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Full Length Research Paper

Effect of *Listeria monocytogenes* inoculation, sodium acetate and nisin on microbiological and chemical quality of grass carp *Ctenopharyngodon idella* during refrigeration storage

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In this study, the microbiological quality and lipid oxidation of the grass carp (*Ctenopharyngodon idella*) fillets treated by dipping in sodium acetate (0, 1 and 3%), nisin (0, 0.1 and 0.2%) or their combination were evaluated during 16 days of refrigeration storage. Antilisterial effect of nisin was enhanced with the increased concentration of sodium acetate. With increasing the concentrations of sodium acetate, mesophilic counts were lower but regarding nisin, better results were obtained by applying 0.1% nisin. Greater inhibition of mesophile bacteria was observed when combination treatment was used. The number of lactobacillus was lower when higher concentrations of sodium acetate and nisin were used. Peroxide, TBA and total viable base nitrogen (TVB-N) values were lower in the samples treated with both nisin and sodium acetate and higher results were obtained in the combination treatments.

Key words: *Listeria monocytogenes*, nisin, sodium acetate, microbial quality, chemical quality, grass carp.

INTRODUCTION

Fish is an excellent protein source with high nutritive value due to a favorable essential amino acid composition (Jannat Alipour et al., 2010) and is one of the most highly perishable food products (Ashie et al., 1996). During handling and storage period, quality deterioration of fresh fish rapidly occurs which limits the shelf life of the product. Microbial contamination can shorten shelf life of meats; reduce the quality of fresh meat and cause economic loss and health hazards (Gram and Huss, 1996). *Listeria monocytogenes* is a new threat to public health and the incidence of food related listeriosis has been increased in recent years (Porto-Fett et al., 2010).

Food borne listeriosis is hazardous to young, old, pregnant and immunocompromised people. *L. monocytogenes* has been isolated from surface fresh waters and coastal marine waters subjected to pollution (Ben, 1994). In processing plants, contamination with this bacterium may occur from direct contact with contaminated processing equipments (Huss et al., 2000). This pathogen is able to survive in a wide range of temperature, pH and NaCl concentration. In refrigerated stored meat, this bacterium can reach hazardous level (Lou and Yousef, 1999). In recent years, consumers demand for safety and fresh refrigerated foods with extended shelf life has increased (Sallam, 2007a). It has been reported that contamination of aquatic products with *L. monocytogenes* cannot be avoided totally, hence in order to inhibit the growth and development of this

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pathogen in the products and to ensure safety, additives are needed (Nykanen et al., 2000).

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 which 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 *L. monocytogenes* (Chen and Shelef, 1992; Nilsson et al., 1997; Nykanen et al., 2000). Nisin can also prevent the growth of Gram-negative bacteria when combined with other compounds such as organic acids (Gogus et al., 2006). The efficacy of sodium acetate and nisin as potential preservatives in grass carp *Ctenopharyngodon idella* has been investigated (Ghomi et al., 2011).

The aim of the present study was to assess the effect of preservatives (sodium acetate, nisin or their combination) on microbial (*L. monocytogenes*, mesophilic bacteria and lactobacillus) and chemical quality (peroxide, thiobarbituric acid, total volatile base nitrogen) of grass carp *C. idella* fillets during refrigeration storage for 16 days.

MATERIALS AND METHODS

Sample preparation

Fresh cultured grass carp (weight = 1000 g; standard deviation (SD) = 38 g; n = 20) were gotten from a local market (Tonekabon, Northern Iran) and transported to the laboratory in ice containing boxes. Upon arrival, the fish were beheaded, gutted and washed with tap water several times to remove the blood and slime. All fish samples were then cut into slices of 0.5 cm thickness. Thereafter, all were homogenized and divided into 9 homogenous batches. *L. minocytogenese* (ATCC 19114) was propagated on sheep blood agar plates (Merck, Darmstadt, Germany) for 24 h at 37°C and then diluted in 0.1% peptone to a concentration of 8 log cfu/g. These inoculums were further diluted in 0.1% peptone resulting in 6 log cfu/g *L. monocytogenes* solutions. Grass carp fillets were inoculated for 60 s at 4°C. The final concentration of *L. monocytogenes* was 3 to 4 log cfu/g in the fillets.

Eight batches were dipped in pre-chilled (4°C) aqueous solution (0, 0.1 and 0.2% nisin and 0, 1 and 3% sodium acetate) as a 3 x 3 factorial design. The final batch was dipped in pre-chilled distilled water as a control sample. Treatments were coded as follow: code 1 (0% sodium acetate + 0% nisin), code 2 (0% sodium acetate + 0.1% nisin), code 3 (0% sodium acetate + 0.2% nisin), code 4 (1% sodium acetate + 0% nisin), code 5 (1% sodium acetate + 0.1% nisin), code 6 (1% sodium acetate + 0.2% nisin), code 7 (3% sodium acetate + 0% nisin), code 8 (3% sodium acetate + 0.1% nisin) and code 9 (3% sodium acetate + 0.2% nisin). Fish to dipping solution ratio was 1:2. After dipping for 15 s, fish slices were allowed to drain for 5 min on a sterile stainless wire mesh screen at 18°C, and then packed with vacuum. Packed slices were subsequently labeled and stored at 4°C.

Microbial analysis

Ten grams of samples were homogenized for 1 min at 230 rpm in a stomacher (stomacher 400 Lab Blender, Seward Medical, UK) which contained 90 ml sterile saline solution (0.85% NaCl). Tenfold serial solution was prepared with a normal saline (0.85% NaCl) and used for the microbiological analysis. For microbial enumeration, 0.5 ml samples of appropriate serial dilutions of homogenates were spread evenly on the surface of dry media using a sterile bent glass rod. Three bags of each treatment were drawn randomly at regular intervals (0, 4, 8, 12 and 16 days) and subjected to microbiological analysis.

Total viable mesophilic count was determined using a plate count agar (PCA). Plates were incubated at 37°C for 48 h (Townley and Lanier, 1981). For lactic acid bacteria (LAB) determination, diluted samples were placed on MRS agar (Merck, Darmstadt, Germany) and incubated at 30°C for 2 to 3 days in anaerobic jars for the generation of an anaerobic medium. Samples inoculated with *L. monocytogenes* were evaluated using listeria selective palcam agar (Merck, Darmstadt, Germany) by the spread plate method. The plates were incubated at 30°C for 48 h.

Peroxide value determination

Peroxide value was measured according to the procedure described by PORIM (Palm Oil Research Institute of Malaysia) (1995). About 0.3 g of fat was put into a 250 ml flask with stopper. Sample was dissolved in 10 ml chloroform-acetic acid mixture by shaking. Then, 1 ml of saturated KI solution was added and immediately, stopped and stand in the dark for 5 min. After that 20 ml distilled water was added and shook. Sample was titrated with 0.01 N, $Na_2S_2O_3$ solution until yellow color almost disappears. Thereafter, 1 ml of 1.5% starch solution was added and titration continued until dark blue color was disappeared. A blank test was carried out, without oil. Peroxide value (meq per 1000 g) was determined by the formula: 1000 (V1 - V2) N/W, where V1 is the volume (in ml) of the sodium thiosulphate solution of normality N used for determination, V2 is the volume of the sodium thiosulphate solution used for the blank test, W is the weight (in g) of the test portion and N is the normality of the sodium thiosulphate solution.

TBA analysis

TBA assay was carried out according to Schmedes and Holmer (1989). Ten grams of fish muscle was mixed with 25 ml of 20% trichloroacetic acid and homogenized in a blender for 30 s. After filtration, 2 ml of the filtrate were added to 2 ml of 0.02 M aqueous TBA in a test tube. The test tube was incubated at room temperature in the dark for 20 h, and then the absorbance was measured at 532 nm by using UV-vis spectrophotometer (UV-1200, Shimadzu, Japan). TBA was expressed as mg malonaldehyde per kg of fish sample.

Total viable base nitrogen (TVB-N)

TVB-N was measured by steam-distillation of the trichloroacetic acid (TCA)-fish extract using the modified method of Malle and Tao (1987). Twenty five milliliter of the filtrate was loaded into a kjeldahl-type distillation unit and the distillate was received into a beaker containing 15 ml of 4% aqueous boric acid solution up to a final volume of 50 ml. The titration was allowed to run against aqueous 0.05 M sulphuric acid solution using an automatic titrator equipped with stirrer and pH electrode.

Statistical analysis

Data were analyzed using the general linear models (GLM) procedure by SPSS 17. A 3 x 3 factorial design was used for investigation of main and interaction effects of nisin and sodium

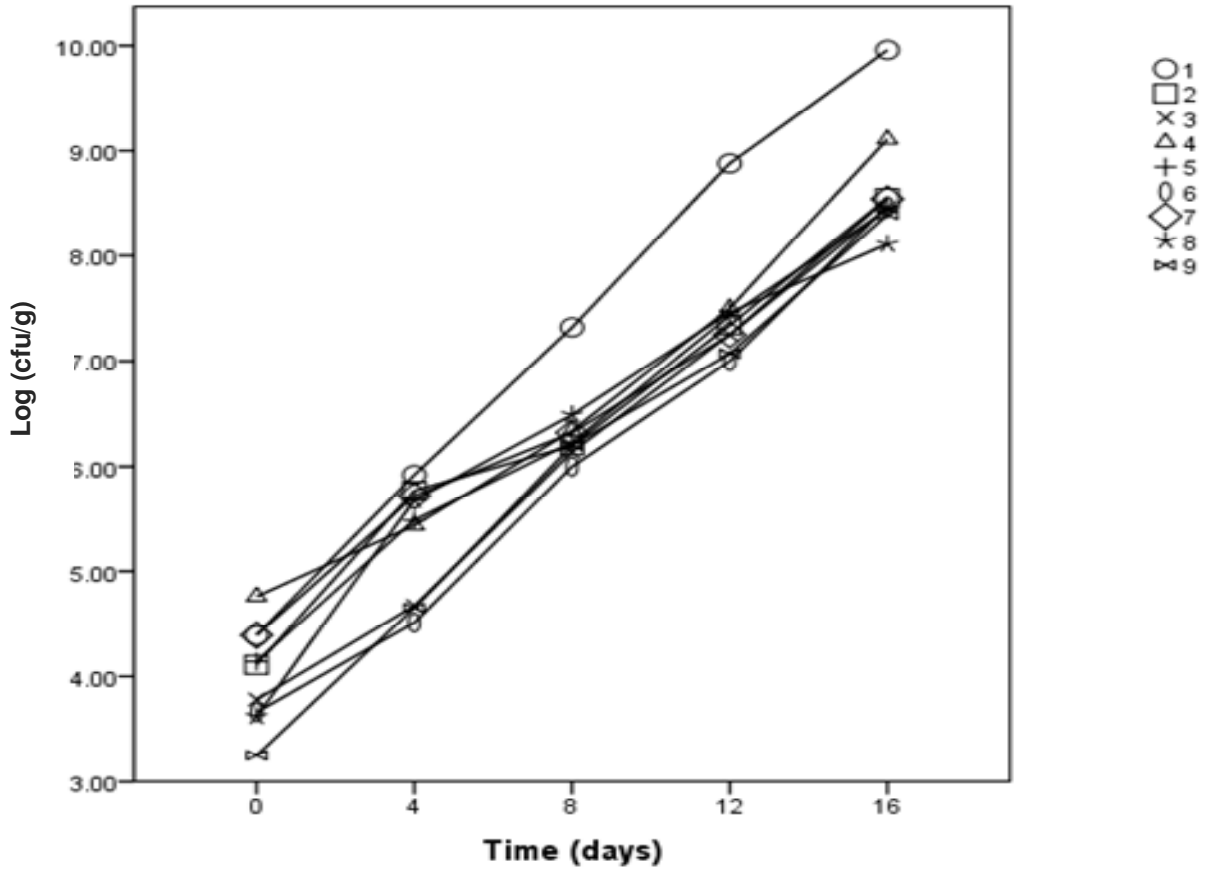

Figure 1. Effect of listeria inoculation, sodium acetate and nisin on total mesophilic bacteria of grass carp fillets during refrigeration storage. Code 1 (0% sodium acetate + 0% nisin), code 2 (0% sodium acetate + 0.1% nisin), code 3 (0% sodium acetate + 0.2% nisin), code 4 (1% sodium acetate + 0% nisin), code 5 (1% sodium acetate + 0.1% nisin), code 6 (1% sodium acetate + 0.2% nisin), code 7 (3% sodium acetate + 0% nisin), code 8 (3% sodium acetate + 0.1% nisin) and code 9 (3% sodium acetate + 0.2% nisin).

acetate on chemical changes. Comparison of differences for mean values was performed by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Total mesophilic bacteria

The initial mesophilic counts were between 3.25 and 4.78 log cfu/g (Figure 1). At the end of the day 8 of refrigerated storage, mesophilic counts in the control (7.32 log cfu/g) exceeded the acceptability levels of 7 log cfu/g (ICMSF 1986), whereas in other treatments, the counts were lower (6 to 6.49 log cfu/g). At the end of days 12 and 16, mesophilic counts were higher than the acceptability levels in all control or treated fillets. Results also showed that with increase of the concentrations of sodium acetate, mesophilic counts were low but regarding nisin, higher results was obtained by applying 0.1% nisin. Greater inhibition of mesophile bacteria was observed when combination treatment was used, indicating a synergy between sodium acetate and nisin. Nisin alone or in combination with lactic acid was effective in reducing the mesophil bacterial count as compared to the control (Gogus et al., 2006). This was further confirmed by Mohan et al. (2010) who found that seer fish treated with sodium acetate showed lower mesophilic counts as compare to that of control. In cold smoked rainbow trout, nisin was effective in reducing the count of total mesophilic bacteria (Nykanen et al., 2000).

Lactobacillus count

Lactobacillus spp. can reduce the quality of fish and fish products and are usually associated with the spoilage of fish. The initial count of this bacterium was between 4.34 and 4.76 log cfu/g. Gradual increase in the counts of lactobacillus was observed with increase of the storage time. At the 8th day of refrigerated storage, lactobacillus counts reached 6.47 log cfu/g which is in close proximity with the maximal recommended limit of 7 $\log_{10}$ cfu/g

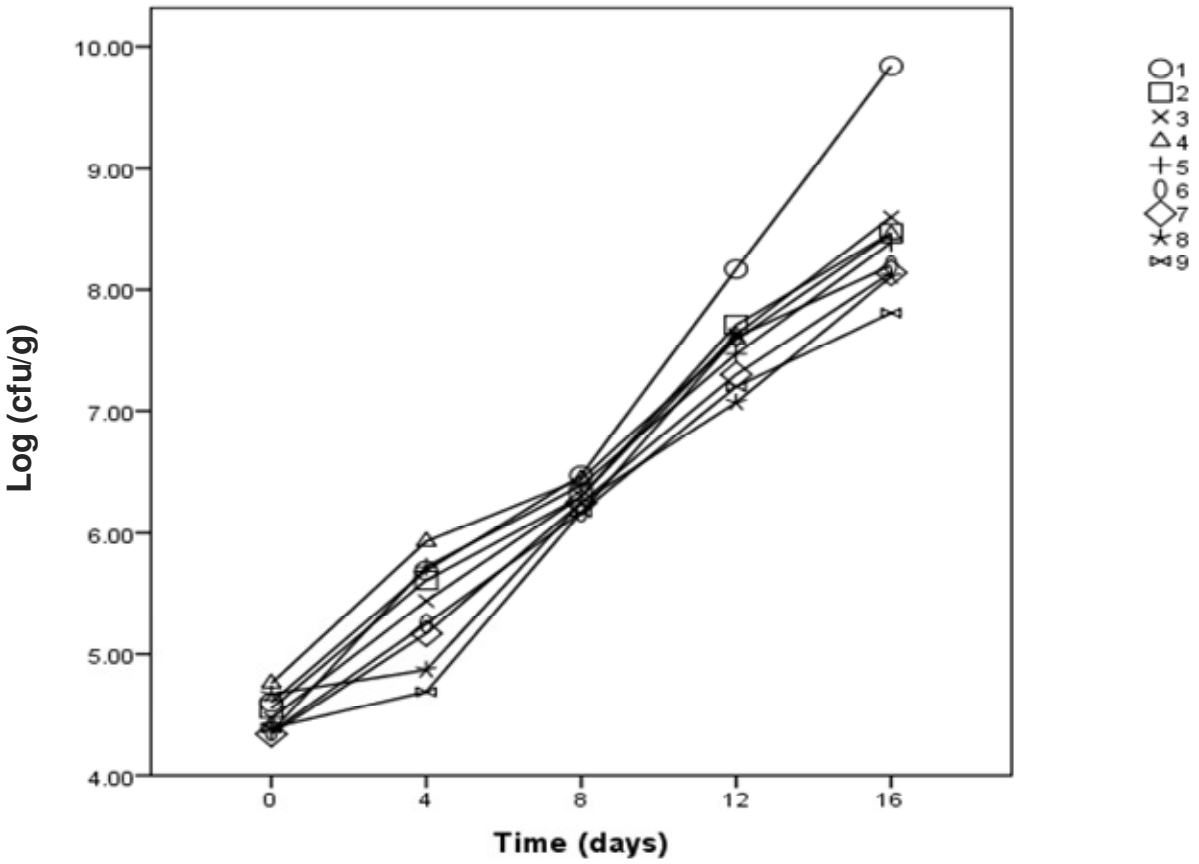

Figure 2. Effect of listeria inoculation, sodium acetate and nisin on lactobacillus counts of grass carp fillets during refrigeration storage. Code 1 (0% sodium acetate + 0% nisin), code 2 (0% sodium acetate + 0.1% nisin), code 3 (0% sodium acetate + 0.2% nisin), code 4 (1% sodium acetate + 0% nisin), code 5 (1% sodium acetate + 0.1% nisin), code 6 (1% sodium acetate + 0.2% nisin), code 7 (3% sodium acetate + 0% nisin), code 8 (3% sodium acetate + 0.1% nisin) and code 9 (3% sodium acetate + 0.2% nisin).

(ICMSF 1986). The number of this bacterium was lower when higher concentrations of sodium acetate (3%) or nisin (0.2%) was used. Moreover, the combination treatments were more effective in reducing the bacterial counts (Figure 2). After 12 days of storage, the counts of this bacterium in control and all treatments were higher than the acceptability level. In sodium acetate treated seer fish (*Scomberomorus commerson*) fillets, the counts of lactobacillus bacterium were significantly lower as compared to that of the control (Mohan et al., 2010).

L. monocytogenes

The initial counts of listeria were between 3.83 and 4.61 log cfu/g (Figure 3). After 4 days of storage, 0.66 log cfu/g increase in the counts of listeria was observed in control but this count was lower for the treated fillets. At day 8, lowest count was observed in treatments 3 (4.36 log cfu/g) and 9 (4.5 log cfu/g). After 12 days of refrigerated storage, the counts of listeria in the control exceeded the maximum acceptability level of 7 log cfu/g (ICMSF, 1986), while in samples treated with sodium acetate, nisin or their combination, the counts were between 5.17 and 5.91 log cfu/g. Moreover antilisterial effect of nisin was enhanced by the increased concentration of sodium acetate. In frozen-thawed salmon, nisin was effective in reducing the counts of inoculated *L. monocytogenes*, but in the control, the counts of *L. monocytogenes* increased from 4.7 to 7.5 log cfu/g after 9 days of storage (Zuckerman and Avraham, 2002). In beef cubes inoculated with *L. monocytogenes* and stored in refrigerator, nisin was effective in reducing the counts of these bacteria by 2.01 log cfu/cm^2 as compared to that of the control (Zhang and Mustafa, 1999). A similar result was obtained by Ariyapitipun et al. (2000) when nisin alone or in combination with organic acids reduced the counts of *L. monocytogenes* in packed beef. In cold-smoked trout, nisin in combination with organic acid was more effective in reducing the listeria counts when compared with the separate use of both preservatives (Nykanen et al., 2000).

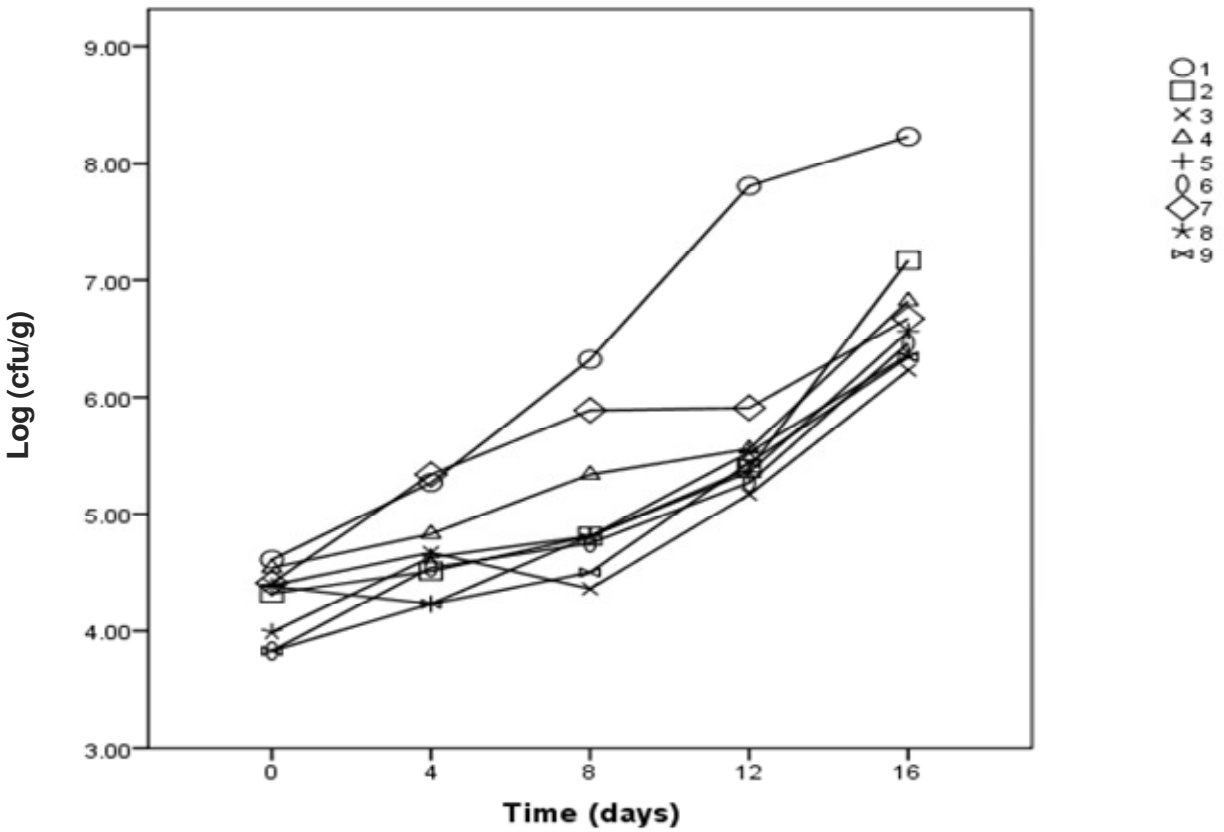

Figure 3. Effect of listeria inoculation, sodium acetate and nisin on *L. monocytogenes* counts of grass carp fillets during refrigeration storage. Code 1 (0% sodium acetate + 0% nisin), code 2 (0% sodium acetate + 0.1% nisin), code 3 (0% sodium acetate + 0.2% nisin), code 4 (1% sodium acetate + 0% nisin), code 5 (1% sodium acetate + 0.1% nisin), code 6 (1% sodium acetate + 0.2% nisin), code 7 (3% sodium acetate + 0% nisin), code 8 (3% sodium acetate + 0.1% nisin) and code 9 (3% sodium acetate + 0.2% nisin).

Total viable base nitrogen (TVB-N)

TVB-N is a useful parameter for spoilage in fresh and lightly preserved seafood (Dalgaard, 2000) and produce as a result of microbial degradation of protein and non-protein nitrogenous compounds (Connell, 1975). A level of 35 to 40 mg TVB-N/100 g of fish muscle is usually regarded as spoiled (Lakshmanan, 2000). At the end of the storage period (day 16), TVB-N values were found to be lower in treated samples than in control (6.6 mg/100 g). The combination treatments (code 7 and 8) were more effective and had the lowest TVB-N values (17.5 to 18.2 mg/100 g). Sodium acetate treated *Etroplus suratensis* fillets had significantly lower TVB-N values during chill storage (Manju et al., 2007). In salmon fillets treated with sodium acetate, lower TVB-N values were observed when compared to that of control at the end of the refrigeration storage (Sallam, 2007a). Lower TVB-N values was found in fish sausage treated with nisin as compared to that of the control (Raju et al., 2003). In all the samples analyzed in this study, the values were found to be within the limit throughout the storage period.

Lipid oxidation

PV is the primary lipid oxidation product and is the most common measure of lipid hydroperoxides (اlafsdottir et al., 1997). In this study, by the end of the storage period (day 16), significant reduction in the PV value was observed in treated samples as compared to the control. Sallam (2007b) also found lower peroxide value in salmon fillets dipped in organic acids. In fish sausage treated with nisin, the peroxide value was lower in comparison with that of control (Raju et al., 2003). TBA assay is usually used as indicator for the assessment of the secondary lipid oxidation. As shown in Table 1, by the end of the storage period, TBA values were significantly lower in the fillets treated with nisin and sodium acetate and the higher results were obtained when 3% of sodium acetate was used. This reduction is in agreement with the findings of Rajesh et al. (2002) and Sallam (2007b) who observed a reduction in TBA values of sodium acetate treated fish slices when compared with the control samples during refrigeration storage. The amounts of PV and TBA in grass carp fillets in this study are lower than

Table 1. Values of TVB-N (g/100 g muscle), peroxide (meq/1000 g) and TBA (mg malonaldehyde/kg muscle) of grass carp fillets treated with sodium acetate and nisin at the end of the storage period (day 16).

Treatment code**	TVB-N	Peroxide	TBA
1	26.6±0.00^{a}	1. 9±0.21^{a}	0.46±0.05^{a}
2	27±1.40^{a}	1.7±0.04^{b}	0.38±0.05^{b}
3	19. 6±1.97^{cd}	1.40±0.00^{c}	0.30±0.00^{cd}
4	23.1±0.98^{b}	1.26±0.05^{cde}	0.26±0.01^{d}
5	23.8±1.97^{b}	1.62±0.07^{b}	0.33±0.03^{bc}
6	22.4±1.97^{bc}	1.30±0.00^{d}	0.26±0.00^{cd}
7	18.2±0.00^{d}	1.08±0.04^{e}	0.19±0.02^{e}
8	17.5±0.98^{d}	1.41±0.08^{c}	0.29±0.02^{cd}
9	21.7±0.98^{bc}	1.12±0.04^{de}	0.19±0.01^{e}

*Mean values with the same letter for each column are not significantly different ($P > 0.05$); **Code 1 (0% sodium acetate + 0% nisin), code 2 (0% sodium acetate + 0.1% nisin), code 3 (0% sodium acetate + 0.2% nisin), code 4 (1% sodium acetate + 0% nisin), code 5 (1% sodium acetate + 0.1% nisin), code 6 (1% sodium acetate + 0.2% nisin), code 7 (3% sodium acetate + 0% nisin), code 8 (3% sodium acetate + 0.1% nisin) and code 9 (3% sodium acetate + 0.2% nisin).

acceptability level of 8 meq O_2/kg of oil for PV and 7 to 8 mg malonaldehyde/kg of oil for TBA (Huss, 1988).

In conclusion, nisin and sodium acetate were effective in reducing the microbial counts, retarding the oxidation process in grass carp fillets under refrigeration storage. The combination of nisin and sodium acetate can be used as effective preservatives to maintain the freshness under refrigeration storage.

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