Arab Gulf Journal of Scientific Research, 20 (1), pp 44-49 (2002)

ORIGINAL PAPER **(REF. 2096)**

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Presence of Vibrios and Aeromonas Strains and Total Psychrotrophs in Hexagonal-Spotted Grouper and Spanish Mackerel Fish

Abstract: This work was conducted on different parts of hexagonal-spotted grouper and Spanish mackerel fish from 10 major fishery shops in Riyadh City to assess the presence of vibrios and aeromonas using the API20E and Biolog system. Also, pH and total psychrotrophs were asesssed as two indexes of quality. Similar data were obtained from both identification systems except for *Aeromonas media-like* and *Vibrio anguillarum* which are not included in the API20E database. *Aeromonas hydrophila*, *A. media-like*, *V. alginolyticus*, *V. anguillarum*, *V. damsela* and *V. fluvialis* were recovered from fish samples with *A. media-like* and *V. damsela* most predominant. Unexpectedly, *V. cholera* and *V. parahemolyticus* were not recovered. The number of skin samples of grouper having more than 7 log CFU/gm were much higher than that of mackerel skin samples. Similar psychrotrophic counts were noticed in gut samples of the two fish types, indicating similarity in growth habitat. Most of the grouper flesh samples had a pH in the range of > 6.40-7.02, whereas 95% of mackerel samples had a pH at 6.40 or below.

Keywords: *Vibrio*, *Aeromonas*, Psychrotrpohs, Grouper, Mackerel.

سلالات بكتيريا الفبريو والإيروموندس والعدد الكلي لمتحملات البرودة في أسماك الهامور والكنعد

مسفر بن محمد الدقل

المستخلص: تم إجراء هذه الدراسة على أجزاء مختلفة من أسماك الهامور والكنعد من عشرة منافذ بيع رئيسية للأسماك بمدينة الرياض، وذلك لتقييم وجود بكتيريا الفبريو والإيروموندس بإستخدام نظامي API 20E و Bilog . كما تم تقدير عدد البكتيريا المتحملة للبرودة وكذا قيم pH للحم السمك. أظهرت النتائج تشابه النظامين في التعرف على أفراد الفبريو والإيروموندس فيما عدا النوعين *Aeromonas media-like* و *Vibrio anguillarum* إذ أنهما غير مضمنين في قاعدة معلومات نظام API 20E. . وفي العموم، أمكن عزل والتعرف على *A. hydrophila* و *A. media-like* و *V. anguillarum* و *V. damsella* و *V. vluvialis*.. من عينات الأسماك . وكان أكثر هذه السلالات شيوعاً *A. media-like* و *V. damsela*. وعلى غير المتوقع، لم يكن من الممكن عزل *V. cholera* و *V. parahaemolyticus*. كما أظهرت الدراسة أن عدد عينات جلد الهامور التي بها أعداد متحملات البرودة <7 لو وم م/جم كان أعلى من تلك لجلد الكنعد، بينما كان عدد متحملات البرودة لعينات القناة الهضمية متشابهة للهامور والكنعد مما يدل على تشابه بيئة نموهما. وأخيراً، اتضح أن معظم عينات لحم الهامور لها pH أعلى من المدى 6.40-7.02 بينما كانت قيم pH في 95% من عينات الكنعد عند 6.40 أو أقل.

كلمات مدخلية: بكتيريا، الفبريو، الإيروموندس، متحملات البرودة، أسماك، الهامور، الكنعد.

Introduction

Seafood is a good source of protein and other important dietary elements such as minerals and vitamins. Consumer awareness of the health benefits of seafood has increased the demand for fish, especially in developing countries (James 1986). In Saudi Arabia, fish production reached 56331 tons in 1998, compared to 46063 tons in 1988 (Anonymous 2000).

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Increasing public, agricultural, and industrial activities near the waters where fish are caught, however, have made seafood one major source of foodborne diseases (Liston 1990). Furthermore, short shelf-life of fish results not only from fish-associated intrinsic parameters, but also from heavy microbial contamination as well as poor handling, especially poor cooling. Generally, microorganisms are present on the skin, in the gills, and in the intestine of live healthly fish (Venugopal 1990).

Vibrio species are among the natural inhabitants of the marine environment, especially coastal waters of the tropics (Thampuran and Surendran 1998, Oliver *et al.* 1983). This genus includes 28 species, of which eleven may cause wound infection, ear infection, septicemia, and foodborne gastroenteritis which is caused by *V. cholera*, *V. parahaemolyticus*,

V. vulnificus, *V. mimicus*, *V. hollisae*, and *V. furnisii* (Hackney and Dicharry 1988, Oliver 1985, Morris and Black 1985).

Aeromonas is a big genus comprising 12 well-defined species, and several ill-defined and unnamed hybrid species (Janda *et al.* 1996). They are recognized as gastrointestinal pathogens (Gelbart et al. 1985), and have been isolated in high numbers from fish samples (Wong *et al.* 1992, Hanninen *et al.* 1997). *Aeromonas* spp., especially *A. hydrophila*, contribute to spoilage of fish from warm waters (Alur *et al.* 1971). The presence of this genus and other mesofiles may be attributed in part to prolonged delay in icing of fish (Barile *et al.* 1985).

Microbiological studies on fresh fish produced and sold in Saudi Arabian markets are limited. This study, therefore, was initiated to assess the significance of *Vibrio* and *Aeromonas* (being similar groups) in fresh fish sold at major fish sales outlets in Riyadh City (Saudi Arabia). In addition, psychrotrophic count (PC) and pH of the fish samples were measured as two important indexes of quality.

Materials and Methods

Samples of fresh hexagonal-spotted grouper (*Epinephelus chlorostigma*) (locally known as *Hamour*) and Spanish mackerel (*Scomberomorus commerson*) (locally known as *Canad*) were collected during summer season from ten major fish sale outlets in Riyadh City. Two samples (on different days) of each of the fish species were drawn from each outlet. Samples were always put in sterile plastic bags, covered with crushed ice, and then transported to the laboratory within 30-45min.

The skin (25gm) of grouper and mackerel was removed from muscle with sterile forceps and scalpel, and was transferred to sterile plastic bags. Gill and the whole gut were obtained for grouper samples only. Whole gut was not readily available for mackerel samples and lining tissues of the gut cavity were obtained instead.

Samples were transferred into sterile plastic bags and 0.85% -saline buffer was added and homogenized with Stomacher homogenizer (Seward Medical, London, U.K.) for 2 min. This 10-fold serial dilution was considered as the original homogenate.

For *vibrio* identification, the original homogenate was cultured in tubes of alkaline peptone water (APW) for 3-tubes most probable number (MPN) (Kaysner *et al.* 1992). This method was used to detect the presence of *vibrios* in each fish sample at any level of dilution, but was not applied to calculate the final count of these microorganisms. Inoculated tubes were incubated at 35°C for 18hr. From the three highest dilution tubes showing growth, tubes were streaked onto thiosulfate citrate-bile salt agar (TCBS) (LAB M, Bury, England). After incubation for 24hr at 35°C, representative colonies (3-5) were transferred onto 3%-NaCl tryptic soy agar (TSA) slant and incubated at 35°C for 24hr, and then were kept refrigerated. At the time of identification, cultures were activated on 3%-NaCl TSA soy agar and a bacterial suspension was prepared based on the manufacturer instructions of both identification systems; API 20E (bioMÈrieux sa, France) and Biolog system (Biolog Inc., California, USA). API 20E and Biolog are identification systems based on biochemical reactions of the microorganisms. The Biolog system has 95 different tests while the API 20E has only 20 tests besides a few more additional tests such as an oxidase test.

Both identification systems were operated first with reference strains of *vibrio* (*Vibrio* hollaisae (ATCC 33563), *V. vulnificus* (ATCC 27562), *V. parahaemolyticus* (ATCC 17802), V. alginolyticus (ATCC 17749), *V. fluvialis* (ATCC 33809), *V. mimicus* (ATCC 33653), and *V. furnissi* (ATCC 35016).

For the psychrotrophic count (PC), samples were plated from the original homogenate on TSA, and then were incubated at 7°C for 10 days. (Cousin *et al.* 1992).

Flesh pH was measured with a pH meter (Sergent Welch 8000, Skokie, IL) after mixing 20gm of the tissue with 20ml of distilled water for 2 min using a Stomacher homogenizer.

Results and Discussion

Selected colonies from TCBS were all gram negative and oxidase positive. All *vibrio* colonies showed a good growth at 6% NaCl (TSA with 6% NaCl), while no apparent growth for *Aeromonas* isolates was evident. This is a key test to differentiate *vibrio* from *Aeromonas* (Palumbo *et al.* 1992).

Table 1 shows that *Aeromonas media-like* was the more predominant in all tested fish samples (both grouper and mackerel). Recent work has shown that *Aeromonas* are ubiquitous in the water environment and common contaminants of fish. It was present in 93% of tested fish samples (Hanninen *et al.* 1997).

Table 1: Incidence of *Vibrios* and Aeromanas (based on Biolog system profile) isolated from Hexagonal-spoted grouper and Spanish mackerel sold in Riyadh City (n = 20 sample, from 10 shops).

Microorganism	Hexagonal-spotted grouper			Spanish mackerel	
	Skin	Gut	Gill	Skin	Gut
Aeromonas hydrophila	5%	ND*	5%	ND	DN
A. *media-like*	45%	30%	45%	5%	5%
Vibrio alginolyticus	ND	10%	5%	ND	ND
V. anguillarum	35%	5%	25%	5%	5%
V. damsela	5%	60%	30%	30%	30%
V. fluvialis	ND	ND	5%	ND	ND

* *ND=not detected*

Likewise, *V. damsela* was the predominant species among *vibrios* especially in the gut and skin of both fish types. This agent is a halophilic bacterium that has been linked with infections in humans (Clarridge and Zighelboim-Daum 1985) and fish such as damselfish (Love *et al.* 1981). It was also recovered from sea water and marine animals (Buck 1990). Careful handling of seafood, therefore, should be practiced to avoid skin puncture.

API and Biolog gave different results for three strains of *Vibrio* and *Aeromonas* isolates. *Aeromonas* hydrophila, *V. fluvialis*, and *Listonella damsela* (identified with the API system) were identified with the Biolog system as *Aeromonas media-like*, *V. anguillarum*, and *V. damsela*, respectively. *A. media-like* and *V. anguillarum* are not included in the database of the API 20E. *V. damsela* (along with *V. anguillarum* and *V. ordalii*) has been transferred into the new genus *Listonella* (MacDonell and Colwell 1985), which is not included in the Biolog database by this name.

V. parahaemlyticus, although of public health concern and largely associated with seafood in different parts of the world (Dalsgaard 1998, Liel 1986, Molitoris *et al.* 1985, Sanjeev and Stephen 1993, Kaysner *et al.* 1990) was not detectable in any of the tested fish samples. This result may be due to low initial contamination with microorganisms. This microorganism, in addition, is cold sensitive and known to be injured by refrigeration (Rusul *et al.* 1991). Other studies, although limited, have reached the same findings (Frenandez *et al.* 1988, Potravnova *et al.* 1988).

V. alginolyticus was present in grouper gut and gill samples, but not in mackerel samples. Studies have shown that this species along with *V. parahaemolyticus* may be distributed world wide in marine environments (Matte *et al.* 1994, Chan *et al.* 1989, Molitoris *et al.* 1985).

The incidence of *V. anguillarum* was relatively high especially in skin and gill samples of grouper. This species is not yet considered in the API 20E system (Santos 1993) and identified with this system as *V. fluvialis* . Pedersen and Larsen (1998) have stated that the Biolog system may be valuable for rapid typing of this microorganism. The main differences between the two species is arabinose fermintation (Kaysner 1992). *V. anguillarum* is considered one of the most important disease causing agents of fish cultured in marine waters (Toranzo and Barja 1990, Pazos *et al.* 1993).

V. fluvialis was present in one sample of grouper gill. One study has found this microorganism among the common *vibrios* in seafood and aquaculture (Wong and others 1992), and it comprised 27% among other *vibrios* isolated from seafood (oyster) samples (Matté *et al.* 1994).

Psychrotrophic Count

Figure 1 shows the total population of psychrotrophs on grouper (skin, gut, and gill) and mackerel (skin and gut lining). The number of mackerel skin samples in the range >6-7 log/CFU were 9 (45%), whereas, one sample only of grouper skin samples fell in this range. On the other hand, 95% of grouper skin samples had counts higher than 7 log CFU/gm. At this limit, fish samples enter the spoilage region (7-8 log CFU/gm) (Ayers 1960, Zhuang *et al.* 1996). A study on grouper produced from Saudi waters have indicated microbiological shelf life of less than six days of ice storage at which the total count was more than 7 log CFU/gm. In one study, the initial count on grouper fish samples two days old was already more than 6 log CFU/gm (Dawood and Abu-Tarboush 1994). Another study on local mackerel skin showed low (4.26 CFU/gm) psychrotrophic count. It took eight days to reach 7.2 log CFU/gm. (Abu-Tarboush *et al.* 1996).

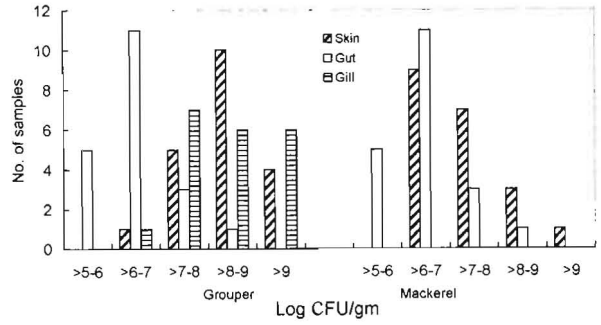

Figure 1: Psychrtrophic populations (number of samples in different ranges of log CFU/gm) in different parts of hexagonal-spotted grouper and Spanish mackerel fish.

The higher counts of microorganisms on grouper skin compared to those of mackerel can be attributed to the smaller size of grouper, which make it vulnerable to extensive handling and contamination throughout the production process.

The same number of gut samples of both fish types fell in all four ranges (>5-6, >6-7, >7-8, and >8-9). This result is a good indication that both fish types were caught from similar habitat. Eighty percent of each fish sample had psychrotrophic counts below 7 log CFU/gm. The number and kind of microorganisms in the gut of fish can be associated with the environmental conditions and the feeding materials (Mayer and Ward 1991).

Grouper gill samples were heavily contaminated. Ninety five percent of the samples exceeded 7 log CFU/gm, and 60% of the samples had psychrotrophic counts higher than 8 log CFU/gm. The gill microorganisms can contaminate other parts of the fish with the aid of melting ice and poor handling.

pH

Figure 2 shows the number of fish samples in different pH ranges. Ninety percent of grouper samples were in the range >6.40 - 7.02, while 95% of mackerel samples were equal to or below pH 7.40. Such difference may be related to the difference in the microbial populations on both fish kinds. Data presented by Dawood and Abu-Tarboush (1994) indicated that pH of grouper flesh reached 7.07, at which samples were rejected based on microbial limits. The pH of mackerel in another study reached 6.02 at the microbial rejection level of 6 log CFU/g, and reached 6.24 and more for extended storage (Bennour *et al.* 1991). These studies and the current one suggest that the changes in pH as related to quality vary depending not only on the number of contaminating microorganisms and type of fish, but also on the type of microorganisms and storage conditions.

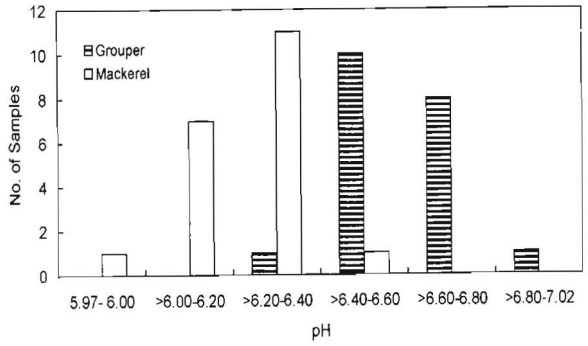

Figure 2: Flesh pH (number of samples in different ranges of pH values) of hexagonal-spotted grouper and Spanish mackerel fish.

Conclusion

Using biochemical-based systems (API20E and Biolog), *Aeromonas* hydrophila, *A. medial-like*, *Vibrio* alginolyticus, *V. anguillarum*, *V. damsela*, and *V. fluvialis* were identified from different parts of Spanish mackerel and hexagonal-spotted grouper fish. *V. cholera* and *V. parahaemolyticus*, however, were not detectable, and this can be attributed to the effects of low temperature.

Extensive handling of grouper fish seems to be the reason behind the higher population of psychtrophs. Similar psychrotophic counts were noticed in gut samples of grouper and mackerel.

Flesh pH of more than 90% of grouper samples was more than 6.40, while 95% of mackerel samples had pH between 6 and 6.40. The pH increase of grouper samples may be associated with the high number of psychrotrophs on the skin.

Acknowledgement

Thanks are expressed to Agricultural Experiment Center, College of Agriculture, King Saud University, for the financial support of this work.

References

Abu-tarboush, H.M., Al-Kahtani H.A., Atia M., Abou-Arab A., Bajaber A.S., and El-Mojaddidi M. (1996) Irradiation and postirradition storage at 2 (2(C of Tilapia (Tilapia nilotica x T. aurea) and spanish mackerel (Scomberomorus commerson): sensory and microbial assessment. J. Food Prot. **59**(10): 1041-1048.

Alur M.D., Lewis N.F., and Kumata, U.S. (1971) Spoilage potential of predominant organisms and radiation survivors in fishery products. Ind. J. Exp. Biol. **9**: 48-51.

Anonymous (2000) Fisheries Statistics of Saudi Arabia. Marine Fishery Department, Ministry of Agriculture and Water, Riyadh, Kingdom of Saudi Arabia.

Ayres, J.C. (1960) The relationship of organisms of genus Pseudomonas to spoilage of meat, poultry and eggs. J. Appl. Bacteriol. **23**: 471-486.

Barile, M.D., Milla, A.D., Reilly, A., and Villadsen, A. (1985) Spoilage pattern of mackerel (Rastrelliger faugggh ni Matsui) 2, Mesophilic and psychrotropic spoilage. Asian Food J. **1**(3): 121-127.

Bennour, M., El Marrkakchi, A., Bouchriti, N., Hamama, A., and El Ouadaa, M. (1991) Chemical and Microbiological assessments of Mackerel (Scomber scombrus) stored in ice. J. Food Prot. **54**(10): 784, 789-792.

Buck J.D. (1990) Potentially pathogenic marine *Vibrio* species in seawater and marine animals in the Sarasota, Florida, aera. J. Coast. Res. **6**: 943-948.

Chan, K.Y., Woo, M.L., Lam L.Y., and French, G.L. (1989) *Vibrio* parahaemolyticus and other halophilic *vibrio*s associated with seafood in Hong Kong. J. Appl. Bacteriol. **66**: 57-64.

Clarridge, J.E., and Zighelboim-Daum, S. (1985) Isolation and characterization of two haemolytic phenotypes of *Vibrio* damsela associated with fatal wound infection. J. Clin. Microbiol. **21**: 302-306.

Cousin, M., Jay, J.M., and Vasavada, P.C. (1992) Psychrotropic microorganisms. In: Vanderzant, C., and Splittstoesser, F. (eds.) Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed. American Public Health Association, Washington, DC, USA., pp. 153-168.

Dalsgaard, A. 1998. The occurrence of human pathogenic *Vibrio* spp.and Salmonella in aquaculture. Int. J. Food Sci. Technol. **33**: 127-138.

Dawood, A.A., and Abu-Tarboush, H.M. (1994) Assessment of shelf-life of sea water and farm fish in the Riyadh area during ice storage. Egypt. J. Appl. Sci. **9**(12): 27-41.

Frenandez, R.E., Nava-Fernandez, L.M., and Mota-de-la-Garaza, L. (1988) Absence of *Vibrio* parahaemolyticus from raw fish. Revista Latinoamerica de Microbiologia **30**(2): 91-96.

Gelbart, S.M., Prabhudesai, M., and Magee, S.M. (1985). *Aeromonas* sobria gastroenteritis in an adult: A case report. Am. J. Clin. Pathol. **83**(3): 389-391.

Hackney, C.R., and Dicharry. (1988) Seafood-borne bacterial pathogens of marine origin. Food Technol. March: 104-109.

Hanninen, M.L., Ovianen, P., and Hirveld-Koski, V. (1997) *Aeromonas* sepcies in fish, fish-eggs, shrimp and freshwater. Int. J. Food Microbiol. **34**(1): 7-24.

James, D.G. (1986) The prospects for fish for the undernourished. Food and Nutrition (FAO) **12**: 20-27.

Janda, J.M., Abbott, S.L., Khashe, S., Kellogg, G.H., and Shimada, T. (1996) Further studies on biochemical characteristics and serologic properties of the genus *Aeromonas*. J. Clin. Microbiol. **34**(8): 1930-1933.

Kaysner, C.A., Abeyta, J.R.C., Stott, R.F., Krane, M.H., and Wekell, M.M. (1990) Enumeration of *Vibrio* species, including *V. cholera* from samples of oyster growing area, Grays Harbor, Washington. J. Food. Prot. **53**(4): 300-301,311.

Kaysner, C.A., Tamplin, M.L., and Twedt, R.M. (1992) *Vibrio*. In: Vanderzant, C., and Splittstoesser, D.F (eds.). Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed. American Public Health Association, Washington, DC, USA, pp. 451-473.

Liel, T.K. (1986) Quality of fish in Kenya and existing problem. FAO Fisheries Rep. No. 329 (Suppl.): 427-432.

Liston, J. (1990) Microbial hazards of seafood consumption. Food Technol. Dec.: 56-62.

Love, M., Fisher, D.T., Hose, J.E., Farmer, J.J., Hickman, F.W., and Fanning, G.R. (1981) *Vibrio* damsela, as a marine bacterium, cause skin ulcers on the damselfish Chromis punctipinnis. Science **214**: 1140-1141.

MacDonell, M.T., and Colwell, R.R. (1985) Phylogeny of the *Vibrio*naceae and recommendation for the new genera *Listonella* and Shewanella. Syst. Appl. Microbiol. **6**: 171-182.

Matté, G.R., Matté, M.H., Rivera, I.G., and Martins, M.T. (1994) Distribution of potentially pathogenic *vibrio*s in oysters from tropical regions. J. Food Prot. **57**(10): 870-873.

Mayer, B.K., and Ward, D.R. (1991) Microbiology of finfish and finfish processing. In: Ward, D.R., and Hackney, C.R. (eds.) Microbiology of Marine Food Products. Van Nostrand Reinhold, pp.3-14.

Molitoris, E., Joseph, S.W., Krichevsky, M.I., Sindhuhardja, W., and Colwell, R.R. (1985) Characterization and distribution of *Vibrio* alginolyticus and *Vibrio* parahaemolyticus isolated in Indonesia. Appl. Envir. Microbiol. **50**(6): 1388-1394.

Morris, J.Jr., and Black, R.E. (1985) Cholerae and other *vibrio*s in the United States. N Engl. J. Med. **312**: 43-350.

Oliver, J.D. (1985) *Vibrio*: an increasingly troublesome genus. Diagan. Med. **8**: 43-49.

Oliver, J.D., Wiarner, R.A., and Cleland, D.R. (1983) Distribution of *Vibrio* vulnificus and other lactose fermenting *vibrio*s in the marine environment. Appl. Envir. Microbiol. **45**: 985-998.

Palumbo, S., Abeyta, C., and Stelma, G.,Jr. (1992) *Aeromonas* hydrophila group. In: Vanderzant, C., and Splittstoesser, F. (eds.). Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed. American Public Health Association, Washington, DC, USA, pp 497-515.

Pazos, F., Santos, Y., Magariños, B., Bandîn, I., Núñez, S., and Toranzo, A.E. (1993) Phenotypic characteristics and virulence of *Vibrio* anguillarum-related organisms. Appl. Envir. Microbiol. **59**: 2969-2976.

Pedersen, K., and Larsen, J.L. (1998) Characterization and typing methods for the fish pathogen *Vibrio* anguillarum. Recent Res. Devel. Microbiol. **2**: 17-93.

Potravnova, R.S., Evgratov, V.G., Baronin, V.V., Strunilin, N.A., Lubskii, V.N., Tyukova, M., and Shovkoplyas, O.A. (1988) Microflora of fish and fish products on board ship. Gigienai Sanitariya **9**: 86-87.

Rusul, G., Dahan, M.M., and Dollah, M. (1991) A survey of microbiological quality of fish sold in two local wet markets. Pertanika **14**(3):243-247.

Sanjeev, S, and Stephen, J. (1993) Incidence of *Vibrio* parahaemolyticus in fish and selfish marketed in Cochin. Ind. J. Mar. Sci. **22**(3): 70-73.

Santos, Y., Romalde, J.L., Bandin, I., Magariños, B., Núñez, S., Barja, J.L., and Toranzo, A.E. (1993) Usefulness of the API-20E system for the identification of bacterial fish pathogens. Aquaculture **116**: 111-120.

Thampuran, N., and Surendran, P.K. (1998) Occurrence and distribution of *Vibrio* vulnificus in tropical fish and shellfish from Cochin (India). Let. Appl. Microbiol. **26**: 110-112.

Toranzo A.E., and Barja, J.L. (1990) A review of the taxonomy and seoepizootiology of *Vibrio* anguillarum with special reference to Aquaculture in the Northwest of Spain. Dis. Aquat. Org. **9**: 73-82.

Venugopal, V. (1990) extracellular proteases of contaminant bacteria in fish spoilage: A review. J. Food Prot. **53**(4): 341-350.

Wong, H.S., Ting, S., and Shieh, W. (1992) Incidence of toxigenic *vibrios* in food available in Taiwan. J. Appl. Baceriol. **73**: 197-202.

Zhuang, R., Hauang, Y., and Beuchat, L.R. (1996) Quality changes during refrigerated storage of packaged shrimp and catfish fillets treated with sodium acetate, sodium lactate or propyl gallate. J. Food Sci. **61**(1): 241-224, 261

Received 08/05/2001, in revised form 04/12/2001