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The Effects of Nettle (*Urtica diocia* L.) on Chemical Properties of Rainbow Trout (*Oncorynchus mykiss*) Fillets

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Abstract: The effects of nettle (*Urtica diocia* L.), containing natural antioxidants, on chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets were investigated. Thiobarbituric acid reactive substance (TBARS), total antioxidant activity, total volatile bases nitrogen (TVB-N) and pH values were measured from rainbow trout fillets treated with nettle at three concentrations (0.4, 0.8 and 1.6), without nettle as control and 0.8% propyl gallate (positive control) during 9 days of storage at 4±1°C. The storage time and antioxidant treatment caused significant ($p<0.05$) changes in TBARS, total antioxidant activity, TVB-N and pH values. TBARS values in fillets of control group exceeded the acceptable level (20 μmol kg^{-1}) on the 5th day. However, fillets treated with nettle at three concentrations and propyl gallate did not reach this degree during the experimental period. The fillets treated with 0.4% of nettle exhibited most effective and powerful antioxidant activity. The antioxidant activity of samples decreased with increasing nettle concentrations. However, the samples treated with 0.4 and 0.8% of nettle showed higher antioxidant activities than that 0.8% of propyl gallate. TVB-N values in fillets of control group reach above 25 mg/100 g on the 3rd day. However, nettle and propyl gallate groups were reached above this level on 5th day. Considering present data, it was concluded that using nettle containing natural antioxidants might cause a distinct beneficial reduction in lipid oxidation and extend storage time of aerobically packaged rainbow trout fillets.

Key words: Nettle, natural antioxidants, fish fillet, lipid oxidation

INTRODUCTION

Fresh fish are highly perishable due to their biological composition. Spoilage of fish muscle causes a short shelf life in fish and other seafood products (Gobantes *et al*., 1998; Gram and Dalgaard, 2002a). Chemical deterioration and microbial spoilage may cause losses up to 25% of gross primary agricultural and fishery products in every year (Gram and Dalgaard, 2002b).

Rancid off-flavors are still the main objections in the production and commercialization of fish and foodstuffs containing fish oils, because unsaturated fatty acids in these oils can easily be oxidized in high temperatures and during storage processing and distribution (Jacobsen *et al*., 1999). The rate and degree of the oxidation are dependent on fish species, presence or absence of activators and inhibitors and treatment or storage (Petillo *et al*., 1998; Undeland *et al*., 1998).

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Various means can be used to control lipid oxidation. Elimination of oxygen from packages by vacuum packaging controls lipid oxidation (Arashisar *et al*., 2004). Addition of antioxidant during processing or packaging is another approach used to delay the oxidation process (Boyd *et al*., 1993). The most commonly used synthetic antioxidants at present are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) (Gülçin *et al*., 2004a; Weilmeier and Regestein, 2004). These antioxidants, however, have limited applications because of their low water solubility and their ability to penetrate into intact muscles (Lee *et al*., 1998) and use of these types of antioxidants is controlled due to their carcinogenic potential (Grice *et al*., 1986).

The natural antioxidants extracted are apparently related to their phenolic content, from teas, spices and herbs, can be used as alternatives to the synthetic antioxidant because of their equivalent or greater effect on inhibition of lipid oxidation (Lai *et al*., 1991). The application of plant extracts to prevent fish oxidative rancidity has been studied in certain fish products like fillets (Weilmeier and Regestein, 2004; Aubourg *et al*., 2004).

Aqueous infusions of Mediterranean herbs including *Urtica dioica* exhibit antioxidant activity towards iron-promoted oxidation of phospholipids, linoleic acid and deoxyribose (Matsingou *et al*., 2001). Therefore, the objective of this study was to investigate the effects of nettle (*Urtica diocia* L.) on chemical changes [pH, total volatile bases nitrogen (TVB-N), lipid oxidation (TBARS) and total antioxidant activity] of rainbow trout (*Oncorynchus mykiss*) fillets during storage at 4±1°C.

MATERIALS AND METHODS

Plant Material and Extraction

The nettle used was collected in 2007 April, from Dumlu country in Erzurum city in Turkey. Then, nettle was dried at room temperature in the shade. The dried sample was chopped into small parts in a blender. One hundred gram of nettle ground into a fine powder in a mill and was stirred with 2 L boiling sterilized distilled water using magnetic stirrer during fifteen minutes. Then the extract was filtered through Whatman No. 1 paper. The filtrate was frozen and lyophilized in a lyophilizator at 5 μmhg pressure at -50°C. The extract of nettle was placed in a sterilized vial and then stored at -20°C until used.

Fish Source, Treatment and Packaging

A total of 50 fresh water rainbow trout with an average weight of 200 g were purchased from Research and Extension Center of Fisheries Department and then transferred to the Meat Processing Laboratory within an hour. They were decapitated and filleted by hand. Two fillets were obtained from each fish by removing the head and bone of fish. The fillets were divided into five batches (n value for each batch was 20). The fillets were dipped in one of 0% (control), 0.4, 0.8 and 1.6% nettle solutions, or 0.8% propyl gallate solution (positive control) for 15 min. After dipping, they were drained at room temperature for 10 min in perforated plastic basins and finally individually wrapped in cling film. The packed fish were stored in a refrigerator at 4±1°C. Three fillets per batch were subjected to chemical analyses on the 0, 1, 3, 5, 7 and 9th days of the storage period.

pH Measurements

The pH of the fish muscle samples was measured using pH meter (Schott, Lab Star pH). Ten gram fish muscle sample was combined with 100 mL distilled water and homogenized in an Ultra turrax (IKA Werk Tp 18-10 20.000 rpm) for 30 sec. Three readings were taken from each of experimental groups.

Lipid Oxidation

Lipid oxidation was determined by Thiobarbituric acid reactive substances (TBARS) method (Lemon, 1975). TBARS value was calculated from a standard curve of malondialdehyde (MDA) prepared by 1, 1, 3, 3 Tetraoxipropan (TEP) and values expressed as μmol kg^{-1} sample.

Total Antioxidant Activity Determination

The antioxidant activity of the fish muscle samples was determined according to the ferric thiocyanate method (Gulcin *et al*., 2004b).

Determination of TVB-N

The TVB-N level is determined by steam distillation method according to Anonymous (1988). The results were expressed as mg TVB-N/100 g.

Statistical Analysis

Data on TVB-N, lipid oxidation (TBARS) and pH values were checked for normal distributions with normality plots prior to one-way analysis of variance (ANOVA) and followed by Duncan's multiple range test to determine significant differences among means at $p = 0.05$ level.

RESULTS AND DISCUSSION

Oxidative changes are very important in the lipids, which are highly vulnerable to oxidation. Storage time x treatment interaction had significant effects ($p<0.01$) on TBARS value. Present data showed that all the nettle and propyl gallate treatments retarded the lipid oxidation compared to the control (Fig. 1a). TBARS value in control group reached to the unacceptable level (20 μmol kg^{-1}) (Arashisar *et al*., 2004) at day 5 and was significantly higher ($p<0.05$) than that of the other groups. TBARS value of fillets treated with nettle and propyl gallate did not reach the unacceptable level during experimental period. Moreover, the differences in TBARS values were not significant between nettle and propyl gallate treatments ($p>0.05$).

It was reported that phenolic antioxidants were effective in reducing lipid oxidation in pork patties and TBARS value of antioxidant treated aerobically and vacuum-packaged irradiated pork patties reduced by about 50 and 20% of the control value. Therefore, it was concluded that the use of antioxidant combinations was more effective in reducing oxidative changes in aerobically packaged than vacuum-packaged irradiated pork patties (Chen *et al*., 1999). Similarly, it was showed that aloe vera (0.25%), fenugreek (0.01%), ginseng (0.25%), mustard (0.10%), rosemary (0.10%), sage (0.05%), soya protein (0.10%), tea catechins (0.25%) and whey protein (4%) were effective in reducing lipid oxidation in patties manufactured from previously frozen pork (McCarthy *et al*., 2001). Bekhit *et al*. (2003) determined that some natural antioxidants (carnosine, quercetin and rutin) retarded the oxidative processes in raw beef patties. It was observed that fermented goat meat sausage with 0.05% rosemary showed the lowest initial TBARS values when compared with the sample containing 0.025% rosemary (Nassu *et al*., 2003). Karpińska *et al*. (2001) observed that addition of natural antioxidants (sage and the mixture of sage, red pepper, black pepper, garlic and marjoram spices) retarded the process of oxidation. Sage proved to be more effective than the mixture of spices. Similarly, it was determined that paprika and garlic were as effective as their mixture with nitrate, nitrite and ascorbic acid in inhibiting lipid oxidation (Aguirrezábal *et al*., 2000). Moreover, it was showed that the exogenous addition of garlic-derived organosulfur compounds (diallyl sulfide, diallyl disulfide, s-ethyl cysteine, n-acetyl cysteine) in ground beef not only enhanced color and minimized lipid oxidation but also enhanced microbial safety of the product (Yin and Cheng, 2003).

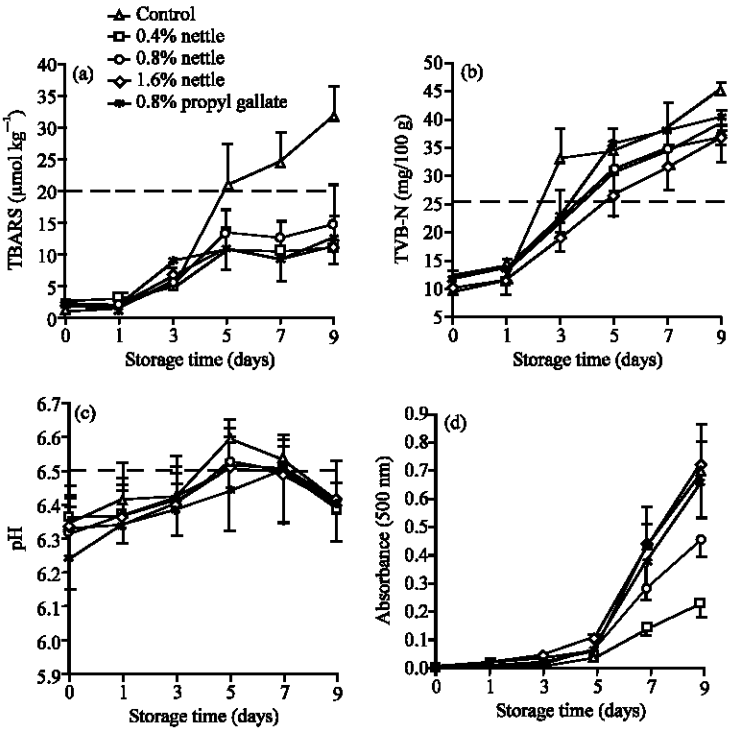

Fig. 1: Effect of the different concentrations of nettle or propyl gallate on TBARS levels (a), TVB-N levels (b), pH values (c) and total antioxidant activity (d) of rainbow trout fillets. Upper areas of horizontal lines are unacceptable in each figure

The effects of various concentrations of nettle and propyl gallate on total antioxidant activity in rainbow trout fillets are shown in Fig. 1d. Storage time x treatment interaction had significant effects ($p<0.01$) on total antioxidant activity. The fillets treated with the 0.4% of nettle exhibited most effective and powerful antioxidant activity. The antioxidant activity of fillets treated with nettle decreased with increasing nettle concentrations. However, the samples treated with 0.4 and 0.8% nettle showed higher antioxidant activities than that of 0.8% propyl gallate.

The antioxidant activity of quercetin and rosemary extracts were studied in minced fish and both extracts showed antioxidant activity after processing. The rosemary extract was more effective in protecting from lipid oxidation (Montero *et al*., 2005). In another study, grape (*Vitis vinifera*) phenolic compounds inhibited the oxidation of fish lipids and the inhibition of formation of hydroperoxides was higher than the inhibition of formation of volatiles (Pazos *et al*., 2005).

The nettle and propyl gallate treatments affected the TVB-N levels of aerobically packaged fish fillets (Fig. 1b). All antioxidants added to fish fillets reduced the amounts of TVB-N as compared to the control. However, the differences between the control and the treatments were statistically not significant in the case of TVB-N values ($p>0.05$). While the TVB-N level reached above 25 mg/100 g in control group on the 3rd day, nettle and propyl gallate groups were reached above this level on 5th day. This value was considered as the highest acceptable level for rainbow trout (Gimenez *et al*., 2002). Similarly, it was reported grape (*Vitis vinifera*) phenolic compounds inhibited the formation of volatiles (Pazos *et al*., 2005). Nam and Ahn (2003) found that addition of antioxidant combinations using sesamol, propyl gallate and α-tocopherol was effective in reducing off-odor volatiles in irradiated pork patties.

No significant effects were observed storage time x treatment interaction on pH values. Although pH value of control group reached above of 6.5 on day 5, differences were also not significant among pH values determined from antioxidants and control group (Fig. 1c). Similarly, McCarthy *et al*. (2001) reported that application of rosemary did not affect the pH values of fresh pork patties held under chilled (4°C) display conditions. However, contrary to the present finding, they also determined significant changes in pH values of pork patties treated with tea catechins, sage, ginseng and aloe vera.

Gülçin *et al*. (2004a, b) reported that water extract of nettle exhibited antimicrobial activity against nine different microbial species. Similarly, it was showed that the exogenous addition of garlic-derived organosulfur compounds in ground beef not only enhanced color and minimized lipid oxidation but also enhanced microbial safety of the product (Yin and Cheng, 2003).

CONCLUSIONS

The addition of three different concentration of nettle was effective in reducing lipid oxidation and TVB-N in aerobically packaged and stored at 4±1°C rainbow trout fillets. Present data showed that addition of 0.4% nettle to rainbow trout fillets competed with propyl gallate in terms of retarding of TBARS and TVB-N values. However, pH values appeared to be unaffected by the application of the different concentration of nettle in the oxidative processes in fish fillets.

The finding of present study suggested that if nettle is used in aerobically packaged fish fillets, it may solve the lipid oxidation problems. Since the findings of the present study is the first records on this topic as far us we know, it is better to be cautious while making generalizations. Further work is needed to give the best suggestions from different fish, especially fatty fish with the addition of other different complementary lipid oxidation indices such as conjugated dienes, peroxides, fluorescent compounds during frozen storage.

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