

## Bacterial Counts in Fresh South-Harvested Fish While Loading in Shiraz

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**Abstract:** Since fish meat, which has a wide range of consumption among people, may harbor pathogens its microbial quality is crucially important in public health. This study focuses on the level of bacterial counts in three kinds of fish (*Scomberomorus commerson*, *Scomberomorus juttatus*, *Otolithes ruber*) harvested from some South coastal areas of Iran (Booshehr, Hormozgan and Sistan-Baluchestan Provinces) subjected to refrigerated-transportation to Shiraz. Hence, this research project was commenced to address whether freshly harvested fish, stored under 5-10 h refrigeration, presented any likely problems such as generating bacterial levels in excess of regulatory standards. In this study, 25 samples of *Scomberomorus commerson*, 45 samples of *Scomberomorus juttatus* and 45 samples of *Otolithes ruber* were studied for total bacteria, coliform and *Vibrio parahaemolyticus* counts. However, none of the samples exceeded the standard value and this study shows that the overall microbial quality of fresh South-harvested fish is acceptable and according to the standard values, while it is loaded in Shiraz.

**Key words:** Total bacterial count, coliform, *Vibrio parahaemolyticus*, fish, coastal areas, Shiraz

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### INTRODUCTION

The importance of fish as a source of animal protein is well understood and the avoidance of quality losses and the contamination of harvested fish with pathogenic bacteria are very important. The number and the nature of the fish bacteria are influenced by many factors such as sea water pollution, temperature, method of capture, preservation methods and handling practices.

Numerous species of fish occur along the Iranian coastline, but this study focuses on three main species, which have a high consumption in Shiraz. These fish species are harvested at several stations along the coasts of the Persian-Gulf and the Oman sea in Iran and are transported at ambient temperatures (approximately, 0°C) prior to loading and marketing in Shiraz. Transportation usually takes 5-10 h and this study was conducted to determine the effect of this transportation period on the bacteriological quality of the fish harvested from these south-coastal areas of Iran.

### MATERIALS AND METHODS

Because of being carried in refrigerated transporting vehicles, it usually took the harvested fish, 5-10 h to reach the markets in Shiraz. In these vehicles, fish were carried in ice-powder in order to keep the temperature around 0°C.

In this study, 25 samples of *Scomberomorus commerson*, 45 samples of *Scomberomorus juttatus* and 45 samples of *Otolithes ruber* were collected randomly, while the fish were being loaded in Shiraz (5-7 o'clock in the morning). Samples were collected in an ice box and taken to the laboratory, where they were immediately examined duplicately for the bacteriological quality.

Fish were analyzed at three different treatment levels: skin, muscle and gills.

Samples of different parts (skin, muscle and gills) of every fish were prepared for analysis according to the Iranian Institute of Standard and Industrial Studies (2006) and examined quantitatively for standard plate count, coliforms and *V. parahaemolyticus*. Standard plate count was performed according to the Iranian Institute of Standard and Industrial Studies (2008), Coliforms and *V. parahaemolyticus* were enumerated using the techniques specified in the Iranian Institute of Standard and Industrial Studies (2005, 2007), respectively. All bacteriological media were obtained from Merck, Germany.

SPSS software (Repeated measure and t-test) were employed to statistically evaluate the data.

### RESULTS

Microbiological standards for fish are prescribed in Iranian Institute of Standard and Industrial Studies (1999).

Table 1: The bacteriological status of the fish samples (cfu g<sup>-1</sup>)

Samples	No. of samples	Sampling area	Total bacterial count average (SV*: 10 <sup>6</sup> -10 <sup>7</sup> )	Coliform count average (SV: 4.0×10 <sup>2</sup> )	<i>Vibrio parahaemolyticus</i> count average (SV: 10 <sup>2</sup> )
Scomberomorus commerson	25	Skin	5.5×10 <sup>4</sup>	2.3×10 <sup>1</sup>	5.7×10 <sup>1</sup>
		Muscle	10 <sup>2</sup>	0.04×10 <sup>1</sup>	1.4×10 <sup>1</sup>
		Gills	5.5×10 <sup>6</sup>	2.5×10 <sup>1</sup>	3.6×10 <sup>1</sup>
Scomberomorus juttatus	45	Skin	5.5×10 <sup>7</sup>	6.0×10 <sup>1</sup>	5.1×10 <sup>1</sup>
		Muscle	10 <sup>4</sup>	1.3×10 <sup>1</sup>	9.0×10 <sup>1</sup>
		Gills	10 <sup>6</sup>	3.5×10 <sup>1</sup>	5.6×10 <sup>1</sup>
Otolithes ruber	45	Skin	10 <sup>8</sup>	3.0×10 <sup>1</sup>	2.7×10 <sup>2</sup>
		Muscle	5.5×10 <sup>2</sup>	0.2×10 <sup>1</sup>	3.1×10 <sup>1</sup>
		Gills	10 <sup>8</sup>	1.8×10 <sup>1</sup>	6.6×10 <sup>1</sup>

\*Standard value (cfu g<sup>-1</sup>)

Table 2: Comparison of the bacteriological status of the fish samples (cfu g<sup>-1</sup>) with the standard value

Grade of samples	No. of samples	Sampling area	Samples exceeded standard value of TBC*		Samples exceeded standard value of Coliform		Samples exceeded standard value of Vibrio	
			No.	%	No.	%	No.	%
Scomberomorus commerson	25	Skin	3	12.0	0	0.0	3	12.0
		Muscle	0	0.0	0	0.0	1	4.0
		Gills	6	24.0	0	0.0	1	4.0
Scomberomorus juttatus	45	Skin	5	11.1	1	2.2	4	8.8
		Muscle	0	0.0	0	0.0	3	6.6
		Gills	4	8.8	1	2.2	4	8.8
Otolithes ruber	45	Skin	5	11.1	1	2.2	2	4.4
		Muscle	0	0.0	0	0.0	1	2.2
		Gills	7	15.5	0	0.0	2	4.4

\*TBC: Total Bacterial Count

It stipulates that the total content of the fish shall not contain a total mesophilic bacteria, faecal coliform and *Vibrio parahaemolyticus* density of >10<sup>6</sup>-10<sup>7</sup>, 4.0×10<sup>2</sup> and 1.0×10<sup>2</sup> Colony Forming Units (CFU)/g, respectively.

Out of total 115 samples, 13 skin samples (11.3%) and 17 gill samples (14.8%) exceeded the standard value of total mesophilic aerobic count, which is 10<sup>6</sup>-10<sup>7</sup> cfu g<sup>-1</sup>. In case of total coliform count, 2 skin samples (1.7%) and 1 gill sample (0.9%) crossed the standard value, which is 4×10<sup>2</sup> cfu g<sup>-1</sup>. Similarly, 9 skin samples (7.8%), 5 muscle samples (4.3%) and 7 gill samples (6%) were found crossing the standard value of *Vibrio parahaemolyticus* count (10<sup>2</sup> cfu g<sup>-1</sup>). The average counts for total mesophilic aerobic bacteria, coliform and *Vibrio parahaemolyticus* were found to be 5.2×10<sup>7</sup>, 3.8×10<sup>1</sup> and 4.5×10<sup>1</sup> cfu g<sup>-1</sup> for skin, 3.5×10<sup>3</sup>, 0.5×10<sup>1</sup> and 4.5×10<sup>1</sup> cfu g<sup>-1</sup> for muscle and 3.5×10<sup>3</sup>, 2.6×10<sup>1</sup> and 5.3×10<sup>1</sup> cfu g<sup>-1</sup> for gills, respectively.

There were not significant differences between the results of the present study with the standard values (p>0.05). And this study shows that the overall microbial quality of fish, encountered to the traditional way of transportation, is found to be satisfactory.

The bacteriological status of the samples are presented in Table 1 and the comparison of the bacteriological status of the samples (cfu g<sup>-1</sup>) with the standard value is shown in Table 2.

## DISCUSSION

This investigation presents the current status of microbial quality of south-harvested fish being sold in Shiraz. From the total analyzed fish samples (n = 115), 13% exceeded standard value of mesophilic aerobic count, 1.3% crossed the standard value of total coliform count and 6.1% were beyond the standard value of *Vibrio parahaemolyticus* count. These results are generally similar to the findings of other authors and can be supported by the research of Koutsoumanis *et al.* (2002) and Alam *et al.* (2006).

It seems that *Vibrio parahaemolyticus*, which is a major cause of seafood-borne human disease occurred at very low frequencies in the samples of the present study. Although, *Vibrio parahaemolyticus* counts in these fish samples were not excessive, fish could pose a risk of *Vibrio parahaemolyticus* food poisoning if harvested in the summer period, when levels of *Vibrio parahaemolyticus* are highest in marine waters and it will be even worse if the summer-harvested fish is eaten raw or semi-raw. This risk would be considerably reduced however, if fish are harvested during winter (Bouchriti *et al.*, 1995). *Vibrio parahaemolyticus* has been shown to be responsible for incidents of food poisoning when present in large numbers (10<sup>6</sup> viable cells g<sup>-1</sup>) in seafood. Also, *V. parahaemolyticus* is a natural bacterial

inhabitant of marine waters and hence its survival pattern in fish during storage is also of interest (Bouchriti *et al.*, 1995).

Vibrios are located in the sediments, but they are resuspended, when the temperature increases (Bryan, 1980; Kaneko and Colweil, 1973) in a previous study, only 11 (2%) of 582 isolates were identified as *V. parahaemolyticus*. The absence of *V. parahaemolyticus* could be attributed to its actual absence or to the relative selectivity of the TCBS agar used for its isolation (Balakrish *et al.*, 1980; Kourany, 1982). Consequently, techniques for isolation of *V. parahaemolyticus* from fish should be evaluated and improved, if necessary (Bouchriti *et al.*, 1995).

The increase in bacterial loads of some of these commercial fish may be attributed to the factors such as contaminated containers and mishandling. The multiplication of bacteria at ambient temperatures may also have contributed to this increase. Both fecal indicator bacteria and pathogens have been shown to multiply in marine products after harvest and prior to marketing (Cook and Rupie, 1989). But, from the present study it is found that the changes in the level of bacterial counts from harvest to sale does not make the South-harvested fish to be exceeded the standard value.

### CONCLUSION

It can be concluded that although there are some defects in the traditional way of fish-transportation in Iran, this way does not lead the product to reach the critical microbial point.

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### REFERENCES

Alam, M., A. Sadique, N.A. Hasan and N.A. Bhuiyan *et al.*, 2006. Effect of transport at ambient temperature on detection and isolation of *Vibrio cholerae* from environmental samples. Applied Environ. Microbiol., 72(3): 2185-2190. DOI: 10.1128/AEM.72.3.2185-2190.  
Balakrish, G.N., A. Martin and R. Natarajan, 1980. Distribution of *Vibrio parahaemolyticus* in finfish harvested from Porto Novo (S. India) environs: A seasonal study. Can. J. Microbiol., 26: 1264-1269. <http://www.ncbi.nlm.nih.gov/pubmed/7214217>.

Bouchriti, N., A.E. Marrakchi, S.M. Goyal and R. Boutaib, 1995. Bacterial Loads in Moroccan Mussels from Harvest to Sale. J. Food Protect., 58 (5): 509-512.  
Bryan, F.L., 1980. Epidemiology of food-borne diseases transmitted by fish and shellfish and marine crustaceans in the United States, 1970-1978. J. Food Protect., 43: 859-876.  
Cook, M.S. and A.D. Rupie, 1989. Indicator bacteria and *Vibrionaceae* multiplication in post-harvest shellstock oysters. J. Food Protect., 52: 343-349.  
Iranian Institute of Standard and Industrial Studies, 1999. Microbiological quality of fish, No. 2394-1.  
Iranian Institute of Standard and Industrial Studies, 2005. Microbiology of food and animal feeding stuffs, detection and enumeration of presumptive *Escherichia coli*, most probable number technique. No. 2946.  
Iranian Institute of Standard and Industrial Studies, 2006. Microbiology of food and animal feeding stuffs, preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 3: specific rules for the preparation of fish and fishery products, No. 8923-3.  
Iranian Institute of Standard and Industrial Studies, 2007. Microbiology of food and animal feeding stuffs, horizontal method for the detection of potentially enteropathogenic *Vibrio* sp. part 1: detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*, No. 9667-1.  
Iranian Institute of Standard and Industrial Studies, 2008. Microbiology of food and animal feeding stuffs, preparation of test samples and initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of initial suspension and decimal dilutions, No. 8923-1.  
Kaneko, T. and R.R. Colweil, 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. J. Bacteriol., 113: 24-32.  
Kourany, M., 1982. Medium for isolation and differentiation of *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. Applied Environ. Microbiol., 45: 310-312.  
Koutsoumanis, K., M. Giannakourou, P.S. Taoukis and G.J.E. Nychas, 2002. Application of SLDs (Shelf life Decision system) to marine cultured fish quality. Int. J. Food Microbiol., 73: 375-382. DOI: 10.1016/S0168-1605(01)00659-6.