967**	0.2284**	0.198**

***p0.01, *p0.05*

Table 2: Effect of processing method on mineral content of cane rat

Treatments		**Minerals**			
Day	**Processing methods**	Ca mg/l	Mg mg/l	K mg/l	Fe mg/l
1	Boiled meat	6.88	8.77	29.98	0.78
	Oven dry meat	6.73	11.67	62.22	1.15
	Roasted meat	6.42	8.03	27.11	0.94
2	Boiled meat	7.62	8.34	32.29	1.78
	Oven dry meat	7.23	9.83	57.67	1.00
	Roasted meat	5.16	7.25	25.11	1.37
3	Boiled meat	8.06	9.82	25.64	1.45
	Oven dry meat	7.88	10.43	59.63	0.93
	Roasted meat	6.90	7.06	30.05	0.89
	LSD	0.320*	0.199*	0.196**	0.200*

***p0.01, *p0.05*

Table 3: Identification of organisms in the processed meat

Days	**Boiled**					**Roasted**					**Oven dried**				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
1	+	-	+	+	-	-	+	-	+	+	+	-	-	+	-
2	+	+	+	-	+	-	+	+	+	-	+	+	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-

NOTE: = Streptococcus spp; = Bacillus spp; = Salmonella spp; = Staphylococcus aureus; = Escherichia coli

Table 4: Rate of change in growth of microbial count

Boiled			**Roasted**		**Oven dried**	
Days	**TBC (Cfu/g)**	**Gradient**	**TBC (Cfu/g)**	**Gradient**	**TBC (Cfu/g)**	**Gradient**
1	1.4	0.6	1	0.2	0.7	0.3
2	2	0.8	1.2	1	1	0.2
3	2.8	0.7	2.2	0.6	1.2	0.25
Mean		0.7		0.6		0.25

TBC - total bacteria count

Total bacteria count of processed meat: The result in figure 1 shows the total bacteria count (TBC) and days. The total bacteria count for boiled meat showed (1.4, 2.0 and 2.8 Cfu/g) for days 1, 2 and 3 respectively, for roasted meat (1.0, 1.2 and 2.2 Cfu/g) for days 1, 2 and 3 respectively while for oven dried meat (0.7, 1.0 and 1.2 Cfu/g) for days 1, 2 and 3.

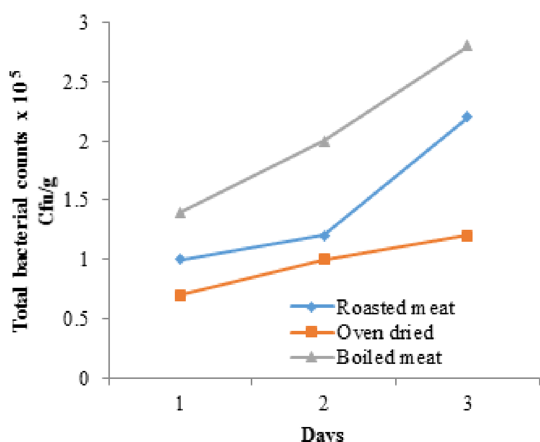

Fig 1: Total bacteria count of processed meat

In the proximate analysis of the boiled, roasted and oven-dried portions of cane rat as shown in the result from day 1 to day 3. Oven dry method produced lower moisture content in comparison with boiled and roasted method. The lowering of the moisture content lowers water activity and the incidence of microbial spoilage that induces zoonotic diseases. This corroborate with the findings of Holley, (2005) who opined that lower water activity in food reduces antimicrobial potency. The percentage of crude protein of oven dry was found to be higher than that of roasted and boiled method. This agrees with the findings of Abulude, (2007) who found the protein content of bush meats to be of higher quality than meat and fish thus making it a major alternative in meeting dietary protein shortages in Africa. The ash content of oven dried meat was slightly higher than that of boiled and roasted. This is in line with the findings of Emelue *et al.,* 2017 who reported lower ash content of smoked and oven dried duiker. The result further revealed that fat content of oven dry cane rat was found to higher than boiled and roasted while lower carbohydrate was recorded in oven dry cane rat. However, the result from mineral composition showed that boiled dried meat sample had highest percentage of calcium while higher percentage of potassium, iron and magnesium was found in oven dry cane rat. Furthermore, there are presence of microorganisms in the meat samples which can cause serious health problems though varies from day 1 to day 3. The difference might be due to disparity in the processing methods, sanitation of the processing area and handling. Clarence *et al.,* (2009)

noted that bacteriological quality of food is important parameter for assessing food safety.

Conclusion: The proximate and mineral analysis revealed that the boiled, roasted and oven dried Cane rat meat had high nutrient profiles. In addition, the result from the microbial count revealed that the longer the days of boiled processed meat, the more the microbial count. However, oven-dried processing methods gave rather better results on the proximate and mineral composition of Cane rat which will encourage the sustainable use of bush meat and reduce microbial spoilage.

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