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Nutritional and shelf-life studies of a ready-to-eat snack developed using by-products from *Tilapia guineensis*

B. Egbedi* and A. O. Osibona

Department of Marine Sciences, University of Lagos, Lagos, Nigeria

*Corresponding author: brakemi.egbedi@unilag.edu.ng
Co-author's email: osibonasola@yahoo.com

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ABSTRACT: Ready-to-eat (RTE) snacks were produced from the head and fins of *Tilapia guineensis*. The snacks were fried using the conventional method and were analyzed for their nutritional composition and shelf-life using biochemical tests (Thiobarbituric Acid (TBA), Peroxide value (PV) and Free Fatty Acid (FFA)), sensory assessment, water activity tests, and microbial analysis. There was no significant difference (p>0.05) in the nutritional composition of the two snacks developed. The snacks developed from the head without gills (PA) and the fins (PC) were low in protein (9.50±0.19 and 10.03±0.71%) and high in carbohydrate (55.33±2.02 and 51.56±2.53%). There was a significant increase (p<0.05) in the PV, TBA and FFA values throughout the storage period. Although the values for FFA exceeded the 0.5-1.5% recommended level for a noticeable rancid taste, the result of the sensory assessment of PA and PC showed that both RTE snacks were acceptable by the taste panel throughout the storage period. The range of water activity values for PA (0.57-0.78) and PC (0.59-0.64) were high and this possibly favoured the survival and outgrowth of microbial spores. The absence of *Salmonella sp, S. aureus* and *E. coli* in PA and PC, showed good manufacturing practice in terms of hygiene and food handling while the presence of low counts of Enterobacteriaceae was indicative of inadequate frying of the snack. The bacteria species identified in both products was *Bacillus sp*. The value for total plate count and mould count in PA was within acceptable limits until the third week of storage while its values for PC were within the acceptable limit throughout the storage period. The results show that the head and fins of *T. guineensis* can be used to make a nutritious snack product that would be accepted by consumers. Although the RTE snacks developed in this study offers a new snack variety and adds value to the fish by-products generated during processing, there is need to conduct more research on optimization studies which would lead to the development of protein rich RTE snacks having a longer shelf-life.

Keywords: Fish by-products, Ready-to-eat snacks, *Tilapia guineensis*, Shelf-life studies, Fried snacks.

1.0 INTRODUCTION

Fish and fishery products are very valuable sources of protein and essential micronutrients (Palmeira *et al.,* 2015) especially for those in developing countries. *Tilapia guineensis* commonly referred to as the Guinean Tilapia, is a euryhaline species found in abundance in most estuaries and lagoons along the West Coast of Africa (Kuton and Adeniyi, 2014). Tilapias are known to be high in protein, low in fat, calories, and carbohydrate. They are also an excellent source of minerals such as Phosphorus, Niacin, Selenium, Vitamin B_{12} and Potassium (Vignesh and Srinivasan, 2012) and are widely accepted and consumed in Nigeria. Depending on the type of fish and level of processing, significant amounts of by-products (about 20- 80%) are generated (Ghaly *et al*., 2013). The solid by-products generated include fish head, viscera,

fins, tails, frames, and skin. In some countries, these wastes are not utilized, but incinerated or dumped at sea thus causing environmental problems (Bozzano and Sarda, 2002). It has been reported by Abimbola *et al.*, (2010) that the by-products generated during the processing of *T. guineensis* make up about 60.5% of its anatomical yield with the head having the greatest yield after the fish's flesh or fillet.

To attain the United Nation Sustainable Development Goal (UN SDG) of eradicating hunger and conserving and utilizing the oceans, seas, and marine resources for sustainable development; there is need to utilize fish by-products. If utilized, some of these by-products can be recovered as human food and in the production of ready-to-eat (RTE) and semi-ready-to-eat products (Adeleke and Odedeji, 2010). Ready-to-eat (RTE) foods are foods which have been processed and can be safely consumed without any further preparation or heat treatment (Rodríguez-Cavallini *et al*., 2010) some of which include snacks. Several RTE and semi-RTE products have been developed using fish by-products some of which include: Fish soup (Stevanato *et al.,* 2007), Surimi (Mello *et al.,* 2010), sausage (Oliveira Filho *et al.,* 2010), fish bouillon cubes (Fabricio *et al.,* 2013), bread fortified with fish flour (Adeleke and Odedeji, 2010), instant soup (Monteiro *et al.,* 2014), red-fish meat based fried snacks (Nawaz *et al.,* 2019). Snacks are light RTE foods which can be eaten anytime or when desired. Snack foods are consumed often and widely and are considered important vehicles for supplying nutrients to its consumers. They can be made via frying, baking or extrusion (Nawaz *et al.,* 2019). Changes in lifestyle and eating habits have led to an increase in the demand for snack foods (Kocherla *et al.,* 2012) worldwide. Snacks are among the most consumed (>65%) products in Nigeria (De Vries-Ten Have *et al.,* 2020**)** which is justified by the convenience in their consumption, their low costs, change of dietary lifestyle and eating habits among Nigerians.

There is a dearth of information on the use of by-products from *T. guineensis* in the development of ready-to-eat snacks in Nigeria. This study aims to develop a ready-to-eat snack using two forms of fish by-products: the head and fins from *T. guineensis* and to determine its acceptability, nutritional composition, and shelf life. The utilization of these by-products in the development of an RTE snack, may contribute to food security and reduce environmental pollution.

2.0 MATERIALS AND METHODS

Fifty-five (55) samples of *T. guineensis* were purchased from local fish mongers at Oluwo market, Epe, Lagos State, Nigeria. The fins were cut off using a pair of sterilized kitchen scissors in such a way that the flesh of the fish was not included. With the aid of a sharp knife, the head was cut off using a round cut to get the lowest flesh loss (Bykowski and Dutkiewicz, 1996) and the gills were removed afterwards.

2.1 PRODUCTION OF FISH BY-PRODUCT POWDER

The production of the fish by-product powder from the head without gills (HWG) and the fins (FIN) is shown in Figure 1.

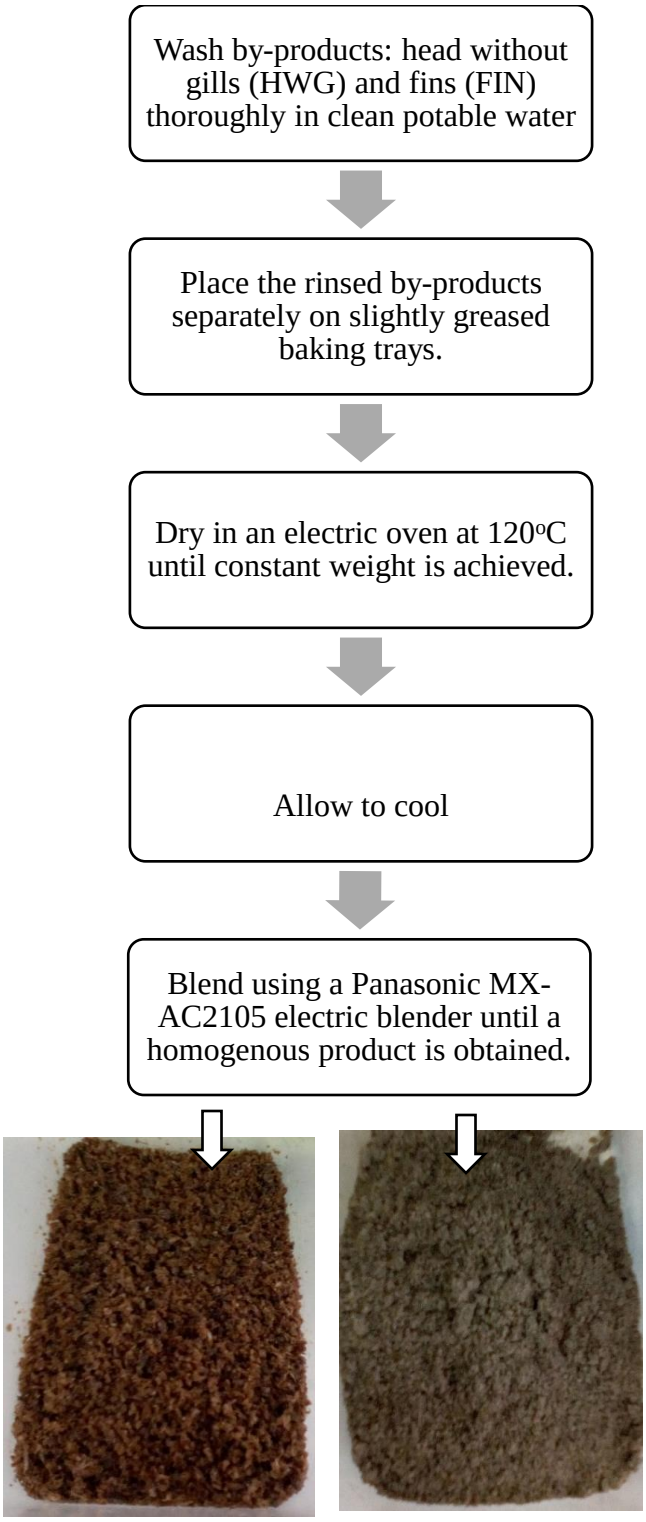

Figure 1: The production of the fish by-product powder

2.1.1 READY-TO-EAT SNACKS PRODUCTION

The production of the ready-to-eat (RTE) snacks in Figure 2, involved the formulation: 12% fish powder, 1% Salt, 87% Flour.

The RTE snacks made from the head without gills (HWG) was labelled as PA while those made using the Fins (FIN) was labelled as PC. The RTE snacks were fried using the conventional frying method (where the frying temperature is moderated manually by the processor) to depict real-time conditions since electricity is a challenge in developing countries and because many poor people cannot afford an electric frying machine.

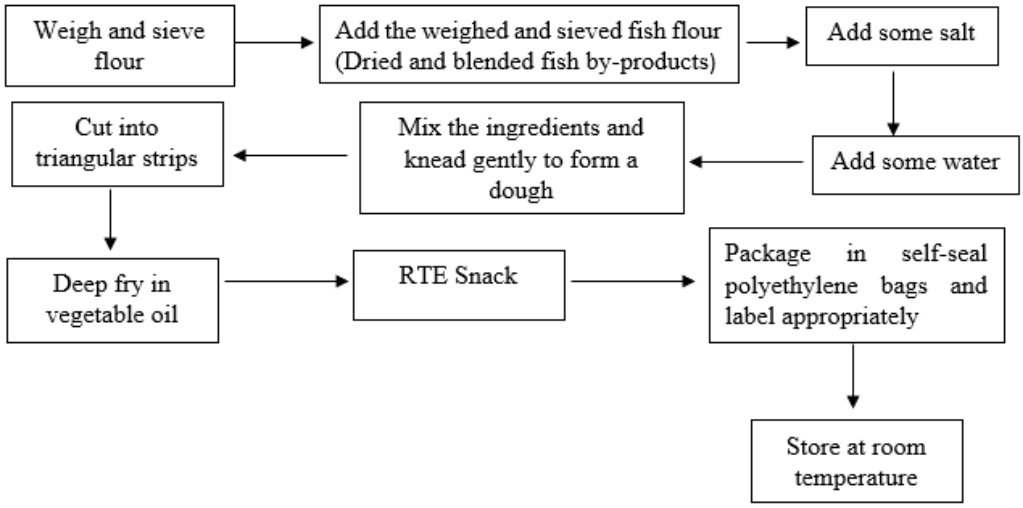

Figure 2: RTE snacks production and storage

2.2 PROXIMTE COMPOSITION OF THE READY-TO-EAT SNACKS

Carbohydrate was calculated by difference using the formula: Percentage carbohydrate (%) = 100 – (% Protein + % Moisture + % Lipid + % Crude fibre). The proximate composition was determined using the AOAC (2002) method. The proximate composition of the RTE snacks were determined in triplicate on the freshly prepared snacks only.

2.3 SHELF-LIFE STUDIES OF THE READY-TO-EAT SNACKS

Shelf-life studies were conducted on the RTE snacks weekly for a storage period of three weeks using biochemical analysis, water activity, sensory and microbial analysis. Samples of the RTE snacks were analyzed on the day of production i.e., day 0 (or week 0), to serve as the control and weekly for a period of three weeks.

2.3.1 BIOCHEMICAL ANALYSIS OF THE READY-TO-EAT SNACKS

The Peroxide Value (PV), Free Fatty Acid content (FFA) of the RTE snacks were determined using the methods described by (AOAC, 2002); the Thiobarbituric acid (TBA) was determined by the method described by Dandago *et al.*, (2004). The analysis for PV, TBA and FFA were determined in quadruplicate.

2.3.2 WATER ACTIVITY (A_W) OF THE READY-TO-EAT SNACKS

Water activity (a_W) of the RTE snacks was measured using a portable Rotronic water activity analyzer (HygroPalm HP23-AW-A) at room temperature. Twenty-five (25g) of the ground RTE snacks samples were put into the water activity measuring cup until it filled the line of the cup. The measuring cups were placed in the a_w chamber in the a_w instrument, and the values were measured automatically after starting the analyzer. The analysis for a_w were determined in duplicate.

2.3.3 SENSORY (ORGANOLEPTIC) ASSESSMENT OF THE READY-TO-EAT SNACKS

The sensory analysis of the developed RTE snacks was carried out using a 9-point hedonic scoring scale ranging from 1"*Dislike Extremely*" to 9 "*Like Extremely*" described by Goes *et al.,* (2015). A group of 7 untrained panelists were selected to evaluate the RTE snacks weekly during the three-week storage period. The panelists were provided with water to rinse their mouths in-between tasting samples. The samples which were labelled using a 3-digit code, were presented to the panelists who evaluated them based on the following attributes: Appearance, taste, odour, texture (crispness), colour and overall acceptability. Scores below 4 were taken as a rejection point for the samples (Vanitha *et al.,* 2015).

2.3.4 MICROBIOLOGICAL ANALYSIS OF THE READY-TO-EAT SNACKS

The microbial load was assayed in duplicate using Tryptone Soya Agar (TSA), Sabouraud Dextrose agar (SDA), Eosine Methylene Blue agar (EMBA), Salmonella / Shigella agar (SSA), Mannitol Salt Agar (MSA) and Mac Conkey agar (MCA) for total plate count, total yeast and mould count, total *Escherichia coli* (*E. coli*) count, total *Salmonella* and *Shigella* count and for Coliform counts respectively as described by Adebayo-Tayo *et al.,* (2006).

2.4 STATISTICAL ANALYSIS

Data obtained for the sensory and biochemical tests were analyzed using One-way ANOVA. Student-Newman-Keuls, Tukey and Duncan post hoc tests were used to check for significant difference in the means at 5% probability level. The data obtained for the proximate composition and water activity were analyzed using Independent sample T-test. The analysis was done using SPSS version 17.0.

3.0 RESULTS

3.1 PROXIMATE COMPOSITION OF THE READY-TO-EAT SNACKS

The products developed using the HWG and FIN of *T. guineensis* labelled PA and PC, are shown in Plate 1. There was no significant difference ($p>0.05$) in the mean proximate composition between the two RTE snacks developed as shown in Table 1.

Table 1: Mean ± Standard Error (SE) proximate composition of PA and PC

Proximate composition (%)	PA	PC
Carbohydrate	55.33 ± 2.02^{a}	51.56 ± 2.53^{a}
Protein	9.50 ± 0.19^{a}	10.03 ± 0.71^{a}
Crude fat	15.50 ± 0.46^{a}	16.14 ± 1.13^{a}
Moisture	13.88 ± 1.17^{a}	17.30 ± 1.15^{a}
Ash	3.02 ± 0.43^{a}	3.67 ± 1.08^{a}
Crude fibre	2.77 ± 0.68^{a}	1.30 ± 0.03^{a}

**Means having letters with identical superscript(s) are not significant ($p>0.05$)*

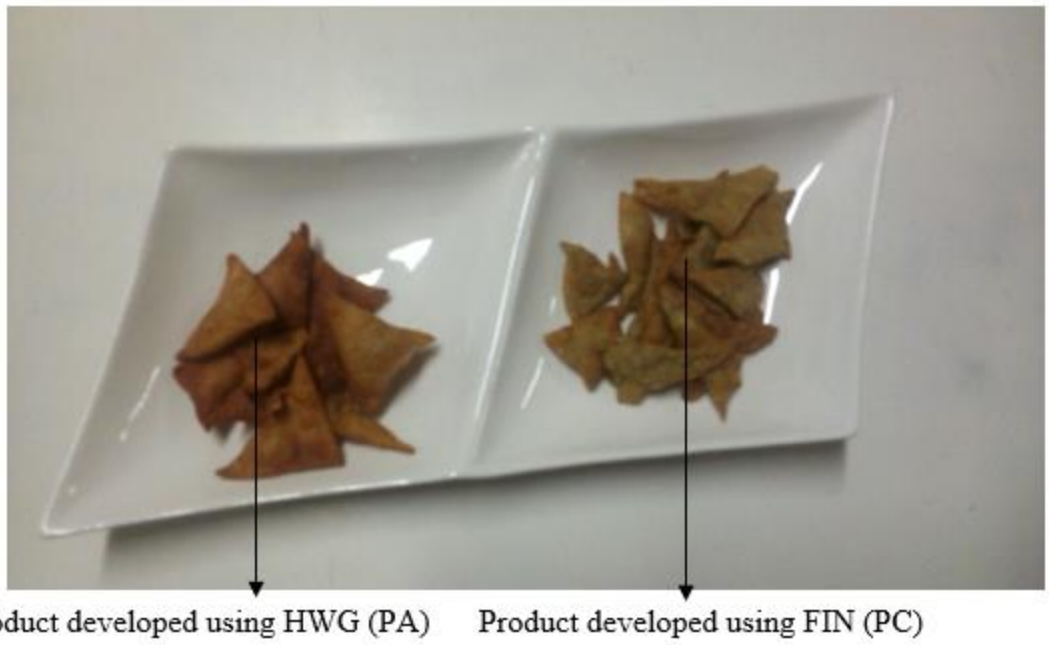

Plate 1: Products PA and PC respectively

3.2 BIOCHEMICAL COMPOSITION OF THE READY-TO-EAT SNACKS

The results of the PV, TBA and FFA of the RTE snacks PA and PC are shown in Table 2.

An increase in PV was observed in PA and PC throughout the three-week storage period. The increase in PV in PA was significant ($p<0.05$) during the first week of storage while the increase in PV in PC was significant ($p<0.05$) during the first two weeks of storage.

The TBA values in PA and PC showed a significant increase ($p<0.05$) during storage as shown in Table 2. The lowest value was recorded on day 0 while the highest value was recorded in week 3 in PA and PC respectively.

The FFA value in PA increased throughout the period of storage. The increase in FFA value was significant ($p<0.05$) during the first and last week of storage while the FFA value in PC increased significantly ($p<0.05$) throughout the period of storage.

Table 2: Mean ± Standard Error (SE) of PV, TBA and FFA in PA and PC during a three-week storage period

PA **Week of storage**	**PV (mEq/kg)**	**TBA (mg malonaldehyde/kg)**	**FFA (%)**
Day 0	5.51 ± 0.57^{a}	1.78 ± 0.30^{a}	3.59 ± 0.33^{a}
Week 1	11.03 ± 2.78^{b}	2.59 ± 0.24^{b}	5.73 ± 0.97^{b}
Week 2	12.81 ± 2.09^{b}	3.42 ± 0.36^{c}	6.86 ± 0.90^{b}
Week 3	12.82 ± 0.42^{b}	4.12 ± 0.14^{d}	11.26 ± 0.72^{c}
PC **Week of storage**			
Day 0	6.86 ± 0.34^{a}	1.78 ± 0.10^{a}	3.58 ± 0.05^{a}
Week 1	10.58 ± 0.57^{b}	2.59 ± 0.14^{b}	6.04 ± 0.25^{b}
Week 2	13.86 ± 1.02^{c}	3.56 ± 0.53^{c}	7.67 ± 1.72^{c}
Week 3	15.80 ± 2.78^{c}	4.75 ± 0.64^{d}	11.77 ± 0.94^{d}

**Means having letters with identical superscript(s) are not significant ($p>0.05$)*

3.3 WATER ACTIVITY (a_W) OF THE READY-TO-EAT SNACKS

The water activity values (a_w) for PA and PC are shown in Figure 3. There was a decrease in the values for water activity in PA throughout the three-week storage period while an obvious decrease in a_w values was observed during the first week of storage in PC.

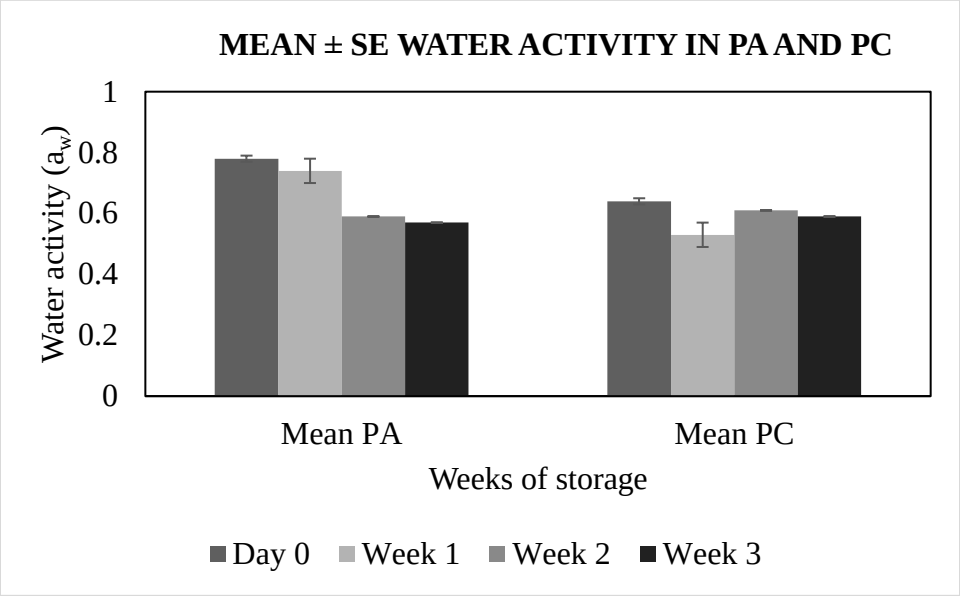

Figure 3: Mean ± SE of water activity in PA and PC during a three-week storage period

3.4 SENSORY EVALUATION OF THE READY-TO-EAT SNACKS

The mean scores for the sensory evaluation of PA and PC are shown in Table 3. There was no significant difference (p>0.05) in the results obtained for appearance, odour, taste/flavour, colour and overall acceptability but there was significant difference in the texture/crunchiness of PA at the third week of storage. There was no significant difference (p>0.05) in the results obtained for appearance, odour, taste/flavour, texture/crunchiness, colour and overall acceptability in PC during the entire three-week storage. PC was most acceptable in the second week of storage (7.43±0.43) and was least accepted in the third week of storage (6.43±0.62).

Table 3: Sensory assessment of PA and PC during the three-week storage period

PA Sensory parameters	**WEEK 1 Mean±SE**	**WEEK 2 Mean±SE**	**WEEK 3 Mean±SE**
Appearance	7.43±0.65[a]	7.14±0.59[a]	7.71±0.42[a]
Odour	7.29±0.47[a]	7.29±0.68[a]	7.00±0.44[a]
Taste/ flavour	6.71±0.57[a]	7.14±0.51[a]	7.57±0.43[a]
Texture/crunchiness	5.71±0.47[a]	5.29±0.52[a]	7.57±0.30[b]
Colour	7.14±0.67[a]	7.00±0.62[a]	8.00±0.38[a]
Overall acceptability	6.14±0.67[a]	6.86±0.63[a]	7.71±0.36[a]
PC Sensory parameters			
Appearance	6.57±0.37[a]	6.86±0.26[a]	6.29±0.61[a]
Odour	7.43±0.53[a]	7.00±0.79[a]	7.00±0.44[a]
Taste/ flavour	7.29±0.42[a]	6.57±0.81[a]	7.43±0.48[a]
Texture/crunchiness	7.86±0.26[a]	7.57±0.43[a]	7.71±0.52[a]
Colour	6.86±0.51[a]	6.57±0.48[a]	6.29±0.61[a]
Overall acceptability	7.29±0.42[a]	7.43±0.43[a]	6.43±0.62[a]

**Means having letters with identical superscript(s) are not significant (p>0.05)*

3.5 MICROBIAL ANALYSIS OF THE READY-TO-EAT SNACKS

The results of the microbial analysis are presented in Table 4. The total plate count (TPC) of PA during storage ranged from 1.0×10^1 cfu g^{-1} to TNTC (Too numerous to count) while the TPC of PC ranged from 0.5×10^1 cfu g^{-1} to 3.80×10^2 cfu g^{-1} as shown in Table 4. *S. aureus, Salmonella sp., Shigella sp.* and *E. coli* were not detected in PA and PC throughout the storage period. Coliforms were detected in PA at day 0 and it was not detected during the remaining weeks of storage. Coliforms were not detected in PC throughout the three-week storage period.

Table 4: Microbiological indicators for PA and PC during storage

PA				
Microbial indicators (in 10g of sample) $CFUg^{-1}$	**Day 0**	**Week 1**	**Week 2**	**Week 3**
Total plate count	3.15×10^2	1.00×10^1	9.05×10^2	TNTC
Total yeast	ND	ND	1.50×10^1	ND
Total mould count	ND	5.0×10^1	ND	TNTC
E. coli detection	ND	ND	ND	ND
Salmonella and *Shigella* detection	ND	ND	ND	ND
Staphylococcus detection	ND	ND	ND	ND
Enterobacteriaceae (Coliforms)	6.5×10^1	ND	ND	ND
PC				
Total plate count	1.00×10^1	0.5×10^1	3.80×10^2	5.00×10^1
Total yeast	ND	ND	ND	ND
Total mould count	ND	1.5×10^1	ND	ND
E. coli detection	ND	ND	ND	ND
Salmonella and *Shigella* detection	ND	ND	ND	ND
Staphylococcus detetion	ND	ND	ND	ND
Enterobacteriaceae (Coliforms)	ND	ND	ND	ND

*ND means not detected *TNTC means too numerous to count

4.0 DISCUSSION

PROXIMATE COMPOSITION OF THE READY-TO-EAT SNACKS

The moisture content in PA and PC were 13.88±1.17% and 17.30±1.15%. Although there was no significant difference, the moisture content in PC was higher than PA. According to Huda *et al.,* (2011) the process of frying causes moisture to evaporate from the snack sample thus causing a decrease in the moisture content. In this study, since an electric frying machine which can thermostatically regulate the frying temperature of the RTE snacks was not used, there is a possibility that some of the batches of snacks may not have been fried at a uniform temperature thus proper evaporation of moisture may have been halted. Another reason for the high moisture content in the snacks developed in this study may be attributed to the high percentage of starch source used. This is in agreement with Netto *et al.,* (2014) who opined that moisture contents in snack products after drying is directly related to the percentage of starch source used as the higher the starch source used, the higher the moisture content of the product. According to Barreto and Beirao (1999) starch sources have a higher water holding capacity when compared to fish sources and this property could have prevented the evaporation of moisture during the drying process. Varying moisture contents in fish snacks ranging from 0.48% to 12% have been reported by King (2002); Nurul *et al.,* (2009, 2010) and Neiva *et al.,* (2011).

The protein content of the developed products in this study was higher than those reported by King (2002) who recorded a value of 8.3% in dried fish crackers with the formulation 40% fish/60% starch. The result of this study is comparable with the results observed by Nurul *et al.,* (2009) who reported a value of 10.12±0.42% in crackers made with tapioca flour and fish using a formulation ratio of 1:1; Goes *et al.,* (2015) who recorded protein values of 9.80±0.49%, 9.21±0.17%, 10.2±0.21% in extruded snacks made with the addition of Tilapia, salmon, and sardine meal respectively. Goes *et al.,* (2015) opined that the variation in protein level among snacks could be as a result of the fish parts used and its level of inclusion. This may be the reason for the difference in protein values between PA and PC.

Another reason for the low protein value in this study may be attributed to the method of preparation of the snacks (frying). King (2002) was also of the opinion that the reason for the lower protein value recorded in fried crackers when compared with that of dried crackers made from Big-eye fish (*Brachydeuterus auritus*) was because of the absorption of oil during frying. In this study, the RTE snacks were below the 12% recommended minimum value for crude protein in non-fried and fried snacks. Although the fried snacks of this study had protein contents below the recommended value, the addition of fish by-products may have an improved nutritional quality of the snack since the main ingredient used in the development of the snacks was a starch source (Flour) which is usually high in fat and carbohydrate but low in protein (Rhee *et al.,* 2004). The findings in this research is in agreement with Netto *et al.,* (2014); Goes *et al.,* (2015) who obtained values less than the recommended FAO crude protein value in the fish snacks developed.

The value obtained for lipid in PA 15.50± 0.46% and PC 16.14±1.13% was higher than the value obtained by Goes *et al.,* (2015) who recorded lipid values 6.44±0.38%, 6.39±0.63%, 6.54±0.80% and 5.93±1.12% in extruded snacks made with Tilapia, Salmon, Tuna and Sardine meals. According to Netto *et al.,* (2014) and Nurul *et al.,* (2010), the variations in lipid in fish snacks may be due to changes in the fat absorbed by the snacks during frying as a result of factors such as type of fish species, fish part used, the level of inclusion of fish: flour and other ingredients; and the snack's expansion during frying (Huda *et al.,* 2011). This is in agreement with the findings of Neiva *et al.,* (2011) who reported a higher lipid value (26.11±0.54%) in fried fish crackers when compared with microwaved fish crackers (0.42± 0.07%) and Huda *et al.,* (2011) who observed higher values in the fried buffalo skin crakers when compared with the pre-fried crackers.

The Ash values recorded in PA and PC were 3.02±0.43% and 3.67±1.08% respectively. The reason for the variation in ash contents between the product PA and PC may be because of the type of fish part used in the production of the RTE snack. This is in agreement with Goes *et al.,* (2015), who stated that the lower mineral content obtained for extruded snacks made using Tuna meals when compared with those

made using Sardine, Salmon and Tilapia meals may be as result of greater muscle inclusion and fewer bones.

The carbohydrate content in PA ranged from 55.33±2.02% and 51.56±2.53% in PC. The values were lower than those obtained by Neiva *et al.,* (2011) who obtained carbohydrate values 78.18% and 59.19% in microwaved and fried fish crackers. According to Martinez *et al.,* (1997), snacks having starch as its major component absorbs oil during frying and this result in the snacks having a high carbohydrate value. In this study, the use of frying as a method of cooking and the starch: fish ratio, resulted in the snacks' high carbohydrate values. The results of this study show that PA and PC are high energy products due to their carbohydrate contents, with relatively low protein levels.

BIOCHEMICAL COMPOSITION OF THE READY-TO-EAT SNACKS

A major problem in the storage of fishery products is the development of rancidity (Jeyasanta *et al.,* 2013*).* Lipid oxidation plays a key role in determining the shelf-life of foods as it often leads to changes in its organoleptic properties (Van Ruth *et al.,* 1999).

The Peroxide values (PV) recorded in this study were within the acceptable limit of 10 - 20mEq/kg of fat (Connell, 1995) throughout the three-week storage period. According to Gulla and Waghray, (2011), a rancid taste is noticeable when the PV range is above 20mEq/kg. This study showed that the PV did not affect PA and PC as the result obtained from the sensory assessments for taste/flavour showed that PA (7.57±0.43: like very much) and PC values (7.43±0.48: like moderately) were still within acceptable limits at the third week of storage.

There was a significant increase (p<0.05) in the TBA values for PA and PC throughout the three-week storage period with week 3 having the highest value. It was observed that the increase in TBA of PA which ranged from 1.78mg malonaldehyde/Kg to 4.12mg malonaldehyde/Kg; was similar to those observed in PC which ranged from 1.78mg malonaldehyde/Kg to 4.75mg malonaldehyde/Kg during the three-week storage period. According to Olafsdottir *et al.,* (1997), TBA factor is responsible for the formation of secondary lipid oxidation and its consequent effects such as the development of rancid flavours, off odour and colour, texture deterioration and nutritional value. Schormuller (1968) proposed a TBA value lower than 3mg malonaldehyde/Kg as the acceptable limit for a fishery sample to be termed as having 'very good quality'; a value of 3-5mg malonaldehyde/Kg as 'good', 5-8mg malonaldehyde/Kg as marketable and a value above 8mg malonaldehyde/Kg as 'spoilt'. The TBA value recorded in PA and PC during the first week of storage were below the 3mg malonaldehyde /kg acceptable limit for a sample to be qualified as 'very good' in terms of quality. The values recorded during the second and third week of storage in PA ranged from 3.42mg malonaldehyde /Kg to 4.12mg malonaldehyde /Kg and 3.56mg malonaldehyde /Kg to 4.75mg malonaldehyde /Kg in PC. These values although above 3mg malonaldehyde/Kg were within the 3-5mg malonaldehyde/Kg limit which signifies that both PA and PC were still of good quality at week 3. The increase in TBA values for PA and PC can be as a result of the development of oxidative rancidity of the products which may be attributed to the packaging material used. This is in agreement with Brewer *et al.,* (1992) who stated that TBA values increase during storage as a result of the permeability of oxygen in packaging materials and Mol (2005) who recommended the use of high barrier packaging materials and proper storage conditions to prevent oxidation of fish products. The absence of seasonings or preservatives in the snack formulation may also have led to the increase in TBA values of the products during storage. According to Shahmohammadi *et al.,* (2016), the addition of seasoning which act as preservatives in products, is a key determinant in the TBA value of snacks during storage.

The formation of FFA was seen to increase with increasing time of storage for PA and PC. The increase in FFA values in this study can be attributed to the frying time, frying temperature and the storage conditions. In this study, since an electrical frying machine was not used, the frying temperature could not be uniformly regulated; thus, it may have made the frying time and temperature for both samples uneven. The increase in FFA value of PA and PC which were stored in self-seal polyethylene bags in this study, is in agreement with the findings of Abong *et al.,* (2011) who recorded a higher accumulation of FFA's in transparent polyethylene bags which allowed for the penetration of more light in the product and

encouraged moisture build up. According to Abong *et al.,* (2011), air is a pro-oxidant agent and proper packaging materials are essential to attain a longer shelf-life for products.

According to Horner (1997), when the FFA value, calculated as oleic acid is about 0.5-1.5%, it becomes noticeable in terms of taste. Although the FFA values in the product developed from PA and PC were above the 0.5-1.5% recommended level, a rancid taste or off- flavour was not noticed in the products as seen in the sensory assessment as shown in Table 3.

SENSORY EVALUATION OF THE READY-TO-EAT SNACKS

Although there were differences in the mean of each sensory parameter measured during the storage period, statistically, there was no significant difference (p>0.05) in the sensory parameters analyzed. The highest value for overall acceptability in PA (7.71±0.36) was observed in week 3 and this can be linked to the product's appearance, taste, texture and colour which also recorded the highest values at the third week of storage. The highest value for overall acceptability in PC (7.43±0.43) was observed during the second week of storage. The colour of the snack PC may have played a role in its acceptance and appearance rating during the sensory assessment. The finding in this research is in agreement with Yu (1991), Huda *et al.,* (2000, 2001) who reported that lighter-coloured crackers were preferred by panelists. Several factors that affect the colour of fish products include the quantity and type of fish used and the quantity and type of starch source added (Huda *et al.,* 2001). In this study, the difference in the mean values in PA and PC for the sensory attribute for colour, can be attributed to the type of fish part used. FIN had a darker colour when compared with HWG as shown in Figure 1; and this colour difference may be linked to the pigmentation in these fish parts. The score values for the overall acceptability for PA and PC were above the rejection score of 4 (Dislike slightly), thus it can be considered an acceptable product.

MICROBIAL ANALYSIS OF THE READY-TO-EAT SNACKS

Microbial growth in food products is due to certain intrinsic and extrinsic factors such as moisture, temperature, water activity (a_w), pH and nutrient content (Gram and Huss, 1996). The values for water activity for PA ranged from 0.57 to 0.78 while those made with the fins ranged from 0.59 to 0.64. The increase in a_w in PA and PC was probably due to the diffusion of water vapour from the surrounding atmosphere through the packaging material. This is in agreement with the findings of Nilsuwan *et al.,* (2016) who observed that fish snack samples stored in air without film covering (which acts as a barrier to water vapour migration), had a higher water activity and moisture content.

Total plate count (TPC) is an important factor used to determine the microbial quality of food products (Khanipour *et al.,* 2014). It can be used to indicate temperature abuse, spoilage or contamination of products (Stannard, 1997). There is no specific regulation for the kind of RTE snack developed in this study in standard regulatory bodies nor did it 'categorically' fall under a specific food category in established guidelines for ready-to-eat foods. Based on the ingredients used and the nature and degree of processing (Centre for Food Safety, 2014), the microbial results of the snacks were interpreted following the microbial specifications for ready-to-eat dried heat processed foods proposed by Stannard (1997) and the Centre for Food Safety (2014) general limits for ready-to-eat foods.

The lowest TPC value in PA was observed at week 1 (1×10^1logcfu/g) while the highest was observed at week 3 (TNTC). The TPC value of PA during the first two weeks of storage were within the acceptable limits ($<10^3$ logcfu/g) for good manufacturing practice for a product of this nature (ready-to-eat dried heat processed foods) by Stannard, (1997) but its value exceeded this limit during the third week of storage. The lowest TPC value for PC was observed at week 1 ($0.5x10^1$log cfu/g) while the highest value was observed at week 2 (3.8×10^2logcfu/g). Although the TPC value was highest at week 2, the TPC values throughout the entire storage period were within the acceptable limit ($<10^3$ logcfu/g) for good manufacturing practice (GMP).

The bacteria species identified in both products was *Bacillus sp*. The members of the family Bacillaceae are spore and vegetative cell formers. The spores produced can withstand high temperatures and adverse conditions such as drying and pasteurization (Stannard, 1997). Some strains of *Bacillus* sp. such as *B. cereus*, *B. subtilis* produce spores which germinate and release enterotoxins. Nausea, vomiting,

abdominal cramps and diarrhoea occur when foods containing these toxins are consumed (Pattison *et al.*, 2003). According to Stannard (1997) levels as high as $>10^5$ per gram are necessary to produce enough toxin to cause illness. Although the *Bacillus* sp. observed in this study was not identified to species level, its value for PA was below the $<10^3$ satisfactory limit value stated by the Centre for Food Safety (2014) for *B. cereus* in ready-to-eat food (in general) during the first two weeks of storage while its value was below this limit throughout the storage period of the snack PC. The use of a large ratio of flour when compared to fish may have also resulted in the development of this bacteria sp. Pattison *et al.*, (2003) stated that *Bacillus* sp. easily gets incorporated into products which contain flour that have been contaminated with its spores. The pH, temperature and water activity play an important role in the vegetative cell growth and spore germination of *Bacillus* spp. (Condon *et al*., 1996). According to Rahman (2009), microbial growth is halted when the a_w value is below 0.6. The aim of lowering the a_w in foods is to reduce or prevent the survival and growth of microbial spores or vegetative cells (Cakmak *et al.*, 2016). In this study the initial a_w value for PA and PC were above the 0.6 recommended limit (0.78 and 0.64 respectively), thus it is possible that the range of a_w values favoured the survival and outgrowth of microbial spores.

Enterobacteriaceae is a large group of biochemically and genetically related bacteria and their presence in foods is used to assess the hygienic level of a food product (Centre for Food Safety, 2014). In this study, Enterobacteriaceae was detected only at day 0 in PA while it was not detected in PC throughout the three-week storage period. According to the Centre for Food Safety (2014), the presence of enterobacteriaceae in heat treated food products indicate inadequate cooking or post-processing contamination. The low value obtained for enterobacteriaceae in this study point to the fact that there might have been inadequate cooking of the snacks during frying owing to the frying method adopted.

"Hygiene indicator organisms" such as Enterobacteriaceae and *E. coli* are used as markers to indicate or reflect the hygienic quality of food. *E. coli* (a faecal coliform) is a commonly used indicator organism used to denote the direct or indirect faecal contamination of foods (Centre for Food Safety, 2014). Its presence in foods (if substantial), suggest poor personal hygiene, poor cleanliness in handling and poor preservation and storage method (Kigigha *et al.*, 2017).

The results of the microbiological analysis showed that *E. coli*, *Staphylococcus aureus* and *Salmonella sp.* were not detected during the entire storage. The absence of *Salmonella sp., S. aureus* and *E. coli* in PA and PC, showed good manufacturing practice in terms of hygiene and food handling. The value for Enterobacteriaceae in PA (6.5×10^1log cfu/g) was within the 10^3 maximum acceptable limits (Stannard, 1997). Similarly, the Enterobacteriaceae count observed in this study was within the $<10^2$ satisfactory level for ready-to-eat- foods in general as reported by the Centre for Food Safety (2014). Based on this, PA and PC can be said to be satisfactory i.e., the tests results indicate good microbiological quality and affirm that products are safe for consumption. This is in agreement with the findings of Coradini *et al.*, (2015) who affirmed that the onion biscuits made with aromatized Tilapia fish meal was fit for human consumption since the microbial results indicated that they were prepared under good hygienic and sanitary conditions.

Yeast was observed in the product PA only at week 2 (1.5×10^1logcfu/g) while it was not detected in PC throughout the three-week storage period. Mould was seen in the product PA at week 1 (5.0×10^1logcfu/g) and week 3, where its growth was too numerous to count (TNTC). Mould was seen in the product PC only at week 2 and its value was 1.5×10^1logcfu/g. Anderson (2004) stated that bacteria, moulds and yeasts are not able to grow in a_w less than 0.60. According to Stannard (1997), dried foods with a water activity less than 0.6 are microbiologically stable. The lower a_w of PC when compared with PA may be the reason for the absence of yeast in the product during the three-week storage. According to Stannard (1997), the GMP value for yeast is $<10^2$ while the maximum microbial limit is 10^5; the GMP value for mould is $<10^2$ while the maximum microbial limit is 10^4 for products which fall under dried heat processed foods. The value for yeast and mould in PA was within the stated GMP value until the third week of storage while those of PC were within the GMP limit throughout the entire storage period. The result of this study is in contrast with the findings of Shahmohammadi *et al.*, (2016), who reported no bacteria, yeast, and mould in corn-fish snack due to its low moisture content and water activity.

5.0 CONCLUSION AND RECOMMENDATION

This study shows that although the RTE snacks developed using the head and fins of *T. guineensis* at an inclusion level of 12% were high in carbohydrates and low in protein; a common nutritional profile of many snacks, the RTE snacks were accepted by the panelists throughout the three-week storage period. The results of the biochemical analysis and microbial studies of PA and PC reveal that the method of processing, the starch: fish ratio, frying temperature and time play a key role in determining the nutritional composition, shelf-life and stability of the RTE snacks during storage.

Further research to improve the protein level, sensory and shelf-life properties of these RTE snacks is needed to encourage the sustainable utilization of fishery resources and to avert the negative consequence their disposal or non-utilization has on humans and the environment.

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