

Full Length Research Paper

A comparative study of the use of radiation, lemon juice, and vinegar for the preparation and preservation of African giant snails (*Achatina* and *Archachatina*)

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African giant snail is a popular alternative source of animal protein in Ghana and many other countries. The meat is high in lean protein and mineral elements. Ready-to-use meat obtained from snails can compete with animal proteins found on the market. After shelling, snails produced some slime that interfered with preparation and processing of the meat. This study aimed to provide consumers with ready-to-use fresh snails conveniently available on the market. The best treatment for eliminating slime from snails was determined using a 2×10×3 factorial design. Vinegar plus salt treatment was the most effective slime removal treatment which led to a significant weight loss. Irradiation at all doses most effectively reduced the microbial load of snails after slime removal. A 6×4×3 factorial design was used for the shelf-life study. Irradiation at 1.5 and 3 kGy extended the shelf life of fresh snails by 14 extra days with the lowest microbial load. Radiation did not affect the fat and mineral content, but the protein content increased. Panelists preferred irradiated snails even though they had different odours and aromas. This study concluded that irradiating fresh snails even at lower doses can extend the shelf-life of fresh snails under refrigeration temperature.

Key words: Contamination, irradiation, *Achatina achatina*, *A. marginata*.

INTRODUCTION

Apart from the conventional protein sources, snail meat is becoming an alternative source of protein for most people in Ghana. Already a delicacy, its consumption increased due to its lean meat nature, being very low in fat and rich in other essential nutrients. According to Cobbinah et al.

(2008), snail is a rich source of iron and calcium but low in fat and cholesterol than other protein sources such as poultry and pork.

Whether cultivated or wild, land snails are usually found in the soil and generally contact soil microorganisms. Soil

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and faecal coliforms are microorganisms of concern associated with snails. Some pathogens associated with fresh land snails included: *Salmonella*, *Staphylococcus*, *Shigella*, and *Arizona*. According to Okafor and Ogbo (2019), 86.7% of achatina obtained from different markets in Nigeria had coliform counts ranging from 10^6 to 10^8 CFU/g.

Despite the growing use of the meat, the studies had not been done on preserving the fresh snail in a ready-to-use state. Snails are still sold in their unshelled state. When shelled, snails contained some amount of slime or mucus naturally present on them. This is a secretory fluid produced from mucus glands located in the skin including the foot plate, mainly used to reduced friction and protected the foot during locomotion in live snails (Wiya et al., 2020).

Medicinally, snail mucus has the ability to facilitate wound healing and to prevent infections due to its many bioactive compounds (Adikwu and Enebeke, 2007).

In food industry however, the slime makes the meat less attractive during processing. According to Etengeneng et al. (2021), the flesh of snails contained some amount of slime and, when removed using the right method, rendered it more attractive and appetizing. Separating the meat from its shell during processing is also time-consuming and provided an opportunity for cross-contamination of the flesh during dressing.

This study aimed to provide consumers with ready-to-use fresh snails readily available to consumers. In doing so, the following will be determined: the best slime removal treatment and its effect on the microbial load of the meat, the shelf life and sensory characteristics of fresh snail meat, and the proximate analyses of irradiated meat snails.

MATERIALS AND METHODS

The study was conducted at the Radiation Technology Centre of Ghana Atomic Energy Commission. Live snail samples approximately of six months were bought from Nsawam farm in the Eastern region of Ghana and used for the study. A $2 \times 10 \times 3$ factorial design was used for this study. Thus, two species of snails were used for the study. The treatment included gamma irradiation (0, 2, 4, 6 kGy), lemon juice (400 and 600 ml), vinegar and salt solution (400 ml for 15 and 30 min) and blanching in water (for 10 and 15 s in three replicates). A $6 \times 4 \times 3$ factorial design was used for the shelf-life study (6 treatments- gamma irradiation at 0, 1.5, 3 kGy; 600 ml of lime juice and a solution of vinegar and salt for 30 min, blanching in water for 15 s; 4 sampling days and 3 replicates).

Removal of slime study

Sample preparation

This study used two snail species (*Achatina achatina* and *Archachatina marginata*). The snails were purged for two days and washed with distilled water to eliminate any soil matter and other debris that may be found on the shell. Shelling was done using a sterile metal pin, and 400g each of the shelled snail samples were

used for the study.

Determination of the effectiveness of the slime removal treatments by the change in weight

Four hundred grams (weight A) each of shelled snail specie were weighed separately into a plastic storage bowl, and different treatments were applied as described below:

Treatment A (Lemon juice): Two concentration of lemon juice were used. Fresh lemon juice of 400 and 600 ml volume were poured on separate snail samples. The snails were immersed in the lemon juice for 15 min, removed, and weighed to obtain weight B (Modification of known Ghanaian traditional method).

Treatment B (Vinegar and salt): Fifty (50) grams of salt were weighed and poured into each beaker containing 300 ml of malt extract vinegar and 100ml tepid water. Each solution was stirred to dissolve the salt. Immersion was done for 15 and 30 min. The snails were removed, and weighed to obtain weight B (A modification of Mezquita et al. (2007).

Treatment C (Blanching): Samples were immersed in boiling water for 10 and 15 s and removed. The weighing was done to obtain weight B.

Treatment D (irradiation): Four hundred grams of shelled snails were put into polyethylene zip-lock pouches and irradiated at 2, 4, and 6 kGy. The snails were weighed after irradiation to obtain weight B (Figure 1).

Chemical analysis

Sample preparation

Four hundred grams of shelled snails were cut into smaller sizes and oven-dried at 60°C overnight till the snails were dried (Babalola and Akinsoyinu, 2009). Dry samples were further broken with a mortar and pestle, and hammer milled to obtain the snail meat powder.

Determination of protein content

Nitrogen was determined using micro-Kjeldahl method. The percentages of nitrogen were converted to protein by multiplying by 6.25 (AOAC, 1990).

Elemental analysis

Atomic absorption spectroscopy

A quantity of 0.5 g of the powdered snail meat sample was weighed into a labelled 100 ml polytetrafluoroethylene (PTFE) Teflon bomb. A 6 ml aliquot of concentrated nitric acid (65%) and 1 ml of Hydrogen peroxide (30%) was added to the sample in a fume chamber. Samples were loaded in a microwave carousel, and the caps securely tightened with a wrench/ forgue. Microwave irradiation of the assembly was done for 18 min in a Milestone Microwave Labstation ETHOS 900 model (report code: 64, INSTR: MLS-200 MEGA) using the following microwave programme (Table 1). Five minutes were allowed for venting.

After digestion, the Teflon bombs were cooled in a water bath to reduce internal pressure and allow volatilized material to restabilise. The digestates were transferred into test tubes and assayed for the



Figure 1. Packaged snails for irradiation B. irradiated snails.
Source: Authors

Table 1. Microwave irradiation of samples.

Step	Time	Power	Pressure	Temp 1	Temp 2
1	00:01:00	250	100	400	500
2	00:01:00	0	100	400	500
3	00:05:00	250	100	400	500
4	00:05:00	400	100	400	500
5	00:05:00	650	100	400	500

Source (Milestone cookbook, update Jan 1, 1996).

presence of Fe, Zn, Mg, Ni, Cu, V, and Mn in Atomic absorption spectroscopy (AAS) on a (VARIAN AA240 FS) in an acetylene-air flame. Reference standards of the elements of interest, blanks, and duplicates of the samples were digested the same way as the actual samples. These served as internal positive controls. Reference standards were from FLUKA ANALYTICAL SIONRA-ALDRICH Chemie GmbH, CH-9471 Buchs product of Switzerland.

Working standards of Fe, Mg, Mn, Cu, Zn, and Ni were prepared by diluting concentrated stock solutions of 1000 mg/l in de-ionized water.

Determination of fat content

A nuclear magnetic resonance (NMR) instrument (Oxford Instruments, U.K.) was used for the analysis. The device was first auto-tuned using acetone. Powdered samples were then poured into a tarred glass tube up to the 4 cm mark. The sample mass was then recorded and transferred into the NMR instrument. The reading of the sample's fat content (%) was recorded.

Microbial analyses

Preparation of serial dilutions

Serial dilutions were done using the general rules for the preparation of initial suspension and decimal dilutions (ISO 6887-1-2017) to estimate the total viable counts of bacteria present in the snail meat. A 10g portion of the sample (snail) removed from the shell was aseptically transferred into 90 ml of sterile buffered peptone water under laminar flow hood to obtain a 1:10 dilution. The suspension was then agitated with a vortex for 5 min to ensure a homogenous mixture. Sevenfold serial dilution was made into McCartney bottles containing 9ml peptone water. 1ml of

the suspension was aseptically transferred into a 90 mm sterile and well labelled Petri dish (double trial for each dilution) beginning with the least dilution. Molten plate count agar and violet, red bile agar all at about 45°C were aseptically poured respectively according to labels; plates were swirled gently to mix and left for about 15 min to solidify. The solidified plates were incubated at 37 C for 18- 24 h in a microbiological incubator. After 24 h of incubation, cultures containing 30-300 colonies were selected and counted using a colony counter (Stuart Scientific).

Total viable count

The average count was calculated (arithmetic mean) with the duplicate plates at two consecutive dilutions. The total aerobic count (N) of CFU/g or ml of the test sample is calculated according to standard protocol (ISO7218:1996, ISO6887-1:2017).

$$\frac{c}{V(n_1 + 0.1n_2)} d$$

Where: C is the sum of colonies on all selected plates counted

V is the volume applied to each plate

n_1 is the number of plates counted at the first dilution

n_2 is the number of plates counted at the second dilution

d is the dilution from which the first count was obtained.

Total coliform count

Sample preparations, serial dilutions, and determination of the CFU/g were made as described for the total aerobic bacterial count. The molten Violet Red Bile Agar was cooled to 50°C and poured into a petri dish. The mixture was swirled gently and allowed to cool. Incubation was done at 37°C for 18-24 h.

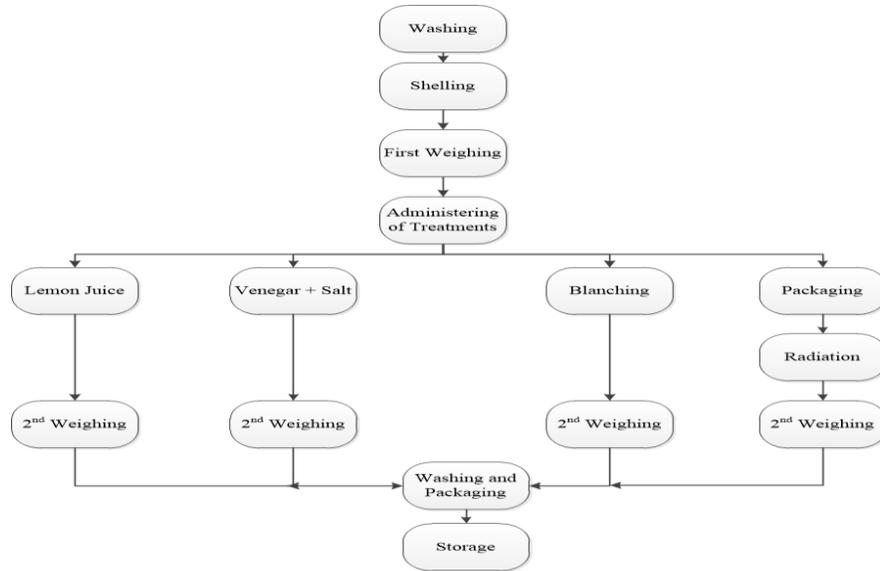


Figure 2. Flow chart for snail processing. Source: Authors

Detection and enumeration of *Staphylococcus*

Serial dilution was made as described above. Plating was done on molten Baird-Parker medium enriched with egg -yolk emulsion, and incubation was done at 37°C for 24 - 48 h. Incubated plates were observed for blackish colonies with mucoid surroundings and counted according to standard methods.

Detection and enumeration of *Salmonella*

Twenty five grams of each snail sample was aseptically transferred into 225 ml of buffered peptone water homogenized with a vortex mixer and incubated at 37°C for 18 to 24 h. 0.1 ml and 1ml of the incubated stock culture were transferred into 10ml of sterile Rappaport-Vassiliadis soya (RVS) peptone broth and Muller-Kauffman Tetrathionate-novobiocin broth (MKTn), respectively. These were then incubated at 37°C for 20 - 24 h proceeded by a subculture onto xylose lysine deoxy-chocolate agar (XLD agar). Inoculated plates were incubated at 37°C for 20 - 24 h and examined for typical blackish colonies. Further biochemical identification was performed using API 20E (BioMerieux).

Detection and enumeration of *E. coli*

Sub-cultures were made from typical pinkish colonies from VRBA agar with a sterile inoculation loop. The cultures were streaked on eosin methylene blue agar plate and incubated at 44 ± 1°C for 18 to 24 h. Further, plates were observed for a shiny metallic sheen (typical of fecal coliforms on EMB agar); Sub-culturing was done to obtain a pure culture. The effect of the slime removal treatment on the microbial load of snails was determined. Immediately after treatment, the Total Viable Count (TVC), coliform count, *Salmonella* and *Staphylococcus* count were determined as described above.

Shelf-life study

Achatina achatina was used for this study. Four hundred grams

each of shelled snails were administered 6 different treatments and observed during a 21-day storage period at a refrigeration temperature of 4°C. Samples were taken at (0, 7, 14 and 21 days) for microbial and sensory analyses during the storage period. All analysis was done in triplicates. Figure 2 shows the flow chart of snail processing.

Sample preparation

A. achatina approximately 6 to 8 months, from a snail farm (Assin Fosu) with the same growth condition was used for the shelf-life study. Shelling and slime removal was done as described above. Irradiation was, however, done at 1.5 and 3 kGy. All samples were stored under a refrigeration temperature of 4°C. Microbial analyses and sensory evaluation, were done within 21 days (0, 7, 14, and 21 days).

Microbiological analyses

Enumeration of total coliforms and total viable counts was done during storage as described above.

Sensory evaluation

Sample preparation

The sensory properties of snails were analysed by 20 trained panelist from the Department of Food Science and Radiation Processing; Ghana Atomic Energy Commission. Samples were cut into equal sizes, boiled in 200ml of water for 15 min and randomly selected for sensory evaluation. Panelists were asked to evaluate cooked snails for colour, taste, flavour, aroma, and tenderness, and fresh snails for colour, firmness, odour, and sliminess. Structured questionnaires aimed at evaluating the effect of each treatment on snails were used and a 9-point hedonic test was used to express their degree of preference for the models. Data from the sensory analysis were pooled and analyzed for the study.

Table 2. Total means weight loss of two species of snail after treatment.

Treatment		<i>A. achatina</i>	<i>A. marginata</i>
Doses of gamma irradiation	0 kGy	-23.0±12.7 ^a	-22.0±9.48 ^b
	2 kGy	-113.8±12.7 ^{cd}	-101.2±9.48 ^d
	6 kGy	-114.3±12.7 ^{cd}	-94.9±9.48 ^d
	4 kGy	-124.5±12.7 ^{cd}	-102.2±9.48 ^d
Vinegar and salt solution	15min	-126.6±12.7 ^{cd}	-101.8±9.48 ^d
	30min	-144.7±12.7 ^d	-95.3±9.48 ^d
Blanching in water	15 sec	-39.3±12.7 ^{ab}	+16.0±9.48 ^a
	10 sec	-48.2±12.7 ^{ab}	+17.5±9.48 ^a
Lemon juice	600ml	-70.7±12.7 ^{bc}	-76.5±9.48 ^{cd}
	400ml	-89.2±12.7 ^{bc}	-57.2±9.48 ^c

Values are means ± standard deviation. Different superscripts within the same columns are significantly different ($p \leq 0.05$).

Source: Authors

Data analysis

Data obtained were analyzed using Microsoft Excel and StatsGraphix Centurion XVI. Means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Effect of various treatments on slime removal

The effects of the various treatments on the slime removal are shown in Table 2. There were significant differences ($p \leq 0.05$) among each treatment. Vinegar + salt had the highest weight loss of -144.7 and -126 for 30 and 15 min dipping time, respectively. Blanching had the lowest weight loss of -48.2 at 10 s and -39.3 at 15 s for the *A. achatina* and a weight gain of 17.5 at 10 s and 16.0 at 15 s for *A. marginata*.

After shelling, snails produced some amount of slime. Burton (2003) compared this slime to blood released when the foot is irritated. It consisted of 95.2% of moisture (Danladi and Haruna, 2020), and the remaining part of mucus without water consisted of a mixture of proteoglycans, glycosaminoglycans, glycoprotein enzymes, hyaluronic acid, copper peptides, antimicrobial peptides, and metal ions (Griestorfer et al., 2017). It is therefore expected that the removal of the slime will be led to some weight loss in the meat.

Vinegar + salt treatments were most effective, achieving the highest mean weight loss. Followed with irradiation at 4, 6, and 2 kGy, then lemon juice and the least was blanching. Irradiation is known to break-down the viscosity properties of polysaccharides such as starch because the β - Glucan components of starch responsible for its gelling properties decreases in viscosity upon irradiation due to the radiolysis of the glycosidic bonds (Eui-Hong et al., 2007). Therefore, it was anticipated that

irradiation might break-down the slime associated with the meat.

The efficacy of these treatments measured as weight loss was less significant in the *A. marginata* and gave in weight gain in the blanched treatments. This may be due to the difference between the components and the slime structure from the *Archachatina* species. The slime of *A. marginata* had a thicker consistency than the *A. achatina*. This increased its tendency to absorb water resulting in weight gain.

Effects of slime removal treatment on the microbial load of snails

The TVC and coliform count of snails before and after slime removal are shown in Table 3. Irradiation at 2, 4, and 6 kGy led to 4, 5, and 5 \log_{10} cycle reductions of TVC; a 6 \log_{10} cycle reduction each in coliform counts. Both levels of blanching were least effective for reducing the TVC and the coliform counts, in which gave a one \log_{10} reduction in both TVC and coliform counts. All doses of irradiation and vinegar + salt treatments led to a 5 \log_{10} cycle reduction in *Salmonella* counts. Blanching for 10 s, however, gave a one \log_{10} reduction of *Salmonella*. Again, vinegar + salt treatment and irradiation at 6 kGy reduced *Staphylococcus* counts by a 6 \log_{10} reduction each. Blanching for 10 s, however, led to a 2 \log_{10} cycle reduction.

Effect of slime removal treatments on the microbiological quality of snails

All treatments had significant effect on the TV and coliform counts of the snail meat. Irradiation at 2, 4, and 6 kGy was most effective for reducing the TVC on the snail

Table 3. Effect of removal of slime treatments on the microbial load of snails.

Treatment	Microbial count log ₁₀ CFU/g			
	TVC	Coliform	Salmonella	S. aureus
0 kGy	7.61±0.07 ^g	6.38±0.53 ^d	5.60 ± 0.07 ^d	6.06 ± 0.51 ^e
2 kGy	3.44± 0.11 ^b	<1	<1	2.68 ± 0.07 ^c
4 kGy	2.18 ± 0.01 ^a	<1	<1	2.11 ± 0.07 ^{bc}
6 kGy	2.06 ± 0.02 ^a	<1	<1	<1
Lemon juice(400ml)	4.48± 0.15 ^c	3.83± 0.34 ^a	3.38 ± 0.42 ^b	1.13 ± 1.24 ^a
Lemon juice(600ml)	4.64± 0.13 ^c	4.05± 0.11 ^{ab}	2.14 ± 0.11 ^a	1.42 ± 1.69 ^{ab}
Vinegar+salt (15m)	4.82±0.10 ^d	4.60±0.42 ^c	<1	<1
Vinegar+salt (30m)	4.55± 0.18 ^c	4.39±0.34 ^{bc}	<1	<1
Blanching (15sec)	6.32±0.45 ^e	6.16±0.84 ^d	2.28 ± 0.35 ^a	4.67 ± 0.07 ^d
Blanching (10sec)	6.71±0.21 ^f	6.24±0.49 ^d	4.15 ± 0.69 ^c	4.23 ± 0.11 ^d

Values are mean count ± standard error.* Values with the different superscripts within the same columns are significantly different (P≤0.05).
Source: Authors

meat samples, and the least was blanching.

The immediate effect of ionizing radiation on the bacterial load of snails confirmed the effective use of ionizing radiation alone in reducing microbial count on food substances. This was observed with a sharp decline in log CFU of the total aerobic and fungal count, and a complete decontamination *Triphala* exposed to gamma-radiation at 5 kGy (Kumari et al., 2009). According to Brewer (2009), a 90% reduction of most vegetative cells can be accomplished with 1-1.5 kGy; hence a higher dose of 2, 4, and 6 kGy was expected to cause higher reduction both in the vegetative parts and some of the sensitive spores. Moini *et al.* (2009) reported that irradiation at 1, 3, and 5 kGy significantly reduced the total viable count of rainbow trout fillets. Radiation of the snail meat, at 2, 4, and 6 kGy, caused complete elimination of coliforms achieving a 6 log₁₀ cycle reduction. The high count of *Salmonella* was eliminated from the sample after irradiation. *Salmonella* is radio-resistant; hence, a treatment designed to eliminate *Salmonella* will ultimately destroy all Gram-negative pathogens (Belle and Tofana, 2010).

Commercially, food substances have been treated with preservatives such as salt and vinegar to reduce the microbial load, prolong the shelf life, and impact the sensory characteristics. According to Eyabi et al. (2001), several studies had been carried out on the microbial effects of treatment with salt and other preservatives such as vinegar, BHT, BHA, citric acid, potassium sorbate etc. of food. Some of these treatments have been used together to have a synergistic effect on the samples. In this study, vinegar + salt treatments not only eliminated the slime associated with the snail but reduced the TVC by a 3-log₁₀ cycle and the coliform count by a 2 log₁₀ cycle. The combination of vinegar and salt had a better effect of eliminating all *Salmonella* and *Staphylococcus* from the samples. Similarly, in a study to

evaluate the impact of various concentrations of vinegar on *Staphylococcus* and other microbial loads of fish, commercial vinegar reduced *Staphylococcus* and other bacterial population due to its acetic acid content (Mohamed et al., 2011). According to Bibek (2005), acetic acid, salts, and vinegar (which contained 5 to 40% acetic acid and many other compounds) are used in different foods for inhibiting growth and reducing the viability of Gram-positive and Gram-negative bacteria, yeasts, and molds.

Lemon juice contained citric acid which has some bactericidal effect. This was confirmed with a 3-log₁₀ cycle reduction in the TVC of snails treated with lemon juice. Both levels of lemon juice did not eliminate all microorganisms of coliform, *Salmonella*, and *Staphylococcus* but resulted in significant decreases in their counts. Similarly, Bingol et al. (2011) observed that treatment of lemon juice for different exposure times caused reduction ranging between 0.1 and 1.7 log₁₀ CFU/g for *Salmonella*. Some bactericidal effect of lemon juice on *Salmonella* inoculated in stuffed mussels was observed by Kisla (2007), where 0.08 to 0.25 log₁₀ CFU/g reductions in mussels treated with lemon juice and 0.22 to 0.78 log₁₀ CFU/g reductions treated with lemon juice dressing were observed. Similar observations were made of carrot samples treated with lemon juice and vinegar, for different exposure times, which caused significant reductions ranging between 0.79 to 3.95 and 1.57 to 3.58 log₁₀ CFU/g, respectively. Lemon juice and other organic acids are usually pre-treatments in many food processing cycles. The initial reduction achieved, therefore, was desirable in most situations.

Pipek et al. (2004) observed that citric acid reduced mesophilic and psychrotrophic microorganisms on pork and beef. When compared to several other mild preservation procedures, lemon juice is inexpensive and uncomplicated as a method of extending shelf-life.

Table 4. Pooled means of microbial counts of snails with different treatment during storage.

Days	Total aerobic plate count	Total Coliform count	Total <i>Salmonella</i> Count	Total <i>Staphylococcus</i> Count
	Log₁₀ CFU/g			
0	5.51 ±0.02 ^a	5.03±0.03 ^a	4.79±0.03 ^c	5.0.8±0.03 ^c
7	6.16 ±0.02 ^b	6.22±0.03 ^c	5.04±0.03 ^d	5.16±0.03 ^c
14	6.82 ±0.02 ^d	5.08±0.03 ^a	4.07±0.03 ^a	3.54±0.03 ^a
21	6.30 ±0.02 ^c	5.17±0.03 ^b	4.58±0.03 ^b	4.04±0.03 ^b
Treatment				
0 kGy	7.97 ±0.03 ^f	8.01±0.03 ^f	7.85±0.04 ^f	7.47±0.04 ^f
1.5 kGy	5.26 ±0.03 ^b	3.13±0.03 ^b	1.96±0.04 ^b	2.19±0.04 ^b
3 kGy	3.91 ±0.03 ^a	1.36±0.03 ^a	1.11±0.04 ^a	1.02±0.04 ^a
Vinegar + salt	6.75 ±0.03 ^d	6.74±0.03 ^d	5.16±0.04 ^d	4.89±0.04 ^d
Lemon juice	6.04 ±0.03 ^c	5.73±0.03 ^c	4.97±0.04 ^c	5.97±0.04 ^e
Blanching	7.28 ±0.03 ^f	7.29±0.03 ^e	6.66±0.04 ^e	4.60±0.04 ^c

*Values are mean count ± standard error. *Different uppercase superscripts within the same columns are significantly different (P≤0.05).

Source: Authors

High-temperature treatments such as blanching have been used in several processing steps as an initial treatment to reduce the microbial load and maintain color or stop enzyme action (Shaheen et al., 2012). Both treatment levels of blanching used in this study led to 1 log₁₀ cycle reduction in the TVC and coliform counts; however, significant decreases were observed in *Salmonella* and *Staphylococcus* counts. These observations were expected since blanching was known to reduce the number of contaminating microorganisms on the surface of foods and assist in subsequent preservation operations (Fellows, 2000). These results indicated that snail meat's initial high microbial load can be significantly reduced by most of these treatments.

Effect of storage on microbial quality of snails

At refrigeration temperatures, most microbial actions are reduced but not completely halted; however, treatments administered before storage influenced the rate at which microorganisms proliferated on the samples. Microbial counts increased in all samples as storage progressed; however, the irradiated samples recorded the lowest counts while blanched samples had the highest count (Table 4).

The lethal effect of ionizing radiation on microorganisms in radiation processed fresh foods usually results from the radiolysis of water and the production of hydrogen peroxide and other free radicals during radiation. This free radical, especially hydrogen peroxide, caused damages to the cells and DNA of microorganism, resulting in mutations and cell death (Lewis et al., 2002). This study showed that gamma radiation was effective in

reducing the TVC on snail samples. A 1.5 kGy led to a 2 log₁₀ cycle reduction in TVC, while a higher dose of 3 kGy led to a 4 log₁₀ cycle reduction (Table 4).

Salmonella population on the snail samples reduced with radiation dose, but there was an increase and some reductions during storage. Radiation at 3 and 1.5 kGy led to a 6 log₁₀ cycle reduction in the *Salmonella* count and a 5 to 6 log₁₀ cycle reduction in *Staphylococcus* count (Table 4). These reductions observed are due to the combined effect of radiation and refrigeration temperatures on the microorganisms. Ionizing radiation alone can be destroyed most microorganisms in food; however, the radio-resistant ones are further eliminated by refrigeration. Similarly, irradiating raw oysters at 1.5 kGy was enough to make them safe from pathogenic bacteria such as *E.coli*, *Shigella*, and *Vibronaceae* (Gelli et al., 2001).

The citric acid content (pH) of lemons acts as a growth inhibitor of many microorganisms. Lemon juice treatment on snail samples led to 1, 3, 3, and 2 log₁₀ cycle reductions in TVC, coliform, *Salmonella*, and *Staphylococcus* counts, respectively (Table 4). Similarly, lemon juice treatment for different exposure times caused a reduction range of 0.1 to 1.5 log₁₀ CFU/g for *Salmonella enteritidis* and 0.1 to 2.1 log₁₀ CFU/g for *E. coli* (Bingol et al., 2011). More than 5 log₁₀ reduction of stationary phase cells of 5 strains of *E. coli* 0157:H7 was achieved in both lemon and lime juice treatment (Enache et al., 2009).

Salts and organic acids are known to cause reductions in microbial loads in foods. Organic acids and extend the shelf life of refrigerated meat, poultry, and fish products by inhibiting the growth of spoilage and pathogenic bacteria (Sallam, 2007). From this study, a combination treatment of vinegar and salts for immersion time of 30

Table 5. Pooled mean scores of multiple comparison of cooked snails with different treatments during storage.

Days	Taste	Flavour	Aroma	Tenderness	Colour
0	6.06±0.05 ^c	6.12±0.05 ^a	5.79±0.05 ^a	6.08±0.51 ^b	6.12±0.04 ^a
7	5.99±0.05 ^c	6.48±0.05 ^a	6.00±0.05 ^b	6.58±0.51 ^c	6.46±0.04 ^c
14	4.53±0.05 ^b	6.12±0.05 ^b	6.04±0.05 ^{bc}	6.06±0.51 ^b	6.26±0.04 ^b
21	4.36±0.05 ^a	6.28±0.05 ^c	6.15±0.05 ^c	5.75±0.51 ^a	6.28±0.04 ^b
Treatment					
0 kGy	2.98±0.07 ^a	6.0±0.07 ^{ab}	5.9±0.06 ^{ab}	5.69±0.06 ^a	5.85±0.06 ^a
1.5 kGy	6.29±0.07 ^c	6.78±0.07 ^d	6.16±0.06 ^d	6.78±0.06 ^c	6.87±0.06 ^d
3 kGy	6.29±0.07 ^c	6.74±0.07 ^d	6.15±0.06 ^d	6.62±0.06 ^c	6.82±0.06 ^d
Lemon juice	6.19±0.07 ^c	6.08±0.07 ^{bc}	5.91±0.06 ^{ab}	6.04±0.06 ^b	6.34±0.06 ^c
Vinegar+salt	6.04±0.07 ^b	6.07±0.07 ^{bc}	6.02±0.06 ^{cd}	6.11±0.06 ^b	6.16±0.06 ^b
Blanching	6.02±0.07 ^b	6.25±0.07 ^c	6.04±0.06 ^{cd}	6.08±0.06 ^b	6.12±0.06 ^b

Values are mean count ± standard error. *Different uppercase superscripts within the same columns are significantly different ($P \leq 0.05$).

Source: Authors

min led to a 1, 2, 2, and 3 log₁₀ cycle reductions in the TV, coliform, *Salmonella*, and *Staphylococcus* counts; however, microbial counts increased during storage (Table 4). The microbial load in pork was reduced during storage by 0.5 to 2 log₁₀ CFU/g after treatment with organic acids and salts mixture under refrigeration (Ratanatriwong et al., 2009).

Even though blanching did not lead to a significant decrease in the TVC of snail samples, coliform, *Salmonella*, and *Staphylococcus* counts have reduced by 1, 1, and 3 log₁₀ cycles, respectively. The main objective of blanching is to inactivate enzymes and surface microorganisms associated with food spoilage (Fellows, 2017). Therefore, it is important to note that microorganisms may be increased during storage after blanching has been done as a pre-treatment.

Sensory evaluation of selected attributes for cooked snail after different treatments

Table 5 show the scores for the attributes measured of cooked snails. Significant differences ($P \leq 0.000$) were recorded in the values for colour, flavour, tenderness, and taste; and significant differences ($P \leq 0.05$) in the aroma. Irradiation at 1.5 and 3 kGy had no significant effect ($P \geq 0.05$) in all attributes measured, but significant differences ($P \leq 0.05$) were observed in samples treated with lemon juice and vinegar + salt. Hedonic test for cooked snails had significant differences ($P \leq 0.05$) in all attributes measured. Pooled mean values for irradiated samples of cooked snails had no significant difference; however, there were significant differences ($P \leq 0.05$) between the irradiated samples and other treatments (Table 7).

Sensory evaluation of raw snail after different treatments

The scores of the attributes measured for raw snails with different treatments are shown in Tables 6 and 8. Values showed significant differences for firmness and sliminess ($P \leq 0.05$) and highly significant differences ($P \leq 0.000$) in the colour and odour for raw snails with different treatments during storage. Samples irradiated at 1.5 and 3 kGy showed no significant effect in the color and sliminess of raw snails but showed a significant difference in both samples' firmness.

Lemon juice, vinegar + salt, and blanched samples had substantial differences in all attributes measured significant differences ($P \leq 0.05$) were recorded in all the attributes in all attributes (Table 8) measured during storage, except aroma which showed a highly significant difference ($P \leq 0.000$). Pooled means of irradiated samples showed no significant difference ($P \geq 0.05$) in all attributes measured, but significant differences ($P \geq 0.05$) existed between irradiated samples and other treated samples. There were significant differences ($P \geq 0.05$) in all attributes measured for samples treated with lemon juice, vinegar + salt, and blanching.

Effect of radiation on elemental, protein, and fat content of *A. achatina*

Snail meat is a rich source of trace elements for human development. Generally, the effect of irradiation on these elements had no specific trend. Iron and manganese concentrations increased with increased radiation dose; however, copper, zinc, magnesium, and nickel

Table 6. Pooled means scores of multiple comparison of raw snails with different treatments during storage.

Days	Colour	Firmness	Odour	Sliminess
0	6.10±0.04 ^a	6.18±0.04 ^b	5.78±0.05 ^a	5.78±0.35 ^a
7	6.10±0.04 ^a	5.81±0.04 ^a	6.52±0.05 ^b	5.97±0.35 ^b
14	6.26±0.04 ^b	6.06±0.04 ^b	6.67±0.05 ^c	5.85±0.35 ^a
21	6.31±0.04 ^b	5.73±0.04 ^a	6.67±0.05 ^c	5.84±0.35 ^a
Treatment				
0 kGy	5.71±0.05 ^a	5.57±0.06 ^a	6.33±0.06 ^a	5.79±0.04 ^b
1.5 kGy	6.83±0.05 ^c	6.68±0.06 ^d	6.39±0.06 ^{ab}	6.0±0.04 ^d
3 kGy	6.85±0.05 ^c	6.47±0.06 ^c	6.42±0.06 ^{ab}	5.99±0.04 ^d
Lemon juice	6.01±0.05 ^b	5.85±0.06 ^b	6.46±0.06 ^{ab}	5.86±0.04 ^{bc}
Vinegar + salt	6.13±0.05 ^b	5.7±0.06 ^{ab}	6.52±0.06 ^b	5.85±0.04 ^{bc}
Blanching	6.06±0.05 ^b	5.8±0.06 ^b	6.44±0.06 ^{ab}	5.95±0.04 ^{cd}

*Values are mean count ± standard error *Different uppercase superscripts within the same columns are significantly different (P≤0.05).

Source: Authors

Table 7. Pooled mean scores of hedonic scale of cooked samples with different treatments during storage.

Day	Aroma	Colour	Flavour	Taste	Tenderness
0	6.34±0.12 ^b	6.59±0.11 ^b	6.37±0.11 ^c	6.69±0.10 ^c	6.41±0.11 ^c
7	5.10±0.12 ^a	5.70±0.11 ^a	5.90±0.11 ^b	5.86±0.10 ^b	5.41±0.11 ^a
14	5.37±0.12 ^a	6.33±0.11 ^{ab}	5.47±0.11 ^a	4.73±0.10 ^a	5.97±0.11 ^b
21	5.28±0.12 ^a	6.21±0.11 ^b	5.50±0.11 ^a	4.66±0.10 ^a	6.04±0.11 ^b
Treatment					
0 kGy	3.7±0.16 ^a	6.04±0.15 ^a	4.68±0.15 ^a	2.77±0.13 ^a	5.53±0.04 ^a
1.5 kGy	6.74±0.16 ^d	6.3±0.15 ^{ab}	6.89±0.15 ^c	6.99±0.13 ^c	6.45±0.04 ^c
3 kGy	6.78±0.16 ^d	6.09±0.15 ^{ab}	6.84±0.15 ^c	7.01±0.13 ^c	6.24±0.04 ^{bc}
Lemon juice	5.92±0.16 ^c	6.31±0.15 ^{ab}	5.98±0.15 ^b	6.16±0.13 ^b	5.94±0.04 ^{ab}
Vinegar + salt	5.65±0.16 ^c	6.5±0.15 ^b	6.21±0.15 ^b	6.33±0.13 ^b	6.08±0.04 ^{bc}
Blanching	5.67±0.16 ^c	6.11±0.15 ^{ab}	5.85±0.15 ^b	6.22±0.13 ^b	5.91±0.04 ^{ab}

Different uppercase superscripts within the same columns are significantly different (P≤0.05).

Source: Authors

concentrations increased or decreased with no particular direction (Table 9). The mean value of iron concentration in the control sample was 7.04. This value fell in the range of 6.79 and 11.09 mg/kg were obtained for *Nucellalapillus* and *Archachatina marginata ovum* (Eneji et al., 2008). The mean values of 0.847, 11.16, and 9.80 mg/kg concentrations were recorded for manganese, copper, and zinc. Although, magnesium values were highest at 13.55 for the control, this value was lower than 28.00 mentioned with Ogbuagu (2011) for *Achatina* samples in Nigeria. The concentration of copper in control was lower than the recommended limit of 10 mg/kg.

Micro-nutrients such as Fe and Mg are essential trace

elements due to the major role played in the metabolic processes of humans and the total well-being of humans. Magnesium functions as a co-factor in enzymatic activities, while the iron is a major component of hemoglobin in human blood. A zinc deficiency is marked by retarded growth, loss of taste, and hypogonadism, leading to decreased fertility (Adebayo-Tayo et al., 2011).

Copper is necessary for the synthesis of haemoglobin, and its deficiency can result in anaemia in children. In snails, trace element content varies due the feeding habits, location (where the snail is found or picked from) and as well as where it feeds from (Ozogul et al., 2005).

The fat content of snails was rather very low. Samples irradiated at 2 and 6 kGy recorded no significant

Table 8. Pooled mean scores of hedonic scale of raw samples with different treatments during storage.

Days	Colour	Firmness	Odour	Sliminess
0	6.52±0.12 ^b	6.60± ^a	6.06±0.13 ^b	5.84±0.12
7	6.17±0.12 ^a	6.16± ^a	5.06±0.13 ^a	5.84±0.12
14	6.36±0.12 ^b	6.23± ^a	5.16±0.13 ^a	5.97±0.12
21	6.19±0.12 ^b	5.98± ^a	5.14±0.13 ^a	5.91±0.12
Treatment				
0 kGy	6.23±0.16 ^{BCD}	5.7±0.17 ^a	2.86±0.17 ^a	5.28±0.16 ^b
1.5 kGy	6.84±0.16 ^e	6.84±0.17 ^b	7.01±0.17 ^e	6.73±0.16 ^d
3.0 kGy	6.67±0.16 ^e	6.65±0.17 ^b	6.78±0.17 ^e	6.69±0.16 ^d
Lemon juice	5.73±0.16 ^a	5.94±0.17 ^a	5.89±0.17 ^{cd}	6.09±0.16 ^c
Vinegar + salt	6.04±0.16 ^{A^b}	5.99±0.17 ^a	5.13±0.17 ^c	5.47±0.16 ^b
Blanching	6.14±0.16 ^{A^{bc}}	6.63±0.17 ^b	5.95±0.17 ^d	6.31±0.16 ^{cd}

Different uppercase superscripts within the same columns are significantly different ($P \leq 0.05$).

Source: Authors

Table 9. Fat, protein, and mineral analysis of *Achatina achatina* before and after irradiation.

Sample	Mineral (elemental) composition (mg/kg)						Proximate composition (g/ 100g)	
	Fe	Mn	Cu	Zn	Mg	Ni	Fat	Protein
0 kGy	7.04	0.847	11.16	9.8	13.55	0.26	0.733±0.004 ^a	16.85±1.21 ^a
2 kGy	18.2	1.48	6.44	6.88	7.73	0.12	0.723±0.004 ^a	30.93±1.21 ^c
4 kGy	22.84	1.36	6.4	6.84	20.44	1.2	0.753±0.004 ^b	22.89±1.21 ^b
6 kGy	20.6	1.32	7.68	7.68	27.75	4.8	0.736±0.004 ^a	26.61±1.21 ^b

*Values are mean count ± standard error *Different superscripts within the same columns are significantly different ($P \leq 0.05$).

Source: Authors

differences in the fat content at 2 and 6 kGy (Table 9). When rainbow trout was irradiated at 1, 3 and 5 kGy, there was no significant change in the fatty acid composition (Oraei et al., 2011) an indication that lower doses and other factors may not have any significant difference in the fat content of food.

The increase in protein content of the snail samples did not follow any trend (Table 9). This is similar literature (Nour et al., 2009), where protein contents in pea nuts increased significantly from 25.84 to 29.75%.

Conclusions

This study shows that irradiation, organic acids, and other substances like salts (which are readily available) can eliminate slime from snails and help in the production of ready-to-use fresh snails. Irradiation effectively reduced

the microbial load of snails and extended the shelf life by 14 days and these samples were accepted by panelist. Gamma irradiation had no significant effect on the fat content of the snails; however, the protein content of the snail increased after irradiation. The findings of this study give a promise for producing packaged ready-to-use snail meat of safe microbiological quality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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