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The effect of nisin Z on growth of *Listeria monocytogenes* in surimi Fish Kilka (*Clupeonella cultriventris caspia*) stored at 4°C

Abdollah Dehbandy[1*], Abbasali Motlebi[1], Vadud Razavilar[1], Reza Poorgholam[2]

[1]*Department of Food Hygiene, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad university, Tehran, Iran*

[2]*Professor of Health Aquatic, Department of Ecology of Caspian Sea, Sary, Iran*

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Abstract

In this study, the antibacterial effect of nisin Z in the form of free and microencapsulated on the growth of *Listeria monocytogenes* in kilka surimi fish (*Clupeonella cultriventris caspia*) on days 0, 3, 6, 9, 12 and 15 holding period were determined. Concentrations which used for the nisin-free and microencapsulated (by Spray Dryer on covered liposomes) two concentrations IU 700 and IU 1000 were on gram that with method of spray were added to surimi kilka and one sample as a control (no preservative) is considered. The results showed that the amount of this index in treatments containing microencapsulated nisin, most significantly towards free treatments as well as free treatments were significantly lower compared to the control treatment. Also treatment with 1000 IU nisin microencapsulated that was exposed to the highest concentrations of microencapsulated nisin form, Most significantly was less treatments from the aspect of reputed index than other treatments, but the amount of bacterial levels were increased in all treatments and did not observe an effect of decreasing of bacterial population but effect of nisin in the form of microencapsulated in reducing bacterial growth rate was higher than the control and free treatments.

* **Corresponding Author:** Abdollah Dehbandy ✉ adehbandy@yahoo.com

Introduction

Fish has a high nutritional value and due to unsaturated fatty acids, especially omega-3 has a special place however, fish is of perishable foods and with exposure to improper temperature start to spoilage and chemical and biological parameters of spoilage can be increased (Rehbein and Oehlenschlager, 2009).

Today's consumers due to the harmful effects of chemical and synthetic preservatives want to use natural preservatives derived from sources of plants, animals and microorganisms so in addition to increasing the shelf life of food be away of the harmful effects of chemical food preservatives, However, research has shown that biological preservative when used individually, don't have positive effects on process of chemical and microbial spoilage and must be mixed with chemical preservatives, but with lower concentrations should be used to give better results (Roller, 1995- Tomé *et al.*, 2006).

One of the biological preservatives that were used in various products, especially dairy products is nisin. This material from Lactococcus lactis bacteria synthesized and is available in two commercially shape Z and A. Nisin is a protein substance and has antimicrobial properties against Gram-positive bacteria, especially lactic bacteria that produce spoilage (Stiles, 1994- Zaerzadeh *et al.*, 2011). Free form of nisin due to interactions with proteins and enzymes release form proteins of fish and different enzymes loses its effects over time, so we need a way to increase the efficacy of nisin (Benech *et al.*, 2002- Kordel and Sahl, 1986).

Today's for increasing the effects of inhibitors as well as to enhance the stability of nisin the Nanocapsulation method was used that in this process of coating material such as zein, liposomes, Gum Arabic, etc were used which causes a slow release of nano form of nisin. The inhibition effects of nanocapsulation form (microencapsulated) nisin against Listeria monocytogenes in cheese has been studied and the results showed that nanocapsulation form has a significant inhibitory effect on this bacteria (Benech *et al.*, 2002- Schmidt, 2009- Xiao, 2010).

Kilka fish is of valuable species from the Caspian Sea and because of having a high nutritional value, especially omega-3 polyunsaturated fatty acids is important (Fazli *et al.*, 2007). Statistical studies from the 2000 to 2007 shows that from 82,128 tons of kilka fish that were caught in the years, 75124, 3413, 2785, 861 tons respectively as fish meal, packaged, canned and fresh were used. This statistics shows represents a lower amount of human consumption of Kilka fish and using various strategies such as transporting of fish with using water and ice system, the addition of preservatives, using modern systems of packing and storing in low temperatures can increase the amount of human consumption of this valuable product (Development, 2004).

One of the most recent advances in the use of economical marine resources are in providing products that now are known as Value-added products. One of the sea products that is of value-added is surimi (Razavi Shirazi, 1994). Surimi is a protein product with high performance properties that are not consumed directly but it can be used as primal material for the production of Sausages, Fish burger, Fish finger, Cutlet, Kebab, Imitation products, (Mousavinasab *et al.*, 2008).

Listeria monocytogenes widely exists in nature, so that the cells of the bacteria without a significant reduction can be alive in wet-dry conditions. Listeriosis disease in humans with symptoms including diarrhea, toxic septicemia, meningitis, encephalitis and brain abscess is associated. This disease in humans primarily through consumption of contaminated food is caused and outbreaks of Listeria monocytogenes at the retail level of silver carp and common carp of fishes in Iran respectively is 10 and 17.5 percent. Also, 8.5 percent of the fishes that were caught from whole of the culturing pond of heat fishes (silver carp and common carp) were contaminated with Listeria monocytogenes. In the world isolated

cases of Listeriosis occurs because of consumption of half-baked fish yearly (Akhondzadeh *et al.*, 2002-Mehdizade *et al.*, 2009).

The aim of this study was to evaluate the effect of nisin Z in two forms, free and microencapsulated with liposomes on the growth of Listeria monocytogenes in surimi fish stored at 4°C at different times.

Materials and methods

Preparation of anchovies fish

Kilka fish prepared from the port of Amir Abad in the province of Mazandaran and in adjacent of ice were transferred to the Institute of Ecology of Caspian Sea. Under study species in this study are typical species of kilka (Clupeonella cultriventris caspia) that has the largest number of fish species that were caught between species of Kilka fish.

Kilka surimi production

To prepare surimi, first kilka fish was washed with water and then manually heads and offal were separated, and after rewashing bones were removed. After grinding, meat was added to a dish with ratio of 1 to 5 (meat to water) and three times washing procedure was performed (The third stage of washing was performed for better dewatering with water containing 0.02% salt and at all stages of the washing the water temperature was below 10 ° C and washing operations were performed without interruption). At the end of the washing procedure the action of dewatering of mixture was done with cleansing manually (Shimizu *et al.*, 1992).

Nisin preparation

Nisin 2.5% (Serva, America) that was used contains 1000IU/mg nisin in dry material, 75% sodium chloride and 22.5 % dry milk.

Preparation of samples that contain encapsulated nisin in liposomes

Nisin solution of 2.5% with concentration 6 mg solids material in 1 mL of solvent (aqueous ethanol 50% v/v) were prepared and Stirred for 6 hours with stirring and then positioned in centrifuged refrigerator for 5 minutes with 1520 rpm (Japan, h-103nr Kokusan). Material that was used for encapsulation was liposome that was prepared in commercially form (Merck, Germany) and at first was stirred with the linoleic acid (Merck, Germany) with amount of 0.130 percent (as surfactant) at temperatures of 40 ° C for 30 minutes.(pH=4.5). After ending of incubation time, nisin was added to in liposomes and surfactant mixture and was stirred for 2 h at room temperature. Then mixture in the Spray dryer instrument (UK, Lab-plant UK Ltd YO14) OPH with a rate of 5.26 ml / min with 100% aeration, inlet temperature 105 ° C and the outlet temperature was 68 ° C degrees was dried (Schmidt, 2009).

Preparation of samples that contain free nisin

Normal clorideric acid 0.02 was used to prepare stock solution of nisin and the obtained solution was sterilized by using 0.22 microns' filter. In the next stage diluted of distilled water was used for providing various dilutions by IU. Solution which was prepared prior to start of the experiment were kept at -20 ° C of freezer (Abdollahzadeh *et al.*, 2012).

Preparation of samples to provide treatment and to perform microbial test

After preparation of the samples (25 g per sample), two concentrations of free and microencapsulated nisin with liposomes (700 IU and 1000 IU) with spray method were added to surimi of kilka and one sample was considered as a control (without any preservative). Samples maintained at refrigerator temperature (4 °C) for 15 days and every 3 days were examined from the aspect of the growing of enumeration of Listeria monocytogenes and based on compliance of these numbers with the standard, acceptable duration of keeping of Listeria monocytogenes was determined which, naturally, the best concentration and type of nisin (free or microencapsulated) was introduced in order to achieve the best time for maintenance and avoid the microbial growth. Including 5 treatments, 3 replicates for each treatment and 6 times for test, totally 90 samples were evaluated (Al-Holy *et al.*, 2005).

Determination and enumeration of Listeria monocytogenes

For enumeration of Listeria monocytogenes from selective medium CHROMagar^{tm} Listeria supplement (chromogenic medium, microbiology company of CHROMEagar of France) and Supplement of it (CHROMagar Listeria supplement) were used. For bacterial counts at each sampling time, 1 g of meat sample was added 9 mL extra saline solution and then was homogenized. Depending on the type of sample, dilutions were variable from 10^{-2} to 10^{-4}. 0.1 ml diluted sample in culture medium CHROMagar^{tm} Listeria supplement was surface cultured and for 24 h at 37 ° C was incubated. Listeria monocytogenes bacteria create colonies of blue with white halos on this medium. For each treatment, two replicates were established that after culturing standard plates were chosen and counted.

Statistical Analysis

Statistical analysis of obtained data was done with SPSS 18 software. For analyzing of quantitative values the two-way ANOVA was used. To determine significant differences in treatment between the averages of at least test (Duncan) was used.

Results

According to changing of amounts of *Listeria monocytogenes* during 15 days period of storage, amounts of numeration of bacteria showed increasing process during storage that this process was statistically significant ($p< 0.05$).

The mean of each treatment and replicates of it ± SD of each treatments and replicates of it. 1. Control treatment (without nisin) inoculated with Listeria bacteria. 2. Treatment with 700 IU of free nisin inoculated with Listeria bacteria 3. Treatment with 1000 IU of free nisin inoculated with Listeria bacteria. 4. Treatment with 700 IU microencapsulated nisin inoculated with Listeria bacteria. 5. Treatment with 1000 IU microencapsulated nisin inoculated with Listeria bacteria.

Different lowercase letters in each row indicate significant differences ($p < 0.05$) on various days of taking samples for one specific treatment and different capital letters in each column indicate significant differences ($p < 0.05$) on a day for various treatments.

Table 1. Differences between mean values of numeration of *Listeria monocytogenes* of various surimi kilka treatments in different storage times.

Group	Storage Time (Day)					
	Zero	3	6	9	12	15
1	3.22±0.03fA	4.25±0.09eA	6.38±0.17dA	8.37±.07cA	9.50±0.04bA	9.81±0.06aA
2	3.20±0.08fA	3.61±0.15eB	5.45±0.11dB	6.76±0.08cB	7.66±0.015bB	7.88±0.10aB
3	3.20±0.07fA	3.37±.03eB	5.38±0.04dB	6.48±0.06cB	7.45±0.08bB	7.82±.04aB
4	3.24±0.09eA	3.30±.06eB	5.18±0.07dC	6.28±0.04cB	7.31±0.04bB	7.66±0.1aB
5	3.23±0.04eA	3.26±0.03eB	5.25±0.11dD	6.36±0.06cB	7.31±0.06bB	7.65±0.09aB

From day 0 to 15 control treatment has higher numeration values of *Listeria monocytogenes* than free and microencapsulated treatments and all the time that this differences were statistically significant ($p<0.05$).

On days 0 to 15, the 1000 IU treatments of microencapsulated nisin, 700 IU microencapsulated nisin, 1000 IU free nisin and 700 IU free nisin respectively have the least numeration value of *Listeria monocytogenes* than control treatment or from the aspect of numeration value of Listeria monocytogenes bacteria was close to lowest treatment and difference between numeration value of *Listeria monocytogenes* bacteria between various treatments in many cases from the aspect of statistics is significant ($p<0.05$).

Discussion

Food borne Listeriosis is a rare but serious illness

caused by *Listeria monocytogenes*. Bacteria are abundant in nature, but only in certain people could cause disease (Mehdizade *et al.*, 2009). Many studies have been done until now that show *Listeria* is found in aquatic ecosystems, both fresh and saline water and sediments. Yet *Listeria monocytogenes* is isolated from processed products such as cooked seafood, frozen marinades fish, surimi products, sushi and smoked salmon. The number of this bacteria can reach more than 100 thousands cells per gram of food during one term of 3-4 weeks (the period of keeping most foods) in the refrigerator foods (Safari and Saeidi Asl, 2011).

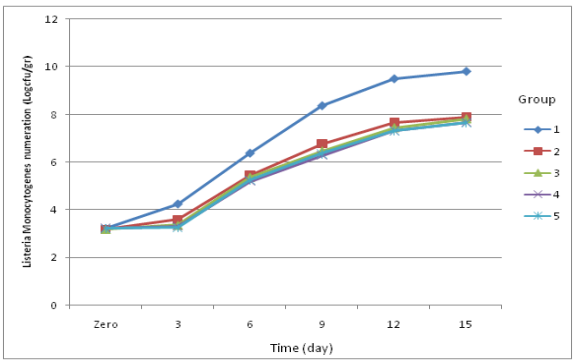

Fig. 1. Changing of *Listeria monocytogenes* bacteria of surimi kilka contaminated with the bacterium during storage time (0, 3, 6, 9, 12 and 15) at 4°C in various treatments.

According to changing of amounts of *Listeria monocytogenes* during 15 days period of storage, amounts of numeration of bacteria showed increasing process during storage that this process was statistically significant ($p < 0.05$). The similar results were obtained in previous studies.

From day 0 to 15 control treatment has higher numeration values of *Listeria monocytogenes* than free and microencapsulated treatments and all the time that this differences were statistically significant ($p<0.05$). The reason can be attributed to the effect of nisin to eliminate or prevent of functioning of Gram-positive bacteria, including *Listeria monocytogenes* (stile, 1994).

On days 0 to 15, the 1000 IU treatments of microencapsulated nisin, 700 IU microencapsulated nisin, 1000 IU free nisin and 700 IU free nisin respectively have the least numeration value of *Listeria monocytogenes* than control treatment or from the aspect of numeration value of Listeria monocytogenes bacteria was close to lowest treatment and difference between numeration value of *Listeria monocytogenes* bacteria between various treatments in many cases from the aspect of statistics is significant ($p<0.05$). Results of studies of various scholars such as Xiao (Xiao, 2010), Benech *et. al.,* (Benech *et al.*, 2002), and Zaerzadeh *et. al.,* (Zaerzadeh *et al.*, 2011) showed that more efficacy of nisin in the microencapsulated form compared to the free form on Gram-positive bacteria in causing food disease including *Listeria monocytogenes* that is confirming the results of present study.

In previous studies it was found that by increasing the concentration of nisin from 500 IU to 1000 IU significant difference on anti-Listeria effect of nisin on the second and fourth day was observed. However, in our study, this significant difference was observed on the twelfth and fifteenth day, and also the results of Vongsawasdi and colleagues studies was showed that the inhibitory effect of nisin on *Staphylococcus aureus* when nisin concentration and incubation time increased, was higher ($p<0.05$), which reflects the effect of increasing the concentration on amplifying of antibacterial effect of bacteriocin (Vongsawasdi *et al.*, 2012).

Researchers in their study evaluate the antibacterial effect of nisin in two forms of free and nano-encapsulated in liposome on decreasing the population of *Listeria monocytogenes* in ultra filtrated Iranian white cheese (Zaerzadeh *et al.*, 2011). Comparison of results of changing of *Listeria*'s population in this study shows significant effect between free nisin's function and pent in nano-capsulated liposomes on decreasing of *listeria's* population, While in our study numeration values of bacterial in microencapsulated treatments compared to control and free treatments were low and in many cases this relationship was significant, but the bacterial values increased in all treatments, and the effectiveness of nisin in microencapsulated form in reducing of bacterial growth rate was higher than the

control and free treatments and effect of decreasing of bacterial population was not observed. The reason for difference between the two studies can be seen in the following cases:

Nisin in liquid foods and homogeneous are more effective than solid and heterogeneous foods because of comfortable distribution of food in matrix. Since that pH of cheese is lower than meats, and also liquidation of nisin at lower pH increases, the molecules pass through the wall of cell facilitate and cause increasing of the efficiency of the preservative in stopping of bacterial growth.Effect of limiting factors on performance of bacteriocin in food such as the composition of bacteriocin with food additives, food processing, dependent factors to bacterial functions such as bacterial and their interplays with food.Since bacteriocin is peptide compounds, it is possible that these compounds because of function of enzymes in meats, especially proteases, or bacteria nisin-ase enzymes meat could be break down. On the other hand in addition to that factors decrease in anti Listeria properties during time periods could be simultaneous with incidence of resistant species of nisin due to their expression hpk1021, pbp2229, Imo2487 genes in bacteria and also related to changes in the composition of the cytoplasmic membrane fatty acids and phospholipids like compounds of bacterial cell wall.

Although nisin in this study, both in free form and in microencapsulated form has anti Listeria effects compared to control but bacteriocin nisin is not able to reduce the number of Listeria bacteria to under acceptable limit for healthy human that it is 100 cells per gram of food. That it was Similar to results of Abdollahzadeh and colleagues (Abdollahzadeh *et al.*, 2012).

References

Abdollahzadeh E, Rezaei M, Hosseini H, Safari R. 2012. Effects of Nisin and Thyme Essential Oil, Individually and in Combination, on Inoculated Populations of Listeria Monocytogenes in Minced Silver Carp. Iranian Journal of Nutrition Sciences & Food Technology **6,** 13-20.

Akhondzadeh A, Zahraie Salehi T, Misaghi A. 2002. The Survey of Listeria Monocytogenes in Fresh Fish and Smoked Fish and Ice Used in Fish Markets for Retaining the Freshness of the Fish in Tehran and Gilan. Journal of Veterinay Research **57,** 9-12.

Al-Holy M, Lin M, Rasco B. 2005. Destruction of Listeria Monocytogenes in Sturgeon (Acipenser Caviar by a Combination of Nisin with Chemical Antimicrobials or Moderate Heat. Journal of Food Protection® **68,** 512-520.

Benech RO, Kheadr E, Laridi R, Lacroix C, Fliss I. 2002. Inhibition of Listeria Innocua in Cheddar Cheese by Addition of Nisin Z in Liposomes or by in Situ Production in Mixed Culture. Applied and Environmental Microbiology **68,** 3683-3690. http://dx.doi.org/10.1128/AEM.68.8.36833690.2002

Fazli H, Zhang C, Hay D, Lee C, Janbaz A, Bourani M. 2007. Population Dynamics and Stock Assessment of Common Kilka(Clupeonella Cultriventris Caspia) in Iranian Water of the Caspian Sea. Iranian Journal of Fisheries Sciences **7,** 47-70.

Kordel M, Sahl HG. 1986. Susceptibility of Bacterial, Eukaryotic and Artificial Membranes to the Disruptive Action of the Cationic Peptides Pep 5 and Nisin. FEMS microbiology letters **34,** 139-144. http://dx.doi.org/10.1016/0378-1097(86)90342-3

Mehdizade M, Rastegar H, Sanaie A, Farshim Rad F. 2009. Foodborne Listeriosis. Journal of Kerman University of Medical Sciences **17,** 181-190.

Razavi Shirazi H. 1994. Marine Products Technology (Preservation Principles and Processing). tehran-Iran, Negar Pars Press.

Rehbein H, Oehlenschlager J. 2009. Fishery Products: Quality, Safety and Authenticity, John Wiley & Sons.

Roller S. 1995. The Quest for Natural Antimicrobials as Novel Means of Food Preservation: Status Report on a European Research Project. International biodeterioration & biodegradation **36,** 333-345. http://dx.doi.org/10.1016/0964-8305(95)00074-7

Safari R, Saeidi Asl MR. 2011. The Effect of Nisin a and Sodium Benzoate on Behavior of Listeria Monosytogenes and Some Microbial and Chemical Parameters in Silver Carp (Hypophtalmichtys Molitrix) Fillet Stored at 4°C. Journal of Food Hygiene **1,** 1-14.

Schmidt SE. 2009. Antimicrobial Efficacy of Liposome Encapsulated Nisin and Nisin's Inhibition against Listeria Monocytogenes in Fluid Milk at Different Storage Temperatures. Master's thesis, Texas A&M University 1.

Shimizu Y, Toyohara H, Lanier T. 1992. Surimi Production from Fatty and Dark-Fleshed Fish Species.

Stiles ME. 1994. Potential for Biological Control of Agents of Foodborne Disease. Food research International **27,** 245-250. http://dx.doi.org/10.1016/0963-9969(94)90091-4

Tomé E, Teixeira P and Gibbs PA. 2006. Anti-Listerial Inhibitory Lactic Acid Bacteria Isolated from Commercial Cold Smoked Salmon. Food microbiology **23,** 399-405. http://dx.doi.org/10.1016/j.fm.2005.05.004

Vongsawasdi P, Nopharatana M, Supanivatin P, Promchana M. 2012. Effect of Nisin on the Survival of Staphylococcus Aureus Inoculated in Fish Balls. Asian Journal of Food and Agro-Industry **5(1),** 52-60.

Xiao D. 2010. Novel Delivery Systems of Nisin to Enhance Long-Term Efficacy against Foodborne Pathogen Listeria Monocytogenes.

Zaerzadeh E, Mortazavi SA, Jafari MR, Afsharnejad S, Tabatabaii F, Nassiri Mahallati M. 2011. Antibacterial Effect of Nano-Encapsulated Nisin in Liposoms in Contrast to Free Nisin in Control of Listeria Monocytogenes in Iranian Feta Cheese (Uf). Iranian Food Science and Technology Research Journal **7,** 191-199.