Microbiological Analysis and Nutritional Constituents of *Achatina achatina* Subjected to various Cooking Methods

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Abstract : *Achatina achatina* harbours many microorganisms which may cause diseases or spoil the snail if not properly cooked. The study investigated the effect of some methods of cooking; frying, boiling, smoke-drying and oven-drying on the nutritional constituents and microorganisms isolated from snail to ascertain the best method which can reduce the or eliminate the unwanted organisms. Microorganisms were isolated from the snail samples using pour plate technique and identified by cultural, morphological and biochemical characterization. Proximate analysis of the snail samples was determined. The bacteria isolated were *Bacillus* sp, *Escherichia coli, Proteus* sp*, Vibro* sp*, Salmonella* sp, *Staphylococcus* sp*, Pseudomonas* sp*, Shigella* sp*, Klebsiella* sp*, Streptococcus* sp and *Citrobacter* sp while *Mucor* sp*, Aspergillus* sp and *Rhizopus* sp. were the fungal isolates. *Salmonella* sp*, Vibrio* sp and *Escherichia coli* were present in all the snail samples whereas *Proteus* sp was inhibited by all the cooking methods. *Bacillus* sp was sensitive to all the cooking treatments except boiling. *Salmonella* sp*, Vibrio* sp *and E. coli* had very high counts. However, the cooking treatments reduced the bacterial and fungal counts appreciably with smoke-drying having the least counts ranging from $1.20x10^{1}$-$2.80x10^{1}$cfu/g respectively. The fat and moisture contents were reduced but reduction of protein and energy contents was minimal. There were varying degrees of reduction in both number and type of organisms inhibited by the different cooking methods. Smoked-drying exhibited the highest potential for the control of microorganisms and still maintained a healthy nutritional composition.

Keywords: *Achatina achatina*, bacteria, cooking method, effect, fungi, proximate analysis

INTRODUCTION

Achatina achatina is a land snail (Mollusc) found mainly in North Coastal area, Central and South Africa where the weather is favourable for their growth (Herbert and Kilburn, 2001). Many species of land snails are recognized but the popular species of interest are the West African giant snails (*Achatina achatina, A. marginata* and *A. fulia*) (Adegbola, 1998). It is composed of about 37-51% protein; 45-59mg/kg iron, lysine, arginine, phosphorus, essential amino acids, Vitamin C and B complex, low in Sodium, fat and cholesterol (Wosu, 2003).

The proteins of *A. achatina* contain all the essential amino acids such as lysine, isoleucine and leucine required by humans (Adeyeye, 1996; Ferhat *et al.,* 2011). The high iron content makes it important in the treatment and prevention of anaemia and are rich in minerals like potassium, phosphorus, copper and iron (Ademolu *et al.,* 2004). Okon et al. (2016) found snails to be high in protein, low in fat and high in minerals such as Ca, Iron, Zn, Phosphorus, Mn, Potassium and Vit. B_{12}.

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They are gastropods and glide on the surface of solids or buried under the soil or leaves which makes the shell and meat to be exposed to quite a number of microorganisms (Nwuzo *et al*., 2016). Snails harbour many gut-bacterial community (Van-Horn *et al*., 2011). A close association is formed between snails and these microorganisms (Fagbuaro, 2006). The organisms may remain in snail as pathogens or as normal flora which can eventually cause diseases if eaten raw or improperly cooked (Adagbada *et al*., 2011). Mudasir, *et al*. (2017) found that there is a host-symbiont relationship between bacterial and snails were the resident bacterial help the host in performing processes such as digestion of complex molecules, generating energy and amino acid metabolism. Pathogenic organisms including microbes of the genera *Escherichia, Citrobacter, Klebsiella,Proteus, Staphylococcus, Serratia and Enterobacter* predominate in snails (Lu *et al*., 2018). Nyaoghe *et al.* (2016) isolated *Salmonella*, S*taphylococcus*, *Bacillus* and *Pseudomonas* from edible snails.

Ekundayo and Fagade (2005) isolated *Staphylococcus sp, Pseudomonas sp, Vibriosp, Escherichia coli, Salmonella sp and Proteus sp* from the gut of edible snail. Microorganisms play useful role in digestion but may eventually find their way to the body of the snail during dressing processes (Wilson, 1981).

Adagbada *et al*., (2011) isolated *Salmonella sp., Shigella* sp*, Aeromonas* sp*, Vibrio* sp*, Pseudomonas* sp*, Enterobacter* sp*, Klebsiella* sp*, Staphylococcus* sp*, Yersinia* sp and *Fusarium* sp from land snails and their eggs. Other microorganisms associated with snail meat include aerobic mesophils, halophilic bacteria, coliforms, faecal coliforms, *Clostridium sp*, *Staphylococcus aureus* and molds (Speck,1984). Some of these organisms produce little or no effect on the snail but others release slimy materials on the snail (Wilson, 1981). Some microorganisms are retained in the body of the snail, reproduce and may spread in the faeces and visceral fluid which serve as agents of diseases in the consumers when the snail is eaten raw or improperly cooked. Agbonlahor *et al.,* (1994) isolated Enterobacteriaceae which included *Salmonella, Proteus, Serratia, Enterobacter* and *Klebsiella* suggesting the public health importance associated with consumption of snails when not subjected to proper cooking.

This research is therefore aimed at studying the effect of some methods of cooking snail on the microorganisms present and proffer the best method of cooking that will prolong the shelf-life and still preserve the food qualities.

MATERIALS AND METHODS

Collection of Samples

Fifteen African Giant Snails (*A.achatina*) of fresh weight of 100± 5.00g were purchased from Abraka main market, Delta State in plastic containers washed with sterile water. The snails were thoroughly washed with distilled water and rinsed with normal saline. The shells were removed and the edible part removed from the visceral mass. They were washed with aluminium sulphate before subjecting to the various cooking methods (Nwuzo *et al.,* 2016).

Cooking of snails: The dressed snails were each subjected to four various methods of cooking;

Boiling: This was done by putting the dressed snail inside an aluminium pot with water and allowed to boil for 10 minutes.

Frying: This was done by deep-frying the dressed snail in hot oil for 10mins.

Smoke Drying: Done on gauze placed on heated charcoal and the dressed snail was placed on the heated gauze for 10mins.

Oven Drying: Snail was placed in an oven (Labtech, India) at 120^0C for 10 minutes.

After subjecting to the different cooking methods, each was ground using an electric blender and kept in the refrigerator at 4^0C for further analyses.

Microbiological Analysis

One gram (1g) of each sample was introduced into a test-tube containing 10ml of sterile 0.1% peptone water and used for the serial dilution (10^{-1}-10^{-5}).

Enumeration and Identification of Microorganisms

One millilitre of each sample (serially diluted sample) was inoculated onto sterile Nutrient Agar and MacConkey agar using the pour plate technique. The plates were incubated at 37^0C for 24hr. Colonies formed were counted and expressed as colony forming units per gram (Cfu/g).

Inoculation was done on potato Dextrose Agar for fungi and plates were incubated at 25^0C for 48hrs. They were observed for growth and recorded as spore forming units per gram (SPU/g). The organisms were identified using appropriate morphological, cultural and biochemical characterization; sugar fermentation, citrate utilization, hydrogen sulphide production, oxidase and catalase tests (Cheesbrough, 2005). V*ibrio sp, Salmonella sp and E. coli* tentatively identified were inoculated onto Thiosulphate Citrate Bile Salts Sucrose Agar, Salmonella-Shigella Agar and Tryptone Bile X-Glucuronide agar respectively, counted and expressed as Cfu/g.

Proximate Analysis of raw and cooked *Achatina achatina*. Determination of Moisture Content, Protein, Crude fat and Energy was done using the method of AOAC (2012).

Moisture content. This was determined by weighing 3.0g of homogenised sample in a clean, dried crucible (W_1). The crucible was kept in an oven at 100^0C -105^0C for 6-12hrs to dry until a constant weight was got. The crucible was then put in a dessicator for 30 minutes after which it was weight (W_2)

$$\% \text{ moisture} = \frac{w1-w2}{weight\ of\ sample} \times 100$$

W1 = Initial weight of crucible + sample

W2 = Final weight of crucible + sample

Crude Fat: Determined using the soxhlet extraction method using petroleum either as the solvent.

$\frac{weight\ of\ fat\ extracted}{weight\ of\ sample}$ x 100 = % crude fat.

Crude protein: This was done using kjedahl's method (% N x 6.25) = crude protein

Total carbohydrate: Calculated by subtracting % amounts of crude-protein, crude fat, moisture and ash from 100%.

Ash content: This was determined by done by incinerating 5.0g of homogenised snail sample at 550^0C overnight in a muffle furnace. Weight before and after ashing was used to calculate ash content (Babalola and Akinyosinu, 2009).

Energy value: This was calculated by multiply the % composition of protein, fat and carbohydrate by the corresponding at water values of 17, 37 and 17 respectively.

RESULTS

The bacteria isolated were mainly Gram positive rods. Some Gram negative cocci and rod were also present (Table 1) which included those of pathogenic importance. The fungal isolates included *Mucor sp, Aspergillus sp and Rhizopus sp*.as shown in Table 2.

The cooking methods did not affect the protein content since the values were within the range of 87.52-90.58 Kcal/g. The raw snail had the highest protein value. There was an appreciable effect on the fat content with the oven-dried *A. achatina* having the least content of 1.68Kcal/g(table 3).

The bacterial load for the raw *A. achatina* was high ranging from $7.2x10^6$ to $2.67x10^8$Cfu/g but there was a high reduction when exposed to the various cooking methods especially oven-dried samples as shown in Table 4a. The fungal isolates showed the same trend of the population being reduced when subjected to the various methods of cooking (Table 5a).

All the organisms identified were present in the raw and boiled *A. achatina.* The occurrence (%) of the isolates in the oven dried *A. achatina* was 36.6%, fried sample was 54.5% while that of the smoked sample was 72.7%.

Table 1: Biochemical characterization of bacterial isolates of *A.achatina*

1	2	3	4	5	6	7	8	9	10	11	12	
+	R	+	+	+	-	-	-	+	+	-	-	*Bacillus* sp
+	C	+	-	+	+	+	-	+	+	-	-	*Micrococcus sp.*
+	C	+	+	+	+	+	+	-	+	-	-	*Staphylococcus sp*
-	R	+	+	+	+	+	+	+	+	-	-	*Escherichia coli*
-	R	+	-	+	+	+	-	-	-	+	-	*Proteus* sp.
-	R	+	+	+	-	-	-	+	+	+	-	*Salmonella* sp.
-	R	+	-	-	+	-	-	+	-	-	-	*Shigella* sp.
-	R	+	-	-	+	-	-	+	+	-	-	*Klebsiella* sp.
-	R	+	-	-	+	-	-	+	-	-	+	*Vibrio* sp
-	R	+	+	-	+	-	-	-	-	-	-	*Bacillus sp.*
-	C	+	+	+	+	-	-	+	-	-	-	*Streptococcus sp*
-	R	+	-	-	+	-	-	+	+	-	+	*Citrobacter* sp
-	R	-	-	-	+	-	-			-	+	*Pseudomonas* sp

1=Gram staining; 2= Morphology; 3=Glucose; 4=Lactose; 5=Citrate; 6=Catalase; 7= Motility 8=Indole; 9 =Acid; 10=Gas; 11=Oxidase; C=coccus; R=rod

Table 2: Characteristics of fungal isolates of *A. achatina*

Fungus	Colour	Structure
Mucor sp.	White	Fluffy dark-grey with sporangia. Sporangia are erect, branches, globose to spherical, multistoried, hyaline and brownish. Sporangia are without apophyses but with well developed collumella
Aspergillus sp.	Black	Mostly consists of dense, erect condiophores. Conidiophores terminate in a vesicle covered with a single palisade-like layer of phialides. Conidia are one-celled, smooth or rough walled, hyaline or pigmented form long chains.
Rhizopus sp.	Yellowish green	Sporangiospores are globose to ovoid one celled, hyaline to brown. Presence of stolons and pigmented rhizoids. Single sporangiophores from nodes directly above the rhizoids. The sporangiospores are columellate and multispored.

Table 3: Proximate composition of the cooked snail samples

Snail sample	Protein (Kcal/g)	Fat (Kcal/g)	Moisture %	Energy (KJ/100g)
Raw snail (control)	90.58	3.68	4.52	1696.90
Boiled snail	88.56	3.20	3.83	1688.58
Oven dried snail	87.90	1.68	2.59	1638.91
Fried snail	87.52	4.00	2.85	1676.35
Smoked dried snail	89.02	2.83	2.78	1654.94

Table 3b: ANOVA with single factor of Proximate composition of the cooked snail samples

Source of Variation	Mean	df	MS	F cal	P-value	F crit
Raw snail (control)	32.93					
Boiled snail	31.86					
Oven dried snail	30.72	4	1.93	**0.01	0.05	3.48
Fried snail	31.46					
Smoked dried snail	31.54					

There wass no significance in between the control and the other variable, this is because f cal (0.01) is less than the f crit (3.48) at p< 0.05 level of significance with df (4).

Table 4: Bacterial count (Cfu/g) of *A. achatina* samples

Snail	*Salmonella* sp	*Vibrio* sp	*Escherichia* sp
Raw snail (control)	7.2×10^6	3.2×10^7	2.67×10^8
Boiled snail	4.3×10^3	6.3×10^3	4.1×10^3
Oven dried	2.9×10^2	4.6×10^1	2.10×10^2
Fried snail	3.4×10^3	4.4×10^3	3.2×10^3
Smoked dried snail	1.60×10^1	2.80×10^1	1.20×10^1

Table 4b: ANOVA with single Factor of Bacterial count (Cfu/g) of *A. achatina* samples

Variation	Mean	df	MS	F cal	P-value	F crit
Raw snail (control)	4.36					
Boiled snail	4.90					
Oven dried snail	3.20	4	1.97	*0.18	0.05	3.48
Fried snail	3.67					
Smoked dried snail	1.87					

**There shows no significance in the between the control and the other variable, this is because f cal (0.18) is less than the f crit (3.48) at p< 0.05 level of significance with df (4).*

Table 5a: Fungal load of *A. achatina* samples (Sfu/g)

Snail	*A. niger*	*M. mucedo*	*R. stolonifera*
Raw snail (control)	4.42×10^8	4.4×10^8	6.6×10^6
Boiled snail	4.0×10^3	4.3×10^3	4.3×10^3
Oven dried	3.2×10^2	4.3×10^2	2.56×10^2
Fried snail	3.5×10^2	4.4×10^3	3.5×10^3
Smoked dried snail	2.50×10^1	1.85×10^1	2.20×10^1

Table 5b: ANOVA with single factor of Fungal load of *A. achatina* samples (Sfu/g)

Variation	Mean	df	MS	F cal	P-value	F crit
Raw snail (control)	5.14					
Boiled snail	4.20					
Oven dried snail	3.35	4	3.56	**6.41	0.05	3.48
Fried snail	3.80					
Smoked dried snail	2.18					

***From the ANOVA table above, there is significance in the between the control and the other variables, since the f cal (6.41) is greater than the f crit (3.48) at p< 0.05 level of significance with df (4).*

Table 6: Occurrence of the isolates in the snail samples

	Snail samples				
Isolate	**Raw**	**Boiled**	**Oven dried**	**Fried**	**Smoked**
Bacillus sp	+	+	-	-	-
Salmonella sp	+	+	-	+	-
Pseudomonas sp	+	+	-	-	+
Staphylococcus sp	+	+	-	-	+
Vibrio sp.	+	+	+	+	+
Proteus sp.	+	+	-	+	-
Escherichia sp.	+	+	+	-	+
Mucor sp.	+	+	+	+	+
Rhizopus sp.	+	+	+	+	+
Aspergillus sp	+	+	-	+	+

Key*: + =*Present; - = *Absent

DISCUSSION

The results of this study showed that the snails harbour a high number and diverse types of microorganisms. Many of these organisms have been known to be pathogenic which could result in food-borne illnesses when contaminated snail is consumed raw or improperly cooked (Adegoke *et al.,* 2010). Occurrence of similar isolates has been observed in *A. achatina* by Adagbada *et al*. (2011) who attributed the presence of pseudomonas and other soil organism to the close association of snails with soil. Ebenso *et al.* (2012) isolated *Salmonella sp, Vibrio sp* and *E.coli* from edible land snail. Efuntoye *et al.* (2011) and Cardoso *et al.* (2012) reported that *Staphylococcus* species resides in the gastro-intestinal tract of snail. The presence of *E.coli* may be attributed to faecal pollution. This is inconformity with the study of Parlapari *et al*. (2014) that isolated *Salmonella sp.* In snail which is an indicating of contact with faecal matter. The snail might be a potential carrier of *Escherichia sp*. since gastropods find mammalian faeces an attractive source of nutrients (Speicer, 2001). The presence of these organisms such as *Bacillus sp* and *Rhizopus sp.*can further be attributed to the fact that snails feed on decaying plant materials which harbor and allow proliferation of microorganisms. Akpomie (2013) in a preliminary study isolated E.coli, Salmonella, staphylococcus sp, shigella, mucor, Rhizopus, Aspegillus, Penicillium and alternaria from land snails.

The raw *A. achatina* was found to harbour a high count of bacteria and fungi. The high occurrence of the fungi in the snail may be attributed to their ability to produce spores which make them able to survive in the soil even under adverse conditions. These spores may be ingested by the snail and eventually germinate to form vegetative cells. Paralapari et al. (2014) stated that efficient processing eliminate microorganism from cooked snail.

Some of the organisms were not present in the snail samples when subjected to the different forms of cooking. This showed that some of the organisms were affected by the cooking methods. This may be attributed to the fact that the heat could be have effected a negative response on their physiological state hence were heat labile. *Salmonella sp., Vibrio sp and Escherichia coli* and all the fungal isolates were found to be present in all the snail samples despite the cooking applications. *Proteus sp*. was not affected by any of the cooking methods. This ability to withstand the heat treatments may be due to the production of spores and/or time of exposure to the heat treatment. This conforms with the findings of Markland and Darlas (2016) and Marjon *et al.* (2016) that bacterial spores pose a challenge in food preservation because of their heat resistance.

Four of the isolates were destroyed by oven-drying which could be due to application of heat combined with dehydration during this process resulting in the reduction of water available to the organisms (A_w) hence the death of the organisms. This conforms with the findings of Vas Pires *et al*.,2003; Huetas,2016.

The reduction in number in smoked drying may have been encouraged by the dual effect of heat and cresol present in smoke which is inhibitory to the growth of some microorganisms. Smoke particles were found to contain compounds which include methanol and ethanol and their acids, resins, tars and other materials which are inhibitory to growth of microorganisms (Ihekoronye and Ngoddy, 1985). The effect of moisture and heat during boiling might have contributed to the control of the microorganisms because vegetative cells of prokaryotes are destroyed at 100^0C but spores may germinate under favourable environment thus the effect of boiling was very limited.

The higher fungal counts could be explained by the fact that moulds tend to be more resistant to adverse situations such as heat and reduced water activity so can survive at lower water activities than bacteria. Girard (1992) stated that the minimum water activity for bacterial growth is between 0.95-0.98, below which bacterial growth is inhibited.

The moisture content, protein and fat levels were affected by the different cooking methods. There was no appreciable difference in the effect on the calories and the protein contents which were reduced minimally by the different methods. The moisture contents of the oven-dried, fried and smoke-dried snail were reduced significantly hence reduction in the water activity level which affected the growth of the microorganisms. The oven-dried and smoke – dried significantly reduced the level of fat whereas there was an increase with frying.

CONCLUSION

The study has shown that raw snail harbours a wide variety of microorganisms many of which are of health importance. However, the organisms were reduced by all the cooking methods especially the smoke-drying and oven-drying. The protein and calorie levels were not affected by all the methods of cooking but the smoked and oven-dried cooking methods reduced the fat content while there was an increase with frying. Smoking and oven drying as a means of cooking snails can serve as a better alternative to the other methods so as to get the benefits of preserving snail, eliminating/reducing pathogenic organisms, enhanced flavour and retaining healthy nutritional content

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