PII: S0956-7135(96)00076-X

PAPER

Bacteriological quality of ice used in Mucuripe Market, Fortaleza, Brazil

Regine H. S. F. Vieira,[†] Oscarina V. de Souza[‡] and Thakor R. Patel[*§]

A close examination of the microbiological quality of the ice made and used in the local markets in Fortaleza, Brazil, revealed it to be of poor quality. Besides the common food spoilage organisms often encountered in sea foods, foodbourne pathogens (streptococcus, listeria and fecal coliforms) were also detected. Gram-negative bacilli/cococacilli outnumbered all other groups by representing 78% of the total 90 isolates. The remaining 12% included gram-positive non-spore forming rods and gram-positive cocci. © 1997 Elsevier Science Ltd.

Keywords: ice quality; fish refrigeration

INTRODUCTION

Fish quality deteriorates rapidly during storage and transportation (Vieira and Vieira, 1989). The effective design and implementation of a hazard analysis and critical control point (HACCP) system for fresh fish is dependent on the identification of those process steps that determine most of the contamination. Although a HACCP programme has recently been adopted by the Brazilian Ministry of Agriculture to improve the quality of fish for export it has yet to be implemented. Moreover, many of the local fish handlers are unaware of the basic rules needed to safeguard quality and safety of the seafoods. Hence, unsanitary practices are common in the market place.

Ice is commonly used to retain freshness of fish. However, if polluted water is used to make ice, it is likely to contaminate the fish during cooling. The objective of the present report is to evaluate the bacteriological quality of the ice in the market place.

MATERIALS AND METHODS

Source and collection of samples

Thirty ice samples were procured from three fish market stalls, designated A, B and C. Ten samples from each were placed in sterilized, large glass beakers. These were then placed in insulated plastic containers (isothermic box) and transported to the laboratory. Each sample weighed about 1 kg and was analysed in the same way.

Aerobic plate counts

Decimal dilutions were prepared, plated on to trypticase soy agar (Difco) incubated at 35°C for 48 h and then examined. Results were recorded as colony forming units/ml.

Most probable number

For a presumptive test for a coliform group the 3-tube MPN method (Hitcheins *et al.*, 1992) was

†Marine Sciences Laboratory, Federal University of Ceara, Fortaleza, CE., Brazil. ‡Marine Science Laboratory, Federal University of Ceara, Fortaleza, CE., Brazil. §*Department of Biology, Memorial University of Newfoundland, St John's, NF., Canada. *To whom correspondence should be addressed.

used. Thawed ice samples were diluted 1:10, 1:100 and 1:1000 in sterile physiological saline. A 1 ml portion of each thawed ice dilution was inoculated into lauryl sulphate tryptose (LST) broth tubes (3 tube MPN) and incubated at 35°C for 48 h. All the tubes were examined for gas production at 24 and 48 h. All tubes showing gas within 48 h were recorded and MPN tables for the 3-tube dilutions were used to estimate the bacterial densities (Oblinger and Koburger, 1984).

Isolation and identification of the isolates

Ninety colonies were isolated from the trypticase soy agar plates and classified according to the published keys (Cardonha *et al.*, 1994; Bergery's Manual, 1974) for (i) gram-negative bacilli/coccobacilli that utilize glucose either oxidatively or fermentatively, (O/F, +/+); (ii) gram-negative bacilli/coccobacilli that metabolize glucose oxidatively, (O/F, +/−); (iii) gram-negative bacilli/coccobacilli that are neither oxidative nor fermentative with respect to glucose; (iv) gram-negative bacilli and coccobacilli based on falgellation; (v) gram-positive bacilli, non-sporulating; and (vi) gram-positive cocci.

RESULTS AND DISCUSSION

Table 1 summarizes the bacterial content of the ice samples collected from the three stalls in the local fish market. The bacterial counts ranged between 100 and 1080, 91 and 2700, and 10 and 2400 in samples from Stalls A, B and C, respectively. The average counts (cfu/ml) for the ten samples from stalls A, B and C were 326, 968 and 743, respectively. Although the average counts cannot be considered relatively high, the diversity of genera (*Table 2*) encountered in the samples raises some concern. The presence of *Streptococcus faecalis*, *Virbio* spp. and *Listeria* spp. is certainly of importance to the Department of Public Health. The marine organisms such as *Flavobacterium*, *Vibrio*, *Aeromonas* and *Moraxella* are important spoilage organisms of concern to the seafood industry.

Table 1 Aerobic plate counts of ice samples obtained from three different stores

Samples	cfu/ml		
	Fish stall A	Fish stall B	Fish stall C
1	180	160	160
2	960	760	100
3	210	2700	770
4	104	280	1900
5	100	760	100
6	1080	249	1220
7	93	91	10
8	320	640	183
9	109	1910	2430
10	108	2130	560

Table 2 Distribution of various strains isolated from ice samples

Gram-negative bacilli/cocbacilli	Strains	
	Number	%
Photobacterium leiognati	28	35.9
Enterobacteriaceae	19	24.3
Vibrio splendidus I	8	10.3
Pseudomonas spp	3	3.8
Chromobacterium violaceum	2	2.6
Vibrio spp	2	2.6
Vibrio gasogenes	2	2.6
Moraxella spp	1	1.3
Aeromonas hydrophyla	5	6.4
Aeromonas sobria	2	2.6
Total	78	100.0

A higher percentage of the bacteria isolated belonged to genera such as *Lactobacillus*, *Streptococcus*, *Planococcus* and *Photobacterium* and the members of the family Enterobacteriaceae. This suggests that perhaps the containers holding the ice may have been contaminated by previous fish. This suggests the potential of transferring bacteria from fish to ice and to fresh fish via the holding containers.

Ice is an excellent medium to prolong the freshness of fish (Sanchez, 1983) provided adequate sanitary conditions are maintained in handling. It is common to use potable water to make ice; however, the use of contaminated water creates problems during processing and/or transport, resulting in poor quality of fish. Ice made under poor sanitary conditions may become a risk factor to consumers. The transfer of cholera-causing *Vibrios* from ice to fish is not uncommon. The MPN of fecal coliforms of the samples ranged between zero and 1100/ml (*Table 3*). Their presence in the ice samples violates the requirements of the National Environmental Council of Brazil (CONAMA). The regulation states that fresh, potable water must be free of total and fecal coliforms.

Due to the lack of technology and better facilities, ice is often transported by manual labour using hand-pushed wheelbarrows. This is not the most desirable or scientific method of carrying ice from factories to the market place. The possibility of fecal coliforms

Table 3 MPN values for fecal coliforms in the ice samples

Samples	Fecal coliforms, MPN/ml		
	Fish stall A	Fish stall B	Fish stall C
1	43	150	150
2	0	0	0
3	1100	460	0
4	0	93	120
5	0	75	0
6	4	0	23
7	240	93	0
8	4	9	23
9	0	11	43
10	0	7	4

Table 4 Isolation of gram-positive rods and cocci from ice samples

	Strains	
	Numbers	%
Gram-positive rods		
Lactobacillus spp.	5	62.5
Listeria	2	25.0
Kurthia	1	12.5
Total	8	100.0
Gram-positive cocci		
Streptococcus faecalis	2	50.0
Planococcus spp.	2	50.0
Total	4	100.0

coming in contact with the ice is increased by contact with soil during transportation. However, the ice used in the present case was transported to the retail fish stalls by the supplier in a truck.

The samples that showed an absence of fecal coliforms may be due either to the use of good quality water in making the ice; and/or there may have been a gradual decline in the mesophilic coliforms during the manufacturing, transportation and storage of the ice. However, post manufacturing contamination cannot be ruled out.

Since chlorine is a potent killer of bacterial cells (McCrady and Longevin, 1932; Cutter and Siragusa, 1995; Reina *et al.*, 1995) it is likely that in the present case the water used in making the ice was probably not chlorinated or was contaminated after leaving the factory. An earlier report (FAO, 1975) strongly emphasized the importance of chiling fish at sea and on land. Another group (Rice *et al.*, 1985) evaluated the concept of washing fresh grouper in ozonized water and storage in ice made from ozonized water. Continuous applications of ozonized water and ice retarded microbial growth.

CONCLUSIONS

The study shows that the microbiological quality of fish sold in the Fortaleza fish market is not in accordance with the standards imposed by the National Environmental Council of Brazil (CONAMA, 1986). These require that fresh water used to process/

preserve fish must be coliform-free. The ice used to preserve the fish examined in this particular study clearly did not conform to the regulations of the National Environmental Council of Brazil.

Therefore, there is an urgent need for local Public Health Services to make more frequent inspections and for standards to be enforced.

REFERENCES

Anonymous (1994) Workshop on sanitation in fisheries: precautions and quality of seafoods. *Higiene Alimentar* **8**(31), 5–10, special edition, Sau Paulo.

Buchanan, R. E. and Gibbon, N. E. (1974) In *Bergey's Manual of Determinative Bacteriology*, 8th edn. eds. R. E. Buchanan and N. E. Gibbon.

CONAMA, Brazil (1986) *National Council of the Environment.* Resolution of CONAMA, No 20, July 1986.

Cardonha, A. M.s., Casimiro, A. R. S. and Vieira, R. H. S. F. (1994) Identification of psychrotrophic bacteria from lobster tails from industrial processing in sodium polyphosphates. *Higiene Alimentar* **8**(31), 29–34.

Cutter, C. N. and Siragusa, G. R. (1995) Application of chlorine to reduce *Escherichia coli* on beef. *Journal of Food Safety* **15**, 67–75.

FAO (1975) *Fisheries Report No. 59.* Rev. 1, FAO, Rome.

McCrady, M. H. and Langevin, E. (1932) The coliaerogenes determination in pasteurization control. *Journal of Dairy Science* **15**, 321–329.

Oblinger, J. L. and Korburger, J. A. (1984) The most probable number technique. In *Compendium of Methods for the Microbiological Examination of Foods*, ed. M. L. Speck. American Public Health Association, Washington, DC.

Reina, L. D., Fleming, H. P. and Humphries, E. G. (1995) Microbila control of cucumber hydrocooling water with chlorine dioxide. *Journal of Food Protection* **58**, 541–546.

Rice, R. G., Otwell, W. S., Blake, N., Sweat, D. E., Marschalk, R. L. and Farquhar, J. W. (1985) Ozonized water and ice to preserve fresh fish, grouper (Mycopteroperca). Intl. Inst. of Refrigeration, Paris, France, **60** (4), 187–193.

Sanchez, L. (1983) Storage of fish products. In *Manual of Storage and Packaging*, eds. Marney Pascoli Cereda and Sanchez Luiz, 153–174.

Vieira, R. H. S. F. and Vieira, G. H. F. (1989) Handling of fish products under refrigeration on fishing boats. In *Science and Technology of Fisheries Products: Methods of Preservation and Transportation of Fisheries Products*, eds Vieira and Vieira, **3**, 3001–3004.