



Effect of Using Tobacco and Cloves Anesthesia in Fish Harvest on the Stress Parameters and Shelf Life of Rainbow Trout Fillet

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A B S T R A C T

In this survey, we compare the effect of two anesthetics, clove and tobacco, on the harvest stress and quality of rainbow trout fillet kept at 4°C for 7 days. The fish sample groups (weighed 200 g) included a control, a group anesthetized with clove (750 mg/l) and a group anesthetized with tobacco (800 mg/l). The results showed that anesthesia with tobacco and clove significantly affects blood lactate and glucose ($p < 0.05$). Fish anesthetized with clove showed less stress during harvesting. Also, the amount of glucose and lactate in their blood was less than those anesthetized with tobacco. There were also some significant changes in Thiobarbituric acid (TBA) and Total Volatile Basic Nitrogen (TVB-N) parameters during the storage of samples in the fridge. The lowest amount of Thiobarbituric acid, Total Volatile Basic Nitrogen, mesophilic, and psychrophilic bacteria during the survey belonged to samples treated with clove. The results also suggest that the fish anesthetized with clove stayed fresh longer in the fridge.

INTRODUCTION

Seafoods are rich in proteins, fat-soluble vitamins, and omega-9 polyunsaturated fatty acids, which are very important in the human diet, in preventing and controlling many diseases and helping to the improvement of various disorders and complications (Perez-Alonso et al., 2004). In many Asian countries, seafood has an important role in people's food basket

and more than 50% of animal protein is provided by eating fish (Arannilewa et al., 2005). However, the susceptibility of fish meat to post-mortem changes and the high perishability of these products compared to other meat products on the one hand, and the increasing people's awareness about the quality of food products on the other hand, make it necessary to pay more attention to the storage and correct processing of these products (Romiani &

Khosravizadeh, 2017). Many factors such as species, size, temperature, physical conditions, methods of catching, processing, and storage affect the shelf life of fish meat during storage (Chytiri et al., 2004). Meanwhile, the methods of catching and killing can play a significant role in changing the quality of fish. The killing of fish by releasing in a dry environment and suffocation, which is accompanied by a lot of struggles, causes severe physiological changes like reduction of adenosine triphosphate in muscle and liver, and an increase in lactic acid in the fish body. Therefore, such activities which cause severe physiological changes before the death of the fish, can lead to the shortening of the time to complete the rigor mortis process and cause qualitative changes in the processed product (Maeda et al., 2014). Before harvesting, stress affects blood and tissue indices. Stressors stimulate the central nervous system, and as a result, the hypothalamic-pituitary-internal axis is activated and releases cortisol and catecholamines into the bloodstream (Stoskopf & Posner, 2008). Cortisol causes hyperglycemia during stress. Also, more energy accumulation and consumption increase muscle activity, anaerobic glycolysis, and plasma lactate (Stoskopf & Posner, 2008).

The quality loss of fish meat after slaughter includes changes in the color and texture, including fragmentation and softening of muscles, weakening of muscle fibers, and changes in water-holding capacity (Erikson & Misimi, 2008). The studies show that increasing the stress and activity of fish at the time of death reduces the quality of fish and creates a suitable environment for the growth of bacteria in the fish after death (Gokoglu et al., 2004). For this reason, reducing fishing stress is one of the important concerns of the aquaculture industry. Efforts are being made to ensure that killing the fish, which is one of the most important stages affecting the quality of fish meat, is done properly. Several methods are used to prevent the intense movements of fish during the fishing process, one of which is the use of anesthetic chemical compounds (Maeda et al., 2014). Nicotine and related alkaloids found in tobacco are generally known as narcotics, and due to the low cost of tobacco and its extensive cultivation in different countries, it can be a suitable option for anesthetizing fish (Agokei &

Adebisi, 2010). The effect of cloves as an anesthetic on reducing the cortisol level and stress response in rainbow trout has been proven (Sink et al., 2007).

So, by considering the cost of chemical drugs and their side effects on some chemical parameters of the fish body, the importance of anesthesia before harvesting, and the use of sustainable methods in aquaculture, the aim of this study was the usage of suitable herbal drugs as an alternative to chemical compounds, to anesthetize the fish in a better way before harvesting.

MATERIAL AND METHODS

In this study, 30 healthy rainbow trout (weighed 200 ± 5 g), were randomly selected and divided into 3 groups: control, treatment 1 (clove (*Eugenia aromatica*) powder 750 mg/l), and treatment 2 (tobacco (*Nicotiana rustica*) powder 800 mg/l). After anesthetizing the fish with clove and tobacco extracts, all of them were caught by the suffocation method. Immediately after the death, blood samples were taken from the heart and after coagulation were centrifuged at 10,000 rpm for 10 minutes. The amounts of glucose and lactate in the serum were measured with the commercial kits (Pars Azmoon Company) and auto-analyzer (model Alycon 300, United States) (Lacerda et al., 2009). Then the fish meat was kept in the refrigerator at 4°C for 7 days and the changes in thiobarbituric acid (TBA) and total volatile nitrogen (TVB-N) parameters were measured during storage (AOAC, 2002). For the microbiological test, the skin of the fish was separated in an aseptic condition. After preparing serial dilutions, the total number of mesophilic bacteria and psychrotrophic bacteria were determined by pour plate method in Plate count agar medium (Merk) after incubation at 37°C for 48 hours for mesophilic bacteria and 7°C for 10 days for psychrotrophic bacteria (Salari-Joo et al., 2012). Statistical analysis was done using Minitab 16 software. After examining the normality, data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. A p-value less than 0.05 was determined as a significant difference.

RESULTS

According to the results, the lactate level in the serum of control samples was significantly higher than the treatment groups ($p < 0.05$). Also, the lactate level in the blood of fish anesthetized with clove was lower than those anesthetized with tobacco (Figure 1).

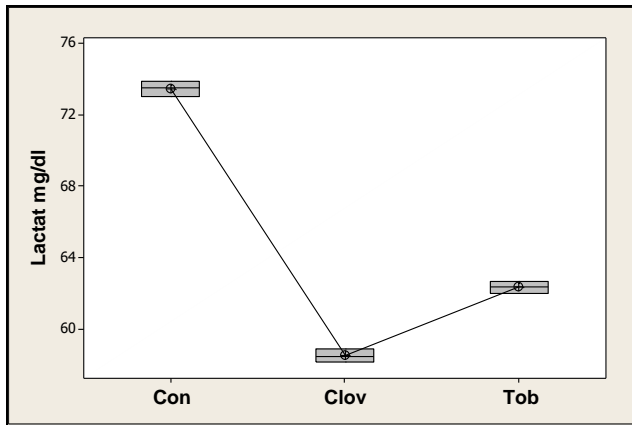


Figure 1. Comparison of plasma lactate in different groups (Con=Control, Clov=Clove, Tob=Tobacco)

In addition, there was a significant difference between the level of plasma glucose in the control and the treatment groups ($p < 0.05$), and the level of glucose in the blood of control samples was the highest. The reduction of glucose level in the case of samples treated with cloves was more than that of samples treated with tobacco (Figure 2).

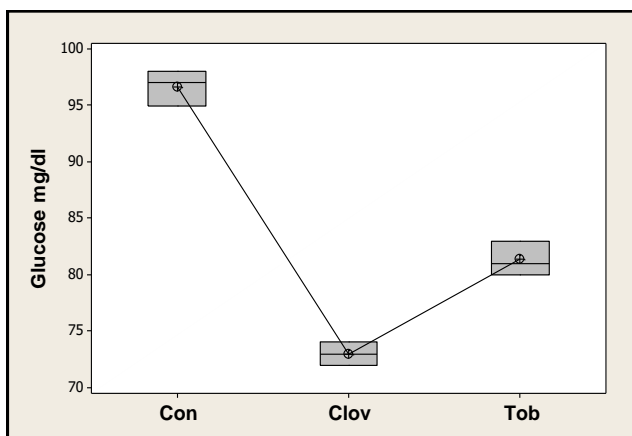


Figure 2. Comparison of plasma glucose in different groups

The results of TBA showed an increasing trend from day 1 to day 7. The amount of TBA in the control sample on the first day was significantly higher than the clove sample ($p < 0.05$), but its difference with the tobacco sample was not significant ($p > 0.05$). On the fourth day, there was no significant difference

between the TBA values in the control and tobacco groups, and despite the apparent difference, there was no significant difference ($p > 0.05$) in the case of the clove and control groups. On the seventh day, there was a significant difference between the amount of TBA in the control and cloves, but no significant difference was observed with tobacco. Therefore, it can be concluded that anesthesia of fish can reduce the TBA value during fish meat storage and compared to tobacco, clove has a better effect on TBA reduction (Figure 3).

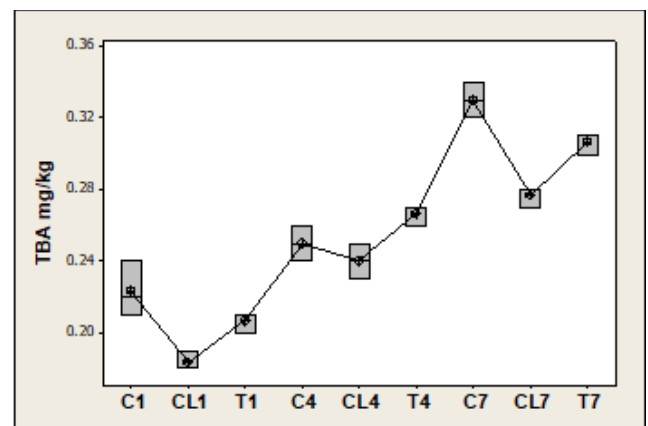


Figure 3. Comparison of TBA values of fish meat samples in groups (C=control, CL=cloves, T=tobacco, 1=1st day, 4=4th day, 7=7th day)

According to the results of the TVB-N test, its value has increased from day 1 to day 7. The amount of TVB-N on the first day in the control sample was significantly different from the clove and tobacco samples ($p < 0.05$). On the fourth and 7th days of meat storage, a significant difference was observed between TVB-N values in the control group and the clove and tobacco groups ($p < 0.05$). It can be concluded that anesthetizing fish before harvesting can reduce the amount of TVB-N production during meat storage and clove compared to tobacco has a better effect in reducing TVB-N value (Figure 4).

The results of microbial count showed that the number of mesophilic bacteria increased from day 1 to 7, but this increase in the groups treated with cloves and tobacco was significantly less than the control group. The lowest number of mesophilic bacteria was seen in the group treated with clove (Figure 5).

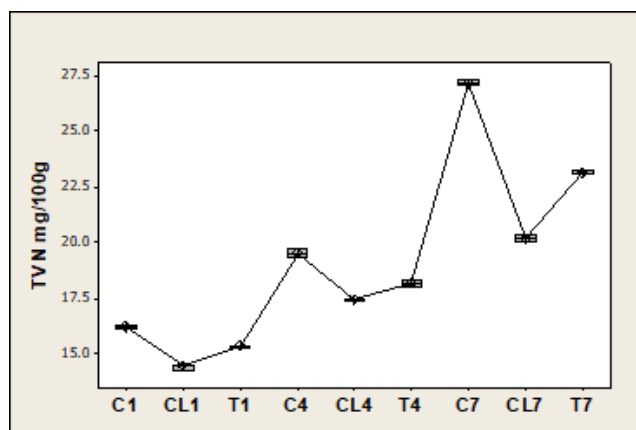


Figure 4. Comparison of TVB-N values of fish meat samples in groups (C=control, CL=cloves, T=tobacco, 1=1st day, 4=4th day, 7=7th day)

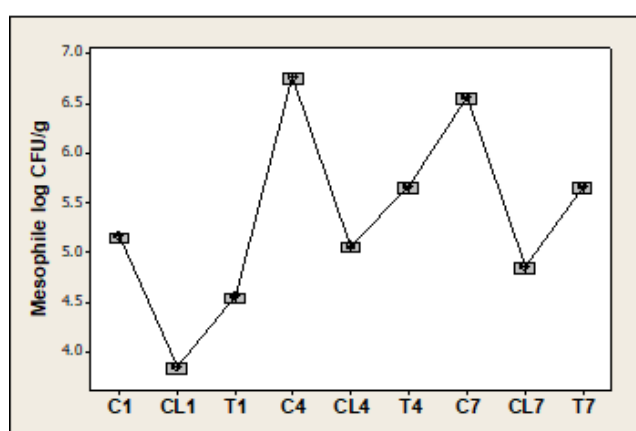


Figure 5. Changes in mesophilic bacteria number in fish meat samples during storage (C=control, CL=cloves, T=tobacco, 1=1st day, 4=4th day, 7=7th day)

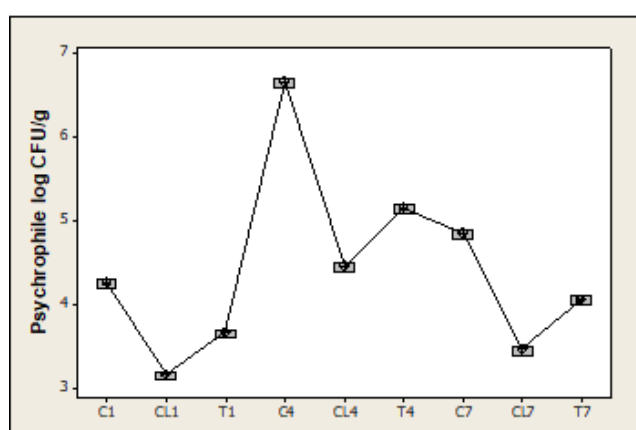


Figure 6. Changes in psychrophilic bacteria number in fish meat samples during storage (C=control, CL=cloves, T=tobacco, 1=1st day, 4=4th day, 7=7th day)

The number of psychrophilic bacteria also increased from day 1 to 4 but then decreased in day 7.

This decrease, in the case of cloves and tobacco was significantly more than the control group. The best result in the reduction of psychrophilic bacteria in day 7 was for samples treated with clove (Figure 6).

DISCUSSION

Currently, because of the possibility of the negative impact of chemical anesthetics and sedatives on fish, their use has decreased (Summerfelt & Smith, 1990). So, essential oils/extracts of different plants have been studied for fish anesthesia. Most of these plants belong to Lamiaceae, Verbenaceae, Lauraceae, and Myrtaceae. In addition, menthol, linalool, myrcene, cineole, globulol, spathulenol, guaial, caryophyllene oxide, terpinene-4-ol, and dehydrofukinone are herbal compounds that their anesthetic effects have been studied in fish. All tested herbal materials compared with clove oil and eugenol (the most common herbal anesthetic) are less efficacious to anesthetize fish (Hoseini et al., 2019). Between the herbal plants, tobacco and cloves have more stated criteria for a suitable anesthetize. In addition, since tobacco and clove extracts are plant compounds, it is expected to be easily decomposed in the environment and have no adverse environmental effects, also in comparison to chemical drugs, they are more available and can be provided at a cheaper price (Marking & Meyer, 1985). Our study showed the effectiveness of these plants on fish anesthetic and fish meat quality.

According to the results, there was a significant difference in lactate level in serum between the control and the treatment groups ($p < 0.05$). The highest amount of lactate was related to control samples and the lowest amount was in treatments anesthetized with cloves. In fact, by anesthetizing the fish with cloves and tobacco before catching, the level of stress in fish can be reduced and this leads to a decrease in blood lactate. The effect of clove on lowering the lactate level was greater than tobacco. In another study, Stress blood indicators in the asphyxia group were significantly higher than other two groups and the control group that this is the same as the data presented in the experiment expressed that fish of the group received clove oil had significantly higher quality and less stress response than other groups (Rahmanifarah et al., 2010).

According to the results, the highest amount of glucose in serum was related to the control samples and then samples treated with tobacco. The fish that were anesthetized with clove had the lowest amount of glucose in the serum. It can be said that by anesthetizing the fish before catching, the stress level of the fish reduced and this led to a decrease in blood glucose. This result is in accordance with another study that examined the stress response of anesthesia and some quality indicators of common carp. In their study, the lowest amount of glucose corresponds to the samples treated with cloves, also the group that received clove oil had significantly higher quality and less stress response than the ice group (Moini et al., 2011).

The results showed that in all samples, the TBA value increased from day 1 to day 7. This may be due to an increase in free iron and peroxides in the muscle. Aldehydes can be produced as a secondary oxidation product from the breakdown of hydroperoxides, which can be a reason for the increasing trend of hydroperoxides (de Azevedo Gomes et al., 2003). However, the TBA index may not express the real degree of oxidation and these reactions change to a great extent according to fish species (Aubourg, 2000). Our results also showed that during storage there was no significant difference in the TBA value of the control sample and samples treated with tobacco but clove had a better influence on reducing TBA value and the amount of TBA in samples treated with clove was lowest among other samples. Studies have shown that the conditions before the slaughter of fish significantly affect the TBA content of meat, which confirms the trend of changes in the results of this study (Lago et al., 2010).

Our study showed an increasing trend in TVB-N value in all samples. According to other studies, during the storage period of fish meat in the refrigerator, a significant increase in the TVB-N content was observed (Duran et al., 2008). TVB-N is mainly produced by the decomposition of proteins and non-protein nitrogenous compounds by bacterial and enzymatic activities. The increase in this factor during storage can be related to the increase in the microbial load of meat (Gram & Huss, 1996). Our results also showed that during storage TVB-N value

in the control sample was significantly ($p < 0.05$) higher than in samples treated with clove and tobacco. In fact, anesthetizing fish before harvesting by clove and tobacco due to their antimicrobial effect reduces TVB-N production during meat storage. Compared to tobacco, clove had better reduction influence. Antimicrobial effects can affect the amount of TVB-N production and keep it at an acceptable level (30 mg/100 g) for fish meat (Özyurt et al., 2009). The values obtained for TVB-N in this study were consistent with the results of other researchers (Ojagh et al., 2010).

In all samples, the number of mesophilic bacteria increased from day 1 to 7. Fish meat has a low amount of carbohydrates and a small amount of lactic acid which are factors that influence the growth of spoilage bacteria. On the other hand, the presence of non-protein nitrogenous compounds such as free amino acids and nucleotides has turned fish meat into a suitable substrate for the growth of microorganisms (Gram & Huss, 1996). According to our results, the increase in the mesophilic bacteria in groups treated with cloves and tobacco was significantly less than the control group, and the lowest number of mesophilic bacteria was seen in group treated with clove. Cloves and tobacco also had an effect on the number of psychrophilic bacteria and the influence of clove on the reduction of psychrophilic bacteria was greater than tobacco. In fact, because of the anesthetic property of cloves, at the time of death, fish activity decreases and the quality of the final product becomes higher than the control treatment. In cases where anesthesia is not applied, increasing the stress and activity of the fish at the time of death will provide a suitable environment for the growth and multiplication of bacteria in the fish body after death, and the quality of the fish will decrease and spoilage will occur sooner (Gokoglu et al., 2004).

Traditional medicines based on plant extracts have been extensively utilized in humans and animals as remedies to control diseases. Their constituents have antibacterial, antifungal, antioxidant, anti-inflammatory, anthelmintic, and digestive medicinal properties. As aquaculture fish are intended for human consumption, it is important to search for new compounds that besides having anesthetic potential

increase the safety of their products. Anesthetic agents are compounds that are widely used to ensure fish welfare, reduce stress and avoid inflicting pain during handling and health interventions. As interest in natural anesthetic products has increased, essential oils and extracts derived from medicinal plants due to their low cost, easy accessibility, efficacy, and environmental safety can be alternative options for fish anesthesia. So, it is necessary to search for new natural compounds with commercial interest and anesthetic effects (Golomazou et al., 2016).

CONCLUSION

In this study, the experiments conducted on the biochemical factors of blood serum in rainbow trout showed that the stress and also the amount of glucose and lactate in the samples anesthesia with clove were lower and the quality of fish samples during storage was better. In fact, spoilage indices like TBA, total volatile nitrogenous bases, psychrophilic and mesophilic bacteria had the lowest amount in clove-treated samples and after clove, tobacco had the best result in fish quality. So, due to the characteristics of these two plants such as availability, low cost, compatibility with the environment, safety for human consumption compared to chemical substances, and sustainability in aquaculture, they can be considered as effective and desirable aesthetic substances for keeping the quality of fish meat during storage.

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Compliance with Ethical Standards

Authors' Contributions

SB: Investigation, Writing – original draft, Methodology

AP: Conceptualization, Supervision

EC: Formal Analysis, Writing – review & editing

ŞSC: Writing – review & editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The IAU Ethics Committee has approved the research with 1403/30 ethical approval number on February 22, 2025.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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