Influence of brine treatment,drying methods and storage conditions on the microbial quality of freshwater snail (*Lanistes libycus)* meat.

Ezeama, C. F.*[1], Keke, E.[3] and Nwachukwu, E.[2]
[1]Department of Food Science and Technology, [2]Department of Microbiology,
Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria.
[3] Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi state, Nigeria.
*Address for correspondence, E-mail: *cfezeama@yahoo.com*

ABSTRACT

Freshwater snail (*Lanistes libycus*) meat samples were subjected to four different preservation treatments (oven-dried, sun-dried, brined oven-dried, brined sun-dried) and assessed for microbiological quality. The microbial quality of fresh or brine (5 % w/v NaCl) treated samples stored at different temperatures (35, 28, 4, -7 ^{o}C) were also investigated. Combining brine treatment with either oven-drying or sun-drying resulted in significant ($p = 0.05$) decrease in total viable counts (TVCs) of the meat with that of brined oven-dried samples being more pronounced ($\log_{10}$ 3.92 cfu g^{-1}) by day 4 of ambient storage. Sun-drying alone could not effectively preserve the meat beyond 2 days. No significant difference ($p = 0.05$) existed in the microbial loads of oven-dried and brined sun-dried samples stored at ambient temperature. The TVCs of all traditionally dried samples were microbiologically unacceptable (>($\log_{10}$ 5.0 cfu g^{-1}) after 4 days of ambient storage with *Bacillus, Clostridium, Staphylococcus sp* and *Aspergillus flavus* being dominant microorganisms isolated. Whereas the fresh meat of *L. libycus* treated with 5 % w/v NaCl and stored at low temperatures (4, -7 ^{o}C) exhibited drastic reduction in microbial load with shelf-life extension to 6 days, that stored at 28 ^{o}C preserved the meat for 4 days.

Keywords: Freshwater snail, brine, microbial quality, drying, shelf life, storage temperature.

INTRODUCTION

The freshwater snail (*Lanistes libycus*) is a popular, seasonal freshwater food widely distributed in Nigeria especially in the Niger Delta and Upper Cross River basins (Arene *et al.*, 1999; Obureke *etal.*, 1987). It serves as a major source of protein as well as generating income to the people (Ezeama, 2000). The spoilage of (*L. libycus*) sets in after about 48h of harvest while shucking accelerates the spoilage rate as the meat cannot be kept in acceptable condition after approximately 12h. Hence, the preservation of the meat has become major concern to both the processors and consumers.

Traditionally, combination of smoke-drying with sun-drying has become a major traditional method of preserving seafoods. But this method had not been assessed if it can achieve much in reducing the microbial quality and increasing the shelf life of *L. libycus* meat. Some workers had combined smoking and potassium sorbate treatment, and dry-curing in reducing the microbial population of freshwater snail and clam meat thereby extending their shelf-life

(Ezeama, 2004; Ekanem *et.al* 1994). However, the effects of the use of brine treatment, sun-drying, oven-drying or combination of these under different storage conditions have not been documented.

The objective of the work therefore, was to assess the effect of using brine treatment in combination with sun-drying, or oven-drying on the microbial quality of *L. libycus* meat stored at ambient temperatures. Microbial profile of traditionally dried samples of the meat as well as the effect of different storage temperatures on the microbial load of fresh or brined treated samples of the meat were also investigated.

MATERIALS AND METHODS:

Collection and processing of samples

Fresh freshwater snails (*Lanistes libycus)* were collected from harvesters in Akaeze, Ebonyi State. The shells were surface sterilized (70 % ethanol), shucked and eviscerated aseptically using hand-glove (Troge Medical GMBH-D Hamburg, Germany). The meats were aseptically removed and pooled into samples of 500g each into sterile beakers. Several sub-samples (25g each) were kept in four sets. One set was dipped into brine solution (5 % w/v NaCl) for 5min and drip-dried before either sun-drying or oven-drying. One sub-set of the treated sample was exposed under the sun intensity for 3 consecutive days while the other was placed in a single layer on a tray in a Gallenkamp oven 300 at 80 ^{o}C for 6 h. One of the subsets was oven-dried while another one was sun-dried. After oven-drying, the meats were cooled to ambient temperature (28 ± 2 ^{o}C) and packaged in polyethylene bags. The untreated fresh samples served as control. The packaged samples (25g each) were stored at ambient temperature for analyses at 2-day intervals.

Similarly, traditionally smoked, sun-dried *L. libycus* meat samples (Fig. 1) used in the study were obtained on prior arrangement from randomly selected local processors at Akaeze, Unwana and Okposi which are important *L. libycus* producing areas of Upper Cross River basin of Nigeria. The samples were similarly packaged and stored at ambient temperature with microbiological analyses carried out initially (day 0) and at 2-day intervals for 6 days.

Storage of sample at different temperatures

One set of brine treated (25g each) and another set of freshly (untreated) prepared samples (25g each) were packaged in sterile polyethylene bags (1.5µm) and stored at different temperature (-7, 4, 28 and 35 ^{o}C) using deep freezer, refrigerator and incubator respectively. The samples were initially analyzed for microbiological quality and after 24h of storage and subsequently at 2 days interval for 6 day.

Microbiological analyses of samples

Twenty-five grams (25g) of either the treated or untreated meat sample were blended (Moulinex, Paris, France) in 225ml of sterile 0.1% (w/v) peptone water (pH 7.2 ± 0.2) to obtain 1:10 dilution. Subsequent dilutions were prepared and total viable counts (TVCs) and coliform counts determined in duplicate using pour plate method on Tryptone Soy Agar (TSA) and MacConkey agar (MA) respectively and incubated at 35 – 37 ^{o}C for 24-48h or on malt extract agar (MEA) at 28 ± 2 ^{o}C for 3-5 days for fungal counts. The colonies that developed on the plates and which were within their suitable ranges of 25-250 (Speck, 1984) were counted and recorded as cfu g^{-1}.

Identification of Isolates

Representative discrete colonies were purified by streaking on TSA or MEA plates. Other media such as MacConkey agar, Triple Sugar Iron agar, Simmon Citrate agar, Litmus milk, Mannitol salt agar, Bacillus cereus selective agar (Biotec, Laboratory Ltd. Suffolks, UK) were used for the identification of the isolates. Following various biochemical tests (oxidase, catalase, coagulase, indole, H_2S production, urease, MRVP and oxidative/ fermentative utilization of glucose, lactose, mannitol, arabinose, sucrose and maltose) and description

(Sneath *et.al*, 1986; Krieg and Holt, 1984) the isolates were identified. Similarly, the fungal isolates were identified based on their cultural and morphological characteristics (Samson and van Reenen – Hoesktra, 1988).

Statistical analysis

The data obtained were statistically analysed using analysis of variance (ANOVA) to determine the mean difference based on least significant (LSD) at $p = 0.005$ (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The microbial counts of brine treated and untreated dried meat of *L. libycus* stored at ambient temperature are presented in Table 1. There was a significant ($p = 0.05$) decrease in total viable counts (TVC) when brine was combined with either oven-drying or sun-drying. Maximum positive impacts on the microbial quality of seafoods seem exerted with combined preservative treatments (Ezeama, 2004; Efiuvwevwere and Isaiah 1998). The significant ($p = 0.05$) decrease in (TVcs) when brine was combined with either oven-drying or sun-drying (Table I) clearly shows the synergistic effect of such treatment. The combined treatment was able to reduce the TVCs to acceptable level (ICMSF, 1986) with that of brined oven-dried sample being more pronounced ($\log_{10}$ 3.92 cfu g^{-1}) by day 4. Sun-drying alone cannot effectively preserve the meat beyond 2 days. Furthermore, the low coliform counts observed ($< \log_{10}$ 2.0) (Table 1) following the combined treatment may be due to the susceptibility to heat and dry cure injury of the coliforms. This observation agrees with the findings of Kemp *et.al* 1980. In addition, there were no significant differences ($p = 0.05$) in TVCs, fungal and coliform counts of the brined sun-dried sample and oven-dried sample during the storage period (Table 1). This finding may be of interest to local processors of the meat who cannot afford modern oven and can therefore preserve the meat by using brine solution that is relatively cheap and then sun-dry.

The microbial qualities of traditionally dried samples of the meat are presented in Tables 2 and 3. The TVCs of all the dried samples by day 4 (Table 2) were above $\log_{10}$ 5.0 (Speck, 1984) indicating questionable safety of the samples after 4 days of ambient storage. Furthermore, the lower and significantly different TVCs and fungal counts of Unwana sampling location from those of other locations may be due to the awareness of Unwana processors (being a Polytechnic town) on the need to maintain good sanitary standards including evisceration of the snail samples. The consistent isolation of *Bacillus cereus* and *Clostridium perfringens* from the locally processed dried samples (Table 3) may be attributed to their ability to survive adverse conditions including high temperature such as 60 ^{o}C and above (Setlow, 1994) during the hot smoking practice. It is also likely that hot – smoking and sun-drying may not have been adequate to eliminate these spore-formers. The results support the findings of Efivwevwere and Isaiah (1998) and Peck *et.al* (1995) on the inability of hot smoking or temperature of 75 ^{o}C for 27 min having little destructive effect on spore-formers. The presence of *Aspergillus flavus, Salmonella* and *Staphylococcus sp* in the samples indicate the level of contamination of the product since grossly contaminated products have been known to frequently contain these pathogens (Speck 1984; Harris *et.al* 1975).

Whereas storage of the fresh meat at 28 and 35 ^{o}C resulted in increased microbial load with time (Table 4), treatment of the meat with 5 % w/v NaCl before storage at 28 and 35 ^{o}C (Table 5) showed reduction in microbial load and hence extended the shelf-life of the meat by 4 days when stored at 28 ^{o}C. However, low temperature (-7 ^{o}C) combination with 5 % w/v NaCl resulted in lower microbial quality and further shelf-life extension to 6 days. These shelf life extension may be as a result of the susceptibility of proteolytic, putrefactive and spore-forming bacteria to relatively low concentrations (3-7 % w/

v NaCl) as earlier reported by Einarsson and Lauzon, 1995; Ihekoronye and Ngoddy, 1985. It is therefore pertinent that local processors who cannot afford low temperature storage can preserve the snail meat samples at ambient temperature (28 ± 2 °C) for a minimum of 4 days following treatment with sodium chloride solution (5 % w/v) that is cheap and relatively available.

CONCLUSION:

The results of the work have highlighted that combining brine (5 % w/v NaCl) with either sun-drying or oven-drying reduces the microbial load of ambient stored *L. libycus* meat to microbiologically acceptable level with that of oven-dried combination being more pronounced. In absence of oven-drying facilities, brine treatment combined with sun-drying can be used to achieve the same result since no significant difference ($p = 0.05$) existed between them in microbial load reduction and shelf life extension of the meat by 4 days. However, treatment of fresh meat of *L. libycus* with 5 % w/v NaCl before storage at 28 °C reduced the microbial load of the meat and extended the shelf life by 4 days. This is of interest to local processors of the meat who cannot afford low temperature storage facilities in the tropics.

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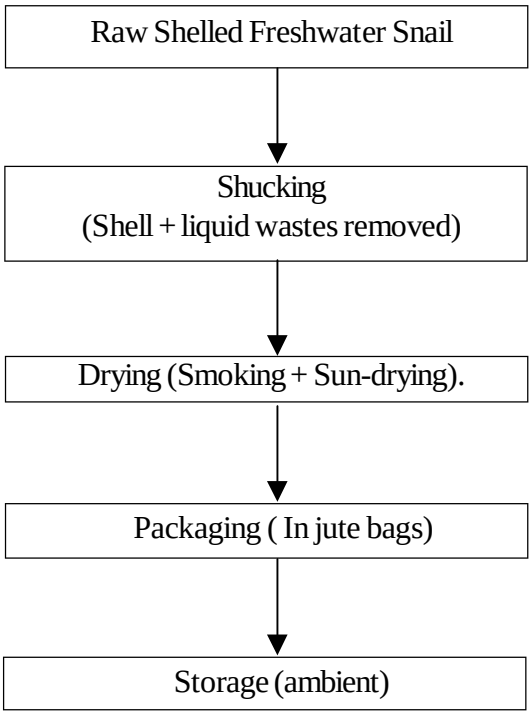

Fig 1 :Flowchart of traditional processing of freshwater snail (*Lanistes libycus).*

Table 1: Microbial counts (log_{10} cfu g^{-1}) of brine treated and untreated dried meat of freshwater snail (*Lanistes libycus*) stored at ambient temperature.

	Total viable count		Total fungal count			Total coliform count			
	Storage time (days)					Storage time (days)			
Treatment	**0**	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**
Brined + sun-dried	4.75c	4.74b	4.62b	2.31c	2.15c	2.43b	1.71b	1.62b	2.83b
Brined + oven-dried	3.60d	3.45c	3.92d	2.52c	1.38d	2.40b	1.61b	1.53b	2.08b
Sun-dried	5.48b	4.96b	6.92a	2.40b	3.43b	4.46b	1.88b	1.66b	2.53a
Oven-dried	4.73c	4.46b	4.82b	2.42c	2.26c	2.38b	1.79b	1.53b	2.75a
Untreated (Control)	6.79a	8.72a	ND	3.48a	4.65a	ND	3.36a	4.81a	ND

ND = Not determined due to obvious spoilage.
Each value represents the mean of 4 determinations.
Values in columns followed by different letters are significantly different at p = 0.05.

Table 2: Microbial counts (log_{10} cfu g^{-1}) of traditionally dried meat of freshwater snails (*Lanistes libycus*) stored at ambient temperature.

Location	Storage time (days)	Total viable count	Total fungal count
Akaeze	0	4.42a	1.75a
	2	4.46a	2.89a
	4	5.83b	3.67c
	6	8.92a	6.81a
Unwana	0	3.92a	1.53b
	2	4.38b	2.83a
	4	5.93b	4.60b
	6	7.99b	5.98b
Okposi	0	4.45a	1.93a
	2	4.58a	2.72a
	4	6.73a	5.81a
	6	8.80a	6.83a

Values represent means of 4 determinations.
Values in columns (within the same storage time) with different letters are significantly different at p = 0.05.

Table 3: Microorganisms isolated from traditionally dried meat of freshwater snail (*Lanistes libycus*).

	Sources of samples		
Bacteria	**Akaeze**	**Unwana**	**Okposi**
Bacillus cereus	+	+	+
Clostridium perfringens	+	+	+
Salmonella enteritidis	-	+	-
Staphylococcus aureus	+	+	+
Moulds			
Aspergillus flavus	+	+	+
Rhizopus nigricans	+	+	-
Penicillium digitatum	+	-	+
Mucor sp	-	+	-

Data was obtained from three replicates.
+ = isolated; - = not isolated.

Table 4: Effects of different storage temperatures on the microbial load (log_{10} cfu g^{-1})* of fresh meat of fresh water snail *(Lanistes libycus)*.

	Storage time (days)			
Storage temp. (°C)	**1**	**2**	**4**	**6**
35	5.45b	ND	ND	ND
28	6.68a	7.73a	ND	ND
4	4.66c	3.56b	2.51a	2.38a
-7	4.56c	2.45c	2.20b	1.11b

**Initial microbial load at day 0 was log_{10} 4.78 cfu g^{-1}.*
ND = Not determined due to obvious spoilage.
Each value represents the means of 4 determinations.
Values in columns followed by different letters are significantly different at p = 0.05.

Table 5: Effects of different storage temperatures on the microbial load (log_{10} cfu g^{-1})* of brine treated meat of freshwater snail (*Lanistes libycus*).

	Brine (5 % w/v NaCl) treatment				
	Storage time (days)				
Storage temp. (°C)	**1**	**2**	**4**	**6**
35	4.36a	3.26a	4.42a	5.38b
28	4.42a	3.36a	3.26b	6.20a
4	4.38a	2.30b	2.08c	2.04c
-7	3.26b	2.08c	2.05c	1.08d

** Initial microbial load at day 0 was log_{10} 4.62 cfu g^{-1}.*
Each value represents the mean of 4 determinations.
Value in columns followed by different letters are significantly different at p = 0.05.