GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 10, NO. 3, JUNE 2004: 363 - 367
COPYRIGHT(C) BACHUDO SCIENCE CO. LTD. PRINTED IN NIGERIA. ISSN 1118-0579

MICROBIOLOGICAL CHARACTERISTICS AND SENSORY QUALITY ATTRIBUTES OF POTASSIUM SORBATE TREATED AND UNTREATED SMOKED FRESHWATER SNAIL *(Lanistes libycus)*

CHIDI F. EZEAMA

(Received 6 May, 2003; Revision accepted 7 April, 2003)

ABSTRACT

Smoked potassium sorbate (KS) treated (0.2% w/v), smoked untreated and raw meat (control) samples of freshwater snail (*Lanistes libycus*) were evaluated for microbiological and sensory quality (aroma, visual appearance) attributes under ambient temperature (28 ± 2°C) storage. There were decreases in total viable counts (TVCs) in smoked KS treated samples till day 4 of storage while smoked untreated samples exhibit population decreases till day 2. Significantly lower counts (P = 0.05) were obtained for smoked KS treated samples than for the control and smoked untreated samples. KS treatment of the smoked samples resulted in fungal population decrease. Most of the gram-negative bacteria present were eliminated following smoking and this resulted in *Bacillus cereus, Staphylococcus aureus* and *Micrococcus spp* being dominant following the treatments. While no fungi were detected in the fresh sample, *Aspergillus niger, A. flavus, Penicillium sp* and *Mucor sp* were detected on the smoked samples. The aroma of the smoked KS treated sample was preferred to that of the smoked sample while the visual appearance of smoked samples were rated higher than the smoked KS treated samples. KS treatment of the smoked sample extended the shelf life of the samples by about 4 days at ambient storage.

KEY WORDS: Smoking, Potassium sorbate, Sensory quality, Shelf-life, Freshwater snail.

INTRODUCTION

Freshwater snails *(Lanistes libycus)* are economically important seafood widely distributed in Nigeria as well as other parts of the world (Arene *et al., 1999*, Brown 1980, Obureke *et al.*, 1987, Ukoli and Asamu,1979). In Nigeria, they are harvested during the rainy season (March – October). One of the major areas where they are harvested and consumed is the Cross River basin of Nigeria (including Akpoha and Akaeze in Ebonyi State) where they provide common source of animal protein for the people. This seasonal and popular seafood unfortunately has considerable limitations, which include unreliable microbial quality and unhygienic handling during processing. Furthermore, it has high spoilage tendency after harvest and accelerated spoilage rate of the meat after shucking, as the meat cannot be kept in acceptable condition after approximately 12 hours at ambient temperature. Despite the smoke-drying method of preservation (as practiced traditionally), the meat still undergoes deterioration, hence the need for combined preservative treatment becomes desirable (Samson and van Reenen-Hoekstra, 1988).

The quality characteristics of seafoods (including freshwater snails) are of interest to researchers. In addition to microbial population, another index of interest is the quality attributes (Jay, 1996, Ward and Baj, 1988). In this regard, extensive information exists on the microbiological and spoilage characteristics of other seafoods such as oysters, clams and fish (Efiuvwevwere and Iweanoge, 1991, Keuh and Chan, 1985), but focus on the combination of potassium sorbate treatment with smoking for shelf-life extension is very limited (Efiuvwevwere and Iweanoge, 1991, Efiuvwevwere and Isaiah, 1998, Jay, 1996). However, published information on the microbiological and shelf life extension of the freshwater snail (*L. libycus*) is lacking.

The objectives of this work therefore, were to evaluate the microbiological attributes of raw freshwater snail (*L. libycus*) meat and assess the effect of smoking, combination of smoking and potassium sorbate treatments on the microbiological and sensory quality attributes of the meat during tropical ambient (28 ± 2°C) storage.

MATERIALS AND METHODS

Samples Collection And Processing

Raw freshwater snails (*Lanistes libycus)* were obtained from local harvesters in Akpoha, Ebonyi State. The shells of the snails were washed with sterile water; surface sterilized (70% ethanol) and shucked aseptically using hand-gloves (Troge Medical GMBH Hamburg, Germany). During evisceration, the meat was aseptically removed, washed in sterile water and placed in a sterile container. Pooled meat samples (500g) each were made and several sub-samples (20g each) dipped into a solution of filter sterilized (0.2 µm sigma filter) potassium sorbate (KS) for 2 min (Chichester and Tanner, 1972). The treated samples were allowed to drip-dry before smoking on a single layer wire rack over a hard wood fire for one hour. The meats were occasionally turned to achieve uniform smoking and avoid charring. The untreated sub-samples were

CHIDI F. EZEAMA, Microbiology/Biochemistry Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, P. M. B. 1007. Afikpo. Nigeria.

TABLE 1: Microbiological quality of potassium sorbate (KS) treated and untreated smoked freshwater snail (*L.libycus*) meat stored at ambient temperature.

Storage time (days)	Total viable count (log_{10} cfu g^{-1})*			Total fungal count (log_{10} cfu g^{-1})+		
	Raw meat (Control)	Smoked	Smoked + KS	Raw meat (Control)	Smoked	Smoked + KS
0	6.64[a]	4.80[b]	4.76[b]	ND	1.34[a]	1.34[a]
2	7.75[a]	4.61[b]	3.79[c]	ND	1.26[a]	1.15[b]
4	8.83[a]	6.93[b]	3.65[c]	ND	1.20[a]	1.11[b]
6	NT	7.38[a]	5.88[b]	NT	1.32[a]	1.04[b]
8	NT	7.84[a]	6.94[b]	NT	1.62[a]	1.45[b]
10	NT	8.08[a]	7.40[b]	NT	1.80[a]	1.61[b]

Each value represents the mean of 4 determinations of two independent experiments.
ND = not detected, NT = not tested due to obvious spoilage.

* Mean in the same row followed by different letters are significantly different at P = 0.05.
+ Mean in the same row followed by different letters are significantly different at P = 0.05.

equally smoked for the same period before both samples were allowed to cool for about 1 hour and then packages in perforated sterile polyethylene bags. The fresh meat without smoking or KS treatment served as control. The packaged samples were stored at ambient temperature (28 ± 2°C) for analyses at two-day intervals.

Microbiological Analysis

Each sub-samples (20g) was blended (Moulinex, France) in 180ml of sterile 0.1 % w/v peptone water (pH 7.2 ± 0.2) to obtain 1:10 dilution. Further 10 – fold dilutions were prepared. The total viable counts (TVCs) and total fungal counts were determined by pour plate technique (1.0ml aliquot) on Tryptone soy agar (TSA) and acidified Malt Extract Agar (MEA) respectively. The TSA plates were incubated anaerobically and aerobically at 35 – 37°C for 18 – 24 hours while the MEA plate were incubated at room temperature (28 ± 2°C) for 2-5 days. The colonies that developed on the plates were within their suitable range of 25-250 (Speck, 1984). These 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, MacConkey agar, Triple Sugar Iron agar, Simmon Citrate agar, Litmus milk, MRVP medium, (Biotec laboratory Ltd Suffolk, UK) were used for the identification of isolates. Following various biochemical tests (oxidase, citrate, catalase, coagulase, indole, urease, hydrogen sulphide production, MRVP and oxidative/fermentative utilization of glucose, lactose, arabinose, and mannitol) an descriptions (Harrigan and McCance 1976, Sneath *et al.* 1986; Krieg and Holt 1984) the bacterial isolates were identified. Similarly, the fungal isolates were identified based on their cultural and morphological characteristics (Samson and van Reenen – Hoekstra 1988).

Sensory Quality Attributes of the Samples

Aroma and visual appearance of both treated and untreated smoked samples were evaluated using a 9 – point hedonic scale (Larmond, 1977). A panel of 10 members consisting of trained staff and students carried out the evaluation.

Statistical Analysis

The data obtained were statistically analyzed using analysis of variance (ANOVA) to determined the mean differences based on the least significant difference (LSD) at P = 0.05 (Snedecor and Cochran 1980).

RESULTS

In Table 1 is shown the microbiological quality of the raw meat, KS treated and untreated smoked meat samples. There was decrease in total viable count (TVC) to the 4th day of ambient storage in smoked KS treated samples while those of smoked samples exhibited population decrease till day 2 (Table 1). However, significantly lower counts (P = 0.05) were observed in smoked KS treated samples than in the control and smoked untreated samples. No fungi were detected in the control sample. KS treatment of smoked samples resulted in decrease of the fungal count from log 1.34 to log 1.04 cfu g^{-1} by day 6 of the storage while that of the untreated smoked samples decreased till day 4 (Table 1).

The microorganisms isolated from the raw meat and KS treated and untreated smoked samples are depicted in Table 2. The bacterial species isolated from the raw meat (control) included *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeroginosa, Bacillus cereus, Micrococcus luteus, Salmonella sp and Klebsiella spp.* While *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Micrococcus luteus* and *Salmonella sp* were the bacteria species isolated from the smoked samples, *Staphylococcus aureus, B. cereus, C. perfringens* and *M. luteus* occurred in the smoked KS treated samples. However, *S. aureus, B.*

Table 2: Microorganisms isolated from raw or potassium sorbate (KS) treatment or untreated smoked freshwater snail (*Lanistes libycus*) meat stored at ambient temperature (day 0).

Raw meat Microorganisms	(Control)	Smoked	Smoked + KS
Bacterial Flora			
Staphylococcus aureus	-	+	+
Escherichia coli	+	-	-
Enterobacter aerogenes	+	-	-
Pseudomonas aeroginosa	+	-	-
Bacillus cereus	+	+	+
Clostridium perfringens	-	+	+
Micrococcus luteus	+	+	+
Salmonella sp	+	+	-
Klebsiella spp	+	-	-
Mycoflora			
Aspergillus niger	-	+	+
Aspergillus flavus	-	+	-
Penicillium sp	-	+	+
Mucor sp	-	+	-

+ = isolated, – = not isolated.

Data were obtained from four replicates.

cereus and *M. luteus* were the dominant bacterial isolated in all treatments (Table 2). Similarly, the moulds isolated from the smoked samples included *Aspergillus niger, A. flavus, Penicillium* and *Mucor* species while *A. niger* and *Penicillium* sp were the only moulds isolated from the smoked KS treated samples (Table 2).

The mean (including the standard deviation) of the sensory attributes (aroma and visual appearance) of the samples are shown in Table 3. The aroma of the KS smoked samples were preferred (6.4 ± 0.6) to the smoked samples till day 6 while the visual appearance was rated higher (6.8 ± 0.7) to the KS smoked samples on the 6th day of storage (Table 3).

DISCUSSION

Seafoods are prone to deterioration despite smoking procedures practiced in the tropics. However, maximum positive impact on the microbial quality seems exerted with combined preservative treatment (Efiuvwevwere and Isaiah, 1998, Okafor and Nzeako, 1985). The decrease in TVC exhibited for 2 days in smoked freshwater snail *(L. libycus)* samples and the population decrease till day 4 in smoked KS treated samples (Table 1) clearly shows the destructive effect of smoking with more pronounced synergistic effect when KS treatment is combined with smoking. Efiuvwevwere and Iweanoge (1991), Jay (1996), and Okafor and Nzeako (1985) had earlier reported corresponding decreases in TVC following such treatments. Nevertheless, the sharp increase in TVC to log 5.0 cfu g^{-1} and above in the smoked samples by the 4th day and in the smoked KS treated samples 6th day of storage (Table 1) indicate the potential danger of consuming such food by the 4th and 6th day respectively as such level of microbial population in food is regarded as unacceptable (ICMSF 1986). The observed decrease in fungal count from log 1.34 to 1.20 cfu g^{-1} in the smoked samples by day 4 and to log 1.04 cfu g^{-1} in the smoked KS treated samples by day 6 of storage may be due to low heat resistance and sensitivity to KS by the fungi. Furthermore, the later increase in fungal count in smoked (day 6) and smoked KS treated (day 8) samples may be attributed to moisture absorption by the samples from the humid tropical environment causing dilution of the preservative thereby leading to waning effect. However, significant difference existed between the two treatments showing the effect of combined preservative treatment on food.

Although there were more bacteria (both gram-positive and gram-negative) isolated from the raw than the

smoked or smoked KS treated samples, the gram-positive bacteria that dominated the smoked and smoked KS treated samples (Table 2) shows that smoking may have eliminated most of the gram-negative bacteria. However, the high occurrence of gram-positive bacteria in the smoked and the smoked KS treated samples (Table 2) may be attributed to the heat-tolerant nature of this group of microorganisms especially spore-formers *(Bacillus and Clostridium spp)* that are known to survive high temperature such 60^{0}C and above (Setlow, 1994, Peck *et al.*, 1995). Similar adaptive mechanism and dominance of Gram-positive organisms have earlier been reported on smoked fish and pasteurized oyster (Efiuvwevwere and Isaiah 1998, Pace *et al.*, 1998). Furthermore, the smoking temperature alone in combination with KS treatment may not have been adequate to eliminate the spores of *Bacillus* and *Clostridium* spp hence their prevalence in the samples. The absence of fungi in the raw meat and their presence in the smoked samples (Table 2) may be due to aerial contamination during smoking and storage periods. However, the presence of *Aspergillus flavus* in the smoked samples (Table 2) is of health significance since *A. flavus* is known to be toxigenic (Speck, 1984).

There was no significance difference in the sensory qualities being tested on day 0 of storage (Table 3). This may be attributed to the masking of the aroma and the visual characteristics of KS by the deposition of phenol and other aromatic compounds associated with smoking practice. Woodsmoke constituents are known to have chemical interactions with foods (Papavergou and Clifford, 1986). However, the sensory impact of the KS treatment became evident from day 2 of storage as shown by significant difference in the hedonic scores of the sensory attributes of the two treatments. Although the visual appearances of smoked samples was better referred (6.8 ± 0.7) by the 6th day of storage than that of smoked KS treated samples (5.0 ± 0.6) it is evident that visual appearance is not a better indicator of the microbial quality of the smoked samples, since by day 6 the microbial load of the smoked sample was not acceptable (Table 1). Aroma therefore, seems to be a better indicator since its score in smoked (6.8 ± 0.4) and smoked KS treated (7.6 ± 0.5) samples by day 4 agrees with the microbial quality of the smoked KS treated sample by day 4. It is therefore pertinent that the combination of smoking with KS (0.2% w/v) exerted positive impact on the microbial quality of the sample and extended the shelf life to about 4 days.

CONCLUSION

This study shows that significantly lower counts were

TABLE 3: Sensory attributes of potassium sorbate (KS) treated and untreated smoked freshwater snail (*Lanistes libycus*) Meat.

Storage time (days)	Treatments	Sensory Attributes: Aroma	Sensory Attributes: Visual Appearance
0	Smoked	8.7 ± 0.7^a	7.5 ± 1.0^a
	Smoked + KS	8.2 ± 0.7^a	7.0 ± 1.6^a
2	Smoked	7.8 ± 0.9^b	7.4 ± 1.0^a
	Smoked + KS	8.6 ± 0.5^a	6.3 ± 0.7^b
4	Smoked	6.8 ± 0.4^b	7.0 ± 0.6^a
	Smoked + KS	7.6 ± 0.5^a	5.6 ± 0.5^b
6	Smoked	4.6 ± 0.8^b	6.8 ± 0.7^a
	Smoked + KS	6.4 ± 0.6^a	5.0 ± 0.6^b
8	Smoked	4.5 ± 0.5^a	5.2 ± 0.5^a
	Smoked + KS	4.8 ± 0.6^a	4.4 ± 0.5^b
10	Smoked	3.8 ± 0.7^a	3.2 ± 0.6^b
	Smoked + KS	4.0 ± 0.6^a	4.2 ± 0.5^a

Each value is the mean ± SD obtained by 10-member panel (10 scores).

Values in columns for each time intervals with different letters are significant

obtained for smoked KS treated samples than for the raw (control) and smoked untreated samples. Smoking alone could not effectively control the microbial load of the sample beyond 2 days at ambient temperature. The aroma of smoked KS treated sample however, was preferred to that of the untreated smoked samples while visual appearance of the smoked untreated samples were rated higher than the smoked KS treated sample. Combining smoking with KS treatment (0.2% w/v) improved the microbial stability of freshwater snail (*L. libycus)* meat and extended the shelf life by about 4 days. With adequate sanitary measures prior to the KS treatment and smoking, the preservation problem encountered by local processors of this seasonal and popular seafood in Nigeria would be solved.

REFERENCES

Arene, F. O. I, Ibanga, E. S. and Asor, J.E 1999. Freshwater Snail and Crab intermediate hosts of *Paragonimus* species in two rural communities in Cross River Basin, Nigeria. Global J Pure & Appl. Sci. 5(2): 184 – 187.

Brown, D. S., 1980. Freshwater snails of Africa and their Medical importance. Taylor and Francis, London. pp 10-30.

Chichester, D. F. and Tanner, F. W. 1972. Antimicrobial Food Additives. In: Handbook of Food Additives. Vol. I Furria, T. E. (ed), CRC Press, Cleveland, Ohio.

Efiuvwevwere, B.J.O and Iweanoge, H.A. 1991. Microbiological and physico-chemical quality of various tissue types of fresh and potassium sorbate treated and untreated smoked mullet *(Mugil cephalus). World J. Microbiol and Biotechnol.* 7:562-566.

Efiuvwevwere, B.J.O and Isaiah, A. U.1998. Effect of hygienic handling in combination with potassium sorbate treatment and smoking on the microbial quality and shelf stability of Croaker *(Micropogonias furnieri). Z. Lebensm Unters Forsch A.* 207:13-17.

Harrigan, W. F. and McCance, M. E. 1976. Laboratory Methods in Food and Dairy Microbiology. Academic Press, London 416 pp.

ICMSF (International Commission on Microbiological Specification for Foods) 1986. Microorganisms in Foods 2. Sampling for microbiological analysis. Principles and Specific Application 2nd edn. Blackwell Scientific Publications, Oxford.

Jay, J.M. 1996. Modern Food Microbiology, 5th edn. Chapman and Hall, New York.

Kreig, N.R. and Holt, J.G. 1984. Bergey's Manual of Systematic Bacteriology Vol. 1. William and Wilkins, Baltimore.

Kueh, C.S.W., and Chan, K. 1985. Bacteria in bivalve Shellfish with special reference to the Oyster. *J. Appl. Bacteriol,* 59:41-47.

Larmond, E. 1977. Laboratory Methods for Sensory evaluation of Foods. Food Research Institute Ottawa. Canada Department of Agriculture Publication 1639.

Obureke, J. U. Arene, P.O.I. and Ufodike, E.B.C. 1987. Occurrence and habitat preference of Freshwater snails of the Rivers State, Nigeria. *Nig. J. Appl. Fisheries and Hydrobiol.* 2:39-43.

Okafor, N. and Nzeako, B.C. 1985. Microbial Flora of fresh and smoked fish from Nigeria freshwater. *Food Microbiol.* 2:71-75.

Pace, J., Wu, C.U. and Chai, T. 1988. Bacteriological flora in pasteurized oyster after refrigerated storage. *J. Food Sci.,* 53:325-327.

Papavergou, E. and Clifford M. N. 1986. Possible chemical interactions between woodsmoke constituents and foods. *Proceeding of the Institute of Food Science and Technology* 19:63-67.

Peck, M. Lund, W., Fairbairn, A.A., Kaspersson, A.S. and Undeland, P. C. 1995. Effect of heat treatment on survival of, and growth from, Spores of non-proteolytic *Clostridium botulinum* at refrigeration temperature. *Appl. Environ. Microbiol.* 61(6): 1789-7985.

Samson, R.A. and van Reenen-Hoekstra, E.S. 1988. Introduction of Foodborne Fungi. 3rd edn. Centralbureau Voor Schimmecultures. Baarn, the Netherland.

Setlow, P. 1994. Mechanisms which contribute to the long-term survival of spores of *Bacillus* species. *J. Appl. Bacteriol. Symposium Suppl.* 76:495-605.

Sneath, P.H.A., Nair, N.S., Sharp, M.E., and Holt, J.G. 1986. Bergey's Manual of Systematic Bacteriology Vol. 2. Williams and Wilkins, Baltimore.

Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods. 7th edn. Iowa State University Press, Ames IA. USA.

Speck, M.L. 1984. Compendium of Methods for the Microbiological Examination of Food. 2nd. edn. American Public Health Association. Washington D.C.

Ukoli, F.M.A. and Asamu, D.I. 1979. Freshwater of the proposed Federal Capital Territory in Nigeria. *Nig J. Natr Sci.* 1(1): 47-59.

Ward, D.R. and Baj, N.J. 1988. Factors affecting microbiological quality of seafoods. *Food Technol.* 42:85-89.