GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 6 NO. 4, SEPTEMBER 2000:623-628
 ISSN 1118-0579

BACTERIAL INHIBITORY EFFECTS OF POTASSIUM SORBATE AND RETARDATION OF SPOILAGE OF OYSTERS *(CRASSOSTREA GASAR)* AT TWO STORAGE TEMPERATURES

B. J. O. EFIUVWEVWERE and G. IZAKPA

(Received 30 November 1998; Revision accepted 17 March, 1999)

ABSTRACT

Shucked oysters (*Crassostrea gasar*) treated with 0.1% or 0.2% w/v potassium sorbate (KS) or untreated (controls) were stored at 5°C or 29°C to evaluate microbial changes and shelf-life. Appreciable variations were observed in the microbial composition, with a Gram-positive to Gram-negative flora ratio of 51:46 occurring in freshly shucked samples. But contrasting ratios of 22:78 and 80:20 occurred in 0.2% KS treated samples stored at 5°C and 29°C respectively. *Pseudomonas* spp. (65%) were most prominent in samples stored at 5°C while Gram positive flora (*Bacillus* spp. 40% and *Micrococcus* spp. 25%) dominated samples held at 29°C. Of the fungi, *Saccharomyces cerevisiae* was most prevalent in the samples regardless of KS treatment and storage temperature. Total plate counts (TPCs) were reduced by approximately 100-fold in 0.2% KS-treated samples stored at 5°C but KS treatment was less effective in samples held at 29°C. The quality indicators (TPCs, coliform bacteria and *Vibrio* spp. population) increased substantially with storage at 29°C. Correlations between total and faecal coliforms or between the latter and *Vibrio* spp. were dependent on KS treatment and storage temperature. pH decreased in all samples especially in controls. Samples stored at 29°C were rejected within 36h of storage but combination of 0.2% KS treatment with storage at 5°C extended the shelf-life by approximately 4 days. The preponderance of *Bacillus* spp., *Staphylococcus* spp. and *Vibrio* spp. with the concomitant high TPCs in samples stored at 29°C have demonstrated the public health risks associated with shucked oysters subjected to inadequate KS treatment and storage temperature.

KEY WORDS: Oysters, microbial inhibition, sorbate, temperature

INTRODUCTION

Oysters are economically important seafoods in various parts of the world including the Niger Delta Region of Nigeria. Unfortunately, they are exposed to several sources of contamination especially in polluted aquatic environments (Jay 1996).

The bacterial diversity of seafoods and the relationship between groups of indicators such as coliforms are of interest to several workers (Son and Fleet 1980; Matte et al. 1994). In addition, since oysters are filter feeders, they tend to accumulate high microbial populations (ICMSF 1986).

Oysters remain in good condition if unshucked but once shucked, they decompose except they are subjected to preservative treatments (Anon, 1980). Several studies have been published on oysters but focus on use of potassium sorbate treatment for shelf life extension is very limited (Jay 1996). Spoilage of oysters is influenced by a number of factors and the indices of deterioration in addition to microbial population include changes in pH and sensory quality attributes (Jay 1996).

The microbiological and spoilage characteristics of oysters from temperate waters have been given much attention (Son and Fleet 1980; Vanderzant and Splittsoesser 1992). In contrast, little research work has been reported on oysters obtained from the Niger Delta Region in spite of their abundance and wide-spread consumption in the region. Consequently, the present investigation was undertaken to study (i) the microbial composition and dynamics of oysters treated and untreated with potassium sorbate and stored under two temperature conditions (refrigerated and ambient) often encountered in Nigeria and (ii) the changes in pH and sensory quality attributes as spoilage indicators.

MATERIALS AND METHODS

Sample Collection and Preparation

Samples of unshucked oysters (*Crassostrea gasar*) harvested from aquatic environment of Bakana (near Eagle Island, Port Harcourt) were purchased from the harvesters, collected in pre-sterilised polyethylene bags and transported to the laboratory. They were then scrubbed, washed, rinsed with 70% ethanol and shucked with a pre-sterilised knife (Vanderzant and

B. J. O. EFIUVWEVWERE, Dept. of Microbiology, Food & Industrial Division, University of Port Harcourt, Port Harcourt - Nigeria
G. IZAKPA, Department of Microbiology, Food & Industrial Division, University of Port Harcourt, Port Harcourt - Nigeria

Splittstoesser 1992). Removal of the flesh was facilitated by heating for 5 min as practised traditionally.

POTASSIUM SORBATE TREATMENT OF SAMPLES

Shucked samples were divided into three sub-samples with each consisting of 72 oysters. Two of the sub-samples were immersed in 0.45 µm filter-sterilised (Gelman Sciences, Ann Arbor, USA) KS solutions (0.1 or 0.2% w/v) for 2 min while the other sub-sample (i.e. controls) were immersed in filter-sterilised deionised water for the same period of time. Following these treatments, they were drained and packaged in sterile polyethylene bags before storage at 5°C or 29°C for analysis at two-day intervals.

MICROBIOLOGICAL ANALYSIS OF SAMPLES

Composite samples (20g each) of 6 aseptically pooled oysters were homogenised in 180 ml 0.1% (w/v) sterile peptone water using a 70% ethanol pre-sterilised Moulinex blender. Further decimal dilutions of 10^{-2} to 10^{-7} of the homogenate were prepared and plated in duplicate as follows: total plate count using spread method on nutrient agar (Oxoid, UK) and incubated at 37°C for 24h; total and faecal coliforms by pour-plating using MacConkey agar (Oxoid, UK) and incubating at 37°C and 44.5° for 18-24h respectively (Harrigan and McCance 1976; Collins and Lyne 1984); *Vibrio* spp. count by spread-plating on Thiosulphate-citrate-bile-salt-sucrose-agar (TCBS, Oxoid, UK) and incubated at 37°C for 18-24h (Lee et al. 1981) and fungi population was determined by pour plating on acidified potato dextrose agar (Oxoid, UK) and incubated at 27°C for 4 days (Harrigan and McCance 1976).

IDENTIFICATION OF ISOLATES

From each primary plate counted, at least four of each colony type were sub-cultured onto nutrient agar for purification and then subjected to Gram- and spore-staining as well as motility test (Harrigan and McCance 1976). The number of the different colony types were recorded and computed as perentage occurrence of the various organisms. After the morphological characterisation, the isolates were subjected to biochemical tests; indole, methyl red, Voges-Proskauer, citrate utilisation (IMViC), oxidase, catalase, coagulase, hydrogen sulphide production (Kliger's iron agar) and oxidative/fermentative utilisation of glucose, lactose, sucrose, mannitol and identified based on earlier descriptions (Harrigan and McCance 1976; Krieg and Holt 1984; Sneath et al. 1986). The fungi were identified using the colonial and morphological characteristics as well as fermentation of sugars (glucose, sucrose,

Table I. Occurrence (%) of Microbial Flora in Potassium Sorbate (KS) Treated and Untreated Oysters During Storage at 5°C or 29°C

		Storage Days	0			2			4			6		
Microflora	Storage Temp (°C)	KS(%)	0.0	0.1	0.2	0.0	0.1	0.2	0.0	0.1	0.2	0.0	0.1	0.2
Baccillus spp.	5		23	23	22	15	18	12	25	24	12	7	10	12
	29		23	23	22	25	36	35	30	15	24	23	21	40
Micrococcus spp.	5		20	19	20	23	16	13	16	10	18	16	3	5
	29		20	19	20	31	24	20	25	33	30	37	38	25
Stapylococcus spp.	5		8	8	9	6	1	1	2	4	10	10	9	10
	29		8	8	9	15	10	14	15	20	14	8	19	15
Enterobacter	5		15	16	15	2	1	1	1	1	3	1	2	ND
	29		15	16	15	3	3	2	10	8	8	5	5	7
Escherichrie coli	5		2	1	1	2	1	1	2	1	3	2	2	4
	29		2	2	2	4	5	3	8	10	13	9	6	5
Pseudomonas spp.	5		17	18	18	35	46	53	46	49	42	51	63	65
	29		17	18	18	8	10	11	2	ND	1	2	ND	ND
Serratia spp.	5		5	4	5	5	3	2	ND	ND	ND	1	3	1
	29		5	4	5	2	1	2	2	1	2	ND	ND	ND
Vibrio spp.	5		7	8	6	12	14	17	8	10	12	10	8	3
	29		7	8	6	9	10	12	6	13	8	15	10	8
Unidentified	5		3	3	4	ND	ND	ND	ND	1	ND	2	ND	ND
	29		3	3	4	3	1	1	2	ND	ND	1	1	ND
Aspergillus flavus	5		34	35	33	1	ND	ND	ND	ND	ND	ND	ND	ND
	29		34	35	32	2	ND	ND	ND	ND	2	1	1	1
Aspergillus niger	5		35	34	36	ND	ND	ND	ND	ND	ND	ND	ND	ND
	29		35	34	36	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fusarium spp.	5		31	32	31	ND	9	ND	ND	ND	ND	ND	ND	ND
	29		31	32	31	ND	ND	ND	2	1	3	1	ND	1
S. cerevisiae	5		ND	ND	ND	99	91	100	100	100	100	100	100	100
	29		ND	ND	ND	98	100	100	98	99	95	98	99	98

Each value represents the mean plates of two independent experiments.
ND = Not detected.

mannitol) by the isolated yeasts (Samson and Reenen-Hoekstra 1988).

DETERMINATION OF CHANGES IN pH AND SENSORY QUALITY ATTRIBUTES OF SAMPLES

Samples were homogenised in deionised water (1:2 ratio) using a Moulinex blender and the pH determined (Pye Unicam model 291 MK2, Cambridge, England).

Samples from the different treatments stored at 5°C or 29°C were respectively boiled for approx. 5 min. and evaluated by a 10 member panel consisting of staff and post-graduate students that have adequate experience with sensory qualities of oysters. The attributes: visual appearance and aroma were scored using a 9-point hedonic scale (Larmond 1977). Overall acceptability based on these two quality criteria was assessed as described previously (Efiuvwevwere and Ezeama 1996).

Analysis of Variance (ANOVA) of the data was carried out to determine the mean differences based on the least significant difference (LSD) (Snedecor and Cochran 1980).

RESULTS

MICROBIAL COMPOSITION OF SAMPLES

The percentage distribution of the microbial flora of samples stored at 5°C or 29°C is presented in Table I. Initially (i.e. day 0), the ratio of Gram-positive to Gram-negative bacterial flora was 51:46 (with 3% unidentified) but it changed drastically to 33:65 (with 2% unidentified), 22:73 and 27:73 respectively for control, 0.1% and 0.2% KS treated samples at the end of storage (6 days) at 5°C with *Pseudomonas* spp. dominating in all treatments (Table I). In contrast, samples stored at 29°C exhibited much higher ratio of Gram-positive to Gram-negative bacterial flora of 68:31 (with 1% unidentified), 76:21 (with 3% unidentified) and 80:20 for controls, 0.1% and 0.2% KS-treated samples respectively (Table I). *Aspergillus* spp. were the dominant moulds isolated from the fresh samples while *Saccharomyces cerevisiae* dominated the samples with storage time (Table I).

Population dynamics of the various bacterial groups as affected by KS treatment and storage temperature are presented in Table II. At higher concentration of 0.2%, a reduction in total plate counts (TPCs) of 1-2 logs as from day 4 in samples stored at 5°C was observed. Conversely, KS treatment exhibited less inhibitory effect in samples held at 29°C thereby resulting in substantially higher TPCs especially as from the 4th day of storage (Table II).

CORRELATIONS BETWEEN BACTERIAL GROUPS

Significant correlations were observed between total and faecal coliforms isolated from samples stored at 5°C but non-significant relationships (except for controls) occurred in samples stored at 29°C (Table III). On the other hand, virtually no significant correlations were observed between faecal coliforms and *Vibrio* species except in samples treated with 0.1% KS and stored at 5°C (Table III).

CHANGES IN pH AND SENSORY QUALITY ATTRIBUTES

There was a significant decrease in pH in all samples as storage time progressed but the

Table II. Population (Log_{10}) dynamics of bacterial groups isolated from oysters subjected to different potassium sorbate concentrations and two storage temperatures

Storage (days)	Treatments	Total plate counts		Total coliforms		Faecal coliforms		Vibrio species	
		5°C	29°C	5°C	29°C	5°C	29°C	5°C	29°C
0*	Control	3.5a	3.5a	3.0a	2.20a	2.20a	2.20a	2.92a	2.92a
	0.1%KS	3.4a	3.4a	3.0a	2.23a	2.23a	2.23a	2.93a	2.93a
	0.2%KS	3.4a	3.4a	2.9a	2.19a	2.19a	2.19a	2.91a	2.91a
2	Control	4.4a	5.1a	3.3a	4.8a	3.26a	2.86a	3.11a	3.51a
	0.1%KS	4.2a	5.0a	3.0a	4.9b	2.78ab	2.89a	2.61ab	3.45a
	0.2%KS	3.6b	4.4b	2.4b	3.9b	2.51b	2.84a	2.48b	3.40a
4	Control	7.15a	9.4a	5.2a	6.3a	4.15a	5.6a	5.04a	4.84a
	0.1%KS	6.95a	8.6b	5.0a	6.0a	3.36b	4.11b	3.51b	4.09b
	0.2%KS	5.68b	6.6c	4.5b	5.91a	3.10b	4.00b	3.23b	3.35c
6	Control	9.61a	10.5a	7.7a	8.2a	4.92a	5.30a	5.76a	6.20a
	0.1%KS	8.57b	9.9a	6.6b	7.2b	380b	4.28b	4.81b	4.63b
	0.2%KS	8.34b	8.7b	5.6c	6.1c	3.71b	4.26b	4.07c	4.28b

* Before storage

Values in columns for the respective time intervals with different letters are significantly different $P = 0.05$. Each value represents the average of 4 determinations of two independent experiments

decrease was more evident in samples stored at 29°C (Table IV). Deterioration was more rapid in samples held at 29°C and were rejected after approx. 36 h of storage. In contrast, only 0.2% KS-treated samples subjected to 5°C storage were considered acceptable at the end of the study (Table IV).

DISCUSSION

The wide variations observed between the microbial flora of the freshly shucked samples and those analysed following storage (Table I) suggests the differential impact of the aquatic environment (with temperatures ranging between 24 and 26°C) and the potassium sorbate treatment as well as the storage temperatures. Since the microbial quality of seafoods is a reflection of their aquatic environment, the comparable ratio of 51:46 Gram-positive to Gram-negative biota in the fresh samples is probably related to the extensive human and industrial pollution of the Nigerian aquatic environments as previously reported by Benka-Coker and Ohimain (1995). However, the dramatic change in the ratio in favour of Gram-negative flora in samples stored at 5°C as opposed to the preponderance of Gram-positive flora in 29°C stored samples clearly indicates the differential effect of temperature of storage. For instance, *Pseudomonas* spp. are psychrotrophic and hence their dominance in 5°C stored samples in comparison with samples held at 29°C (Table I). On the other hand, the high occurrence of Gram-positive flora in samples stored at 29°C may be attributable to their enhanced survival following the shucking (heating) process. A similar adaptative phenomenon and dominance of Gram-positive flora in pasteurised oysters and smoked fish have earlier been reported (Pace et al 1988; Efiuvwevwere and Isaiah 1998). In addition, microbial responses to KS-treatment varies (Efiuvwevwere and Isaiah 1998) and must have contributed to these differential changes. For example, *Bacillus* spp. have minimum inhibitory concentration of KS ranging from 50-100 ppm while *Serratia* spp have approximately 50 ppm (Gould 1989). Similarly, fungi have a wide range of growth requirements (Samson and van Reenen-Hoekstra 1988; Jay 1996) and these may explain the fungal diversity found in the different samples (Table I). However, the *Vibrio* spp. exhibited comparable populations at both temperatures (Table II), thus indicating their psychrotrophic characteristics (Singleton et al. 1982; Mate et al. 1994).

Based on the reference values of total plate counts (TPCs) and faecal coliforms especially

Table III. The relationship (r) between bacterial groups isolated from oysters subjected to different potassium sorbate (KS) treatments and storage temperatures.

	BACTERIAL GROUPS			
	Total versus faecal coliforms		Faecal coliforms versus Vibrio species	
Treatments	5°C	29°C	5°C	29°C
Controls (% KS)	0.9585***	0.5713*	0.1724^{NS}	0.4273*
0.1%KS	0.9429***	-0.1685^{NS}	0.9171***	-0.0319^{NS}
0.2%KS	0.5825*	0.1587^{NS}	0.0814^{NS}	-0.334^{NS}

Correlation coefficients (r) were computed using the obtained data (n = 12 i.e. 4 mean data multiplied by values 3 treatments). Significance levels: NS = Not significant

*P = 0.05; **P = 0.01; ***P = 0.001

Table IV. Changes in pH and sensory quality (overall acceptibility) of oysters subjected to different potassium sorbate (KS) treatment and storage temperatures

		pH		Overall acceptability	
Storage (days)	Treatments	5°C	29°C	5°C	29°C
0*	Control (0%KS)	6.7a	6.7a	8.8a	8.8a
	0.1%KS	6.8a	6.8a	8.7a	8.7a
	0.2%KS	6.7a	6.7a	8.7a	8.7a
2	Control (0%KS)	6.4c	6.2b	7.1b	3.5b
	0.1%KS	6.5bc	6.3ab	7.6a	4.0ab
	0.2%KS	6.7a	6.4a	7.9a	4.2a
4	Control (0%KS)	5.3b	5.0b	5.0b	2.4b
	0.1%KS	5.9a	5.5a	6.4a	2.3b
	0.2%KS	5.6a	5.6a	6.5a	3.1a
6	Control (0%KS)	4.6b	4.5c	3.9b	1.6a
	0.1%KS	4.9b	4.8b	4.2b	1.8a
	0.2%KS	5.4a	5.1a	5.7a	2.0a

Values inn columns (for the respective time intervals) with different letters are significantly different at P = 0.05

Each value represents the average of 4 determinations from two independent experiments. Higher values (i.e. for overall acceptability) represent better sensory quality.

from day 4 (Table II), most of the samples would be considered rejected from the microbiological standpoint since they showed much higher microbial levels than the recommended TPCs and FCs of $\log_{10}$ 5.7 and 2.3 cfu respectively for bivalve molluscs (ICMSF 1986). Consequently, it will be unwise to consume these products except they are adequately processed.

The poor and non-significant correlations observed between the total and faecal coliforms from samples stored at 29°C which contrasted with the strong positive correlations found in samples held at 5°C (Table III) demonstrate the consequences of storage temperature in relation to growth behaviour of these indicators. Similarly, the poor relationships observed between the faecal coliform and *Vibrio* species (Table III) clearly suggest variations in the growth requirements of organisms contained within the microbial groups (Jay 1996). Thus, the use of one microbial group to predict the presence or absence and behaviour of another group of indicator is complex and highly variable as observed previously (Edberg and Smith 1989).

The significant decrease in pH of the samples from 6.74 to 4.47 at the end of storage (Table IV) is a clear indication of spoilage since pH of 5.2 and below is associated with "souring" (i.e. spoilage) of oysters (Banwart 1981). However, samples treated with 0.2% potassium sorbate and stored at 5°C exhibited shelf-life of about 4 days indicating the antimicrobial benefit of KS. These samples also showed enhanced overall sensory quality but not those stored at 29°C (Table IV).

ACKNOWLEDGEMENTS

The senate Research grant to BJOE for this work by the University of Port Harcourt is gratefully acknowledged.

REFERENCES

Anon. 1980. Microbial Ecology of Foods, Vol. 2, Food Commodities. Academic Press, New York.

Banwart, G. J. 1981. Basic Food Microbiology. Avi Publishing Co; Westport

Benka-Coker, M. O. and Ohimain, E. I. 1995. Investigation of Spatial Variations in Bacterial Distribution in Surface waters of Ikpoba River. Nigerian Journal of Microbiology 10: 27-32.

Edberg, S. C. and Smith, D. B. 1989. Absence of Association between Total Heterotrophic and Total Coliform Bacteria from a Public Water Supply. Applied and Environmental Microbiology 55: 380-384.

Efiuvwevwere, B. J. O. and Isaiah, A. U. 1998. Effects of Hygienic handling in Combination with Potassium Sorbate Treatment and Smoking on the Microbial Quality and Shelf-Stability of Croaker (*Micropogonias furnieri*). Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung A. 207: 13-17.

Efiuvwevwere, B. J. O. and Ezeama, C. F. 1996. Influence of fermentation time and an 'indigenous tenderiser' (Kanwa) on the Microbial Profile, Chemical attributes and Shelf-life of rice masa (a Nigerian fermented product). Journal of the Science of Food and Agriculture 71: 442-448.

Gould, C. W. 1989. Mechanisms of Action of Food Preservation Procedures. Elsevier Applied Science, London.

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

International Commission on Microbiological Specifications for Foods (ICMSF). 1986 Microorganisms in Foods 2, Sampling fo Microbiological Analysis. Principles an Specific Applications. 2nd edn. Blackwe Scientific Publications, Oxford.

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

Krieg, N. R. and Holt, J. G. 1984. Bergey' Manual of Systemic Bacteriology. Vol. Williams and Wilkins, Baltimore.

Larmond, E. 1977. Laboratory Methods f Sensory Evaluation of Food. Cana Department of Agriculture, Ottawa.

Matte, G. R,, Matte, M. H., Sato, M. I. Z.; Sanchez, P. S., Rivera, I. G. and Marti M. T. 1994. Potentially Pathogenic Vibr associated with mussels from a trop region on the Atlantic coast of Bra Journal Applied Bacteriology 77: 281-28

Pace, J., Wu, C. U. and Chai, T. 19 Bacteriological flora in pasteurised oys after refrigerated storage. Journal of F Science 53: 325-327.

Samson, R. A. and van Reenen-Hoekstra, E Van 1988. Introduction to Food-b

Fungi, 3rd edn. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

Singleton, F. L., Attwell, R., Jangi, S. and Colwell, R. R. 1982. Effects of temperature and salinity on *Vibrio cholerae* growth. Applied and Environmental Microbiology 44: 1047-1058.

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

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

Son, N. T. and Fleet, G. H. 1980. Behaviour of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during depuration, re-laying and storage. Applied and Environmental Microbiology 40: 994-1002.

Vanderzant, C. and Splittstoesser, D. F. 1992. Compendium of Methods for the Microbiological Examinations of Foods. 3rd edn. American Public Health Association. Washington D. C