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Investigation Of Oncorhychus Mykiss (Walbaum, 1792) Deep Immersion in Resveratrol Suspension Before Fishing On The Level Of Meat Malondialdehyde in During Refrigerated Storage

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Abstract

Rainbow trout is one of the most important species of farmed fish in Iran, due to the presence of protein, essential fatty acids, minerals, and vitamins, a valuable contribution to the human food supply. It is so crucial that we can prevent the rapid oxidation of this flesh. Resveratrol is a fat-soluble compound and has been shown to have good effects on oxidative damage and oxidative stress. In this study, its antioxidant activity in rainbow trout meat during storage at $4^{\cdot c}$ was investigated. For this experiment, 18 fish with an average weight of 180 g were randomly selected and divided into three groups. The treatment groups were immersed for 3 and 30 minutes in the concentration of 0.5 gr/L resveratrol suspensions. After catching and cutting off the head and peeling and emptying the abdomen cavity, the samples were transferred to the refrigerator (4°C). On the seventh and fourteenth days, malondialdehyde (MDA) level was measured. The results showed that MDA levels in the treatment group 3Min and treatment group 30Min were significantly different from the control group ($p<0.05$). The MDA level in treatment group 3Min on the seventh and fourteenth days of refrigeration was lower than that of treatment group 30Min, but it was only significantly different in 14 days ($p<0.05$). Therefore, we can say that resveratrol has a better effect in lower doses on reducing the MDA in rainbow trout during the storage period. The MDA level of treatment groups 3, 7, and 14 days was much less than control and treatment group 30. As the evaluation of these results, it can be concluded that resveratrol can be less effective in decreasing the MDA in rainbow trout meat and, consequently, meat survival for a long time.

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INTRODUCTION

Resveratrol is a fat-soluble polyphenolic compound found in large quantities in grape seed and other medicinal plants such as peanuts and raspberries it has antioxidant and anti-inflammatory effects and it has shown good effects in preventing oxidative damage that paying attention this useful plant substance in modern medicine can be effective in the prevention and treatment of some diseases, Grape, and its products are the main source of this substance in the human diet (Kursvietienea, et al 2016).

Rainbow trout (*Oncorhynchus mykiss*) has a valuable contribution to the human food supply and is one of the important species of fish farms which is considered an essential food due to its rich sources of protein, fat, and energy (Ahmed and Ahmad, 2020).

The body tissues of fish putrefied faster than those of mammals and birds after death, due to the significant amount of fatty acids with multiple double bonds and their oxidation. Corruption in seafood is influenced by internal and external factors such as the concentration of oxidation-sensitive compounds, internal iron compounds, myoglobin, enzymes, pH, temperature, ionic strength, and the presence of oxygen. There are several solutions to prevent or delay the spoilage of fish and its products; these include temperature control and reduction, vacuum packaging, and the addition of antioxidants (Bazargani-Gilani and Pajohi-Alamoti, 2020).

Today, due to the adverse effects such as mutagenesis, poisoning, and carcinogenesis caused by synthetic antioxidants, the use of natural antioxidants as a substitute for synthetic antioxidants is highly recommended. Plant extracts are known as a natural source of antioxidants. The use of natural antioxidants of plant origin in the fisheries and food industries has been proven to be an effective factor in delaying chemical and oxidative changes and increasing the shelf life of fishery products (Ural et al, 2015).

Natural antioxidant additives such as various essential oils and herbal extracts are a new approach of food researchers for prolonging the shelf life of meat products. The major problems of their application in foods are organoleptic conversion of food, their potential toxicity, application costs, and vigorous aroma (Bazargani-Gilani and Pajohi-Alamoti, 2020).

In other animal species, the antioxidant effects of resveratrol have been investigated and confirmed. For example, the administration of resveratrol to diabetic rats resulted in a significant reduction in the amount of MDA in their red blood cells, which confirms the occurrence of oxidative stress in diabetes and the antioxidant effect of resveratrol (Sedlak et al, 2018).

Fish, like other animals, have antioxidant mechanisms, including specific antioxidant defense enzymes (SOD, CAT), which, along with some other enzymes, are a set of antioxidant defenses. They form a cell and play the role of eliminating free radicals (Halliwell and gutteridge, 1990).

Despite all the antioxidant defense mechanisms, the capacity of cells to produce such enzymes is limited and exposure to fish with subacute amounts of various contaminants in the long run poses a serious threat to their life. In such cases, non-enzymatic antioxidant compounds exert their antioxidant effects by preventing the formation of free radicals as well as inhibiting them. (Attia and El-Demerdash, 2002)

Among the different farmed species, rainbow trout has high annual production, accessibility for the consumer and proper distribution is of great importance among breeders and consumers, and often in the form of whole fish from retail stores or it can be prepared in the form of fillets and on an empty stomach from big shops. (Lakashmanan, 2000)

Feeding rainbow trout with a resveratrol diet led to reduced feed intake and slightly increased protein utilization but did not significantly affect the whole-body fatty acid (FA) composition. Overall, feeding the fish oil-free diet supplemented with the phytochemicals resulted in more pronounced effects on fish performance. In terms of FA composition, resveratrol could be a potentially useful complement to fish oil. (Torno et al, 2019).

Considering the economic and nutritional value of this fish, its high production and temporary storage methods and supply percentage, as well as the antioxidant effect of resveratrol and the limited oral use of resveratrol in the rainbow trout diet, the purpose of current study is investigation of bathing resveratrol effect on the MDA level in fish meat was select.

MATERIALS AND METHODS

This study was conducted in 2016. In this study, 18 healthy rainbow trout with an average weight of 300 g were selected. Three identical pans were considered as control and treatment groups 1 and 2. In each of the pans, 20 liters of water with suitable physical and chemical characteristics for trout was poured and continuously aerated. Resveratrol was prepared by Danesh Bonyan Novin Company in the form of powder with 98% purity. Based on the concentration of 0.5 g / l resveratrol, the appropriate amount for 20 liters of water was calculated and added to the treatment pans and stirred thoroughly. The fish were randomly transferred in equal

numbers to the pans. The fishes in treatment groups 2 and 3 were immersed in resveratrol for 3 and 30 minutes, after which all fish were caught. Immediately after the death of the fish, emptying of the viscera was performed and the meats were transferred to the refrigerator at 4 ° C. MDA levels of all groups were measured on the seventh and fourteenth days of refrigerated meat. To measure the MDA level of meat, the colorimetric method was used in which 222 mg of minced fish sample was transferred to a 25 ml balloon, then 0.5 ml of this mixture was introduced into the dry-sealed tubes and 5 ml of thiobarbituric acid (TBA) reagent was added to it. TBA reagent was dissolved in 222 mg of reagent in 122 ml of 1 butane solvent after filtering. The capped tubes were placed in a hot water bath at 35 ° C for 2 hours and then cooled to ambient temperature. Then, the absorbance value was read at 592 nm before the distilled water control with Herolab model UVT-20 M / L. The amount of MDA per mg/kg of fish meat was calculated based on the following equation. (Kirk and Sawyer, 1991)

MDA (mg/kg) = Sample absorbance – Control absorbance ×50/200

MDA values obtained from fish meat were entered in the statistical software Minitab (16) and, after examining the normal distribution of data with one one-way ANOVA method analyzed using the Tukey test.

RESULTS AND DISCUSSION

All the changes in MDA of fish meat on day 1 to day 14 and treatment 3 Min to treatment 30Min shown below (Tab1).

Based on the results of comparing the means, Changes in meat MDA in all fish on day 7 show an upward trend from treatment 3Min to treatment 30Min, also the amount of MDA in fish meat of treatment 3Min and treatment 30Min was less than the control group and there is a significant relationship between them ($p<0.05$). On the seventh day of storage, despite the increase in MDA from treatment 3Min to treatment 30Min, there was no statistical relationship between them ($p>0.05$). The trend of changes in meat MDA on day 14 was almost the same as on day 7. Based on the results of comparing the means, the amount of MDA in fish meat of treatment 3Min and treatment 30Min was less than the control group and there is a significant relationship between them ($p<0.05$). On the 14th day of maintenance, a statistically significant relationship was observed between all groups ($p<0.05$). However, as on the 7th day, the amount of MDA in treatment 3Min was the lowest, which confirms the results of the seventh day of maintenance. (Fig. 1)

The cells of the body are always exposed to various oxidants. Reactive oxygen species, which contain highly active oxygenated molecules, are common oxidizing compounds. These compounds attack various molecules and oxidize them to produce by-products. Lipids are the most important group of biomolecules that are the target of reactive oxygen species called lipid peroxidation. Lipid peroxidation usually occurs on unsaturated fatty acids and the end product is active aldehydes such as MDA, which can be measured to determine the degree of oxidation of food and its shelf life (Ozyurt et al, 2009)

To maintain the quality of caught fish and prevent its oxidative spoilage, especially in fatty fish such as rainbow trout, the effect of antioxidants on increasing the shelf life of the meat of this fish can have a great effect on maintaining its quality and nutritional value and marketability. Features that cause the consumer not to accept meat products, like as undesirable or an unpleasant odor of it. Also, consumer demand for high-quality products and environmental concerns due to the non-recovery of materials used. In packaging, it can be considered as one of the effective factors in the development of preservatives for meat products (Gennadios et al, 1997)

Basically, any method that slows down lipid oxidation will increase the shelf life of products (Antoniewski et al, 2007)

Studies have shown that resveratrol has an effect on reducing the inflammatory effects and regulating the immune system of fish, and its antioxidant, anti-inflammatory, and immune-balancing effects are similar to those of salmon in mammals, and its oral administration is approved (Smith and Charter, 2010)

Although taking resveratrol orally can be a potentially useful supplement to increase the quality of fatty acids in the body of the fish, however, disturbances in growth and functional parameters limit its use in the diet of rainbow salmon (Torno et al, 2019)

Therefore, because resveratrol was soluble in fat, its use was tested by immersion and the results of this study showed that this substance can penetrate the gill epithelium and create its effects in a short time. The amount of MDA in all treatments on the seventh and fourteenth days was significantly lower than the control group. Changes in MDA of meat after 7 days of refrigeration in all groups have an increasing trend, which indicates the occurrence of natural oxidation in all samples. Although the amount of MDA in both groups of treatments 3Min and 30Min on the seventh day was significantly lower than the control group, but its low level was not significant for treatment 3Min compared to treatment 30Min and the cause may be related. To high doses of antioxidants and adverse effects on their antioxidant properties, in other words, in treatment 30Min, despite the longer the fish has been in contact with resveratrol and more of this substance enters the body, the opposite effect in reducing the amount of MDA is formed which can be due to the reduction of antioxidant effects of these substances in higher doses.

The results of the fourteenth-day assay also confirmed the findings of the seventh day, so treatments 3 and 30 both showed significantly lower amounts of MDA than the control group, but unlike the seventh day, the difference between MDA of treatment 3Min With treatment 30Min, it was statistically significant and it can be said that in a longer period, the effects of antioxidants increased and although the amount of resveratrol entered the body on the seventh day was less and showed better effects, but more of this The female in treatment 30Min controlled the continuation of the oxidation process after the seventh day and in the long run,

less oxidation occurred in the meat.

Obviously, the oxidation process of fish meat cannot be prevented during storage until consumption, but by taking measures it can be reduced. In this study, the process of MDA changes from days 1 to 7 and 7 to 14 showing that although the oxidation process is continuous. However, with the use of antioxidants, this process is much slower and the slope of the chart can be reduced. It is also possible to maintain and supply the materials needed by humans with less cost and more food security.

In this regard, the effect of immersion of silver carp fillets in garlic extract was performed and the results of this study clearly indicated the significant effect of garlic extract on the freshness of fish fillets. Accordingly, the best results were related to the use of garlic extract 1.5%. Thus, the index of the total number of visible bacteria, the total number of psychrophilic bacteria, peroxide number, thiobarbituric acid index, amount of volatile nitrogen, pH, and amount of free fatty acids showed optimal values (Ghiasvand and Changizi, 2016).

Also, the addition of antioxidants during the life of a living organism causes the spread of this substance in almost the same amounts in muscle tissue and certainly, the method of fillet immersion will have different effects, which usually needs further investigation.

CONCLUSION

Considering the above and the solubility of resveratrol in fat, we can have a positive view of the effect of resveratrol dipping in increasing the shelf life of meat and its role in balancing oxidative stress. Oxidation and potency of plasma antioxidant properties and its protective effect on cell damage, therefore, its use can be recommended to increase the shelf life of rainbow trout meat when stored in the refrigerator and its role in increasing the quality and health of fish meat and ultimately the health of society has been used. However, the effect of lower amounts of resveratrol in the fish body is greater in reducing oxidation and it is necessary to use controlled amounts and lower doses. In this regard, the effect of water and environment parameters on the uptake of resveratrol by fish should be investigated and the effects of immersion of fish fillets in resveratrol should also be evaluated in future studies.

COMPLIANCE WITH ETHICAL STANDARDS

a) Authors' Contributions

Each of the authors contributed 50%

b) Conflict of Interest

The authors declare that there is no conflict of interest.

c) Statement on the Welfare of Animals

The study protocol was approved by the IAU Local Ethics Committee

d) Statement of Human Rights

This study does not involve human participants.

e) Acknowledgements

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Figures

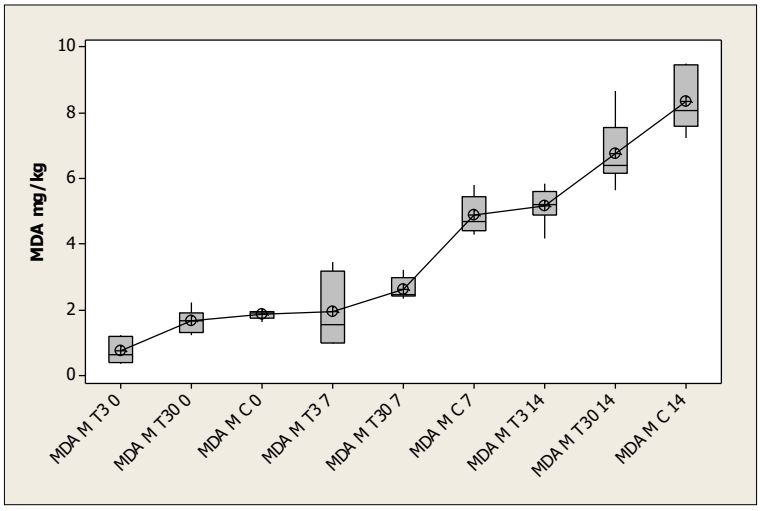

Fig1: Comparing the amounts of malondialdehyde in the meat of all control and treatment groups

*(MDA M T3 0= amount of meat malondialdehyde in the treatment 3Min on 0 days), (MDA M T30 0= amount of meat malondialdehyde in the treatment 30Min on 0 days), (MDA M C 0= amount of meat malondialdehyde in the control on 0 days),(MDA M T3 7= amount of meat malondialdehyde in the treatment 3Min on 7 days), (MDA M T30 7= amount of meat malondialdehyde in the treatment 30Min on 7 days), (MDA M C 7= amount of meat malondialdehyde in the control on 7 days), (MDA M T3 14= amount of meat malondialdehyde in the treatment 3Min on 14 days), (MDA M T30 14= amount of meat malondialdehyde in the treatment 30Min on 14 days), (MDA M C 14= amount of meat malondialdehyde in the control on 14 days)

Tables

Tab1: Amounts of MDA in fish meat on day 1 to day 14

Groups	MDA Mg/l
MDA M T3 0*	0.7145±0.3744 ^{B**}
MDA M T30 0	1.6382±0.3576 ^{A}
MDA M C 0	1.8440±0.1355 ^{A}
MDA M T3 7	1.9140±1.0697 ^{B}
MDA M T30 7	2.6133±0.3455 ^{B}
MDA M C 7	4.8658±0.5783 ^{A}
MDA M T3 14	5.1687±0.5569 ^{C}
MDA M T30 14	6.7737±1.0524 ^{B}
MDA M C 14	8.3483±0.9524 ^{A}

*(MDA M T3 0= amount of meat malondialdehyde in the treatment 3Min on 0 days), (MDA M T30 0= amount of meat malondialdehyde in the treatment 30Min on 0 days), (MDA M C 0= amount of meat malondialdehyde in the control on 0 days), (MDA M T3 7= amount of meat malondialdehyde in the treatment 3Min on 7 days), (MDA M T30 7= amount of meat malondialdehyde in the treatment 30Min on 7 days), (MDA M C 7= amount of meat malondialdehyde in the control on 7 days), (MDA M T3 14= amount of meat malondialdehyde in the treatment 3Min on 14 day), (MDA M T30 14= amount of meat malondialdehyde in the treatment 30Min on 14 days), (MDA M C 14= amount of meat malondialdehyde in the control on 14 day)
** Different letters in the column indicate statistically significant differences ($P \leq 0.05$).