Short Paper

Effects of *Scrophularia striata* water extract on quality and shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets during superchilled storage

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Summary

The purpose of this study was to evaluate the effects of *Scrophularia striata* water extract on the quality and shelf life of the rainbow trout fillet during superchilled storage. Fish samples were treated with 1% and 3% *S. striata* water extract and then stored at -2°C for 20 days. The samples were analyzed periodically for chemical, microbial and sensory characteristics. Results indicated that incorporation of *S. striata* water extract on rainbow fillets caused the delay of lipid peroxidation and hydrolytic spoilage in 3% treated sample in comparison with the control sample at the last day of the experiment ($P<0.05$). Moreover, fish fillets containing 3% *S. striata* water extract showed lower bacterial count than the control and 1% water extract supplemented samples ($P<0.05$) during the experiment. According to sensory analysis results, 3% treated samples were acceptable even at the end of the 20-day storage. It was concluded that the effect of *S. striata* extract on fish samples was to retain their good quality characteristics and extend the shelf life during superchilled storage.

Key words: Quality, Rainbow trout, *Scrophularia striata*, Superchilled storage, Water extract

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important freshwater fishes with high acceptability for consumers and its production was 30.3% of total aquaculture products from 2005 to 2007 (Kalbassi *et al*., 2013). However, fish are more susceptible than other muscle foods to both microbial and chemical deterioration due to the abundance of polyunsaturated fatty acid and protein present in their flesh (Vidya Sagar Reddy and Srikar, 1996).

Since freezing modifies the organoleptic properties of the product, storing food just above the initial freezing temperature (e.g. at -2°C/-3°C for fish meat) or super-chilling has recently been considered as an innovative technology for retarding spoilage of fish meat. Nevertheless, this method does not completely inhibit microbial and chemical reactions that lead to quality deterioration of fish due to its muscle chemical composition (Vidya Sagar Reddy and Srikar, 1996).

The *Scrophulariaceae* is a large angiosperm family, which is widely distributed in deciduous and coniferous forests of central Europe, Asia, and North America, especially in the Mediterranean area (Ardeshiry Lajimi *et al*., 2010) and a large number of them are well known for their antitumoral, hepatoprotective, anti-inflammatory, antioxidant and antimicrobial properties (Vahabi *et al*., 2011).

Since extending superchilling condition would be critical point for increasing the quality of the fish during storage, using *Scrophularia* genus species can be considered as a suggestive method toward this goal. Therefore, the aim of the present study was to evaluate the effects of *Scrophularia striata* water extract on the quality and shelf life of the rainbow trout fillet during superchilled storage.

Materials and Methods

S. striata extraction

Water extract *S. striata* was prepared using soxhlet. The extract was filtered and evaporated to dryness in vacuum (Kamkar *et al*., 2010).

Sample preparation and dipping

Freshwater rainbow trout was purchased from a local aquaculture farm located near Shahmirzad city (Semnan province). The fish were immediately headed, gutted and cut into a 0.5 × 3 × 4 cm sized fillets. After washing, fish samples were given a dip treatment in 1% and 3% *S. striata* extract solution (treatment groups) and in distilled water as control, respectively for 120 min and then well

drained. After that, they were individually packed in plastic trays and airproofed with polyvinyl dichloride (PVDC) and then all the packs were kept in a refrigerator maintained at -2°C for 20 days (Fan *et al*., 2009). Fish samples were taken randomly every 5 days for microbial and chemical evaluation.

Proximate analysis

Moisture content was determined by oven drying of samples (AOAC, 1995). Total crude protein was determined using the Kjeldahl method (James, 1995). The ash content was determined by ashing of 5 g of minced fish in a furnace at 550°C (AOAC, 1995). The total lipids were extracted using chloroform: methanol (2:1 by vol) extraction solution (James, 1995).

Determination of pH

A 10 g sample of the fish muscle was homogenized in 100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a digital pH meter (Jenway, UK).

Fat extraction and peroxide value measurement

Fat extraction was done using the method of Zouari *et al*. (2010). The PV was determined by titrating the iodine liberated from potassium iodide and expressed as meq of peroxide per kg of lipid (Zouari *et al*., 2010).

TBARS value measurement

TBARS was determined using the method of Botsoglou *et al*. (1994), with some modifications (Jebelli Javan *et al*., 2012).

Determination of total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVBN) was determined according to the method described by Goulas and Kontominas (2005).

Microbiological analysis

Samples of raw rainbow trout were plated on PCA (plate count agar) for enumeration following the rules reported by Siskos *et al*. (2007).

Sensory evaluation

The sensory quality of fish sample was evaluated by a seven member trained panel from the laboratory staff (Fan *et al*., 2009). Panelists scored for sensory characteristics using a nine-point hedonic scale (1, dislike extremely to 9, like extremely). The median of panel members' scores was calculated to evaluate the statistical analyses.

Statistical analysis

Analyses were run in triplicate. Statistical analyses were done by SAS software version 6.12 (SAS, 1997). The descriptive statistics are described by mean ± SEM. For quantitative data One Way ANOVA and Tukey Post Hoc tests were used, and for qualitative data (sensory evaluation), Kruskal-Wallis and Mann-Whitney U tests were used. A p-value less than 0.05 was considered statistically significant.

Results

Flesh proximate composition

Proximate composition of rainbow trout fillets was 71.8 ± 1.1, 4.7 ± 0.7, 18.9 ± 0.5, and 1.2 ± 0.3% for water, lipid, protein, and ash, respectively.

Determination of pH

Variations in values of pH during storage are depicted in Fig. 1. The pH values in all samples showed a trend to increase from the beginning of the storage period to the end of experiment except day 5, when a initial decrease of pH values can be seen. For the control and 1% treated samples the gradual increase in pHs from day 10 to the end of the storage period were higher than the 3% treated samples; although the differences between control and 3% treated samples were statistically significant ($P<0.05$) but the difference between the 1% and 3% did not show significant difference ($P>0.05$).

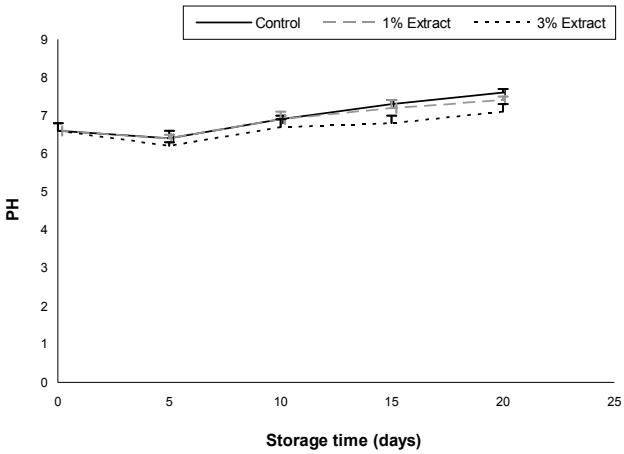

Fig. 1: Changes in pH of fish sample during the storage. The error bars show standard error of the mean (SEM)

Peroxide value and TBARS measurement

Figures 2 and 3 show the PVs and TBARS levels during the storage of fish fillets over a 20-day period. The initial PVs and TBARS levels in the fresh fillets were 2.6 ± 0.2 meq/kg and 0.51 ± 0.03 mg MDA/kg, respectively. In all samples PVs showed a trend to increase from the beginning of the storage period to the end of experiment. For the control and 1% treated sample, the gradual increase in PVs from day 5 to end of the storage period was significantly ($P<0.001$) higher than the 3% treated sample. In this respect, we have found that PVs of control and 1% treated sample from the beginning until the end of storage period were similar ($P>0.05$).

Similar to PV results, TBARS values (Fig. 3) in 3% treated sample were lower than the control and 1% treated sample from day 5 until the end of storage period

(P<0.001) and there was no difference between control and 1% treated samples (P>0.05). As can be seen in Fig. 3, TBARS values in all the cases increased gradually up to a certain point during storage; followed by a decrease in values at the end of experiment.

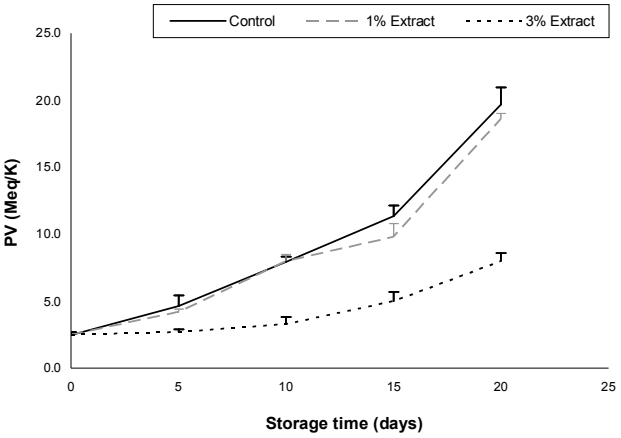

Fig. 2: Change in peroxide values (PVs) of fish samples during the storage. The error bars show standard error of the mean (SEM)

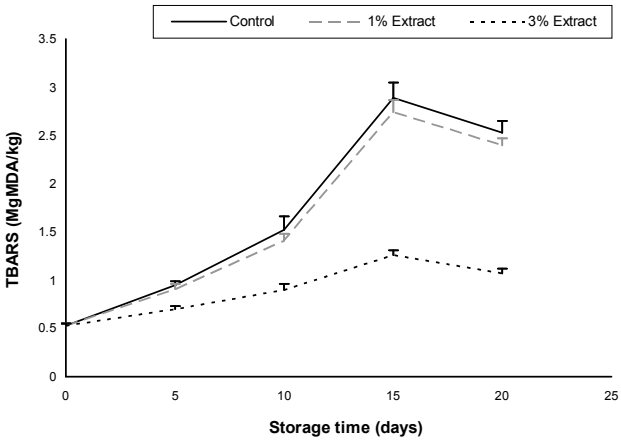

Fig. 3: Change in TBARS values of fish samples during the storage. The error bars show standard error of the mean (SEM)

Total volatile basic nitrogen (TVB-N)

Changes in TVB-N value are shown in Fig. 4. The data showed that TVB-N increase was significantly lower in 3% treated sample than 1% treated one and control from day 10 until the end of the storage (P<0.05). In this regard, there was no difference between control and 1% treated sample throughout the entire storage period (P>0.05).

Changes in the total viable count

Changes in the total viable microbial count of the rainbow trout fillets during storage at -2°C are shown in Fig. 5. In this test, 3% treated sample gave a significant reduction (P<0.05) in the TVC immediately after treatment as compared to the control and 1% treated groups. Accordingly, the control and 1% supplemented samples did not show any difference (P>0.05) between each other from the start of the storage period to the end of the experiment.

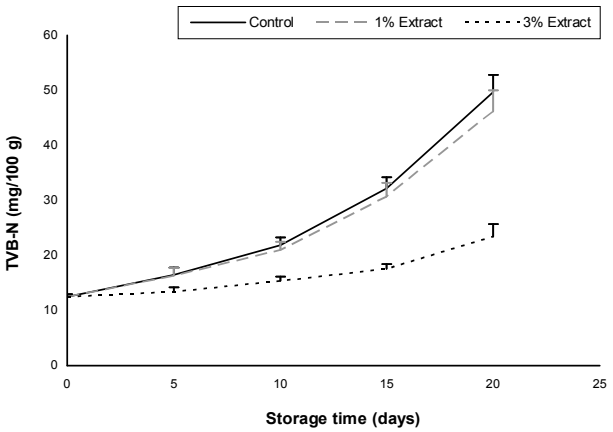

Fig. 4: Changes in TVB-N values of fish samples during the storage. The error bars show standard error of the mean (SEM)

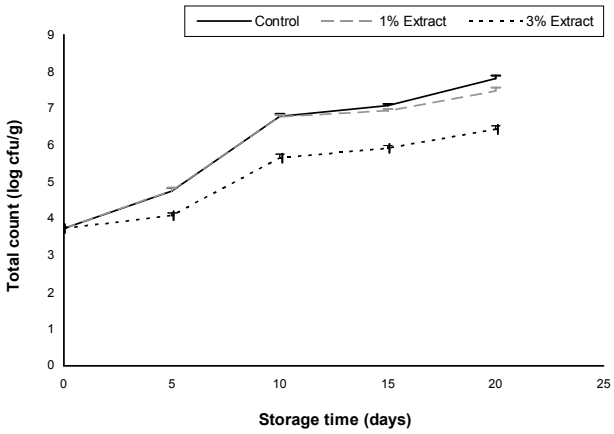

Fig. 5: Changes in total viable count of fish samples during the storage. The error bars show standard error of the mean (SEM)

Sensory evaluation

The results of the sensory evaluation of samples are given in Fig. 6. Sensory scores showed a significant decline in both treated and control samples with increasing storage period and, also, the 3% treated sample earned a higher score than did the 1% treated sample and control (P<0.05).

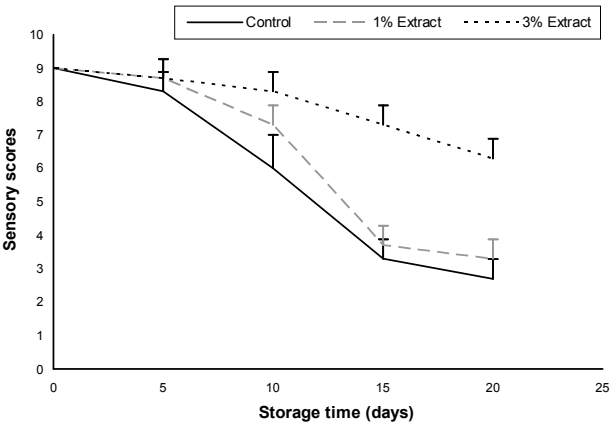

Fig. 6: Changes in sensory scores of fish samples during the storage. The error bars show standard error of the mean (SEM)

Discussion

The results of proximate composition are in good agreement with those reported by Rezaei *et al*. (2011) except the fat content that was higher in this research as compared with Rezaei *et al*.'s (2011) work (1.28 ± 0.42%).

In all fish samples, the values of pH decreased initially and then increased. Similar observations were made by Manju *et al*. (2007). The initial pH decrease was probably due to dissolution of CO_2 in aqueous phase of the fish sample, while the increase of pH was postulated to be due to an increase in volatile bases produced, e.g. ammonia and trimethylamine, by either endogenous or microbial enzymes (Manju *et al*., 2007). The lower pH of 3% treated sample can be because of microbial inhibition and inhibiting the activity of the endogenous proteases (by *S. striata* water extract).

In the present study, we showed that water extract of *S. striata* is able to inhibit both primary and secondary oxidation of dipped fish fillets during storage. Peroxide values and TBARS levels of 10 meq/kg and 2 mg/kg of flesh are respectively regarded as the maximal permissible limit in the fish muscle (Connell, 1990). In this study, the initial PV and TBARS values of fresh samples were 2.6 ± 0.2 meq/kg and 0.51 ± 0.03 mg MDA/kg, respectively. In control samples these parameters reached to maximal permissible limit after 10 and 15 days in PV (11.3 ± 0.8 meq/kg) and TBARS (2.89 ± 0.2 mg MDA/kg) test respectively; however, the final PVs and TBARS values of 3% treated samples were within the limit value after 20 days from the beginning of the experiment. The data revealed that the *S. Striata* treated samples indicated preservation of fish flesh by inhibiting the oxidation of lipid. Given that TBA value is a measurement of MDA content, decrease in MDA at the end of the storage may be caused by interaction between MDA and amino acids, proteins, glucose and other fish constituents and production of secondary metabolites that include carbohydrates, furfural, alkenals, alkadienals and other aldehydes and ketones (Fernandez *et al*., 1997). This observation is in agreement with previous reports (Fernandez *et al*., 1997). In this regard, according to Botsoglou *et al*. (1994) TBARS values may not reveal the actual rate of lipid oxidation.

These characteristics of the water extract of the *S. striata* can be attributed to its phenolics, flavonoids and terpenoids constituents. These compounds have been shown in Sharafati-Chaleshtori *et al*.'s (2010) phyto-chemical analysis. In this regard, Jebelli Javan *et al*. (2013), discussed how phenolic contents and flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals. Moreover, Joshi *et al*. (2008) showed a potent antioxidant activity for terpenoids. In this regard, Kamkar *et al*. (2010) showed that these antioxidative components are more soluble in water in comparison with other polar solvents.

Total volatile basic nitrogen (TVB-N) is widely used as an indicator of meat deterioration. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Fan *et al*., 2009).

During storage at -2°C, the TVB-N of the control and 1% treated sample increased to reach the acceptable limit of rainbow trout reported by Arashisar *et al*. (2004; 25 mg TVB-N/100 g) on day 15. But, 3% treated sample did not exceed the upper acceptability limit after 20 days of superchilled storage and TVB-N increase in this group was significantly lower than control and 1% treated one from the day 5 of the experiment, indicating either a faster reduction of bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (or both) due to the effect of *S. striata* water extract in the fish samples (Sharafati-Chaleshtori *et al*., 2010).

The maximum acceptable count for rainbow trout is 10^6 cfu g^{-1}, as recommended by Arashisar *et al*. (2004) and Rezaei *et al*. (2008). So, according to the Fig. 5, control groups, 1% and 3% treated samples reached acceptable limits of TVC in 10, 10 and 20 days, respectively. The results indicated that dipping in 3% *S. striata* water extract can cause the retardation of the growth of TVC bacteria in the stored rainbow trout samples. This is in line with the previous reports on *in vitro* antimicrobial activity of *S. striata* extracts (Sharafati-Chaleshtori *et al*., 2010; Vahabi *et al*., 2011). The significantly ($P<0.05$) lower total bacterial count of sample containing *S. striata* water extract may be attributed to the antibacterial properties of this plant and more specifically to its phenolic constituents (Burt, 2004).

The sensory qualities of fish samples were evaluated in terms of colour, odour, general acceptability and texture, using a nine-point hedonic scale (1, dislike extremely to 9, like extremely). The fish samples were considered to be acceptable for human consumption until the sensory score reached 4 (Truelstrup Hansen *et al*., 1995). The samples treated by 1% *S. striata* water extract and control were acceptable up to 15 days while 3% treated sample was in good and acceptable condition during the entire 20 days of storage. This may be attributed to *S. striata*'s functional properties, e.g. antioxidant, antimicrobial and oxygen barrier, and this conclusion is supported by our results of microbial and chemical quality analyses.

The results of chemical and microbial analysis and also sensory evaluations indicate that *S. striata* water extract can lead to retention of the good quality characteristics and extension of the shelf life of the dipped rainbow trout (*O. mykiss*) during superchilled storage. In this respect, the sample supplemented with 3% water extract was more potent compared with the 1% one and could extend the minimum shelf life of the fish fillets by 5 days.

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