Nutritional and Sensory Properties of Formulated Biscuits Supplemented with *Vernonia amygdalina* Del Leaves And *Heteroclarias* Spp Viscera Oil

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ABSTRACT

The high prevalence of nutrition-related health issues has spurred a growing interest in developing healthier snack alternatives. The aim of this study therefore was to develop and assess the sensory as well as the nutritional properties of biscuits supplemented with *Vernonia amygdalina* leaf (VA) and *Heteroclarias* spp viscera oil. Four biscuit samples were developed: one without *Vernonia amygdalina* and fish oil (labeled BWVF) and three others containing increasing quantities of VA (BWVF-1 g, BWVF-2 g, and BWVF-3 g). A commercially purchased conventional biscuit (CB) was utilized as a control. Physicochemical parameters of fish viscera oil, proximate analysis and sensory evaluation using a 9-point hedonic scale was carried out on the formulated biscuits. Results showed that the physicochemical properties of the fish viscera oil were within range recommended for edible oil. Carbohydrate was significantly lower ($p < 0.05$) in BWVF- 1 g, BWVF- 2 g and BWVF- 3 g in a dose dependent manner compared to the CB, while percentage crude protein, lipid and ash contents increased significantly ($p < 0.05$) compared to CB. The rating of appearance, texture/consistency, crunchiness, and overall acceptability of the test biscuits were not significantly different ($p > 0.05$) to CB. However, there was a dose-dependent decrease in the ratings for the taste of BWVF-1 g, BWVF-2 g and BWVF-3g compared to CB and BWVF. In conclusion, *Vernonia amygdalina* and fish oil-supplemented biscuits have good sensory evaluation and contain nutrients that can contribute to the daily nutritional and caloric needs of consumers.

Keywords: Biscuit; Nutrients; Trained panelist; *Vernonia*; Viscera oil

INTRODUCTION

Biscuits are popular and convenient snacks consumed in large amounts worldwide (Ayensu *et al*., 2019, Goubgou *et al.*, 2021). Their widespread appeal have been attributed to their portability, long shelf life, and enjoyable taste. They are however often considered as unhealthy because of their high sugar, fat, salt and calories contents (Malik *et al*., 2010). They have also been

reported to contain low essential nutrients like fiber, vitamins, and minerals. Their high sugar content can contribute to dental caries, weight gain, and an increased risk of chronic diseases such as type 2 diabetes and heart disease in consumers (Malik *et al.*, 2010). Many snacks, such as chips and crackers, are high in unhealthy fats, such as trans fats and saturated fats, which can contribute to an increased risk of heart disease (Mozaffarian *et al.*, 2006, Iqbal, 2014). The Malmö Diet and Cancer cohort study indicated that higher intake of biscuits was positively related with increased risk of non-aggressive prostate cancer (Drake *et al.*, 2012).

The high prevalence of nutrition-related health issues, including metabolic disorders, has spurred a growing interest in developing healthier snack alternatives. Functional foods, enriched with beneficial ingredients, have emerged as a promising solution to address this challenge. In a study in India, biscuit composed of 30% soy flour was developed to boost its nutritional value without changing the physical properties (Ansari and Kumar, 2012). Similarly, vitamins B_{12}, C, and folic acid, as well as prebiotic fibre, were added to biscuits transforming it into functional food. Human volunteers who ate these fortified cookies had decreased homocysteine and glucose levels in their blood, implying that the biscuits may help reduce risk factors for heart diseases such as myocardial infarction, stroke, and venous thrombosis (Boobier *et al.*, 2006). Rababah *et al.* (2006) also fortified biscuits with chickpea flour, broad bean flour, and soy protein to make high-protein biscuits.

Vernonia amygdalina, commonly known as bitter leaf, is a leafy green vegetable found in many parts of Africa and Asia (Soji-Omoniwa *et al.*, 2023). It has gained recognition for its rich nutritional content and potent bioactive compounds such as flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and sesquiterpene lactones (Quasie *et al.*, 2016; Luo *et al.,* 2017) with various health benefits. These include anti-inflammatory, antioxidant, and antimicrobial properties, making it a valuable candidate for fortification in food products (Oyeyemi *et al.*, 2018, Soji-Omoniwa *et al.*, 2023).

Heteroclarias spp catfish, is a notable source of fish oil known for its high omega-3 fatty acid content (Afia and David, 2019). Omega-3 fatty acids have been extensively studied for their role in promoting heart health, reducing inflammation, and supporting cognitive function (Mozaffarian and Wu, 2011). For many years, fish oil is associated with good health effects. The American Heart Association recommend to patients with coronary heart disease, the consumption of 1 g of fish oil per day, preferably by eating fish (Jude *et al.*, 2006). Epidemiological studies revealed that there is an inverse relationship between high fish oil consumption and the low mortality following coronary heart disease, possibly through the changes in prostaglandin metabolism (Jude *et al.,* 2006). Similarly, daily use of fish oil was associated with reduced risk of death in patients with solid tumors (Skeie *et al.*, 2009) and lower risk of diabetes mellitus insulin-dependent (Type I) (Stene *et al.*, 2000).

Incorporating *Heteroclarias* spp viscera oil into food products presents an opportunity to enhance the nutritional profile of snacks like biscuits. The convergence of both *Vernonia amygdalina* leaves and *Heteroclarias* spp oil in biscuit production holds significant promise for creating functional biscuits with enhanced health benefits. However, it is imperative to assess the sensory attributes of these biscuits to ensure consumer acceptability, as well as evaluate their nutritional composition and potential health-promoting properties.

The aim of this study therefore was to evaluate the sensory qualities and nutritional

content of biscuits fortified with *Vernonia amygdalina* leaves and *Heteroclarias spp* viscera oil. By doing so, it seeks to provide valuable insights into the feasibility of producing a novel and healthier snack option that may contribute to improved public health by addressing both taste preferences and nutritional needs. Such research is critical in the ongoing effort to combat nutrition-related health issues and promote healthier dietary choices among consumers.

MATERIALS AND METHODS
Plant collection and preparation

Fresh leaves of *Vernonia amygdalina* were harvested from a garden at *Ajowa* Street, *Ilorin* 240101, Kwara state, Nigeria (8˚31'37.12116''N 4˚35'19.21848''E) in January, 2023. The identification and authentication of the plant was carried out at the herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara state. A voucher specimen no UILH/001/1324/2023 was assigned to the *Vernonia amygdalina* sample.

Vernonia amygdalina leaves collected were rinsed with tap water and air-dried to constant weight at room temperature. The dried leaves were then pulverized using an electronic blender, stored in air-tight container and kept in air-tight container prior to usage.

Heteroclarias viscera oil extraction

The viscera of *Heteroclarias* spp catfish was used for this experiment. The viscera were purchased from the local market in Ilorin (Unity road catfish Market). The fish viscera were extracted from the fish manually by the market vendors. They were bought fresh and washed to remove any blood residue. The viscera oil was extracted according to the wet (boiling) extraction method as described by Vidotti *et al.* (2011) with slight modification by skipping the centrifugation step. A weight of 1000 g

chopped viscera was placed into a cooking bowl with 1000 ml of water and cooked at 100 °C for 50 minutes. The cooked viscera was left to cool down for 3 hours and the oil allowed to float on the surface of the cooked mixture. The floating oil was then collected and placed in a separating funnel in order to separate the oil from the cooked mixture. The clear oil was decanted into a reception container, sieved and heated to remove moisture in the oil.

Characterization of purified oil

Iodine value, was estimated by the Hanes method as described in Association of Official Analytical Chemists (AOAC, 2000). Peroxide value was assessed according to the method of Pearson (1976) where the results were expressed in milliequivalent (meq) of peroxide per kilogram of oil. The acid value, saponification and Thiobarbituric acid (TBA) values were also estimated according to the method described by Pearson (1976) and free fatty acids were estimated based on method described by Stoffel *et al.* (1959).

Biscuit ingredients and preparation

Wheat flour, butter, milk, baking powder, salt and sweetner (Merisant UK Limited, Dublin, Ireland), were purchased from local vendors in Ilorin, Kwara State, Nigeria. The biscuits were formulated following the recipe of Whitley (1970) with slight modifications in the percentages of the ingredients. Four biscuits samples were formulated, namely; biscuit without *Vernonia amygdalina* and fish oil (BWVF), biscuit with 1 g of *Vernonia amygdalina* and fish oil (BWVF-1 g), biscuit with 2 g of *Vernonia amygdalina* and fish oil (BWVF-2 g) and biscuit with 3 g of *Vernonia amygdalina* and fish oil (BWVF-3 g). The control biscuit labelled CB (conventional biscuit) (a product of Yale Foods Industrial LTD, plot 1B, Block1, Oluyole Estate. P.O. Box 2033, Ring Road Ibadan, Oyo State,

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Nigeria) was purchased from a local vendor in Ilorin.

BWVF was prepared by mixing 240 g of wheat flour with 15 g of sweetener, 1 g of salt, 4 g of baking powder, 60 g of butter and 60 g of milk, to form a dough. The dough was kneaded and rolled carefully with a rolling pin, cut into round shapes and baked in the oven for 15 minutes at 175 ºC.

BWVF-1 g was prepared by mixing 240 g of wheat flour with 15 g of sweetener, 1 g of salt, 4 g of baking powder, 69.5 g of fish oil, 60 g of milk and 1 g of pulverized *Vernonia amygdalina* leaves, to form a dough. The dough was kneaded and rolled carefully with a rolling pin, cut into round shapes and baked in the oven for 15 minutes at 175 ºC.

The process used for the formulation of BWVF-1 g was repeated for the formulation of BWVF-2 g and BWVF-3 g with variation in the quantity of *Vernonia amygdalina*. Two (2 g) of *Vernonia amygdalina* was used for the formulation of BWVF-2 g while 3 g was used for the formulation of BWVF-3 g. Afterwards, the biscuits were cooled at room temperature and kept in ziplock bags.

Proximate analysis of experimental biscuits

The proximate nutrient contents of the formulated biscuits were carried out using the procedures described by Association of Official Analytical Chemists (AOAC, 2005).

Determination of moisture content

Briefly, 2.00 g of each sample was placed into already dried and weighed moisture dishes and dried in an oven at 105 °C for 6 hr, after which the dishes were placed in a desiccator to cool to room temperature, weighed, and placed in the oven and dried overnight till a constant weight was obtained. The moisture content was expressed as a percentage of the average weight loss after drying the sample according to the formula:

$$\frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where W_1 is the weight of the dish; W_2 is the weight of dish and wet sample; and W_3 is the weight of dish and dried sample.

Determination of ash content

The ash of a foodstuff is the inorganic residue left after the organic residue has been burnt away. In this study, the total ash content of the biscuits was determined by incinerating samples overnight in a muffle furnace according to the AOAC (2005) method. Briefly, 2 g of each sample was transferred into dry and weighed crucibles. The sample in the crucible was charred for 3 hr in an oven and incinerated overnight in a muffle furnace at 550 °C. Samples were then allowed to cool to room temperature in a desiccator and weighed afterward. The difference in weight was expressed as the percentage total ash of the biscuit samples.

The percentage of ASH was calculated according to the formula below:

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

where A is the weight of the crucible; B is the weight of crucible and raw sample; and C is the weight of crucible and dried sample.

Determination of crude protein

The Kjeldahl method was used for determining the crude protein content of biscuits in this study. Briefly, 1 g each of homogenized sample was weighed and transferred into digestion tubes and 0.5 g of Kjeldahl tabs was added to each sample. After this, 12 ml of H_2SO_4 was added and the tubes were agitated to wet the samples. The sample was digested at 420 °C for an hour when a clear solution was obtained. The samples were then cooled to room temperature and distilled, after which the

bluish green distillate was titrated with 0.1 M HCl solution. The acid was added until the first color change was observed. The volume of acid used for titrating each sample and the blank was recorded. The percentage nitrogen was estimated using the formula:

$$\% \text{ Total nitrogen} = \frac{100 \times (VA - VB) \times NA \times 14}{W \times 1000}$$

where: VA is the volume in ml of standard acid used in titration, VB is the volume in ml of standard acid used in blank, NA is the normality of acid (HCl), and W is the weight in grams of the sample.

Determination of crude fat

The Soxhlet extraction method was used for crude fat determination. In the experiment, 2 g of dried samples from moisture determination was transferred into extraction thimbles and covered with balls of cotton wool. The thimble and its content were placed in a Soxhlet extractor and connected to a weighed dry 500 ml round bottom flask containing 250 ml of petroleum ether. The apparatus was then connected to a quick fit condenser and refluxed for 16.5 hr on an electrothermal extraction unit. The flask was removed after extraction was completed and the petroleum ether evaporated on a rotary water bath. After the evaporation, the flask with the fat was heated in an oven for 1 hr at 103 °C, cooled in a desiccator, and weighed. The crude fat content was calculated according to the formula below:

$$\frac{\text{Weight of flask and fat - Weight of flask}}{2} \times 100\%$$

Determination of crude fiber

The defatted sample from the crude fat determination was transferred into a 500-ml round bottom flask, and 200 ml of boiling 1.25 % H_2SO_4 was added and then connected to a condenser. The sample was boiled for 30 min after which the content of the flask was filtered. The residue was washed with boiling water until it was no longer acid, and boiled again for 30 min with 200 ml 1.25 %

NaOH, and filtration was repeated until the residue had no base. The residue was transferred to a Gooch crucible and the remaining particles washed into the crucible with 15 ml 10 % HCl and filtered. The crucible and its content were weighed and dried overnight at 105 °C, cooled in a desiccator, and reweighed. This was then ashed in a muffle furnace at 550 °C for 3 hr. The crucible was cooled in a desiccator and reweighed. The difference in the weight of crucible and its content before and after ashing was expressed as the percentage crude fiber.

The formula used for crude fiber determination.

$$\frac{(X - Y)}{W} \times 100$$

where:
X is the weight of crucible and dried sample before ashing, Y is the weight of the crucible and sample after ashing, W is the weight of the sample used in the fat determination.

Determination of carbohydrate and energy contents

The carbohydrate composition of the biscuits was determined by difference (100 - the sum of the other five determinations. The energy content of the biscuits was also calculated by Atwater general factor system (AOAC, 2005).

Sensory evaluation of experimental biscuits

The sensory evaluation of prepared biscuits was carried out by 15 trained panelists from Department of Biochemistry, University of Ilorin. The following parameters were evaluated: appearance, taste/flavour, texture/consistency, crunchiness, aroma/smell and overall acceptability. The sensory evaluations were collected on the 9- point Hedonic Scale (Yahaya *et al.,* 2012) where the quality characteristics of each sample were rated as, 9- like extremely, 8- like very much, 7- like

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moderately, 6- like slightly, 5- neither like nor dislike, 4- dislike slightly, 3- dislike moderately, 2- dislike very much and 1- dislike extremely. Samples were evaluated in a day after baking under daylight illumination and in isolated booths within a sensory laboratory. The presentation of the biscuit samples was performed in a blind fashion, coding each sample with a random numeric code of five. The ration of consumption was one whole biscuit of each reference, maintained at a temperature of 25 °C. Water was served to the evaluators for cleaning of the mouth between the different samples.

Statistical analysis
Data were expressed as mean of 3 replicates ± standard deviation (SD). The data were subjected to statistical analysis using the IBM® statistical package for social sciences (SPSS) software version 20. All significant differences were determined by one way analysis of variance (ANOVA). Post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95 %).

RESULTS AND DISCUSSION
Physichochemical properties of *Heteroclarias* spp fish oil
Table 1 shows the results of the physicochemical properties of the extracted *Heteroclarias* spp fish oil. Results showed that the oil contain 0.68 % Oleic acid/100g free fatty acid, 3.48 mg/L TBA value, acid value (0.48 mg KOH/g oil), peroxide value (3.61 meq/kg) and iodine value of 79.95 g/100g oil.

In this study, the utilization of catfish viscera-derived oil from *Heteroclarias* spp served as a replacement for butter, which is known for its undesirable trans-fatty acid content, in the formulation of three different types of biscuits, denoted as BWVF-1 g,

BWVF-2 g, and BWVF-3 g. To ensure the quality of the edible oil used, various physical and chemical parameters were monitored, in accordance with established standards (Ceriani *et al.*, 2008; Mousavi *et al.*, 2012).

These parameters included the iodine value (IV), saponification value (SV), peroxide value (PV), thiobarbituric acid (TBA), free fatty acids (FFAs), and acid value. The peroxide value (PV) serves as an indicator of the extent of rancidity reactions that may occur during storage, thereby reflecting the quality and stability of fats and oils (Ekwu and Nwagu, 2004). The study's results, as presented in Table 1, indicated that the peroxide value was below the standard threshold of 10 meq/kg set by SON (2000) and NIS (1992). Consequently, the oil used in the biscuits exhibited relatively stable fatty acids and good quality.

SV is associated with the average molecular mass of fatty acids in the oil sample. In this study, the SV value fell below the expected range of 195–205 mg KOH/g of oil, as specified for edible palm oils by SON (2000) and NIS (1992). This suggests either a lower mean molecular weight of fatty acids or a lower number of ester bonds. Such results may indicate limited interaction between fat molecules (Denniston *et al.*, 2004).

The IV was employed to measure the degree of unsaturation in the oil, reflecting its susceptibility to oxidation. The high IV values recorded in this study suggests increase unsaturation and higher vulnerability to oxidation. The acid value provides insights into the quality of fatty acids within the oil, indicating the presence and extent of hydrolysis by lipolytic enzymes and oxidation (Ichu and Nwakanma, 2019). Low acid values recorded in this study suggest oil stability over an extended period, guarding against rancidity and peroxidation. The maximum acceptable levels are 0.6 mg

KOH/g for refined oils and 4.0 mg KOH/g for cold-pressed and virgin oils (CODEX, 2005).

TBA testing is commonly employed to assess secondary oxidation products in oils, measuring the degree of malondialdehyde (MDA) and related aldehyde compounds formed during oil oxidation (Yeboah *et al.*, 2021). The reference range of TBA for fish oil is 7-8 mg of malondialdehyde/kg sample (IFOMA, 1998), hence, the TBA value for the oil is within the recommended standard. FFA on the other hand, are the result of oil hydrolysis, not bound to glycerol molecules (Fatimah *et al.*, 2019). FFA content in edible oils is undesirable as it can reduce the oxidative stability of food products, elevate acidity, and lead to off-flavor development. Based on the standards set in International Fishmeal and Oil Manufacturers Association (IFOMA, 1998), the good free fatty acid content in coarse fish oil is 1-7%, but in certain industries the standard of free fatty acid content is 2-5%. Therefore, the low FFA recorded in this study further confirm the oxidative stability of the oil.

Nutrient composition of *Vernonia amygdalina* and fish oil-enriched biscuits

Results showed that the formulated biscuits contain the major classes of nutrients (Table 2). Carbohydrate was significantly lower ($p < 0.05$) in the test biscuits BWVF- 1 g (59.46 ± 0.04), BWVF- 2 g (54.47 ± 0.00) and BWVF- 3 g (54.47 ± 0.00) in a dose dependent manner compared to the CB (76.67 ± 0.06). Percentage crude protein, lipid and ASH compositions increased significantly ($p < 0.05$) compared to CB, while there was no significant difference ($p > 0.05$) in the moisture contents of BWVF-1 g (2.58 ± 0.25), BWVF-2 g (2.68 ± 0.06), BWVF-3 g (2.62 ± 0.03) compared to CB (2.50 ± 0.13). A significant increase ($p < 0.05$) in the moisture content of BWVF (8.74 ± 0.17) was however recorded while fibre

content in the formulated biscuits were significantly low compared to CB.

The nutritional analysis of the formulated biscuits, as presented in Table 2, revealed that the biscuits contain the essential nutrient categories. Nevertheless, it's notable that the carbohydrate content in the *Vernonia amygdalina* and fish oil-enriched biscuits was lower than that in the commercially purchased conventional biscuit (CB). This reduction in carbohydrate content is likely due to the replacement of sugar in CB with artificial sweeteners in the test biscuits (BWVF-1 g, BWVF-2 g, and BWVF-3 g). Several studies have linked the frequent consumption of sugar-rich snacks, particularly those laden with refined added sugar, to an elevated risk of cardiovascular disease (CVD) (Wang *et al.*, 2014; Malik and Hu, 2019). The high energy content, palatability, and elevated levels of refined carbohydrates and sugar in snacks may lead to reduced satiety and increased appetite, potentially contributing to excess weight gain, particularly when consumed when not hungry (Mekary *et al.*, 2012; Denniston *et al.,* 2016).

The noticeable increase in the percentage of crude protein, lipids, and ash content in the test biscuits compared to both the CB and BWVF suggests that incorporation of *Vernonia amygdalina* and fish oil into the biscuits plays a role in elevating their protein, lipid, and ash contents (Oboh, 2006; Abiona, 2021). Dietary fat is a vital macronutrient serving various functions in nutrition, including the provision of essential fatty acids, contributing to satiety, and acting as an energy source (Montmayeur and Le Coutre, 2009, Carreiro *et al.*, 2016). Fish oil, renowned for its omega-3 fatty acids, significantly impacts the lipid content and nutritional profile of products (Bonilla-Méndez and Hoyos-Concha, 2018). These fatty acids are well-regarded for their numerous health benefits, encompassing

cardiovascular protection and anti-inflammatory properties (Calder, 2015). Similarly, total ash content is a pivotal parameter as it encompasses both organic and inorganic components, such as essential minerals. The potential contribution of minerals like calcium, iron, and potassium from *Vernonia amygdalina* leaves can enhance the overall nutrient profile of the biscuits (Garba and Oviosa, 2019).

The assessment of moisture content in food products is a fundamental parameter crucial for gauging product quality, shelf life, and stability. Moisture content significantly impacts the texture, appearance, and overall sensory characteristics of food items, including biscuits (Bakare *et al*., 2020). Excessive moisture content can lead to undesirable textural changes such as softening or sogginess, whereas excessively low moisture content can result in overly dry and crumbly biscuits (Bakare *et al*., 2020). In the context of this study, the moisture content of the formulated samples, namely BWVF-1 g, BWVF-2 g, and BWVF-3 g, was found to be favorable in comparison to the conventional biscuit (CB), indicating a potentially longer shelf life and positive organoleptic properties. This finding aligns with the results of a study by Usunomena *et al*. (2016), where it was observed that *Vernonia amygdalina* leaves exhibited excessively low moisture content. Overall, the formulated biscuits provided more energy arising from the inclusion of fish oil compared to the control biscuits.

Sensory evaluation of *Vernonia amygdalina* and fish oil-supplemented biscuits

Table 3 shows the result of the sensory evaluation of the formulated biscuits. The rating of appearance, texture/consistency, crunchiness and overall acceptability of the formulated biscuits (BWVF-1 g, BWVF-2 g, and BWVF-3 g)

were not significantly different ($p < 0.05$) to CB. However, there was a dose-dependent decrease in the ratings for the taste of BWVF-1 g (3.87 ± 0.19), BWVF-2 g (3.40 ± 0.35), and BWVF-3g (2.87 ± 0.16) compared to CB (4.97 ± 0.35) and BWVF (4.33 ± 0.15). A similar trend was observed for the aroma/smell rating, CB (4.80 ± 0.41), BWVF (4.60 ± 0.51), BWVF-1 g (4.07 ± 0.96) and BWVF-3 g (3.40 ± 0.21).

The sensory attributes of the samples subjected to evaluation were; Appearance, Taste, Texture/Consistency, Crunchiness, Aroma/Smell, and Overall Acceptability (Table 3). These attributes were essential in assessing how the inclusion of *Vernonia amygdalina* leaves and fish oil influenced the sensory qualities of the biscuits. A noteworthy observation was the dose-dependent decrease in taste ratings for BWVF-1 g, BWVF-2 g, and BWVF-3 g in comparison to CB and BWVF. A similar decrease was observed for the aroma/smell rating. These reductions can be attributed to the inherent bitterness of *Vernonia amygdalina* and the fishy odor of the fish oil incorporated into the biscuits. Numerous studies have reported the bitterness of *Vernonia amygdalina* due to the presence of bioactive compounds like sesquiterpene lactones and flavonoids (Ugbogu *et al.,* 2021; Djeujo *et al*., 2023). This inherent bitterness has been known to impact the overall palatability and acceptability of food products which in further study could be addressed. However, the ratings for appearance, texture/consistency, crunchiness, and overall acceptability of the formulated biscuits (BWVF-1 g, BWVF-2 g, and BWVF-3 g) were favorable in comparison to the conventional biscuit, suggesting that *Vernonia amygdalina* and fish oil-supplemented biscuits can be a suitable alternative snack contributing to the daily nutritional and calorie need of consumers.

CONCLUSION

Vernonia amygdalina and fish oil-supplemented biscuits formulated in this study can be a suitable alternative snack contributing to the daily nutritional and calorie need of consumers. We therefore recommend further study into its impact on health needs of consumers.

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Table 1. Physicochemical parameters of *Heteroclarias spp* fish oil

Parameters	Quantity
Free Fatty Acid (% Oleic acid/100g)	0.68 ± 0.00
Saponification Value (mg KOH/g oil)	4.49 ± 0.00
Acid Value (mg KOH/g oil)	0.48 ± 0.04
Peroxide Value (meq/kg)	3.61 ± 0.73
Iodine Value (g/100g oil)	79.95 ± 0.00
Thiobarbituric Acid (mg/L)	3.41 ± 0.02

Values are mean of 3 replicates $\pm$ SD

Table 2. Nutrient composition of *Vernonia amygdalina* and fish oil-enriched biscuits

Nutrients (%)	CB	BWVF	BWVF-1g	BWVF-2g	BWVF-3g
Crude protein	4.81 ± 0.31^a	4.65 ± 0.08^a	5.69 ± 0.31^b	6.13 ± 0.30^b	6.67 ± 0.16^c
Crude Lipid	8.24 ± 0.07^a	13.84 ± 0.15^b	20.88 ± 0.11^c	20.70 ± 0.01^d	20.33 ± 0.04^e
Moisture	2.50 ± 0.13^a	8.74 ± 0.17^b	2.58 ± 0.25^a	2.68 ± 0.06^a	2.62 ± 0.03^a
Total ASH	1.49 ± 0.01^a	1.67 ± 0.01^b	2.00 ± 0.01^c	2.44 ± 0.02^d	2.46 ± 0.04^d
Crude Fibre	6.30 ± 0.18^a	0.65 ± 0.01^b	2.43 ± 0.23^c	3.26 ± 0.20^d	3.44 ± 0.00^d
Carbohydrate	76.67 ± 0.06^a	67.07 ± 0.01^b	59.46 ± 0.04^c	54.47 ± 0.00^d	54.47 ± 0.00^d
Energy(Kcal)	400.08 ± 1.64^a	411.44 ± 1.05^b	448.52 ± 0.37^c	428.70 ± 0.94^d	427.53 ± 0.13^d

Values are mean of 3 replicates $\pm$ SD; Values carrying different alphabets across the row are significantly different at $p < 0.05$; CB – Conventional biscuit; BWVF – Biscuit without *Vernonia amygdalina* and fish oil; BWVF-1g - Biscuit with 1 g *Vernonia amygdalina* and fish oil; BWVF-2 g - Biscuit with 2 g *Vernonia amygdalina* and fish oil; BWVF-3 g - Biscuit with 3 g *Vernonia amygdalina* and fish oil; Kcal – Kilocalorie

Table 3. Organoleptic parameters of Experimental biscuits

Parameters	CB	BWVF	BWVF-1 g	BWVF -2 g	BWVF-3 g
Appearance	4.67 ± 0.82^a	4.33 ± 0.23^a	4.27 ± 0.46^a	3.80 ± 0.86^a	3.80 ± 0.94^a
Taste	4.97 ± 0.35^a	4.33 ± 0.15^b	3.87 ± 0.19^c	3.40 ± 0.35^c	2.87 ± 0.16^d
Texture/ Consistency	4.67 ± 0.49^a	4.40 ± 0.91^a	4.40 ± 0.74^a	3.80 ± 0.77^a	3.80 ± 0.77^a
Crunchy	4.87 ± 0.35^a	4.53 ± 0.52^a	4.60 ± 0.83^a	3.93 ± 0.88^a	4.40 ± 0.63^a
Aroma/ Smell	4.80 ± 0.41^a	4.60 ± 0.51^a	4.07 ± 0.96^a	3.50 ± 0.21^b	3.40 ± 0.21^b
Overall Acceptability	4.73 ± 0.59^a	4.60 ± 0.51^a	4.33 ± 0.92^a	4.28 ± 0.12^a	4.20 ± 0.11^a

n = 15 ± SD; Values carrying different alphabets across the row are significantly different at $p < 0.05$; CB – Conventional biscuit; BWVF – Biscuit without *Vernonia amygdalina* and fish oil; BWVF-1g - Biscuit with 1 g *Vernonia amygdalina* and fish oil; BWVF-2 g - Biscuit with 2 g *Vernonia amygdalina* and fish oil; BWVF-3 g - Biscuit with 3 g *Vernonia amygdalina* and fish oil