Antimicrobial and antioxidant effects of nisin Z and sodium benzoate in vacuum packed Caspian Kutum (*Rutilus frisii*) fillet stored at 4°C

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Abstract

This study was done to evaluate the antimicrobial and antioxidant effects of nisin Z (0.02 %) and sodium benzoate (1.5 and 2.5 %) in vacuum packed Caspian Kutum (*Rutilus frisii*) fillet stored at 4°C. Microbial changes [aerobic plate counts (APC), psychrotrophic counts (PTC) and lactic acid bacteria] and chemical indices [peroxide value and total volatile basic nitrogen (TVB-N)] were determined in days 0, 4, 8, 12 and 16. Results showed that PV and TVB-N in control samples (the sample dipped in prechilled distilled water) were deteriorated after 12 days compared to preserved samples which were acceptable after 16 days. Microbial tests indicated that control samples contained APC and PTC bacteria in day 16 more than standard limit, whereas treatment samples were in the acceptable range. In case of lactic acid bacteria, after 16 days, all samples were in the acceptable range. Results of chemical and microbial analysis showed that simultaneous use of nisin Z and sodium benzoate could increase the shelf life of vacuum packed *R. frisii*.

Keywords: *Rutilus frisii*, Microbial quality, Lipid oxidation, Nisin Z, Sodium benzoate

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Introduction

Owing to rapid spoilage of fish, using of various processing methods, transportation and preservation such as chemical and biological material in vacuum packaging, primary processing and keeping in low temperature, is considered essential (Perez-Alonso and Auborg, 2003). Whether preservation in refrigerator temperature cause in slowing down the rate of enzymatic, chemical and microbial activities, any adverse changes such as fat oxidation, hydrolysis and deterioration of quality will occur (Yin and Cheng, 2003). Therefore, in order to maintain the meat quality, meat shelf life and also prevention of economic losses, application of antibacterial and antioxidant agents seems useful (Yin and Cheng, 2003). There are many researches, which investigated the application of biological additives to control pathogens and spoilage microorganism (Tome *et al*., 2006). Antimicrobial activity of organic acid and their salts could be increased when it used in combination of other additional inhibitors such as bacteriocins or other natural antimicrobial agents (Samelis *et al*., 2005; Vescovo *et al*., 2006). Sodium benzoate is one of the most widely used organic acids in food products (Ogiehor *et al*., 2004; Lopez Malo *et al*., 2005). Antimicrobial activity of lactic acid bacteria (LAB) is attributed to organic acid production (lactic acid, free fatty acids), H_2O_2 and bacteriocin production (Chen and Hoover, 2003). *Lactococus lactis* produce two bacteriocins including nisin Z and nisin A, which have inhibitory effects on common bacteria in refrigerated fish including *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas* sp. In refrigerated foods, for increasing the antimicrobial properties, nisin Z and nisin A are used with combination of organic acids including sodium propionate, sodium citrate, sodium benzoate (Sallam, 2007). Antimicrobial agents such as sodium acetate and nisin are found to be effective in preventing microbial growth and improving quality of food under storage condition. Results of Sallam (2007) showed that using sodium acetate, sodium lactate, and sodium citrate with nisin Z could improved the antimicrobial and antioxidant indices in refrigerated sliced salmon. Behnam *et al*. (2015) showed that treatment of the vacuum packaged rainbow trout with nisin resulted in improvement of quality and extension of shelf life of the fish from 12 to 16 days at 4°C. Also, Ghomi *et al*. (2011) suggested that treatment of slices using 3% sodium acetate in combination with 0.2% nisin is the best condition for maintaining the quality of grass carp (*Ctenopharyngodon idella*) slices stored under the refrigerated condition.

Rutilus frisii is one of the most important economic fish in the Caspian Sea, it allocated about 13200 tons of catching bony fishes in 2014 (Iranian Fisheries Organization, 2014). The main objective of this study was to investigate using a chemical (sodium benzoates) and biological (nisin Z) preservatives, in order to assess the

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shelf life of vacuum packed fillets stored at 4°C.

Material and methods

Fish sample

Approximately 45 fish with average weight of 450 ± 10 g were prepared from Mazandaran fisherman and transported to the Caspian Sea Ecology Research Center laboratory in boxes full of ice. Transmission and filleting were immediately started for an hour.

Proximate composition

Moisture was determined by drying the samples in an oven (Behr, Germany) at 100°C (AOAC, 2005). Fat was determined by chloroform methanol extraction (2:1, v/v); ash was measured by incineration in a muffle furnace at 500°C for 6 h and crude protein was determined (Kjeldahl procedure: N× 6.25) using an automatic Kjeldahl system (AOAC, 2005).

Preparation of fish samples

Prepared fillets were weighted and dipped in 1.5 and 2.5 % (w/v) aqueous solution of sodium benzoate and after 15 minutes fillets were removed (Sallam, 2007). Then, nisin Z solution with concentration of 0.2 (g/kg body weight of fish) was sprayed on samples (Ghomi *et al*., 2011). All treated samples were packaged in vacuum condition (machine BOSS N84) and stored at 4 °C. On days 0, 4, 8, 12 and 16 of storage periods, three fillets were randomly selected and tested for evaluation of microbial and chemical factors.

All measurements were carried out in triplicate (n=3) and followed as below:

1- control samples (the sample dipped in prechilled distilled water) packaged in vacuum condition

2- samples contain 0.02% w/v aqueous solution of nisin Z

3- samples contain 1.5 % w/v aqueous solution of sodium benzoate

4- samples contain 2.5 % w/v aqueous solution of sodium benzoate

5- samples contain a combination of 0.02% w/v aqueous solution of nisin Z and 1.5 % w/v aqueous solution of sodium benzoate

6- samples contain a combination of 0.02% w/v aqueous solution of nisin Z and 2.5 % w/v aqueous solution of sodium benzoate

Measurement of peroxide value (PV)

PV was measured according to the AOAC method (2005). The sample (5 g) was weighed in a 250-mL glass container and heated in a water bath at 60 °C for 3 min, then thoroughly agitated for 3 min with 30 ml acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman paper. Saturated potassium iodide solution (0.5 mL) was added to the filtrate, which was transferred into titrator equipped with stirrer and pH electrode. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/l). PV was calculated

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and expressed as milliequivalent peroxide per kg of sample:

PV (meq / kg) = (S ×N) / W × 1000

where: S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N=0.01), and W the sample weight (kg) (Namulema *et al*., 1999).

Determination of total volatile basic nitrogen (TVB-N)

TVB-N was determined by distillation after the addition of MgO to fillet sample (AOAC, 2005). The distillate was collected in an Erlenmeyer containing 3% aqueous solution of boric acid and a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.05 g of methylene blue to 100 ml of ethanol 96%. Finally, the boric acid solution was titrated with 0.1 N hydrochloric acid solution (Namulema *et al*., 1999).

Microbiological and chemical analyses

Ten grams of fish samples were aseptically removed from the trays and homogenized for 1 min in a stomacher (VRN-200, Taiwan) containing 45 ml of physiological saline solution (0.85% NaCl) (Merck, Darmstadt, Germany). After resuscitation (for 30 min at 25°C) further decimal serial dilutions were prepared from this homogenate in the same sterile diluents. The appropriate dilutions were subsequently used for enumeration and differentiation of microorganisms and particular microbial genera in the samples, at each of the pre-determined time intervals, during refrigerated storage (Sallam, 2007).

Aerobic plate count (APC)

APC were determined by inoculating 0.1 ml of the sample homogenate onto duplicate sterile plates of dried Tryptic Soy Agar (Merck, Darmstadt, Germany) using the surface spread technique, then the plates were incubated for 48 h at 35 °C (Sallam, 2007).

Psychrotrophic count (PTC)

PTC counts were measured by inoculating 0.1 mL of the sample homogenate onto duplicate sterile plates of dried Tryptic Soy Agar (Merck, Darmstadt, Germany) using the surface spread technique, then the plates were incubated at 7 °C for 10 days (Cousin *et al*., 1992).

Lactic acid bacterial count (LAB)

For counting of LAB, diluted samples were plated on deMan, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany) and incubated at 30°C for 48 h in anaerobic jars with disposable anaerocult C bags (Merck, Darmstadt, Germany) for the generation of an anaerobic medium (Sallam, 2007).

Statistical analysis

Normal of data and homogeneity of variance was evaluated using Kolmogorov-Smirnov and Levene's test, respectively. The obtained data were subjected to one-way analysis of variance using SPSS statistical software (release 18.0). Duncan's multiple range test was performed to determine the significant differences between means at the 5% probability level ($p< 0.05$).

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Results

Proximate composition

Body composition of *R. frisii* fillet is shown in Table 1. Variation in chemical composition may be related to the nutrition, catching season (spawning cycle), sexual variation, fish size, living area, as well as the other environmental conditions (Pacheco Aquilar *et al*., 2000).

Chemical examination

Peroxide value

Changes in the PV revealed that the PV (meq peroxide/kg fish sample) of control in day 12 of storage was 10.25 (Table 2). On day 16 of storage, PV in nisin Z, 1.5 and 2.5 sodium benzoate treatments were 10.36, 10.21 and 10.30, respectively. These values in combined treatments (nisin plus sodium benzoate) were in the range of 8.36 to 9.25 that was in the standard ranges (10-20 meq peroxide/kg fish sample).

Total volatile base nitrogen (TVB-N)

TVB-N in the control group on day 12 was 31.25 mg/100g. In samples with nisin and benzoate individual in day 16 were 30.26 and 31.25 mg/100g, respectively (Table 3).

Microbiological examination

Total viable count (TVC)

Results showed that TVC in control samples on day 16 was 7.39 log cfu and in treatment samples were in standard limit which was lower than 7 logs (Table 4).

Psychrotrophic counts (PTC)

Changes in pshycrophilic bacteria are shown in Table 5. Result of PTC in control samples indicated 7.62 in day 16, whereas in treatment samples were in the standard limit (lower than 7 $\log_{10}$ CFU/g) (Sallam, 2007). PTC value in nisin and sodium benzoate groups were lower than control samples at different times ($p< 0.05$) (Table 5).

Lactic acid bacteria

Based on the results that shown in Table 6, with increasing of storage time, LAB counts in the control samples progressively increased ($p<0.05$) and reached to 6.25 ± 0.14 log cfu/g at day 16.

Discussion

PV is an indicator of the primary lipid oxidation product and is the most common index of lipid hydroperoxides (Ólafsdottir *et al*., 1997). Nisin Z and benzoate changes in individual treatments in comparison to control and combined samples were significant ($p<0.05$). Results indicated that changes in PV in controls were higher than the other treatments. This reduction could be attributed to the effect of nisin on lipolytic bacteria (such as Pseudomonas) (Nykänen *et al*., 2000) and also antimicrobial effect of sodium benzoate on some bacteria such as *L. monocytogenes* and *Pseudomonas* sp. (Jamuna *et al*., 2005; Stanojevic *et al*., 2009). The results were compatible with reports of Pacheco Agailar *et al*.

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(2000) in sardines. Similar results were reported for sodium and nisin by Manju *et al*. (2007) in *Etroplus suratensis* fillets, Asghari *et al*. (2009) in silver carp (*Hypophthalmichthys molitrix*) fillet, Ghomi *et al*. (2011) in grass carp slices and Behnam *et al*. (2015) in vacuum packaged of rainbow trout (*Oncorhynchus mykiss*).

TVB-N is a useful parameter for spoilage in fresh and lightly preserved seafood (Lakshmanan, 2000). Generally, the volatile bases are produced as a result of microbial degradation of protein and non-protein nitrogenous compounds (Lakshmanan, 2000). A level of 35–40 mg TVB-N/100 g of fish muscle is usually regarded as spoiled (Ozoqul *et al*., 2005).

Table 1: Proximate composition (%) of *Rutilus frisii* fillet.

Ash	Moisture	Crude lipid	Crude protein
1.09 ± 0.13	73.68 ± 0.65	6.16 ± 0.31	18.96 ± 0.42

Table 2: Effect of nisin Z and sodium benzoate on PV (meq peroxide/kg fish sample) of *Rutilus frisii* fillets at 4°C (Mean ± *SD*).

Nisin Z + sodium benzoate 2.5%	Nisin Z + sodium benzoate 1.5%	Sodium benzoate 2.5%	Sodium benzoate 1.5%	Nisin Z	Control	Treatment Time intervals (day)
1.25±0.26 ^{Ea*}	1.55±0.11 ^{Ea}	1.32±0.55 ^{Da}	1.62±0.25 ^{Da}	1.50±0.11 ^{Ea}	1.25±0.35 ^{Da}	Zero
3.14±0.50 ^{Db}	4.29±0.21 ^{Da}	3.11±0.29 ^{Cb}	4.11±0.13 ^{Ca}	3.25±0.15 ^{Db}	3.11±0.25 ^{Cb}	4
6.25±0.16 ^{Cb}	5.11±0.50 ^{Cc}	5.41±0.20 ^{Bc}	6.35±0.17 ^{Bb}	5.11±0.17 ^{Cc}	7.26±0.317 ^{Ba}	8
7.11±0.17 ^{Bc}	7.35±0.16 ^{Bc}	8.35±0.30 ^{Ab}	7.45±0.15 ^{Ac}	8.29±0.13 ^{Bb}	10.25±0.45 ^{Aa}	12
8.36±0.80 ^{Ac}	9.25±0.13 ^{Ab}	10.21±0.35 ^{Dd}	10.30±0.16 ^{Ed}	10.36±0.11 ^{Aa}	10.25±0.35 ^{Aa}	16

*Different small and capital superscript letters within each row and column, respectively, represent significant differences (p<0.05).

Table 3: The effect of nisin Z and sodium benzoate on TVN-B (mg/100g) index of *Rutilus frisii* fillet at 4°C (Mean ± *SD*).

Nisin Z + sodium benzoate 1.5%	Sodium benzoate 2.5%	Sodium benzoate 1.5%	Sodium benzoate 1.5%	Nisin Z	Control	Treatment Time intervals (day)
6.11±0.60 ^{Ea*}	6.25±0.70 ^{Ea}	6.35±0.60 ^{Ea}	6.55±0.70 ^{Ea}	6.37±0.60 ^{Ea}	7.35±0.60 ^{Ea}	zero
11.22±0.50 ^{Db}	11.25±0. 60 ^{Db}	13.25±0. 17 ^{Da}	13.13±0.60 ^{Da}	14.51±0.60 ^{Da}	14.32±0.80 ^{Da}	4
18.25±0.90 ^{Cd}	17.35±1.12 ^{Cd}	20.25±0.70 ^{Cc}	22.36±0.50 ^{Cb}	25.11±1.40 ^{Ca}	24.11±0.90 ^{Ca}	8
24.36±1.12 ^{Bc}	25.11±1.36 ^{Bc}	24.26±1.25 ^{Bc}	25.30±1.35 ^{Bc}	28.25±1.20 ^{Bb}	31.25±1.36 ^{Ba}	12
28.35±1.35 ^{Ac}	29.25±1.11 ^{Ac}	30.26±1.01 ^{Ac}	31.25±0.70 ^{Ac}	33.11±0.80 ^{Ab}	36.11±1.11 ^{Aa}	16

*Different small and capital superscript letters within each row and column, respectively, represent significant differences (p<0.05).

On day 16, TVB-N of combined treatments (nisin plus benzoate) was 28.35 to 29.25, which were allocated in standard range (p>0.05). Gimenez *et al*. (2002) recommended a value of 30 mg N/100 g muscle as the highest

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acceptable level. Microbial activity during storage is considered as one of the sources of nitrogen productions (Ozoqul *et al*., 2005). The increase in TVB-N value in fish muscle during storage at 4°C is probably due to amino acid deamination (Mangalassary *et al*., 2005; Rodriquez *et al*., 2008). According to age, sex, environmental condition and season, TVN-B value varies in various species. The antibacterial effect of bacteriocin on proteolytic bacteria could reduce the volatile amine production (Pacheco Agailar *et al*., 2000). Based on our results, TVB-N and PV values in nisin or sodium treatments and the combination treatments did not show significant differences with other (p>0.05), but significant differences was observed in TVB-N and PV value between individual and combined treatments with control group (p<0.05). Similar results was reported by Behnam *et al*. (2015) when they used nisin in vacuum packaged of rainbow trout or Asghari *et al*. (2009) that survived the effects of sodium acetate on shelf-life of silver carp fillet during refrigerator storage.

TVC is of very doubtful value in the examination of frozen fish products (Gram and Huss, 1996). Total number of bacteria on fish rarely closely indicates sensorial quality or storage characteristics.

TVC value is one of the useful indicator for measuring the condition of raw material, effectiveness of procedures and hygiene conditions during processing, sanitary conditions of equipment and utensils and interaction of time and temperature profile during storage and distribution (Gram and Huss, 1996). The difference of TVC in nisin and benzoate during different days were significant compare to control group. Also, significant differences were observed in nisin and benzoate individually in comparison with combined groups (p<0.05). It could be mentioned that Maximal Recommended Limit (MRL) of fish TVC is considered 7 log cfu/g (Mohan *et al*., 2008). Studies of Krizek *et al*. (2004) in vacuum packaged and non-vacuum packaged flesh of carp (*Cyprinus carpio*) stored at 3°C for 4 days, Savvaidis *et al*. (2002) in vacuum packaged flesh of rainbow trout stored in refrigerator for 8 days and Chytiri *et al*. (2004) in vacuum packaged fillet of rainbow trout on ice for 6 days, had been shown the MRL. The effect of nisin Z and sodium benzoate on microbial growth of processed fish products are depends on concentration of used nisin and sodium benzoate, methods of application of nisin, immersion time, fish species, type of product, microbial load and storage condition (Kim *et al*., 1995). Based on the current study, lower values of TVC were detected in the treated fish with nisin and benzoate compare to control group, which might be due to antibacterial activity of the nisin and subsequently, bacterial growth

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inhibition. Hampikyan and Ugur (2007) suggested that nisin is active against gram positive organisms including bacterial spores, but it is not generally active against gram negative bacteria, yeasts and fungus. Also, in Nattress *et al*. (2001) study, the ability of nisin to control meat spoilage bacteria including *Brochothrix thermosphacta* B2 and *Carnobacterium* sp. is confirmed. In similar study, Scannell *et al*. (1997) showed that a combination of 2% sodium lactate and 500 IU/g nisin was particularly effective in reducing the TVC counts at the end of 10 days of storage fresh pork sausage in refrigerated storage. The same result was found in seer fish, *Scomberomorus commerson* (Mohan *et al*., 2010), cold smoked rainbow trout (Nykanen *et al*., 2000) and grass carp (Ghomi *et al*., 2011) treated with sodium acetate or nisin in which treated samples had lower total viable bacterial count compared to the control.

In this study, an increase in PTC values were observed in all samples, but the rate of increase was small in nisin Z- sodium benzoate 2.5% treated samples compared with the control (p<0.05). These results were similar to Raju *et al.* (2003) that showed nisin at 25 and 50 ppm level reduce the PTC by 5 log and spore count by 2 log over that of control at the end of 30 days of storage fish sausage in refrigerated storage. These results were in agreement with reports of Behnam *et al*. (2015), Kallinteri *et al*. (2013) and Sallam (2007). The same results were achieved in researches on shrimp and catfish fillets (Zhuang *et al*., 1996), *Lethrinus lentjan* fillets (Shalini *et al*., 2000), Pearspot (*Etroplus suratensis*) (Manju *et al*., 2007) and Pacific salmon (*Onchorhynchus nerka*) (Sallam, 2007).

Lactic acid bacteria (LAB) are gram-positive pathogens, facultative anaerobic bacteria that can grow under both anaerobic and aerobic conditions (Mexis *et al*., 2009). LAB value in control samples on day 16 was higher than standard limit (7 log cfu/g), this data were in the standard limit for other treatment samples. In Gram and Huss (1996), the elimination of LAB by nisin resulted in the shelf life extension of smoked salmon. Also, in Ghomi *et al*. (2011) LAB count of 6.76 log cfu/g was found in the control sample at the end of the storage of grass carp slices (day 8), whereas the samples treated with nisin in combination with sodium acetate had significantly lower LAB count (5.07–6.07 log cfu/g) (p<0.05). Moreover, in Sallam (2007) study, LAB bacteria count for sodium acetate treated salmon fillets was 0.85 log lower than the control group. All mentioned studies confirm the current results.

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Table 4: The effect of nisin Z and sodium benzoate on TVC (log $_{10}$ CFU/g) of *Rutilus frisii* fillets at 4°C (Mean ± *SD*).

Nisin Z + sodium benzoate 2.5%	Nisin Z + sodium benzoate 1.5%	Sodium benzoate 2.5%	Sodium benzoate 1.5%	Nisin Z	Control	Treatment Time intervals (day)
3.25±0.20 ^{Da*}	3.11±0.10 ^{Da}	3.17±0.20 ^{Da}	3.15±0.20 ^{Da}	3.11±0.10 ^{Da}	3.20±0.30 ^{Ea}	zero
3.11±0.40 ^{Db}	3.55±0.40 ^{Db}	3.43±0.30 ^{Db}	3.73±0.40 ^{Db}	3.66±0.40 ^{Db}	4.11±0.20 ^{Da}	4
4.12±0.85 ^{Cb}	4.23±0.14 ^{Cb}	4.41±0.12 ^{Cb}	4.65±0.11 ^{Cb}	4.36±0.10 ^{Cb}	5.13±0.40 ^{Ca}	8
5.26±0.43 ^{Bb}	5.45±0.17 ^{Bb}	5.66±0.50 ^{Bb}	5.86±0.14 ^{Bb}	5.60±0.40 ^{Bb}	6.14±0.50 ^{Ba}	12
6.20±0.70 ^{Ab}	6.39±0.13 ^{Ab}	6.46±0.40 ^{Ab}	6.50±0.30 ^{Ab}	6.49±0.26 ^{Ab}	7.39±0.30 ^{Aa}	16

*Different small and capital superscript letters within each row and column, respectively, represent significant differences ($p< 0.05$).

Table 5: The effect of nisin Z and sodium benzoate on PTC (log $_{10}$ CFU/g) of *Rutilus frisii* fillets at 4°C (Mean ± *SD*)

Nisin Z + sodium benzoate 2.5%	Nisin Z + sodium benzoate 1.5%	Sodium benzoate 2.5%	Sodium benzoate 1.5%	Nisin Z	Control	Treatment Time Intervals (day)
3.20±0.30 ^{Da*}	3.26±0.20 ^{Da}	3.16±0.30 ^{Da}	3.13±0.30 ^{Da}	3.18±0.10 ^{Da}	3.12±0.20 ^{Ea}	zero
3.26±0.20 ^{Db}	3.45±0.30 ^{Db}	3.59±0.20 ^{Db}	3.29±0.13 ^{Db}	3.80±0.30 ^{Db}	4.36±0.15 ^{Da}	4
4.20±0.16 ^{Cb}	4.29±0.17 ^{Cb}	4.50±0.14 ^{Cb}	4.60±0.12 ^{Cb}	4.50±0.13 ^{Cb}	5.25±0.23 ^{Ca}	8
5.43±0.15 ^{Bb}	5.52±0.14 ^{Bb}	5.70±0.30 ^{Bb}	5.29±0.17 ^{Bb}	5.82±0.30 ^{Bb}	6.29±0.30 ^{Ba}	12
6.29±0.30 ^{Ab}	6.45±0.12 ^{Ab}	6.65±0.17 ^{Ab}	6.73±0.30 ^{Ab}	6.68±0.25 ^{Ab}	7.62±0.17 ^{Aa}	16

*Different small and capital superscript letters within each row and column, respectively, represent significant differences ($p<0.05$).

Table 6: The effect of nisin Z and sodium benzoate on LAB (log $_{10}$ CFU/g) of *Rutilus frisii* fillets at 4°C (Mean ± *SD*).

Nisin Z + sodium benzoate 2.5% w/v	Nisin Z + sodium benzoate 1.5% w/v	Sodium benzoate 2.5% w/v	Sodium benzoate 1.5% w/v	Nisin Z	Control	Treatment Time intervals (day)
1.29±0.10 ^{Da}	1.32±0.30 ^{Da}	1.26±0.20 ^{Ea}	1.30±0.10 ^{Ea}	1.32±0.20 ^{Ea}	1.30±0.07 ^{Ea}	zero
2.20±0.10 ^{Cb}	2.30±0.10 ^{Cb}	2.30±0.10 ^{Db}	2.32±0.10 ^{Db}	2.35±0.20 ^{Db}	3.85±0.10 ^{Da}	4
3.17±0.40 ^{Bb}	3.29±0.30 ^{Bb}	3.65±0.40 ^{Cb}	3.85±0.20 ^{Cb}	3.87±0.20 ^{Cb}	4.25±0.30 ^{Ca}	8
3.72±0.30 ^{Bbc}	3.87±0.30 ^{Bbc}	4.11±0.30 ^{Bb}	4.32±0.40 ^{Bb}	4.25±0.10 ^{Bb}	5.46±0.17 ^{Ba}	12
4.25±0.31 ^{Ac}	4.35±0.30 ^{Ac}	5.26±0.15 ^{Ab}	5.42±0.15 ^{Ab}	5.46±0.17 ^{Ab}	6.25±0.14 ^{Aa}	16

*Different small and capital superscript letters within each row and column, respectively, represent significant differences ($p<0.05$).

The difference of LAB in nisin and sodium benzoate groups via control samples at different times were significant; otherwise changes in LAB in nisin and benzoate groups individually were significant in comparison to combined groups ($p<0.05$). This could be attributed to an equal bactericide effect of nisin and also sodium benzoate on LAB (Castellano *et al*., 2008). LAB tend to grow slowly at refrigeration temperatures and are under aerobic condition generally out-competed by Pseudomonas (Chen and Hoover, 2003). In contrast, the contribution of LAB as the major spoiling microorganisms had been reported in

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fresh vacuum-packed Atlantic salmon portions stored at 4°C (Rasmussen *et al*., 2002).

Overall, combination of nisin and sodium benzoate on days 0, 4, 8, 12 and 16 showed the best effect in increasing of *R. frisii* shelf life. Quality chemical indices (TVB-N, PV) in combined treatments were lower than control and nisin samples. Microbial analysis revealed a significant reduction in TVC, PTC and LAB in all treatments except the control. Finally, it could be concluded that the combination of nisin Z and sodium benzoate increase the shelf life of *R. frisii* fillets stored at 4°C for about 16 days and this can be recommend for the storage.

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