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Process Time Evaluation of African Giant Snail (*Achachatina achatina*) Based Products

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Abstract: The thermal resistances of *Clostridium sporogenes* (ATCC 19404) in African giant snail based formulated low acidity (pH 6.8) products comprising Snail in Brine (SIB), Snail in Sauce (SIS) and Snail in Egusi Soup (SES) were investigated using Thermal Death Time (TDT) technique within lethal temperature range 104.4-121.1°C. Established decimal reduction times (D-values) were used for canning the products in 300/208L/L round sanitary cans based on 5D-concept with respect to the test microbe. The D-values (min) of canned African giant snail ranged from 5.4-0.88 min for snail in sauce, 6.8-1.2 min for snail in egusi and 5.6-0.84 min for snail in brine within 104.4-121.1°C, the z-values ranged from 8.8°C for SIB to 9.6°C for SES product. The established thermal process schedules of temperatures 110, 115, 120 and 121.1°C and at times of 4.2, 5.6, 20.7, 76.7 min are recommended for commercial production of canned snails in brine, sauce and egusi soup, respectively.

Key words: African giant snail, thermal resistance, commercial sterilization schedule, canned snail, brine

INTRODUCTION

The African giant snail (*Achachatina achatina*), the vineyard snails (*Helix pomatia*) and *Helix lucorum* are unconventional sources of meat (Sotelo *et al*., 1993). The snail meat is a major source of protein in the diet of some people living in the forest region of Nigeria. Snails are also consumed as a rare delicacy by many Nigerians living outside the forest region.

Unlike the conventional meat sources such as beef, mutton and poultry, the snails have very low cholesterol and saturated fatty acids contents (Ikeme, 1985). The consumption of snails therefore offers nutritional benefits since cholesterol and saturated fatty acids have been implicated in coronary heart diseases and arteriosclerosis (Leisner and Gram, 2000). Because of their feeding habits, snails may act as vectors of some parasitic diseases (Ariahu and Ilori, 1993). Therefore, snails require adequate processing in order to assure safety of the meat from public health point of view. The processing options include freezing, irradiation, dehydration and canning. Canning of the snails holds the advantage of commercial sterility while offering the products in ready to eat forms. Such products would enjoy wider geographical distribution and long term preservation without dependence on power supply.

The production of canned snails requires commercial sterilization of the meat in metal containers by the application of the botulinum cook. This is the minimum heat treatment that all low acid foods (pH>4.5) must receive in order to assure safety from *Clostridium botulinum*. This microbe is spore forming, putrefactive, anaerobic and capable of growing in low acid foods. *C. botulinum* and its spores are highly heat resistant. The microbe is capable of producing the deadly neurotoxin that is responsible for botulism. Commercial sterility is normally verified by inoculated pack studies. These studies involve the use of substantial quantities of equally highly heat resistant and spore forming but non-pathogenic putrefactive anaerobes for process times determination. Such bacteria include *Clostridium sporogenes* and *Bacillus stearothermophilus*.

Information on commercial sterilization of the African giant snail in various media is lacking. The objective of the study was to evaluate the process time of African giant canned snail in brine, snail in sauce and snail in egusi soup products.

MATERIALS AND METHODS

African giant snails (*Achachatina achatina*) of 200 g average weight were purchased from a local market (Modern market) in Makurdi, Nigeria. About 15 kg of the

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mollusc were transported to the laboratory in moistened jute bags. After cracking the shells, the meats were shucked, washed with 5% alum solution to remove slime, packaged in polythene bags and kept under frozen storage (-18°C) until utilized.

Lacquered 208×300 round sanitary cans and lids were donated by Metal Box Nigeria Limited, Ogba, Ikeja, Lagos.

Media formulation and selection: Egusi soup and a sauce were prepared as described by Vincent. The egusi soup was prepared from fresh water leaves (*Talinum triangulare*), ground melon seeds, pepper, salt, fermented locust bean, onion and vegetable oil. The sauce consisted of tomatoes, pepper, salt, fresh onions and vegetable oil, neutralized hydrolyzed vegetable protein (Maggi cubes, Nestle Foods Plc., Lagos, Nigeria) and potable water. The brine consisted of 0.4% sodium tripolyphosphate and 4% table salt and potable water. The pH of each formulation was varied from 5.2-6.9 with citric acid. The sauce, egusi soup or brine selection was by a preliminary sensory evaluation which suggested that products with an equilibrium pH of 6.8 had optimum quality based on flavour and appearance. The flow charts for the production of the various canned snail products are shown in Fig. 1-3.

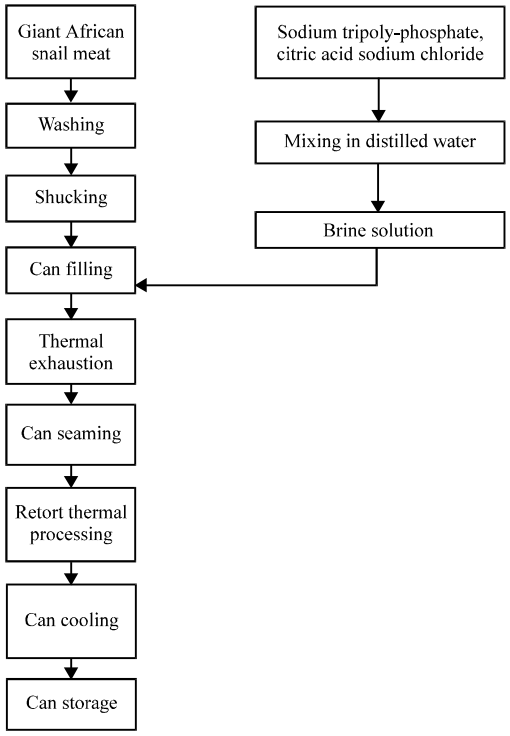

Fig. 1: Flow chart for the production of canned African giant snail in brine

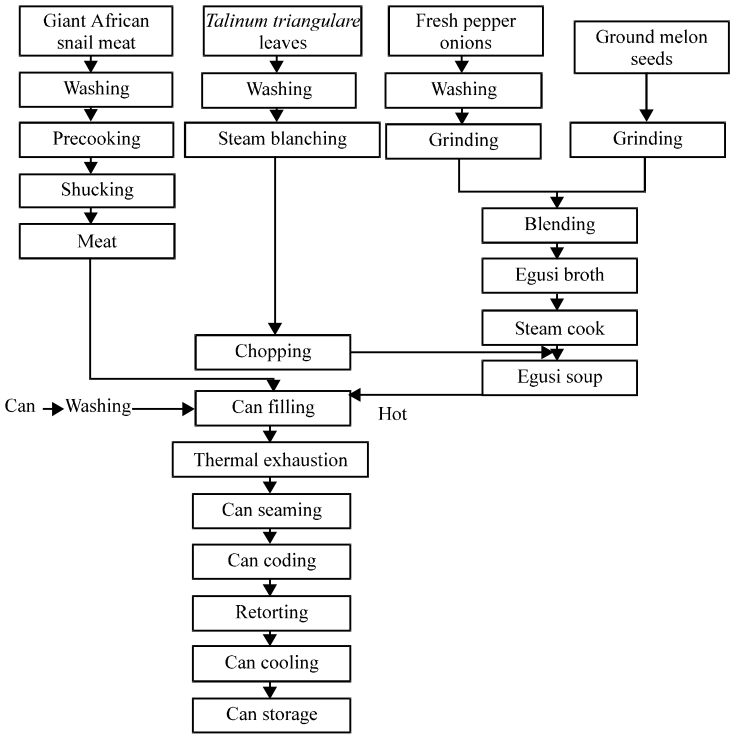

Fig. 2: Flow chart for the production of canned snail in egusi soup

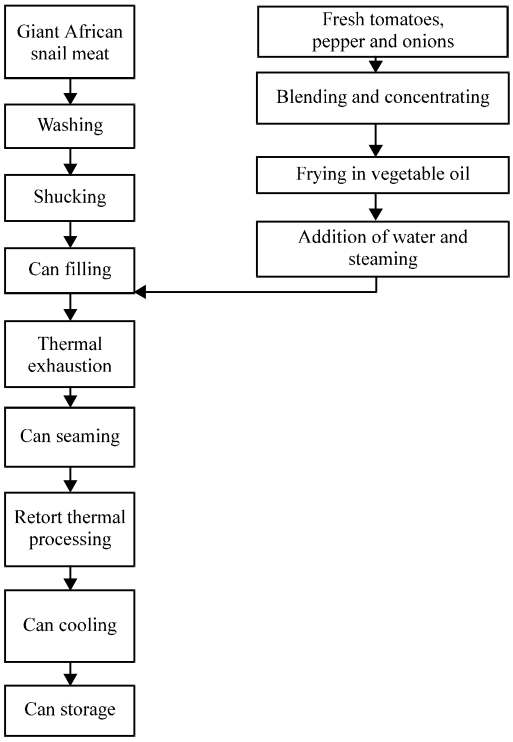

Fig. 3: Flow chart for the production of canned snail in sauce

Preparation of spores: Spores of *Clostridium sporogenes* (ATCC 19404) were prepared in a beef infusion broth using a multiple stage inoculation procedure of Goldoni. Colony counts were made in fluid thioglycolate medium (oxoid) solidified with 1.4% agar, stratified with vaspar in Pricket tubes using 1.0, 0.1, 0.01 and 0.001 mL portions of heat activated (80°C, 30 min harvested spores stock as described by Cameron *et al*. (1980). The harvested spores were suspended in M/15 phosphate buffer (pH = 7.0) and adjusted to a final concentration of 10^6 CFU mL^{-1} and stored refrigerated at 6±2°C.

Thermal resistance studies

Heating cycles: The National Canners Association (NCA) Thermal Death Time (TDT) Can technique (NCA, 1968) was used for investigating heat resistance of *Clostridium sporogenes* spores in the products. The 208×300 L/L round sanitary cans were aseptically filled with lobes of snail meat (170 g can^{-1}). The aductor muscles in each can were infected with a 1 mL 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 250 g which yielded about 10^6 spores/can of each product.

After gentle agitation to expel entrapped air, 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 and 20 min) for temperatures 104.4, 110, 115.6 and 121.1°C (220, 230, 240 and 350°F) for each products. 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 (Millwalls, J.S. Fraser and Sons, London) equipped with an automatic temperature control. Before each run, the retort was vented for 10 min. Come Up Times (CUT) were determined using plug-in copper-constantan thermocouples (0.1 mm diameter needles) with the probes carefully placed inside the muscles at central axis of at least three cans for each product.

The signals from the thermocouple junction were recorded using an Ellab type Z9CTF recorder (Electrolaboratoriat, Copenhagen). At the end of each heating cycle, cold water was fed into the retort to cool it as quickly as possible. Heating and cooling times were measured by stopwatch and corrected for CUT as described by NCA (1968). Cans removed at CUT were considered to contain initial *C. sporogenes* concentration (N_o). The other cans were held for desired times at each of the temperatures. The control cans were heated for 13 min at 80°C to activate the spores and were incubated for 6 weeks at 30°C.

Microbiology: The cans were opened aseptically after flooding the lids with a 100 ppm chlorine solution as recommended by Deny (1972). The contents of each run were 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. The tubes were incubated at 30°C 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 NCA (1968).

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

$$D_T = t\ (\text{Log}\ N_o - \text{Log}\ N) \quad (1)$$

Where:

t = The heating time at temperature T (°C)

N_o = Initial number of microorganism spores/sample multiplied by the number of replicate samples

N = 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 (Ariahu *et al*., 2004).

Thermal process schedule: The NCA (1968) suggested a 5D reduction as the minimum process for commercial sterilization of low acid foods with respect to *C. sporogenes*. Using 121.1°C as reference temperature, the equivalent heating times to achieve commercial sterilization effects at selected temperatures (110, 115 and 121°C) were evaluated using the following relationship:

$$U_T = 5D_{121.1}F_T \quad (3)$$

Where:

F_T = Log^{-1} (121.1-T)/Z

U_T = Equivalent heating time to achieve commercial sterility at temperature T (°C) assuming instantaneous heating and cooling

The established thermal process schedules (Temperature/Time combination) were validated by inoculated pack studies as described by Ogunsua *et al*. (1991). Ten cans of each product were processed at each of the temperature/time combination followed by incubation for 5 weeks at 37°C together with unprocessed canned controls. The cans were observed for swells and positive cans subjected to microbiological, cultural and biochemical tests as described by Harrigan and McCance (1976).

RESULTS AND DISCUSSION

Proximate composition: 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 1. The crude protein ranged from 89.3 g/100 g solids, solids for snail in brine to 90.1 g/100 g solids for snail in sauce product. The fat content varied from 2.0 g/100 g solids in snail in brine to 5.0 g/100 g solids for snail in egusi soup. The ash contents were 1.6, 1.6 and 1.5 g/100 g solids for SIB, SIS and SES, respectively.

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

	Products		
Nutrient (g/100 g 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[e]	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

Table 2: Regression parameters and derivatives from phantom thermal death curves for *Clostridium sporogenes* in canned snail products

	Products		
Parameter/Derivative	SIB	SES	SIS
n	4	4	4
r^2	0.999	0.999	0.998
SE	0.008	0.065	0.078
Intercept (Loge 0°C)	13.5783	12.7895	13.0283
gradient	-0.1136	-0.1041	-0.1086
z-value (°C)	8.8	9.6	9.2

n = Number of points, r^2 = Coefficient of regression, SE = Standard Error of estimate; SIB = Snail in Brine, SES = Snail in Egusi Soup, SIS = Snail in Sauce

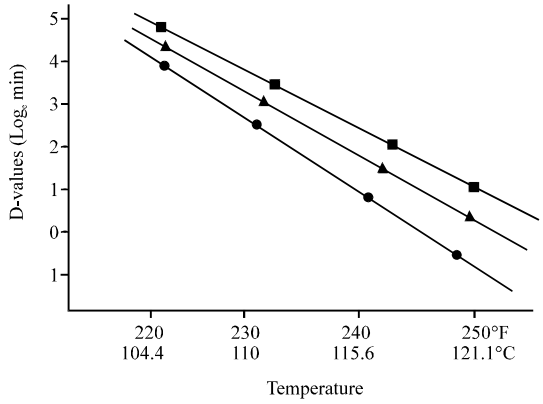

Fig. 4: Phantom thermal death time curves for *Clostridium aporogens* ATCC 19404 in (●) African giant snail in brine; (▲) African giant snail in sauce and (■) African giant snail in egusi soup by most probable number method

Influence of media on the heat resistance of the microorganism: The decimal reduction curves for *Clostridium sporogenes* ATCC 19404 spores in the canned snail products are shown in Fig. 4. The D-values (min) ranged from 5.4-0.88 min for snail in sauce, 6.8-1.2 min for snail in egusi and 5.6-0.84 min for snail in brine within 104.4-121.1°C, the z-values ranged from 8.8°C for SIB to 9.6°C for SES product (Table 2). The

Table 3: Equivalent heating times (ut) for commercial Sterilization ($5D_o$) of canned snail products with respect to *C. sporogenes*

Products	Temperature (°C)	U_T (min)	Equation
SIB	121.1	4.2	$LogU_T = 14.3856-0.1136T$
	120.0	5.6	($r^2 = 0.998$)
	115.0	20.7	
	110.0	76.7	
SIS	121.1	4.4	$Log\ U_T = 13.8076-0.1087T$
	120.0	5.8	($r^2 = 0.999$)
	115.0	20.3	
	110.0	77.8	
SESs	121.1	6.0	$Log\ U_T = 13.3962-0.1042T$
	120.0	7.8	($r^2 = 0.998$)
	115.0	25.9	
	110.0	86.0	

$U_T = 5D_oF_T$; where $F_T = Log^{-1}(121.1-T)/Z$, $D_o = D_{121.1}$

Table 4: Regression parameters for semi-log relationship between equivalent heating times and temperature for commercial sterilization of African giant snails based low acid (pH = 6.9) products

	Products		
Regression parameters	SIB	SIS	SES
n	4	4	4
Coefficient of regression (r^2)	0.998	0.999	0.998
Standard error of estimate	0.034	0.056	0.064
Gradient ($°C^{-1}$)	-0.1136	-0.1087	-0.1042
Intercept	14.3856	13.8076	13.3962
Log U_T	14.3856-0.1136T	13.8076-0.1087T	13.3962-0.104T

SIB = Snail meat in Brine, SIS = Snail meat in Sauce, SES = Snail meat in Egusi Soup, n = Number of points

equivalent heating times for commercial sterilization of the snail products are shown in Table 3. The regression parameters for semi-log relationship between equivalent heating times and temperature are shown in Table 4. It can be observed from the curves that the D-values were lowest in SIS and highest in SES. Again, the curves were closer at lower than at higher temperatures implying that the influence of substrate was greatest at higher than at lower lethal temperatures. For example, the heat resistance of the spores in SIS at 121.1°C was 1.6 and 1.3 times lower than in SES and SIS, respectively. At 104.4°C, the heat resistance of the spores was about 1.1 times higher in SES and SIS than in SIB. As expected, heat resistances were higher at lower than at higher lethal temperatures. Apparently, certain characteristics of the egusi soup and sauce attributable to the ingredients such as oil, proteins and starch gave more protection to the microbe than in the brine medium. This is consistent with the observations of Ogunsua *et al.* (1991) who reported higher heat destruction rates for *C. sporogenes* in Periwinkles canned in brine than in egusi soup or sauce. However, the D-values and hence thermal processing times for the snails were higher than those reported by the earlier workers for periwinkles (Ogunsua *et al.*, 1991) and mussels (Ariahu *et al.*, 2004) in the same media. These could be due to differences in the size, shape and chemical composition of the molluscs. This implies that the use of an established thermal schedule of a mollusc for others may be risky from commercial sterilization point of view. The z-values (°C) of 9.5 (SIB), 10.13 (SIS) and 10.6 (SES) were close to the values of 9.8-10.3°C reported earlier by Ariahu *et al.* (2004) for *C. sporogenes* ATCC 19404 in freshwater mussels based low acidity products. These values are comparable with the range of 8-10°C reported for *C. botulinum* in low acid foods (Myrseth, 1985) thereby justifying the use of *C. sporogenes* ATCC 19404 for thermal process times evaluation in African giant snail meat based low acid food products. The relatively lower z-value in SIB indicates that High Temperature Short Time (HTST) processes would favour greater destruction of the spores in SIB than in the other products.

Commercial sterilization schedules: The NCA suggested a 5D reduction as the minimum process for commercial sterilization of low acid foods with respect to *C. sporogenes*. Using 121.1°C as the reference temperature, the equivalent heating times to achieve commercial sterilization effects at other temperatures were evaluated using the following relationship:

$$U_T = 5D_{121.1}\,F_T \quad (7)$$

Where:

F_T = $Log^{-1}(121.1-T)/z$

U_T = Equivalent heating time to achieve commercial sterilization at temperature T (°C) assuming instantaneous heating and cooling

The log of U_T values as a function of temperatures gave linear curves. The equations relating U_T to temperatures can easily be used by processors for commercial sterilization of the snail meat in the various media at specified conventional heating temperatures. The processing times to deliver these specified sterilization values would depend on the size, initial product temperature, retort temperature, fill-in weight and mode of heating among other factors. These processing times can easily be computed using either the Improved General Method (Myrseth, 1985), the Trapezoidal Integration Method (Patashnik, 1953) or the formula method (Lund, 1977).

In order to validate the thermal process schedules, inoculated pack studies of the products were carried out using the determined times at 121.1°C. The processing times were challenged by inoculation of the products with viable (10^2 cfu g^{-1}) *Clostridium sporogenes* spores. The processed and non-processed control cans were stored at 37°C and monitored at weekly intervals for >4 weeks (Table 5). It was observed that the test cans did not show

Table 5: Inoculated pack tests for canned snail products at 37°C

	No. of cans swollen		
Incubation time (weeks)	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
2			
Test cans	0/10	0/10	0/10
4			
Test cans	0/10	0/10	0/10
5			
Test cans	0/10	0/10	0/10

Control = Non heat processed cans; Text cans = Heat processed cans; SIS = Snail in Brine; SIB = Snail in Brine; SES = Snail in Egusi Soup

any swelling unlike the control (non-heat processed) cans that were all swollen within 1st week of storage at 37°C. Positive bacteria growth was evident from the swelling due to gas production and distinctive putrid odour produced by the microbe. Cultural and biochemical tests confirmed that spoilage of the control cans was due to *Clostridium sporogenes*. The cultural tests included shape (rods with rounded ends), arrangement of cells (mainly single, occasional short chains), oxygen requirement (anaerobic), endospores (present), gram stain (positive). The biochemical tests included Starch and maltose utilization (fermentative with acid production). Gelatin liquidfaction (positive), indole production (negative), meat utilization (positive) (reddened then darkened and digested with foul odour) and catalase (positive). The non-swelling of the heated cans indicated that the processing times were adequate for commercial sterilization of the snail products.

CONCLUSION

C. sporogenes (ATCC 19404) spores are suitable for evaluation of thermal processes for African giant snail meat based low acid foods. The thermal resistance of the strain depends on the media used. It is lower in brine than in sauce or egusi soup. The established thermal process schedules of temperatures 110, 115, 120 and 121.1°C and at times of 4.2, 5.6, 20.7 and 76.7 min are recommended for commercial production of canned snails in brine, sauce and egusi soup, respectively. The thermal process schedules developed in this study should be used by regulatory bodies as standard operating procedures for commercial sterilization of canned snail products.

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