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# Antibacterial activities of nisin encapsulated in zein and modified atmosphere packaging on rainbow trout (*Oncorhynchus mykiss*) fillet during chilled storage 4°C

**Shamloofar M.[1]; Hoseini E.[2]*; Kamali A.[1]; Motalebi Moghanjoghi A.A.[3]; Poorgholm R.[4]**

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**Abstract**

Nisin is a widely used naturally occurring antimicrobial effective against many pathogenic and spoilage microorganisms. It has been proposed that reduced efficacy of nisin in foods can be improved by technologies such as encapsulation to protect it from interferences by food matrix components**.** This study was carried out to evaluate the microbiological quality of fresh trout slices treated with $N_1$ (nisin 0.15 g/kg) and $N_2$ (nisin 0.25 g/kg), $NE_1$(encapsulated nisin 0.15 g/kg), $NE_2$ (encapsulated nisin 0.25 g/kg ) and were then packaged under Modified Atmosphere Packaging (MAP) (45% CO2, 50% N2 ,5% O2) and stored at 4±1 °C for 20 days. The results revealed that nisin in both forms of free and encapsulated was efficient against the proliferation of various categories of spoilage microorganisms; including aerobic and psychrotrophic populations and lactic acid bacteria. The shelf life of the treated products was extended by 4–7 days more than that of the control. As a consequence, nisin, in particular encapsulated nisin, might be considered as an effective tool in preventing the quality degradation of the fillet, resulting in an extension of their shelf life.

**Keywords:** Encapsulated nisin, Antibacterial activities, Modified Atmosphere Packaging (MAP), Rainbow trout (*Oncorhynchus mykiss*), Chilled storage

1-Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran
2-Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran
3- Agricultural Research, Education and Extension Organization, Iranian Fisheries Science Research Institute, P. O. BOX: 14155-6116 Tehran, Iran
4- Agricultural Research, Education and Extension Organization, Iranian Fisheries Science Research Institute, Caspian Sea Ecology Research Center, Sari, Iran
*Corresponding author's email: Ebhoseini@srbiau.ac.ir

## Introduction

Fish is an excellent protein source with high nutritive value due to its favorable essential amino acid composition and is one of the most highly perishable food products (Jannat Alipour *et al*., 2010). During handling and storage period, quality deterioration of fresh fish rapidly occurs which limits the shelf life of the product (Kashiri *et al*., 2011). Because of consumer's demand for fresh refrigerated foods with extended shelf life, considerable research has been directed toward using various preservation strategies to prolong the shelf life, while ensuring the safety of fresh foods including fishery products (Sallam, 2007). The antimicrobial additives can suppress the growth of bacteria during storage with minor effects on the quality of meat products (Zhu *et al*., 2005). Nisin is the most commercially important bacteriocin that is produced by *Lactococcus lactis*, and is used extensively as a safe food preservative (Zhu *et al*., 2005). It inhibits the growth and development of many gram positive bacteria such as *Listeria monocytogenes* (Nykanen *et al*., 2000; Thomas *et al*., 2005). However, numerous studies have reported much reduced antimicrobial efficiency of nisin when applied to foods than in a growth medium. A reduction of nisin activity was reported because of nonspecific binding of nisin with lipids and proteins (Delves-Broughton, 1990; Bhatti *et al*., 2004). Rose *et al*. (2008) found that the compromised antimicrobial activity of nisin in fresh meat was caused by the complexation with glutathione. Incorporation of nisin within capsules of edible polymers may reduce the interaction of nisin with food components and minimize its dysfunction in foods (Teerakarn *et al*., 2002; Xiao and Zhong, 2011). Salmoso *et al*. (2004) demonstrated that sustained release of nisin from poly-(L-lactide) nanocapsules inhibited the growth of *Lactobacillus delbrueckeii* over 45 days, in comparison to ca. 4 days for un-encapsulated nisin. Much work is needed to utilize GRAS (Generally Recognized As Safe), sustainable, and inexpensive ingredients as delivery systems of antimicrobials and low-cost and scalable processes. Spray drying is a quick, simple, low-cost, and one-step method to obtain a powdered product and is a popular choice in the food industry to encapsulate bioactive compounds in food biopolymers (Xiao and Zhong, 2011). Zein, which is a class of alcohol-soluble proteins (prolamins) extracted from maize kernel (Zhong and Jin, 2009), was used for spray-dried encapsulating of nisin in our study. MAP, is a protecting technique used to extent the shelf-life of fish and fish products (Özogul *et al*., 2006) MAP, eliminates the oxygen from inside the package and fills it with different concentrations of $CO_2$ and $N_2$ (Kılınç and Çaklı., 2004). There are a lot of research related to the extension of the shelf life of fish with MAP, including chub mackarel (Erkan *et al*., 2007), rainbow trout (Çaklı *et al*., 2006; Oguzhahan and Angis, 2012), bass (Torrieri *et al*., 2006), herring (Lyhs *et al*., 2007). The aim of this research is to determine the combined effects of encapsulated nisin and MAP on the shelf life of refrigerated (4ºC) rainbow trout fillets by evaluating certain microbiological parameters.

## Materials and methods

*Prepration of encapsulated nisin*

The 2.5% nisin was purchased from (Serva, USA). The product specifications indicate a nisin content of 2.5% and 1,000 IU/mg solids, 75% sodium chloride, and 22.5% denatured milk solids (Xiao, 2010). Zein was purchased from (Fluka, BCBG 3298, Germany). Other chemicals were obtained from (Merck, Germany).

*Encapsulation by spray drying*

The 2.5% nisin was suspended at a concentration of 6 mg solids per mL of 50%v/v aqueous ethanol. After mixing for 6 hours using a stirring plate, the suspension was centrifuged at 1,520×*g* for 5 min (Refrigerator Centrifuge Kokusan. h-103nr, Japan). The transferred supernatant (extract) was constituted to 70%v/v ethanol to dissolve zein at a concentration of 2%w/v. Spray dryer (Lab-plant UK Ltd YO14 OPH, England ) was used to dry the solution at a feed rate of 5.26 ml/min and an aspirator setting of 100% (Xiao and Zhong, 2011 ) . The inlet and outlet temperature in this study was 105 and 68 °C, respectively.

*Evaluation of encapsulation properties*

Spray-dried samples were evaluated for these parameters (Xiao and Zhong, 2011):

$$(1)\ \text{Encapsulation efficiency } \% = \frac{\text{total nisin units in the feed}}{\text{total nisin units in a collected product}} \times 100$$

$$(2)\ \text{Mass yield } \% = \frac{\text{non solvent mass in the feed}}{\text{mass of collected product}} \times 100$$

*In vitro release kinetics*

1.5 mL micro-centrifuge tubes were used for release studies by suspending 4 mg spray-dried particles in 1 mL of 20 mM sodium phosphate buffer that was pre-adjusted to pH 6.0. Micro-centrifuge tubes were attached to an end-to-end shaker (Fater Rizpardaz, Iran) and were continuously rotated at room temperature. Then, samples were centrifuged at 14,500×*g* for 5 min (Refrigerator Centrifuge Kokusan. h-103nr, Tokyo, Japan), and 700 µL supernatant was sampled to determine nisin activity using the method below. 700 µL of the corresponding fresh phosphate buffer was supplemented to the remaining sample that was then re-suspended for continued release studies. The cumulative release of nisin at a certain time point was calculated using the following (Xiao and Zhong, 2011):

$$(3)\ \text{Rti}(\%) = \frac{\sum_{n-1}^{i-1} a_n + \frac{10}{7} a_i}{u_o} \times 100\%$$

Where *R*ti (%) is the cumulatively released nisin at time *t*i, the *i*th time of sampling; *a*i is the nisin concentration (IU/mL) in the supernatant at the sampling time *t*i; and *U*o is the total nisin activity units in 4 mg powder (corresponding to 100% release).

*Determination of nisin activity*

Nisin activity of samples was determined by the standard agar diffusion assay (Wolf and Gibbons, 1996) using *Micrococcus luteus* ATCC 10240 as an indicator

microorganism. To constitute nisin standard solutions, a stock solution was prepared by dissolving 0.1 g of the 2.5% nisin preparation in 10 mL of 20 mM HCl, i.e., 10,000 IU/mL. 20 mM HCl or different volumes of ethanol and sterile water were used to dilute the stock solution to a nisin concentration of 50-1500 IU/mL. Nisin solutions were loaded into wells of agar gels and incubated at 35 ºC for 24 h. Inhibition zone diameters in agar gels corresponding to standard solutions were measured and used to generate a semi-log plot, and a linear regression from the plot resulted in a standard curve taking the form of:

(4) $D = a \log10 [Nisin] + b$

Where *D* is the diameter of the inhibition zone after baseline subtraction; [Nisin] is the concentration of nisin in IU/ml; a and b are the slope and intercept from the linear regression, respectively. Nisin samples prepared from encapsulation products were incubated together with standard solutions at 35ºC for 24h. Two sample replicates were used, each loaded in 4 well replicates in agar gels. The average of 8 inhibition zone diameters from each sample was used to estimate nisin activity using an appropriate standard curve (Xiao, 2010).

*Preparation and treatment of fish samples*

Rainbow trout *(O. mykiss)* (250±25g) were obtained from a local market (Sari, North of Iran) and transported to the laboratory in boxes containing ice. Upon arrival, the fish were beheaded, gutted and washed with tap water several times to remove the blood and slime. Then the fish were filleted manually. Solutions corresponding to each treatment of nisin for the experimental design were prepared, and 1 ml of each was sprayed uniformly on the surface of the fillets. Treatments included*:* C (control samples), $N_1$ (nisin 0.15 g/kg) and $N_2$ (nisin 0.25 g/kg), $NE_1$ (encapsulated nisin 0.15 g/kg), and $NE_2$ (encapsulated nisin 0.25 g/kg). Each group included 18 fillets. All filleted samples, including the control, were packaged in 15x25 cm low density polyethylene/polyamide/low density polyethylene (LDPE/PA/LDPE) barrier pouches. The MAP gas mixture used was 45%/50%/5% ($CO_2/N_2/O_2$). Pouches were heat sealed using a vacuum sealer (Multivac, Germany) and kept under refrigeration (4±1°C) and samples were subjected to microbiological (aerobic plate count, psychrotrophic bacteria, lactic acid bacteria,) analyzes on certain days (0, 4, 8, 12, 16 and 20th days) of storage.

*Microbiological Analysis*

A sample (5 g) was taken from each trout fillet, transferred aseptically into a stomacher bag containing 45 ml of 0.1% peptone water, and the mixture was homogenized for 60 s using a Stomacher (Lab Stomacher Blender 400-BA 7021 Seward medical, England) at room temperature. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1% peptone water) of fish homogenates were spread on petri dish of various agar materials.

*Aerobic plate count*

Aerobic plate counts (APC) were determined by inoculating 0.1 ml of the sample homogenate, at selected dilutions, onto duplicate sterile plates of pre-poured and dried Standard Method Agar (Nissui

Pharmaceutical Co,.Ltd., Tokyo, Japan) using the surface spread technique, then the plates were incubated for 48 h at 35°C based on the method described by American Public Health Association, APHA (1992).

*Psychrotrophic count*

Psychrotrophic counts (PTCs) were determined in a similar method to that for APC except that plates were incubated at 7°C for 10 days (Sallam, 2006).

*Lactic acid bacterial count*

To determine the lactic acid bacteria (LAB), diluted samples were plated on (deMan, Rogosa, and Sharpe) MRS agar (Merck, Darmstadt, Germany) and incubated at 30°C for 2–3 days in anaerobic jars with disposable Anaerocult C bags (Merck, Darmstadt, Germany) in order to generate an anaerobic medium. (Chytiri *et al.*, 2004). Micribiological examinations were done as triplicates and were expressed as $\log_{10}$ cfu/g.

*Statistical Analysis*

All the data were presented as means±standard error. The experimental design was a factorial 5×6×3 (4 treatments and a control, six storage times and three replicates). All obtained data from this study were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significant differences among means at ($p$<0.05) level, using SPSS.

## Results

*Encapsulation properties*

Parameters defined to evaluate encapsulation performance of samples produced by spray-dryer are summarized in Table 1. The encapsulation efficiency of nisin encapsulated in zein in this study, was estimated at about 49 %. Another parameter to evaluate encapsulation performance is mass yield that is defined in Equation 2. The mass yield of spray- dried zein capsules co-encapsulated with nisin in this study was 62 %. The release kinetics of nisin from zein capsules produced by spray-drying is shown in Fig 1. The values for this factor at time intervals of 0, 24, 48, 72 and 200 hours was 42, 49, 61, 72 and 76 %, respectively.

**Table 1: Encapsulation performance of samples produced by spray-dryer.**

| Inlet temperature (C°) | Mass yield (%) | Encapsulation efficiency (%) |
|---|---|---|
| 105 | 62±0.93 | 49.89±0.76 |

*Aerobic plate count*

Table 2 presents the changes of APCs in all treatments through the storage time. The initial APC (log10 CFU/g) in sliced trout ranged from 3.77 in (N1) to 3.93 in control treatment and there was no significant difference ($p$<0.05) between all of the treated samples and the control. By day 12 of storage, however, APCs in sliced trout for all of the different treatments were still below 6 log10 CFU/g, while that in controls attained a count of 6.83, which is in close proximity to the maximal recommended limit of 7 log10 CFU/g for APC in raw fish by International Commission on Microbiological Specification for Foods, ICMSF (1986), indicating a microbiological shelf life of less than 16 days (13-14) days for the non-treated

control samples that were packed under MAP.

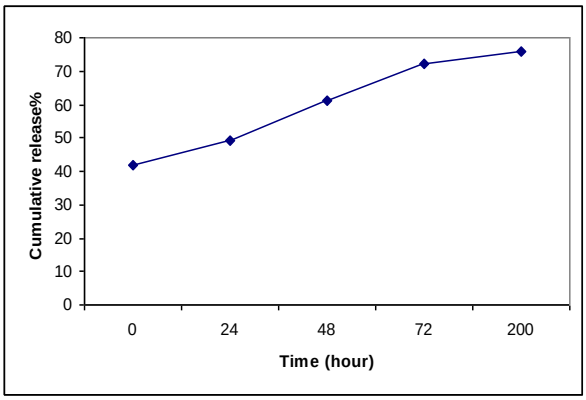


**Figure 1: Release kinetics of nisin from zein capsules produced by spray-drying.**

At the end of day 20, aerobic plate counts were higher than the acceptability levels in all control or treated fillets. Results also showed that with increase of the concentrations of nisin, APCs were lower than in the control but greater inhibition of total bacteria was observed when encapsulated nisin treatment was used. The reductions of APC for N1, N2, NE1 and NE2 compared to the control were 0.43, 0.59, 0.95 and 1.48 log CFU/g on day 20 respectively. Significant differences ($p$<0.05) were detected between all of the treated samples and the control, and although there was no significant difference between $N_1$ and $N_2$, but significant differences were detected among the two treatments and NE1 and NE2. According to the results of the APCs for all the treatments and the maximal recommended limit of 7 log10 CFU/g for APC in raw fish the shelf life of the control, $N_1$, $N_2$, $NE_1$ and $NE_2$ was approximately 12, 16, 17, 19 and 20 days, respectively.

**Table 2: Effects of $N_1$(nisin 0.15 g/kg) and $N_2$ (nisin 0.25 g/kg), $NE_1$(encapsulated nisin 0.15 g/kg ), and $NE_2$ (encapsulated nisin 0.25 g/kg ) on aerobic plate counts (APC) of filleted rainbow trout during storage at 4°C. Values represent means±SE of three replicates.**

| | Storage time (day) | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **0** | **4** | **8** | **12** | **16** | **20** |
| **Control** | 3.93± .16 $^{a}$ | 5.08± .042 $^{a}$ | 6.10± .063 $^{a}$ | 6.83± .113 $^{a}$ | 7.80± .091 $^{a}$ | 8.60± .113 $^{a}$ |
| **$NE_1$** | 3.90± .08 $^{a}$ | 4.21± .077 $^{b}$ | 4.98± .084 $^{b}$ | 6.15± .657 $^{ab}$ | 6.40± .028 $^{c}$ | 7.65± .035 $^{c}$ |
| **$NE_2$** | 3.88± .04 $^{a}$ | 4.13± .021 $^{b}$ | 4.88± .035 $^{b}$ | 5.76± .106 $^{b}$ | 6.17± .084 $^{d}$ | 7.22± .035 $^{d}$ |
| **$N_1$** | 3.83± .12 $^{a}$ | 3.93± .16 $^{b}$ | 5.04± .091 $^{b}$ | 6.00± .12 $^{ab}$ | 6.76± .35 $^{b}$ | 8.17 ± .049 $^{b}$ |
| **$N_2$** | 3.77± .91$^{a}$ | 4.24± .176$^{b}$ | 4.90± .077$^{b}$ | 5.84± .162$^{b}$ | 6.64± .014$^{b}$ | 8.01± .134$^{b}$ |

Different superscripts within a column indicate significant differences ($p$< 0.05)

*Psychrotrophic bacteria*

Changes of PTCs in all treatments through the storage time are shown in Table 3. In this study, the initial PTCs (day 0) of filleted trout ranged from 3.8 log10 CFU/g, in $NE_1$ and control to 3.66 log10 CFU/g in $N_2$. Additionally, the growth pattern of PTC showed same behavior as that of APC with the control being the highest on day 20 (8.55 log10 CFU/g), followed by samples treated with $N_1$ (7.79 log10 CFU/g), $N_2$ (7.65 log10 CFU/g) and $NE_1$ (7.61 log10 CFU/g), while a lower count (7.28 log10 CFU/g) was detected in samples treated with NE2. The results did not reveal any significant differences ($p$>0.05) in the

initial PTC among different treatments or between the treated and control samples. However, by the end of storage (day 20), significant differences ($p$<0.05) were detected between all the treated trout samples and the control. Although there was a significant difference between $NE_2$ and other treatments, no significant differences were detected among the PTC in $N_1$, $N_2$ and $NE_1$ treated samples. According to the results of the PTCs for all the treatments and the maximal recommended limit of 7 log10 CFU/g for PTC in raw fish the shelf life in the control, $N_1$, $N_2$, $NE_1$ and $NE_2$ was approximately 12, 16, 16, 18 and 19 days, respectively.

**Table 3: Effects of $N_1$ (nisin 0.15 g/kg) and $N_2$ (nisin 0.25 g/kg), $NE_1$ (encapsulated nisin 0.15 g/kg ), $NE_2$ (encapsulated nisin 0.25 g/kg ) on psychrotrophic counts (PTC) of filleted rainbow trout during storage at 4°C. Values represent means±SE of three replicates.**

| | Storage time (day) | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **0** | **4** | **8** | **12** | **16** | **20** |
| **Control** | 3.80±.063 $^{a}$ | 4.84±.014$^{a}$ | 5.88±.056$^{a}$ | 6.885±.035$^{a}$ | 7.83±.042$^{a}$ | 8.655±.091$^{a}$ |
| **$NE_1$** | 3.80±.0141$^{a}$ | 4.69±.014$^{bc}$ | 5.325±.077$^{d}$ | 6.225±.120$^{c}$ | 6.775±.021$^{d}$ | 7.615±.063$^{b}$ |
| **$NE_2$** | 3.68±.091$^{a}$ | 4.63±.056$^{c}$ | 5.25±.176$^{d}$ | 6.025±.049$^{d}$ | 6.525±.169$^{e}$ | 7.285±.063$^{c}$ |
| **$N_1$** | 3.66±.091$^{a}$ | 4.78±.084$^{ab}$ | 5.62±.049$^{b}$ | 6.465±.063$^{b}$ | 7.085±.035$^{b}$ | 7.790±.098$^{b}$ |
| **$N_2$** | 3.74±.049$^{a}$ | 4.75±036$^{abc}$ | 5.49±.028$^{c}$ | 6.365±.035$^{b}$ | 6.950±.042$^{c}$ | 7.655±.049$^{b}$ |

Different superscripts within a column indicate significant difference ($p$< 0.05)

*Lactic acid bacterial count*

Table 4 presents the changes of LABs in all treatments through the storage time. The initial count of this bacterium was between 2.26 and 2.47 log cfu/g. Until the 20th day of storage, lactic acid bacteria counts did not reach 7 log cfu/g which is the maximal recommended limit for LABs count in fish fillets (ICMSF, 1986 ; Sallam, 2007) except in controls (7.26 log cfu/g). The LAB counts at the end of the storage time in the samples treated with $N_1$, $N_2$, $NE_1$ and $NE_2$ was (6.53, 6.27, 5.89 and 5.93 log10 CFU/g), respectively. The results did not reveal any significant ($p$>0.05) differences in the initial LAB count among the different treatments or between the treated and control samples; however by the end of storage (day 20), significant ($p$<0.05) differences were detected between all of the treated trout samples and the control. Although there was a significant difference between $N_1$, $N_2$ and $NE_1$ and $NE_2$, no significant differences were detected among the LAB counts in $NE_1$ and $NE_2$ treated samples.

**Table 4: Effects of $N_1$ (nisin 0.15 g/kg) and $N_2$ (nisin 0.25 g/kg), $NE_1$(encapsulated nisin 0.15 g/kg ), $NE_2$ (encapsulated nisin 0.25 g/kg ) on lactic acid bacteria counts (LAB) of filleted rainbow trout during storage at 4°C. Values represent means±SE of three replicates.**

| | Storage time (day) | | | | | |
|---|---|---|---|---|---|---|
| **Treatments** | **0** | **4** | **8** | **12** | **16** | **20** |
| **Control** | 2.41±.014$^{a}$ | 3.725±.049$^{a}$ | 4.71±.028$^{a}$ | 5.14±.0141$^{a}$ | 6.41±.233$^{a}$ | 7.26±0.162$^{a}$ |
| **$NE_1$** | 2.46±.063$^{a}$ | 3.075±.049$^{b}$ | 4.03±.077$^{c}$ | 4.58±.056$^{c}$ | 5.52±.091$^{c}$ | 5.89±.021$^{d}$ |
| **$NE_2$** | 2.39±.028$^{a}$ | 2.8±.056$^{c}$ | 3.81±.063$^{d}$ | 4.42±.035$^{c}$ | 5.17±.084$^{d}$ | 5.93±.014$^{d}$ |
| **$N_1$** | 2.33±.169$^{a}$ | 3.16±.084$^{b}$ | 4.27±.049$^{b}$ | 4.81±.155$^{b}$ | 5.94±.049$^{b}$ | 6.535±.212$^{b}$ |
| **$N_2$** | 2.65±.063$^{a}$ | 3.10±0141$^{b}$ | 4.09±.028$^{c}$ | 4.76±.049$^{b}$ | 5.81±.028$^{bc}$ | 6.27±.049$^{c}$ |

Different superscripts within a column indicate significant difference ($p< 0.05$)

## Discussion

The results of this study in the field of encapsulation efficiency was in accordance with that of Xiao (2010) who studied encapsulation of nisin in zein microcapsules at four inlet temperatures of spray- dryer between 75 and 120°C. The results revealed that spray drying is an efficient and simple method to encapsulate nisin. At 95°C and above, no apparent loss of nisin activity was noticed after spray drying and the capsules produced at an inlet temperature of 105°C showed the most sustained release of nisin at pH 6.0. As we wanted to use encapsulated nisin as an antimicrobial agent to extend the shelf life of rainbow trout fillets and the pH of fish fillets was close to 6, we selected an inlet temperatue of 105°C for the spray drier. The two important factors impacting release profiles, are capsule structure and molecular interactions between the carrier polymer and the encapsulated compound (Zhong and Jin, 2009). The isoelectric point (pI) of zein is 6.8 (39) and that of nisin is 8.8 (Miserendino *et al*., 2008). In this study, when pH was 6.0, less than 100% of nisin release was observed for all capsules, possibly due to the significance of hydrophobic interactions because both nisin and zein are more hydrophobic at pH 6.0.

It has been suggested that nisin disrupts cell membrane activity via pore formation and may have additional effects on electron transfer chain components (De Vuyst and Vandamme, 1995, Shirazinejad *et al.*, 2010**)**. Moreover, the outcome of nisin activity within a food system depends on numerous factors; Nature of the food, other preservative hurdles such as heat treatments, low water activity, modified atmosphere, low temperature, and pH enhanced activity (Szabo and Cahill, 1998; Shirazinejad *et al.*, 2010). The increase in net charge of bacteriocins at low pH might facilitate translocation of bacteriocin molecules through the cell wall. The solubility of bacteriocins may also increase at lower pH, facilitating diffusion of bacteriocin molecules.

It should be noted that the antimicrobial properties of $CO_2$ under MAP are attributed to the reduction of pH below the level at

which the growth of many bacteria is inhibited (Samelis *et al*., 2005). Undissociated weak acids possess the ability to cross membranes of microorganisms and become dissociated inside the cell and acidify the cell interior. A moderately high $CO_2$ concentration in the atmosphere of packages interacts positively with nisin, thereby enhancing the effectiveness of this additive. However, several interactions between $CO_2$ and another additive have been demonstrated *in vitro* ($CO_2$ –nisin) (Nillson, 2000) and *in vivo* ($CO_2$ – nisin), (Cabo, 2005). A possible hypothesis to explain the interactions between $CO_2$ and nisin may relate to a gradual change in the distribution of Gram-positive and Gram-negative organisms in the population. Considering that nisin is more active against Gram-positive bacteria (Cabo *et al*., 2005) and that microbial flora change from Gram-negative (initially predominant) to Gram positive when refrigerated fish is packaged under high $CO_2$ concentrations, an increase in their effect with increasing $CO_2$ is expected (Cabo *et al.*, 2005).

The initial quality of fish used in this study was good, as indicated by a low initial number of bacteria (<4 log10 CFU/g) before the fish slices were subjected to the different treatments. Chytiri *et al*. ( 2004), report an initial APC of whole ungutted and filleted rainbow trout were ca. 2.5 and 3.8 log cfu/cm2, respectively (day 0). In their study APC reached ca. 7.0 log cfu/$cm^2$ after 18 days of storage for whole ungutted trout and after 10 days for filleted samples. The results indicated that nisin alone was less effective on APCs growth when compared with encapsulated nisin with zein capsules. Nisin alone or in combination with lactic acid was effective in reducing the APC when compared to the control (Gogus *et al*., 2006). In cold smoked rainbow trout, nisin was effective in reducing the count of total plate count (Nykanen *et al*., 2000). This greater inhibition of $NE_1$ and $NE_2$, compared to the N and $N_2$, may be due to the reduction of free nisin activity, and it was due to the nonspecific binding of nisin with lipids and proteins (Bhatti *et al*., 2004).

The Gram-negative psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Mohan *et al.*, 2010). The results of this study were in accordance with that of Hozbor *et al*. (2006), who revealed similar growth patterns for psychrotrophic population in sea salmon during refrigerated storage. The results indicated that nisin alone was less effective on PTCs growth when compared with encapsulated nisin with zein capsules. Rose *et al*. (2008) found that the compromised antimicrobial activity of nisin in fresh meat was caused by the complexation with glutathione. Incorporation of nisin within zein capsules in this study may have caused a reduction in the interaction of nisin with food components, and thus encapsulated nisin produced better antimicrobial results.
Lactic acid bacteria can reduce the quality of fish and fish products and are usually associated with the spoilage of fish (Mohan *et al*, 2010). The low LAB count in this study was expected since lactic acid bacteria tend to grow slowly at refrigeration temperatures and are under aerobic conditions generally out-competed by

pseudomonas (Shirazinejad *et al.*, 2010). In contrast, the contribution of LAB as the major spoiling microorganism had been reported in fresh vacuum-packed Atlantic salmon portions stored at 4°C (Rasmussen *et al*., 2002) Gradual increase in the counts of lactobacillus was observed with increase in the storage time. This result was in accordance with that of Faghani *et al*. (2011), who revealed similar growth patterns for LABs in grass carp *Ctenopharyngodon idella* treated with nisin and sodium acetate during refrigerated storage. The results showed that encapsulated nisin treatments were more effective in reducing the LAB counts, compared with free nisin treatments.

In conclusion, nisin in the form of free and encapsulated was effective in reducing microbial counts and retarding the oxidation process in rainbow trout fillets under refrigeration storage and also the results of this study have clearly shown that interactions between $CO_2$ and nisin are an advantage in the application of MAP in food preservation.

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