Effect of chitosan and cinnamon essential oil on a food-borne pathogen and antioxidant activity in frozen rainbow trout (Oncorhynchus mykiss)

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ABSTRACT

Food industries use synthetic preservatives to improve the quality and enhance the shelf life of food products during storage. However, the most common industrial strategies for preservation may not always bring the desired protection for curbing corruption. Furthermore, consumer demands for safer foods encourage researchers to find natural and effective preservatives. In this study, the antioxidant and antibacterial activity of chitosan in combination with cinnamon were investigated in frozen condition. Rainbow trout were combined with 2% chitosan in combination with different concentration of cinnamon essential oils (0.125, 0.25 and 0.5 ml). The samples were kept at -18 ºC. Oxidative stability of samples was assessed by measuring lipid peroxidation level using thiobarbituric acid reactive substances (TBARS) method. The bacterial test was assayed by counting colony forming unit. The evaluation of statistical differences between groups was analyzed using the student’s T-test by SPSS software according to the statistical facts, the difference of more than 95% (P ≤0.05) was considered significant. Combination of chitosan with 0.25 and 0.5 ml cinnamon significantly decreased lipid peroxidation level compared to control group and chitosan combination with 0.5 ml cinnamon showed the synergistic effect. The antimicrobial activity of chitosan in combination with 0.5 ml cinnamon was higher than other concentrations and control groups. Chitosan in combination with cinnamon oil could considerably increase the oxidative stability and decrease the total count of bacteria in frozen fish. These results may suggest that these edible coatings can be used instead of artificial preservatives and non-edible coatings.


1. Introduction

Fish and other kinds of marine-derived foods have been recognized as valuable sources of high nutritional components. These types of food are key ingredient in many countries’ diets. They are considered as good sources of long-chain polyunsaturated fatty acids (PUFAs) belonging to the Omega-3 family, including EPA (20:5n3) and DHA (22:6n3). DHA has a positive effect on preventing and curing several diseases such as coronary heart disease, atherosclerosis and some cancers (1). Freezing is a general preservation method to control or decrease biochemical changes that occur during storage in fish determined by chemical indicators of spoilage such as Thioobarbituric acid (TBA) values, Total Volatile Base Nitrogen (TVB-N) values and the pH values (2). However, frozen storage does not completely prevent chemical reactions (e.g., lipid oxidation) which lead to quality deterioration of fish tissue. Food industries commonly used antioxidants such as butylated hydroxyanisole (BHA) and butylated...
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2. Materials and methods

2.1. Preparation and treatments of fish samples

Rainbow trout fish were prepared from a fish farm (Alborz Caspian Fish Reproduction & Breeding) with an average weight of 700 gr and the average length of 30 cm. The fillets were prepared with 25 g weight. The fish fillets were divided into three treated groups and one control group. Cinnamon essential oils were obtained from Givaudan Company (Switzerland). Cinnamon essential oil solutions were prepared at concentrations of 0.5, 0.25 and 0.125 ml in the solution of 2% of chitosan (2 g chitosan was dissolved in 100 ml of glacial acetic acid). Then fish fillet was covered with the solution. The samples were randomly assigned into four treatment lots, one control group without chitosan and cinnamon essential oil and three groups with 2% chitosan and different concentrations of cinnamon (0.125, 0.25 and 0.5 ml). Then the fish fillets were dried and stored at -18 °C for 40 days.

2.2. Bacterial test

At first 25 g of meat was homogenized with 225 ml of distilled water. Then, further dilutions up to 10-7 were made. In order to culture and measure total count of bacteria, 1 cc of 10-1 to 10-7 dilutions was poured into sterile nutrient agar medium plates in sterile conditions and was incubated at 37 °C for 24-48 h. Afterward, the entire colony that grew on the surface was counted regardless of the detection of species and was reported as cfu/g.

2.3. Chitosan and cinnamon effect on the oxidative stability

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were measured. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex which has peak absorbance at 532 nm. The chitosan and cinnamon essential oil, Copper sulfate that was obtained from Merck Company (CuSo4), rainbow trouts (without antioxidant) were mixed and shaken for 3 h in room temperature. The capacity of the mixture was centrifuged. Then, 2ml thiobarbituric acid was added to the 2ml supernatant and heated in water bath for 10 min at 90°C. The absorbance of the supernatant was measured at 532 nm. The values were expressed in nM.
of malondialdehyde, using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ (12).

2.4. Statistical Analysis

The evaluation of statistical differences between groups was analyzed using the student’s T-test by SPSS software. According to the statistical facts, the difference of more than 95% ($P \leq 0.05$) was considered significant. The data values are presented as mean±SD and significance values related to pair groups.

3. Results
3.1. Antimicrobial Properties

Total count was measured after 20 and 40 days from freezing. Our results indicated that chitosan in combination with different concentration of cinnamon significantly decreased total count of bacteria in comparison with the control group after 20 and 40 days from freezing ($p < 0.001$, table 1).

Table 1. The antibacterial effect of cinnamon and chitosan on storage fish

<table>
<thead>
<tr>
<th>day</th>
<th>groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
</tr>
<tr>
<td></td>
<td>0.125 ml cinnamon essential oils and 2% chitosan</td>
</tr>
<tr>
<td></td>
<td>0.25 ml cinnamon essential oils and 2% chitosan</td>
</tr>
<tr>
<td></td>
<td>0.5 ml cinnamon essential oils and 2% chitosan</td>
</tr>
<tr>
<td>TCC after 20 days (cfu/g)</td>
<td>6.5×10^5±30.0a</td>
</tr>
<tr>
<td></td>
<td>1.2×10^5±64.29b</td>
</tr>
<tr>
<td></td>
<td>1.4×10^5±63.51b</td>
</tr>
<tr>
<td></td>
<td>1.4×10^5±1c</td>
</tr>
<tr>
<td>TCC after 40 days (cfu/g)</td>
<td>1.2×10^5±5.69a</td>
</tr>
<tr>
<td></td>
<td>4.7×10^5±1b</td>
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<tr>
<td></td>
<td>4.1×10^5±7.02b</td>
</tr>
<tr>
<td></td>
<td>3.53×10^5±0.25c</td>
</tr>
</tbody>
</table>

TCC= Total Count of Bacteria

3.2 Antioxidant activity

The results of antioxidant activity are given in figure 1. This figure shows the results of the efficacy of chitosan in combination with cinnamon essential oils on the quality and improvement shelf life of frozen rainbow trout in the period of 40 days. In the first stage (after 20 days), there was significant difference between the oxidative stability of control sample and chitosan in combination with cinnamon essential oils at concentrations of 0.25 and 0.5 ml ($p < 0.05$) but there was no significant difference between the oxidative stability of control sample and chitosan in combination with cinnamon essential oils at 0.125 ml concentration.

Figure 1. The oxidative stability of cinnamon essential oils and chitosan in fish meat

Key: a=control group, b=0.125 ml cinnamon+chitosan, c=0.25 ml cinnamon+chitosan, d=0.5 ml cinnamon+chitosan

In this figure, the oxidative stability level (nmol/gr) of chitosan and cinnamon essential oils in fish meat are shown. The groups observed in this figure are a,a'=control sample b,b'=0.125 ml cinnamon essential oils and 2% chitosan c,c'=0.25 ml cinnamon essential oils and 2% chitosan and d,d'=0.5 ml cinnamon essential oils and 2% chitosan on days 20 and 40 respectively. Also after 40 days, a significant difference was shown between the oxidative stability of control sample and chitosan in combination with cinnamon essential oils at 0.25 and 0.5 ml concentrations ($p < 0.01$ and $p < 0.001$, respectively) but there wasn’t a significant difference between the oxidative stability of control sample and chitosan in combination with cinnamon essential oils at 0.125 ml concentration. Furthermore, there was a significant difference between the control sample in the first stage and the control sample after 40 days ($p < 0.001$) (table 2).

Table 2. The oxidative stability of cinnamon essential oils and chitosan in fish meat

<table>
<thead>
<tr>
<th>day</th>
<th>level of lipid peroxidation (nmol/gr)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>0.125 ml cinnamon essential oils and 2% chitosan</td>
</tr>
<tr>
<td></td>
<td>0.25 ml cinnamon essential oils and 2% chitosan</td>
</tr>
<tr>
<td></td>
<td>0.5 ml cinnamon essential oils and 2% chitosan</td>
</tr>
<tr>
<td>after 20 days</td>
<td>2.9±0.84</td>
</tr>
<tr>
<td>after 40 days</td>
<td>5.5±0.5</td>
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4. Discussion
The present study investigated the effect of chitosan in combination with cinnamon essential oils on quality and improving shelf life of frozen rainbow trout in the period of 40 days. The obtained results revealed that in both control samples and chitosan in combination with cinnamon essential oils, lipid peroxidation increased from the first to the second stage, so during storage lipid oxidation definitely had an ascending trend. The level of peroxidation in treated samples was less than the control sample. Therefore, treated samples have more oxidative resistance and quality than control sample. PUFAs are vulnerable to free radical damages and oxidation. In the current study, considerable change in the level of lipid oxidation was evaluated between 20 days and 40 days of storage in control groups. Presence of antioxidants plays an important role in preventing oxidative changes of these valuable components. Regarding previous reports investigating the oxidative stability of frozen shrimp in the presence of carotenoids and chitosan, both chitosan and carotenoids coating are compared to other samples and control group. It indicates that chitosan in combination with cinnamon essential oils provide good oxidative stability in frozen fish.

Recent studies on antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils have shown that chitosan and cinnamon essential oils have synergistic effects in decreasing the total viable count (4). Therefore, coating of chitosan in combination with cinnamon oil provides an active type of coating that can be utilized as a safe preservative for fish under refrigerated storage (5). Previous studies have also identified trans-cinnamaldehyde as the major antibacterial constituent of cinnamon oil (14).

The antimicrobial properties of chitosan coating have been reported in the literature (2). Ojagh et al investigated the effects of a chitosan coating enriched with cinnamon oil on quality of rainbow trout (Oncorhynchus mykiss) during refrigerated storage, over a period of 16 days. They reported that chitosan in combination with cinnamon oil coating is able to retain longer and extend the shelf life of fish samples during the refrigerated storage (6). Our study showed that chitosan in combination with cinnamon could reduce total count of bacteria in freezing conditions after 40-days period and 0.5 ml cinnamon had the greatest influence. Effective for preserving frozen shrimp during refrigerated storage.

Gram-negative psychotropic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (13). The total count of bacteria increases during storage. It has been shown that chitosan alone and in combination with cinnamon essential oils has an antibacterial effect (3). The obtained results from our study revealed that chitosan in combination with cinnamon essential oils could decrease the level of microbial count in the samples stored at -18°C after 20 days. After 40 days, total count was measured again. Our findings show the microbial load had sharply dropped because of the coating of chitosan in combination with cinnamon as well as freezing conditions. After the 40 days, our data showed that 0.5 ml concentration of cinnamon essential oils and chitosan were more effective to reduce total count of bacteria.

5. Conclusion
Current results revealed that coating of chitosan in combination with cinnamon oil can decrease the level of lipid peroxidation as well as total count of bacteria in frozen fish leading to extend the shelf life of fish samples. These results may suggest that this edible coating can be used instead of artificial preservatives and non-edible coatings.

Conflict of interest
The authors declare that they have no conflict of interest.

Acknowledgement
None

References