EFFICACY OF DIFFERENT PROCESSING ADDITIVES ON MICROBIAL LOADS AND ANTIBIOTIC SENSITIVITY PARTERN OF GIANT AFRICAN LAND SNAIL *(Archachatina marginata)*

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ABSTRACT

Fifteen samples of *Archachatina marginata* were collected from Melege village, Ose local Government area, Ondo State, Nigeria. The foot and head were analysed microbiologically for bacterial loads before and after processing with five cleansing additives: Lime, Alum, Vinegar, Salt and Ash. Lime proved most effective reagent for decontaminating snail meats, it had the highest reduction of microbial load after processing (57.1%).The mean microbial load on the head ranged between 9-22cfu/cm^2 before and 4-12 cfu/cm^2 after processing, while foot, ranged between 13-19 cfu/cm^2 before and 8-14 cfu/cm^2 after processing. Eight isolates belonging to nine genera including *Micrococcus luteus* [10(21.31%)] most predominant, followed by *Escherichia coli* [8(13.11%)], and least was *Enterobacter spp* [4(6.56%)] respectively. The presence of higher numbers of pathogenic *Klebsiella spp* and *E. coli* among others, encountered in *A. marginata* is alarming and indicates public health hazard, a warning signals for possible occurrence of food borne infection. The antibiotic susceptibility patterns of the bacterial isolates showed that all the bacteria isolated were susceptible to Gentamicin (GEN), only *P. vulgaris* and *Bacillus spp* are susceptible to Ceftazidime (CAZ) and Cloxacillin (CXC) respectively and all are resistant to Ampicillin (AMP).

Keywords: *Archachatina marginata,* Cleansing reagents, Snail meats, Microbial load, Public health.

INTRODUCTION

Increasing human population coupled with rising standards of living has placed great pressure on existing conventional sources of animal protein (Babalola *et. al.,* 2009). Finding new meat sources to solve the shortage of meat protein is a major challenge to the food industry worldwide. Therefore, need to source for other non-conventional meat protein sources that are equally nutritious and capable of bridging the demand gap. Micro-livestock such as the giant land snail has been domesticated to solve the problem of protein insufficiency (National Research Council, 2012). Snails are harvested for food in many parts of the world as delicacy and constitute an important source of animal protein, vitamins, minerals in many coastal communities of Nigeria and other parts of Africa Ebenso and Ebenso, (2011).Snails are prone to environmental contaminations by pollutants and continually ingest bacteria from the moist soil and their environments (Walker *et al.,* 2000).Based on this, and if not properly processed, it will lead to various diseases. Molluscs have been reported to serve as vehicles for human infections from different *Bacteria spp. Escherichia coli*, *Enterococcus spp* and *Samonella spp* are used as hygiene indicators (Frahm and Obst, 2003, Mani- Lopez, 2012). Additives are chemicals that are added to food to improve it in some way, while food processing is the use of series of mechanical or chemical operation to change or preserve food. However, providing a safe nutritious diets in order to maintain health remain the major acceptable objective for food processing (Obatolu, 2016). Therefore, the present study focused on investigating the prevalence of microorganisms on *A. marginata* before and after washing with additives and determining the antibiotic sensitivity patterns.

MATERIALS AND METHODS

Collection of samples and media preparation: Samples of *A. marginata* were obtained from Melege village market in Owo. The samples were kept in a clean polythene bag and taken to the laboratory in Department of Microbiology, Adekunle Ajasin University, Akungba Akoko, Ondo State. All media used were prepared according to Adewole *et al,* (2013).

Sample preparation, microbial analysis for isolation, identification and sensitivity test: Three samples of snail were used for the five different processing reagents; lime, alum, vinegar, salt solution and ashes. The snails were washed with water, dissected and a part of about 1cm on the body was swabbed and dropped into the prepared peptone water labelled body-before (BB) corresponding to the reagent to be used in processing the snail sample. For Lime reagent: L^1FB signifying Lime reagent, foot and before processing for the first snail. The snails were processed by washing with the other reagents and swabbed just like before processing the snails for both foot and body libelled as FA and BA respectively. The microbial counts of the snails and growth of the colonies were observed and recorded as reported by Adewole *et al,* (2013).The standard biochemical tests were conducted to identify bacterial isolates using Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).The bacteria isolates were used for testing their susceptibility patterns to different antibiotics as described by Bauer *et al.* (2002). The zone of inhibitions for each antibiotic disc were determined and interpreted using methods of Clinical and Laboratory Standards Institute, (2008).

RESULTS AND DISCUSSION

The total bacterial count in cfu/cm^2 obtained from the foot and body of the snails before and after processing with different reagents as shown in (Table 1). It revealed that the total bacteria count ranged from $9cfu/cm^2$ - $22cfu/cm^2$. Highest percentage reduction from the processed snail body was from lime (57.1%), followed closely by salt (44%) and the least was from the snail foot washed with vinegar (10%)respectively. The frequency of occurrence of the bacteria isolated from the snail samples as shown in (Table 2) revealed that *Micrococcus luteus* to be the most abundant with frequency of occurrence of 13 (21.31%), closely followed by *Klebsiella spp* 10 (16.39%) and the least from*Enterobacterspp*4 (6.56%) respectively. The antibiotic sensitivity tests showed the varied degree of susceptibility/sensitivity/resistance/ to the different antibiotics evaluated as shown in Table 3.

Swabs from both the foot and the body of the giant African snail, *A. marginata* both before and after processing with additives yielded marked growth of bacteria. The presence of these organisms could be attributed to the fact that snails are voracious feeders and eats almost all kinds of foods bringing them in contact to different kinds of surfaces. The high total viable counts recorded in this study showed the microbial diversity (difference in form and species) in this snail, environmental condition and season during collection of snails and hygienic practice employed by the local dwellers of the area where snails are been collected showed correlation with the work of Eze, *et. al.,* 2006 where a higher number of bacteria was gotten after handling by the local market women compared to when it is yet to get to the market. The difference in the bacterial loads after washing with reagents showed the efficacy of these reagents. This is obviously established by greatest percentage reduction from snail washed with lime. The higher the percentage reduction of a reagent, the more the efficient the reagent as a decontaminating. Several additives such as sodium hydroxide and organic acids have been reported to be used in decontamination of food products such as beef, pork and poultry products (Mani- Lopez *et.al*, 2012; McKee, *et al.,* 2008). The emergence of resistance to some of the antibiotics tested is an indication of declining in clinical effectiveness of these drugs in Nigeria.

CONCLUSION AND RECOMMENDATION

Microbial loads of snail coupled with sensitivity test in this study showed that the snail samples harbour quite a number of highly pathogenic bacteria of potential public health hazard to the dependants' as protein source. The difference in the bacterial load after washing with reagents shows the efficacy of these reagents on the bacteria. The use of lime as an organic compound is therefore recommend for snails' meat decontamination.

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Table 1. Total bacterial count in cfu/cm^2

Reagents	Sample sites	Before	After	Difference	Percentage Reduction (%)
Lime	Body	22	6	16	57.1
	Foot	13	8	5	23.8
Alum	Body	18	12	6	20
	Foot	19	14	5	15.2
Vinegar	Body	9	4	5	38.5
	Foot	11	9	2	10
Salt	Body	18	7	11	44
	Foot	15	8	7	30.4
Ash	Body	16	10	6	23.1
	Foot	18	13	5	16.1

Table 2, Frequency and % of occurrence of bacterial isolates

Isolates	Frequency of occurrence	% of occurrence
Micrococcus luteus	13	21.31%
Klebsiella sp	10	16.39%
Proteus vulgaris	8	13.11%
E. coli	8	13.11%
Aeromonas sp	7	11.48%
Bacillus sp	6	9.84%
Streptococcus pyogenes	5	8.20%
Enterobacter sp	4	6.56%
Total	**61**	**100%**

Table 3, Antibiotic susceptibility patterns of the bacterial isolates

Isolates	CAZ	CXC	GEN	CPR	OFL	AUG	NIT	AMP	CTR	ERY	CLX	COT
E. Coli	0(8)	0(8)	5(8)	7(8)	6(8)	4(8)	0(8)	0(8)	NT	NT	NT	NT
Enterobacter spp	0(4)	0(4)	2(4)	3(4)	3(4)	0(4)	2(4)	0(4)	NT	NT	NT	NT
Klebsiellaspp	0 (10)	0 (10)	7(10)	8(10)	8(10)	0 (10)	6 (10)	0 (10)	NT	NT	NT	NT
Aeromons spp	0 (7)	0 (7)	4(7)	5(7)	5(7)	4(7)	7(7)	0 (7)	NT	NT	NT	NT
Proteus spp	6 (8)	0(8)	4(8)	7(8)	7(8)	0(8)	5(8)	0(8)	NT	NT	NT	NT
Bacillus spp	0 (6)	1(6)	4(6)	NT	NT	2(6)	NT	NT	0(6)	3(6)	0(6)	2(6)
Micrococcus luteus	0 (13)	0(13)	10 (13)	NT	NT	0(13)	NT	NT	0(13)	7(13)	6(13)	9(13)
Streptococcus pyogenes	0 (5)	0(5)	3(5)	NT	NT	0(5)	NT	NT	0(5)	4(5)	0(5)	3(5)

KEY: CAZ= Ceftazidime, **CXC**= Cloxacillin, **GEN**= Gentamicin, **CPR**= Ciprothoxacin, **OFL**= Oflaxacin, **AUG**= Augmentin, **NIT**= Nitrofurantoin, **AMP**= Ampicillin, **ERY**= Erythromycin, **CLX**= Cloxacillin, **COT**= Co-Trimoxazole, **NT**= Not sensitive. Figures in brackets represent the total number of isolates.