Microbiological quality of pre-cooked seafood marketed in Santa Catarina Island, Brazil

Qualidade microbiológica de frutos do mar pré-cozidos comercializados na Ilha de Santa Catarina, Brasil

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ABSTRACT

The present study assessed the microbiological quality of pre-cooked and refrigerated seafood marketed in Santa Catarina Island, Brazil. Forty-eight samples of crabs, mussels, shrimp and clams were purchased at fish markets in Santa Catarina Island from June to September 2008. Microbiological analysis were conducted for total counts of psychrotrophic, coliforms at 45 °C, *Enterococcus* spp., *Escherichia coli*, coagulase-positive staphylococci and *Salmonella* spp. detecting. All of analyzed samples showed absence of *Salmonella* spp. in 25g. Two (4.2%) samples exceeded the counting limits of coliforms at 45 °C (one of mussel and one of crab meat), and nine (18.75%) samples (five of clams and four of crab meat) exceeded the limits for coagulase-positive staphylococci. The psychrotrophic counts were high in all products analyzed. Positive correlations were found between coliforms counting at 45 °C and *Escherichia coli*, but no correlation was found between *Enterococcus* spp. and coliforms at 45 °C or *Escherichia coli*. This study evidenced that about 20% of the samples were not comply with the sanitary standards established by the Brazilian legislation.

Keywords. pre-cooked seafood, risk assessment, shelf life.

RESUMO

Este estudo teve por objetivo avaliar a qualidade microbiológica de frutos do mar pré-cozidos e refrigerados comercializados em Florianópolis, Santa Catarina, Brasil. Quarenta e oito amostras de carne de siri, mexilhão, camarão e berbigão foram adquiridas em peixarias, entre os meses de junho e setembro de 2008. Foram realizados ensaios microbiológicos para contagem total de psicrotróficos, coliformes a 45 °C, *Enterococcus* spp., *Escherichia coli*, estafilococos coagulase positiva e detecção de *Salmonella* spp. Todas as amostras foram negativas para *Salmonella* spp. em 25 g. Duas (4,2%) amostras ultrapassaram os limites estabelecidos de coliformes a 45 °C, sendo uma de mexilhão e outra de carne de siri. Nove (18,75%) amostras, cinco de berbigão e quatro de carne de siri, ultrapassaram os limites estabelecidos para estafilococos coagulase positiva. As contagens de microrganismos psicrotróficos foram elevadas em todos os produtos analisados. Foram observadas correlações estatísticas positivas ($p < 0,05$) entre as contagens de coliformes a 45 °C e *Escherichia coli*, mas não foi observada correlação ($p>0,05$) entre *Enterococcus* spp. e coliformes a 45 °C ou *Escherichia coli*. O presente estudo demonstrou que 20% das amostras não atendiam aos parâmetros higiênico-sanitários estabelecidos pela legislação vigente no Brasil.

Palavras-chave. frutos do mar pré-cozidos, análise de risco, vida de prateleira.

INTRODUCTION

World production of fish products is estimated in 100 million tons/year, being 70 tons for human consumption[1]. Both production and consumption of fish products have increased in the last years, being the main animal protein consumed in several countries[1-3]. Ready-to-eat seafood, as cooked shrimp, requires a high microbiological safety once it is often consumed with no subsequent thermal treatment[4,5]. The control of microorganism growth aims to obtain healthier food products, resulting on the elimination or reduction of risks to consumer health[6]. The refrigeration process does not present sterilizing action, just retards the microbial activity and prevents the growth of new spoiling agents, while thermal treatment can physically modify the meat and promote changes in several constituents[7].

Generally, seafood microbiota reflects the quality of the water where such animals live and, besides water, microorganisms are acquired in the several processing steps, such as peeling, deshelling, descale, evisceration, among others[8]. According to Huss[9], the real incidence of foodborne diseases caused by seafood is unknown and the majority of cases are not reported. However, there are evidences that the seafood and the seafood products are frequently associated with foodborne diseases. The microbiological quality of post-processing seafood reflects the original hygienic-sanitary conditions of the product, equipments, staff, thermal treatment, time/temperature control, good manufacturing practices, as well as caution in packaging and freezing[10].

Transmition of enterical pathogenic bacteria from human or animal wastewaters by seafood, including *Salmonella* spp., has been reported[11]. The contamination of seafood by *Streptococcus* spp. and *Staphylococcus aureus* (both of human origin) can appear as a direct consequence of inappropriate handling. Both species are found in mucosa and skin surface, being seafood a suitable environment for growing[12-14]. The detection of coliforms at 45 °C and *Escherichia coli* provides information about the hygienic-sanitary conditions and indicates the presence of possible pathogenic enterobacteria[8].

The aim of the present work was to evaluate the microbiological quality of pre-cooked and chilled seafood commercialized in the Island of Santa Catarina.

MATERIAL AND METHODS

Samples

Samples were acquired in seafood stores from June to September, 2008. Samples of crab (*Callinectes danae*), mussel (*Perna perna*), shrimp (*Litopenaeus vannamei*) and clams (*Anomalocardia brasiliana*) were analyzed. All samples were found pre-cooked and stored under refrigeration. Once collected, samples were transported to the Food Microbiology Laboratory, located in the Department of Food Science and Technology of the Federal University of Santa Catarina in isothermal boxes containing ice. Analyzes were performed within a period of 2 hours.

Microbiological analysis

Microbiological analyses were performed to evaluate total psychrotrophic count, coliforms at 45 °C, *Enterococcus* spp., *Escherichia coli*, positive coagulase *Staphylococcus* and *Salmonella* spp. All analyses were carried out according to American Public Health Association[15].

For each sample, two portions of 25 g were weighted aseptically, one portion for *Salmonella* spp. analysis and the other for the remaining analyses. The 25 g were placed in a sterile pack, added of 225 mL of buffered peptone water and peptone water 1%, respectively. The samples were disintegrated on Bagmixer® (Interscience, France). The serial decimal dilutions were prepared from these solutions. All analyses were performed in duplicate.

Statistical analysis

Statistical analyses were performed in *Statistica 7.0*® software, Stat-Soft, Inc., USA[16]. Averages of microbiological counts were compared using Tukey's test at a level of significance of 5%. In order to evaluate the correlation among the hygienic-sanitary indicators, the Spearman non parametric test was used.

RESULTS AND DISCUSSION

The Brazilian legislation, by RDC 12/2001 – ANVISA[17], establishes microbiological parameters for different types of food. For cooked and frozen seafood, the limits are 5 x 10 MPN/g for coliforms at 45 °C, 10^3 CFU/g for positive coagulase *Staphylococcus* and absence of *Salmonella* spp. in 25 g of sample.

Among the different seafood analyzed in the present work, *Salmonella* spp. was not detected in none sample of shrimp, crab, clam and mussel, being

Table 1. Counts of different microorganisms in seafood sold pre-cooked on the Island of Santa Catarina

Microorganism	Seafood (n)	Minimum count	Maximum count	Average count
Coliforms 45 °C		**(MPN/g)***	**(MPN/g) ***	**(MPN/g)***
	Mussels (12)	< 3.0	2.4×10^2	6.0 [a]
	Clams (12)	< 3.0	2.3 x 10	3.8 [a]
	Crabs meat (12)	< 3.0	2.4×10^2	3.7 [a]
	Shrimp (12)	< 3.0	9.0	2.6 [a]
Escherichia coli		**(MPN/g)***	**(MPN/g)***	**(MPN/g) ***
	Mussels (12)	< 3.0	2.4×10^2	5.4 [b]
	Clams (12)	< 3.0	2.3 x 10	3.5 [b]
	Crabs meat (12)	< 3.0	4.0	2.2 [b]
	Shrimp (12)	< 3.0	4.0	2.1 [b]
Psychrotrophic		**(CFU/g)****	**(CFU/g)****	**(CFU/g)****
	Mussels (12)	1.3×10^3	1.1×10^7	3.8×10^5 [c]
	Clams (12)	1.0×10^4	1.7×10^8	2.2×10^6 [c]
	Crabs meat (12)	7.2×10^5	2.7×10^9	2.8×10^7 [c]
	Shrimp (12)	1.5×10^6	2.9×10^8	3.3 x 107 [c]
***Enterococcus* spp.**		**(CFU/g)****	**(CFU/g)****	**(CFU/g)****
	Mussels (12)	1.6×10^2	1.5×10^5	7.9×10^2 [d]
	Clams (12)	< 1.0 x 10	8.9×10^5	1.2×10^4 [d]
	Crabs meat (12)	< 1.0 x 10	5.4×10^5	1.5×10^4 [d]
	Shrimp (12)	< 1.0 x 10	2.8×10^5	7.4×10^2 [d]
Positive coagulase *staphylococci*		**(CFU/g)****	**(CFU/g)****	**(CFU/g)****
	Mussels (12)	$< 1.0 \times 10^2$	1.5×10^2	1.0×10^2 [e]
	Clams (12)	$< 1.0 \times 10^2$	1.9×10^5	1.6×10^3 [e]
	Crabs meat (12)	$< 1.0 \times 10^2$	3.6×10^3	3.6×10^2 [e]
	Shrimp (12)	$< 1.0 \times 10^2$	1.0×10^3	2.0×10^2 [e]

*Note: * MPN/g = Most probable number per gram; **CFU/g = Colony forming unit per gram*
Average counts of each microorganism presenting the same letter were not statistically different (p > 0.05) among the different seafood samples

in agreement with Brazilian legislation. Vieira et al[18], evaluating microbiological quality of crab meat processed in Antonina, Paraná, did not detected this microorganism in the samples analyzed. Sombrio et al.[19] did not detected *Salmonella* spp. by analyzing samples of pre-cooked mussel and canned mussel. Kumar et al.[20], evaluating the incidence of *Salmonella* spp. in 247 samples of non processed seafood in India, found contamination in 20% of samples. Aveiro[21], evaluating the quality of *in natura* clams collected in Pirajubaé, Florianópolis, Santa Catarina, did not detect *Salmonella* spp. in none analyzed sample.

Evaluating the counts of the main hygienic-sanitary indicators in the different samples of seafood pre-cooked it was observed that only two out of 48 samples were not in agreement with Brazilian legislation for coliforms at 45 °C, i.e., presenting counts higher than 50 MPN/g. Regarding to these two samples, one was of mussel (it also presented high count for *E. coli*) and one was of crab meat (it presented count for *E. coli* lower than for coliforms at 45 °C). The presence of bacteria from fecal coliforms group is interpreted as an indicative of fecal contamination, i.e., unsuitable hygienic-sanitary conditions, because populations from this group are constituted of high proportions of *Escherichia coli*. Thus, the occurrence of enteric pathogens is possible[22]. It can be observed that average counts of coliforms at 45 °C presented lower values than the limit fixed by legislation, suggesting that these products present a suitable hygienic-sanitary quality. A strong positive correlation was observed between counts of coliforms at 45 °C and *E. coli* (Spearman, 0.87, $p < 0.05$). Significant statistical differences ($p > 0.05$) were not observed for counts of coliforms at 45 °C and *E. coli* in seafood samples analyzed (Table 1).

Vieira et al.[18], evaluating the quality of processed crab meat, found low counts for coliforms at 45 °C (4 MPN/g). Similar results were found by Sombrio et al.[19], which evaluated canned mussel. Also, Cordeiro et al.[23], evaluating cooked mussel stored for 90 days, did not detect coliforms at 45 °C.

Minimal, maximal and average counts for coliforms at 45 °C and *Escherichia coli* are presented in Table 1.

Hagler et al.[24] suggested the quantification of *Enterococcus* spp. instead of coliforms for epidemiological studies, mainly because that these microorganisms are resistant to several environmental conditions such as high levels of salinity, dehydration, pollution with detergents and disinfectants, low pH values and moderate thermal treatment. *Enterococcus* spp. consist of lactic bacteria, Gram positive, coccus or cocobacillus-shaped and, generally, are not present in non polluted water or soil[8]. In the present study high counts of *Enterococcus* spp. were observed in the different pre-cooked seafood samples, suggesting high tolerance to the cooking process and even to an unsuitable thermal treatment or recontamination after cooking. Correlations between *Enterococcus* spp. and coliforms at 45 °C (Spearman 0.03, $p > 0.05$) and between *Enterococcus* spp. and *Escherichia coli* (Spearman 0.00, $p > 0.05$) were not observed. Comparing the analyzed products (Figure 1), it can be notice that crab meat and clams presented higher *Enterococcus* spp. counts than mussel and shrimp, with a difference of 2 log cycles. However, significant differences ($p > 0.05$) were not observed for average counts of *Enterococcus* spp. in the different seafood analyzed (Table 1).

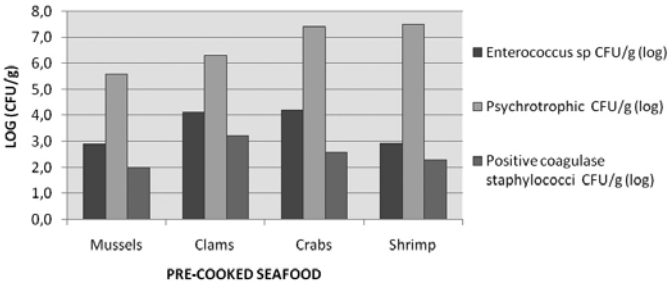

Figure 1. Average counts (log) of psychrotrophic microorganisms, *Enterococcus* spp. and positive coagulase staphylococci in samples of pre-cooked seafood commercialized in the Santa Catarina Island

Observing the averages of psychrotrophic counts (Figure 1) it can be notice high counts in all analyzed products, with crab meat and shrimp reaching more than 7 log cycles and mussel and clams reaching 5 and 6 log cycles, respectively. Table 1 shows that the lowest counts, minimal and maximal, were observed for mussels, while highest minimal and maximal were observed for shrimp and crab meat, respectively. Significant differences ($p > 0.05$) were not observed for average counts of psychrotrophic in the analyzed seafood samples (Table 1).

According to Huss[25], the lost in seafood quality occurs when counts of aerobic bacteria on the fish skin reach 10^8-10^9 CFU/g. Fresh seafood products are highly perishable and susceptible to spoiling caused by the increasing of psychrotrophic population. Storage of such products in ice or under refrigeration during distribution and commercialization results in a small shelf life, between 5 and 10 days[26]. These products did not present any kind of information regarding to expiration date and it is possible that some samples were already near the end of its expiration date or even over once, two clams samples, five crab meat samples and four shrimp samples presented counts higher than 8 log cycles. Ready-to-eat seafood, as pre-cooked peeled shrimp, demands microbiological safety because of the consumption of these products which occurs without any subsequent thermal treatment[5].

Cordeiro et al.[23], evaluating counts of psychrotrophic bacteria on *in natura* and pre-cooked mussels found values lower than those found in the present study, 9.3 x 10^3 CFU/g and 3.1 x 10 CFU/g, respectively. It is possible that such difference in counts occurred once mussels were cooked under controlled conditions of time and temperature and counts were carried out right after cooking process, not being stored or exposed for sale, avoiding a recontamination or growth of remaining viable cells. Such conditions were very different from those observed in the present study.

Regarding to positive coagulase staphylococci, nine out of 48 analyzed samples presented counts above the limit established by legislation, being five clams samples and four crab meat samples, representing 18.8 % of samples presenting unsuitable hygienic-sanitary conditions. Besides, two shrimp samples presented counts reaching the maximum limit, 1 x 10^3 CFU/g. Staphylococci are commonly found in humans (airways and hair) being transferred to food by unsuitable handling and, due to this, the presence of such microorganisms reflects unsuitable handling and storage[27]. It can be point out that this kind of food is very handled, once meat is manually separated after cooking process and the lack of good manufacturing process can put consumer health at risk. Vieira et al.[18], evaluating microbiological quality of crab meat processed in Antoninha-PR, found three out of 11 (27.3%) samples with positive coagulase staphylococci counts above the limit established by Brazilian legislation. Valdimarsson et al.[5], on the other hand, evaluating the quality of 1913 samples of cooked and peeled irish shrimps, found staphylococci in less than 0.2% of analyzed samples.

Figure 1 shows that clams samples presented counts with more than three log cycles, above the limit established by legislation (1.0 x 10^3 CFU/g), while samples of mussels, crab meat and shrimp presented counts ranging from 2 to 3 log cycles, being in agreement with Brazilian legislation[17]. Significant differences ($p > 0.05$) were not observed for average counts of positive coagulase staphylococci in the analyzed seafood samples (Table 1).

Table 1 shows the minimal and maximal counts, as well as averaged values for the contamination of different seafood samples. The lowest value found was of < 1 x 10^2 CFU/g, while the highest value was found for clams samples, 1.9 x 10^5 CFU/g. Such high value allows the production of a thermostable staphylococcal enterotoxin, which remains in food after cooking process and can leads to food intoxication[28].

Ten (20.8%) out of 48 analyzed samples were not in agreement with the microbiological standards established on RDC 12/2001-ANVISA[17]. One sample of crab meat presented counts higher than 1.0 x 10^3 CFU/g for positive coagulase staphylococci and higher than 5.0 x 10 MPN/g for coliforms at 45 °C, being disapproved for these two parameters.

CONCLUSION

Considering the current legislation it can be observed that approximately 20% of the analyzed samples presented unsuitable hygienic-sanitary conditions. This should alert the Municipal Health Surveillance Agency, once pre-cooked seafood are consumed without any subsequent thermal treatment, or even sufficient thermal treatment in order to eliminate pathogenic microorganisms, being a risk to consumers food safety. High counts of psychrotrophic microorganisms observed in the present study show lack of control on the shelf life of these products, which were exposed for selling with no information about their expiration date.

REFERENCES

1. Huss HH, Reilly A, Embarek PKB. Prevention and control of hazards in seafood. Food Control. 2000;11:149-56.
2. Feldhusen F. The role of seafood in bacterial foodborne diseases. Microbes Infect. 2000;2:1651–60.
3. Ababouch L. Potential of *Listeria* hazard in African fishery products and possible control measures. Int J Food Microbiol. 2000;62:211-5.
4. Miget R. Microbiology of crustacean processing: Shrimp, crawfish and prawns. *In*: Ward DR,Hackney CR, Editors. Microbiology of Marine Food Products. New York: Van Nostrand Reinhold; 1991.p.65–87.
5. Valdimarsson G, Einarsson H, Gudbjornsdottir B, Magnusson H. Microbiological quality of Icelandic cooked-peeled shrimp (*Pandalus borealis*). Int J Food Microbiol. 1998;45:157-61.
6. Alves CL, Carvalho FLN, Guerra CG, Araújo WMM. Comercialização de pescado no Distrito Federal: avaliação das condições. Hig aliment. 2002;16:41-9.
7. Ben AM. Effect of freezing and microbial growth on myoglobin derivates of beef. J Agric Food Chem. 1999;47:4093–9.
8. Jay JM. Microbiologia de Alimentos. 6a ed. Porto Alegre: Editora Artmed; 2005.
9. Huss HH. Control of indigenous pathogenic bacteria in seafood. Food Control. 1997;8:91-8.
10. 10. Liston J. Microbial hazards of seafood consumption. Food Technol. 1990;44:56-62.
11. International Commission on Microbiological Specifications for Foods – ICMSF. Ecologia Microbiana de los Alimentos II, Productos Alimenticios. Zaragoza: Acribia Publisher; 1985.
12. Adams AM, Leja LL, Jinneman K, Beeh J. Anisakid parasites, *Staphylococcus aureus* and *Bacillus cereus* in sushi and sashimi from Seattle area restaurants. J Food Prot. 1994;57: 311–7.
13. Cardonha AMS, Casimiro, ARS, Vieira RHSF. Identificação de bactérias psicotróficas em caudas de lagosta, durante processo industrial com tripolifosfato de sódio. Hig aliment. 1994;8: 29-34.
14. Germano PML, Oliveira JCF, Germano MIS. O pescado como causa de toxinfecções bacterianas. Hig aliment. 1993;7: 40–5.
15. American Public Health Association - APHA. Compendium of methods for the microbiological examination of foods, 4th ed. Washington: USA; 2001.
16. Statistica, version 7.1 [Data Analysis Software System].Stat-Soft, Inc.; 2006. Disponível em: [http://www.statsoft.com].
17. Brasil. Ministério da Saúde. Resolução RDC nº 12, de 02 de janeiro de 2001. Aprova o Regulamento Técnico sobre Padrões Microbiológicos para Alimentos. Diário Oficial [da] Republica Federativa do Brasil. Brasília, DF, 10 jan. 2001. Seção 1, nº7-E. p.45-53.
18. Vieira DM, Naumann CRC, Ichikawa T, Cândido LMB. Microbiological characteristics of meat of siri benefited in Antonina (PR) before and after the adoption of good measures of practical. Scientia Agraria. 2006;7: 41-8.
19. Sombrio PS, Prudêncio ES, Amboni RDMC, Barreto PLM, Amante ER. Avaliação tecnológica, microbiológica, química e física de conservas de mexilhões (*Perna perna*) embaladas a vácuo. B Ceppa. 2008;26: 277-86.
20. Kumar R, Surendran PK, Thampuran N. Detection and characterization of virulence factors in lactose positive and lactose negative *Salmonella* serovars isolated from seafood. Food Control. 2009;20: 376–80.
21. Aveiro MV. Análise nutricional, microbiológica e histológica do berbigão *Anomalocardia brasiliana* da Reserva Extrativista Marinha do Pirajubaé (REMAPI), Florianópolis/SC [dissertação de Mestrado]. Florianópolis (SC): Universidade Federal de Santa Catarina; 2007.

22. Brito G, Cordeiro LN, Josino AS, Melo ML, Coutinho HDM. Avaliação da qualidade microbiológica de hambúrgueres e cachorros – quentes comercializados por vendedores ambulantes no município de Juazeiro do Norte, CE. Rev. Hig aliment. 2003;17: 90- 4.

23. Cordeiro D, Lopes TGG, Oetterer M, Porto E, Galvão JA. Qualidade do mexilhão *Perna perna* submetido ao processo combinado de cocção, congelamento e armazenamento. B Ceppa. 2007;25: 165-79.

24. Hagler AN, Mendonça-Hagler LC, Santos EA, Farage S, Silva-Filho JB, Schrank A. Microbial pollution indicators in brazilian tropical and subtropical marine surface waters. Sci Total Environ. 1986;58: 151-60.

25. Huss HH. Fresh fish: quality and quality changes. A training manual prepared for the FAO/DANIDA Training Programme on Fish Technology and Quality Control. Roma: Food and Agriculture Organization of the United Nations Publisher; 1988. (FAO Fisheries Series, 29).

26. Reddy NR, Paradis A, Ropman MG, Solomon HM, Rhodehamel EJ. Toxin development by *Clostridium botulinum* in modified atmosphere-packaged fresh tilapia fillets during storage. J Food Sci. 1996;61:632-5.

27. Galvão JA, Furlan EF, Sálan EO, Porto E, Oetterer M. Características físico-químicas e microbiológicas (*Staphylococcus aureus* e *Bacillus cereus*) da água e dos mexilhões cultivados na região de Ubatuba, SP. Ciênc agrotec. 2006;30: 1124-9.

28. Cunha Neto A, Silva CGM, Stamford TLM. *Staphylococcus* enterotoxigênicos em alimentos *in natura* e processados no estado de Pernambuco, Brasil. Ciênc Tecnol Aliment. 2002;22: 263-71.