

Antioxidant Activities of *Pistacia atlantica* on Meat of the *Oncorhynchus mykiss* Kept at 4°C

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Research Article

Abstract

Background: Fish, meat is prone to lipid oxidation. The present study was done to research the antioxidant effects of *P. atlantica* on meat of the *Oncorhynchus mykiss* kept at 4°C.

Methods and findings: *P. atlantica* was purchased, dried and its methanolic extract was extracted. Meat samples were dipping on the *P. atlantica* extract for 30. Samples were kept at 4°C for 20 days. Chemical properties were analyzed. DPPH radical scavenging of *P. atlantica* was $34.3 \pm 5.92\%$. The pH was pointedly higher in control group than *P. atlantica* on days 15 and 20. Highest levels of PV were determined on the 20th day of storage for control group (5.21 ± 0.44 meq O₂/kg fat). Fish slices treated with *P. atlantica* had the lower incensement of TBA and TVB-N during storage.

Conclusion: Chemical oxidation of the meat of *Oncorhynchus mykiss* can decrease using the essential oil of the *P. atlantica*.

Keywords: *Pistacia atlantica*; Antioxidant activities; Rainbow trout; Refrigerator.

1. Introduction

Meat of the Rainbow trout (*Oncorhynchus mykiss*) is an excellent food choice because of presence of a lot of components such as essential fats like omega-6 and omega-3, proteins, vitamins like niacin, thiamin and vitamins B12, B6 and E, minerals including potassium, phosphorus, calcium, magnesium and selenium and amino acids. Besides, this product has a low content of harmful fats such as cholesterol, monoenoic and trans polyenoic fatty acids and also calories which make it suitable for all age groups [1-3].

Meat of the *Oncorhynchus mykiss* is generally marketed at cold temperatures (about 4°C). However, this type of storage causes a lot of types of harmful changes in the chemical components of fish meat which cause corruption, quality discount

and financial losses. Chemical changes occurred in the meat of the rainbow trout are usually removed by application of synthetic antioxidants. Inappropriately, use of synthetic and chemical anti-oxidative agents had so many harmful effects including deficiency in supporting properties, mutagenicity effects and financial issues [4-7]. However, using from natural anti-oxidants like the essential oils of medicinal plants solves this issue.

Pistacia atlantica (*P. atlantica*) (Bene) is an innate plant and/or fruit of Iran which has a rich history in an ancient medicine. Essential oil of the *P. atlantica* has been recently presented as an extremely steady complex with anti-oxidative property [8-11]. Its main components are carotenes, tocopherols, unsaponifiable matter and alcohols which have considerable antioxidant actions equal to vitamin E [8-11]. Numerous surveys have been described considerable anti-oxidative action of *P. atlantica* essential oil [8-11]. It is also improve the nutritional index of foods [8-11].

Rendering to the considerable anti-oxidative properties of *P. atlantica*, its considerable rate in Iran and deficiency of available information on its application on the meat of Rainbow trout, the current examination was done to investigate the antioxidant actions of *P. atlantica* on the meat of the *Oncorhynchus mykiss* kept at 4°C.

2. Materials and Methods

2.1 Fruits and extraction of essential oil

From August to September 2015, fruits of *P. atlantica* were purchased from the groceries of Khouzestan, Iran. Fruit's identification was done at the herbal plant research center (Islamic Azad University, Shahrekord Branch, Shahrekord, Iran) by an skillful professor. *P. atlantica* fruits were dried using oven (40°C, 24 h) and crowded in paper bags. Samples were then kept for further applications at Food Science Research Center, Islamic Azad University, Shahrekord Branch, Iran. Powder of dried fruits was achieved by crushing. Each gram of powder were then solved on 20 ml of

solvent and then lightly enthused for 48 h. Extracts were then filtered (paper filter, No. 1, Whatmann) and a rotary evaporator (60°C) was used for solvent elimination. Crude extract of fruit was finally dissolved in 3 ml of methanol.

2.3 Classification of rainbow trout

Fifty healthy and equal size *Oncorhynchus mykiss* were bought from the farms of the Chaharmahal Va Bakhtiari province, Iran. All of them were transported using the cold water. Fishes were then killed by cold shock. Head and fins of samples were cut and their abdominal contents came out. They were cut into slices with approximately weigh of 110 g and were then divided into two groups of treatment and control. Fish slices of the treatment group were dipping in 1:2.5 proportion of methanol extract (with concentration of 1000 ppm) for 30 min and then packed in vacuum bags (low-density polyethylene, 75 µm thickness) using the Boos N84 device. Then they were kept at 4°C for 20 days. Fish slices of the control group were packed in similar conditions deprived of any plunging process. Fish slice samples of groups were arbitrarily taken on 0th, 5th, 10th, 15th and 20th days of holding and examined for chemical properties.

2.4 Chemical and anti-oxidative examination

pH analysis

The pH was determined using digital pH meter (Model 420A; Orion Research, Beverly, Massachusetts, USA) regarding to the process designated recently [12]. For this, 10 g of samples was homogenized with 50 ml of distilled water and the electrode was dipped into the suspension to note down the pH.

DPPH radical scavenging

The DPPH radical scavenging of *P. atlantica* methanol extract was perfumed using the method described by Yen et al. [13]. Acid ascorbic was the standard component of reaction [14]. Radical scavenging activities of extract were achieved using to the below formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Peroxide value analysis

Peroxide value (PV) was achieved by the assay to the AOAC International instruction [15]. 5 g of samples were heated at 60°C for 3 min using the water bath. Materials were then agitated with 30 ml of acetic acid–chloroform solution (3:2 v/v). Whatman filter paper was then used for vacuum filtration of samples. A total of 0.5 ml saturated potassium iodide

solution was added to the materials of the previous stage in a burette of an automatic titrator (DL 25 Titrator, Greifensee, Switzerland) contained stirrer and pH electrode. PV was achieved using the below formula.

$$PV \text{ (meq/kg)} = \frac{\text{Titration in ml} \times 0.01 \text{ (normality of sodium thiosulfate)}}{\text{Weight of sample (kg)}} \times 100$$

Analysis of total volatile based-nitrogen

Total Volatile Nitrogen (TVB-N) was achieved by the instruction of FOSS (2002) [16]. Distillation after adding magnesium oxide to homogenized slices was used for the micro diffusion. TVB-N values in mg N/100 g slices were distinguished using the Kjeltec 2300.

Analysis of thiobarbituric acid

The 2-thiobarbituric acid (TBA) method was done using the assay determined by Schmedes and Holmer (1989) [17]. 10 g of slices were mixed with 25 ml of trichloroacetic acid (w/v) with purity of 20% and homogenized in blender. Samples were then filtered and added to equal volume of aqueous TBA (2 ml) in a purity of 0.02 M and incubated in dark place at room temperature for 20 h. Finally, absorbance of samples was measured at 532 nm using spectrophotometer (Perkin Elmer, USA). The unit of measurement was mg malonaldehyde (MA) per kg sample slice.

Arithmetical examination

All examinations were done for four times. Arithmetical examination was done using the SPSS.ver 20, ANOVA and Duncan's tests. P<0.05 was determined as arithmetical level of significance.

3. Results

3.1 DPPH radical scavenging activity

The percent of the DPPH radical scavenging of methanol extract of *P. atlantica* was 34.3 ± 5.92% (Table 1).

3.2 pH analysis of samples

Changes in the levels of pH in meat of the *Oncorhynchus mykiss* preserved with methanol extract of *P. atlantica* through maintenance period were shown in Table 2. Levels of pH in control and *P. atlantica* treatment groups had the ranges of 6.65 to 6.74 and 5.65 to 6.16, respectively. The pH of control and *P. atlantica* did not show any momentous alteration up to 10th day of maintenance, but it was significantly higher (P<0.05) in control than *P. atlantica* on days 15 and 20.

3.3 Peroxide value analyses

Table 3 signifies variations in the levels of PV of meat

Table 1: Average of DPPH radical scavenging effects of the methanolic extract of *Pistacia atlantica*.

Samples	Inhibitory effects of the DPPH free radicals (%)
Methanolic extract (with concentration of 1000 ppm)	34.3 ± 5.92

Table 2: Changes in the levels of pH in rainbow trout meat samples treated with methanolic extract of *Pistacia atlantica* during 20 days.

Groups	Average levels of pH				
	0 day	5 th day	10 th day	15 th day	20 th day
Control	5.65 ± 0.14a	5.37 ± 0.07a	5.78 ± 0.02a	6.25 ± 0.12a	6.74 ± 0.06a
<i>Pistacia atlantica</i>	5.65 ± 0.09a	5.19 ± 0.08a	5.28 ± 0.04a	5.39 ± 0.14b	6.16 ± 0.07b

Different letters in the same column indicate significant differences ($p < 0.05$)

Table 3: Changes in the levels of peroxide value of rainbow trout meat samples treated with methanolic extract of *Pistacia atlantica* during 20 days.

Groups	Average levels of PV (meq O ₂ /kg fat)				
	0 day	5 th day	10 th day	15 th day	20 th day
Control	1.14 ± 0.013a	6.85 ± 0.21a	3.69 ± 0.26a	4.13 ± 0.36a	5.21 ± 0.44a
<i>Pistacia atlantica</i>	1.14 ± 0.011a	3.72 ± 0.018b	2.84 ± 0.2a	2.08 ± 0.14b	4.15 ± 0.39b

Different letters in the same column indicate significant differences ($p < 0.05$)

Table 4: Changes in the levels of thiobarbituric acid of rainbow trout meat samples treated with methanolic extract of *Pistacia atlantica* during 20 days.

Groups	Average levels of TBA (mg malonaldehyde equivalent/kg tissue)				
	0 day	5 th day	10 th day	15 th day	20 th day
Control	0.24 ± 0.017a	1.06 ± 0.076a	1.36 ± 0.098a	2.28 ± 0.21a	2.19 ± 0.17a
<i>Pistacia atlantica</i>	0.24 ± 0.016a	1.23 ± 0.082a	1.09 ± 0.091b	1.06 ± 0.010b	1.05 ± 0.076b

Different letters in the same column indicate significant differences ($p < 0.05$)

Table 5: Changes in the levels of total volatile nitrogen of rainbow trout meat samples treated with methanolic extract of *Pistacia atlantica* during 20 days.

Groups	Average levels of TVB-N (mg N/100 g)				
	0 day	5 th day	10 th day	15 th day	20 th day
Control	11.47 ± 1.18a	15.39 ± 1.28a	16.90 ± 1.32a	25.92 ± 1.96a	38.16 ± 2.77a
<i>Pistacia atlantica</i>	11.47 ± 1.23a	13.71 ± 1.21a	14.12 ± 1.27a	15.23 ± 1.45b	14.07 ± 1.24b

Different letters in the same column indicate significant differences ($p < 0.01$)

of the *Oncorhynchus mykiss* treated with methanol extract of *P. atlantica* through maintenance period. There were no statistically significant changes for the PV among two groups in the first day, but it was significantly higher ($p < 0.05$) in control than *P. atlantica* on days 5 to 20. The highest level of PV was determined on the 20th day of maintenance for the control group (5.21 ± 0.44 meq O₂/kg fat), while the lowest was on the first day (1.14 meq O₂/kg fat in both groups).

3.4 Levels of thiobarbituric acid

Changes in the levels of TBA of meat of the *Oncorhynchus mykiss* treated with methanol extract of *P. atlantica* through maintenance period are shown in Table 4. The highest and lowest levels of TBA were determined on 15th maintenance day for the control group (2.28 ± 0.21 mg malonaldehyde equivalent/kg tissue) first day (0.24 mg malonaldehyde equivalent/kg tissue), respectively. There were no statistically significant changes for the TBA value between control and treatment groups up to day 5, but it was significantly higher ($p < 0.05$) in control than *P. atlantica* on days 10 to 20.

3.5 Levels of total volatile based-nitrogen

Changes in the levels of TVB-N of rainbow trout meat

samples treated with methanolic extract of *P. atlantica* during 20 days are shown in Table 5. First day (11.47 mg/100 g) and 20th day (38.16 mg/100 g) had the lowest and highest levels of TVB-N, respectively. There were no statistically significant changes for the TVB-N value between control and treatment groups up to day 10, but it was significantly higher ($p < 0.01$) in control than *P. atlantica* on days 15 and 20.

4. Discussion

Fallouts of current study displayed that plummeting of meat slices of the *Oncorhynchus mykiss* in 1:2.5 relation of *P. atlantica* methanol extract for 30 min can lessen their chemical corruption. As far as we know, there were no beforehand available items about the anti-oxidative properties of *P. atlantica* on meat of the *Oncorhynchus mykiss*.

Oncorhynchus mykiss has slight carbohydrate in its meat. So, the levels of lactic acid are low in its meat [18]. This item caused its high range of final pH which prepares suitable conditions for its chemical and even microbial corruption. We found that the pH value was enhanced throughout the maintenance period. In addition, the levels of TVB-N have been increased during the storage period. The most important reason for incensement in the levels of pH during storage is

due to the increase in the levels of TVB-N occurred by enzymes of bacteria and endogenous [19,20]. The pH of meat of the *Oncorhynchus mykiss* is depend on the post mortem variations and environmental circumstances including coldness and warmness of weather, diet, seasons and stress [21]. Enhancement of the pH in the meat of *Oncorhynchus mykiss* has so many unwanted effects on odor, taste, color and other sensorial features which cause decrease in its the quality and shelf life [18,19]. The pH of treated group containing methanolic extract of *P. atlantica* was reliably lower than control group through the maintenance period which is due to the antimicrobial effects of *P. atlantica*. Similar findings have been reported by Sallamm et al. [22] and Raeisi et al. [23].

The PV is the most common measure of lipid hydroperoxides. It is also known as primary lipid oxidation product. The initial PV in the sliced rainbow trout analyzed was 1.14 (meq O₂/kg fat) which was low. Treatment groups had the lower level of PV. However, the PV in all samples was well below the proposed acceptable level of 10–20 meq peroxide/kg fish fat through maintenance period [24].

TBA value is expected as a good marker for oxidation of lipids. High amount of unsaturated fatty acids and their facing with oxidative conditions cause increase in the level of TBA. This change may cause bitterness of fish meat which may lead to unwanted effect on quality of sea-food products [25]. The results of our study indicated that plummeting of meat slices of *Oncorhynchus mykiss* in *P. atlantica* extract diminish lipid oxidation which is derived from the antioxidant activities of *P. atlantica*. Another reason for this claim is decrease in penetrability of oxygen to the lipids of *Oncorhynchus mykiss*. Our conclusions are similar with other researches [26,27].

Presence of TVBN is an important indicator for corruption of meat of the *Oncorhynchus mykiss* [28]. Growth of some proteolytic bacteria increase through maintenance of meat off the *Oncorhynchus mykiss*. Activity of these bacteria leads to squalor of non-protein nitrogen components and proteins. Gathering of TVB-N has unwanted effect on odor, taste, color and other sensorial features of *Oncorhynchus mykiss* [28]. Our findings revealed that plummeting of *Oncorhynchus mykiss* slices on methanol extract of *P. atlantica* had prohibitory effects on production of TVB-N.

5. Conclusion

In swift, we recognized a great quantity of anti-oxidative activities of the methanol extract of *P. atlantica* on meat slices of the *Oncorhynchus mykiss*. Levels of pH, PV, TBA, TVB-N markers in treated samples with *P. atlantica* were completely lesser. It is shown that application of methanol extract of the *P. atlantica* as an anti-oxidant agent can protect from the chemical corruption of *Oncorhynchus mykiss* and increase its shelf-life.

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