The use of rosemary extract in combination with nisin to extend the shelf life of pompano (Trachinotus ovatus) fillet during chilled storage

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A R T I C L E   I N F O

Article history:
Received 1 June 2013
Received in revised form 5 September 2013
Accepted 6 September 2013

Keywords:
Pompano fillet
Rosemary extract
Nisin
Shell life
Chilled storage

A B S T R A C T

The effects of rosemary extract (RE) combined with nisin (N) on the quality of pompano (Trachinotus ovatus) were assessed throughout 15 days of storage at 4 ± 1 °C. Physicochemical (peroxide value, thiobarbituric acid, total volatile basic nitrogen, trimethylamine, pH, K value, texture, and color), sensory, and bacteriological characteristics of fish fillet were all periodically analyzed. RE + N treatment effectively improved physicochemical quality parameters and the sensory, and reduced microbial growth as compared with either treatment of RE or N alone or the control, which resulted in a significant extension in the shelf life of pompano fillet. Therefore, rosemary extract combined with nisin treatment may be a promising method of maintaining the storage quality and extending shelf life of pompano fillet during chilled storage.

1. Introduction

Pompano (Trachinotus ovatus), an important marine-cultured fish species in China, is enthusiastically welcomed by Southeast Asia and North America markets due to the palatability and nutritional quality of its flesh (Ji, 2011, pp. 1–10). Because of active and aggressive characters, pompano is highly susceptible to die during the transport, so it is commonly stored and sold as death condition. But the high levels of nutrient content and moisture in pompano render it as an easy perishable product. Chilled storage is an ordinary preservation method that is used to control the quality of fish during storage; however, it could not completely inhibit biochemical reaction and bacteriological activity, which result in affecting odor, flavor, color, texture, and nutritional values of fish. Previous studies about the pompano focused on the food and feeding activity (Batistic et al., 2005), growth rhythm (Tutman, Glavić, Kožul, Skaramuca, & Glamuzina, 2004), and early development (He, Ou, & Li, 2009). In contrast, little work has been conducted on the preservation and the postmortem quality changes of pompano during the chilled storage.

Synthetic preservatives (e.g., antioxidants, chelating agents, antimicrobial compounds etc.) have been used as food additives to extend shelf life of foods, but they are strictly regulated due to toxicological concerns and some health problems (Wilson & Bahna, 2005). So, it is increasingly attractive to find out effective and nontoxic measures to delay spoilage and to extend the shelf life of fish.

Rosemary (Rosmarinus officinalis) is a plant species of the Labiatae family, and its major and most active extract components (e.g., carnosol, carnosic acid, carnosol, rosmarinic acid etc.) have been proved to be against cancer and inflammation diseases in experimental animals and humans (Johnson, 2011; Ngo, Williams, & Head, 2011). Besides, Zhang et al. (2010) found that carnosic acid exhibited stronger antioxidant activity than butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), while less than that of tert-butyl hydroquinone (TBHQ) in sunflower oil; moreover, Jiang et al. (2011) identified that rosemary extract had strong antibacterial activity against Gram-positive and -negative bacteria. Thus, rosemary extract has been commonly used to preserve manufactured meat and fish in food industry owing to these meritorious characters (GBT 2760-2011, 2011; Li, Li, et al., 2012).

Nisin, the most widely used bacteriocin, is the only natural antimicrobial peptide as a food preservative approved by the FDA/WHO. It is highly active against many Gram-positive bacteria by...
interacting with phospholipids in the cytoplasmic membrane, disrupting membrane function, and inhibiting the swelling process of germination. Thus, nisin has been widely used to inhibit microbial growth in sausages, beef, poultry etc. (Marcos, Aymeric, Garriga, & Arnau, 2013; Tiwari et al., 2009).

Previous study demonstrated that the food additives used alone could not provide a sufficient protection against spoilage, which showed an improved but limited shelf life in the presence of either rosemary extract or nisin (Li, Li, et al., 2012; Tiwari et al., 2009). To our knowledge, there are no published data on the use of natural antioxidant (rosemary extract) combined with natural antimicrobial (nisin) for enhancing the shelf life of pompano. Therefore, the objective of this study was to investigate the combination effects of rosemary extract and nisin treatments on physicochemical, sensory, and bacteriological characteristics of pompano, and to extend the shelf life of pompano fillet during chilled storage.

2. Materials and methods

2.1. Preparation of materials

Live cultured pompano (T. ovatus), from 400 g to 500 g (wet weight), were purchased from finjiang Aquatic Market (Hangzhou City, Zhejiang province, China). They were then transported to the lab in Zhejiang Gongshang University within one hour and kept alive before being processed. The fish were killed by immersion in an ice-water mixture (1:1, w/v) for 20–30 min, and then were decapitated and filleted by hand (5 × 6 cm). Two fillets were obtained from each fish and kept at 0–2 °C until use. Rosemary extract was used as our previously described (Li, Li, et al., 2012). Nisin was purchased from Zhejiang Silver Elephant Bioengineering Co., Ltd. (Zhejiang province, China).

2.2. Treatment of fish samples

Fillet samples were randomly divided into four treatment groups: (1) rosemary extract (RE), (2) nisin (N), (3) rosemary extract combined with nisin coating (RE + N), and (4) the control. For RE or N treatment, fish fillets were given a dip treatment in 0.2% RE solution (w/v) or 5% N solution (w/v) at 4 ± 3 °C for 30 min (Li, Li, et al., 2012; Zhang, Wang, Wang, & Li, 2006). As for the RE + N treatment, RE treatment samples were drained at 4 ± 3 °C less about 1 h, and then treated with 5% N solution (w/v) at 4 ± 3 °C for 30 min. Fish fillets of the control were immersed in distilled water at 4 ± 3 °C for 30 min. After treatment, all of them were drained at 4 ± 3 °C less about 1 h, and then individually packed in air-proofed polyethylene packs, which were stored at 4 ± 1 °C for subsequent quality assessment. Physicochemical, sensory, and bacteriological analyses were performed at 3-day intervals to measure the quality of fish fillets. Each of the analyses was repeated three times with three fillet samples.

2.3. Proximate composition analyses

Proximate compositions (moisture, crude protein, lipid, and ash contents) analysis was performed on 6 fish with the day 0 of storage according to AOAC procedures (AOAC, 2005).

2.4. Peroxide value, thiobarbituric acid, total volatile basic nitrogen, and trimethylamine, pH, K value analyses

The values of peroxide value (PV, peroxide meq/kg lipid), thiobarbituric acid (TBA, mg malonaldehyde equivalents/kg tissue), total volatile basic nitrogen (TVB-N, mg of TVB-N/100 g tissue), trimethylamine (TMA-N, mg TMA-N/100 g tissue), and pH were determined as our previously described methods (Feng, Jiang, Wang, & Li, 2012). ATP and its breakdown products (ADP, AMP, IMP, HxR, and Hx) were measured by a reverse phase high-performance liquid chromatography method (Ryder, 1985). Compounds were identified using standard samples and the retention time. The K value was defined as the percent of the sum of HxR and Hx divided by the sum of ATP and its degradation products as follows:

\[
K\% = \frac{\text{HxR} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx}} \times 100
\]

2.5. Texture and color analyses

Texture profile analysis (TPA) was carried out to analyze the texture of fillets using a TA-XT2i Texture Analyzer (Stable Micro System) equipped with a 5 mm cylindrical probe (P/5). Two consecutive cycles at 25% compressions were applied to construct texture profile analysis parameters. The trigger force was 0.05 N, and the testing speed was 3 mm/s. The parameters (hardness, gumminess, chewiness, adhesiveness, springiness, cohesiveness, and resilience) were calculated by the Expression PC V.2.1 software (Feng et al., 2012).

The surface fresh color of raw pompano fillets was determined using a Minolta Chroma Meter CR400 (Minolta, Osaka, Japan) and reported as L*, a*, and b* as CIELab coordinates. Parameters of L*, a*, and b* indicate the lightness (the scale range of 0–100 points from black to white), red (+) or green (−), and yellow (+) or blue (−), respectively. The whiteness was calculated as follow (Sathivel, 2005):

\[
\text{Whiteness} = 100 - \left[ \left( 100 - L^* \right)^2 + a^{*2} + b^{*2} \right]^{1/2}
\]

2.6. Sensory evaluation

As our previously described method (Feng et al., 2012), the sensory of raw and cooked fish samples was evaluated by 10 experienced panelists (five males and five females and ranged in age from 28 to 48 years), and voting number was set at k, k ∈ (1, 10). Fish quality was divided into m levels, and the score of a specific level was set at hj, j ∈ (1, m). Fish attributes were divided into n elements, and a specific element was set at ui, i ∈ (1, n). The contributory weight was determined by pairwise comparison of each attribute based on the significance of attributes, and a specific contribution weight of attributes was set at ki (∑ki = 1). If there was a specific relationship between two objects of hj and ui, the relation set (matrix) of f was calculated as follows:

\[
F = \begin{bmatrix}
    f_{11}/k & f_{12}/k & \ldots & f_{1m}/k \\
    f_{21}/k & f_{22}/k & \ldots & f_{2m}/k \\
    \vdots & \vdots & \ddots & \vdots \\
    f_{n1}/k & f_{n2}/k & \ldots & f_{nm}/k
\end{bmatrix}
\]

Thus, the overall acceptability of fish was calculated by the weight grade method as follows:

\[
Z = \sum_{i=1}^{n} x_i \cdot \sum_{m=1}^{l} f_{ij} \cdot k_j
\]

2.7. Bacteriological analyses

A sample (25 g) was taken aseptically in a vertical laminar-flow cabinet, and then was transferred to a stomacher bag; 225 mL of NaCl solution (0.85%) was added and the mixture was homogenized...
for 1 min using a Stomacher 400 (Seward). The decimal dilutions were made, and 1 mL of each dilution was pipetted into an empty sterile petri dish. Subsequently, 15–20 mL of melted plate count agar (PCA, Qingdao Hope Bio-Technology Co., Ltd., cool down in a water bath at 45 °C) was poured into the petri dish, then mixed thoroughly by gentle titling and swirling the dish. After 20 min, they were inverted and incubated at 30 °C for 72 ± 3 h. Total viable counts (TVC) were determined by counting the number of colony-forming unit followed with the Chinese National Standard (GBT 4789.2-2010, 2010).

2.8. Statistical analyses

All analyses were carried out in triplicate. Mean values with standard deviations (SD) were reported for each case. Data were subjected to one-way analysis of variance (ANOVA), and mean separations were performed by Tukey’s multiple range test (SPSS 13.0). Differences were considered significant at the $P < 0.05$ level.

3. Results and discussion

3.1. Proximate composition

Proximate compositions of moisture, crude protein, lipid, and ash in the cultured pompano (T. ovatus) were 68.42 ± 1.53%, 17.27 ± 0.33%, 10.31 ± 0.15%, and 1.83 ± 0.11%, respectively. The proximate compositions of fish are closely related with fish size, sexual variation, nutrition, living circumstance, catching season, as well as other environmental conditions (Alasalvar, Taylor, Zubcovb, Shahidic, & Alexisd, 2002). Lech and Reigh (2012) found that the lipid content of the cultured Florida pompano (Trachinotus carolinus) ranged from 5.2% to 9.8% by changing fed compounded diets. As a warm-water fatty fish, high-lipid content in the cultured pompano is more likely to induce fat oxidation and microbial growth during storage. Thus, it is worthwhile to find out effective measures to retard oxidative degradation of lipid and inhibit activity of microorganisms, and thereby to improve the quality and nutritional values of fish and extend its shelf life during the storage.

3.2. Peroxide value and thiobarbituric acid analyses

Both peroxide value (PV) and thiobarbituric acid (TBA) have proved to be valuable indicators to assess the degree of lipid oxidation during the food storage (Barbosa-Pereira et al., 2013; Ojagh, Rezaei, Razavi, & Hosseini, 2010). In this study, both PV and TBA values in all treatment groups increased gradually during the storage (Fig. 1A and B). All fish started with very low PV and TBA values of 0.42–0.46 meq/kg and 0.16–0.19 mg MDA/kg samples, respectively. On the 9th day, fish samples treated with N suffered a rapid increase of PV value, reaching numbers close to 3.2 meq/kg sample or above, which was significant higher than both RE and RE + N treatment groups ($P < 0.05$), and TBA value in N treatment group was also higher than both RE and RE + N treatment groups. During the entire storage period, both PV and TBA values of fish treated with N were close to the control, suggesting nisin has almost no effect on reducing the lipid oxidation of fish. Rosemary extract, an excellent antioxidant, prevents lipid oxidation by scavenging free radicals to terminate the free radical chains, and has been widely used in the preservation of food items, such as vegetable oils, animal fats, shortenings (Choe & Min, 2009; GBT 2760-2011, 2011). In this study, lipid oxidation of pompano fillets was strongly inhibited by RE; both PV and TBA values of fish containing RE were significantly lower than the control and nisin treatment groups during the storage ($P < 0.05$). Although nisin has little effect to inhibit lipid oxidation, both PV and TBA values of RE + N treatment were statistically lower than RE treatment at the end of storage time (Day 15) ($P < 0.05$), suggesting nisin has a good synergistic effect to reduce lipid oxidation when combined with rosemary extract, as well as ascorbic acid, sodium lactate, ascorbyl palmitate, and citric acid (Hrás, Hadolin, Knez, & Bauman, 2000).

3.3. Total volatile basic nitrogen and trimethylamine analyses

Protein and non-protein nitrogenous compounds of fish are degraded by several enzymatic processes and microbial activity, which result in the productions of total volatile basic nitrogen (TVB-N) and trimethylamine (TMA). The content of these two productions are the most extensive indicators to assess the quality of fish products (Delbarre-Ladrat, Cheret, Taylor, & Verrez-Bagnis, 2006). Changes in the TVB-N content of fillet over storage time are shown in Fig. 2A. At Day 9, the TVB-N level in the control (34.21 ± 3.32 mg of TVB-N/100 g) exceeded the acceptable level of 30 mg of TVN-N/100 g of flesh suggested for fish products (Ocañoa-Higuera et al., 2011), while TVB-N levels of both RE and N treatment groups were reached the acceptable level after 12 days. At the end of a 15-days storage period, TVB-N content of fish samples treated with RE (39.57 ± 1.85 mg of TVN-N/100 g) or N (43.76 ± 5.57 mg of TVN-N/100 g) increased by 5–6 folds as compared to 7–8 folds increase for the control (50.66 ± 4.71 mg of TVN-N/100 g), reflecting a 14–38% reduction in the formation of TVB-N in the treated samples (RE or nisin treatment groups). Rosemary extract combined with nisin treatment reduced TVB-N formation during the entire storage period, and TVB-N value in RE + N treatment group did not exceeded the acceptable level at Day 15, indicating
RE and nisin also resulted in a significant reduction in TMA production of fish during the storage (P < 0.05) (Fig. 2B). At the end of storage (Day 15), TMA content of fish samples coated with RE and/or nisin was 25–54% lower than that of the control. The level of 5–10 mg of TMA/100 g of flesh, an indication rejection limit for fish product (Ocaño-Higuera et al., 2011), was reached after 12 days in untreated samples (control), whereas in all treated samples (RE, N, and RE + N), the levels were below this rejection limit during the storage period. Moreover, TMA level of RE + N treatment was observed significant lower (P < 0.05) than that of RE or nisin treatment after 12 days storage. Several factors such as age, sex, culture method, and locality may influence the content of protein and non-protein nitrogenous compounds in fish muscle (Delbarre-Ladrat et al., 2006). Increase in TVB-N and TMA levels in fish may result from deamination of free amino acids, oxidation of amines, and degradation of nucleotides by autolytic enzymes and microbial activity (Ocaño-Higuera et al., 2011). Our results indicated that the combination of rosemary extract and nisin was more effective inhibiting enzyme and microbial activity than either treatment alone.

3.4. pH value analyses

The pH values of fillet ranged from 6.56 to 6.77 during the postmortem storage, and the lowest value was at the 9th day within each of treatment groups (Fig. 3). The decreasing pH might result from the glycolysis of muscle that the residual glycogen broken down to pyruvic acid and then to lactic acid (Delbarre-Ladrat et al., 2006). However, there was no significant difference (P ≥ 0.05) of pH values between treatment groups (RE, N, and RE + N) and the control during the postmortem storage. Similar results were also found in the black sea bream (Sparus macrocephalus) (Feng et al., 2012) and the yellow grouper (Epinephelus awoara) (Li et al., 2011). Gram and Huss (1996) suggested that little carbohydrate residue and small content of lactic acid produced in muscle tissue during the storage was the main reason.

3.5. K value analyses

K values are depicted in Fig. 4. Previous studies suggested that a fish product with K value less than 20% is very fresh, and less than 50% is moderately fresh, while greater than 70% is not fresh, and K value is one of the most important indicator to monitor freshness and shelf life of fish muscle (Delbarre-Ladrat et al., 2006; Ocaño-Higuera et al., 2011). The control suffered a rapid increase of K value, reaching number above 20% at the 6th day of storage, when treated groups (RE, N, and RE + N) were less than it. On Day 9, RE + N treatment group showed the lowest K value (19.6%), followed by RE and N treatment groups (25.1% and 26.8%, respectively); on Day 15, mean K values were 48.0%, 53.2%, 36.4%, and 62.9% for treatment of RE, N, RE + N, and control treatment groups, respectively. Thus, RE + N treatment effectively reduced the
degradation of ATP and extended the shelf life of pompano fillet, suggesting a synergistic effect of RE combined with N treatment to inhibit the microbial activity that degraded ATP and its breakdown products.

3.6. Texture analyses

Food texture covers some related physical properties, including resistance to hardness, gumminess, chewiness, adhesiveness, springiness, cohesiveness, resilience; fillet texture is demonstrated through complexity of events in fish muscle, where will be influenced by seasonality and carbohydrate dynamics (Mørkøre et al., 2010). Texture analyses results during storage of pompano fillets are shown in Table 1. During the storage period, the average values for hardness, gumminess, chewiness, adhesiveness, springiness, cohesiveness, and resilience display significant variation within each treatment groups (P < 0.05), but not cohesiveness and resilience. Besides, in each treatment group, the average values of hardness, gumminess, and chewiness decreased sharply in the first 3 days, when the fish muscle lost its firmness after the rigor mortis due to enzymatic degradation of muscle proteins (Ochoa-Higuera et al., 2011); however, there was no significant difference between treatment groups (RE, N, and RE + N) and the control in these three properties at the 3rd day of storage (P > 0.05). On the 15th day, the average values of hardness, gumminess, and chewiness in RE + N treatment groups were higher than those treated with RE, N, and control groups, while no significant differences were observed between treatment groups and control on the values of adhesiveness, springiness, cohesiveness, and resilience (P ≥ 0.05). Previous studies indicated that the fish death triggers autolysis and subsequently the muscle becomes softer and less elastic, and the process is accelerated by microbial activity (Delbarre-Ladrat et al., 2006). We proposed that texture properties of hardness, gumminess, and chewiness in pompano can be improved by RE + N treatment during chilled storage.

3.7. Color analyses

The color of fresh meat product is also reflected by another aspect of major importance from a consumer viewpoint. Hence, color stability during storage and retail display is important to meat processing and the retailers. Surface color parameters for the muscle throughout storage (from days 0–15) are shown in Table 2, where it can be noted that all of the samples suffered a progressive lightness (L*) and whiteness decrease (P < 0.05), while a gradual blueness increase (P < 0.05). On Day 15, fish samples treated with RE + N had higher values of lightness (L*) and whiteness and lower value of blueness (b*) than RE, nisin, and control (P < 0.05). On the contrary, no significant effects of all treatments were observed on the red color (a* value). Mørkøre et al. (2010) suggested the color of fish muscle was influenced by both pigment concentrations and muscle structure characteristics, besides Li, Hu, et al. (2012) and Li, Li, et al. (2012) reported that color loss in fish muscle is also attributed to the oxidation of protein. Our results showed that RE

### Table 1

<table>
<thead>
<tr>
<th>Analyse parameters</th>
<th>Days of storage</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
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<tbody>
<tr>
<td><strong>Hardness (N)</strong></td>
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<tr>
<td>RE</td>
<td>932.05 ± 11.53</td>
<td>635.70 ± 19.41</td>
<td>639.27 ± 17.36</td>
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<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>N</td>
<td>923.57 ± 12.69</td>
<td>636.03 ± 6.07</td>
<td>619.27 ± 3.67</td>
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<tr>
<td>RE + N</td>
<td>926.10 ± 10.10</td>
<td>663.87 ± 18.47</td>
<td>625.93 ± 9.40</td>
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<td>...</td>
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<tr>
<td>Control</td>
<td>935.13 ± 23.85</td>
<td>650.77 ± 18.61</td>
<td>628.97 ± 11.10</td>
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<td><strong>Gumminess (N)</strong></td>
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<tr>
<td>RE</td>
<td>388.47 ± 16.67</td>
<td>276.45 ± 25.59</td>
<td>211.58 ± 16.40</td>
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<tr>
<td>N</td>
<td>373.76 ± 20.06</td>
<td>284.05 ± 12.34</td>
<td>239.85 ± 28.01</td>
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<tr>
<td>RE + N</td>
<td>378.11 ± 10.12</td>
<td>290.08 ± 15.38</td>
<td>264.28 ± 11.29</td>
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<tr>
<td>Control</td>
<td>387.09 ± 10.78</td>
<td>266.38 ± 7.53</td>
<td>177.52 ± 13.38</td>
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<td><strong>Chewiness (N mm)</strong></td>
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<tr>
<td>RE</td>
<td>289.27 ± 34.41</td>
<td>211.19 ± 21.64</td>
<td>184.16 ± 37.80</td>
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<tr>
<td>N</td>
<td>291.11 ± 25.99</td>
<td>220.56 ± 27.23</td>
<td>169.80 ± 24.29</td>
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<td>...</td>
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<tr>
<td>RE + N</td>
<td>281.77 ± 26.70</td>
<td>232.90 ± 25.18</td>
<td>215.47 ± 18.72</td>
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<tr>
<td>Control</td>
<td>282.81 ± 25.46</td>
<td>216.98 ± 24.93</td>
<td>145.00 ± 11.61</td>
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<td><strong>Adhesiveness (N%)</strong></td>
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<tr>
<td>RE</td>
<td>–53.73 ± 6.86</td>
<td>–71.90 ± 6.46</td>
<td>–42.80 ± 5.37</td>
<td>...</td>
<td>...</td>
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<tr>
<td>N</td>
<td>–47.87 ± 5.37</td>
<td>–45.10 ± 6.78</td>
<td>–34.60 ± 4.26</td>
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<tr>
<td>RE + N</td>
<td>–35.53 ± 2.07</td>
<td>–52.77 ± 6.21</td>
<td>–49.93 ± 3.61</td>
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<tr>
<td>Control</td>
<td>–47.17 ± 2.39</td>
<td>–48.03 ± 6.68</td>
<td>–35.53 ± 2.41</td>
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<td><strong>Springiness (mm)</strong></td>
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<tr>
<td>RE</td>
<td>0.97 ± 0.02</td>
<td>0.93 ± 0.01</td>
<td>0.92 ± 0.02</td>
<td>0.86 ± 0.02</td>
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<tr>
<td>N</td>
<td>0.98 ± 0.01</td>
<td>0.93 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.88 ± 0.01</td>
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<tr>
<td>RE + N</td>
<td>0.98 ± 0.02</td>
<td>0.94 ± 0.02</td>
<td>0.95 ± 0.01</td>
<td>0.92 ± 0.03</td>
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<tr>
<td>Control</td>
<td>0.97 ± 0.01</td>
<td>0.93 ± 0.03</td>
<td>0.93 ± 0.02</td>
<td>0.88 ± 0.04</td>
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<td><strong>Cohesiveness</strong></td>
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<tr>
<td>RE</td>
<td>0.37 ± 0.02</td>
<td>0.36 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>0.31 ± 0.01</td>
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<tr>
<td>N</td>
<td>0.38 ± 0.04</td>
<td>0.34 ± 0.03</td>
<td>0.36 ± 0.03</td>
<td>0.34 ± 0.02</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>RE + N</td>
<td>0.34 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.39 ± 0.02</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Control</td>
<td>0.38 ± 0.04</td>
<td>0.35 ± 0.02</td>
<td>0.34 ± 0.05</td>
<td>0.36 ± 0.03</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td><strong>Resilience (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RE</td>
<td>0.17 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.12 ± 0.03</td>
<td>0.15 ± 0.02</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>N</td>
<td>0.18 ± 0.03</td>
<td>0.13 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>RE + N</td>
<td>0.14 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.01</td>
<td>0.17 ± 0.02</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Control</td>
<td>0.19 ± 0.01</td>
<td>0.12 ± 0.04</td>
<td>0.13 ± 0.03</td>
<td>0.17 ± 0.04</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Mean values and standard deviations from three replicates are presented.

1 Means in row with different small letters are significantly differently (P < 0.05).

2 Means in column with different capital letters are significantly differently (P < 0.05).
combined with nisin had protecting effects on lightness ($L^*$), blueness ($b^*$), and whiteness of pompano fillets during chilled storage.

### 3.8. Sensory analyses

Sensory attributes of fish were divided into 6 elements, whose preference levels were scored from 1 to 5; the higher preference level, the higher element score (Table 3). The evaluation of sensory attributes of raw and cooked fillets is shown in Fig. 5. All samples started with score of 5. There were no significant differences among the different samples within 6 days of storage ($P > 0.05$). However, large significant differences were evident after 9 days of storage; samples without treatment had scores significantly lower at that time than any other treated samples ($P < 0.05$). Similar results were obtained in our previous studies, in which the unpleasant odors and loss of fresh appearance in untreated yellow grouper (E. awara) and black sea bream (Sparus macrocephalus) began to emerge from the 9th day of storage (Feng et al., 2012; Li, Hu, et al., 2012). At days 9–12, all treatment samples had values of about 3 or below, a limit of acceptance according to Martínez, Djeneke, Cilla, Beltrán, and Roncaldés (2005), with the exception of RE + N that had significantly higher preference score ($P < 0.05$); furthermore, fillets treated with RE + N given scores above 3 even at the end of storage (Day 15). Therefore, due to an inhibitory action on both pigment and lipid oxidation, rosemary extract in combination with nisin delay color and odor decay, and extend the shelf life of pompano fillets during chilled storage.

### 3.9. Bacteriological analyses

Bacteria in a living fish are generally present in the skin and gut, but are prevent from entering the muscle. Once a fish dies and its autolysis begins, then bacteria can enter and decompose the muscle (Aberoumand, 2010). Changes of total viable counts (TVC) on fillets throughout storage are shown in Fig. 6. All samples started with low microbial counts of near 1.5 log10 cfu/g, which indicated that the pompano fillet studied was of good quality. There were no significant differences among the different samples within 6 days of storage ($P > 0.05$). On the 9th day of storage, fillets without treatment had rapidly growing counts, and reached 6.54 ± 0.31 log10 cfu/g, which was significant higher than treated groups (RE, N, and RE + N) ($P < 0.05$). On Day 12, both RE and nisin treatment groups had significantly higher TVC values (6.60 ± 0.27 log10 cfu/g and 6.24 ± 0.27 log10 cfu/g, respectively) than the RE + N group (5.00 ± 0.28 log10 cfu/g) ($P < 0.05$), while the control was 7.46 ± 0.26 log10 cfu/g, more than the maximal recommended limit (7 log10 cfu/g) in raw fish (Ojagh et al., 2010). Samples treated with RE + N had lower counts throughout storage, and they did not reach 7 log10 cfu/g even at the end of storage (Day 15) and had significantly lower TVC values than the RE, N, and control groups ($P < 0.05$). These results clearly demonstrated that

### Table 3

<table>
<thead>
<tr>
<th>State of fish</th>
<th>Attributes</th>
<th>Attribute degree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Raw fish</td>
<td>Appearance of the skin</td>
<td>Very bright</td>
</tr>
<tr>
<td></td>
<td>Clarity of eyes</td>
<td>Very clear</td>
</tr>
<tr>
<td></td>
<td>Color of Gills</td>
<td>Dark red</td>
</tr>
<tr>
<td></td>
<td>Odor</td>
<td>Neutral</td>
</tr>
<tr>
<td>Cooked fish</td>
<td>Taste</td>
<td>Delicious</td>
</tr>
<tr>
<td></td>
<td>Odor</td>
<td>Neutral</td>
</tr>
</tbody>
</table>
the rosemary extract had strong inhibitory effect on microbial growth in storage fish fillet as nisin, and extended the shelf life of the fish fillet, in accordance with previous reports of Li, Hu et al. (2012) and Li, Li et al. (2012), who reported that microbial deterioration was delayed about 5 days in crucian carp containing 2% rosemary extract, and Özyurt et al. (2012), who showed that rosemary extract was effective in maintaining low bacteriological numbers to extended shelf life of sardine (Sardinella aurita) during chilled storage. Moreover, RE + N treatment group had the lowest counts throughout the storage, and increased the shelf life by a minimum of 6 days as compared with the control, suggesting RE + N effectively inhibiting microbial growth due to the synergistic effect of RE combined with nisin treatment.

4. Conclusions

This study showed that rosemary extract in combination with nisin treatment could effectively inhibit lipid oxidation, protein decomposition, nucleotide breakdown, and microbial growth, and could improve texture, color, and sensory attributes within acceptable limits during the 4 °C storage period, and could extend the shelf life of fish fillets for 6 days compared with the control. Therefore, natural antioxidant (rosemary extract) combined with natural antimicrobial (nisin) may be a promising method of maintaining the storage quality of pompano and extending its shelf life.

Acknowledgments

This work was financially supported by the National Key Technologies R & D Program of China during the 12th Five-Year Plan Period (No. 2012BAD29B06), the National Natural Science Foundation of China (No. 31301566), the Program for the Science & Technology of Zhejiang Province (No. 2012C22049), the Zhejiang Provincial Natural Science Foundation of China (No. LQ12C20005), and the State Key Laboratory of Freshwater Ecology and Biotechnology (No. 2012FB24).

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