# Incidence and control of *Listeria monocytogenes* in fishery products

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**Abstract**

The target of the present work was exploring the incidence of listeria species in different fish products and to evaluate the inhibitory impact of chitosan coating on *Listeria monocytogenes* and sensory attributes of basa fillets *(Pangasius bocourti)*. Total number of 319 samples (frozen, smoked and canned) fish products obtained from local fish (Giza markets- Egypt) over 2 years. The incidence of *Listeria monocytogenes* in the first year was 13.7% while other listeria species was 6.8% with total incidence 20.6% , while in the second year the incidence of *Listeria monocytogenes* was 0% while other listeria species was 9.09 % with total incidence 9.09 %. Morovere inhibitory effcts of chitosan coating was evaluated experimentally to control the growth of *Listeria monocytogenes* in basa fillets (*Pangasius bocourti*). Four groups of fish basa fillets were prepared with experimental inoculation of *Listeria monocytogenes* then three groups were coated with chitosan (1, 1.5 and 2%) beside control group. Incorporation of the highest concentration of chitosan 2% revealed significant inhibitory effect on experimentally inoculated basa fillets *(Pangasius bocourti)* with *Listeria monocytogenes*. Moreover, sensory panel scores of chitosan coated basa fillets show no significant difference between treated and control group expect for raw samples treated with 2% chitosan which show slimmy textute to some extent.

In a conclusion chitosan could achieve benefits for the industry and for the safety of consumer. Therefore, Industry of food may use chitosan concentrations as a natural source of antioxidants, antibacterial and extending shelf life in fish processing.

**Keywords:** Chitosan- Basa fillets- *Listeria monocytogenes* – *Pangasius bocourti* – Bacterial quality.

## 1.Introduction

Fish and fish products intake continuously increased as a result of consumers awareness about integration of diet and health care. Consumers recognize fish and fishery products as healthful and complete foods as it contain valuable lipids especialy polyunsaturated fatty acids. Also they are a good source of high-quality proteins so fish and its products has great effect on reduction of cardiovascular disease risk and hypertension. Morovere they are tender, easy to digest and a brilliant source of many essential minerals and vitamins ***(Ghanbari et al. 2013)***.

Despite the aforementioned essential properties, Fish and fish products are subjected to various hazardous bacteria, such as *Listeria monocytogenes* ***(Kadam and Prabhasankar 2010)*** which one of most significant causes of food outbreaks which may resulted from consumption of different products as fish, meat, poultry, milk and dairy products ***(Parihar et al. 2008)***. In the United States, annual listeriosis accounts for approximately 2500 cases of illness at a cost of about US$ 200 million ***(CDC 2002)***, with an extraordinarily high mortality rate (20–40%) ***(Slutsker and Schuchat 1999)***.

*Listeria monocytogenes* is able to grow and multiply under cooling temperatures and thrive in various food-processing conditions **(Jemmi and Stephan 2006)**. Also This microorganism has a major risk to public health at temperatures as low as 1 °C and up to 10 per cent water phase salt.Also it can be isolated from domestic animals, fish and humans ***(NACMCF 1991, Dillon and Patel 1992, Ryser and Marth 1999)***. According to ***(FAO 1999)*** fish and its products are ideal vectors for the transmission of Listeria monocytogenes to humans, thus reducing the occurrence of highly significant foodborne listeriosis of great importance.

There are several methods to control contamination of fish and fish products with *Listeria monocytogenes* such as adding high concentrated salt solution more than 10%, freezing during storage or adding of inhibitory substances such as lactates with or without a carbon dioxde atmosphere, lactic acid bacteria and/or their bacteriocins ***(Vogel et al. 2010)***. In food processing, using of antimicrobial agents and edible films such as chitosan is also a recent trend to preserve the quality of the product, enhance sensory properties and increase the storage life of the products in order to improve the safety of product ***(Beverly 2004)***. So

incorporation of chitosan as a protective material seems to be a good alternative. Chitosan is polysaccharide originating from the N-acetyl group in chitin alkaline hydrolysis which is the key ingredient of crustacean shells. It is a recognized biopolymer that forms film and has significant antimicrobial and antifungal activities ***(Duan et al. 2010)***, which has been widely applied in extending shelf life of fish and fish products ***(Fan et al. 2009, Duan et al. 2010, Li et al. 2013, Ojagh et al. 2010)***. Chitosan has great antibacterial effect against Gram-positive bacteria ***(Raafat and Sahl 2009)***. Chitosan's antimicrobial efficacy is improved by reducing pH of medium where chitosan is ionised at low pH ***(Khan et al. 2017)***.

The current research was therefore organized to explore the incidance of Listeria species in imported different fishery products (frozen, smoked and canned) in addition to evaluate the role of chitosan as a natural antimicrobial agent in basa fillets (*Pangasius bocourti*) to suppress the growth of *Listeria monocytogenes*.

## 2.Material and methods

### 2.1. Samples collection

A total number of 319 fishery products were collected over two years as shown in **(Table 1)** including: Frozen fish products (62 samples of basa fillets *(Pangasius bocourti*), 63 samples of horse mackerel, 62 samples of shrimp and 39 samples of calmari), smoked fish products (13 samples of smoked salmon and 54 samples of smoked herring), canned fish products (4 samples of anchovies and 22 samples of sardine) which were obtained from local fish (Giza markets- Egypt) and transported in an iceboxes to Food Microbiology Department, Central Public Health Laboratories, Abdien, Egypt.

***2.2. Isolation of listeria species:*** was carried out according to **ISO 11290– (1996)**

Twenty five grams were taken from each sample and added to 225 mL of half Fraser broth in a sterilized bag left to stand for 1 hour±2 minutes/20℃±2℃ to resuscitate the stressed microorganism then mixed in stomacher for 30 seconds. Then 0.1 ml of initial suspension was moved to oxford agar then incubate at 37℃ aerobically 24-48 hours, Listeria monocytogens' typical colonies are somewhat large ,dark with a greenish reflection and a black halo effect.

### *2.3. conformation of suspected isolated Listeria monocytogens*

The typical colonies were taken on to Tryptose soya-yeast extract agar for 18–24 hours at 35 $^{o}$C. Typical colonies were collected and transferred on to nutrient agar for catalase test, gram staining and to blood agar of haemolysis. Catalase positive, gram positive, oxidase negative colonies that grow in the umbrella appearance in motility solid media assume Listeria species as a characteristic morphology. Differentiation of Listeria species is conducted on the basis of the CAMP phenomenon, with beta-haemolytic strain Staphyloccocusaureus and Rhodococcusequi).

### *2.4. preparation of Listeria monocytogenes strain*:

Couple days before expremint frozen beads of *Listeria monocytogenes* culture (NCTC7973/ATCC®35152) was replicated by dublicate inculation in 10 ml TSB (tryptic soy broth) and incubated at 32ºC over night. One ml of activated culture were transfered to 100 ml flask of tryptic soya broth and cultivated at 32ºC over night to obtain 8.0 logs CFU/ml (determined by plating on oxford agar). From the flask 1 ml was decimally diluted to 4.0 logs CFU/ml in 10 ml TSB and transferred to three plates of oxford agar media to detect the true value of *Listeria monocytogenes*.

### *2.5. Preparation of basa fillets (Pangasius bocourti)*

Frozen basa fillets *(Pangasius bocourti)* were obtained from a local fish (Giza markets-Egypt) and transported in an iceboxes to Food Microbiology Department, Central Public Health Laboratories, Abdien, Egypt. Fillets were cutted into pieces (10 g each) and dived into four groups. All groups were dipped for 10 minutes in solution of *Listeria monocytogenes* (4.0 logs CFU/ml), then allow to dry for 20 min in the laminar air flow for attachment.

### *2.6. Preparation of chitosan*

Acid soluble, 350K Da molecular weight, food grade chitosan was obtained from local source (boichemsitry department, Animal Research Institute- Cairo – Egypt) and dissolved in 0.5% of acetic acid solution (v/v) in distilled water. Concentrations of 1%, 1.5 % and 2% chitosan (w/v) were prepared.

### *2.7. Coating of basa fillets with different chitosan concentrations*

Each chitosan solution (1%, 1.5 % and 2%) was used as a dipping solution for inoculated group of fillets for 15 min, to obtain 1%, 1.5% and 2% chitosan in addition to control group (dipped in water), followed by dryness period for 15 minutes. The experiment was repeated twice. Reduction rate of chitosan was evaluated by isolation of *Listeria moncytogens* from control group and coated basa fillets as in market samples.

### *2.8. Sensory asseaament*

Sensory analysis was performed by a 9 panelists team from the staff members of the Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University Prior to the analysis, panelists were trained to identify the intensities and rating of sensory attributes of raw fish products (appearance, odour, colour, texture) and cooked fish products (by cooking in hot air oven at 180 $^{o}$C for core temperature 68$^{o}$C) include (falvour, texture, color, juiciness and overall acceptability). The panelists were asked to rate various samples on a 9-point hedonic scale sensory evaluation where (9:extremely like; 8:very much like, 7:moderately like; 6:slightly like, 5:don't like or dislike, 4:a little dislike, 3:moderately dislike, 2:very much dislike and 1:highly dislike) was used to evaluate basa fillets samples. Five or below score was considered unacceptable.

### *2.9. Statistical analysis*

For the three independent replicates, statistical analysis was completed with SPSS statistics 17.0 for windows. The difference between means of values of sensory characteristics and bacteriological investigation between different treatments were calculated using one-way variance analysis (ANOVA) and multiple means comparisons were made with least square difference test, LSD method. Differences at ($p$ <0.05) level were significant.

## 3.Discusion

### *Incidence on listeria species in different fishery products*

The incidence of *Listeria monocytogenes* in examined fish products as shown in **(Table 2)** where Listeria species and *L.monocytogenes* can only be detected in frozen basa fillets *(Pangasius bocourti)* with incidence in the first year was 13.7% while other listeria species was 6.8% with total incidence 20.6% ,While in the second year the incidence of *Listeria*

*monocytogenes* was 0% while other listeria species was 9.09 % with total incidence 9.09 %. The total number of isolates of listeria species in the examined basa fillets through 2 years was 9 isolates in which 6 isolates was isolated in the first year and 3 isolates was isolated in the second year. listeria species which can be isolated from basa fillets as shown in **(Figure 1)** *Listeria monocytogenes,* Listeria *grayi* and *Listeria welshimeri* with incidence 6.45%, 3.20%, 4.80% respectively. These results agree with results recorded by ***Rahimi et al (2012)*** who can detect listeria species from frozen fish with incidence 4.2%. Thses results can be explained by the ability of listeria species to survive under freezing temperature and low water activity ***(Ghanbari et al, 2013).*** However ***Jalali and Abedi (2008)*** and ***Zarei et al (2012)*** not found *L.monocytogenes* in frozen fish and shrimp. On the other hand ***Rahimi et al (2012)*** can isolate listeria species from frozen shrimp with incidence 6.6%.

Frozen (Horse mackerel,shrimp and calamari), Smoked (salmon and herring) and canned (anchovies and sardine). fish products were negative for listeria species and *L.monocytogenes*. The results in harmony with those recorded with ***(Abdellrazeq et. al, 2014)*** who can not detect listeria species in frozen horse mackerel and herring. Also (***Kwiatek 2004)*** can not found *L.monocytogenes* in smoked fish products. However ***(Chau et al 2017)*** can isolate *L.monocytogenes* from prepacked smoked salmon with incidence 21.6% . On the other hand ***Rahimi et al (2012)*** can isolate listeria species from frozen shrimp with incidence 6.6%. Also ***(Farber 1991)*** documented that canned salmon was negative for *L.monocytogeges*. Generally, Fish caughted from open water not commonly contaminated with *L. monocytogenes*. While, fish captured from coastal water and surface water of lackes may possibly contaminated with *L.monocytogenes* ***(FAO, 1999)***. Nowadays researches begin to use natural antimcribial agents to control growth of bacteria and extend shelf life time of fish products. In food science, chitosan and its derivatives are of great interest because it has unique functional characteristics, such as antioxidant activity and antimicrobial ability ***(Mohan et al. 2012, Niladri et al. 2015, Georgantelis et al. 2007)***.

***Sensory examination of chitosan treated basa fillets (Pangasius bocourti)***

The key preferences affecting consumer acceptance of raw fishery products are appearance, colour, odour and texture. The results of sensory analysis of raw and cooked basa fillets treated with chitosan are described in **(Tables 3 and 4)** respectively. Coating of basa fillets with chitosan (1%, 1.5 % and 2%) showed no significant ($p$<0.05) change in appearance, colour, odour sensory scores among treated groups and control. Conecerning texture, 2% chitosan coated basa fillets proved signicicant ($p$<0.05) change than 1%, 1.5 % and control groups, where silmness on fillets surface could be detected. This could be attributed to the viscous nature of chitosan solution. However, after cooking no slimness could be recognized by assessors. Similar results are recorded by ***(Taher et al. 2018)***. Moreovre, cooked chitosan treated (1%, 1.5% and 2%) basa fillets and control groups presented no significant ($p$< 0.05) difference in their sensory characteristics (flavor, color, juceness and texture). These results proved that treatment with chitosan didn't impaired the sensory properties of basa fillets. These results are in harmony with that obtained by ***(Fan et al. 2009)*** who found that there were no major effect between sensory scores within the sliver carp samples treated with chitosan and nontreated fish samples but Chitosan lead to a major increase in silver carp shelf life during storage in freezing condition. Also ***(Fernandez et al. 2010)*** found no differences in aroma and flavor between the control fish soup got from fish and fresh vegetables without chitosan and treated fish soup samples with chitosan.

***Chitosan's inhibitory impact on the growth of Listeria monocytogens***

Due to its non-toxic nature, antibacterial and anti-oxidative activity, film-forming property, biocompatibility and biodegradability, chitosan has concerned a lot of consideration as a natural food additive (***Majeti and Ravi 2000)***. Many researches on the antimicrobial and antioxidant impact of chitosan have been carried out (***Fan et al. 2009, Fernandez et al. 2010, Jeon et al. 2002, Ojagh et al. 2010, Souza et al. 2010)***.

Inhibitory effect of chitosan treatments (1%, 1.5% and 2%) on growth of *Listeria monocytogens* (log CFU/g) in basa fillets are shown in **(Table (5)**. In general, coating of basa fillets by chitosan in different concentrations significantly ($p$<0.05) reduce *L. monocytogens* count than control group. However, no significantly ($p$< 0.05) difference

could be noticed between fillets coated by 1% and 1.5% chitosan in reduction rate of *L. monocytogens* count,where both chitosan concentrations reduces *L. monocytogens in* fillets by about half log CFU\gm. Proportional releationship between inhibitory effect of chitosan on *L. monocytogens* and its concentration is observed from **figure (2)** in While basa fillets treated with 2 % chitosan showed the highest reduction rate in *Listeria monocytogens* when compared with control non treated basa fillets. Chitosan's antimicrobial activity may be attributable to electrostatic interaction between positive charge in chitosan molecules on the amino group of glucosamine monomer and negative charge in the microbial cell membrane that resulted in outflow of intracellular components ***(Dutta et al. 2009)***. Application of chitosan in food processing is prospective due to broad-spectrum antimicrobial activity, in addition to it is nontoxic, biodegradable, and has no allergy effect ***(Aider 2010 and Kong et al. 2010)*** furthermore, it is GRAS by United States Food and Drug Administration.

The findings were consistent with the results stated by ***(Fan et al. 2009)*** who found that 2% chitosan solution fish coating was successful in extending the storage life of refrigerated fish for 30 days..

Results also agree with ***(Ye and chen 2008)*** who showed that for 8 weeks storage of refrigerated cold smoked salmon under vaccum with chitosan film coated has long-term antilisterial efficacy.

***4. Conclusion***

Listeria species and *Listeria monocytogenes* only be detected in frozen fish fillets. Coating basa fillets *(Pangasius bocourti)* with chitosan has no adverse effect on sensory quality and consumer acceptability. Moreover, the highest concentration of chitosan 2% resulted in the highest reduction rate on *Listeria monocytogens* which ensure the product safety and prevent great health hazard. These achieved benefits encourage the industry of fish processing to use this natural bioactve antibacterial and health promoting functional ingredient.

**5. Acknowledgments**

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**6. References**

***Abdellrazeq GS, Kamar AM and El-Houshy SM, 2014.*** Molecular characterization of Listeria species isolated from frozen fish. Alexandria Journal of Veterinary Sciences, 40(1), 1-15.

***Aider M, 2010.*** Chitosan application for active bio-based films production and potential in the food industry: review. LWT - Food Science and Technology 43: 837-842.

***Beverly RL, 2004.*** "The control, survival, and growth of Listeria monocytogenes on food products" (2004). LSU Doctoral Dissertations. 1184. https://digitalcommons.lsu.edu/gradschool_dissertations/1184.

***CDC 2002.*** Multistate outbreak of listeriosis – United States. Morbidity Mortality Weekly Report 51: 950–951.

***Chau ML, Aung KT, Hapuarachchi HC, Lee PSV, Lim, PY, Kang JSL, LC Ng Y, Yap HM and Yuk HG, 2017.*** Microbial survey of ready-to-eat salad ingredients sold at retail reveals the occurrence and the persistence of *Listeria monocytogenes* Sequence Types 2 and 87 in pre-packed smoked salmon.*BMC microbiology*,*17*(1), 1-13.

***Dillon MR and Patel RT, 1992.*** Listeria in Seafoods: A Review. Journal of Food Protection 55(12): 1009-1015.

***Duan J, Cherian G and Zhao Y, 2010.*** Quality enhancement in fresh and frozen lingcod (Ophiodon elongates) fillets by employment of fish oil incorporated chitosan coatings. Food chemistry 119(2): 524-532.

***Dutta PK, Tripathi S, Mehrotra GK and Dutta J, 2009.*** Perspectives for chitosan based antimicrobial films in food applications. Food chemistry 114(4): 1173-1182.

***Fan W, Sun J, Chen Y, Qiu J, Zhang Y and Chi Y, 2009.*** Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food chemistry 115(1): 66-70.

***FAO 1999.*** FAO Fisheries Report. No. 604. Report of the FAO Expert Consultation on the Trade impact of Listeria in Fish Products. FAO Fish Utilization and Marketing Service; FAO Food Quality and Standard Service. Amherst, MA, USA, 34 p.

***Farber JM, 1991.*** Listeria monocytogenes in fish products.Journal of Food Protection,54(12), pp.922-924.

***Fernandez-Saiz P, Soler C, Lagaron JM and Ocio MJ, 2010.*** Effects of chitosan films on the growth of *Listeria monocytogenes, Staphylococcus aureus* and Salmonella spp. in laboratory media and in fish soup. International journal of food microbiology 137(2-3), 287-294.

***Georgantelis D, Ambrosiadis I, Katikou P, Blekas G and Georgakis SA, 2007.*** Effect of rosemary extract, chitosan and a-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 Cº. Meat Sci ence 76:172–181.

***Ghanbari M, Jami M, Domig KJ and Kneifel W, 2013.*** Seafood bio preservation by lactic acid bacteria – a review. LWT – Food Science and Technology 50(2):315–24.

***ISO 11290-1:1996*** Microbiology of food and animal feding stuffs-Horizontal method for the detection and enumeration of Listeria monocytogenes-Part 1:Detection method.

***Jalali M and Abedi D, 2008.*** Prevalence of Listeria species in food products in Isfahan, Iran. Int J Food Microbiol;122:336–340

***Jemmi J and Stephan R, 2006.*** *Listeria monocytogenes*: food-borne pathogen and hygiene indicator. Review Science and Technology, office Intenational Epizootics 25(2): 571-580.

***Jeon YJ, Kamil JY Shahidi F, 2002.*** Chitosan as an edible invisible film for quality 411 preservation of herring and Atlantic cod. Journal of Agriculture and Food Chemistry 50: 5167-5178.

***Kadam SU and Prabhasankar P, 2010.*** Marine foods as functional ingredients in bakery and pasta products. Food Research International 43: 1975–1980.

***Khan I, Tango CN and Oh DH, 2017.*** Development and evaluation of chitosan and its derivative for the shelf life extension of beef meat under refrigeration storage. International Journal of Food Science & Technology 52(5): 1111-1121.

***Kong M, Chen XG, Xing K and Park HJ, 2010.*** Antimicrobial properties of chitosan and mode of action: a state of the art review. International Journal of Food Microbiology 144: 51-63.

***Kwiatek K, 2004.*** Occurrence of Listeria monocytogenes in selected food of animal origin. BULLETIN-VETERINARY INSTITUTE IN PULAWY,48(3), 269-272.

***Li T, Li J, Hu W and Li X, 2013.*** Quality enhancement in refrigerated red drum (*Sciaenops ocellatus*) fillets using chitosan coatings containing natural preservatives. Food Chemistry 138(2-3): 821-826.

***Majeti NV and Ravi k, 2000.*** A review of chitin and chitosan applications Reactive and Functional Poly mers 46: 1-27.

***Mohan CO, Ravishankar CN, Lalitha KV Gopal STK, 2012.*** Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (Sardinella longiceps) during chilled storage. Food Hydrocolloids 26:167–174.

***National Advisory Committee on Microbiological Criteria for Foods (NACMCF) 1991.*** *Listeria monocytogenes*: recommendations by the NationalAdvisory Committee on Microbiological Criteria for Foods.Inter-national Journal of Food Microbiology 14:185-246.

***Niladri SC, Panda SK, Navitha M, Asha KK, Anandan R and Suseela M, 2015.*** Vanillic acid and coumaric acid grafted chitosan derivatives: improved grafting ratio and potential application in functional food. Journal of Food Science and Technology 52(11):7153–7162

***Ojagh SM, Rezaei M, Razavi SH and Hosseini SMH, 2010.*** Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food chemistry 120(1): 193-198.

***Parihar VS, Barbuddhe SB, Danielsson-Tham ML and Tham W, 2008.*** Isolation and characterization of Listeria species from tropical seafoods. Food Control 19(6): 566-569.

***Raafat D and Sahl HG, 2009.*** Chitosan and its antimicrobial potential – a critical literature survey. Microbial Biotechnology 2(2): 186–201.

***Rahimi E, Shakerian A and Raissy M, 2012.*** Prevalence of Listeria species in fresh and frozen fish and shrimp in Iran. Annals of Microbiology, 62(1), pp.37-40.

R***yser ET and Marth EH(Eds.), 1999.*** Listeria: listeriosis, and food safety. CRC Press.

***Slutsker L and Schuchat A, 1999.*** Listeriosis in humans. In E. T. Ryser & E. H. Marth (Eds.), Listeria, listeriosis, and food safety. New York, US:Marcel Dekker, Inc.

***Souza BWS, Cerqueria MA, Ruiz HA, Martins JT, Casariego A, Teixeira JA and Vicente AA, 2010.*** Effect of chitosan-based coatings on the shelf life of salmon (Salmo salar). Journal of Agriculture and Food Chemistry 58(21):11456-11462.

***Taher Salma T, El-Khawas KM, Elmossalami MK and Kassem Gehan MA, 2018.*** Extending shelf life of chilled chicken fillets using chitosan and lactic acid. Egyptian Journal of food safety 3(1): 35.

***Vogel BF, Hansen LT, Mordhorst H Gram L, 2010.*** The survival of *Listeria monocytogenes* during long term desiccation is facilitated by sodium chloride and organic material. International journal of food microbiology 140(2-3):192-200.

***Ye M, Neetoo H and Chen H, 2008.*** Effectiveness of chitosan-coated plastic films incorporating antimicrobials in inhibition of *Listeria monocytogenes* on cold-smoked salmon. International journal of food microbiology 127(3): 235-240.

***Zarei M, Maktabi S, and Ghorbanpour M, 2012.*** Prevalence of Listeria monocytogenes, Vibrio parahaemolyticus, Staphylococcus aureus, and Salmonella spp. in seafood products using multiplex polymerase chain reaction. Foodborne Pathogens and Disease,9(2), 108-112.

## Results

**Table (1) Total number of examined different fishery products over two years**

| Types of fishery products | | First year | Second year | Total |
|---|---|---|---|---|
| **Frozen fishery products** | **Basa fillets *(Pangasius bocourti)*** | 29 | 33 | 62 |
| | **Horse mackrel** | 38 | 25 | 63 |
| | **shrimp** | 44 | 18 | 62 |
| | **calamari** | 7 | 32 | 39 |
| **Smoked Fishery products** | **salmon** | 6 | 7 | 13 |
| | **Herring** | 30 | 24 | 54 |
| **Canned fishery products** | **Anchovies** | 0 | 4 | 4 |
| | **Sardine** | 14 | 8 | 22 |
| **Total** | | 168 | 151 | 319 |

**Table (2) Incidence of listeria species in examined fishery products over two years**

| | | First year | | Second year | | Total | |
|---|---|---|---|---|---|---|---|
| | | **Number** | **%** | **Number** | **%** | **Number** | **%** |
| **Frozen fishery products** | **Basa fillets *(Pangasius bocourti)*** | 6 | 20.6 | 3 | 9.09 | 9 | 14.51 |
| | **Horse mackerel** | 0 | 0 | 0 | 0 | 0 | 0 |
| | **shrimp** | 0 | 0 | 0 | 0 | 0 | 0 |
| | **calamari** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Smoked fishery products** | **salmon** | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Herring** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Canned fishery products** | **Anchovies** | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Sardine** | 0 | 0 | 0 | 0 | 0 | 0 |

**Table (3) Sensory examination of chitosan treated raw basa fillets *(Pangasius bocourti)***

| | Appearance | Colour | Odour | Texture |
|---|---|---|---|---|
| **Control** | $8.67^{a}$±0.33 | $8.83^{a}$±0.17 | $8.83^{a}$±0.17 | $9.00^{a}$±0.00 |
| **Chitosan 1%** | $8.83^{a}$±0.17 | $9.00^{a}$±0.00 | $9.00^{a}$±0.00 | $8.83^{a}$±0.17 |
| **Chitosan 1.5%** | $8.92^{a}$±0.08 | $8.83^{a}$±0.17 | $8.92^{a}$±0.08 | $7.00^{b}$±0.58 |
| **Chitosan 2%** | $9.00^{a}$±0.00 | $8.92^{a}$±0.08 | $8.83^{a}$±0.17 | $6.67^{b}$±0.33 |

$^{a–b}$ Means with different superscripts within the same row are significantly ($P< 0.05$) different.

* Values represent the mean of 3 independent replicates ± SE.

**Table (4) Sensory examination of chitosan treated cooked basa fillets *(Pangasius bocourti)***

| | Flavour | Texture | Colour | juiciness | overall acceptability |
|---|---|---|---|---|---|
| **Control** | $8.67^{a}$±0.33 | $8.73^{a}$±0.15 | $8.83^{a}$±0.17 | $8.75^{a}$±0.14 | $8.75^{a}$±0.14 |
| **Chitosan 1%** | $8.50^{a}$±0.29 | $8.90^{a}$±0.10 | $8.93^{a}$±0.07 | $8.60^{a}$±0.21 | $8.87^{a}$±0.09 |
| **Chitosan 1.5%** | $8.92^{a}$±0.08 | $8.83^{a}$±0.17 | $8.92^{a}$±0.08 | $8.67^{a}$±0.33 | $8.73^{a}$±0.15 |
| **Chitosan 2%** | $8.83^{a}$±0.17 | $8.92^{a}$±0.08 | $8.75^{a}$±0.14 | $8.77^{a}$±0.12 | $8.90^{a}$±0.10 |

$^{a}$ Means with different superscripts within the same row significantly ($P< 0.05$) different.

* Values represent the mean of 3 independent replicates ± SE.

**Table (5) Inhibitory effect of chitosan application on *Listeria monocytogens* (log CFU/g) in chitosan treated raw basa fillets *(Pangasius bocourti)***

| **Treatments** | ***Listeria monocytogens* (log CFU/g)** |
|---|---|
| **Control** | $4.18^{a}\pm0.10$ |
| **Chitosan 1%** | $3.69^{b}\pm0.02$ |
| **Chitosan 1.5%** | $3.54^{b,c}\pm0.00$ |
| **Chitosan 2%** | $3.41^{c}\pm0.02$ |

$^{a–c}$ Means with different superscripts within the same row significantly ($P< 0.05$) different.

* Values represent the mean of 3 independent replicates ± SE.

**Figure (1) Incidence of isolated listeria species in basa fillets (*Pangasius bocourti*) over two years**

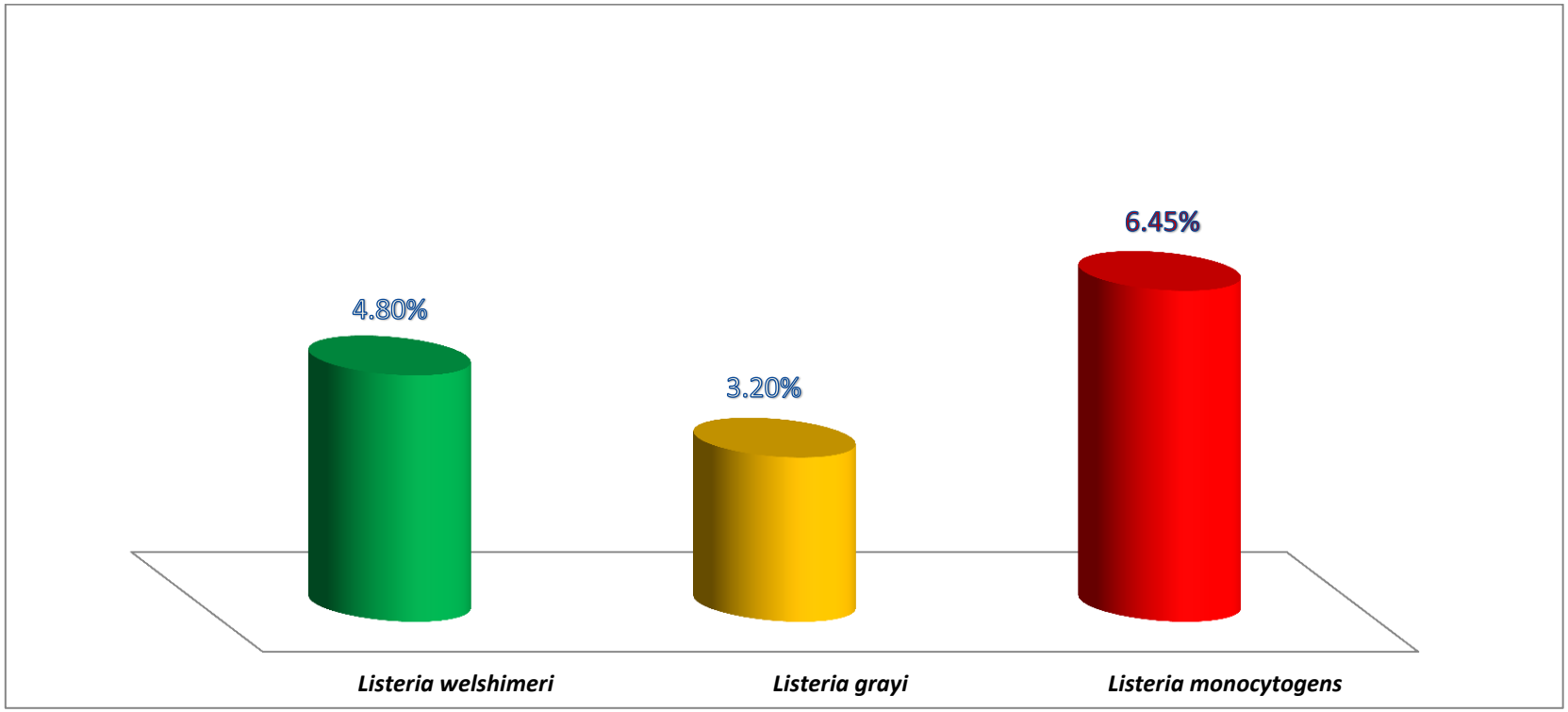

**Figure (2) Reduction rate of different chitosan concentrations on *Listeria monocytogens* in basa fillets *(Pangasius bocourti)***