British Biotechnology Journal
12(4): 1-6, 2016, Article no.BBJ.24119
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
www.sciencedomain.org

Nutrients and Microbial Evaluations of Ginger Pre-treated Smoke-dried African Lungfish (*Protopterus annectens* Owen, 1883)

L. A. Agbabiaka[1*], B. C. Umeh[1], O. A. Kuforiji[2] and A. O. Atanda[3]

[1]Department of Fisheries Technology, Federal Polytechnic Nekede Owerri, Imo State, Nigeria.
[2]Department of Agricultural Technology, Federal Polytechnic, Ekowe, Bayelsa State, Nigeria.
[3]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Author's contribution

This work was carried out in collaboration between all authors. Author LAA designed the experiment, wrote and prepared the draft and interpreted the results, while authors OAK, BCU and KOA prepared the spice, laboratory works and carried out the statistical analysis respectively. All authors read the manuscript and agreed on the results.

Article Information

DOI: 10.9734/BBJ/2016/24119
Editor(s):
(1) Cosmas Nathanailides, Department Aquaculture & Fisheries, TEI of Epirus, Greece.
Reviewers:
(1) Kumbukani Mzengereza, Mzuzu University, Malawi.
(2) Anonymous, University of Missouri-St. Louis, USA.
Complete Peer review History: http://sciencedomain.org/review-history/13740

Original Research Article

Received 5th January 2016
Accepted 15th January 2016
Published 17th March 2016

ABSTRACT

The nutrients assay and microbial evaluation of ginger pre-treated smoked African lungfish (*Protopterus annectens*) stored for a week at room temperature were studied. Forty five fish samples weighing 1.0 kg each were purchased, killed, eviscerated and washed thoroughly under tap water and grouped into three treatments of fifteen fish each coded as treatments A, B, and C respectively. Fish in treatment A were immersed in 5% brine without spice (ginger) pre-treatment served as the control, treatment B was soaked in mixture of 5% brine and 2.5% ginger extract while treatment C was immersed in a solution containing mixture of 5% brine and 5% ginger extract. Each batch was smoke-dried for a period of six hours. The samples from each batch were subjected to proximate composition within 48 hours and microbial analysis on 7th day of storage at ambient temperature. The results of the proximate analysis showed a significant difference ($P<0.05$) among the pre-treated samples. Sample A had the highest moisture content (11.85%).

**Corresponding author: E-mail: adegokson2@yahoo.com;*

The highest concentration of crude protein was obtained in sample C (63.94%) while samples B and A recorded the values of 63.8 and 59.51% respectively ($p<0.05$). The highest crude fat concentration was obtained in sample C (8.46%), followed by sample B (8.44%) and the least was recorded in sample A (5.78%). The microbiological analysis showed that sample A (control) favoured the proliferation of microorganisms such as *E. coli*, *Staphylococcus aureus*, *Bacillus spp* and *Micrococcus spp* more than samples treated with ginger extract. The overall microbial population ranged between $3.0x10^4$ and $2.0x10^5$ which are within the safety limit ($\leq 10^5$ cfu/g) for total bacterial plate count for microbiological food. This result therefore has indicated that the use of brine with ginger extract could be suitable for prolonging the shelf-life of smoked lungfish without negative effects on the nutrients composition and storage quality under ambient conditions.

Keywords: Evaluation; microbial-load; spices; nutrients; Protopterus annectens.

1. INTRODUCTION

Fishes are very vital and major source of high quality protein and constitute important part of man's nutrition, providing about 16% of the animal protein consumed by the world's population as reported by FAO [1] especially in regions where livestock is relatively scarce. Fish supplies less than 10% of animal protein consumed in North America and Europe, 17% in Africa, and 26% in Asia [2]. It was estimated that about one billon people world-wide rely on fish as their primary source of animal protein [2].

Fishes are very perishable commodity, hence, soon after fish is dead, it begins to spoil; therefore freshly caught fish pass through various stages of deterioration until they become putrid and unfit for human consumption. Clucas and Ward [3] noted that after a fish dies, it remains in its premium quality only for a short while. One of the challenges facing the fish industry is that the fish deteriorate very fast if not properly handled and preserved [4,5]. Spoilage is caused by lipid oxidation and microbial proliferation at high ambient temperatures typical of the tropical environment, poor wet handling and storage devices. Lipids oxidation and microbial proliferation causes reduction in nutritional quality of fish, and also imparts offensive odour on the fish which affects its acceptability to the consumers even after processing hitherto fish [6].

Smoking of fish reduces moisture content in the product, and imparts desirable colouration as well as taste and aroma in the fish product, additionally, it provides a longer shelf-life through its anti-bacterial and anti-oxidative effect, lowering the pH, as well as accelerating the drying process and acting as antagonist to spoilage agents [6,7,5]. Eyo [6] identified the common microbes associated with smoked fish as bacteria (*Staphylococcus aureus*), yeasts (*Saccharomyes cerevisiae*) and moulds (*Penicillium* and *Aspergillus spp*).

Spices are edible plant materials such as leaf in onion and garlic, rhizome in ginger that have anti-oxidative, antiseptic and bacteriostatic properties [8]. They are added to food such as fish and meat to delay the onset of deterioration such as rancidity and reduce microbial proliferation [6]. Spices also function as seasonings to foods as well as impart flavour to the foods [6,8].

Experts in human nutrition/pathology have reported that components of spices such as phenols are endowed with properties that stimulate the immune system and protect cells against diseases and fight free radical oxygen molecules that can damage the cells leading to diseases such as cancer and heart disease. Spices consumption are also reported to help normalize blood sugar after meals and can reduce the amount of triglycerides in the blood [9]. Fish nutrients help the memory and keep our immune system and eyesight in peak condition as it is a good source of vitamin A and D [10]. There is paucity of information on the effect of spice pre-treatments in smoked fish in general and West African lungfish in particular, hence, this study was aimed at providing the baseline information on the effect of spice pre-treatment on nutrients assay and microbial evaluation on processed (Smoked) and stored *Protopterus annectens* respectively, which is a delicacy in South-Eastern Nigeria.

2. MATERIALS AND METHODS

2.1 Sources and Collection of Samples

Forty five freshly caught Africa lungfish (each weighing 1000 g) were purchased from Oguta

market while the spice, ginger (*Zingiber officinale*) was purchased at Ekeuku market Owerri, both at Imo State, Nigeria.

2.2 Preparation of Samples

The fresh rhizome of ginger samples was crushed with kitchen blender and known weights were dissolved in distilled water inside two containers overnight, sieved to produce 2.5 and 5% ginger extracts respectively. The forty five freshly caught African Lungfish (*Protopterus annectens*) weighing 1.0 kg each were selected and randomly divided into three (3) batches of fifteen fish coded as A,B, and C respectively. The fish samples were gutted and washed thoroughly with clean water to remove slime and blood. The first batch (A) was immersed in 5% brine solution only without ginger treatment and served as the control. The second batch (B) fish were immersed in mixed solution of 5% brine and 2.5% ginger extract while batch C were soaked in solution containing the mixture of 5% brine and 5% ginger extract for 30 minutes respectively within ambient temperature of 28°-30°C. Thereafter, the pre-treated samples were transferred into clean baskets differently for proper draining of water prior to smoke-drying at a temperature range of 70-80°C in the smoking kiln for six hours using charcoal as fuel. All the processed fish were cooled and stored at room temperature for 7 days in sterilized trays and labelled accordingly.

2.3 Microbiological Evaluations

Microbiological analysis of each of the three (3) batches of smoked fish samples were carried out on 1g sample of each treatment (in triplicates) which were blended with 10ml of sterile 0.1% peptone water as described by Cheesbrough [11]. Pour plates were prepared from 10-fold dilutions on Nutrient Agar (NA) for total bacterial count, MacConkey Agar (MAC) for total coliform counts and Sabourand Dextrose Agar (SDA) for fungal counts as outlined in Oxoid [12]. After incubation at 37°C for 24 hours for bacteria counts and at ambient temperature for 3-5days for fungal counts, the plates were inspected and colonies were selected randomly. Bacteria cultures were characterized and identified using various morphological and biochemical tests such as gram stain, spore stain, motility, catalase, coagulase, indole, MR-VP, urease, citrate, oxidase and sugar fermentation tests. The isolates were identified according to the methods of Cheesbrough [11] and Fawole and Oso [13].

2.4 Proximate Analysis

Samples of smoke-dried lungfish were collected from the treatments within 24hours on replicate basis and the nutrients assay such as crude protein (Kjeldhal procedure), lipid (Soxhlet method), Ash (flame photometric) were determined according to AOAC [14].

3. RESULTS AND DISCUSSION

The result of the weight characteristics of spice pre-treated smoked Africa Lungfish (*Protopterus annectens*) is presented in Table 1. The average moisture loss (58.7%) from the smoked African lungfish (*Protopterus annectens*) is less than the value of 65.00% recommended by Cardinal et al. [15] which may be due to the relatively high adipose tissue of *Protopterus annectens* impairing water dehydration. Table 2 presents the data on proximate composition of the pre-treated smoked African lungfish; the highest concentration of crude protein (63.94±0.03%) was recorded from sample C, while values for samples B and A were (63.83±0.13% and 59.59±0.28%) respectively. The crude protein contents recorded in this study agrees with values reported by Ogbonna and Ibraheem [16], Olayemi et al. [17] and Agbabiaka et al. [5] on processed African catfish.

The improved nutrient concentration linearly with ginger fortification might be due to additional nutrients inherent in ginger especially crude protein (8.60%), Ether extract (15.21%) and minerals (Ash= 5.21%) according to Nwinuka et al. [8] and Meadow [18] respectively. This also might have affected the crude fat values which were 8.46±0.01, 8.44±0.02 and 7.78±0.02% for samples C, B and A respectively indicating that the spice had positive effect towards the nutrients. There was a significant differences ($p<0.05$) in nutrients composition between the processed fish in treatment A and those pre-treated with ginger extract in batches B and C.

Microbial analyses showed that sample A had the highest microbial count (TNTC), followed by sample B (2.0×10^5) while sample C recorded the least 3.0×10^4 cfu/ml on nutrient agar (Table 3). Also, microbial count on SDA revealed that sample B recorded the highest fungal count (2.0×10^5 cfu/ml) followed by sample A (1.2

x10^5cfu/ml) and sample C (3.0 x10^4cfu/ml) respectively. The cultural and Biochemical characteristics of bacteria isolates from the pre-treated smoked *Protopterus annectens* is presented in Table 4. The organisms identified were *Staphylococcus aureus*, *Bacilius spp* and *Micrococcus spp* perhaps due to their salt tolerant nature [19]. *Staphylococcus aureus* have been found to be relatively resistant to drying which is a property that favours their transmission from one host another; furthermore, Prescot et al. [20] corroborated by Oduor-odote and Obiero [21] have reported that *Staphylococcus species* were able to grow in concentration of sodium chloride up to 15%. The presence of this particular microbe in these three fish samples might also be through handling as a normal flora of the skin [22]. Nevertheless, the occurence of this organism could also be as a result of its prevalence in the environment especially in water, soil, plants, insects, animals, human [23].

Table 1. Weight characteristics of spice pre-treated smoked African Lungfish

Samples	Live weight of fish (kg)	Dressed weight (kg)	Weight after smoking (kg)	Total weight loss (kg)	% weight loss
A	15.00	13.65	5.85	9.00	61
B	15.00	13.50	6.00	9.15	60
C	15.00	13.50	6.75	8.25	55

Average weight loss in percentage = 58.7%; A - Fish sample treated with 5% brine solution only, B - Fish sample treated with mixture of 5% brine and 2.5% ginger extract, C - Fish sample treated with mixture of 5% brine and 5% ginger extract

Table 2. Proximate composition of spice pre-treated smoked African Lungfish

Samples	Moisture content (%)	Crude protein (%)	Crude fat (%)	Ash (%)	NFE (%)
A	11.74±0.01^a	59.59±0.28 ^{a}	7.78±0.01^a	10.46±0.02^a	10.43±0.14^c
B	11.85±0.01^a	63.83±0.13 ^{b}	8.44±0.02^b	8.99±0.02^b	6.89±0.23^a
C	10.28±0.02^a	63.94±0.03 ^{b}	8.46±0.02^b	9.22±0.01^b	8.52±0.05^b

a,b,c Mean within column with same superscript are not significantly different (p>0.05)

Table 3.Total bacterial and fungal counts on spice pre-treated smoked fish (*Protopterus annectens*)

Samples	Total viable count on NA	Total viable count on MAC	Total fungal count on SDA
A	TNTC	1.0 x 10^5	1.2 x 10^5
B	2.0x 10^5	2.0x 10^4	2.0x 10^5
C	3.5 x 10^4	3.0 x 10^4	3.0 x 10^4

Key: A - Fish samples pre-treated with 5% brine only, B - Fish samples pre-treated with mixture of 5% brine and 2.5% ginger extract, C - Fish samples pre-treated with mixture of 5% brine and 5% ginger extract, TNTC-Too Numerous to Count, NA- Nutrient Agar, MAC- MacConkey Agar, SDA- Sabourad Dextrose Agar

Table 4. Cell morphology and Biochemical characteristics of bacteria isolated from spice pre-treated smoked *Protopterus annectens*

Sample	Cell morphology	Gram reaction	Motility test	Indole test	Catalase test	Coagulate test	Citrate test	Glucose test	Sucrose test	Lactose test	H_2S	Identified organism
MFS_A	Rod	+	-	-	+	NA	+	A/G	A	A	-	*Bacillus spp*
MFS_B	Cocci	+	-	-	+	+	+	A/G	A/G	A	+	*S. aireus*
MFS_C	Rod	-		+	+	+	-	A/G	-	A/G	-	*E. coli*

Key: - = Negative, + = Positive, H_2S =Hydrogen sulphide, A =Acid , G=Gas, MFS_A =Fish sample treated with 5% brine solution (control), MFS_B= Fish sample treated with mixture of 5% brine and 2.5% Zingiber officinale, MFS_C =Fish sample treated with mixture of 5% brine and 5% Zingiber officinale

Though, it was observed that, there were significant differences ($p<0.05$) in terms of microbial load between sample A (control) and samples B, C pre-treated with ginger extract; however, these results are in accordance with the safety limit (≤ 10^5 cfu/g) for total bacterial plate count for microbiological food [24]. It is also generally accepted that fish with microbial load exceeding 10^6cfu/g is likely to be at the stage of being unacceptable from the microbiological point of view and unfit for consumption. The relatively low microbial load in samples B and C corroborates the report by Tagoe et al. [4] that ginger extract possesses anti-microbial and anti-oxidative characteristics when added to foods.

4. CONCLUSION AND RECOMMENDA-TION

From the above results, it appears that ginger as a spice has shown to have a good potential as an antimicrobial agent; however, pretreatment of smoked fish with mixture of 5% brine and 5% ginger extract appeared the best concentration, without deleterious effect on nutrients. I strongly recommend further studies on the use of natural spices on similar type of fishes which are prone to oxidation after cropping and or processing/preservation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. The State of food security in the world (SOFI). Food and Agriculture Organisation (FAO). Rome Italy; 2003.
2. FAO. Projected population and fish demand in Nigeria (1997 - 2025). FAO Fisheries department country profile, Nigeria. FID/CP/NIR. Rev. 4th March, Rome Italy; 2000.
3. Clucas IJ, Ward AR. Post harvest fisheries development. In: A guide to Handling preservation, processing and Quality. Chatham Maritime, Kent; 1996.
4. Tagoe DNA, Nyako HD, Akpaka R. A comparison of anti-fungi properties of *Allium cepa,* Ginger (Zingiber *officinale*) and garlic (*Allium sativum)* against *Aspergillus flavus, A. niger* and *Cladosporum herbarum.* Res. J. Med. Plant. 2011;5:281-287.
5. Agbabiaka LA, Amadi AS, Madubuko CU, Nwankwo FC, Ojukannaiye AS. Assessment of nutrients and sensory qualities of brine pre-treated catfish smoked with two different woods. African Journal of Food Science. 2012;6(7):245-248.
6. Eyo AA. Fish processing technology in the tropics. University of Ilorin Press, Nigeria. 2001;155-164.
7. Sengor GF, Kalafatoglu H, Gun H. The determination of microbial flora, water activity and chemical analysis in smoked Mussels (*Mystus galloprovincialis* L). Turkish J. Anim. Sci. 2004;28:793-797.
8. Nwinuka NM, Ibeh GO, Ekeke GI. Proximate composition and levels of some toxicants in four commonly consumed spices. Journal of Applied Sciences and Environmental Management. 2005;9(1): 150-155.
9. Oakenful D, Sidhu GS. Saponins. In: Cheeke PR (ed), toxicants of plant origin. Acad. Press, New York. 1989;2:78-113.
10. Agbabiaka LA. Aquaculture and pond management in the tropics. Vintage Books Limited Owerri, Nigeria. 2010;111.
11. Cheesbrough M. Medical laboratory manual for tropical countries. Cambridge University Press, ME44TB, United Kingdom. 2000;1:64-140.
12. Oxoid: The oxide manual of cultural media ingredients and other laboratory services. Oxford Ltd. Bareagstate; 1982.
13. Fawole MO, Oso BO. Laboratory manual on microbiology. 2nd Edn., Spectrum Books Ltd., Ibadan, Nigeria. 1995;77-78.
14. A.O.A.C. Association of official analytical chemist. Official methods of Analysis. 17th edition Washington D.C; 2000.
15. Cardinal M, Knockaert C, Torrissen O, Sigurgisladottir S, Morkore T, Vallet JL. Relation of smoking Parameters to the yield colour and sensory quality of smoked Atlantic Salmon (*Salmo salar*). Food Res. Int. 2001;34:537–550.
16. Ogbonna C, Ibrahim MS. Effect of drying methods on proximate composition of catfish (*Clarias gariepinus).* World J. Agric. Sci. 2009;5(1):114-116.
17. Olayemi FF, Adedayo MR, Baminshaiye I, Awagu EF. Proximate composition of catfish (*Clarias gariepinus*) smoked in Nigeria Stored Products Research Institute (NSPRI) developed Kiln. Inc. J. Fish. Aquacult. 2011;3 (5):96–98.

18. Meadows AB. Ginger processing for food and industry. Federal Institute of Indus. Res., Oshodi, Lagos, Nigeria. 1988;1–30.
19. Adams MR, Moss MO. Food microbiology. Royal society of chemistry. Cambridge. 1999;40-44.
20. Prescot LM, Harley JP, Klein DA. Microbiology seventh edition. The McGraw-Hill Companies Inc; 2008.
21. Odour- Odote PM, Obiero M. Lipid and organoleptic response during shelf storage of some smoked marine fish in Kenya. African Journal of Food Agricultural Nutrition and Development. 2009;9(3): 885-900.
22. Okonko I, Ogunnusi O, Ogunjobi TA, Adedeji AA, Adejoye AO, Babalola OD. Microbial studies on frozen shrimps processed in Ibadan and Lagos, Nigeria. Scientific Research. 2008;3:543-545.
23. Brissses P, Grimont F, Grimont PAD. Prokaryote. NY Springer. New York. 2006;159-196.
24. Public Health Laboratory Services “PHLS”. Provisional microbiological guideline for some ready to eat foods sampled at point of sales. Notes for PHLS food examiners, PHLS Microbiology Digest. 1992;9:98-99.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13740