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Shelf-life Prediction Modeling and Physicochemical Changes of Canned African Giant Snail (*Achachatina achatina*) Based Products during Storage Using Sensory and Kinetic Data

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Abstract

Canned African giant snails (brine, egusi and sauce) of low acidity (pH 4.5) were produced using Thermal Death Time (TDT) techniques. Sixty cans of each product were stored for 6 months at normal temperature (30^{o}C). The physicochemical and sensory properties of these products were evaluated monthly. And also, 28 cans of each product were equally evaluated at accelerated storage temperatures stress (50, 60, 70 & 80^{o}C) for 6 weeks. Changes in pH and overall acceptability were described by first and second order kinetics respectively. The extent of proteolysis, peroxidase activities, and extent of fat hydrolysis i.e peroxide and thiobabituric acid values did not change significantly ($P > 0.05$) during storage. Regression analysis was used to fit models for Gibbs free energy of activation for physicochemical changes as a function of temperature and to predict models for overall acceptability as a function of pH. Shelf-life prediction models were fitted based on sensory and pH kinetic data. Shelf-lives of 27.6 months, 27-25 months and 18-16.8 months were estimated for snail in brine (SIB) snail in egusi (SES) and snail in sauce (SIS) respectively during storage at 30^{O}C.

Keywords: snails, unconventional, cholesterol, canning, *Clostridium botulinum*, physicochemical changes

1. Introduction

African giant Snail (*Achachatina achatina*) is a terrestrial shell bearing animal of approximately 100,000 species of molluscs grown in the forest region of Nigeria and other countries in the humid /Semi Arid region of Africa. Snail is an unconventional source of cheap and affordable animal protein that is required for growth and repairs of body tissues .Inadequate supplies and high shortages of food in the world, particularly in developing countries necessitate the search for new sources of food (Mahala & Mohhammed, 2010). According to Ariahu (2012), the conventional meat sources such as beef, broiler meat, goat meat, mutton, pork and horse meat, have high amount of cholesterol and fatty acid contents. Nutritionally, the African giant snail has high content of iron, phosphorus and calcium; hence, it is useful in treatment of anemia, hypertension and other fat related diseases.

Previous studies on African giant snail in Nigeria have reported that, snail rearing operations can be approached with much convenience, since the business could be accommodated within a family backyard and are often eaten cooked in stew or soups or fried as snacks once they are harvested. The implications of this, is that, the African giant snail lacks long term preservation techniques such as canning, irradiation, dehydration, or even use of low temperatures (e.g. freezing and refrigeration). Canning offers convenience for retail and consumer handling without dependence on low/ erratic power supply as witnessed in developing countries. Snails require adequate processing in order to ensure its preservation and keeping qualities.

The production of canned snails needs the commercial sterilization of the meat in metal or glass containers by the application of the *"Botulinum* cook". This is the minimum heat treatment that all low acid foods (pH>5) must receive in order to assure safety from *Clostridium botulinum.* Commercial sterility is normally verified by inoculated pack studies (Ariahu *et al*., 2004).

During storage of commercially sterile low acid foods, physicochemical reactions may occur. These reactions may lead to changes in sensory and nutritional qualities such as taste, aroma, colour, proteolysis, loss of vitamins

and break down of carbohydrates. Changes in physicochemical properties of commercially sterile products during storages may be a treat to its shelf-life, more than microbiological activities. Shelf-fife of a food product is the period before microbiological, physical, chemical or sensory qualities deteriorate beyond a set of limit (Ismail *et al.*, 1996). Reaction kinetics may be applied to quantify and predict extent of physicochemical changes during processing and storage of foods (Rustom *et al.*, 1996).

Information on changes in the physicochemical parameters of the African giant snail in various media during canning and sterilization and the shelf-life prediction models are lacking. The general objective of this study was to make the African giant snail available in ready-to-eat forms that are safe from the public point of view.

The specific objectives of this study were to produce canned snail-in-brine, snail-in-sauce and snail-in-egusi soup products, develop shelf-life prediction models for the products and also to analyse its physicochemical changes during storage based on sensory and kinetic data.

2. Materials and Methods

2.1 Sample Collection

Locally grown African giant snails *(Achachatina achatina)* of 300g average weight were purchased from a local market (Ogige market) in Nsukka Nigeria. About 18kg of the mollusc were transported to the laboratory in moistened jute bags. After cracking the shells, the meats were removed, washed with 5% alum solution to remove slime, packaged in polythene bags and kept under frozen storage (-18 ℃) until utilized.

Lacquered 208 x 300 round sanitary cans and lids were donated by Metal Box Nigeria Limited, Oshodi, Lagos.

2.2 Media Formulation and Selection

Egusi soup and a sauce were prepared as described by Vincent (1980). The *egusi* soup was prepared from fresh pumpkin leaves, ground melon seeds, pepper, salt, fermented locust bean, onion and palm vegetable oil. The sauce consisted of tomatoes, pepper, salt, fresh onions, vegetable oil, neutralized hydrolyzed vegetable protein (Maggi cubes, Nestle Foods Plc., Lagos Nigeria) and pure water. The brine consisted of 0.5% sodium tripolyphosphate and 4% table salt and potable water.

The pH of each formulation was varied from 5.7 to 7.9 with citric acid. The sauce, *egusi* soup or brine selection was done by a preliminary sensory evaluation which suggested that products with an equilibrium pH of 6.9 had optimum quality based on flavour and appearance. The flow charts for the production of the various canned snail products are shown in Figures 1, 2 and 3 below.

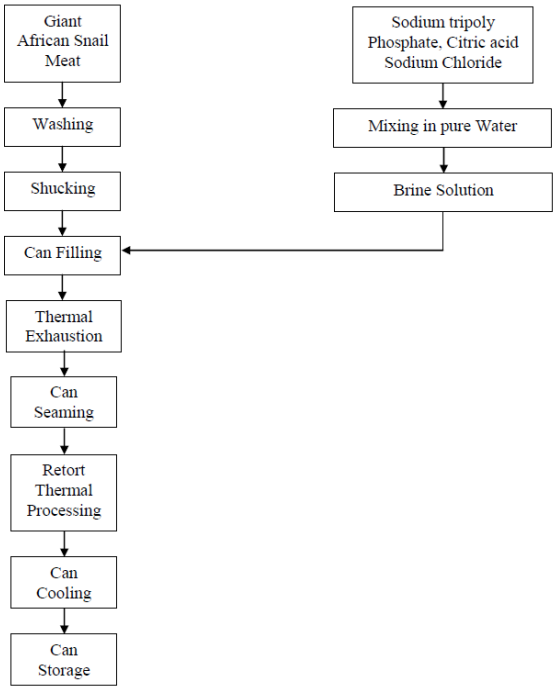

Figure 1. Flow chart for the production of canned African giant snail in brine (Ariahu *et al.*, 2012)

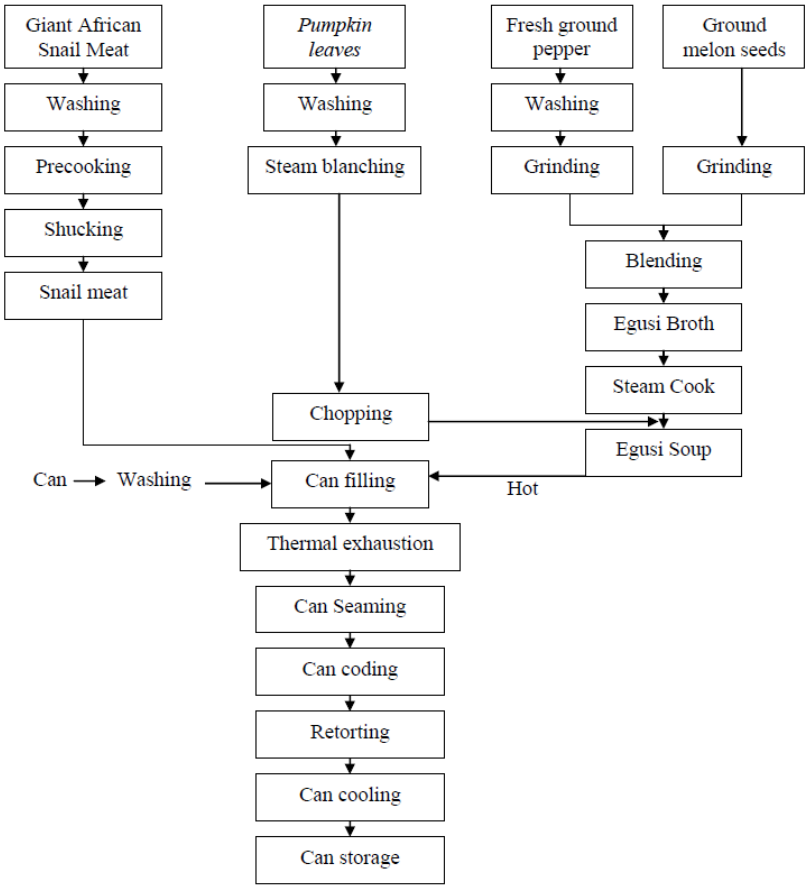

Figure 2. Flow chart for the production of canned snail – in – *egusi* soup

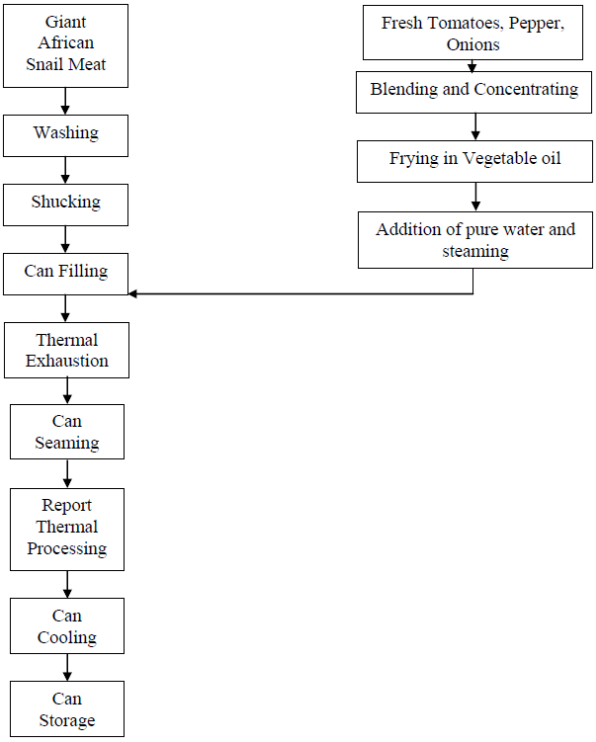

Figure 3. Flow chart for the production of canned snail – in – sauce

2.3 Heat Resistance of Microorganisms

The 208x300L/L round sanitary cans were aseptically filled with lobes of snail meat (160g/can). The abductor muscles in each can were infected with a 1ml spore suspension by multiple injection using a disposable hypodermic syringe and needle. Enough cooking brine or sauce or *egusi* soup was then added to a fill-in weight of 250g which yielded about 10^6 spores/can of each product. After gentle agitation to eliminate air bubbles, the cans head spaces were thermally exhausted (10 min) using saturated steam followed by steaming with a semi-automatic can seaming machine.

Thermal resistance was determined at four times intervals (5, 10, 15, 20 minutes) at temperatures of 104.4, 110, 115.6 and 121.1 ℃ (220, 230, 240 and 350 ℉) for each product. For each time interval, fourteen cans were prepared, ten heated and four used as control. The cans were heated in a laboratory scale batch retort (Mill walls, J.S. Fraser and Sons, London) equipped with an automatic temperature control. Before each run, the retort was vented for l0 mins. Come-Up-Times (CUT) were determined using plug-in copper-constantan thermocouples (0.1mm diameter needles) with the probes carefully placed inside the muscles at central axis for at least three cans for each product.

2.4 Microbiology

The cans were opened aseptically after flooding the lids with a l00 ppm chlorine solution as recommended by Deny (1972). The contents of each sample was transferred into a sterile mixer and diluted ten-fold by weight with sterile water. The resulting mixture was macerated (4 min) and neutralized with 4M NaOH solution. Serial ten-fold dilutions were made in liver broth and the surviving spores counted with the aid of the 5-tube Most Probable Number (MPN) technique (Mayou, 1976). The tubes were incubated at 30 ℃ for 15 days together with their controls. Positive growth was identified by turbidity and gas and was confirmed to be due to *C. sporogenes* using series of cultural and biochemical tests described by Nigerian Canners Association.

2.5 Thermal Data Analysis

The decimal reduction times (D_T) were calculated by the partial sterilization technique (Stumbo, 1973) from:

$$D_T = t\ (\text{Log}\ N_0 – \text{Log}\ N) \quad (1)$$

where t is the heating time at temperature, T (℃); N_0 is initial number of microorganism spores/sample multiplied by the number of replicate samples and N is MPN of microorganism spores which survived. The D_T values (i.e., the times required to reduce the *C. sporogenes* spores population in each product by 90% at the various temperatures) were described as direct exponential function of temperatures:

$$\text{Log}\ D_1/D_2 = (T_2 – T_1)/z \quad (2)$$

where D_1 and D_2 are decimal reduction times at temperature T_1 and T_2 respectively with z (negative reciprocal slope of the TDT curve) representing slope index of the line generated via least square linear regression.

2.6 Physicochemical Analysis

2.6.1 pH

The pH was measured at 30 ±1 ℃ with an electronic pH meter (M62, Radiometer, Copenhagen, Demark). Duplicate samples (l0g each) of product were macerated with 40mL chilled phosphate buffer (pH = 6.88) for 5 minutes in a household electric blender. The pH of the homogenate was measured using a previously referenced electrode of the pH meter.

2.6.2 Proximate Analysis

The proximate compositions of the canned products were determined by AO AC (2010) standard procedure 14.064 for moisture content, 14.067 for protein. Carbohydrate was calculated by difference. Energy values were estimated by the Atwater factor method (4xg protein:, 9xg fat and 4xg carbohydrate).

2.6.3 Extent of Proteolysis

The total nitrogen (TN) in each product was fractionated into non-protein nitrogen (NPN) and protein nitrogen (isoelectric precipitate). Extent of proteolysis was calculated from the difference between relative amounts of NPN to TN as percentage at 0 and 24 weeks Storage. NPN was determined according to a modification of the procedure described by Ismail *et al.* (1996). About l0g of each product was diluted (1:10, w/w) with distilled water and blended using a household electric blender. The pH was adjusted to 4.6 (with 1 M HCl) to precipitate protein. The nitrogen in the original sample (TN) and in the supernatant (NPN) was determined with a kjeltic Auto 1030 Analyzer (Tecator AB, Hoganas, Sweden).

2.6.4 Peroxidase Activity

Peroxidase activity was measured by the method described by Adams (1978).Assays were performed on samples (10-100mg) of the homogenate weighed directly into a spectrophotometer (Spectro-20) sample cuvette without prior extraction of the peroxidase.Two ml of acetate buffer (pH 5.6) was then pipette into the cuvette which was shaken and held for 5 mins at 30 ^{O}C in a constant temperature bath. 0.7 mL of substrate (0.5% guaiacol +0.1 % hydrogen peroxide in acetate buffer, mixed 1:1) was added and the assay carried out with the reference cuvette containing an equal weight of homogenate shaken in 2.7 mL of acetate buffer. The absorbance of brown colour formed was measured at 420 nm against the blank. Peroxidase activity was expressed in terms of maximum rate of increase in absorbance (Usually 20 minutes).

2.6.5 Extent of Fat Hydrolysis

Peroxide and thiobarbituric acid (TBA) values were determined as described by Pearson (1976). For peroxide value, ten grams of each prepared sample were weighed into a Kenwood blender followed by 8mL of distilled water, 20 mL of methanol, 10 mL of chloroform and 0.1mL of butylated hydroxyl toluene (BHT). The mixture was blended for 2 mins. 10mL of chloroform was then added followed by blending for 30 seconds. Additional 10mL of distilled water was added followed by homogenization for 30 seconds. The resultant mixture was then filtered with Buchner-filter under wet vacuum and the filtrate transferred to a separator funnel. After allowing the filtrates to settle for about 10 mins, the lower fracture was transferred to a weighed flask. 5ml of the organic solvent ‘extract were evaporated on a steam bath and the amount of fat recorded

Peroxide values were estimated on 0.5g extracted fat as follows: to 0.5g of extracted fat in 250 mL Erlenmeyer flasks were added 30mL acetic chloroform solution(1:1 v/v) and the flasks swirled until samples dissolved, then 0.5mL of saturated potassium iodide(KI) was added to each flask and the solutions allowed to stand for exactly 1 minute. 30mL of distilled water were then added and the solutions titrated gently with 0,1M with constant shaking to a very faint yellow colour.0.5mL of starch indicator was then added and the titrations continued with vigorous shaking near the end point to liberate all the iodide from the chloroform layer. Several drops of the thiosulphate solution were added until the blue colour vanished.

$$\text{Calculations: } \frac{\text{S X M X 100}}{\text{Weight of sample}}$$

Where: S = mL of thiosulphate; M= molarity of $Na_2S_2O_3$ solution.

For thiobarbituric acid, 10g of each prepared food sample were macerated with 50mL of distilled water for 2 mins. The macerate was then washed into a distillation flask with 47.5 mL distilled water and 2.5mL of 4M HCl added .A few beads of anti bumps of glass were added and the flask heated in an electric mantle (1000 volts).50 mL of the distillate was collected after boiling began within 10 mins. Then 5mL of each distillate was pipette into a glass-stoppered tube followed by 5mL thiobarbituric acid reagent (0.2883/100mL of 90% glacial acetic acid). The tubes were tightened, shaken and heated in boiling water for 35 mins. A blank was prepared similarly using 5mL distilled water and 5mL of the reagent. The tubes were cooled in water for 10min and the optical densities measured against the blank at53nm using 1cm cells in a spectroplus spectrophotometer.

Thiobarbituric acid No. (mg malonaldehyde/1000g sample): = 7.8D

Where: D = optical densities.

2.6.6 Drained Weights

The drained weights of each product was determined according to the United States Department of Agriculture (USDA) standard method as reported by Lopez (1988).Essentially it was performed by emptying the contents of product can evenly upon an 8-mesh screen (2.5mm opening) and inclining of the sieve at an angle of about 30.Exactly 2 mins after placing on the screen, the drained solids were weighed by placing the screen containing the drained solids directly on a balance and weighing. The weight of the draining screen was then subtracted to obtain drained weight of the product.

2.7 Sensory Evaluation

Sensory evaluation was performed using descriptive analysis and affective testing (Stone and Sidel, 1985). Forty panelists participated. The panelists consisted of staff members and graduate students from the Department of Food Science and Technology, University of Nigeria, Nsukka. A 5-point unstructured descriptive scale was used to rate attribute of sensory appearance (1= bright, 5 = dark), taste (1 = bad, 5 = good), bitterness (1 = no bitter after-taste, 5 = strong bitter after-taste). Perception of off-flavors was judged quantitatively as "present" or

"absent". The panelists asked to identity off-flavors as rancid, putrid, or others. A 7-point structured hedonic scale (1= dislike extremely, 4 = neither like nor dislike, 7 = like extremely) was used to score overall acceptability of the canned products. The panels were conducted under fluorescence light in a special room. The three products were judged on monthly basis by each of the panelists. Slices of the snail meat with the brine, sauce or egusi soup were served in 100ml colorless transparent plastic cups coded with 4-digit random numbers. Colorless plastic spoons were also provided. About 25g sample was served warm (40-50 ℃). Fresh water was provided to rinse between evaluations.

2.8 Statistical Analysis

STATGRAPHICS software (STATGRAPHICS, 1991) was used (multivariate methods module) to calculate Pearson product-moment correlation coefficients between sensory attributes and physicochemical properties. Regression analysis (Kramer and Twigg, 1970) was used to fit models for Gibb's free energy of activation for physicochemical changes as a function of temperature and to predict models for overall acceptability as a function of pH. Significant ($p<0.05$) differences in chemical composition and kinetic data were determined by analysis of variance (Kramer and Twigg, 1970). Tukey's tests as described by Ihekoronye and Ngoddy (1985) were used for separating the means.

2.9 Kinetics and Thermodynamic Calculations

Simplifying assumptions were made for calculation of kinetic parameters of physicochemical changes in the canned products during storage. Irreversible, non cyclic, unimolecular reaction mechanism (A—f B) was assumed for all physicochemical changes.

Reaction order for change in physicochemical parameters at constant temperatures was calculated (Ariahu and Ogunsua, 2000) with the following equation (4):

$$dC/dt = \pm kC^n \quad (4)$$

where C = value of physicochemical parameters at time, t (weeks), k = reaction rate constant (wk^{-1}), n = reaction order.

Reaction rate constant was calculated by linear regression using equation (5)

$$C_0 - C = kt \quad (5)$$

for zero order reactions and, equation (6)

$$\ln (C_0/C) = kt \quad (6)$$

for first order reactions with C_0 - initial value of physicochemical parameter.

Temperature dependence of the rate constant was expressed by Arrhenius equation:

$$k = k_o \exp. (Ea/RT) \quad (7)$$

where k_0 = frequency factor (independent of temperature), E_a = activation energy (kJ/mol), R = universal gas constant (0.008314 kJ/mol ℃K), T = absolute temperature (℃K).

3. Results and Discussion

3.1 Proximate Composition of the African Giant Snail Meat

The proximate composition of the canned snail in brine (SIB), snail in sauce **(**SIS**)** and snail in *egusi* soup **(**SES**)** products are shown in Table l. The crude protein ranged from 89.3 g/l00g solids, solids for snail in brine to 90.1 g/l00g solids for snail in sauce product. The fat content varied from 2.0 g/l00g solids in snail in brine to 5.0 g/l00g solids for snail in egusi soup. The ash contents were 1.6, 1.6 and 1.5 g/l00g solids for SIB, SIS and SES respectively.

Table 1. Proximate composition of canned African giant snail meat in brine, *egusi* and sauce

Nutrient	**Products**		
(g/100g solids)%	**SIB**	**SES**	**SIS**
Crude Protein	84.3^a	90.1^a	89.3^a
Crude Fat	2.0^b	5.0^c	3.3^d
Ash (%)	4.8^n	1.6^f	1.5^f
Carbohydrate*	8.9^g	3.3^h	5.9^k

*By difference.

Values with common superscript letters are not significantly ($p>0.5$) different within each row. Data are mean of

triplicate determinations. SIB = snail in brine, SES = snail in *egusi* soup, SIS = snail in sauce.

3.2 Inoculated Pack Studies for the Canned Snail Products

Tests for commercial sterility were carried out by inoculated pack studies. The results are presented in Table 2. For the canned products, it can be observed that the test cans did not show any swelling unlike the control (non-heat processed) cans that were all swollen within first week of storage at 37℃. Microbiological tests indicated that spoilage was due to *Clostridium sporogenes.*

Table 2. Inoculated pack tests for canned snail products at 37^0C

Incubation	**Number of cans swollen**		
Time (wks)	**SIB**	**SIS**	**SES**
0. Control	0/10	0/10	0/10
Test cans	0/10	0/10	0/10
1. Control	10/10	10/10	10/10
Test cans	0/10	0/10	0/10
3. Test cans	0/10	0/10	0/10
5. Test cans	0/10	0/10	0/10
8. Test cans	0/10	0/10	0/10

Control = non heat processed cans, test cans = heat processed cans

SIS = snail in brine, SIB = snail in brine, SES = snail in *egusi* soup.

3.3 Sensory Properties

Typical variance data for taste, appearance and bitterness of the canned products are shown in Tables 5, 6 and 7 respectively. Since the variance ratio for sensory score for taste of the samples is smaller than tabulated (2.85) for judges, likewise for the appearance (1.27) and bitterness (1.09). There were no significant ($p>0.05$) changes in sensory properties during the six months of storage.

Table 5. Analysis of variance chart for sensory score for taste of canned snail-in-brine

Sources of variation	Df	Ss	ms	F
Samples	6	4.94	0.82	0.38
Judges	14	4.71	0.33	2.85
Error	84	179.49	2.13	
Total	104	189.14		

df = degree of freedom, ss = sum of square, ms = mean squares, F = variance ratio.

Table 6. Analysis of variance chart for sensory score of appearance of canned snail in brine

Sources of variation	df	Ss	ms	F
Samples	6	1.84	0.30	1.66
Judges	14	3.24	0.23	1.27
Error	84	15.63	0.18	
Total	104	20.10		

df = degree of freedom, ss = sum of square, ms = mean squares, F = variance ratio.

Table 7. Analysis of variance chart for sensory score for bitterness of canned snail-in-brine

Sources of variation	df	Ss	ms	F
Samples	6	1.66	0.28	1.27
Judges	14	3.35	0.24	1.09
Error	84	18.35	0.22	
Total	104	23.56		

df = degree of freedom, ss = sum of square, ms = mean squares, F = variance ratio.

3.4 Accelerated Storage Tests

Within a period of 6 weeks, the canned snail products were subjected to temperature stress by storage at 50, 60, 70 and 80 ℃ respectively. During this period, the pH and overall acceptability of the products were determined. See tables 8 and 9 below.

Table 8. Changes in pH with storage time for canned snail products at elevated temperatures

		Storage temperature(^{o}C)			
Storage Time (weeks)	**Products**				
		50	60	70	80
0	**SIB**	6.85	6.80	6.85	6.86
1		6.74	6.74	6.74	6.22
2		6.66	6.62	6.66	6.07
3		6.58	6.55	6.58	5.82
4		6.54	6.51	6.54	5.64
5		6.48	6.58	6.48	5.45
6		6.42	6.42	6.92	5.53
0	**SES**	6.85	6.89	6.48	6.66
1		6.70	6.47	6.77	6.54
2		6.62	6.39	6.96	6.79
3		6.49	5.98	6.59	6.53
4		6.51	6.59	6.97	6.21
5		6.70	5.99	6.38	6.79
6		6.78	6.98	6.39	6.58
0	**SIS**	6.34	6.45	6.76	6.65
1		6.32	6.43	6.79	6.65
2		6.59	6.23	6.75	6.63
3		6.48	6.12	6.74	6.62
4		6.76	6.32	6.73	6.43
5		6.89	6.12	6.65	6.12
6		6.88	6.11	6.54	6.32

N =4 number of points, S.E standard error of estimate, r^2 = coefficient of regression, E_a =Activation energy

Table 9. Mean overall acceptability scores for canned snail products

		Storage temperature(^{o}C)			
Storage Time (weeks)	**Products**				
		50	60	70	80
0	**SIB**	6.8	6.8	6.8	6.8
1		6.7	6.7	634	6.2
2		6.6	6.6	6.6	6.7
3		6.5	6.5	6.5	5.2
4		6.5	6.5	6.4	5.4
5		6.4	6.5	6.8	5.5
6		6.4	6.4	6.2	6.2
0	**SES**	6.8	6.0	6.48	6.6
1		6.7	6.4	6.77	6.4
2		6.6	6.3	6.96	6.9
3		6.4	5.9	6.59	6.3
4		6.5	6.9	6.97	6.1
5		6.7	5.9	6.38	6.7
6		6.7	6.8	6.39	6.8
0	**SIS**	6.9	6.8	6.8	6.85
1		6.65	6.45	6.54	6.89
2		6.76	6.11	6.12	6.70
3		6.99	6.90	6.09	6.44
4		6.51	6.40	6.39	6.09
5		6.43	6.47	6.45	6.49
6		6.89	6.79	6.75	6.44

N =4 number of points, S.E standard error of estimate, r^2 = coefficient of regression, E_a =Activation energy

3.5 Shelf-life Prediction

A relationship between pH and overall acceptability of each product was established. The pH of each product was predetermined by varying the values from 6.8 to 4.5 (within low acidity range) using citric acid. The samples were then subjected to sensory evaluation on a scale with 7 as like extremely and 1 as dislike extremely. The average overall acceptability scores from a panel of 15 judges for the products are presented. These data were subjected to least square linear regression analysis to obtain relationships between pH and overall acceptability as provided in Table 10. The predicted shelf-lives using pH (via first order reaction kinetics) or overall acceptability (via zero order kinetic model) are presented in Table 10.

Table 10. Arrhenius regression derivatives for changes in pH of canned snail products

	Products		
Arrhenius			
Parameter	**SIB**	**SES**	**SIS**
N	4	4	4
r^2	0.940	0.868	0.977
SE	0.08	0.07	0.05
Intercept	10.2317	7.3250	7.4885
K_0	$27.77x10^3$	$1.52x10^3$	$1.787x10^3$
Slope	-93.7997	-71.4970	-73.4131
$\therefore E_a$ (kJ/mol)	0.78	0.59	0.61

n = number of points, S.E standard error of estimate, r^2 = coefficient of regression, E_a = activation energy, k_0 = frequency factor, SIB = snail-in-brine, SIS = snail-in-egusi soup, SES = snail-in-sauce.

4. Conclusion

Changes in pH were best described by first order kinetics ($r^2 \geq 0.988$) while overall acceptability was best described by zero order kinetics ($r^2 \geq 0.997$). The reaction rate constants were then fitted with the Arrhenius kinetic model to verify temperature dependence and prediction of reaction rate constants at selected ambient

temperatures.

Models were fitted to depict the relationship between sensory attribute of acceptability scores and changes in pH of the canned products.

On the assumption of a minimum sensory score of (5-like much) on a scale with 6 (like very much) and 7 (like extremely), the shelf-life of the canned products were evaluated at various ambient storage temperature. These models predicted shelf-lives of 27.6 months for Snail in brine, 27 to 25 months for Snail in egusi soup and 18 to 16 months for snail in sauce of the canned products.

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