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Effect of smoking, polythene and vacuum packaging on the proximate composition, heavy metals concentration, physicochemical and sensory properties of periwinkle

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ABSTRACT

This study investigated the effect of smoking, polythene and vacuum packaging on the proximate composition of freshly harvested periwinkles (FHPs) (*Tympanotonus fuscatus*). Heavy metals concentration was also determined using standard methods. Periodically, physicochemical and sensory properties of vacuum packaged smoked periwinkle (VPSP) and polythene packaged smoked periwinkle (PPSP) stored at ambient temperature (28±2°C) were assessed. FHP had 75.09±0.05% protein, 12.51±0.05% lipid, 6.87±0.05% fiber, 2.34±0.05% carbohydrate, 3.23±0.05% dry base ash and 70.89±0.05% moisture while the corresponding values for smoked periwinkle either vacuum-packaged, polythene-packaged or non-packaged were within the range of 65.63±0.05 - 72.91±0.05%, 15.75±0.05 - 25.41±0.15%, 4.64±0.05 - 6.69±0.05%, 0.96±0.05 - 1.96±0.05%, 2.09±0.05 - 4.03±0.05% and 10.65±0.17 - 28.30±0.05%, respectively. Among the samples, there was significant difference (P<0.05) between the proximate parameters. The lowest and highest energy value was recorded for FHP (422.31 kcal/100 g) and non-packaged smoked periwinkle (NPSP) (495.05 kcal/100 g), respectively. The concentration of lead and mercury in both samples were within acceptable limits. During storage, the pH and sensory characteristics of VPSP were relatively stable compared to PPSP. However, both samples experienced steady increase in total volatile nitrogen (TVN) and peroxide value (PV). The values recorded for both parameters indicate that VPSP is more resistant to spoilage and rancidity compared to PPSP. Thus, from the findings of this study, it could be concluded that vacuum-packing showed high preference than polythene-packing in preserving the sensory attributes of smoked periwinkle during storage at ambient temperature, hence it is recommended for public usage.

INTRODUCTION

Periwinkle meat is a cheap source of animal protein to many poor families in Nigeria when compared with other sources such as meat, fish, egg, chicken and meat (Ogungbenle and Omowole, 2012; Johnnie et al., 2020). According to Inyang et al. (2018), seafood which include periwinkle offers consumers superior quality protein than what is obtainable in meat and poultry. A study carried out by Ogungbenle and Omowole (2012) revealed that periwinkle (*Tympanotonus fuscatus* var. radula) contains 74.74% protein, 1.32% fat, 0.18% carbohydrate, 9.56%

ash, 0.74% crude fibre and 13.45% moisture. Periwinkle is also rich in minerals, essential amino acids and some vitamins (Ogungbenle and Omowole, 2012; Ekop et al., 2019).

Omega 6 fatty acids present in periwinkle in reasonable amount could offer some health benefits such as brain development, reduction in dementia linked to Alzheimer's disease, symptoms of osteoarthritis and rheumatoid arthritis. Low prevalence of heart diseases among people of the Niger Delta could be as a result of high intake of periwinkle (Nrior et al., 2017). The consumption of periwinkle is known to boost reproduction and help in the treatment of endemic goiter. There is vitamin A in periwinkle which could be beneficial to the eyes and skin. Also found in periwinkle is vitamin D important in the development of bones and teeth. Nutritional benefits of periwinkle is not limited to the meat. Ground shell of periwinkle provides calcium for animal feed (Jimmy and Okonkwo, 2016; Asemota et al., 2019). The shell of periwinkles are also used for medicinal purposes, bait for catching small fish, gravel supplements, production of scouring powder, ornamentals and cosmetics, powder for pimples and fertilizers (Okpeku et al., 2013).

Most of the soups prepared in the country Nigeria particularly some native soups in the Niger Delta region such as '*ekpang nkwukwo*', '*edikang ikong*' '*afia efere*', '*afang*' and '*asa*' are incomplete without the addition of periwinkle which makes it more delicious and nutritious. *Tympanotonus* species is known as '*isam*', '*mfi*' and '*udekana*' by the Igbos and the people of Akwa Ibom and Niger Delta, respectively. Irrespective of social status, many people relish eating soup prepared with periwinkle and other seafood (Inyang et al., 2018; Chika and Mercy, 2019; Ekop et al., 2019; Adesanya et al., 2021).

In recent years, the demand for periwinkle is increasing due to its affordability, availability and rise in human population (Ogungbenle and Omowole, 2012). Various anthropogenic activities especially in the Niger Delta region where periwinkles are in abundant supply predisposes them to heavy contamination by toxic heavy metals (Chioma et al., 2021). Although *T. fuscatus* var. radula could withstand polluted environment to some extent, a lot of concern have been raised concerning decline in size, population and availability of periwinkles (Bob-manuel, 2012; Okpeku et al., 2013). The use of mercury in medicine, electrical fittings, pesticides and other agrochemicals predisposes the environment to pollution. Some activities associated with pollution of the environment with lead include crude oil exploration, pipeline transportation and corrosion inhibition (Otitoju and Otitoju, 2013). According to Elekima et al. (2020), periwinkles have met several criteria for selection as an ideal bioindicator of metals. The accumulation of mercury and lead in periwinkle over time as a result of biomagnification, cross reactivity between the heavy metal and radioactive agent might lead to food poisoning in humans (Davies et al., 2006; Freeman and Ovie, 2017). High level exposure to mercury causes permanent damage to the brain, kidney and fetuses still undergoing development. There is high risk of miscarriages in pregnant women due to exposure to high amounts of lead. Severe damage to the brain and kidney which ultimately lead to death is caused by lead. Intake of food with high level of lead can elicit vomiting, abdominal pain, drowsiness and convulsion (Otitoju and Otitoju, 2013; Abiaobo et al., 2020).

Commonly practiced processing method of freshly harvested periwinkle (FHP) is by boiling this edible food with or without the shell (Omenwa et al., 2011). Despite numerous nutritional and health benefits derived from eating periwinkle, one of the major limitations is short shelf life (Inyang et al., 2018). Traditionally, periwinkle is preserved by drying or smoking (Ngozi et al., 2020). According to Nrior et al. (2017), periwinkle is best preserved by sun-drying, oven-drying, storage at constant temperature or kept inside a refrigerator. Research findings have shown that different processing and preservation methods to some extent influences the level of nutrients and other properties of food including shellfish species (Devi, 2015; Venugopal and Gopakumar, 2017).

An important aspect of food processing and preservation is packaging (Meena et al., 2017). Vacuum packing of different food products such as pastrami, pork, beef and other meats have been reported (Kumar and Ganguly, 2014). In 1940s, vacuum packs were invented for the purpose of storing foods. After two decades, a German citizen known as Karl Busch invented and put into use home vacuum sealers and later introduced industrial sealers in 1963 (Patil et al., 2020). According to Meena et al. (2017), vacuum packing simply involves the removal of air from a product pouch and seals it hermetically. The essence of vacuum packaging is to create an unfavourable environment for the growth of aerobic microorganisms, prevent oxidation and discolouration of foods as well as protect its nutrients. The use of polyethylene bags helps to maintain oxidative stability of vacuum packaged products. It is very important to take into consideration the state of the food prior to vacuum packaging as well as regulate the storage temperature of the packaged product (Kumar and Ganguly, 2014).

Vacuum-packing and storage under chilling condition is among the recent technologies used in extending the shelf life of seafood (Kumar and Ganguly, 2014; Patil et al., 2020; Kontominas et al., 2021). There is dearth of information regarding the use of polythene bags and vacuum packing to preserve smoked periwinkle. Although food products subjected to vacuum-packing are better stored under chilling condition, there is need to evaluate

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such products stored at room temperature because of erratic power supply being experienced in most parts of Nigeria. Therefore, this study is aimed at determining the effect of smoking, vacuum and polythene packaging on the nutritional composition, heavy metals' concentration, physicochemical and sensory properties in periwinkle.

MATERIALS AND METHODS

Sample collection

Sterile transparent high density polyethylene (HDPE) bags were used to harvest fresh periwinkles (*T. fuscatus*) from Buguma creek located in Asari Toru Local Government Area, Rivers State, Nigeria. The samples obtained were quickly taken to the Food and Industrial Microbiology Laboratory, University of Port Harcourt for analysis.

Processing of periwinkle

The procedure described by Inyang et al. (2018) and Kumolu-Johnson et al. (2010) with slight modifications were adopted in processing the FHP. The length of the periwinkles (with the shell) were within the range of 2.5 - 4.0 cm; the weights were between 3.70 -5.30 g. Thorough washing of the whole body of the periwinkles was carried out using potable water in order to remove mud and other foreign materials adhered to the body.

Smoking process

'Hot smoke drying' method described by Abu and Eli (2019) with some modifications was adopted in smoking the FHPs. Approximately 2.4 Kg of shucked periwinkles were poured inside a sterilized removable wire mesh tray and kept on a rack. Mangrove wood was put inside a locally manufactured smoking kiln (improved design) and ignited to preheat the chamber for 15-17 min. Afterwards, the periwinkles were placed at the central chamber for smoking to commence. The temperature attained inside the smoking chamber was within the range of 65 - 70°C. The temperature inside the smoking chamber was regulated manually by removing or adding logs of mangrove. The smoking process lasted for 3½ h. The hot smoked periwinkles were allowed to cool to ambient temperature (28±2°C) and reweighed. The weight of the smoked periwinkle is 370 g.

Packaging and storage of periwinkles

Aseptically, the hot-smoked periwinkles which were allowed to cool to ambient temperature (28±2°C) were divided into three portions labelled 'VP', 'PP' and 'NP'. The portion labelled 'VP' was vacuum-packaged using a vacuum food sealer Model VS230-IUK and stored at ambient temperature (28±2°C). Before the smoked periwinkles were sealed inside sterile transparent HDPE bags (2.25 mm thickness, 20 × 30 cm in size, 0.5 g), air trapped inside the packaging material was expelled using the vacuum machine. Three (3) samples of smoked periwinkle vacuum packaged separately inside the sterile HDPE bags were prepared. The second portion labelled 'PP' was separately sealed with sterile HDPE bags (2.25 mm thickness, 20 × 30 cm in size, 0.5 g) without being vacuum-packed which amounted to three (3) samples. The third portion of smoked periwinkle labelled 'NP' was further divided into three (3) samples and left open without any packaging. A total of nine (9) samples of vacuum packaged, polythene packaged and non-packaged smoked periwinkle (NPSP) were used for analyses. Depicted in Figures 1 and 2 are polythene packaged and vacuum packaged smoked periwinkle (VPSP), respectively.

Proximate analysis

The AOAC (2006) method was adopted in determining the proximate composition of periwinkle subjected to different treatments.

Moisture content

Two grams (2 g) of periwinkle was weighed into a dry pre-weighed crucible and dried in an oven (Astell Hearson, England) at 105°C for 18 h to a constant weight. The moisture content of the sample was calculated using the formula below:

$$\text{Moisture content} = \frac{\text{Weight of fresh sample (g)} - \text{Weight of dry sample (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

Carbohydrate content

Exactly 0.1 g of periwinkle was weighed into a 25 ml volumetric flask. One milliliter (1 ml) of distilled water and 1.3 ml of 62% perchloric acid was added to the content of the flask. Thereafter, it was swirled for 20 min to achieve complete homogenization. Thereafter, the content of the flask was made up to 25 ml mark using distilled water and stoppered. The resulting solution was filtered through a glass filter paper. One milliliter (1 ml) of the filtrate was collected and transferred into a 10 ml test tube. The content of the test tube was diluted to volume using distilled water. One milliliter (1 ml) of the working solution

Figure 1. Polythene packaged smoked periwinkle.

Figure 2. Vacuum packaged smoked periwinkle.

was pipette into a clean test tube and 5 ml Anthrone reagent was added. One millilitre (1 ml) of distilled water and 5 ml Anthrone reagent was mixed. The whole mixture was read at 630 nm wavelength with the aid of UV visible spectrophotometer (Model 754 Ningbo Hinotek Tech. Co.) using 1 ml distilled water and 5 ml Anthrone reagent prepared as blank. Glucose solution (0.1 ml) was also prepared and was treated as the sample with Anthrone reagent. Absorbance of the standard glucose was read and the value of carbohydrate as glucose was calculated using the formula below:

$$\%\ \text{CHO as glucose} = \frac{25 \text{ x absorbance of sample}}{\text{Absorbance of standard glucose}}$$

Crude protein

Exactly 0.5 g of periwinkle was transferred into Kjeldhal digestion flask. Then, one and half tablets of catalyst was added into a flask containing 10 ml concentrated H_2SO_4. The flask with its content was gently heated in an inclined position in a fume cupboard until frothing ceases and boiled briskly until digestate is clear. The content was cooled and diluted to 100 ml with distilled water. Ten milliliter (10 ml) of the digestate was added to another flask with 45% NaOH solution (10 ml) and connected to a distillation apparatus. The ammonia was subjected to steam distillation into boric acid (5 ml) indicator in a 100 ml conical flask and 50 ml distillate was collected. The distillate was titrated against the standard acid (0.05 N) HCl.

Crude protein (%) = N × Protein factor

$$\% \text{ N} = \frac{\text{Sample titre} - \text{Blank titre X N of acid X 1.4 X 6.25}}{\text{Weight of sample}}$$

Ash content

Porcelain crucible was weighed and 1.0 g of periwinkle was placed in the porcelain crucible. The sample on the crucible was ignited. Thereafter, it was dried on a mantle in a fume cupboard until the smoking ceased. The crucible containing 1.0 g of periwinkle sample was taken into a muffle furnace to ash for 3 h at 500°C. The ash left in the crucible was allowed to cool in a desiccator, weighed and the result obtained was expressed as the percentage ash using the formula below.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Crude fat (lipid) content

Exactly 0.5 g of periwinkle that has been previously dried in an oven was used to extract crude fat using petroleum ether in a Soxhlet apparatus and the process lasted for 3 h. Two gram (2 g) of sample was carefully wrapped in a filter paper and placed in a soxhlet extractor. Dried distillation flask was weighed and the extractor was placed inside it. Slowly, solvent (acetone) was introduced into the distillation flask through the condenser attached to the soxhlet extractor. A retort stand clamp was used to hold the set up. Cooled water jet was allowed to flow into the condenser. What results is a heated solvent. During the process of continuous refluxing, the lipid in the solvent chamber was extracted. When it was observed that fat in the sample has been completely extracted, the condenser and the extractor was disconnected. Then, the solvent was allowed to evaporate in order to concentrate the fat.

The flask was then dried in the air oven to constant weight and re-weighed to obtain the weight of fat in the sample.

$$\% \text{ lipid} = \frac{\text{Weight of flask and extract} - \text{Weigt of empty flask}}{\text{Weight of sample extracted}} \text{ x } 100$$

Crude fibre content

The material without fat and sulphuric acid 1.25 % (w/v) was placed in a 200 ml beaker and 50 ml of NaOH (1.25 % v/w) which was then covered with a watch glass. NaOH (1.25 % v/w) was used to mix the residue that was washed with 50 ml of water to the initial flask and then boiled for 30 min which gave an insoluble material. The insoluble material was placed on a filter paper, washed very well with hot water, and finally with 15 ml of 95% ethanol. Thereafter, the filter paper was dried for 1 h at 100°C to a constant weight and incinerated to ash at 500°C for 1 h.

After allowing the ash to cool, the weight was subtracted from the increase in weight of the filter paper because the insoluble material and the difference expressed as the fiber.

$$\% \text{ Fiber} = \frac{\text{Weight of fiber}}{\text{Weight of sample}} \text{ x } 100$$

Determination of energy value

The energy value of FHP and smoked periwinkle subjected to different treatments were calculated using the Atwater general factors for the energy density of fat (9 kcal/g) and protein (4 kcal/g) (Kiczorowska et al., 2019). The relationship between the crude values of protein, fat and carbohydrate obtained from proximate analysis as earlier described by Oginni (2019) was used to calculate the energy value of the samples.

Energy value (kcal/100g) = P × 4 (protein) + C × 4 (carbohydrate) + F × 9 (fat)

Determination of pH

The procedure described by Efiuvwevwere and Izakpa (2000) was adopted. Ten gram (10 g) of periwinkle meat was homogenized in 20 ml sterile distilled water using Benton electric blender (China). The pH of the homogenate was determined using a calibrated digital pH-meter (Hi-98107 pH, India).

The glass electrode was calibrated using buffer solution (pH 7.0). Ten milliliters (10 ml) of homogenized periwinkle was dispensed into 100 ml beaker. Thereafter, the electrode of the pH meter was inserted into the sample. After 30 s of inserting the electrode into the sample, a stable figure seen on the digital display of the pH meter was recorded.

Determination of lead and mercury concentration

The procedure described by Moslen et al. (2017) using atomic absorption spectrophotometer (AAS) was adopted. The smoked periwinkle tissue was dried to a constant weight at 90°C for 2 days using clean acid washed Petri dishes. On completion of drying, the samples were kept in a desiccator and allowed to cool. The samples were then crushed into fine powder using porcelain mortar and pestle. The samples were digested by microwave digestion method. It involves adding nitric acid (Analar grade) and hydrogen peroxide (Analar grade) in the ratio of 3:1 to the samples. The mixtures were then digested at 150°C for 30 min using a microwave oven. The hydrogen peroxide and nitric acid added reduces nitrous vapour and speeds up digestion of organic substances by increasing the temperature of reaction in the digestion process. The digested samples were filtered with 20 ml of deionized water. The filtrates were collected with clean acid-washed and appropriately labeled 30 ml polyethylene containers for analysis by Atomic absorption spectrophotometric method.

Determination of total volatile base nitrogen (TVBN)

The method described by Idakwo et al. (2016) was adopted. One hundred gram (100 g) of periwinkle was weighed and blended with 300 ml of 5% tricholoroacetic acid. To obtain a clear extract, the blend was centrifuged at 3000×g for 1 h. Five millilitre (5 ml) of the extract was pipetted into Markhan apparatus. Thereafter, 5 ml of 2 M sodium hydroxide (NaOH) was added. It was steam-distilled into 15 ml of standard 0.01 M hydrochloric acid (HCl) containing 0.1 ml rosolic indicator. After distillation is completed, the excess acid was then titrated in the receiving flask using standard 0.01 M NaOH until a pale pink end point was reached.

A procedural blank was obtained using 5 ml trichloroacetic acid without the sample and titrated as earlier stated. The water content (W) of the sample was obtained by drying an initial weight of periwinkle at 77°C in an oven to a constant weight. At that temperature (77°C), the material was completely dehydrated as to limit the vaporization of volatile materials. The concentration of TVB-N (mg N/100g sample) was computed as follows:

$$\text{TVB-N (mg N/100g sample)} = \frac{(\text{M})(\text{VB}-\text{VS})(14)(300+\text{W})\text{TVBN}}{5}$$

Where, VB, ml NaOH used for blank titration; W, water content of sample in g/100g; M, molarity of NaOH standard solution; VS, ml NaOH used for sample titration.

Determination of peroxide value

Exactly 0.5 g of periwinkle (dried and blended) was weighed into a 250 cm^3 conical flask containing 10 ml of chloroform and 15 cm^3 of acid acetic. The mixture was stirred and 1 cm^3 of 10% (w/v) KI was added before shaking the corked flask for 1 min. The flask and its content were placed in the dark for 5 min. Thereafter, 75 cm^3 of water was added to the flask. The iodine liberated was titrated with standard sodium thiosulphate solution until yellow colour almost disappeared. 0.5 ml of (1 %) starch solution was introduced and the titration continued until the blue colour disappeared. Blank determination was performed. The PV was calculated using the formula below.

$$\text{PV (meq}O_2\text{/Kg oil)} = \frac{\text{Vo} - \text{V}}{\text{Weight of sample}} \times \text{C}$$

Where, C, Concentration of sodium thiosulphate used; Vo, volume of sodium thiosulphate used for blank; V, volume of sodium thiosulphate used for periwinkle sample.

Sensory evaluation

The organoleptic properties of polythene packaged smoked periwinkle (PPSP) and VPSPs (*T. fuscatus*) were evaluated by ten (10) experienced panelists drawn from academic staff and students of University of Port Harcourt. Sensory analysis of both samples were performed using the method described by Huss (1995). The panelists were requested to evaluate the general appearance, dryness, smell and colour of the samples using a 7-point Hedonic scale (7= Like very much, 6= Like moderately, 5=Like slightly, 4= Neither like nor dislike, 3=Dislike slightly, 2=Dislike moderately, 1= Dislike).

Statistical analysis

All the analyses were carried out in triplicate. Statistical analysis was carried out with the aid of Statistical Package for Social Sciences (IBM, SPSS) version 23. Data generated from the analyses were subjected to Analysis of Variance (ANOVA) to determine significant difference as $P<0.05$. Duncan's multiple range test was used in evaluating the differences between means.

RESULTS

Presented in Table 1 is the proximate composition of FHP, NPSP, PPSP and VPSP. Protein content of NPSP, PPSP and VPSP is lower than what was obtainable in FHP. This trend was also reported for crude fiber, carbohydrate and moisture content. On the contrary, lipid content of FHP is lower than the values recorded for NPSP, PPSP and VPSP, respectively. Although the moisture content of PPSP and VPSP were approximately the same, the values obtained in FHP and NPSP were higher (Table 1).

Figure 3 shows the energy value of FHP and smoked periwinkle subjected to different treatments. Among the samples, the FHPs (422.31 kcal/100g) and NPSPs (495.05 kcal/100g) had the lowest and highest energy value, respectively.

Tables 2 and 3 shows the sensory score assigned to

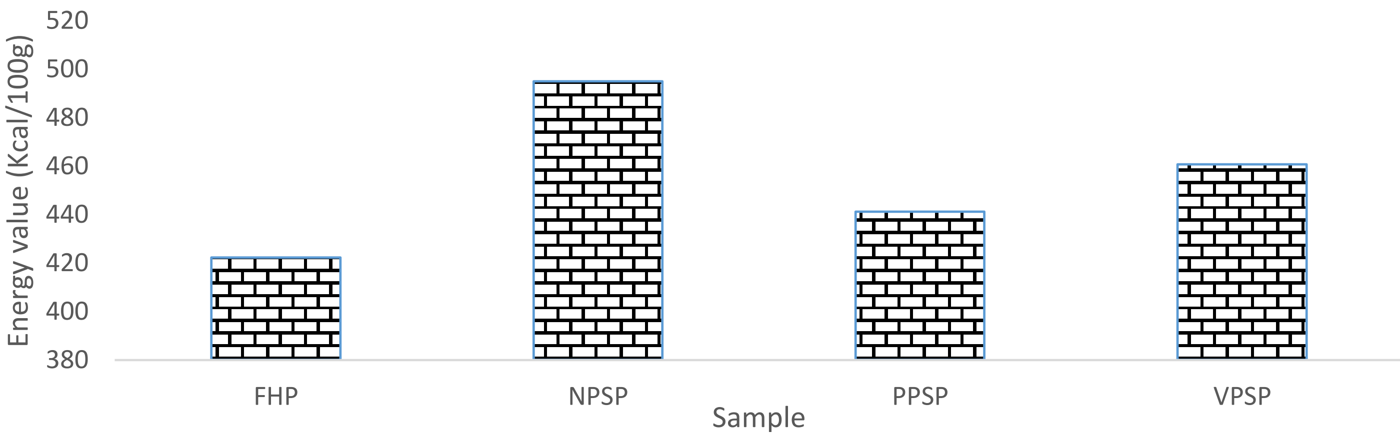

Figure 3. Energy value of FHP and smoked periwinkle subjected to different treatments. Key: FHP, Freshly harvested periwinkle; NPSP, non-packaged smoked periwinkle; PPSP, polythene packaged; VPSP, VPSP.

Table 1. Proximate composition of FHP, non-packaged, polythene packaged and VPSP.

Sample	Crude protein (% DB)	Lipi (% DB)	Crude fiber (% DB)	Carbohydrate (% DB)	Ash (% DB)	Moisture (% WB)
Freshly harvested periwinkle	75.09 ± 0.05^{d}	12.51 ± 0.05^{a}	6.87 ± 0.05^{c}	2.34 ± 0.05^{d}	3.23 ± 0.05^{c}	70.89 ± 0.05^{c}
Non-packaged smoked periwinkle	65.63 ± 0.05^{a}	25.41 ± 0.05^{d}	5.91 ± 0.05^{b}	0.96 ± 0.05^{a}	2.09 ± 0.05^{a}	28.30 ± 0.05^{b}
Polythene packaged smoked periwinkle	72.91± 0.05^{c}	15.75 ± 0.15^{b}	6.69 ± 0.05^{c}	1.96± 0.05^{c}	2.69 ± 0.05^{b}	10.94 ± 0.17^{a}
Vacuum packaged smoked periwinkle	70.62 ± 0.05^{b}	19.09 ± 0.15^{c}	4.64 ± 0.05^{a}	1.62 ± 0.05^{b}	4.03 ± 0.05^{d}	10.65 ± 0.17^{a}

All values are triplicate means ± SD. Non-identical superscripts along the columns indicate a significant difference at $P < 0.05$. Key: DB, Dry basis; WB, wet basis.

Table 2. Sensory evaluation of PPSP monitored during storage.

Week	Colour	Smell	Dryness	General appearance
0	6.3 ± 0.05^{c}	5.9 ± 0.05^{c}	5.6 ± 0.05^{d}	6.3 ± 0.05^{d}
2	4.2 ± 0.05^{b}	5.2 ± 0.05^{c}	4.6 ± 0.05^{c}	4.5 ± 0.05^{c}
4	3.9 ± 0.05^{b}	3.2 ± 0.05^{b}	2.9 ± 0.05^{b}	3.2 ± 0.05^{b}
6	2.1 ± 0.05^{a}	1.7 ± 0.05^{a}	1.4 ± 0.05^{a}	1.7 ± 0.05^{a}

All values are means ± SD of sensory score of ten panelists. Non-identical superscripts along the columns indicate a significant difference at $P < 0.05$. Key: 7, Like very much; 6, like moderately, 5, like slightly; 4, neither like nor dislike; 3, dislike slightly; 2, dislike moderately; 1, dislike).

each sensory attribute of PPSP and VPSP during storage, respectively. The sensory evaluation report shows that lower sensory score was assigned to PPSP and VPSP as the storage time of both samples increased with few exceptions. Depicted in Figure 4 is the concentration of mercury and lead in fresh periwinkle and NPSP. The result obtained showed that fresh periwinkle had higher concentration of mercury and lead than what was obtainable in NPSP.

Figure 5 shows the pH, PV and TVN of FHP and NPSP.

Table 3. Sensory evaluation of VPSP monitored during storage.

Week	Colour	Smell	Dryness	General appearance
0	6.3 ± 0.05^{c}	5.9 ± 0.05^{a}	5.6 ± 0.05^{bc}	6.3 ± 0.05^{d}
2	6.0 ± 0.05^{c}	5.6 ± 0.05^{b}	5.7 ± 0.05^{bc}	6.3 ± 0.05^{d}
4	5.9 ± 0.05^{c}	5.6 ± 0.05^{b}	5.9 ± 0.05^{c}	6.0 ± 0.05^{cd}
8	5.3 ± 0.05^{b}	5.6 ± 0.05^{b}	5.3 ± 0.05^{ab}	5.6 ± 0.05^{bc}
12	5.0 ± 0.05^{b}	5.3 ± 0.05^{b}	5.3 ± 0.05^{ab}	5.3 ± 0.05^{ab}
16	4.2 ± 0.05^{a}	5.2 ± 0.05^{b}	4.8 ± 0.05^{a}	4.9 ± 0.05^{a}

All values are means ± SD of sensory score of ten panelist. Non-identical superscripts letter within columns indicate a significant difference at $P < 0.05$. Key: 7, Like very much; 6, like moderately; 5, like slightly; 4, neither like nor dislike; 3, dislike slightly; 2, dislike moderately; 1, dislike.

The PV and TVN of the NPSP are higher than value reported for fresh periwinkle. In contrast, the pH of fresh periwinkle is slightly higher than what was obtainable in NPSP.

Figure 6 shows the pH, PV and TVN of PPSP, while the corresponding values for VPSP (Figure 7). The result obtained showed that PV and TVN of both products increased as their storage period increased. On the contrary, the pH of VPSP is relatively stable during the period of storage while that of PPSP is on the decrease.

DISCUSSION

Proximate composition and energy value

The result obtained from this study shows that fresh periwinkle (*T. fuscatus*) harvested from Buguma creek is rich in protein, but low in lipid, carbohydrate, ash and crude fibre. The protein content (75.09±0.05%) of FHP is in agreement with the result (74.74%) earlier reported by Ogungbenle and Omowole (2012). High moisture content of fresh periwinkle (70.89±0.05%) is a strong indication of poor shelf life. Therefore, food preservation methods of sea foods such as smoking of the periwinkles popularly known for reducing moisture content of foods were applied. The results obtained showed a reduction in moisture content of vacuum packaged, polythene packaged and NPSP s which were within the range of 10.65±0.17 - 28.30±0.05%. The reduction in moisture content of fresh periwinkles after smoking is consistent with the report from related studies which involved smoking freshly harvested shellfish and other seafood (Kumolu-Johnson et al., 2010; Obire et al. 2017; Abu and Eli, 2018). After smoking the FHPs and carrying out proximate analysis of the samples subjected to vacuum and polythene packing, worthy to note that the amount of protein in VPSP, PPSP and NPSP ranges between 65.63±0.05 - 72.91±0.05% and is lower than what was recorded for FHP (75.09±0.05%) (Table 1). This could be attributed to decrease in the quantity of soluble protein as a result of smoking the periwinkles. The intensity of heat used in the smoking process could have resulted to denaturation of protein and amino acids contained in the samples. According to Abraha et al. (2018), reduction in protein content of fish after smoking could be attributed to smoke compounds such as pyrene and benzo in vapour form generated during smoking process which dissolve in the liquid present on the surface of the fish. The results obtained showed that crude fiber, carbohydrate and moisture content of FHPs were lower than the values recorded for smoked periwinkles subjected to different treatments. On the contrary, the lipid content of FHP (12.51±0.05%) is lower than the values recorded for VPSP (19.09±0.15%), NPSP (25.41±0.05%) and PPSP (15.75%). In a related study, Tiwo et al. (2019) attributed increase in lipid content of fresh fish after undergoing smoking process to reduction in moisture content based on the inverse correlation between fat and water level. According to Abraha et al. (2018), dripping of fats and water which occur during smoking of fish physically reduces the amount of micronutrients, lipids and amino acids in the product. Thus, this study found that the lipid content of VPSP, NPSP and PPSP were not in conformity with the findings of Abraha et al. (2018). Also, the moisture contents of VPSP (10.65±0.17%), PPSP (10.94±0.17%) and NPSP (28.30±0.05%) were far below the fresh periwinkle's moisture content (243.52±0.05%). This could be attributed to the effect of smoking the periwinkles which involves application of heat and subsequent reduction of moisture content and prolongation of shelf life in periwinkles (Abraha et al., 2018). Slightly higher ash content of VPSP compared with FHP is an indication of higher amounts of minerals could be attributed to loss of humidity (Kumolu-Johnson et al., 2010). This study has clearly shown that periwinkle is a very poor source of energy. The carbohydrate content of all the samples is within the range of 0.96±0.05 - 2.34±0.05%. This result is in agreement with Vengopal and Gopakumar (2017) which reported that shellfish have low carbohydrate content.

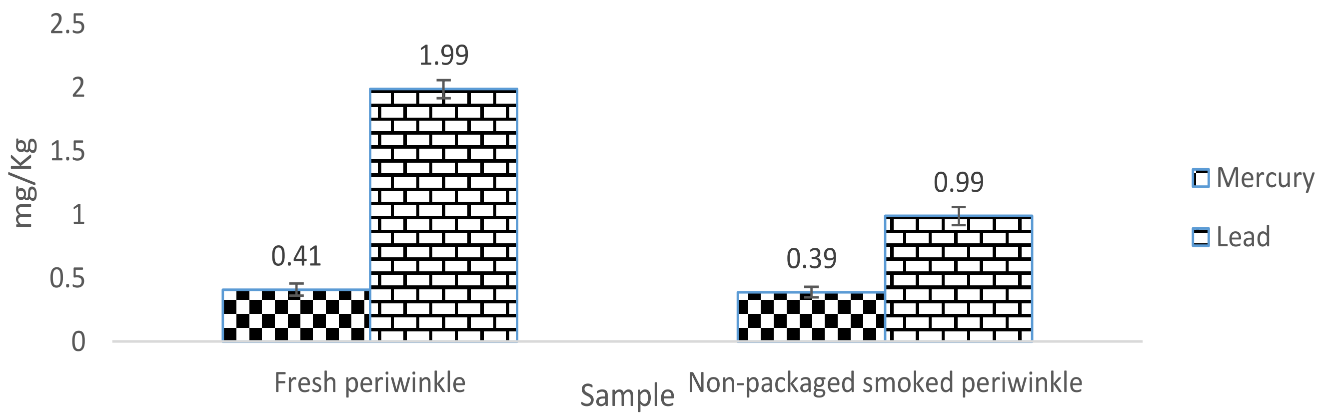

Figure 4. Concentration of mercury and lead in fresh periwinkle and NPSP.

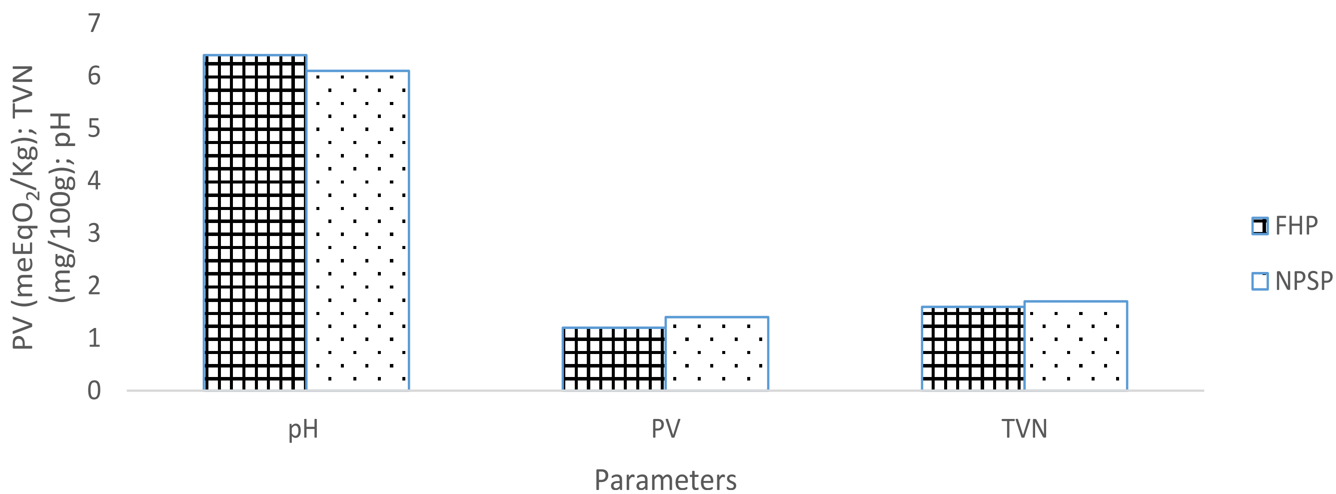

Figure 5. pH, PV and TVN of fresh periwinkle and NPSP. Key: PV, Peroxide value; TVN, total volatile nitrogen; FHP, freshly harvested periwinkle; NPSP, non-packaged smoked periwinkle.

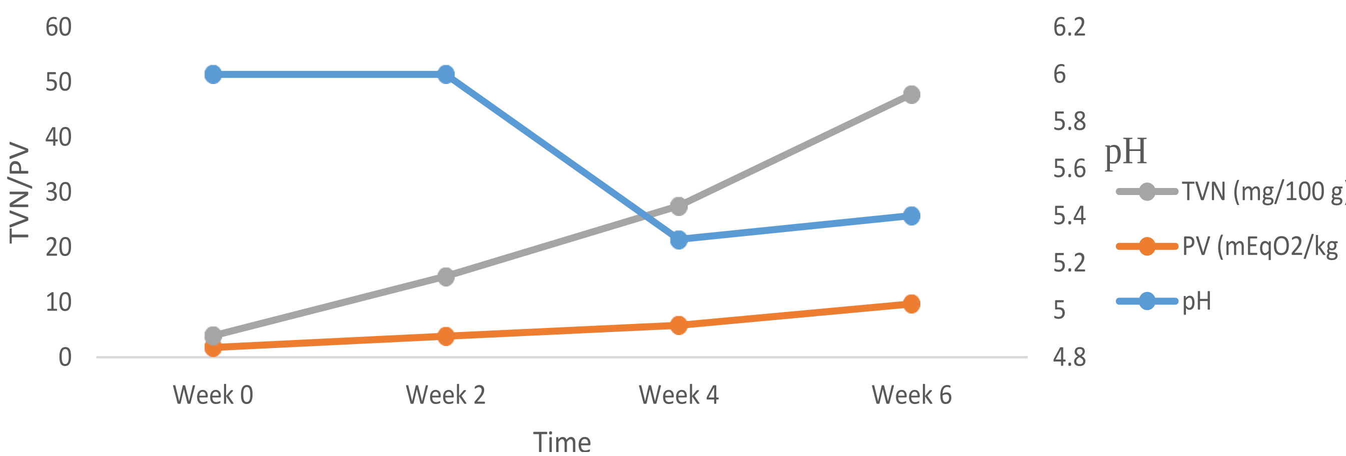

Figure 6. pH, PV and TVN of PPSP.

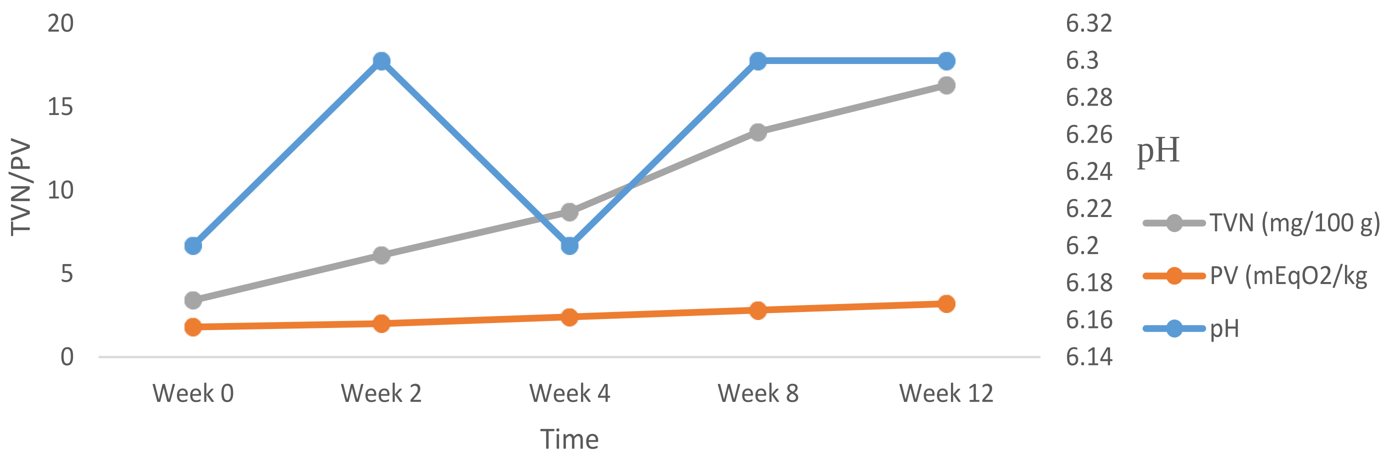

Figure 7. pH, PV and TVN of VPSP.

According to Oginni (2019), carbohydrate is considered first among all the organic nutrients utilized by the body for energy generation. The results obtained showed that energy value of FHP (422.31 kcal/100g) is lower than the values recorded for smoked periwinkles subjected to different treatments which were within the range of 441.23 - 495.05 kcal/100g). Worthy to note is that significant differences ($p<0.05$) were reported for crude protein, lipid, carbohydrate and ash content of FHPs, PPSP, non-packaged periwinkle (NPSP) and VPSP. This result is an indication that smoking process, polythene and vacuum-packing significantly influenced the proximate parameters of FHP. As for moisture content, there was no significant difference ($p>0.05$) between the values assigned to PPSP and VPSP. This could be attributed to the properties of the packaging material used in wrapping both samples. In contrast, there is a significant difference ($p<0.05$) between moisture content of FHP and NPSP which could be attributed to smoking process well-known to reduce moisture content of shellfish and other seafood.

Sensory evaluation

According to Obire et al. 2017, wood smoked periwinkles have improved sensorial characteristics especially flavour as a result of certain phenolic compounds present in wood smoke which include guaiacol, 4-methylguaiacol, and syringol. Abu and Eli (2018) reported that breakdown of components in smoke generated as a result of burning wood/charcoal is responsible for improvement in taste and colour of fish. This is attributed to a wide range of phenolic compounds, nitrites and formaldehyde present in the smoke. During storage of PPSP at ambient temperature (28±2°C), sensory report of PPSP monitored at two (2) weeks interval shows a steady reduction in sensory score assigned to sensory characteristics of the sample. At Week 0, the general appearance and colour of PPSP was liked moderately while the smell and dryness of the samples were liked slightly by the sensory panelists. At Week 6, the colour of PPSP was disliked moderately while the other sensory parameters (general appearance, smell and dryness) of the product were disliked by the sensory panelists. Depreciation in sensory characteristics of PPSP during storage could be attributed to microbial activities favoured by air trapped by the polythene bag used in packaging smoked periwinkles. The values separately assigned to two sensory attributes (dryness and general appearance) of PPSP during the period of storage were significantly different ($p<0.05$). Dryness of a food product which is usually associated with moisture loss might have occurred as a result of low relative humidity of the environment where the sample was stored and properties of the packaging material used in wrapping the sample. To some extent, both factors might also have influenced the general appearance of the sample. Thus, significant differences were observed in the sensory scores assigned to dryness and general appearance of the sample during the storage period. Slow biochemical reactions or metabolic activities of microorganisms present in the sample during the early period of storage might be responsible for no significant difference ($p>0.05$) in smell reported by the panelists at Week 0 and 2. As the storage time increased, probably the metabolic activities of spoilage microorganisms present in the sample increased leading to further breakdown of PPSP which resulted in significant difference ($p<0.05$) in smell reported by the panelists at Week 4 and 6. To some extent, this condition might also have influenced the significant difference ($p<0.05$) in the values assigned to the colour of the sample during the storage period. However, no significant difference in colour was reported in the sample (PPSP) at Week 2 and 4.

As storage time of VPSP increased, sensory report of the sample monitored at intervals showed that slightly lower sensory scores were assigned to sensory characteristics of the sample. At Week 16, the sensory panelists reported that the smell of VPSP was slightly liked whereas other sensory parameters were neither liked nor disliked. The sensory report shows that the values assigned to VPSP within the storage period of 4 - 16 weeks with respect to smell were not significantly different ($p>0.05$). A similar result was observed with respect to colour of the sample between Week 0 - 4; Week 8 - 12. This could be attributed to relatively stable biochemical or microbial activities influenced by vacuum-packing of the smoked periwinkle. According to Kumar and Ganguly (2014), vacuum packing suppresses the development of oxidized flavours during storage of seafood. The significant differences ($p<0.05$) observed in the values assigned to two sensory attributes (dryness and general acceptability) of VPSP could be attributed to microbial or biochemical activities, properties of the packaging material and humidity of the environment where the sample was stored. A comparison between the sensory report of PPSP and VPSP indicate that vacuum-packing preserved the sensory characteristics of smoked periwinkle better than packing smoked periwinkle inside polythene bags without subjecting it to vacuum-packing.

Heavy metal concentration

Detection of lead and mercury in periwinkles could be attributed to inhabitation of the bottom of rivers and oceans by periwinkles where these heavy metals accumulate (Ogundiran and Fasakin, 2015). The results shows that the amount of lead (1.99±0.54 mg/Kg) and mercury (0.41±0.80 mg/Kg) in FHP is higher than the corresponding values reported in NPSP which were 0.99±0.44 and 0.39±0.80 mg/Kg, respectively. Both results for lead content of FHP and NPSP were within the maximum limits (2.0 mg/Kg dw) recommended by FAO/WHO for seafood. Similarly, the results for mercury content of FHP and NPSP were within the maximum limit of 0.5 mg/Kg dw recommended by FAO/WHO for seafood (Elekima et al., 2020). Lower concentration of lead and mercury in NPSP compared with FHP could be attributed to the effect of smoking the periwinkles. This result is in agreement with the study carried out by Cieślik et al. (2017) which reported that amount of lead and mercury in fresh water fish reduced after being subjected to smoking process and attributed it to conversion of heavy metals to other compounds. In a related study, Asemota et al. (2020) observed that the amount of heavy metals present in periwinkle was reduced after boiling it for a period of 5 minutes at 100°C.

Naturally, mercury is found in the soil. However, the use of pesticides and herbicides, industrial processes and discharge of industrial liquid waste and fossil fuel combustion add to the amount of mercury present in the soil. Mercury is known to be exceedingly bio-accumulative and highly toxic (Ezeilo et al., 2020). According to Elekima et al. (2020), mercury has useful applications in the industries. In medicine, silver-mercury tooth amalgam is used for tooth filling. However, damage to the lungs could occur after an individual is exposed to inorganic mercury. Symptoms such as restlessness, tremor, anxiety, depression and sleep disturbance could manifest as a result of chronic exposure to mercury. Damage to the kidney is associated with exposure to elemental mercury. Being an allergen, elemental mercury might cause contact eczema.

Lead is naturally present in the earth crust. It is usually released as a result of anthropogenic activities which include mining, combustion of fossil fuel, paint and battery products. The human body is capable of absorbing lead and stores it in the bones, blood and tissues. Increase blood pressure experienced by adults is associated with accumulation of lead in the body (Ezeilo et al., 2020). According to Chioma et al. (2021), lead is a neurotoxin. In humans, high amount of lead is associated with symptoms such as loss of appetite, fatigue, chronic anemia, low sperm count, renal dysfunction and death.

Physicochemical parameters

Free hydrogen ions (H^+) in a solution are measured using a parameter known as pH (Raji et al., 2018). The results obtained from this study showed that reduction in pH from 6.0±0.01 to 5.4±0.01 in PPSPs occurred during storage. During storage of VPSP at ambient temperature (28±2°C), reduction in pH of the samples (pH 6.2) occurred at Week 4. The slight reduction in pH observed in this study was earlier reported by Ochieng et al. (2015) from a related study. However, there was a slight increase in pH (6.2±0.01 - 6.3±0.01) in VPSPs during storage of the product. This observation is in agreement with a related study carried out by Ochieng et al. (2015) which involved monitoring the effects of vacuum packaging on the chemical properties of solar rack dried sardines during chill storage. The increase in pH reported in this study during storage of VPSP could be as a result of ammonia (volatile base compound) released by spoilage bacterial activities during storage of the product.

PV is an index of biochemical lipid oxidation useful in monitoring the development of rancidity in food. Aldehydes constitute the major lipid peroxidation product responsible for rancidity and food deterioration (Raji et al., 2018). The PV of NPSP (1.4±0.05 $mEqO_2$/kg) is slightly higher than PV of 1.2±0.05 $mEqO_2$/kg reported in FHP. During the storage period of PPSP, the PV of the product increased from 1.8±0.01 - 9.7±0.06 $mEqO_2$/kg. Although increase in PV of VPSP was also recorded during storage of the sample, the values were within a shorter range (1.8±0.01

- 3.2±0.01 $mEqO_2$/kg) when compared with the values obtained during storage of PPSP. According to Raji et al. (2018), acidity and rancid taste of food can be noticed when its PV is within the range of 20 - 40 $mEqO_2$/kg.

TVN is a very important parameter used in evaluating the quality of seafood. It is used in monitoring spoilage of fresh and lightly preserved seafood. The TVN of seafood increases as spoilage of the product progresses (Akusu et al., 2019). The TVN of fresh periwinkle and NPSP is 1.6±0.05 and 1.7±0.05 mg/100g, respectively. Thus, slight increase in TVN is an indication that spoilage of FHPs had commenced but it was arrested by smoking the periwinkles. During storage of PPSP at ambient temperature (28±2°C), the TVN increased from 2.1±0.01 - 38.1±0.11 mg/100g. Similarly, the TVN of VPSPs stored at ambient temperature (28±2°C) increased from 1.6±0.01 - 13.1±0.06 mg/100g. According to Akusu et al. (2019), TVN within a range of 30 mg-35 mg/100g is the maximum permissible limit for seafood. Therefore, the TVN of VPSP monitored for 12 weeks is satisfactory. However, the TVN of PPSP stored for 6 weeks exceeded the maximum permissible limit. This result is an indication that vacuum packaging is more effective than polythene packaging in slowing down spoilage of smoked periwinkles.

RECOMMENDATIONS

Since the risk of consuming periwinkle contaminated with pathogenic microorganisms is high, microbiological analysis of FHP, VPSP, PPSP and NPSPs are recommended. Also important is the shelf life study of the samples subjected to different preservation methods. In addition to mercury and lead, the concentration of other heavy metals in the samples should be evaluated. Safety of the products for human consumption is strictly based on the results obtained with reference to standards recommended by International Commission on Microbiological Specifications for Food (ICMSF), World Health Organization (WHO), Food and Agricultural Organization (FAO) and European Food Safety Authority (EFSA).

Conclusion

The result of proximate analysis shows that crude fibre, protein, carbohydrate, and moisture contents of FHP had higher values when compared with the values encountered in VPSP, PPSP and NPSP. In contrast, the lipid content of FP is higher than the values obtained from PPSP, NPSP and VPSP. Among all the samples, the value recorded for ash content of VPSP is the highest. The sample that had the highest and lowest energy value was NPSP and FHP, respectively. Although the concentration of lead and mercury in FHP and NPSP were within the FAO/WHO limits, the process of smoking fresh periwinkle resulted in the reduction of concentration of both heavy metals in the samples. The pH of FHP is slightly higher than the value recorded for NPSP. In contrast, the PV and TVN of NPSP is slightly higher than the values recorded for FHP. During storage of VPSP and PPSP at ambient temperature, there was steady increase in PV and TVN of the samples. However, during the storage period, the pH of VPSP was relatively stable whereas that of PPSP was on the decrease. Sensory report shows that vacuum packing of smoked periwinkle preserved the sensory characteristics of the product better than polythene packing of smoked periwinkle.

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