

# Evaluating the antimicrobial activity of nisin on the inhibition of *Staphylococcus aureus* bacteria in silver carp mince

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## Abstract

*Staphylococcus aureus* is a pathogen in food products that poses a great danger for the human food industry. This study was performed for evaluating the effect of the antimicrobial activity of nisin on the inhibition of *Staphylococcus aureus* bacteria inoculated in silver carp mince. The effect of different concentrations of nisin at 0, 2.5, 3.5 and 5 micrograms per gram on the growth behavior of the said bacterium was examined at 4°C for 21 days. The results showed that the growth of the bacteria at different concentrations of nisin in silver carp mince was inhibited and possessed significant statistical differences compared to the control treatment. Based on the findings of this research it can be said that nisin has strong<sup>1</sup> bactericidal properties and is introduced as a natural food preservative instead of its chemical and industrial variations.

**Keywords:** fish, nisin, *Staphylococcus aureus*, silver carp, mince

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## 1. Introduction

Food infected by pathogens or their enterotoxins cause illnesses most common in the world. Enterotoxin produced by *staphylococcus aureus* causes gastrointestinal toxicity and gastroparesis symptoms. Moreover, food spoilage microorganisms cause economic losses (Sandri *et al.*, 2007; D'Mello, 2003). The use of antimicrobial preservatives in many food

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products for prevention of food spoilage after production is common and increases food durability and preserves its quality (Ojagh *et al.*, 2010). Among these antimicrobials their biological variations can be mentioned with lactic acid bacteria (LAB) and the varieties of microbial metabolites produced from this group or associated groups, being some of them. LABs secrete a wide variety of bacteriocins like nisin, pediocins, lactucin, divergent bacteriocin CDSs, diplocin, lactostrepcins, etc. Nisin is the only bacteriocin allowed to be used in food products, and it is a GRAS (Generally Regarded as Safe) food preservative (FDA, 1998). Nisin, a polypeptide amphipathic bacteriocin consisting of 34 amino acids produced by certain levels of *Lactococcus lactis* which are subspecies of *lactis* and prevent gram-positive bacteria growth and *Clostridium* and *Bacillus* spores' creation (Thomas and Wimpenny., 1996; Kuwano *et al.*, 2005). Several studies have been done in laboratory environments about the antibacterial effects of nisin. Some studies have been performed on the antimicrobial effect of nisin in liquid foods. But few studies have been performed about the antibacterial effect of this bacteriocin in raw meat products. However, it is necessary to study about the antimicrobial effects of natural preservatives specially nisin in solid foods specially minced fish meat which are quickly infected during processing. Given the necessity of using natural and healthy preservatives and the quick spoilage of some food products such as minced fish meat, it seems necessary to find nontoxic preservative compounds to decrease the microbial load and inhibit the growth of pathogen microorganisms in food and fishery products to enhance the health level of food products and consumers. This study was performed to evaluate the effect of different levels of nisin including 2.5, 3 and 5 micrograms per gram of silver carp mince inoculated with *Staphylococcus aureus* in different storage days during 21 days at 4°C.

## 2. Materials and methods

### 2.1. Preparation and inoculation of *Staphylococcus aureus*:

In the present study, *Staphylococcus aureus* bacteria (ATCC 1113) obtained from the Department of Food Hygiene, Faculty of Veterinary Medicine, Zabol University, was used. Lyophilized bacteria were activated twice via culturing for 18 hours in the BHI Broth (Merck co., Germany) overnight at 37°C. Next, after the second culture the bacteria were mixed with a 1 to 5 ratio with Glycerin sterile and stored in Eppendorf two-mL microtubes at -20°C. Next, the bacteria stored in the Eppendorf microtubes were re-cultured two consecutive times in the BHI Broth for 18 hours at 37°C. For measurement of the quantity of the bacteria required ( $1 \times 10^3$  CFU/g) for inoculation in the fish mince, a spectrophotometer instrument (made by Pharmacia UK. Ltd) set to 600nm wavelength with the use of a standard curve, and also the stationary phase of bacterial growth obtained through bacterial count using spread plate count, were used (Basti *et al.*, 2007).

## 2.2. Preparing the nisin solution

Nisin (nisaplin) made by Sigma, Aldrich, UK, was used to prepare the nisin solution. For this purpose, nisin powder was dissolved in 37.5% chloridric acid (Merck, Germany) 0.02 Molar and after filtration, it was sterilized twice using 0.22micrometer Microbiological Millipore filters and stored in glass containers impenetrable to sunlight and artificial light and kept in a refrigerator.

## 2.3. Preparing the minced meat and the treatments

After separating the fish meat in sterilized conditions and mincing it using an Iranian made Pars Khazar M.G.1400 meat grinder with 4mm diameter pores, it was used for the treatments. The minced fish meat divided by the number of the treatments as required, were stored in blue lid autoclavable containers. Then they were inoculated with ( $1 \times 10^3$  CFU/g) staph bacteria in day one and fully homogenized. Spread Plate Count was used to examine the growth and presence of the bacteria. For this purpose, in days 0, 1, 3, 6, 9, 12, 15, 18 and 21 sampling was performed under sterile conditions, and for decimal dilution, 1% peptone water and sterile physiological serum each with ratios of 50% were used. The bacteria were inoculated using spread plate count in the BHI medium and stored at a hothouse at 37°C. The colonies were counted after 48 hours. Each test was repeated thrice.

## 2.4. Statistical analysis

Since bacteria grow exponentially over time and bacteria distribution in different samples gradually deviates from normal, therefore for normalization of the data and avoidance of large means and standard deviations, in the analyses the logarithm of the number of bacteria, base-10 was used. For comparison of the treatments mean every day, the one-way ANOVA technique was used. Also, Tukey post hoc test was used for the comparison of pairs of treatments every day. For evaluation of the changes of bacteria logarithm in different days of the experiment, a repeated measures statistical test was used. The degree of significance was considered as P-Value <0.05. The SPSS statistical software version 18 was used for data analysis.

The logarithm of the number of the bacteria in silver carp mince affected by different concentrations of nisin in different storage days, is shown in table 1. The results of the statistical test showed that the different concentrations of nisin compared to the control treatment, had significant effects on the inhibition of the bacteria under study ( $p < 0.05$ ).

**Table 1:** the effect of nisin on inoculated silver carp mince during storage at the refrigerator at 4°C

Day 21	Day 18	Day 15	Day 12	Day 9	Day 6	Day 3	Day 1	Day 0	Time(day)
7.9±0.17b	7.2±0.22b	6.9±0.08c	6.6±0.11c	6.3±0.03c	6.2±0.03d	6.1±0.1d	4.9±0.09c	3±0.02a	0
0±0a	0±0a	3.4±0.02b	3.7±0.02b	3.8±0.03b	4.7±0.25c	4.8±0.04c	4.9±0.09c	3±0.05a	2.5
0±0a	0±0a	0±0a	0±0a	0±0a	2.6±0.02b	2.6±0.05b	3.4±0.03b	3±0.03a	3.5
0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	3.9±0.1a	3±0.05a	5

The different letters (a, b, c, d) in each column, show a significant difference ( $P < 0.05$ ) between the treatments in different days.

### 3. Result

The results in table 1 show that different concentrations of nisin could in different days of the storage, inhibit the growth of the bacteria ( $p < 0.05$ ). In day 0, not all the treatments showed significant statistical differences ( $p < 0.05$ ). Among the treatments, the nisin treatment at 2.5 micrograms per gram had less effect compared to the treatments at 3.5 and 5 micrograms. However, the 5 micrograms per gram nisin treatment was the most effective treatment of this research for the inhibition of staphylococcus aureus bacteria. The 5 µg/g nisin treatment, inhibited the bacteria growth from day 3 ( $p < 0.05$ ). The 3.5 µg/g nisin treatment, did the same from day 9 ( $p > 0.05$ ). The lowest effect of nisin on the inhibition of the bacteria growth was observed at 2.5 µg/g which inhibited the growth from day 18.

### 4. Discussion

Nisin is the only bacteriocin approved by the WHO for use in the food industry (Delves-Broughton and Gasson., 1994). The effect of nisin in gram-positive and gram-negative bacteria occurs in the phospholipid of the cell membrane. It disrupts the polarity of the cell membrane and by creating pores, causes an accumulation of amino acid, prevention of its transference and exit of its cytoplasmic vital organs which result in the destruction of the gram-positive bacteria (Van Heusden *et al.*, 2002; Breukin *et al.*, 1999). Nisin has no effect on gram-negative bacteria, which is due to the structure of their external layer consisting of lipopolysaccharide in the external and phospholipid glycerophosphate in the internal part. According to Shirazinejad *et al.* (2010), the effect of nisin on the microbial growth in processed fish products, probably depends on several factors such the concentration of the nisin being used, the method of using it, fish species, product type, degree of microbial contamination and storage conditions. There is some controversy on the effect of nisin on meat. Some say that the phospholipids in meat, limits nisin activity, and that the best activity of nisin is in an aqueous and homogenized medium, and these bacteriocins are deactivated by proteolytic enzymes in foods such as fresh meat (Juncioni de Arauz *et al.*, 2009). Whereas our research results showed that nisin was effective at inhibiting the growth of

staphylococcus aureus bacteria in minced fish meat. Mobseri *et al.* (2008) in a study evaluating the effect of nisin on the reduction of the concentrations of foods' chemical preservatives, specified the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the preservative substances of sodium nitrate, benzoic acid and nisin for the bacteria of staphylococcus aureus and listeria monocytogenes. Their MICs for staphylococcus aureus were 350, 200 and 25ppm and for listeria monocytogenes were 100, 200 and 10ppm respectively. When chemical preservatives were used along with nisin, it reduced their MIC (200 and 50ppm for staphylococcus aureus and 25 and 25ppm for listeria monocytogenes, respectively). This study showed that nisin can be used as a safe preservative in food microbiology and that it can reduce the concentration of chemical preservatives.

Pawar *et al.* (2000) performed a research on the inhibition of listeria monocytogenes in buffalo minced meat at refrigerator temperature, and their results showed that with an increase in the nisin concentration from 400 to 800IU/g, its anti-listeria activity in the minced meat is strengthened. Choobkar *et al.* (2010), evaluated the effect of different nisin concentrations (0, 0.15, 0.25, 0.75, 1.5 $\mu$ g/g) on the growth behavior of staphylococcus aureus in salted fillets of silver carp in inappropriate refrigerator conditions (10 degrees Celsius), which showed that with increased salt and nisin, particularly in high concentrations of nisin like 0.75 and 1.5 $\mu$ g/g, the logarithm of the growth of the bacteria reached its lowest value. Asghari *et al.* (2011), evaluating the effect of nisin bacteriocin on the durability of silver carp fillet during storage at a refrigerator, using 0.2g of nisin per each kilogram, could increase the durability time of silver carp fillet up to 3 days at 4°C. This is while the nisin used in our research at 3.5 and 5microgram per gram, increased the durability time of the silver carp mince by 12 and 18 days respectively.

## 5. Conclusion

The results of this study indicate the high antimicrobial effects of nisin against the staphylococcus aureus bacteria. At 5 microgram per gram, nisin completely stopped the growth of staphylococcus aureus from day 3 and preserved the quality of the minced fish meat up to a useable level. Nisin can replace its chemical and synthetic variations in the fish processing industry.

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