Original Article

Journal of Food Safety and Hygiene

journal homepage: http://jfsh.tums.ac.ir

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

Tahereh Mohajerfar [a,*], Ahmad Erfanmanesh[a], Parisa Sadighara[b], Mostafa Mohajerfar[c], Afsaneh Mohajer[c,d]

[a] *Academic Center of Education, Culture and Research (ACECR), Tehran, Iran*
[b] *Department of Environmental Health Engineering, Division in Food Safety and Hygiene, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Department of Basic Sciences, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran*
[d] *Student's Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran*

ARTICLEINFO

Article history:
Received 25 Mar. 2017
Received in revised form
29 May. 2017
Accepted 07 Jun. 2017

Keywords:
Chitosan;
Cinnamon;
Lipid peroxidation;
Antibacterial;
Antioxidant

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% ($P \leq 0.05$) 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.

Citation: Mohajerfar T, Erfanmanesh A, Sadighara P, Mohajerfar M, Mohajer A. **Effect of chitosan and cinnamon essential oil on a food-borne pathogen and antioxidant activity in frozen rainbow trout (Oncorhynchus mykiss).** J Food Safe & Hyg 2017; 3(1-2): 16-20.

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 Thiobarbituric 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

* Corresponding author. Tel.: +982166930415
E-mail address: t.mhjr@yahoo.com

hydroxytoluene (BHT) to improve the quality and increase the shelf life of food products during storage. Numerous studies have currently focused on using natural ingredients to enhance food quality and shelf life to meet consumer demands for safer foods in order to avoid the use of synthetic preservatives (3). Chitosan, the second most abundant natural polymer after cellulose, is used in foodstuff, agriculture, cosmetic, pharmaceutical industries and wastewater treatment (4). Chitosan extracts from waste of shrimp, about 50 percent of total body weight of shrimp is waste (5). Antioxidant activity is one of the well-known functions of chitosan. Several mechanisms about the antioxidant action of chitosan have been proposed (3). One of the features of chitosan is antimicrobial activity. The degree of this activity depends on the methods involved in the production of chitosan (5). In order to control spoiling and pathogenic microorganisms in foods, antimicrobial substances can be added in edible films (6). Cinnamon belongs to the Lauraceae family and the genus of Cinnamomum which contains about 250 species. Cinnamon is also a traditional herbal medicine that is distributed in China, India and Australia (7). The bark yields an essential oil containing cinnamaldehyde and eugenol in which biological activities have been ascribed to cinnamaldehyde (8). It has been used in food, seasonings, cosmetics and medical industries because of its antimicrobial, antioxidant and anticarcinogenic activities (9). The present studies have shown that the methanolic extract of Cinnamon essential oil contains a number of antioxidant composites which can efficiently scavenge reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals as well as other free radicals under in vitro conditions (10). Furthermore, many spices and herbs possess antimicrobial and antioxidant activity, which minimizes questions regarding their safety in food products. Usually, compounds with phenolic groups are most effective. Among these, the essential oil of clove, thyme, cinnamon, rosemary, sage and vanillin have been found to be effective against microorganisms. These compounds have more inhibitory effects against gram-positive bacteria in comparison with gram-negatives (11).

Current work was therefore carried out to investigate the quality of frozen fish with an edible coating of chitosan in combination with cinnamon after 40 days' storage.

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 chitosan and cinnamon to inhibit MDA formation against CuSo4 was assayed. Briefly, the samples of rainbow trouts were mixed with 20% trichloroacetic acid solution (20gr of trichloroacetic was dissolved in 100 ml distilled water) and 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 ×105 M-1 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 ≤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

day	groups			
	control	0.125 ml cinnamon essential oils and 2% chitosan	0.25 ml cinnamon essential oils and 2%chitosan	0.5 ml cinnamon essential oils and 2% chitosan
TCC after 20 days (cfu/g)	$6.5\times10^5\pm300a$	$1.2\times10^5\pm64.29b$	$1.4\times10^5\pm63.51b$	$1.4\times10^3\pm1c$
TCC after 40 days (cfu/g)	$1.2\times10^3\pm5.69a$	$4.7\times10^3\pm1b$	$4.1\times10^3\pm7.02b$	$3.53\times10^2\pm0.25c$

TCC= Total Count of Bacteria

3.2 Antioxidant activity

The results of antioxidant activity are given in figure1. 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.

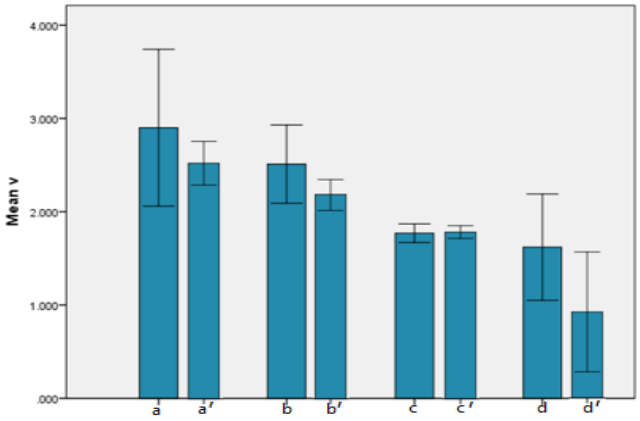

Figure 1. The oxidative stability of cinnamon essential oils and chitosan in fish meat
Key: a=control group, b=0.125 ml cinnamon+chitosan, c=0.25 ml cinnamon+chitosan, d=0.5 ml cinnamon+chitosan

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 group 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 ($p < 0.001$) (table2).

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

day	**level of lipid peroxidation (nmol/gr)**			
	Control	0.125 ml cinnamon essential oils and 2% chitosan	0.25 ml cinnamon essential oils and 2%chitosan	0.5 ml cinnamon essential oils and 2% chitosan
after 20 days	2.9±0.84	2.51±0.42	1.77±0.1	1.62±0.57
after 40 days	5.51±0.51	4.8±0.35	3.9±0.15	2.01±1.4

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

1. Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. J Food Microbiol 2005; 22: 273-92.
2. Ninan G, Lalitha KV, Zynudheen AA, et al. Effect of chilling on microbiological, biochemical and sensory attributes of whole aquacultured rainbow trout (Oncorhynchus mykiss Walbaum, 1792). J Aqua Res Dev 2011; 5 doi:10.4172/2155-9546.S5-001.
3. Sathivel S, Liu Q, Huang J, et al. The influence of chitosan glazing on the quality of skinless pink salmon (Oncorhynchus gorbuscha) fillets during frozen storage J Food Engin 2007; 83: 366-373.

4. Sadighara P, Erfanmanesh A, Haghi E, et al. The Antibacterial Activity of Chitosan between Different Extraction Method. J Chem Pharma Sci 2015; 9: 1325-8.
5. Hosseini MH, Razavi SH, Mousavi MA. Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. J Food Process Preserv 2009; 33: 727-43
6. Ojagh SM, Rezaei M, Razavi SH, et al. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout Food Chem 2010; 120: 193-198
7. Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Volatile constituents from Cinnamomum zeylanicum fruit stalks and their antioxidant activities. J Agri Food Chem 2003; 51: 4344-8
8. Bullerman LW, Liew FY, Seier SA. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J Food Sci 1977; 42: 1107–1109
9. Li YQ, Kong DX, Wu H. Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy. J Ind Crops Products 2013; 41: 269-78
10. Mathew S, Abraham TE. Studies on the antioxidant activities of cinnamon (Cinnamomum verum) bark extracts, through various in vitro models. J Food Chem 2006; 94: 520-8
11. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 2004; 94: 223-253
12. Sicinska P, Bukowska B, Michalowicz et al. Damage of cell membrane and anti-oxidative system in human erythrocytes incubated with microcystin-LR in vitro. J Toxicol 2006; 47: 387-397
13. López-cervantes J, Sánchez-machado DI, Bueno-solano C, et al. fatty acid composition and total lipid content in protein hydrolysates of shrimp heads. Int J Pharm Bio Sci 2013; 4: 464 – 470
14. Shan B, Chi Y, Brooks J, et al. Antibacterial properties and major bioactive components of cinnamon stick (Cinnamomum burmannii): activity against foodborne pathogenic bacteria. J Agri Food Chem 2007; 55: 5484-5490