LAUTECH Journal of Engineering and Technology 18 (2) 2024: 65-79
10.36108/laujet/4202.81.0260

Optimization of the effects of process parameters on nutritional and microbial qualities of canned snail meat using Taguchi method

[1]Oyesola L. A., [2]Adejumo A. L, [2]Jimoh M.O. and [1]Solomon, B.O.

[1]*Department of Chemical Engineering Obafemi Awolowo University, Ile-Ife. Nigeria*
[2]*Department of Chemical Engineering, Osun State University, Osogbo, Nigeria.*

Article Info

Article history:

Received: March 10, 2024
Revised: May 21, 2024
Accepted: May 25, 2024

Keywords:

Optimization,
Microbial Qualities,
Snail Meat,
Taguchi Method

.

Corresponding Author:
ayoade.adejumo@uniosun.edu.ng

ABSTRACT

Snail farming, being a low-cost farming process that requires minimal professional skill requirements, has gained more attention in recent times. Snail meats are rich in protein but are vulnerable to microbial contamination due to their habitat. Smoking and drying are the most common methods of preserving snail meat in Africa, especially in Nigeria. The quest to retain the nutritional value of snail meat while preserving it fresh has necessitated further investigation. Fresh snail meats were preserved by canning in three packaging media (brine, vinegar, and brine-vinegar). The effects of canning time, temperature, nitrite concentration and packaging media on protein content, fat content, moisture content, ash and pH were investigated using the Taguchi model. Statistical analysis was carried out. Process parameters were also optimized and validated for effective snail meat canning. Temperature was discovered to be the most controlling parameter for the variation in protein, fat and moisture content. Carbohydrate and ash content were not significantly affected by the process parameters. The optimum canning process parameters, 50 mins, 121°C, brine and 100 ppm sodium nitrite concentration gave 18.98% ±0.11 protein content, 1.51% ±0.09 ash content, 1.40% ±0.04 fat content, 1.88% ±0.13 carbohydrate content, 76.23% ±0.08 moisture content and pH of 5.93 ±0.06. The absence of heterotrophic bacteria, fungi, coliform and Staphylococci in the validation experiment confirmed the efficiency of the optimum conditions and its suitability in the commercial preservation of canned fresh snail meat.

INTRODUCTION

Snail meat is a rich source of protein and has low fat content (Adeyeye *et al*. 2020). It is consumed in almost every part of the world. Its consumption holds various economic, cultural and nutritional importance in some regions (Achaglinkame *et al*. 2020). Being a sustainable protein source with a unique taste and high nutritional value, enhancing its processing to preserve its nutritional qualities became imperative. Heliciculture (snail farming) is a low-capital investment farming that requires fewer professional skills and labour requirements. Snail farming is characterized by low mortality, high productivity and readily available local and international markets hence it is a viable alternative source of protein especially for women and children in countries where access to proteins is rather difficult (Munonye and Moses, 2019).

The shells and meat of snails are exposed to several microorganisms due to their usual habitat. Snails live on or near the ground and feed on decaying plants matters. Snails are always in close contact with soil and their feeding patterns are unrestrained. Hence, they are vulnerable to various microbial contamination. The hurdle effect technology is a process developed for safe, nutritious, stable and economical food production. The technology ensures that pathogens in food products are controlled or eliminated thereby ensuring safe consumption and improving its shelf life. The most vital hurdles in food preservation include increased acidity, the addition of preservatives, low and high temperatures as well as reduced water activity and reduced redox potential (Tanyitiku *et al*., 20222). Canning is the most popular method of preserving food items while retaining their nutritional content over a specified period. Liquid antimicrobial preservatives (packing media) are used in canned food including meat to prevent microbial growth. The composition of the packing media plays a significant role in the microbial content of canned food (Okafor-Elenwo and Imade, 2019). Commonly used packing media in canned food items include brine, alcohol and vinegar (Simpson *et al*., 2013).

Taguchi method is a design method often used as an initial screen technique to identify the most influential factors and their optimal values. The design of the experiment proposed by Taguchi involves using orthogonal arrays to arrange input parameters (factors) affecting the process and the level at which they should be set. The method allows for the selection of a subset of a combination of multiple factors. The number of experimental runs is reduced, hence the reduction in time and resources needed to identify the most optimal factor. Response Surface Methodology (RSM) is a statistical and mathematical technique used in the design of experiments and analysis to optimize processes. It is used to improve and enhance product quality and understand the relationship between the factors (input variables) and response variables of interest.

Utilizing the efficient screening capabilities of Taguchi's method coupled with the detailed modelling and optimization technique of RSM, researchers and engineers can achieve robust and efficient improvements for various processes. Especially, when there is a desire for optimal results with limited experimentation resources.

As far as it can be ascertained, research outputs regarding the optimization of process parameters for improved microbial qualities of canned snails are very diminutive. In most African countries, especially Nigeria, conventional methods of processing and preserving (smoking and oven drying) snail meat are a major concern. Snail meat, when consumed immediately after processing retains most of its nutritional value. However, for economic viability, it's imperative to preserve snail meat such that its nutritional value is meat and the consumer to various pathogens, diminishes its microbial qualities and ultimately its nutritional value. To this end, this study is aimed at processing and preserving snail meat via canning in selected packaging media. The optimum process conditions for microbial-free canned snail meat will be investigated using the Taguchi method.

METHODOLOGY

Materials

Achatina achatina (African Giant Snails) was purchased from Oja Oba market in Ile-Ife, Nigeria. Media and reagents used were NaCl pickling salt for brine, alum, vinegar (5% acetic acid strength), distilled water, Nutrient Agar, Malt Extract agar, mannitol salt agar, bromocresol green solution, Methyl red solution, conc. sulphuric acid, hydrochloric acid, sodium hydroxide, n-hexane, lactose broth (MacConkey broth), boric acid, and sodium nitrite salt. All reagents are analytical grade. The media (brine, alum and vinegar) are food-grade.

Snail processing and packaging media

Snail meat was cleaned using alum and distilled water to effectively remove all slime. Cleaned snail meat was blanched in hot water. Blanching is done to inactivate microbes, retain flavour and maintain texture firmness. The packaging media used in the study are brine, alum and vinegar. Brine solution (5%), vinegar (100%) concentrations with 5% acetic acid, and brine-vinegar mixture (5% brine + 100% vinegar) were prepared. The selected concentrations were similar to the study of Ugbede-Ohuoba *et al*. (2021). 180g of blanched snail meat were closely packed in a 250 ml sterilized gas jar. 100ml of pre-determined concentration of media (brine or vinegar or brine-vinegar) solution was added to the jar. Snail meat was left in the jar while checking for microbial growth and pH at intervals according to the method of Jayantilaljay *et al*. (2017).

Proximate analysis

Proximate analyses of the fresh and canned snail meat were carried out following the recommended standard method of the Association of Official Analytical Chemists (AOAC, 2000). Parameters investigated include protein yield, crude fat, moisture content and carbohydrates.

Crude protein determination

The Kjeldahl technique (AOAC, 2000) was employed here to determine the protein content. 0.2 g was weighed in a Kjeldahl flask and 10 ml of concentrated H_2SO_4 and one Kjeltec tablet (Kjeltec-Auto 1030 Analyzer, USA) were added. The mixture was digested on a heating racket to achieve a clear solution while the digestate was cooled to make up the 75 ml with distilled water. This was later delivered onto the Kjeldahl distillation setup up followed by the addition of 50 ml of 40 % of NaOH solution. The NH_3 formed in the mixture was distilled into 25 ml, 2 % boric acid solution containing 0.5 ml of the mixture of 100 ml of bromocresol green solution and 70 ml of methyl red solution indicators. The distillate obtained was then titrated with 0.05M HCl while blank determination was determined by excluding the sample using the procedure below:

$$Cp = \frac{1.401 \times M \times F \text{ (ml titrant-ml blank)}}{\text{sample weight}} \quad (1)$$

Where,

Cp= Crude protein (%)

$M = \text{Molarity of acid used} = 0.05 \left(\frac{mol}{dm}\right)$

$F = kjeldahl\ factor = 6.25$

Crude fat determination

Crude fat was determined by the AOAC (2000) method using a soxhlet apparatus. Approximately 2 grams of the ground sample was placed into a thimble, which was placed inside a soxhlet extractor and n-hexane was poured into a pre-weighed round bottom flask, used to extract the oil from the sample. The extraction was carried out for about 6 hours. The solvent was removed from the extracted oil by distillation. The oil in the flask was further dried in a hot-air oven at 90 ºC for 30 minutes to remove residual organic solvent and moisture. This was cooled in a desiccator and flask and its content weighed. The quantity of oil obtained was expressed as a percentage of the original sample used using the equation given below (AOAC, 2000)

$$\text{Ether extract (\%)} = \frac{W_1 - W_2}{W_3} \times 100 \quad (2)$$

W_1 = weight of flask+oil

W_2 = weight of empty flask

W_3= weight of sample

Ash determination

Ash content (Ac) was determined by the official AOAC (2000) method using a muffle furnace (Carbolite AAF1100, United Kingdom). One gram of the sample was weighed into an ashing crucible with a known weight and placed in the muffle furnace chambers at 700 ºC until the samples was ashed within 3 hours. The crucible was removed, cooled in a desiccator and weighed. The Ash content is then expressed as the percentage of the weight of the original sample as stated in the equation below (AOAC, 2000).

$$Ac = \left(\frac{W_1 - W_2}{W_3}\right) \times 100 \quad (3)$$

Where,

Ac = Ash content (%),
W_1 = mass of crucible+ash (g)
W_2 = mass of empty crucible (g),
W_3 = mass of sample (g)

Crude fibre determination

Crude fibre was determined as described by AOAC (2000). 200 ml of 1.25 % (*v/v*) sulphuric acid was added and the flask was placed on a hot plate and boiled for 30 minutes. The content was filtered using filter paper (Whatman No.1) and the residue on the filter paper was washed with 50-70 ml of distilled water. The washed residue was transferred back into the flask and about 200 ml 1.25 % (w/v) NaOH was added and boiled for 30 minutes. The content was then filtered as described earlier and the residue obtained was washed with distilled water and filtered again using filter paper (Whatman No.1). The residue was later poured into an ashing dish and dried at 130 ºC for 2 hours, cooled in a desiccator and weighed. This was ashed at 550 ºC inside the muffle furnace chamber (Carbolite AAF1100, United Kingdom) for 30 minutes, cooled and reweighed. The ash obtained was subtracted from the residue and the difference was expressed as a percentage of the starting material as shown in Equation 4 (AOAC, 2000).

$$Cf = \left(\frac{W_1 - W_2}{W_3}\right) \times 100 \quad (4)$$

Cf = Crude fibre (%)
W_1=*mass of crucible with the dried residue* (*g*),
W_2=*mass of crucible with the ash* (*g*),
W_3=*mass of sample*(*g*)

Moisture content

The moisture content of the samples was determined by the standard AOAC (2000) official method by drying 1 g of the sample in a hot air oven at 105 ± 1 ºC until a constant weight was obtained, the samples were then removed from the oven, cooled in a desiccator and weighed. The results were expressed as a percentage of dry matter as shown in the equation below:

$$\text{Moisture content} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad (5)$$

w_1=*mass of snail meats before drying*(*g*),
w_2=*mass of snail meats after drying (g)*

Carbohydrate determination

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)

pH determination

A small sample of meat was cut off and minced to ensure total sampling of the internal and external parts of the meat and placed on the sensitive sensor of the pocket pH meter and measured. When sampling are repeated, the sensor was washed with diluted soap water and pat dry with a paper tissue while the new meat sample is placed on it to repeat the process (Horiba, 2002).

Design of Experiment (Taguchi method)

Design parameters and the chosen levels considered for the Taguchi experiment are shown in Table 1. Variables investigated were the processing times (30, 40 and 50 minutes), sterilization or heat treatment temperatures (115 ℃, 121 ℃ and 126 ℃) sodium nitrite concentrations (0, 50 and 100 ppm) and packing media concentrations (brine, vinegar, and brine-vinegar mixtures). Responses selected protein yield, crude fat, ash content, moisture content, carbohydrate content and pH. The canning of the snail meats was carried out according to the Design of Experiment based on the Taguchi model. Table 2 shows the Taguchi orthogonal array for experiments based on actual levels. A total of 9 experimental runs were conducted. The Taguchi orthogonal array based on the actual level is generated using the following equation 1.

No of Taguchi experiment= 1+ NV (L – 1)

Where; NV = Number of parameters; L = Number of levels. In this study, the NV = 4, L = 3, Hence:

No of Taguchi experiment= 1+ 4 (3 – 1) = 9

Table 1: Design Parameters and the Chosen Levels Considered for the Taguchi Experiment

Design parameter	Level 1	Level 2	Level 3
Temperature (°C)	115	121	126
Time (mins)	30	40	50
Nitrite concentration (ppm)	0	50	100
Packaging media	Brine	distilled white vinegar	brine-vinegar

Table 2: Taguchi orthogonal array for experiments based on Actual levels

Test Run	Time (min)	Temp. (°C)	Nitrite conc.(ppm)	Packing media
1	50	121	0	brine-vinegar
2	40	121	100	Brine
3	50	115	100	Vinegar
4	30	115	0	Brine
5	30	126	100	brine-vinegar
6	50	126	50	Vinegar
7	40	126	0	Vinegar
8	40	115	50	brine-vinegar
9	30	121	50	Vinegar

Statistical analysis and Optimization of process parameters

Experimental results were subjected to statistical analysis. Analysis of Variance (ANOVA) was conducted at 95% confidence intervals. Regression models were generated, and the effects of factors (time, temperature, nitrite concentration and packaging media) on the factors (protein yield, crude fat, ash content, carbohydrate content, moisture content and pH) were investigated. Optimum operating parameters for snail meat canning were determined and validated.

RESULTS AND DISCUSSION

Experimental results

Experimental results showing the protein content, moisture content, crude fat, carbohydrate and pH are shown in Table 3. Expectedly, moisture content is high in all the experimental runs, while the % carbohydrate content is less than 2% for all the experiments. Snail meat is rich in protein with values ranging from 18.76 – 19.66 %. However, a reduction in protein content was observed in all experimental runs with the highest reduction at experimental run 3 (40 mins, 126 ℃, 0 ppm nitrite, 100% vinegar). The fat content of 1.89% was the highest at experimental run 7 (process parameters of 126 ℃, 0 ppm nitrite concentration and vinegar as the packing media). The least fat content (1.13%) was however recorded at Run 1 (operating condition: brine-vinegar, 121 ℃ and 0 ppm nitrite concentration). Rashid and Khidhir, (2021) reported in a similar work on meat canning that as temperature increases it affects the physical properties of meat fat. The decrease in pH value was significant for snail meats in vinegar and brine-vinegar mixtures, ranging from 5.58 to 6.01. The decrease in pH may be due to the acid content of vinegar. Meat products are mostly low-acid. Increasing the temperature of the heat treatment of meat was found to increase the pH value of meat. The ash content for all experimental runs after canning (Table 3). Expectedly, carbohydrate content was low for both the fresh snail and canned snail meat, since snail meat is primarily animal protein. However, a slight increase in carbohydrate content was observed in all the canned snail meat. Ervika *et al.* (2016) reported that using brine solution as a packing medium can significantly increase the ash content of canned catfish. The results obtained in this study were also similar to the investigations of Okafor-Elenwo and Imade (2019). Moisture contents were high for fresh snail and canned snail meat contrary to the investigation of Engmann, *et al* (2013). The authors analyzed for moisture content after drying the snail meat for 6 hours at 80 ℃. However, Analysis of Variance (ANOVA) and Optimization of the Taguchi model is required to determine the interactive effect of process parameters on the properties of the canned snail meat and the optimum operating condition to achieve the best quality snail meat during and after canning.

Statistical Analysis

The interactive effect of the operating parameters (time, temperature, nitrite concentration and packing media) on the chemical composition of canned snail meat was investigated by subjecting experimental data to statistical analysis. The significance of the model terms is based on R^2, Fisher value and P-statistics. A p-value less than 0.05 indicates that the model term is significant with a confidence level of 95%. Statistical summary of the fitted using Taguchi experimental data for the dependent variables (protein, fat, ash, carbohydrate, moisture and pH) are shown in Tables 4 and 5. The Taguchi models generated from the ANOVA for the responses are shown in Equations 2-7.

The predominant factors controlling the protein yield are the temperature and nitrite concentrations with a percentage contribution of 81.1% and 14.9% respectively while the process time is the least significant factor with a percentage contribution of only 3.84%. Packaging media did not significantly influence the protein quality (Table 4). The model F-value of 47.17 implies the model is significant. Furthermore, it can be seen that there is only a 2.09% chance that an F-value this large could occur due to noise. The predicted R^2 of 0.8579 reasonably agrees with the Adjusted R^2 of 0.9719; i.e. the difference is less than 0.2. Adequate precision measures the signal-to-noise (S/N) ratio and when the ratio is greater than 4, the model is usually desirable. The S/N ratio of 19.286 indicates an adequate signal. The R^2 is close to unity signifies models sufficiently describe the experimental data. $A(1)$ and $A(2)$in Equation 2 are the times at 30 and 40 minutes, $B(1)$ and $B(2)$ are temperatures at 115 and 121 ℃ while $C(1)$ and $C(2)$ are 0 and 50 ppm nitrite concentrations respectively.

Table 3: Protein, moisture content, crude fat, pH of canned snail meat at various operating conditions

Run	Time (min)	Temp. (^{0}C)	Nitrite conc (ppm)	Packing media	Protein (%)	Moisture content (%)	Ash (%)	Fat (%)	Carbohydrate (%)	pH
1	30	115	0	Brine	19.66	76.49	1.81	1.13	1.61	6.00
2	40	115	50	brine-vinegar	19.50	76.11	1.58	1.52	1.89	5.96
3	40	126	0	Vinegar	18.84	75.70	1.32	1.19	1.81	5.87
4	50	121	0	brine-vinegar	19.30	76.15	1.48	1.17	1.2	6.15
5	30	126	100	brine-vinegar	18.76	76.02	1.86	1.47	1.89	5.90
6	50	126	50	Brine	18.96	76.00	1.43	1.75	1.86	6.04
7	40	126	0	Vinegar	19.06	76.13	1.45	1.89	1.90	5.90
8	40	121	100	Brine	19.55	76.17	1.46	1.19	1.74	6.07
9	30	121	50	Vinegar	19.04	76.19	1.44	1.47	1.86	5.89
FS	-	-	-	-	20.08	75.95	1.00	1.37	1.30	6.65

FS = fresh snail

Fats are a group of chemical compounds that contain fatty acids needed in daily diet for proper growth. Temperature is the most significant factor controlling the fat content with a p-value of 0.0264 while time and packaging media are the least significant (Table 4).

Percentage contributions of temperature, packaging media and process time are 70.89%, 19.20% and 9.9% respectively. Equation 3 is the Taguchi model generated for the % crude fat of the canned snail meat. A(1) and A(2) are 30 and 40 minutes process times, B(1) and B(2) are temperatures 115 °C and 121 °C while D(1) and D(2) are brine and vinegar respectively (Equation 3). The Model F-value of 17.34 implies there is a 5.55% chance that an F-value this large could occur due to noise which is the process temperature.

The Predicted R^2 of 0.6180 is not as close to the Adjusted R^2 of 0.9245 as expected. Adeq Precision measures the signal-to-noise ratio of 11.683 and it indicates a good signal.

The Model F-value of 2.10 as shown in Table 4 implies the model is not significant relative to the noise for carbohydrate content. Temperature, time and packaging media did not have a significant effect on the carbohydrate content of the canned snail meat.

This is evident in the p-values (0.2728, 0.2862 and 0.4679) which are far greater than the confidence interval of 0.05. Taguchi model generated for carbohydrates is shown in Equation 4. B (1) and B (2) are process temperatures of 115 ^{0}C and 121 ^{0}C, C (1) and C (2) are 0 ppm and 50 ppm nitrite concentrations and D (1) and D (2) are brine and vinegar packing media. The results are in agreement with the study of Iwanegbe et al. 2019, the authors reported that snail carbohydrate content is not significantly affected by the processing method and temperature. Jayantilal *et al.* (2017) reported a similar result for canned green peas.

Table 4: Statistical summary from Analysis of Variance of protein, crude fat and carbohydrate

	Protein		**Crude fat**		**Carbohydrate**	
Source	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Model	47.17	0.0209	17.34	0.0555	2.10	0.3574
A – Time	5.43	0.1556	5.15	0.1625	2.49	0.2862
B – Temp	114.87	0.0086	36.87	0.0264	2.67	0.2728
C – Nitrite conc	21.21	0.0450			1.14	0.4679
D – Packaging media			9.98	0.0911		
R^2	0.9930		0.9811		0.8629	0.8629
Adjusted R^2	0.9719		0.9245		0.4518	0.4518
Predicted R^2	0.8579		0.6180			

$$Protein\ yield = +19.19 - 0.0322A(1) - 0.0522A(2) + 0.3844B(1) - 0.1256B(2) + 0.1544C(1) - 0.0189C(2) \quad (2)$$

$$Fat = +1.42 - 0.0500A(1) + 0.1133A(2) - 0.2367B(1) - 0.0467B(2) + 0.0600D(1) + 0.0967D(2) \quad (3)$$

$$Carbohydrate = +1.75 - 0.1678B(1) + 0.00356B(2) - 0.1811C(1) + 0.0689C(2) - 0.1011D(1) + 0.1056D(2) \quad (4)$$

Process time, temperature and packing media are significant factors for variation in moisture content while nitrite concentration is the least significant for the process variation (Table 5). Model F-value of 430.42 implies the model is significant for moisture content. There is only a 0.23% chance that an F-value this large could occur due to noise (Table 5). The model obtained for the moisture content of the canned snail meat is given in Equation 5. $A(1)$ and $A(2)$ are process times at 30 and 40 minutes, $B(1)$ and $B(2)$ are temperatures at 115 ℃ and 121 ℃ while $D(1)$ and $D(2)$ are brine and vinegar packing media respectively. The predicted R^2 of 0.9843 reasonably agrees with the adjusted R^2 of 0.9969. An adequate precision ratio greater than 4 is desirable and as such the ratio of 77.576 proves a good model. Percentage contribution of temperature, process time and packing media in ascending order of significance to the moisture model is 54.63%, 25.34% and 20.07% respectively.

The percentage of ash in the snail meat sample indicates the number and quantities of elements present. Equation 6 is the Taguchi model generated for ash content from the ANOVA. $B(1)$ and $B(2)$are temperatures of 115 ℃ and 121 ℃, $C(1)$ and $C(2)$ are sodium nitrite concentrations of 0 ppm and 50 ppm while $D(1)$ and $D(2)$ are brine and vinegar packing media respectively. The Model F-value of 5.46 implies the model is not significant relative to the noise. There is only 16.28% chance that an F-value this large could occur due to noise. The ratio of 6.359 indicates an adequate signal and the packing media had a higher percentage of contribution to the process (59.29%). Maha *et al.* (2015) reported that there were no significant differences among treatments for ash response in the canning of beef sausage.

Nitrite concentrations and the packing media are the most significant factors for variation in pH value, while temperature and time were the least significant (Table 5). The percentage contributions are 55.53%, 26.39% and 18.08 % for packaging media, nitrite concentration and temperature respectively. Equation 7 shows the Taguchi model for pH. B, C, D are significant model terms, temperature, packing media and nitrite concentrations respectively with $p<0.05$. $B(1)$ and $B(2)$ are process temperatures of 115 ℃ and 121 ℃, $C(1)$ and $C(2)$ are Nitrite concentrations of 0 ppm and 50 ppm while $D(1)$ and $D(2)$ are brine and vinegar packing media. Model F-value of 158.71 implies the model is significant. There is only a 0.63% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. The predicted R^2 of 0.9576 is in reasonable agreement with the Adjusted R^2 of 0.9916. Adequate Precision measures the signal-to-noise ratio and a ratio greater than 4 (34.714) indicates an adequate signal.

Table 5: Statistical summary from Analysis of Variance of Moisture content, ash content and pH

	Moisture content		**Ash content**		**pH**	
Source	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Model	47.17	0.0209	5.46	0.1628	158.71	0.0063
A-Time	5.43	0.1556	4.10	0.1961	85.86	0.0115
B-Temp	114.87	0.0086	2.57	0.2800	127.00	0.0078
C-Nitrite conc	21.21	0.0450	9.71	0.0934	263.29	0.0038
R^2	0.9930		0.9425		0.9979	
Adjusted R^2	0.9719		0.7699		0.9916	
Predicted R^2	0.8579				0.9576	

$$MC=+76.11+0.1267A(1)-0.1133A(2)+0.1367B(1)+0.0633B(2)+0.1133D(1)-0.1D(2) \quad (5)$$

$$Ash= +1.54-0.1167B(1)+0.0733B(2)+0.0433C(1)-0.0933C(2)-0.0400D(1)-0.1333D(2) \quad (6)$$

$$pH=+5.98+0.0054B(1)-0.0256B(2)+0.0411C(1)+0.0244C(2)+0.0744D(1)-0.0889D(2) \quad (7)$$

Interactive effects of factors

The interactive effects of the operating conditions (time, temperature and nitrite concentrations) on the properties of canned snail meat were further studied using a 3-D bar plot as shown in Figures 1-6. The interactions were studied at various nitrite concentrations and packaging media.

Figure 1 shows 3-D interaction bar plots for protein at 0 % nitrite concentration and brine (Figure 1a), 50 % nitrite concentration and brine (Figure 1b) and 100 nitrite concentration and brine (Figure 1c). Protein yield was higher at lesser nitrite concentrations and lower time and temperature combinations (Figure 1a - 1c). Protein yield decreased with increasing nitrite concentration. Similar results of a nutritional loss of protein were reported by Belloso and Barriobero (2001) during the canning of grass-cutter meats. The 3-D plot in Figure 2a-2c indicated fat increased with increasing packaging media. However, the increasing rate was higher with vinegar compared to brine and brine-vinegar media. The results obtained are in agreement with the study of Maha *et al*. (2015) where fat content increased with increasing nitric and citric acid content for canned beef sausage.

Carbohydrate contents are higher in vinegar than in brine or brine-vinegar packing media (Figure 3a-c). Interaction between the variables was not significant and the results are in agreement with the results reported by Sejani *et al.* 2017 in the canning of bottle gourd cubes. It also agrees with the results (Jayantilal *et al.*, 2017) in the canning of green peas.

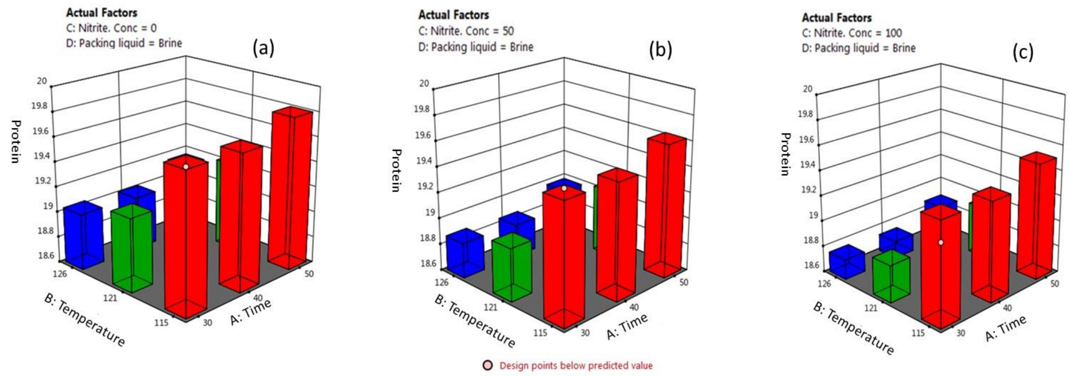

Figure 1: Interactive effect of parameters on protein yield (a) 0% nitrite and brine (b) 50% nitrite and brine (c) 100% nitrite and brine

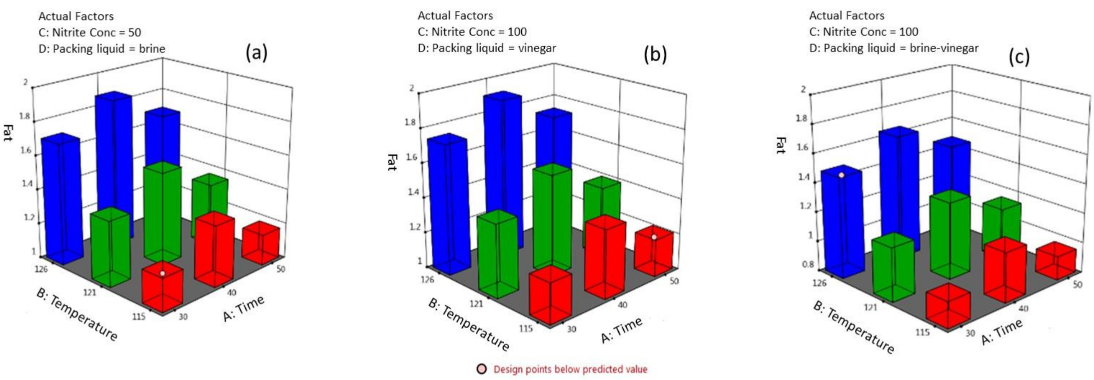

Figure 2: Interactive effect of parameters on fat (a) 50% nitrite and brine (b) 100% nitrite and vinegar (c) 100% nitrite and brine-vinegar

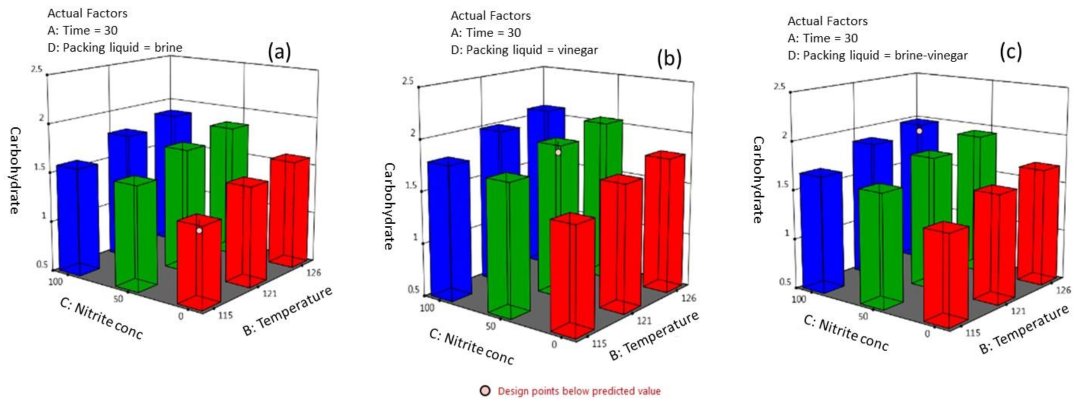

Figure 3: Interactive effect of parameters on carbohydrate (a) 30 mins and brine (b) 30 mins and vinegar (c) 30 mins and brine-vinegar

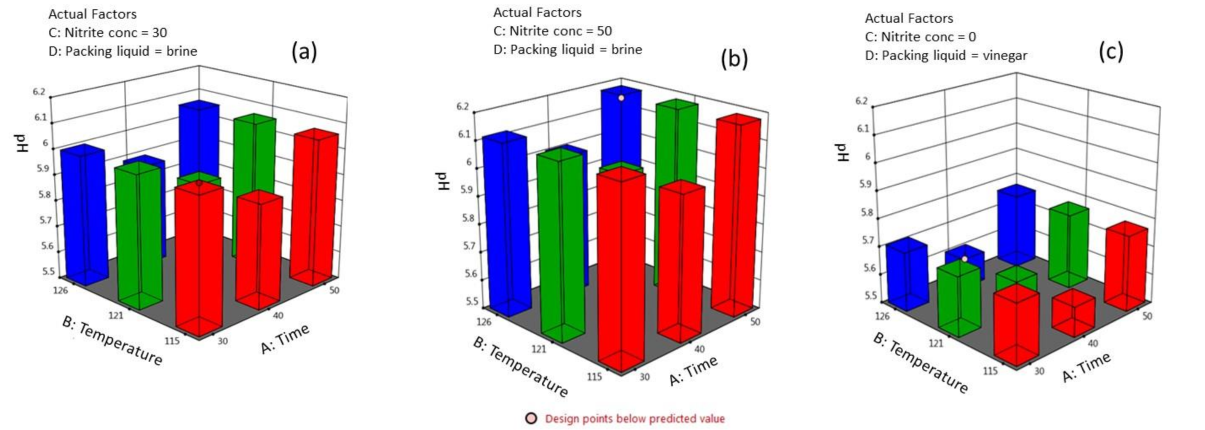

Figure 4: Interactive effect of parameters on pH (a) 30% Nitrite conc and brine (b) 50% Nitrite conc and brine (c) 0% Nitrite conc and vinegar

Figures 4 (a-c) show the interactive effect of parameters on pH at different packing media and nitrite concentrations. They show that the pH was mostly affected by the packing media with the highest nitrite concentration and nitrite concentration was the other significant factor too. The pH as shown on the graph in Figures 4.8 (a-c)- Figures 4.10 (a-c) indicates that it is lower when in vinegar than in brine or brine-vinegar packing media. They also show that it is higher in the presence of sodium nitrite. This could be a result of the mildly basic nature of the salt. Percentage ash content was highest with brine-vinegar or brine than vinegar irrespective of the nitrite concentrations at equal time-temperature combinations (Figures 5 a-c). Moisture content decreased during high thermal time and temperature treatments. It shows that at constant nitrite concentrations (0 ppm), there is an increase or retention of moisture with the usage of brine than brine-vinegar mixture and vinegar as packing media (Figure 6 a-c).

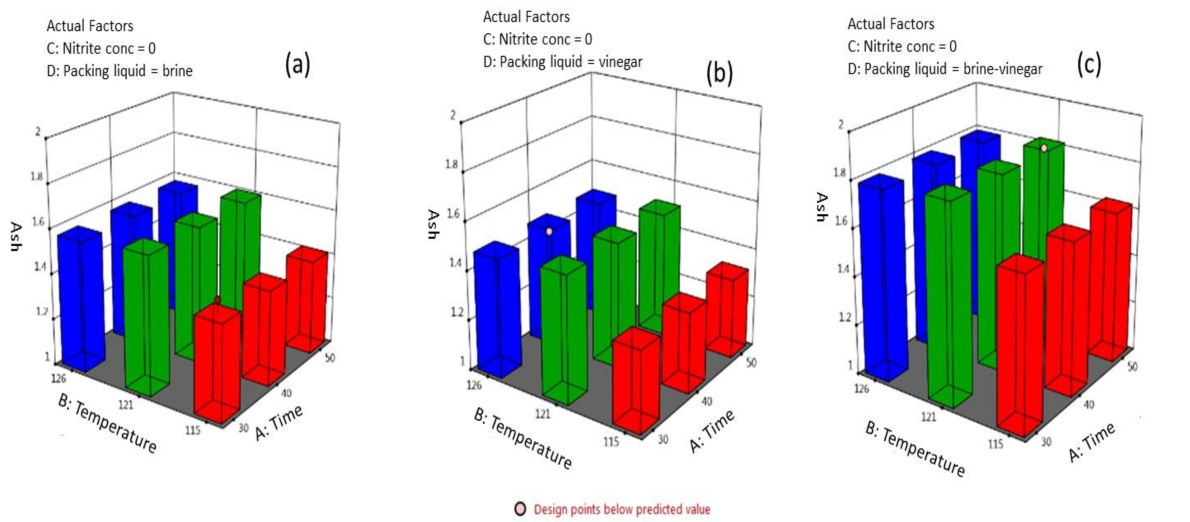

Figure 5: Interactive effect of parameters on Ash content (a) 0% Nitrite conc and brine (b) 0% Nitrite conc and vinegar (c) 0% Nitrite conc and brine-vinegar

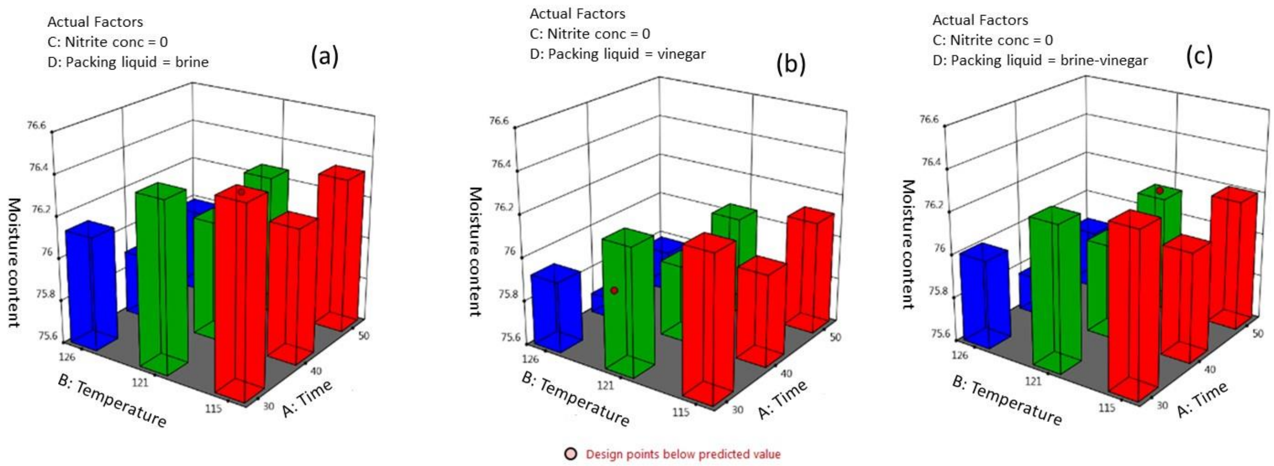

Figure 6: Interactive effect of parameters on moisture content (a) 0% Nitrite conc and brine (b) 0% Nitrite conc and vinegar (c) 0% Nitrite conc and brine-vinegar

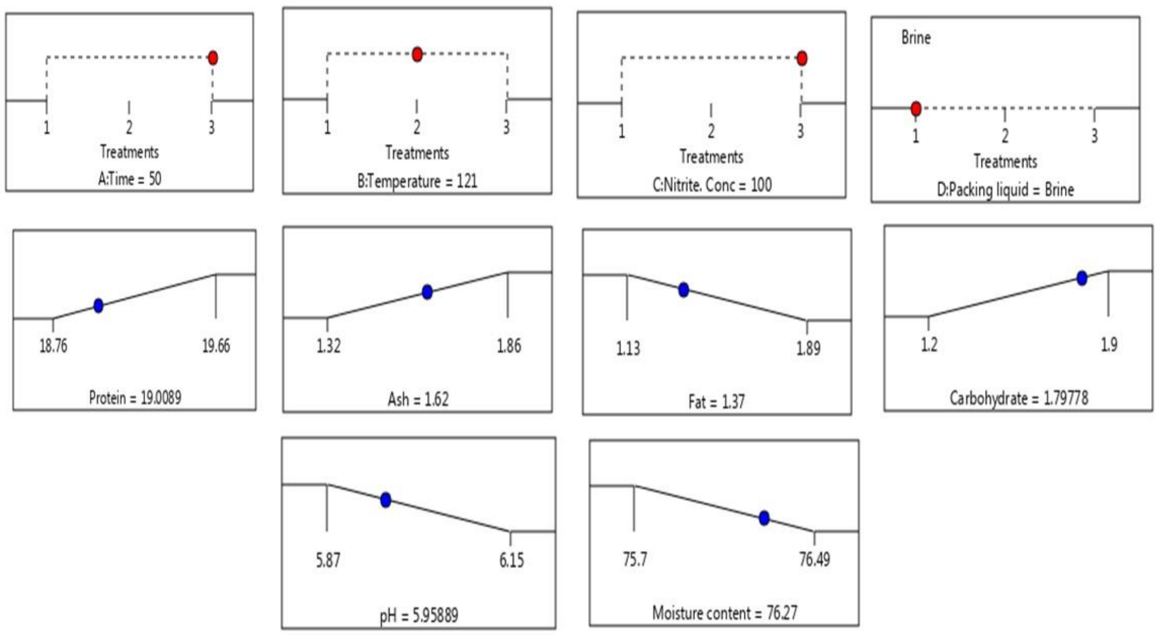

Figure 7: Optimum values and the predicted response by the Taguchi model

Optimization and model validation

Experimental data were subjected to optimization to achieve maximum protein yield and minimum pH. The red colour on the graphs shown in Figure 7 indicates the selected optimum levels while the predicted values for the response are indicated with the blue colour pointer. The optimized conditions are 50 minutes, 121 ℃ brine and 100 ppm nitrite for time, temperature, packing media and nitrite concentrations, respectively. The predicted values of biochemical parameters as shown on Figure 7 are 19.0089%, 76.27%, 1.62%, 1.37%, 1.79778% and 5.958 for protein, moisture content, ash content, fat, carbohydrate and pH respectively. In the commercial production of canned beef, the optimum processing conditions given in the technical specifications by Codex (2010) recommended 100 ppm $NaNO_2$ sodium nitrite as the level of additive to be used. The optimum factors generated here are similar to work carried out by Anna *et al*. (2013) on the commercial canning of Gudeg.

Validation experiments were carried out to confirm the reproducibility of the optimization predictions. Experiments were carried out in duplicates. The result of the confirmatory experiment on Table 6 revealed an average protein content of 18.98% ±0.11, ash 1.51% ±0.09, fat 1.40% ±0.04, carbohydrate 1.88% ±0.13, moisture content 76.23% ±0.08 and pH of 5.93 ±0.06. The mean values of protein, fat, ash, carbohydrate, moisture content and pH at the optimum conditions stated earlier were projected by Taguchi design, as 19.009%, 1.37%, 1.58%, 1.62%, 76.27% and 5.96. The quality response of the biochemical parameters was carried out in duplicates and the result was similar to the values predicted by the Taguchi model developed as shown in Figure 4.11. None of the microbial parameters investigated were detected in this confirmatory experiment and this further proves that in the development of microbial-free canned snail meat in a jar, the process conditions of 121 ℃, 50 minutes, brine as the packing media and 100 ppm nitrite concentration can be employed.

Table 6: Confirmatory Experiment for Taguchi Optimal Predictions

Run	Parameter	Mean	SD
1	Protein content (%)	18.98	±0.11
2	Moisture content (%)	76.23	±0.08
3	Fat content (%)	1.40	±0.04
4	Ash (%)	1.51	±0.09
5	Crude fiber (%)	-	_
6	Total carbohydrate content (%)	1.88	±0.13
7	pH	5.93	± 0.06
8	THB	-	
9	THF	-	
10	*Staphylococci*	-	

Analysis was carried out in duplicate, THB = Total heterotrophic bacteria; THF = Total heterotrophic fungi

CONCLUSIONS

In this study, the effect of process parameters (time, temperature, packaging media and nitric concentration) on the physicochemical properties (protein content, fat content, carbohydrate content, moisture content, ash content and pH) of canned fresh snail meat was investigated using Taguchi model. Experimental results indicated that temperature and nitrite concentration are the predominant factors controlling the protein content, while the fat content was significantly affected by temperature and packaging media. The percentage contributions of temperature on the protein, fat, and moisture content of the canned snail meat are 81.1%, 70.89%, 54.63% respectively. Carbohydrate and ash contents were not significantly affected by the process parameters. pH contents were mostly affected by nitrite concentration and packaging media. pH was affected most by the packaging media with the highest nitrite concentration (50 ppm). Taguchi models obtained from the statistical analyses were significant for protein, fat, moisture content and pH. The optimum canning process parameters, 50 mins, 121℃, brine and 100 ppm sodium nitrite concentration for process time, process temperature, packaging media and nitrite concentration respectively. Model predictions after optimization are 19.00% protein, 76.27% moisture content, 1.80% carbohydrate, 1.37% fat, 1.62% ash, and pH of 5.96. Model prediction validation experiments are 18.98% protein, 76.23% moisture content, 1.88% carbohydrate, 1.40% fat, 1.51% ash, and pH of 5.93. The microbial parameters such as heterotrophic bacteria, fungi, coliform and *Staphylococci* investigated were not detected in this validation experiment.

REFERENCES

Achaglinkame M.A, Owusu-Mensah E., Boakye AA, Oduro I. Effect of size and drying time on the rehydration and sensory properties of freeze-dried snails (Achatina achatina). *International Journal of Food Science*, 2020:1-5.

Adeyeye S, Bolaji O, Abegunde T, Adesina T.O. (2020). Processing and utilization of snail meat in alleviating protein malnutrition in Africa: a review. *Nutr. Food Sci.* 50: 1085-97.

Munonye, J.O. and Moses, P. (2019), "Profitability analysis of snail farming in Owerri agricultural zone Imo state", Contributed Paper presentation at the 93rd Annual Conference of the Agricultural Economics Society, University of Warwick, England, pp. 15-17.

Tanyitiku M.N., Nicholas, G., Sullivan, J.J., Njombissie Petcheu, I.C.N. and Stephen L.W. (2022). Snail Meat Consumption in Buea, Cameroon: The Methodological Challenges in Exploring Its Public Health Risks. *International Journal of Qualitative Methods,* 21: 1–12

Adeyeye S.A.O, Bolaji O.T, Abegunde T.A. Microbial safety of intermediate moisture smoked African giant snail (Achatina achatina) meat. 44th annual conference and annual general meeting of Nigerian Institute of Food Science and Technology 14th-15th October, 2020.

Okafor-Elenwo, E. J., & Imade, O. S. (2019). Estimation of pathogenic exposure levels and associated baseline bio-risks in edible terrestrial snails (Achachatina marginata) sold in Nigerian markets. Journal of *Applied Science & Environmental Management,* 23(4), 673.

Simpson R.J., Almonacid, S.F., Teixeira, A.A. (2013). 18 - Automatic control of batch thermal processing of canned foods In Woodhead Publishing Series in Food Science, Technology and Nutrition, Robotics and Automation in the Food Industry, Woodhead Publishing, Pages 420-440,
https://doi.org/10.1533/9780857095763.2.420 .

Ugbede-Ohuoba E., Okonkwo, T.M., Nkama, I., Eze, J. I. and James S. (2021). Shelf-life Prediction Modeling and Physicochemical Changes ofCanned African Giant Snail (*Achachatina achatina*) Based Products during Storage Using Sensory and Kinetic Data. *Journal of Food Research,* 10(12): 11-20

.Association of Official Analytical Chemists (2023). Official methods of analysis of AOAC, 22nd Edition, Washington DC.

Okafor-Elenwo E.J. and Imade OS. (2019). Estimation of pathogenic exposure levels and associated baseline biorisks in edible terrestrial snails (Achachatina marginata) sold in Nigerian markets. *Journal of Applied Science and Environmental Management,* 23: 673-80.

Maha, M. M. A., Abogroun, H. A., & Ibraheem, M. T. (2015). Effect of nitrite and citric acid on the chemical composition and ph of the canned beef sausages. *Online Journal of Animal and Feed Design*, *5*(1), 1–8.

Rashid N.J and Khidhir Z.K. (2021). Impact of different storage temperatures on canned meat Characteristics. *Euphrates Journal of Agriculture Science*, 13 (3): 28-37.

Ervika R.N.H., Angwar, AgusSusanto, Kurniadi. (2016) .Effect of Brine Concentration on the Nutrient Content and Fatty Acid Profile of Canned Catfish (*Pangasiussutchi.*). 2nd International Symposium on Aquatic Products Processing and Health ISAPPROSH 2015. *Aquatic Procedia,* 95-91

Engmann F.N., Afoakwah, N.A. Darko, P.O. and Sefah W. (2013). Proximate and Mineral Composition of Snail (Achatina achatina) Meat; Any Nutritional Justification for Acclaimed Health Benefits? *J. Basic. Appl. Sci. Res.,* 3(4)8-15.

Sejani V.M., Cholera S.P and V. A. Naliapara (201.). Studies on canning of bottle gourd pulp and cubes. *International Journal of Pure and Applied Bioscience,* 5(4): 867-876

Jayantilaljay, H. N., Cholera, S. P., Vaishali, C. C., & J, H. N. (2017). Effect of Canning Variables on Physical Biochemical and Microbial Parameters of Peas. *International Journal of Pure and Applied Bioscience, 5*(5), 1470–1477.

Anna A.K., Purwiyatno H., Eko H. (2013). Effect of sterility value at different canning temperatures on the physical properties of canned gudeg. *Journal of Food Science,* 34: 20-50.

Codex (2010). Technical specifications for the manufacture of canned beef. 30th March, 2010. Food and Agriculture Organization of the United Nations.