Proximate and Biological Properties of Dried Tympanotosus Fuscatus and Pachymelania Aurita Periwinkle Meat

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Abstract:
Periwinkles are small edible snails commonly available for sale in markets, most especially in the southern part of the country. Its consumption has drastically increased in sub-sahara Africa due to the recent awareness on avoiding red meat for health reasons. Also,it is useful for general health and brain development. However, periwinkles are known to harbor various microorganisms that they consume during feeding. Despite it's enormous importance, there is limited literature on the biological and proximate properties of tympanotosus fuscatus and pachymelania aurita periwinkles. This study aims to determine the effect of cabinet and oven drying methods on periwinkle meat's proximate and microbiological properties. The washed periwinkle samples were sorted manually to remove dirt and other extraneous materials. It was then blanched in hot water (100ºC for 10mins), using a sterilized needle to extract the meat. The initial microbiological and proximate properties of the two periwinkle meat varieties extracted were then determined. Afterward, the periwinkle meat samples were dried using the cabinet and oven drying methods to a safe storage moisture content level of 8.69% and 13.5%. The results of the microbial analysis reveal that fresh periwinkles have higher microbial loads than the oven and cabinet-dried samples. The bacterial load of pachymelania aurita and tympanotosus fuscatus for freshly extracted meat samples ($4.23x10^4$ cfu/g, $4.05x10^4$ cfu/g) was reduced to $1.15x10^3$cfu/g, $1.21x10^3$ cfu/g after oven-drying and $1.26x10^3$ cfu/g, $1.31x10^3$ cfu/g for cabinet dried samples. The fresh samples' fungi count, coliform count, and salmonella count were drastically reduced in the oven and cabinet dried samples with statistically significant differences ($P< 0.05$). Generally, the analysis revealed that oven-dried periwinkles are nutritionally richer than the fresh and cabinet-dried periwinkle samples due to its higher proximate values. The generated data will help design and develop efficient processing, drying, transportation, and storage of periwinkle meat.
Keywords: Periwinkle, drying, proximate,biological, oven, cabinet

1. Introduction

Periwinkle is the common name of tiny edible snails in Littorinidae. The two common small edible sea snails in Nigerian brackish and freshwater bodies are tympanotosus fuscatus and pachymelania aurita. These seafoods areprevalent in Nigerian markets, and the sales are always high, particularly in the Southern geo-political zone. Almost all soup preparations require periwinkle, particularly in native foods like Ekpang and Nkukwo. Also,with their large populations in Eastern and Western Africa, many snails are consumed, sold for income, and exported to other countries [1] and [2]. Periwinkles harbor a wide variety of microorganisms consumed during feeding; they use their radulae to pull algae from rock surfaces, pick up algae from cord grass, or biofilm that covers the surface of mud in estuaries [3].

Notwithstanding the enormous importance of periwinkle, there is limited research on the biological and proximate properties of tympanotosus fuscatus and pachymelania aurita periwinkles. Assessment of selected mollusk shells are some of the past studies undertaken on periwinkle [1]. The nutritional benefits of periwinkle meat cannot be overemphasized because it offers the required protein needed by man. Periwinkle meat is high in protein and low in fat [3] and [2]. Periwinkle consumption has increased in sub-sahara Africa largely because more people are avoiding red meat for health reasons [4]. It is helpful for body and mental development and also boost brain lipid contents with Omega 6 fatty acids [1].

The essence of drying includes preserving food and increasing its shelf life by reducing the water content. Drying helps to limit or prevent the need of refrigeration systems for transport and storage of food materials; it also aids in reducing space requirements for their storage and transport. Finally, to produce foods with different flavors and textures by offering the consumers choice of foods to buy [5]. This paper aims to determine the effect of cabinet and oven drying on the microbial and proximate properties of periwinkle samples (tympanotosus fuscatus and pachymelania aurita). It will benefit the food processing industry and relevant stakeholders by providing viable knowledge on extension of shelf life of these varieties and conservation of their nutritional quality.

2. Materials and methods

The materials used for this study include; fresh samples of tympanotosus fuscatus and pachymelania aurita, oven dryer (MINO/50), cabinet dryer, digital weighing balance (Wensar-TTB3), mercury in glass thermometer, digital vernier caliper (Digital LD-6inH), Glass cylinders, colorimeter (Sherwood-

260UV), plywood, glass, metal, calibrated protractor, gas cylinder, sterilized needle, graduated cylinder, water, thermocouple.

Sample preparation

The two periwinkle samples were procured from Itu-waterfront market in Akwa Ibom State, Nigeria. The periwinkle samples were cleaned with water, graded and taken to the laboratory for analysis. The two periwinkle samples were kept in a jute bag each and stored in a cool environment with shields and sandy grounds, which were water sprayed morning and evening to avoid dehydration of the snails. The washed periwinkle samples were sorted manually to remove dirt and other extraneous materials. It was then blanched in hot water (100ºC for 10mins), using a sterilized needle to extract the meat. The initial microbiological and proximate properties of the two periwinkle meat varieties were determined. Afterward, the samples were dried to a safe moisture content storage level (8.69% and 13.5%) using the cabinet drying and the oven drying methods. An existing cabinet dryer in the Department of Agricultural and Bioresources Engineering, Michael Okpara University, Umudike, was used in carrying out the drying. The cabinet dryer is made of galvanized sheet metals (length 105cm, width 65cm, and depth 85cm). It consists of flat metal trays (length 100cm, width 60cm, and depth 10cm), the drying cabinets, and a heat exchanger. The blower circulates heat in the chambers and also has an exit vent. The cabinet dryers` primary heat source was cooking gas to regulate burning heat pressure. The maximum temperature of the dryer was 60ºC, and the drying rate was checked with a time interval of two hours for each periwinkle meat sample until a constant moisture content was achieved.

The oven drying of the two periwinkle meat samples was done using a conventional electric oven dryer at a temperature of 105ºC for about 3 hours. The drying process was checked at a time interval of one hour for each periwinkle meat sample until constant moisture content was achieved. After drying was completed, the samples were subjected to microbiological and proximate analyses. The flow chart for the spiked periwinkle meat sample preparation is shown in Fig 1:

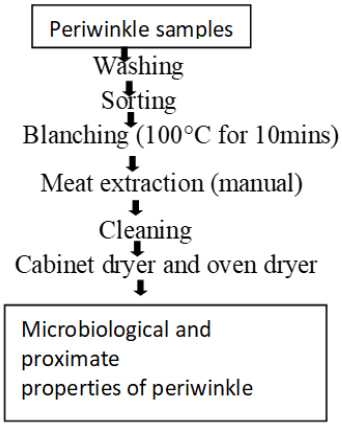

Fig. 1 Flow chart for spiked periwinkle meat processing

2.1 Microbiological Analysis

Microbiological analysis of freshly extracted, oven-dried, and cabinet-dried periwinkle meat samples was carried out. 10g of each periwinkle variety sample (fresh, oven-dried and cabinet dried) blended with 90.0ml of sterile saline were used to prepare a stock solution. The stock was diluted (1:10) by adding 100ul normal saline in Eppendorf's tubes. Exactly 100ul of stock was inoculated on nutrient agar (N.A.) and sabouraud dextrose agar (S.D.A.) media following the spread plates method and incubated at 37^0C for 18h except for S.D.A. which was incubated at 25^0Cfor 48h. Pour plates were prepared from ten fold dilutions in agar (oxoid) for total bacteria count after incubation at 37^0C for 24h and 25^0C for 72h for fungal growth. Bacteria cultures were characterized and identified using various morphological and biochemical tests.

2.2 Proximate Analysis

The proximate composition of the two periwinkle meat samples (moisture, total ash, crude protein, crude fat, and crude fibre) was determined using the methods of the AOAC (2015).

2.3 Moisture Content Determination

One gram of each sample was reweighed (W_1) in a beaker and placed in a cabinet dryer and oven dryer at 60^0C for 10h and 105^0C for 3h respectively. Afterward, the sample was removed from the cabinet and oven dryers, cooled in a desiccator and reweighed (W_2). The percentage moisture content was calculated using the formula:

$$Moisture\ content\ (\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where: W_1 = weight in beaker (g)
W_2 = weight after drying (g)

2.4 Total Ash

The determination of the total ash content of the periwinkle samples was carried out by incinerating the sample at 600^0C as total inorganic matter. Two grams of samples were placed in a pre-weighed porcelain crucible and charred in an electric heater.And after that, taken to a muffle furnace at 600^0C for 2hours. The crucible was removed from the muffle furnace, cooled in a desiccator, and weighed for the fresh, oven-dried, and cabinet-dried periwinkle meat samples. The weight of ash obtained was determined by difference and calculated as a percentage of the weight of the periwinkle sample analyzed thus:

$$Moisture\ content\ (\%) = \frac{W_1 - W_2}{\text{weight of sample}} \times \frac{100}{1} \quad (2)$$

Where:
W1 = weight of empty crucible (g)
W2 = Weight of crucible + Ash (g)

2.5 Crude Fat

Crude fat contained in the sample was determined by solvent extraction using a soxhlet extraction unit. One gram of each periwinkle sample was weighed into an extraction thimble and covered with absorbent cotton. 50ml petroleum ether was added to a pre-weighed cup. Both thimble and cup were attached to

the extraction unit. The sample was subjected to extraction with solvent for 30min, followed by rinsing for 1½ hours. The

W = Weight of sample (0.5 g),

N = Normality of titrant (0.02 N H_2SO_4)

Vt = Total digest volume (100 mL)

Va = The volume of digest analyzed (10 mL)

T = Sample titre value

B = Blank titre value

petroleum ether was evaporated from the cup passing through the condensing column for the three samples (fresh, oven, and cabinet).

The residual fat extract in the cup was calculated using the formula:

$$Crude\ fat(\%) = \frac{extracted\ fat\ (g)}{sample\ \text{weight (g)}} \times 100 \quad (3)$$

2.6 Crude Fibre

One gram of each periwinkle sample was placed in a glass crucible and attached to the extraction unit. 140ml of boiling sulphuric acid solution (1.25%) was added. The periwinkle sample was digested for 35 minutes, and the acid drained off. The sample was washed in boiling distilled water. This was followed by adding 150ml of sodium hydroxide (1.25%). The periwinkle sample was digested for 30minutes. After that, the alkali was drained, and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and dried in the cabinet dryer at 60^0C and oven dryer at 105^0C. The periwinkle sample was ashed at 550^0C in a muffle furnace for 2hours, cooled in a desiccator, and reweighed (W_2). The resulting fibre extract was expressed as a percentage of the original undefatted.

$$Crude\ fibre\ (\%) = \frac{(w_1)-(w_2)}{\text{weight}\ of\ sample} \times 100 \quad (4)$$

Where:

W_1 = Weight of crucible +sample after washing, boiling and drying (g)

W_2 = Weight of crucible +sample of ash (g)

2.7 Crude Protein Determination

This was carried out by the Kjeldahl method as described by [6] to obtain protein content of the periwinkle sample; the total nitrogen was determined and multiplied by 6.25. Each periwinkle sample (0.5 g) was mixed with 10mL of concentrated H2SO4 in the digestion flask. A tablet of selenium catalyst was added before being heated under a fume cupboard until a clear solution (the digest) was obtained. The digest was diluted to 100mL in a volumetric flask and used for the analysis. The 10mL of the digest was mixed with an equal volume of 45% NaOH solution in a Kjeldahl distillation apparatus. The mixture was distilled into 10mL of 40% boric acid containing 3 drops of mixed indicator which is Bromo cresol green/methyl red. 50mL of distillates was collected and titrated against 0.02 N EDTA from green to a deep red endpoint. A reagent blank was also digested, distilled, and titrated. The nitrogen content and hence the protein content was calculated for the fresh, oven-dried, and cabinet-dried samples using the formula below:

$$N_2(\%) = \frac{100}{w} \times \frac{N \times 14}{1000} \times \frac{Vt}{Va} \times T.B \quad (5)$$

1 mL of 1 N H_2SO_4 = 14 mg

Protein (%) = N_2 (%) x6.2

2.8 Carbohydrate Determination

The total carbohydrate content of the fresh, oven-dried, and cabinet-dried samples was determined by difference [6].

$$\%\ \text{carbohydrate} = [100 - \%(\text{moisture} + \text{protein} + \text{fat} + \text{ash} + \text{fibre})] \quad (6)$$

Determination of Total Energy or Caloric Value

Samples are shown in Fig. 2.

(a) (b) (c)

Fig. 2: (a) Fresh periwinkle meat sample (b) Oven-dried periwinkle meat sample (c) Cabinet dried periwinklemeat sample

3. Results and Discussion

The microbial analysis of the periwinkle samples showed that the total bacterial count (TBC) of the freshpachymelania aurita and tympanotosus fuscatus ($4.23x10^4$ cfu/g, $4.05x10^4$ cfu/g) was reduced when oven-dried to $1.15x10^3$ cfu/g, $1.2x10^3$ cfu/g and $1.26x10^3$cfu/g, $1.31x10^3$ cfu/g when cabinet dried. The total fungi count (TFC) for the fresh sample ($2.7x10^5$ cfu/g, $2.5x10^5$ cfu/g) was reduced when oven-dried to $1.3x10^4$ cfu/g, $1.45x10^4$ cfu/g and $1.6x10^4$ cfu/g, 2.05x104 cfu/g when cabinet dried. Results showed that oven and cabinet drying of periwinkle samples reduced the total bacterial count and total fungi count; and extended the shelf life of the periwinkle meat. The high bacterial count in fresh periwinkle meat samples could be because they are deposit feeders that ingest sediments, use organic matter and microorganisms in sediments as food [7]. [1] reported a high level of microbial count in periwinkle from different creeks and have reported that the degree of the microbial count of shellfish depends on the pollution of the waters since the water contains untreated and industrial wastes. As filter feeders, there is a great tendency that these periwinkles will accumulate several pathogenic organisms from waters influenced by sewage pollution and biotoxins produced by phytoplanktons that they feed on [8]. Both cabinet and oven drying effectively reduced the microbial loads in the two periwinkle meat samples,which was also reflected in the coliform and salmonella count reduction(Table 1). Statistically, there wasa significant difference ($P< 0.05$) in the microbial loads of the fresh periwinkle samples to the oven and cabinet dried samples (Table 2).

Table 1. Total bacteria countand fungal count of periwinkle samples

S/NO	Sample	Total Bacterial Count (cfu/g)	Total FungiCount (cfu/g)	Coliform count (cfu/g)	Salmonella count (cfu/g)
1	Fresh P.aurita	$4.23x10^4$	$2.7x10^5$	$2.43x10^5$	$5.33x10^5$
2	FreshT.fuscatus	$4.05x10^4$	$2.5x10^5$	$2.52x10^5$	$5.25x10^5$
3	Oven-dried P.aurita	$1.15x10^3$	$1.3x10^4$	$1.2x10^4$	$2.5x10^4$
4	Oven-dried T.fuscatus	$1.21x10^3$	$1.45x10^4$	$1.6x10^4$	$2.3x10^4$
5	Cabinet-dried P.aurita	$1.26x10^3$	$1.6x10^4$	$1.5x10^4$	$2.6x10^4$
6	Cabinet-dried T.fuscatus	$1.31x10^3$	$2.05x10^4$	$1.2x10^4$	$2.4x10^4$

Table 2 ANOVA of the Microbial composition of the periwinkle samples

S/NO	Nutrient	Sample	Mean (cfu/g)	F	Sig.
1	Total Bacterial Count (cfu/g)	Fresh P.aurita Oven-dried P.aurita Oven-dried T.fuscatus Fresh T.fuscatus Cabinet-dried P.aurita Cabinet-dried T.fuscatus	$4.23x10^4$ $1.15x10^3$ $1.21x10^3$ $4.05x10^4$ $1.26x10^3$ $1.31x10^3$	35686.14	1.36E-24
2	Total Fungi Count (cfu/g)	Fresh P.aurita Oven-dried P.aurita Oven-dried T.fuscatus Fresh T.fuscatus Cabinet-dried P.aurita Cabinet-dried T.fuscatus	$2.7x10^5$ $1.3x10^4$ $1.45x10^4$ $2.5x10^5$ $1.6x10^4$ $2.05x10^4$	56.30769	6.49E-08
3	Coliform-count (cfu/g)	Fresh P.aurita Oven-dried P.aurita Oven-dried T.fuscatus Fresh T.fuscatus Cabinet-dried P.aurita Cabinet-dried T.fuscatus	$2.43x10^5$ $1.2x10^4$ $1.6x10^4$ $2.52x10^5$ $1.5x10^4$ $1.2x10^4$	61.26154	4.01E-08
4	Salmonella-count (cfu/g)	Fresh P.aurita Oven-dried P.aurita Oven-dried T.fuscatus Fresh T.fuscatus Cabinet-dried P.aurita Cabinet-dried T.fuscatus	$5.33x10^5$ $2.5x10^4$ $2.3x10^4$ $5.25x10^5$ $2.6x10^4$ $2.4x10^4$	423.3133	4.67E-13

The chart showing the comparative difference in the microbial loadcomposition of the fresh, oven, and cabinet dried *pachymelania aurita*periwinkle is shown in Fig 3. In contrast, the*tympanotosus fuscatus* periwinkle meat sample is shown in Fig 4.

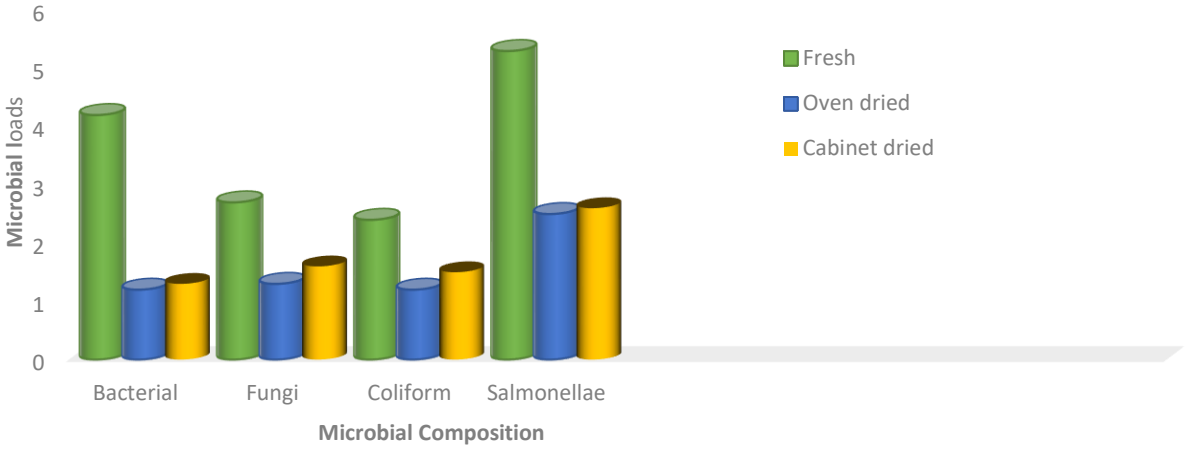

Fig 3: Microbial composition of fresh, oven, and cabinet dried *Pachymelania aurita* periwinkle meat sample

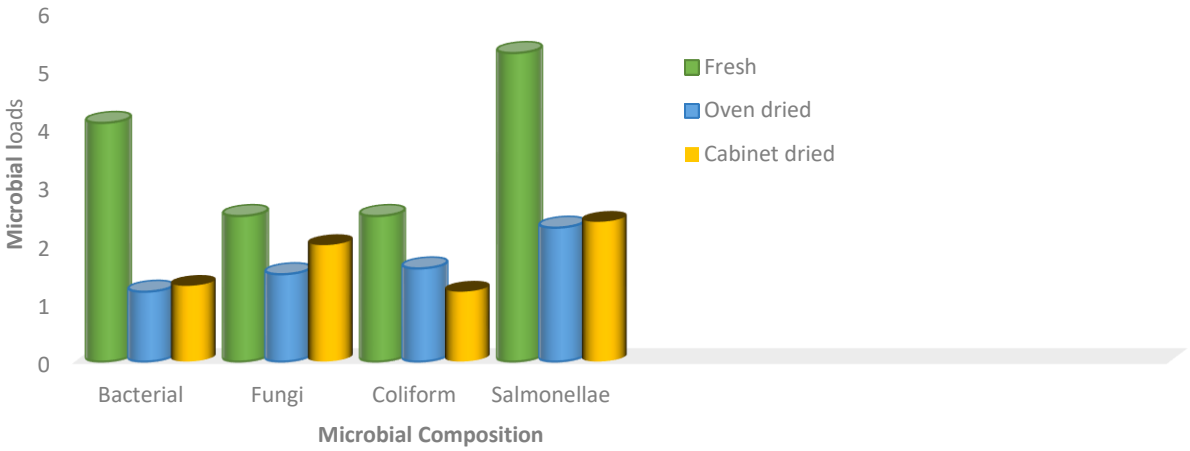

Fig 4: Microbial composition of fresh, oven, and cabinet dried *tympanotosus fuscatus* periwinkle meat sample

The comparative effect of the oven and cabinet drying on the periwinkle samples' proximate composition shows that the protein content of oven-dried and cabinet dried samples ranged from 72.12 – 70.53 and 64.74 – 61.97%, respectively. This finding is similar to that of [1]. It was observed that both periwinkle meat samples had higher moisture content in the fresh than in the oven and cabinet dried samples. This result compared favorably with 45-55% moisture content of periwinkle reported by Kiin-[9]. There was an increase in the crude fibre content of the oven and cabinet-dried periwinkle samples thanin the fresh sample. The percent crude fat content was observed to reduce in the oven and cabinet dried compared to the fresh sample with a higher value.The protein was not lost during drying compared with the fresh periwinkle samples, which could be attributed to the drying which concentrated proteins, according to [2].

Ash content was recorded to possess a higher value in the oven-dried sample than in the cabinet and freshmeat samples. The reduction in crude fat is similar to the finding of [10] and [11], who reported a decreased fat content of oven-dried shellfish shrimp (Penaeusmonoden Fabricius). Total carbohydrate for the two periwinkle types was recorded to have a higher value in the cabinet-dried periwinkle meat sample than in the fresh and oven-dried sample,as shown in Table 3. The statistical significance differences ($P< 0.05$) are shown in Table 4. The total energy value of the two periwinkle samples(pachymelania aurita and tympanotosus fuscatus) was determined, the oven-dried sample had a higher value than the cabinet-dried and fresh samples. Generally, the proximate analysis revealed that oven-dried periwinkles are nutritionally richer than the fresh and cabinet-dried periwinkle samples (Fig. 5 and 6).

Table 3: Proximate composition of periwinkle samples

Species	Chemical property	Fresh	Oven dried	Cabinet dried
Pachymelania aurita	Moisture content (%)	50.05	8.69	13.5
	Crude protein (%)	35.21	72.12	64.74
	Fat (%)	2.25	1.44	1.34
	Crude fibre (%)	2.10	2.76	2.52
	Ash (%)	3.38	9.36	7.72
	Carbohydrate (%)	7.01	7.66	8.18
	Total energy (%)	184.42	332.06	319.76
Tympanotosus fuscatus	MC (%)	50.12	9.79	15.61
	Crude protein (%)	34.14	70.53	61.97
	Fat (%)	2.20	1.25	1.13
	Crude fibre (%)	2.01	2.67	2.45
	Ash (%)	3.74	8.86	7.64
	Carbohydrate (%)	7.79	8.89	13.23
	Total energy (%)	184.59	328.97	310.85

Table 4: ANOVA for proximate composition of periwinkle samples

S/NO	Nutrient	Sample	Mean (cfu/g)	F	Sig.
1	Moisture content (%)	Fresh P.aurita	50.05	97869.35	3.19E-27
		Oven-dried P.aurita	8.69		
		Oven-dried T.fuscatus	9.79		
		Fresh T.fuscatus	50.12		
		Cabinet-dried P.aurita	13.5		
		Cabinet-dried T.fuscatus	16.61		
2	Crude protein (%)	Fresh P.aurita	35.21	449.249	3.28E-13
		Oven-dried P.aurita	72.12		
		Oven-dried T.fuscatus	70.53		
		Fresh T.fuscatus	34.14		
		Cabinet-dried P.aurita	64.74		
		Cabinet-dried T.fuscatus	61.97		
3	Fat (%)	Fresh P.aurita	2.25	26.2697	4.51E-06
		Oven-dried P.aurita	1.44		
		Oven-dried T.fuscatus	1.25		
		Fresh T.fuscatus	2.20		
		Cabinet-dried P.aurita	1.34		
		Cabinet-dried T.fuscatus	1.13		
4	Crude fibre (%)	Fresh P.aurita	2.10	1.976923	0.154701
		Oven-dried P.aurita	2.76		
		Oven-dried T.fuscatus	2.67		
		Fresh T.fuscatus	2.01		
		Cabinet-dried P.aurita	2.52		
		Cabinet-dried T.fuscatus	2.45		
5	Ash (%)	Fresh P.aurita	3.38	961.622	3.48E-15
		Oven-dried P.aurita	9.36		
		Oven-dried T.fuscatus	8.86		
		Fresh T.fuscatus	3.74		

		Cabinet-dried P.aurita	9.36		
		Cabinet-dried T.fuscatus	7.64		
6	Carbohydrate (%)	Fresh P.aurita	7.01	641.9956	3.89E-14
		Oven-dried P.aurita	7.66		
		Oven-dried T.fuscatus	8.89		
		Fresh T.fuscatus	7.79		
		Cabinet-dried P.aurita	8.18		
		Cabinet-dried T.fuscatus	13.23		
7	Total energy (%)	Fresh P.aurita	184.42	11646.34	1.12E-21
		Oven-dried P.aurita	332.06		
		Oven-dried T.fuscatus	328.97		
		Fresh T.fuscatus	184.59		
		Cabinet-dried P.aurita	319.76		
		Cabinet-dried T.fuscatus	310.85		

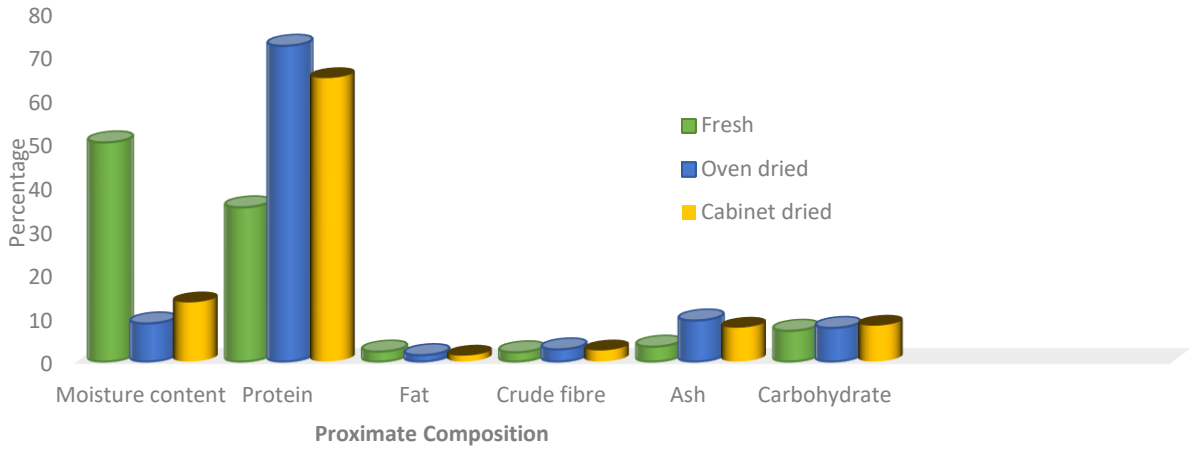

Fig 5: Proximate composition of fresh, oven, and cabinet dried *Pachymelania aurita*periwinkle meat sample

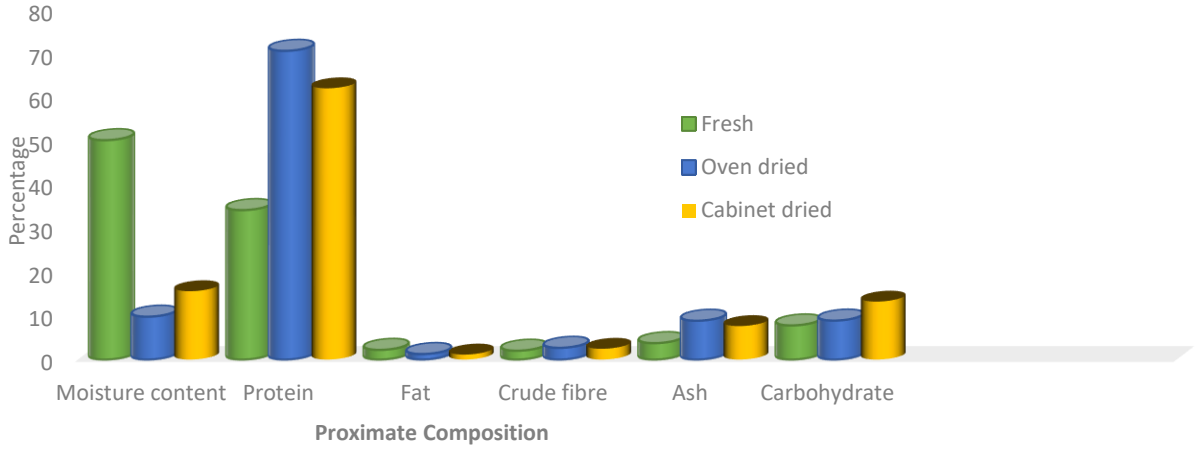

Fig 6: Proximate composition of fresh, oven,and cabinet dried *Tympanotosus fuscatus* periwinkle meat sample

4. Conclusion

The results of the microbial analysis reveal that fresh periwinkles have higher microbial loads than the oven and cabinet-dried samples. The bacterial load of pachymelania aurita and tympanotosus fuscatusfor fresh samples (4.23x104, 4.05x104) reduced to (1.15x103, 1.21x103) after oven-dried and (1.26x103, 1.31x103) for cabinet dried samples. The fresh samples' fungi count, coliform count, and salmonella count were drastically reduced in the oven and cabinet dried samples with statistically significant differences ($P< 0.05$).

The proximate analysis results reveal that fresh periwinkle meat contains more moisture than oven and cabinet dried samples. Considering the massive consumption and demand for periwinkle, preservation by oven and cabinet drying increased the nutritional quality, safety, and shelf life of the periwinkle types.Generally, the analysis revealed that oven-dried periwinkles are nutritionally richer than the fresh and cabinet-

dried periwinkle samples. The generated data will help in the design and development of efficient processing, drying, transportation, and storage of periwinkle meat.

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