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MICROBIOLOGICAL QUALITY OF SALMON (*Salmo salar*) SOLD IN CITIES OF THE STATE OF SÃO PAULO, BRAZIL

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ABSTRACT

The present paper evaluated the microbiology of salmon by quantifying mesophilic heterotrophic microorganisms, total and thermotolerant coliforms, and the presence of *Vibrio parahaemolyticus, Staphylococcus aureus, Salmonella* sp., *Escherichia coli* and *Aeromonas* sp. in the meat. This study can provide technical support for the suggestion of a new regulation of a Brazilian legislation through specific microbiological standards concerning the consumption of raw fish. A number of 31 (16 cooled and 15 frozen) samples of salmon were collected in the retail market network of a few cities in the State of São Paulo, Brazil. Results presented populations of mesophilic heterotrophic microorganisms ranging from 1.0 x 10 and 3.9 x 10^6 CFU/g, total and thermotolerant coliforms in 32.25% and 19.35% of the samples, respectively, and *Aeromonas* sp. in 41.95% of the samples with a populational variation ranging from 2.0 x 10^2 to 8.0 x 10^3 CFU/g. *Staphylococcus aureus* was found in one sample whereas *Vibrio parahaemolyticus*, *Salmonella* sp. and *Escherichia coli* were not found. These results demonstrated the presence of potencially pathogenic microorganisms in fresh fish consumed in Brazil, highlighting the necessity of control measures to avoid public health problems related to the consumption of raw fish.

Key words: fish, salmon, microbiology, pathogenic bacteria.

INTRODUCTION

Fish is a highly digestible food and a source of high biological value and polyunsaturated fatty acids. It can be consumed by people at any age as well as by convalescent patients. It also plays an important role for fetuses and newborns regarding brain cell development. On the other hand, such outstanding protein compound with its high water content turns fish into an excellent substratum for microbial development (22, 34, 35).

Among the potentially pathogenic microorganism that can be conveyed through fish are *Salmonella* sp., *Vibrio parahaemolyticus, Escherichia coli, Staphylococcus aureus,* and *Aeromonas* sp., which reach fish through environmental contamination during food processing processes, ranging from capture to its preparation for consumption (9, 12, 16).

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Fish consumption – including fresh raw fish – has increased due to the findings provided by nutrition and food science. Salmon (*Salmo salar*) is among the fish whose consumption as a fresh raw food has gradually increased, mainly presented as *sushi* and *sashimi*. As a result, hygienic-sanitary quality should be a matter of greater concern for this kind of food consumption as exposing consumers to different pathogenic microorganisms might lead to simple gastroenteritis and even death (16, 32).

Several studies reveal the importance of keeping fish in quality hygienic-sanitary conditions as well as the concern with pathogenic microorganisms that might affect the human being (5, 6, 9, 10, 15, 20, 23, 24, 36, 37, 38).

Therefore, the present study was developed towards an evaluation of salmon microbiology through quantifying mesophilic heterotrophic microorganisms, total and thermotolerant coliforms, in addition to the risk of infection by *Vibrio parahaemolyticus, Staphylococcus aureus, Salmonella* sp., *Escherichia coli* and *Aeromonas* sp. that might be found in the fish.

MATERIALS AND METHODS

From June 2007 to April 2008, a number of 31 samples of salmon (*Salmo salar*) were collected from the local retail market in the cities of Araraquara, Jaboticabal, Ribeirão Preto and São Carlos, in the Northeast of the State of São Paulo. The samples were taken in fillets or portions weighing approximately 500 g. From the total amount of samples, 16 were cooled whereas 15 were frozen samples. The samples were properly packed in ice, placed inside isothermal boxes. Next, they were taken to the laboratory to be analyzed. According to the APHA methodology (3), 50 g of each sample were immersed in 450mL of 0.1% peptone water (Himedia, Mumbai, India). After a 1-minute homogenization process, an initial 10^{-1} dilution was obtained. Next, decimal dilutions were prepared up to 10^{-5} using 0,1 mL of the dilution used before. The standard count in mesophilic heterotrophic microorganism plate counts was performed after the dilutions as well as the determination of the most probable number (MPN) for total coliforms/g and thermotolerant coliforms/g, test for *Escherichia coli*, *Staphylococcus* sp. count, *Staphylococcus* coagulase-positive count and test for *Staphylococcus aureus*, test for *Salmonella* sp. and determination of MPN for *Vibrio parahaemolyticus* (3).

For *Aeromonas* sp. isolation, 25 g of each sample were immersed in 225 mL of tryptone soya broth (TSB) (Himedia, Mumbai, India) with ampicillin (30 mg/L) (Sigma, Steinheim, Germany) under incubation at 28ºC for 24 hours. After that, an aliquot of this broth was streaked in phenol red-starch ampicillin agar (19, 25) and ampicillin-dextrin agar (13), both containing antimicrobial at a 10 mg/L concentration. The incubation was performed in BOD incubator (Tecnal-390, Brasil) at 28ºC for 24 hours. From both medium, five colonies suggestive of the genus were taken and streaked in tubes with tryptone soya agar (TSA) (Himedia, Mumbai, India), which were incubated at a temperature of 28ºC for 24 hours. After checking morphology and staining through the Gram's method, straight and short rod-shaped bacteria, in pairs, isolated or in short chains and Gram-negative were replicated in triple sugar iron agar (TSI) (Oxoid, Basingstoke, UK) (30). After incubation, cultures presenting acid reaction both in the butt and in the slant, either with or without gas formation, were inoculated in TSA slants and incubated at a temperature of 28ºC for 24 hours. As the oxidase test was performed, the positive cultures were considered as pertaining to the genus known as *Aeromonas*. The scheme proposed by Popoff (29) and updated by Furuwatari *et al*. (11) was used for species characterization, supplemented by a few tests recommended by Abott *et al*. (1), which is made up by performing the following tests: indol production; esculin and arginine hydrolysis; lysine decarboxylase and ornithine decarboxylase; inositol, salicin, sucrose, mannitol, and arabinose fermentation; acetoin production (VP) and gas production from glucose, growth in nutrient broth at 37ºC with 0%, 3%, and 6% of sodium chloride and nitrate reduction (18). For the counting of *Aeromonas*,

phenol red-starch ampicillin agar plate counts were used, prepared as previously described in this paper. The streak was made in the surface in volumes of 0.1 mL of each of five dilutions and incubated in BOD at 28ºC for 24 hours. The counting of colonies presenting features of the gender *Aeromonas* followed. Up to five characteristic colonies were isolated to confirm the genus. The colonies underwent identification tests as previously presented. The final result for the *Aeromonas* count was obtained upon the result of the gender identification tests in proportion to the number of colonies found in the plate count, multiplied by ten and by the dilution factor. The results referring to the mesophilic heterotrophic microorganism population underwent the Student's *t*-test (31) and the results from the other determinations were analyzed through the nonparametric chi-square test (8).

RESULTS AND DISCUSSION

As presented in Table 1, the population of mesophilic heterotrophic microorganisms varied from 10 to 3.9 x 10^6 CFU/g, with 16.13% of the samples presenting populations above 10^5 CFU/g. The population varied from 1.1 x 10^3 to 3.9 x 10^6 CFU/g for the cooled product and from 10 to 4.2 x 10^4 CFU/g for the frozen product.

Table 1. Number of samples and population variation gap concerning mesophilic heterotrophic microorganisms and *Staphylococcus* sp. in salmon (*Salmo salar*) purchased from public markets in the cities of Araraquara, Jaboticabal, Ribeirão Preto and São Carlos, State of São Paulo, Brazil.

Population variation gap (CFU/g)	Number of samples (%)	
	Mesophilic heterotrophic microorganisms	*Staphylococus* sp.
< 50	—	13 (41.93)
1.0 x 10 —| 1.0 x 10^2	5 (16.13)	—
1.0 x 10^2 —| 7.0 x 10^2	5 (16.13)	10 (32.25)
1.0 x 10^3 —| 9.8 x 10^3	10 (32.26)	5 (16.13)
1.0 x 10^4 —| 6.0 x 10^4	6 (19.35)	3 (9.68)
1.0 x 10^5 —| 9.2 x 10^5	3 (9.68)	—
2.0 x 10^6 —| 3.9 x 10^6	2 (6.45)	—
Total	31 (100.00)	31 (100.00)

While studying frozen freshwater fish, Aquino *et al*. (5) found higher values pointing to a variation from 3.0 x 10^3 to 2.5 x 10^7 CFU/g. In comparison to the results obtained in the present study, such variations indicate a reasonable condition presented by frozen salmon as well as the importance of such conservation method in order to maintain the initial microbiological features of the product so as to avoid deterioration. Populations above 10^5 CFU/g have also been studied by Muratori *et al*. (23) in 55.9% of fresh samples taken from the so-called branquinha fish (*Curimatus ciliatus*).

The mesophilic population average among the cooled and frozen samples amounted respectively to 5.0 x 10^5 CFU/g and 4.4 x 10^3 CFU/g, leading to a statistically significant difference ($p<0.01$), i.e., freezing was more efficient to retard the growing of microbiological population than cooling. The importance of low temperatures for product conservation was also observed by Soares & Germano (33) in salmon samples used in *sashimi* and by Lourenço *et al*. (17) while examining the meat of the crab known as caranguejo-uçá in two localities in the State of Pará, Brazil.

In the absence of parameters in the Brazilian law concerning mesophiles in ready-to-use meals based on fresh raw fish (4), the creation of a standard is recommended for fresh raw fish, helping the authorities with hygienic-sanitary inspection either for seafood or seafood-derived meals.

Table 2 shows that the total coliform population variation

ranged from < 3.0 to 1.1 x 10^3 MPN/g while thermotolerant coliforms ranged from < 3.0 to 4.6 x 10^2 MPN/g. The results point to the good hygienic-sanitary quality of the raw material referring to fecal contamination as values < 3.0 MPN/g were observed in 80.64% of the samples for thermotolerant coliforms. The conservation method influenced microorganism population as well as product conservation: among 21 samples presenting a total coliform population < 3.0 MPN/g, 8 were cooled and 13 were frozen. Among 25 samples with the same thermotolerant coliform population, 11 were cooled and 14 were frozen.

Table 2. Number of samples and population of total and thermotolerant coliforms in salmon (*Salmo salar*) purchased from public markets in the cities of Araraquara, Jaboticabal, Ribeirão Preto and São Carlos, State of São Paulo, Brazil.

Population (MPN/g)	Number of samples (%)	
	Total Coliforms	Thermotolerant Coliforms
< 3.0	21 (67.74)	25 (80.64)
0.4 x 10	0	2 (6.45)
0.7 x 10	2 (6.45)	0
0.9 x 10	2 (6.45)	1 (3.22)
4.3 x 10	0	1 (3.22)
9.3 x 10	2 (6.45	0
1.5 x 10^2	0	1 (3.22)
2.9 x 10^2	1 (3.22)	0
4.6 x 10^2	2 (6.45)	1 (3.22)
1.1 x 10^3	1 (3.22)	0
Total	31 (100.00)	31 (100.00)

Fang *et al*. (10) also found low total coliform populations ranging from 2.3 to 6.1 CFU/g in 80.0% of seafood samples and, unlike the present study, the authors found *E. coli* in 6.0% of the samples.

Soares & Germano (33) evaluated the hygienic-sanitary quality in *sashimi* sold in shopping malls. Total coliform populations ranged from 1.25 to 7.0 x 10^4 CFU/g and thermotolerant coliforms varied from < 10 to 4.0 x 10^3 CFU/g, where 66.4% of the samples presented thermotolerant coliforms, unlike the present study, where such group was not found in 80.64% of the samples. In another study, the same authors found total coliform populations ranging from 35 to 1.1 x 10^6 CFU/g in fresh salmon used in *sashimi* (34), unlike the present study, where this group reached a maximum 1.1 x 10^3 MPN/g.

By relating the groups of total and thermotolerant coliforms with the storage and marketing temperatures, it was possible to notice that, for the cooled samples, the total coliform populations varied from < 3.0 to 1.1 x 10^3 MPN/g, whereas lower values were found in the frozen samples, which varied from < 3.0 to 4.6 x 10^2 MPN/g. Considering samples with a result < 3.0 MPN/g as negative ones for total coliforms, the nonparametric chi-square test was applied to the averages that were found and such test revealed a significant difference ($p<0.05$) between the results, which confirms that freezing is the best conservation procedure for the product.

The population of thermotolerant coliforms in the cooled product varied from <3.0 to 1.5 x 10^2 MPN/g. In the frozen product, the variation ranged from <3.0 to 4.6 x 10^2 MPN/g. In this group, the difference in the number of positive and negative samples between the frozen and cooled products did not prove to be statistically significant ($p>0.01$).

Pursuant to the results and considering the Resolution RDC no. 12 (4) issued by the Brazilian National Health Surveillance Agency (ANVISA), concerned with raw fish-based, ready-to-use meals, which allows the amount of 10^2 MPN/g for thermotolerant coliforms, specific legislation should be suggested in Brazil regarding the consumption of fresh raw fish with a more stringent standard for thermotolerant coliforms.

The importance of changing the current standards into specific ones has been also advocated by Martins (20) while evaluating the hygienic-sanitary quality of *sushi* and *sashimi*. Martins found thermotolerant coliform populations above 10^2 MPN/g in 50% of the samples and *E. coli* in 45%.

In a similar study, Soares & Germano (33) and Meldrum *et al*. (21) observed the absence of *E. coli* in the microbiological analysis of fish and fish-derived products. On the other hand, Taulo *et al*. (36) found *E. coli* in 49% of the samples of home-prepared fish in Malawi, where 5.0% presented *E. coli* O157:H7.

The *Staphylococcus* sp. population varied from < 50 to 1.3 x 10^4 CFU/g (Table 3), with a variation of < 50 to 1.3 x 10^4 CFU/g for the cooled product and amounting to < 50 a 2.6 x 10^3 CFU/g for the frozen product, which presents freezing as the best conservation procedure. A single cooled sample with 5.5 x 10^2 CFU/g presented coagulase-positive *Staphylococcus*, which shows the importance of practicing healthy habits during product manipulation in order to avoid contamination.

Table 3. Species of *Aeromonas* detected in salmon samples (*Salmo salar*) purchased from public markets in the cities of Araraquara, Jaboticabal, Ribeirão Preto and São Carlos, State of São Paulo, Brazil.

Number of samples					Species identified	Number of samples in which the species was found
Conservation method	**Analyzed**	**Positive**	**%***	**%****		
Cooled	16	10	76,90	32,25	*A. caviae*	7
					A.caviae and *A. sobria*	1
					A. caviae and *A. trota*	1
					A. sobria	1
Frozen	15	3	23.10	9.70	*A. caviae*	3
Total	31	13	100.00	41.95		

*Percentage in relation to total positive samples.
**Percentage in relation to total analyzed samples.

Similar results such as < 10^2 and 3.0 x 10^4 CFU/g have been found by Cunha Neto *et al*. (9) when analyzing fresh shrimp and processed food, including previously cooked fish. The authors quantified coagulase-positive *Staphylococcus* in a sample of cooked fish and in a sample of raw shrimp amounting to 9.5 x 10^2 CFU/g and 4.0 x 10^2 CFU/g, respectively, which is similar to the sample analyzed by the present paper.

Supporting the data presented by this study, one sample containing coagulase-positive *Staphylococcus* with a population of 80 CFU/g was found. Albuquerque *et al*. (2) also found coagulase-positive *Staphylococcus* in *sushi* sold in the State of Ceará, Brasil, with a population ranging from < 10^2 and 1.4 x 10^6 CFU/g.

The presence of potentially pathogenic microorganisms in raw fish destinated to the human consumption suggests the necessity of a specific regulation for fresh raw fish. Such regulation should contains stringent microbiological standards because several foodborne microrganisms present very low infectious dose, while other pathogens can multiply in fresh fish exposed to abuse temperature.

Aeromonas sp. were identified in direct quantification in 11 samples with populations ranging from 2.0 x 10^2 and 8.0 x 10^3 CFU/g – all of them under refrigeration. The population average was 3.2 x 10^3 CFU/g and, among 11 samples, eight (72.73%) presented populations exceeding 10^3 CFU/g, which means high populations that could endanger people´s health, mainly through the intake of the product in its natural state, now an increasing habit in Brazil. It was not possible to quantify *Aeromonas* in the frozen samples, which suggests the absence or a reduced population of such microorganism and shows this conservation procedure as the most adequate to preserve the product and impair contamination through meat consumption.

One analysis performed with fish sold in public markets identified *Aeromonas* sp. in 62% of 50 samples, with a population variation between 1.95 x 10^2 and 1.5 x 10^7 CFU/g (14), unlike the present study, where 64.51% of the 31 salmon samples presented *Aeromonas* sp. with populations below 10^2 CFU/g.

Ullmann *et al*. (37) also found *Aeromonas* sp. in populations smaller than 10^4 CFU/g in most (67.9%) of the 84 fish samples, including salmon sold in public markets of Berlin.

Pursuant to the results presented, the importance of seafood as a source of protein for adequate nourishment, mainly when eaten in its fresh, raw state, and the absence of parameters for quantification and research of *Aeromonas*, it is required to create specific laws.

After selective enrichment, 41.95% of the 31 samples presented *Aeromonas* sp., among which 76.90% were cooled and 23.10% were frozen. This reinforces the presence of bacteria at low temperatures and the importance of keeping the product frozen.

Azevedo *et al*. (6) obtained higher results revealing the presence of *Aeromonas* sp. in 87% of 26 fish samples and in 93% of 28 water samples, which represents risk of serious diseases for human beings.

Similar results for *A. caviae* (26.1%) and *A. sobria* (6.0%) were found by Ullmann *et al.* (37) in a study about the isolation and characterization of *Aeromonas* sp. in fish samples, including salmon.

Lower results for *A. caviae* (14.80%) and *A. sobria* (4.20%), and higher for *A. trota* (4.20%) were obtained by Pereira *et al*. (26) while evaluating the presence of emerging pathogens in mussels (*Perna perna*). The authors identified *A. media*, *A. hydrophila, A. schubertii* and *A. jandaei* as well. A result that surpasses the one found by the present study was also obtained by Azevedo *et al*. (6), who identified *A. veronii*, *A. allosaccharophila* and *A. trota* as the most frequent species isolated from fish samples. Herrera *et al*. (14) found similar results for *A. sobria* in fish samples commercialized in Spain, where 16% were *A. caviae* and 6.5% were *A. sobria* among the 31 positive samples for *Aeromonas* sp.

Considering that some *Aeromonas* species have been reported as emerging pathogens isolated from human and animal sources, it is extremely important to supervise their presence in seafood and evaluate their risks for Public Health.

None of the samples presented *Salmonella* and *V. parahaemolyticus,* which fits the standards set forth by Resolution RDC no.12 (4), regarding ready-to-use meals made from raw fish, in the absence of *Salmonella* and a maximum 10^3 CFU/g of *V. parahaemolyticus*/g. In agreement with the present study, Aquino *et al*. (5), Basti *et al*. (7), Martins (20), and Pereira *et a*l. (27), while analyzing seafood, fish, food made from raw fish, and oysters, respectively, did not find *Salmonella* either. Basti *et al*. (7), Martins (20), and Pereira *et al*. (27) also researched *V. parahaemolyticus*, found only by Basti *et al*. (7) at high levels in salted fish and at lower levels in fresh fish. On the other hand, Lourenço *et al*. (17) identified *Salmonella* sp. in crab meat samples. Pereira *et al*. (28) noticed the presence of *V. parahaemolyticus* in 7.7% of mussel samples (*Perna perna*) collected in Rio de Janeiro.

Pursuant to these results and the increase in the consumption of fresh raw salmon, it is extremely important to adopt Good Manufacturing Practices (GMP) during every fish processing stage in order to minimize the risk of contamination by deteriorating or pathogenic bacteria that may cause harm to the health of consumers. Therefore, the participation of government entities through the creation of specific laws concerned with the fresh, raw-consumed fish and through the inspection of such products in every production stage is fundamental to improve the microbiological quality of the fish that is commercialized in Brazil.

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REFERENCES

1. Abbott, S.L.; Cheung, W.K.W.; Janda, J.M. (2003). The genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. *J. Clin. Microbiol.* 41 (6), 2348-2357.
2. Albuquerque, W.F.; Barreto, N.S.E.; Silva, A.I.M.; Vieira, R.H.S.F. (2006). Ocorrência de *Vibrio parahaemolyticus* e estafilococos coagulase positivo, em *sushis* comercializados em alguns estabelecimentos de Fortaleza – CE. *Hig. Aliment*. 20 (146), 58-61.
3. American Public Health Association (APHA). Compendium of methods for the microbiological examination of foods. v. 4. D.C., Washington, 2001, 676p.
4. ANVISA. Agência Nacional de Vigilância Sanitária. Resolução – RDC n°12, de 2 de janeiro de 2001. Available at: http://www.anvisa.gov.br/legis/resol/12_01rdc.htm. Accessed 11 October 2001.
5. Aquino, J.S.; Vasconcelos, J.C.; Inhamuns, A.J.; Silva, M.S.B. (1996). Estudo microbiológico de pescado congelado comercializado em Manaus – *AM. B. CEPPA*. 14 (1), 1-10.
6. Azevedo, V.M.; Dropa, M.M.M.; Cabianca, M.A.A.; Esteves, K.E.; Matté, G.R.; Matté, R.H. (2003). Ocorrência de *Aeromonas* spp. e *Vibrio cholerae* em pesque-pagues da região metropolitana de São Paulo. *Revista Eletrônica de Epidemiologia das Doneças Transmitidas por Alimentos.* 3 (4), 114-119. Available at: http://www.ftp.cve.saude.sp.gov.br/doc_tec/hidrica/revp03_vol3n4.pdf. Accessed 11 June 2008.
7. Basti, A.A.; Misaghi, A.; Salehi, T.Z.; Kamkar, A. (2002). Bacterial pathogens fresh, smoked and salted Iranian fish. *Food Control*. 17 (3), 183-188.
8. Beiguelman, B. (2002). *Curso Prático de Bioestatística*. FUNPEC, Ribeirão Preto, 272p.
9. Cunha Neto, A.; Silva, C.G.M.; Stamford, T.L.M. (2002). *Staphylococcus* enterotoxigênicos em alimentos in natura e processados no estado de Pernambuco, Brasil. *Ciênc. Tecnol. Aliment*. 22 (3), 263-271.
10. Fang, T.J.; Wei, Q.K.; Liao, C.W. ; Hung, M.J.; Wang, T.H. (2003). Microbiological quality of 18°C ready-to-eat food products sold in Taiwan. *Int. J. Food Microbiol.* 80 (3), 241-250.
11. Furuwatari, C.; Kawakami, Y.; Akahane, T.; Hidaka, E.; Okimura, Y.; Nakayama, J.; Furihata, K.; Katsuyama, T. (1994). Proposal for an Aeroschem (modified Aerokey II) for the identification of clinical *Aeromonas* species. *Med. Sci. Res.* 22 (9), 617-619.
12. Germano, P.M.L.; Germano, M.I.S.; Oliveira, C.A.F. (1998). Aspectos da qualidade do pescado de relevância em saúde pública. *Hig. Aliment.* 12 (53), 30-37.
13. Havelaar, A.H.; Vonk, M. (1988). The preparation of ampicilin dextrin agar for the enumeration of *Aeromonas* in water. *Lett. Appl. Microbiol*. 7 (6), 169-171.
14. Herrera, F.C.; Santos, J.A.; Otero, A.; Garcia-Lopez, M.L. (2006). Occurence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. *J. Appl. Microbiol.* 100 (3), 527-536.
15. Hozbor, M.C.; Saiz, A.I.; Yeannes, M.I.; Fritz, R. (2006). Microbiological changes and its correlation with quality indices during aerobic iced storage of sea salmon (*Pseudopercis semifasciata*). *LWT-Food Sci. Technol*. 39(2), 99-104.
16. Huss, H.H.; Reilly, A.; Embarek, P.K.B. (2000). Prevention and control of hazards in seafood. *Food Control*. 11 (2), 149-156.
17. Lourenço, L.F.H.; Oliveira, M.L.; Pinto, C.M.C.; Pereira, D.X.P. (2006). Análises físico-químicas e microbiológicas de carne de caranguejo-uçá *Ucides cordatus* (linnaeus, 1763), comercializada nos municípios de São Caetano de Odivelas e Belém, PA. *Hig. Aliment*. 20(142), 90-95.
18. Mac FADDIN, J. F. (1976). *Biochemical tests for identification of medical bacteria*. The Williams e Wikins Co, Baltimore, 312p.
19. Majeed, K.N.; Egan, A.F.; Mac Rae, I.C. (1990). Production of exotoxins by *Aeromonas* spp at 5°C. *J. Appl. Bacteriol*. 69 (3), 332-337.
20. Martins, F.O. (2006). *Avaliação da qualidade higiênico-sanitária de preparações (sushi e sashimi) a base de pescado cru servidos em bufês na cidade de São Paulo*. São Paulo, Brasil, 142p. (M. Sc. Dissertation. Faculdade de Saúde Pública. USP).
21. Meldrum, R.J.; Smith, R.M.M.; Ellis, P.; Garside, J. (2006). Microbiological quality of randomly selected ready-to-eat foods sampled between 2003 and 2005 in Wales, UK. *Int. J. Food Microbiol*. 108 (3), 397-400.
22. Moraes, F.P.; Colla, L.M. (2006). Alimentos funcionais e nutracêuticos: definições, legislação e benefícios à saúde. *Revista Eletrônica de Farmácia*. 3 (2), 109-122.
23. Muratori, M.C.S.; Costa, A.P.R.; Viana, C.M.; Podesta Júnior, R.L. (2004). Qualidade sanitária do pescado "in natura". *Hig. Aliment*. 18 (116-117), 50-54.
24. Normanno, G.; Parisi, A.; Addante, N.; Quaglia, N.C.; Dambrosio, A.; Mantagna, C.; Chiacco, D. (2006). *Vibrio parahaemolyticus, Vibrio vulnificus* and microorganisms of fecal origin in mussels (*Mytilus galloprovincialis*) sold in the Puglia region (Italy). *Int. J. Food Microbiol*. 106 (2), 219-222.
25. Palumbo, S.A.; Maxino, F.; Willians, A.C.; Buchanan, R.L.; Thayer, D.W. (1985). Starch-ampicilin agar for the quantitative detection of *Aeromonas hydrophila*. *Appl. Environ. Microbiol*. 50 (4), 1027-1030.
26. Pereira, C.S.; Possas, C.A.; Viana, C.M.; Rodrigues, D.P. (2004). *Aeromonas* spp. e *Plesiomonas shigelloides* isoladas a partir de mexilhões (*Perna perna*) in natura e pré-cozidos no Rio de Janeiro, RJ. *Ciênc. Tecnol. Aliment*. 24 (4), 562-566.
27. Pereira, M.A.; Nunes, M.M.; Nuerberg, L.; Schulz, D.; Batista, C.R.V. (2006). Microbiological quality of oysters (*Crassostrea gigas*) produced and commercialized in the coastal region of Florianópolis – Brazil. *Braz. J. Microbiol*. 37 (2), 159-163.
28. Pereira, C.S.; Rodrigues, D.P.; Viana, C.M. (2007). Isolamento de *Vibrio*

spp. em mexilhões (*Perna perna*) coletados na região de Ponta de Itaipu, Niterói, RJ. *Hig. Aliment*. 21(137), 94-97.

29. Popoff, M. (1984). Genus III. *Aeromonas* Kluyver and Van Niel. *In*: Drieg, N. (eds). *Bergey´s Manual of systematic bacteriology*. The Williams & Wilkins Co., Baltimore, p. 545-548.
30. Saad, S.M.I.; Iaria, S.T.; Furlanetto, S.M.P. (1995). Motile *Aeromonas* spp in retail vegetables from São Paulo, Brazil. *Rev. Microbiol*. 26 (1), 22-27.
31. SAS Institute. (2005). *User´s guide: Statistics*. Cary.
32. Sato, N.H.; Usui, K.; Kobayashi, T.; Imada, C.; Watanabe, E. (2005). Quality assurance of raw fish based on HACCP concept. *Food Control*. 16(4), 301-307.
33. Soares, C.M.; Germano, P.M.L. (2004). Análise da qualidade microbiológica de *sashimis*, comercializados em *shopping centers* da cidade de São Paulo, Brasil. *Hig. Aliment.* 18(116/117), 88-92.
34. Soares, C.M.; Germano, P.M.L. (2005). Características microbiológicas e físico-químicas do salmão (*Salmo salar*) utilizado em *sashimis*. *Hig. Aliment*. 19 (135), 59-63.
35. Souza, A.T.S. (2003). Certificação da qualidade de pescados. *Biológico.* 65 (1/2), 11-13.
36. Taulo, S.; Wetlesen, A.; Abrahamsen, R.; Kululanga, G.; Mikakosya, R.; Grimason, A. (2008). Microbiological hazard identification and exposure assessment of food prepared and served in rural households of Lungwena, Malawi. *Int. J. Food Microbiol*. 125 (2), 111-116.
37. Ullmann, D.; Krause, G.; Knaber, D.; Weber, H.; Beutin, L. (2005). Isolation and characterization of potencially human pathogenic, cytotoxin producing *Aeromonas* strains from retailed seafood in Berlin, Germany. *J. Vet. Med*. 52 (2), 82-87.
38. Vernocchi, P.; Maffei, M.; Lanciotti, R.; Suzzi, G.; Gardini, F. (2007). Characterization of mediterranean mussels (*Mytilus galloprovincialis*) harvested in Adriatic sea (Italy). *Food Control*. 18 (12), 1575-1583.