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EVALUATION OF THE QUALITY OF CANNED SNAIL (*ACHINATTA ACHINATTA*) MEATS BASED ON BIOCHEMICAL AND MICROBIAL PARAMETERS.

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ABSTRACT

In this research work, snail meat was canned. Microbial and proximate analyses of fresh and canned snail meat were carried out. The purpose was to investigate the effects of brine, vinegar, and combinations of different concentrations of brine and vinegar on the microbial counts and proximate composition, aimed at establishing the appropriate method of blanching during canning. The snail meat was blanched with different concentrations of brine of 1%, 3% and 5%; vinegar concentrations of 50%, 75% and 100%; and a mixture of 3% brine + 50% vinegar, 3% brine + 75% vinegar, 5% brine + 75% vinegar and 5% brine + 100% vinegar. The initial number of microorganisms in the treated samples after blanching determines the efficiency of the chemical preservative based on their viable count. The result showed both 1% brine and 3% brine concentrations had 20% and 28% percentage reductions in microbes of the snail meat respectively by the end of the first day. However, there was a progressive increase in the total viable count after the first day until the projected 7th day. 5% brine recorded a 51.57% reduction and was more effective in reducing viable organisms of the sample for the first two days but also increased progressively until the seventh day. The 50% vinegar concentration showed a higher viable count at the end of the seven days of test with a 57.89% reduction in the viable count. The 75% vinegar was also effective, with a 60.53% reduction in the viable count, and 100% vinegar, with a 68.4% reduction, as the best. It was evident from this study that the optimum brine concentration of 5% can be established as brine media concentration according to its high microbial reduction ability and lowest pH of 5.9.

Keywords: Brine, vinegar, snail meats, blanching.

INTRODUCTION

The major task facing the present world is giving adequate nourishment of sufficient quality for the alarmingly expanding human population. Food shortage is further marked with protein deficiency when contrasted with the accessibility of other food classes. Over time, the Food and Agriculture Organization (FAO) has reported that the average animal protein intake in Nigeria is low, calling for deliberate endeavours towards alleviating this problem of protein shortage. Sadly, the regular and conventional sources of animal protein in the nation like pork, beef, goat meat, fish, and poultry are getting out of the reach of common populace because of their high costs (Olayide *et al*. 2004). To bridge this gap, different non-conventional animal protein sources like snail, cricket, and winged termite are presently being investigated. Snail meat is accounted for to be high in protein and low in fat (Ademolu *et al*. 2007), and was reported by Ariahu *et al*. (2012) that snail meat is a major source of protein in the diet of some people living in the forest region of Nigeria and are consumed as a rare delicacy by many Nigerians living outside the forest region.

The consumption of land snails by humans has been practised for many years because of their typically low fat and cholesterol contents (in contrast with other protein sources like poultry) as well as their high protein, water and iron contents. They represent food of high nutritive value, and they likewise possess anti-cancer properties and boost the immune system due to their anti-inflammatory and anti-oxidant effects (Cabbinah *et al*., 2008). The low fat content and cholesterol levels make snail meat a great antidote for vascular diseases like high blood pressure, heart attack, hypertension, and other fat-related diseases (Lucy *et al*., 2016).

Snail meat is prevalent nourishment broadly conveyed in Nigeria, particularly in the Niger Delta and Upper Cross River Basins. It is likewise a noteworthy source of income to the general population (Anthony *et al*., 2010). Nonetheless, decay sets in after forty-eight hours of collection, and the deterioration rate of the meat quickens, as it can't be kept in satisfactory conditions after roughly half a day. This is the reason the conservation of snail meat has turned into a noteworthy worry to the processors and consumers. They also convey a few parasites because of their feeding habits. When not cleaned or cooked appropriately, the parasites could influence the human body and deliver lethal conditions (Ariahu *et al*. 2012). Its close contact with soil and unrestrained pattern of feeding makes them vulnerable to microbial contamination and therefore require adequate processing to assure safety of the snail meat from the public health point of view (Dokuzlu et al. 2016).

The processing options usually employed include canning, freezing, salting, pickling, pasteurization, irradiation, dehydration etc. Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container with a simple aim of keeping bacteria away from the food product (Afoakwa and Yenyi, 2006). The problem that could emanate from improper canning of low acid meats like snails includes a danger of botulism, thus a requirement for proper sterilization and the nutrients maintenance (USDA, 2014). The proper canning of the snails holds an advantage of commercial sterility while offering the products in ready-to-eat forms. Such products would enjoy wider geographical distribution and lengthy preservation without dependence on power supply (Ariahu *et al*. 2012). Sengor *et al*. (2008) reported that the process of canning using the heating temperature and time can affect the final product's nutrient content and fatty acid profile. The medium used

in the canning process also can affect the nutrient content and fatty acid profile of the final product. The sale of canned snail meats is not available in Nigeria as no active industries are associated with its production. As far as we know, especially through the internet shuffling, there is no research carried out on canned snail meats in Nigeria. Thus, this study was carried out to establish the suitability of chemical pickling (Vinegar/ brine solution) as a preservation method for blanched *Achinatta achinatta* (snail) meats by evaluating the effect of different brine and vinegar concentrations on the microbiological quality, as well as proximate and mineral contents of snail meats.

MATERIALS AND METHODS

Materials

African Giant snails of different sizes (big and medium-sized) were purchased from local markets in Ile-Ife, Nigeria. The snails were bought from market women who got them directly from the forest.

Methods

Snail Meat Processing

The African Giant snails of different sizes (big and medium-sized snails) were transferred to a bowl while alum was added to effectively remove slimes. The snails were transferred into hot water and covered immediately. After 3-4 minutes, the lid was opened, and the dirt was poured from the hot water. It was later cooled off under running water and, with ease, shook off from its shell into clean cool water. The internal organs (offal) were removed and rinsed in clean water. The snail meats were prepared and transported on ice in an ice chest to the Laboratory within two hours.

Media Formulation for Packing and Analysis

The packing media were of food-grade quality, and they conformed to international food standards for molluscs (FAO, 2010). Blanching was applied to the snail meats to inactivate microorganisms, firm the texture and moderate, strong flavours to have good effects on meats, similar to what was done by Biji *et al*. (2015). The pH was allowed to equilibrate (stabilize) thoroughly by adding sufficient packing media. The packing media of different concentrations were analysed for their microbial qualities via total viable counts and their respective pH.

Preparing packing media with brine

The glass jars were washed with distilled water and sterilized to eliminate, as much as possible, the initial microbes that could be present. Equal weights of 180.0 ± 10 g of the blanched fresh snail meats were closely packed into the glass jars while the hot brine solution of predetermined concentrations was added. The blanched snail meats were not immediately subjected to sterilization but were checked for their viable counts and the resulting pH for a period of three days and an extended period to the seventh day. This was done to effectively get the optimum concentration of the packing liquid media, as the sterilization process would have rendered it impossible. Freshly prepared hot brine solutions of 1%, 3% and 5% concentrations were made and poured into different glass jars of the same size (pint). This was similar to a study by Aljawad and Bowers (1988) for experimental investigations on lamb-soy mixtures where brine concentrations of 1%, 2%, and 3% were chosen in the experimental test. Each glass jar was filled with 100ml of the solution measured with a sterile measuring cylinder. The snail meat were left in the brine solutions during which regular checks for microbial growth and pH were carried out. The pH measurement was useful for evaluating the meat quality and control of acidity.

Preparing packing media with vinegar

The aforementioned procedure was applicable for the media solutions containing vinegar of 50%, 75% and 100% concentrations and its addition to low-acid snail meat was in controlled proportions to conform with specific formulations (Theron and Lues, 2007), with a minimum of 5% acetic acid-vinegar. The chosen concentrations employed are the recommended concentrations of vinegar of minimum acidity which should be 50% or greater in the formulation of media for canning snail meats.

Preparing packing media with brine-vinegar mixtures

Similar procedural steps were applied for brine-vinegar mixtures while considering the minimum and maximum concentration values for various mixing ratios. Fresh snail meats are closely packed into the glass jars while the hot brine-vinegar mixtures are added. The snail meats were left in the mixture during which regular checks for microbial growth were performed.

Total Viable Count of Samples and pH of Media

Table 1 shows the sample identities of media formulation. The initial number of microorganisms in the blanched snail meats was determined to know the efficiency of the chemical preservative on the basis of their viable counts. The viable counts of the samples were determined by taking 1ml each sample to make the first dilution (10^{-1}), which was transferred to a second test tube containing 9 ml. of sterile distilled water. The 2nd dilution or 10^{-2} was then made from the second test tube while 1 ml was also transferred to the third tube (3rd dilution or 10^{-3}) and so on till the 4^{th,} 5th or 6th dilution. The inoculation was done according to the pour plate method by putting the sample into the petri dish while 15 ml agar (liquefied in a boiling water bath at 40-45°C) were poured into the plate afterwards. The agar and the sample were then thoroughly mixed by rotating the petri dish. Incubation for 48 hours at 35°C was followed while the colony forming units (CFU) got counted.

Microbiological Parameters

The microbial analyses for total heterotrophic bacteria, total heterotrophic fungi (THF), coliform bacilli by coli most probable number (MPN) presumptive test and estimation of *Staphylococci* were the standards used for microbial analysis of the canned snail meats. These parameters were initially determined for the fresh snail meats before and after the canning.

Total heterotrophic bacteria

The snail meat sample was aseptically weighed and 10g of the homogenate was used in serial dilutions up to the 10^{-6}. One millitre of the diluent was placed on nutrient agar and rapidly mixed at a cold temperature. The ground sample was kept in glass or similar containers, which were air and liquid tight. To mitigate the effect of time delay, the sample was chilled to inhibit decomposition. Hundred folds serial dilution of each sample was carried out respectively six times in a set of test tubes, each containing 9.9 ml sterile distilled water. Then one millilitre of each dilution was plated in duplicates using a nutrient agar medium (sterile), which was kept in molten form as the pour plate method was adopted. The culture plates, having allowed the agar medium to set were incubated inverted and aerobically at 35 ^{0}C for 48 hours. The plates were observed for growth and selected for heterotrophic bacteria count after the expiration of the incubational period. The count obtained was multiplied by the dilution factor at their dilution and expressed as colony forming unit (cfu) per gram of the original sample.

Total heterotrophic fungi

The procedural steps for the total heterotrophic bacteria were repeated here for the total heterotrophic fungi count but there are a few differences noted. The culture medium used here was a malt extract agar and the culture plates were incubated inverted and aerobically at 30 ^{0}C for 4-7 days (until the plates showed no further increase in the number of fungal colonies).

Table 1: Sample Identities of Media Formulation

Sample	Identity
1	1% brine
2	3% brine
3	5% brine
4	50% vinegar
5	75% vinegar
6	100% vinegar
7	3% brine + 50% vinegar
8	3% brine + 75% vinegar
9	5% brine + 75% vinegar
10	5% brine + 100% vinegar

Estimation of *Staphylococci*

The same procedure for the total heterotrophic bacteria was repeated to estimate *Staphylococci*. The differences however, were the mannitol salt agar employed as the culture medium while the culture plates were incubated inverted and aerobically at 35 ^{o}C for 48 hrs. Only small, regular, raised colonies surrounded with or without yellow zones were observed, counted and recorded.

Estimation of coliform bacilli by coli MPN presumptive test

The most probable number (MPN) of coliform was carried out on each test sample by planting three portions in each of three dilutions in a geometric series. The use of sterile single and double strengths Lactose broth (MacConkey broth) was employed. Two sets of three test tubes, each containing 5 ml double strength broth and 5ml, sterile single strength broth was required. Five millilitre of a sample was inoculated into each tube of the double strength broth. 1.0 ml of the sample was inoculated into each of the first three sets of tubes of a known strength and 0.1ml of the same sample was inoculated into each of the other set of three tubes of the single broth. The cultured tubes were carefully agitated to mix the inoculum with the broth medium. They were incubated at 35 ^{o}C for 48 hours and each tube was observed for growth and gas production. A tube in which acid and/or gas is produced is referred to as positive tube. The combined numbers of positive tubes in each set arranged in order of least diluted to the most diluted tubes and read out from the appropriate standard MPN table to obtain the estimated number of coliform cells present in the original sample.

Proximate Analysis of the Canned Snail Meats

The chemical or proximate compositions of the snail meat were determined for the fresh and the canned snail meats samples to observe the differences between the values before and after canning. The methods of the Association of Official Analytical Chemists (AOAC, 2000) were used to determine the crude protein, crude fat, ash, and moisture content of the snail meats. Nitrogen was determined by the Kjeldahl method, and the percentage nitrogen was converted to crude protein by 6.25. The crude fat content of the snail sample was determined using the Soxhlet extraction apparatus to thoroughly extract crude fat from 2.0 g of milled snail sample using n-hexane in the Soxhlet method of fat determination. The weight of fat extracted divided by the weight of the sample multiplied by 100 % gave the percent

crude fat content. Ash was determined by incinerating 1.0 g of the milled snail samples at 700 ^{0}C in the muffle furnace chambers until the samples were ashed within 3 hours. The weights before and after ashing were used to calculate the percent ash content.

The Carbohydrate content was expressed as the percentage difference between the addition of other proximate chemical components and 100%.

Carbohydrate = 100 - (protein + crude fat + ash + fibre + moisture)

RESULTS

Viable Count and pH of Formulated Media

In this research work, the initial number of microorganisms in the treated samples after blanching determines the efficiency of the chemical preservative on the basis of their viable count. The total viable count gives a quantitative estimate of concentration of microorganisms such as bacteria, yeast or mold spores present in the snail meat sample. The count, represents the number of colony forming units (per ml) of the sample. With an average initial microbial count of 4.75 x 10^3cfu/ml, both 1% brine and 3% brine samples subdued the number of microbes to 3.8 x 10^3 cfu/ml and 3.42 x 10^3cfu/ml, respectively. Hence, resulting in 20 % and 28 % percentage reductions in microbes of the snail meats respectively by the end of the first day (Table 2). It showed a progressive increase in total viable count after the first day until the projected 7th day. This further proves that the snail meats being a low acid-food cannot be packed in glass jars without a further heat treatment by sterilization. Hence, brine concentrations of 1% and 3% (wt/vol) did not make a significant difference in the number of viable cells obtained. It was also shown in Table 2 that

Table 2: Changes in total viable counts (cfu/ml) of snail meat samples with brine

		DAYS						
S/N	**Sample**	**0 (BLANCHED)**	**1**	**2**	**3**	**7**	**% Reduction**	**pH (After the first Day)**
1	1% brine	4.75×10^3	3.8×10^3	6.0×10^4	9.8×10^4	2.48×10^6	20%	6.3
2	3% brine	4.75×10^3	3.42×10^3	1.2×10^4	2.8×10^4	1.3×10^6	28%	6.1
3	5% brine	4.75×10^3	2.3×10^3	3.7×10^3	1.76×10^4	6.2×10^5	51.6%	**5.9**

the pH of the first two sampled jars (6.3 and 6.1) were the highest among the set of brine concentrations tested, as a lower pH was required to formulate the desired media.

The result also shows that after the first day, 5% brine recorded a 51.57 % reduction and was more effective in the reduction of viable organisms of the sample for the first two days but also increased progressively until the seventh day. It also recorded a higher level of acidity with a pH value of 5.9 which is similar to the results obtained by (3) where it was suggested that about 5 % brine is sufficient for media formulation. A wrong conclusion could be made if it is assumed that an increase in concentration of the brine could be suitable for packing, as higher salt concentration above 10 % could be detrimental to its texture, structure and the microbial quality of food. The optimum brine concentration of 5 % can be established as brine media concentration according to its high microbial reduction ability and lowest pH of 5.9.

The maintenance of pH 5.7 or less prevented the outgrowth of spores in a study carried out by Ito and Chen (1978) on the inhibitory effects of pH on the growth of *Clostridium botulinum* spores, in figs packed in various styles of syrup. Table 2 shows the changes in total viable microorganisms with vinegar as the packing media. The initial number of microorganisms in the treated samples after blanching was 3.8 x 10^3 cfu/ml. There were significant reductions in the total viable account for the three cases except that the 50 % vinegar concentration showed a higher viable count at the end of the seven-day period of test with a 57.89 % reduction in viable count. The 75% vinegar was also effective with a 60.53 % reduction in viable count and these tested ranges of vinegar concentrations were found to be markedly effective after two days in all cases but 100 % vinegar, with a 68.4% reduction, was found to be the best. The pH value of the meat sample with 50 %vinegar was 6.0 while the pH values of meat samples of 75 % and 100 % vinegar were similar with an equal value of 5.8. The growth of viable organisms was inhibited with a 5% concentration of acetic acid in the vinegar.

Woodburn (2006) studied the pH of Oregon-grown figs in the acidification of figs required for boiling-water canning. The average pH of fresh figs was 5.51, and an increase in pH occurred with progressive ripening or mold growth. It was recommended that acidification should be carried out with one metric 4% lemon juice per pint of figs before covering with syrup and processing.

Table 4 indicates a strong synergistic effect of sodium chloride in brine and vinegar in antimicrobial effectiveness. A brine concentration of 3% salt solution alone has no apparent effect on the reduction of microbes as shown in the previous tests. However, when the low concentration of vinegar was paired with brine, a strong antimicrobial effect resulted. Vinegar has a significant antimicrobial effect on food-borne pathogenic bacteria and this action against microorganisms was synergistically enhanced with the sodium chloride in brine.

The calculated % reductions for the first three brine-vinegar mixtures in Table 4 were 58.97 %, 64.10 % and 61.54 %, respectively. There was only a 0.2 difference in the pH values of tested samples and on this note; any of the formulation media could be applicable as there was no significant disparity in their levels of acidity. However, the formulation of 5 % brine + 100 % vinegar (71.794 % reduction) was most effective in reducing the microbial count of the snail meats. It can be seen in Tables 2 - 4 that the blanching technique alone was only effective at subduing the viable microorganisms to a range of 3.78 x 10^3 to 4.75 x 10^3 cfu/ml.

Table 3: Changes in total viable counts (cfu/ml) of snail meat samples with vinegar

		DAYS						
S/N	**Sample**	**0 (BLANCHED)**	**1**	**2**	**3**	**7**	**%Reduction**	**pH (After the first Day)**
1	50% vinegar	3.8×10^3	1.6×10^3	4.2×10^3	8.0×10^3	9.8×10^5	57.89%	6.0
2	75% vinegar	3.8×10^3	1.5×10^3	3.6×10^3	4.5×10^3	5.6×10^5	69.53%	5.8
3	100% vinegar	3.8×10^3	1.2×10^3	1.4×10^3	3.7×10^3	3.5×10^5	68.42%	5.8

Table 4: Changes in total viable counts (cfu/ml) of snail meat samples with brine-vinegar mixture

		DAYS						
S/N	**Sample**	**0 (BLANCHED**	**1**	**2**	**3**	**7**	**%Reduction**	**pH (After the first Day)**
1	3% brine + 50% vinegar	3.9×10^3	1.6×10^3	2.0×10^4	4.7×10^4	8.0×10^5	58.97%	5.9
2	3% brine + 75% vinegar	3.9×10^3	1.4×10^3	1.8×10^3	2.16×10^4	6.7×10^5	64.10%	5.9
3	5% brine + 75% vinegar	3.9×10^3	1.5×10^3	2.3×10^3	1.94×10^4	3.8×10^5	61.54%	5.8
4	5% brine + 100% vinegar	3.9×10^3	1.1×10^3	1.6×10^3	3.96×10^3	2.0×10^5	71.79%	5.7

Microbial Parameters Assessment of Fresh Snail Meats

The microbial parameters of the fresh snail meat were determined on the basis of the total heterotrophic bacteria, total heterotrophic fungi (THF), coliform bacilli by coli MPN presumptive test and the estimation of *Staphylococci* via standards as shown on Table 4.17. Fecal contamination of the snails' natural habitat indicates high levels of coliforms a result of the negative human activities carried out in the forest where these snails are picked and sold to the markets.

The snail meat purging process had a significant effect on the reduction of the total heterotrophic bacteria count. It was very effective in reducing the initial microbial load of 2.67 $x10^7$ cfu/g in the fresh state to 1.53$x10^5$ cfu/g (Table 4.17). Purging is important because it reduces the microbial load of the snails apart from emptying the intestinal tract of any food material, which might be harmful to humans when eaten. The blanching process also reduced the microbial load further from 1.53 $x10^5$ cfu/g to 4.72 $x10^3$ cfu/g for bacteria and this process was done at a temperature of 80 °C for about five minutes to prevent overcooking. This blanching temperature and time combination was effective at subduing the viable microorganisms to a range of 3.78 x 10^3 to 4.75 x 10^3 cfu/g.

Table 5: Measured Microbial parameters of fresh snail meats

Microbial Parameter	Count
THB@35^{0}C	2.67 x 10^7
MPN(CELLS/100ml)	1.86 x 10^5
STAPHYLOCOCCI (CFU/ML)	4.9 x 10^5
THF@30^{0}C	6.0 x 10^6
THB of fresh snail meat	2.67 x 10^7
THB after purging	1.53 x 10^5
TVC after blanching	4.72 x 10^3

Proximate Analysis of Fresh Snail Meats

Table 6 shows the mean values of the proximate compositions of the fresh and canned snail meats. Experiments were carried out in duplicate to determine their average values. The protein content with their standard deviation was found as 20.075 % ± 0.50. The mean moisture content of fresh snail meats was found to be 75.945 % ±0.08 while the average carbohydrate content of fresh snail meats was determined to be 1.30 % ±0.07. The table also reveals the mean value pH of the snail meats as 6.65 ±0·21 being a low acid food. Fat content of fresh snail meats was 1.37% ±0·04 while the mean value of the ash contents of the fresh snail meats was 1.0 %.±0·76. Fiber was not detected in all the samples of snail meats. These values are similar to the findings by Kalio and Etela (2011) and Fagbuaro *et al*. (2006) on the proximate compositions of fresh snail meat samples. For canned snail meats on the other hand, the protein content was found to be 19.19 ±0·22. Moisture content was 76.11 ±0·77. Total carbohydrate, fat, ash and pH were found to be 1.54 ±0.32, 1.42 ±0.52, 1.54 ±0.07 and 5.92 ±0.13, respectively. The snail meats are a low acid-food; thus, from this result, it was observed that the acidity of the snail meats slightly increased after canning from the pH of 6.65 to 5.92 after canning. This might be as a result of effect of processing chemical that were used for canning. Also, the value of protein content also slightly decreased after canning from 20.08 to 19.19. Other parameters: moisture content, carbohydrate, fat and ash were found to be slightly increased.

Table 6: Mean Values of Measured Biochemical Compositions of Fresh Snail Meat

Run	Parameters (%)	Fresh meats	Canned meats
1	Moisture content	75.95 ±0.08	76.11 ±0.77
2	Protein content	20.08 ± 0.50	19.19 ±0.20
3	Total carbohydrate content	1.3 ±0.07	1.54 ±0.32
4	Fat Crude	1.37 ±0.04	1.42 ±0.52
5	Fiber content	-	-
6	Ash	1.00 ±0.76	1.54 ±0.07
7	pH	6.65 ±0.21	5.92 ±0.13

CONCLUSION

The chemical pickling method investigated for the best packing media indicated that 5% brine, 100% vinegar (of 5% acetic acid) and brine-vinegar mixture (5% brine+ 100% vinegar) are the best media concentrations for the snail meats canned in jars. They recorded high percentage reduction of viable count within the time frame of tests and lower pH values. A salt concentration of 10 % is the optimum suitable for packing, as higher salt concentration above 10% could be detrimental to its texture, structure and the microbial quality of food.

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