International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 1169-1173
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Effect of Different Types of Salts on Brining of Sea Foods and their Preservation Efficiency

Ramabhai V[1], Pushpa Latha R[1], Krishnaraj M.,[2], Jayaprakashvel M[1]*
[1]Department of Marine Biotechnology, AMET Deemed to be University, Kanathur, Chennai – 603112, Tamil Nadu, India.
[2]ABTRI Biotech Private Limited, T.S. 89, SIDCO Industrial Estate, Ekkatuthangal, Guindy, Chennai 600 032, India

Received: 10 Jan 2019 / Accepted: 19 Mar 2019 / Published online: 1 Apr 2019
***Corresponding Author Email:** jayaprakashvel@gmail.com

Abstract
Salting method for fish processing is commonly practiced for centuries. The use of brining is an intermediate step in marinating, smoking and canning of fish sample. Brining helps to improve the quality of fish and fish products. Instead of using the traditional dry salting method, salt brining can be followed to extend the shelf life of the product by delaying microbiological growth and the chemical changes. The aim of this study is to know the effect of different types salts like Iodized salt, Non-Iodized Salt and Himalayan rock salt for bringing the selected fish sample stored at both room temperature (35±2°C) and refrigerated temperature (35±2°C). Brined fish sample was kept for 15 days both at room temperature (35±2°C) and refrigerated temperature (35±2°C) after incubation, microbial population of heterotrophic bacteria, *Salmonella* and *Shigella*, Coliforms, *Vibrio* sp., counts were evaluated and tabulated. The result showed that the Himalayan rock salt brining had reduced the microbial load in comparison to other iodized salt and non-iodized salt. Effect of 10% Himalayan Rock Salt on microbial population of heterotrophic bacteria, *Salmonella* and *Shigella*, Coliforms, *Vibrio* sp., counts in sea foods brined at room temperature was found to be $15x10^7$, $2x10^3$, $13x10^3$, $5x10^3$ cfu/mL respectively. However, the quality of the final products is affected by the conditions of the raw material, the type, quality and concentration of the salt as well as the salting method. Comparing the salt brining method penetration rate of salt is higher in dry salting method into the fish flesh.

Keywords
Brining, Iodized salt, Non-Iodized Salt, Himalayan rock salt, salt brining, dry salting.

*****

INTRODUCTION
Salting or brining is one of the most simple and efficient method of food preservation especially sea foods. Traditionally salting is practiced all over the world to preserved sea foods and commercially sold as dried fish. There are different salts available with

varying chemical composition. It is understood that because of their different in chemical composition, different salts may perform differently during brining process. Iodized salts are in use for the past few decades. Iodine is a known antimicrobial agent. Hence a research study is essential to compare the brining effect of both iodized and non iodized salts. This proposal is made to study the effect of iodized salt, non-iodized sea salt and Himalayan rock salt on the microbial load in the commercially available fishes.

Seafood quality and safety are of great concern in the present world. Most of the cephalopods that are caught are stored and marketed in their frozen state, spoilage caused by autolysis of the muscle is particularly intensive in cephalopods because of the high level of proteolytic activity in the muscle produced by their highly active metabolism,[1-2] and this favors rapid microbial growth. The spoilage of cephalopods in chilled storage is governed mainly by Gram-negative bacteria.[3] In vacuum-packed samples, there are qualitative and quantitative changes due to the growth of anaerobic microbial flora as aerobic organisms are inhibited,[4] and spoilage is largely dominated by Gram-positive bacteria.[5] Of the latter type, lactic bacteria are favored, to the extent that they can become the dominant flora.[6]

For improving the quality of whole muscle beef, pork and poultry products, many studies have been grown to incorporate the low salt brines using multi needle injection technology. To impact the whole muscle protein functionality without altering flavor/appearance low salt brines are formulated typically for less than 5% NaCl. Thus, low salt brines are found to have high potential in reducing drip loss during retail display, reducing cool-loss, improving palatability (perceived juiciness) and extending shelf-life.[7]

Researchers had focused on the use of high salt brines through fish injection majorly for the products like salted cod or cold-smoked salmon. Studies on low salt injection in cod and catfish and have demonstrated their use in uncooked fillet firmness improvement and thaw drip reduction. The fish evaluated in that research, however, does not experience the type of texture degradation during cooking that occurs with Pacific whiting.[8]

Salting is one of the oldest preservation methods for foods. However, despite many other processes being used today, the most common of which is freezing, salting of fish remains a very important process for Iceland where the products are, primarily, exported. In the production of salted fish, the raw fish is filleted, or butterfly split and then heavily salted. Various traditional methods like pickling/brining and stacking (Kencing) are used for curing of salted fishes. Instead of layers of fish and dry salt, split fishes are piles in kench salting method. Liquid diffuses from the fish as it takes up salt and the resulting brine is allowed to drain off. In pickle salting the fish is also dry salted but the fish is kept in vats and the liquid, which diffuses from the fish, forms a strong brine solution as the salt dissolves.[9]

There are many factors which affect the quality of the final product, including the condition of the raw material, the type, quality and concentration of salt as well as the salting method.[10-11] The salting method has an influence on the structural and mechanical properties of the fish muscle. Akse et al.,[12] had studied that the rate of salt penetration into the fish muscle is higher than that obtained by dry salting, when the fish is brined it is believed to render both better quality and higher weight yield[11] The concentration of salt in the brine affects the rate of salt diffusion into the muscle and the quantity of water and proteins extracted. Salt rate and water diffusion rate were positively correlated with increasing salt concentration of the brine.[13-14] Protein extraction from the muscle is one of the functions of salt concentration, with a maximum at 6–9% NaCl. Pure water or higher salt concentration brines were used for the low quantity extractions.[14] This is clearly an influence of (salt/water) on inter and intra-molecular bonds of the muscle protein and will affect the structure and denaturation of the proteins and thereby the water holding capacity of the muscle.

In light of the reviewed literature it is found that a comprehensive attempt to find the effect of different types of salts to enhance the efficiency of brining of sea foods is yet to be undertaken. Hence, it is aimed to study the effect of iodized salt, non-iodized sea salt and Himalayan rock salt on the microbial load in the commercially available fishes.

MATERIALS AND METHODS

Collections of fish sample

Fish sample collected from the local market near Kanathur (12.8663°N, 80.2447°E) Chennai was washed with the running tap water to remove the sand and dust particles, and then it was cut into pieces for further studies.

Immersing in brine

The collected fish sample was taken for salt brining with three different types of salts like iodized salt, non-iodized salt and rock salt and was dissolved in Distilled water. Dissolve 10g of three different types

of salt to three different conical flasks of 100mL distilled water and maintain a conical flask as control without adding salt. Weigh 10gm of fish sample to each conical flask expect the control. After 15 days brining of fish sample at both room temperature (35 ±2°C) and refrigerated (35 ±2°C) temperature, inhibitory effect of salt on the microbial growth were evaluated using the spread plate technique on the Nutrient Agar medium, *Salmonella* and *Shigella* Agar medium, Eosin methylene blue agar and Thiosulfate Citrate Bile salt Sucrose agar medium.

Enumeration of microbial population

Sample is serially diluted and plated out on various respective agar surfaces in such a manner that single isolated bacteria form visible isolated colonies by the plate count method. The number of colonies can be used as a measure of the number of viable (living) cells in that known dilution. Thus, the bacterial sample is diluted by factors of 10 and plated on agar. After incubation, the number of colonies on a dilution plate showing 30 and 300 colonies is observed, from the plate count data and calculate the concentration of bacteria in the original sample. Each Colony Forming Unit (cfu) represents a single cell or a group of cells attached together and inseparable by shaking. Therefore, the number of cfu in the original sample is determined by multiplying the number of colonies on a dilution plate by the corresponding dilution factor.

The number of CFUs per mL of sample → **The number of colonies (30-300 plates) X The dilution factor of the plate counted**

RESULT AND DISCUSSION

Salting is one of the traditional preservation methods for food. Liquid diffuses from the fish as it takes up salt and the resulting brine is allowed to drain off. In this fish was kept in conical flasks and the liquids, which diffuses from the fish forms a strong brine solution as the salt dissolves. The method used for salting of sea food has preceded salt brining using various salts like ionised salt, non-ionised salt and rock salt. This method of preservation had enhanced the quality of the final product of various sea foods in day-to-day life. The effect of the salt concentration in the brine has been a matter of controversy in their microbial load may be obtained by using lower salt concentrations than by using a fully saturated brine solution.

Gormley[15] explains that there will be the different metabolic rate for different strains of same species at the same temperature. However different group of bacteria could grow with the increase above 0°C. Importantly gram-positive flora will increase above the temperature 5°C. Since halophytic bacteria are adapted to salinity, 2%-8% sodium chloride required for its growth. They are highly populated to the outer skin layer, on the gills and intestine of marine fish. Bacteria are adapted to even lower refrigerated sea water (RSW) system in presence of NaCl.[16]

Many factors are believed to affect the quality of the final products like the condition of the raw material, the type, quality and concentration of the salt as well as the salting method. The rate of salt penetration into the fish flesh during the salt brining will be higher comparative to the dry salting.[17]

Effect of salt on microbial population in the selected sea fish sample brined at room temperature (35 ±2°C) and the total bacterial population & pathogenic bacteria population were assessed as follows (Table No-1); The total heterotrophic bacterial count in the selected fish brine was found to be $42x10^8$, $32x10^8$, $19x10^8$ and $15x10^7$cfu/mL; *Salmonella* and *Shigella* count in the selected fish brine was found to be $4x10^4$, $2x10^4$, $1x10^4$ and $2x10^3$ cfu/mL; *Vibrio* sp., count in the selected fish brine was found to be $10x10^4$, $35x10^3$, $25x10^3$ and $13x10^3$ cfu/mL; Coliforms count in the selected fish brine was found to be $12x10^4$, $7x10^4$, $6x10^3$ and $5x10^3$ cfu/mL.

Table No-1: Effect of salts on microbial population in sea foods brined at room temperature:

Treatment	Microbial count (cfu)			
	Total Heterotrophic bacteria(cfu/mL)	*Salmonella* and *Shigella* (cfu/mL)	*Vibrio* (cfu/mL)	Coliforms (cfu/mL)
Control	$42x10^8$	$4x10^4$	$10x10^4$	$12x10^4$
10% Iodized Salt	$32x10^8$	$2x10^4$	$35x10^3$	$7x10^4$
10% Non-Iodized Salt	$19x10^8$	$1x10^4$	$25x10^3$	$6x10^3$
10% Himalayan Rock Salt	$15x10^7$	$2x10^3$	$13x10^3$	$5x10^3$

Effect of salt on microbial population in the selected sea fish sample brined at refrigerator temperature (35 ±2°C) and the total bacterial population & pathogenic bacteria population were assessed as follows (Table No-2); The total heterotrophic bacterial count in the selected fish brine was found to be $30x10^7$, $21x10^{7,}$ $22x10^7$and $19x10^7$ cfu/mL; *Salmonella* and *Shigella* count in the selected fish brine was found to be $28x10^3$, $12x10^4$, $18x10^4$ and $12x10^3$ cfu/mL; *Vibrio* sp., count in the selected fish brine was found to be $25x10^4$, $13x10^3$, $17x10^3$ and $13x10^3$ cfu/mL; Coliforms count in the selected fish brine was found to be $24x10^4$, $11x10^4$, $16x10^2$and $15x10^3$ cfu/mL.

Table No- 2: Effect of salts on microbial population in seafoods brined at refrigerator

Treatment	Microbial count (cfu)			
	Total Heterotrophic bacteria (cfu/mL)	*Salmonella* and *Shigella* (cfu/mL)	*Vibrio* (cfu/mL)	Coliforms (cfu/mL)
Control	$30x10^7$	$28x10^3$	$25x10^4$	$24x10^4$
10% Iodized Salt	$21x10^7$	$12x10^4$	$13x10^3$	$11x10^4$
10% Non-Iodized Salt	$22x10^7$	$18x10^4$	$17x10^3$	$16x10^2$
10% Himalayan Rock Salt	$19x10^7$	$12x10^3$	$13x10^3$	$15x10^3$

By increasing the effect of salt, the period of bacterial development can be reduced. However, the total bacterial count increases along the ripening period[18] were found to have the Bacterial value of $7.5x10^5$-$7.5x10^6$ cfu/g [19] and between $6.5x10^6$-$1.67x10^8$ cfu/g [20]. The total mesophilic aerobic bacterial count according to the Patir et al.,[21] in salted mullet (*Chalcalbulrnus tarichii*) stored at 4 ± 1°C ranged from 2.0 to 5,0 log cfu/g, with average 3.94 log cfu/g wereas Al-Asous & A-Harbu[22] study reports to be of range 3.62 to 4.15 cfu/g for the salted wild mullet (*Valamugil seheli).* All the above studies were in evidence for the present study.

Therefore, to test this hypothesis, the effect of different brine salting methods was studied by traditional brine salting as maintaining a constant brine concentration and increasing the salt concentration gradually during brining. Fish samples collected from the local market of kanathur (12.8663°N, 80.2447°E), Chennai. Analyses were carried out on the fish-brine salting with Iodized salt, Non-Iodized salt and Himalayan rock salt, stored at room temperature (35±2°C) and refrigerated temperature (35±2°C). Microbial population of total heterotropic bacteria, *Salmonella* and *Shigella*, Coliforms and *Vibrio* sp., were enumerated by spread plate technique on Nutrient Agar medium, *Salmonella* and *Shigella* Agar medium, Eosin methylene blue agar and Thiosulfate Citrate Bile Salt Sucrose agar medium. Plates were incubated for 24 hours at room temperature and refrigerated temperature for observation. Colonies on plates were counted and counts were multiplied with the relevant dilution factor. As per the colony count of three different salt concentration, ionized salt showed abundant growth of microorganisms and the rock salt showed a smaller number of colonies. The result indicates that the rock salt showed the better inhibitory effect on the growth of microorganisms and was significant in sea food preservation comparing with other ionized and non-iodized salt brining.

CONCLUSION

The method used for salting of sea food has preceded salt brining using various salts like iodized salt, non-iodized salt and rock salt. This method of preservation had enhanced the quality of the final product of various sea foods in day-to-day lifes. The effect of the salt concentration in the brine has been a matter of controversy in their microbial load may be obtained by using lower salt concentrations than by using a fully saturated brine solution. Therefore, to examine this hypothesis, the effect of different brine salting methods was studied; traditional brine salting, maintaining a constant brine concentration and increasing the salt concentration gradually during brining. The results indicated that the effect of rock salt in seafoods brined at room temperature and refrigerator storage had reduced the microbial load and was significant comparing with other ionized ans non-iodized salt.

ACKNOWLEDGEMENT

Authors are thankful to the Administration and Management of AMET deemed to be University, Kanathur, Chennai, for their continuous encouragement and constant support. Author VR acknowledges the financial support provided by AMET deemed to be University.

REFERENCES

1. Stanley, D.W. and Hultin, H.O. 1984. Proteolytic activity in North America1 Squid and its relation to quality. Can.Inst.Food Techno1.J. 17(3): 163-7.
2. Hurtado, J.L., Borderias, J., Montero, P., 1999a. Characterization of proteolytic activity in octopus (Octopus vulgaris) arm muscle. J. Food Biochem 23, 469–483.
3. Paarup, T., Ruiz-Capillas, C., Morales, J., Lopez, E., Moral, A., 1996. „ Gravad" – type rainbow trout, packed in vacuum and modified atmosphere: microbiological, biochemical and sensory aspects. Refrigeration and aquaculture, in Bordeaux (France) colloquium, Proceedings IIR Conference-Commission C2. pp. 333-337.
4. Bahr, S. and Wolf, J.R. 1985 Postnatal development of axosomatic synapses in the rat visual cortex: morphogenesis and quantitative evaluation. J. Comp. Neurol., 233: 405-420.
5. Stammen, K., Gerdes, D., Caporaso, F., Martin, R.E. 1990. Modified atmosphere packaging of seafood. Crit. Rev.Food Sci. Nutr. 29, 301–331.
6. Leroi, F., Arbey, N., Joffraud, J.J., Chevalier, F., 1996. Effect of inoculation with lactic acid bacteria on extending the shelf life of vacuum-packed cold-smoked salmon. Int. J. Food Sci. Technol. 31, 497–504.
7. Parsons, A.N., Vanoverbeke, D.L., Goad, C.L., and Mireles Dewitt, C.A. 2011. Retail display evaluation of steaks from Select beef strip loins injected with a brine containing 1% ammonium hydroxide. Part 1: fluid loss, oxidation, color and microbialplate counts. J. Food Sci., 76, 63-71.
8. Kin, S., Schilling, M. W., Smith, B. S., Silva, J. L., Jackson, V. and Kim, T. J. 2010. "Phosphate type affects the quality of injected catfish fillets," Journal of Food Science, vol. 75, no. 1, pp. S74–S80, 2010.
9. Van Klaveren, F.W. & Legrende, R. 1965. Salted cod. In: Fish as Food (edited by G. Borgstrom), Vol III. Pp. 133–160. New York: Academic press.
10. Zaitsev, V., Kizevetter, I., Lagunov, L., Makarova, T., Minder, L. and Podsevalov, V. 1969. Salting and marinating. In: Fish curing and processing. Moscow: MIR Publishers. pp.198–256.
11. Beraquet, N.J., Iaderoza, M., Jardim, D.C.P. and Lindo, M.K.K. 1983. Salting of mackerel (Scomber japoonicus) II. Comparisonbetween brining and mixed salting inrelation to quality and salt uptake.Coletaneado Instituto de Tecnologia deAlimentos 13: 175–198.
12. Akse, L., Gundersen, B., Lauritzen, K., Ofstad, R. and Solberg, T. 1993. Saltfisk: Salt modning,Utproving av Analysemetoder, Misfarget Saltfisk (in Norwegian). Tromso: Fiskeri forskning. pp. 1–61.
13. Poernomo, A., Fawzya, Y.N. and Ariyani, F. 1992.Salting and drying of mackerel. Asian FoodJ. 7: 141–146.
14. Lawrie, R.A. 1998. The storage and preservation of meat II. Moisture control. In: Lawrie'sMeat Science, 6th edn. (edited by R.A.Lawrie). Cambridge, UK: Wood headPublishing Limited. pp. 191–194.
15. Gormley, T.R. 1990. Chilled foods, The state-of-the-art England: Elsevier science.
16. Silva, C.C.G., Ponte, D.B.J.D., Dapkevicius, M.L.N.E. 1998. Storage temperature effect on Histsmine Formation in Big eye Tuna and Skipjack. Journal of food science vo, 63, No. 4, 644-646.
17. Thorarinsdottir K.A., Sigurjon Arason, Sigridur G. Bogason, and Kristberg Kristbergsson, 2004. The effects of various salt concentrations during brine curing of cod (Gadus morhua). University of Iceland.
18. Todorov, L. 1975. Bacterial contamination of salted atlantic mackerelduring processing (Abst. Only). Veterinarnomeditsinski. Nauiki,12(2), 51-57.
19. Kucukoner, E., & Akyüz, N. 1992. Microbiological, physical, chemical and sensory characteristics determination of the micronutrient pearlmullet fish prepared with different brine method of Van - Ercisregion. Y.Y.Ü. Fen Bilimleri Enstitüsü Dergisi, 1(1), 39-50.
20. Nino De Onshuus, Y. 1974. Studies a salted fish (Sbst. Only). Revista del Institudo de Investigaciones Technologicas, 16(91), 33-43.
21. Patır, B., Gürel İnanlı, A., Öksüztepe, G., & İlhak, O. I. 2006. Microbiological and chemical qualities of salted grey mullet (Chalcalburnus tarichii Pallas, 1811). International Journal of Science & Technology, 1(2), 91-98.
22. Al-Asous, A. I., & Al-Harbı, A. H. 2016. Microbiological and physicochemical quality of salted bluespot mullet (Valamugil seheli) stored at different temperature. Journal of Food Safety. http://dx.doi.org/10.1111/jfs.12291.