



## Effect of Traditional Marinating on Bacterial and Chemical Characteristics in Frozen Rainbow Trout Fillet

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### Abstract

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**Background:** In recent years, there has been an increasing interest in using food additives from natural sources to improve taste and also extend shelf life of semi-preserved food. The aim of this paper was to examine chemical and microbiological changes promoted by a traditional marinating process in rainbow trout fillets during frozen storage.

**Methods:** Fish fillets were immersed in traditional marinades and stored at  $-18^{\circ}\text{C}$  for 56 days. Some chemical and microbial characteristics like total volatile basic nitrogen (TVN), thiobarbituric acid (TBA), water holding capacity (WHC), pH, mesophilic, and psychrophilic bacterial count were performed with 7 days interval. The results were analyzed by repeated measure ANOVA test using SPSS v. 16.0 software.

**Results:** Data showed that although the value of TVN, TBA and, WHC in marinated samples were higher than control ones, but the differences were not significant ( $p>0.05$ ). The numbers of bacteria in marinated samples were lower than control groups and for psychrophilic bacteria was significant ( $p<0.05$ ).

**Conclusion:** The most obvious finding to emerge from this study is that rainbow trout flesh could be marinated and stored at  $-18^{\circ}\text{C}$  for at least 56 days with no major unfavorable changes.

### Introduction

The rainbow trout (*Oncorhynchus mykiss*) is one of the most widely introduced fishes on a global basis. This fish has the ability to culture in most parts of Iran and has been one of the most desirable farmed fish in the last two decades (FAO, 2010; Iranian Fisheries Organization, 2009). Rainbow trout is very closely related to salmon which having the highest content of polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid compared to other fish and seafood (Cunnane and Griffin, 2009). Fatty fish such as rainbow trout has limited shelf life and quality deterioration of this species is mainly caused by rapid growth of microorganisms and

lipid oxidation (Gram et al., 1990). So, the off-odor and off-taste of the products affect the consumer acceptability (Moini et al., 2009; Rostamzad et al., 2010). Today, the addition of synthetic preservatives, antioxidants, colorants to extend shelf life has been revised by authorities due to certain health problems. Use of natural preservative has been the subject of many investigations (Ahmadi et al., 2014; Attouchi and Sadok, 2012; Halliwell et al., 1995; Nirmal and Benjakul, 2012).

Marinades are the solutions, including sugar, spices, oil and vinegar or fruit juice that already has been used to improve the tenderness, juiciness, flavor and aroma (Cadun et al., 2008). Marinating process slows down the

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bacterial and enzymatic activity and provides test tenderness, textural and structural changes with a prolonged shelf life (Sallam et al., 2007). To the best of our knowledge, several studies regarding seafood marinating by tomato sauce (Kilinc and Cakli, 2005), rosemary extract (Cadun et al., 2008), holy basil, paper-garlic (Pakawatach et al., 2009) and acetic acid with NaCl (Kilinc and Cakli, 2004) have been documented.

People in south-west of Iran made a local marinade by immersion of fish fillets in a mixture of lemon juice, grated fresh garlic, salt, turmeric, red chili powder and black pepper. This marinade has a great reputation and the public interest as well. This method could be used as an industrial process in order to provide a semi-preserved and ready to cook fish for consumers using natural antioxidants. The aim of the present study was to investigate the chemical and microbiological changes promoted by the local marinating process in rainbow trout fillets during frozen storage.

## Materials and methods

### Raw materials and process

This experimental study consisted of three trials and in every trial, 20 fish fillets (approximately weight 100-120 g) were used. Similar to a fish processing plant, the fillets were washed under tap water and placed in a clean basket to dry. Fillets were separated in two groups. The first group was immersed in marinated mixture as described below for 3 h at 4 °C. Second group was left in the same condition without marinating as the control group.

### Preparation of marinating mixture

In a bowl, 500 ml industrial lemon juice, 250 g grated fresh garlic, 60 g table salt, 3 g turmeric, 1 g hot chili powder, 3 g black pepper and 100 ml distilled water were mixed well as marinating mixture.

### Sample preparation

All marinated and normal fish samples separately were placed in a plastic container with lid and incubated in a household freezer (-18 °C) for 56 days and examined on days 0, 7, 14, 21, 28, 35, 42, 49 and 56 in terms of microbiological and chemical features.

### Chemical analysis

Fish samples were defrost in a domestic refrigerator and then homogenized using a normal blender. Total volatile basic nitrogen (TVN) were measured according to method with Malle and Poumeyrol (1989), briefly 10 g fish flesh, 1 g magnesium oxide and 60 ml distilled water

were put in distilling flask (Bakhshi, Iran). The samples were boiled and distilled into 40 ml of boric acid containing methyl red as indicator. After the distillation, the contents of conical flask were titrated with H<sub>2</sub>SO<sub>4</sub> and expressed as mg of TVN/100 g muscle. The value of thiobarbituric acid (TBA) was determined according to Wrolstad et al. (2005). About 5 g sample were blended with 100 ml of 10% TBA for 3 min and filtered. The solution content was increased to 100 ml by adding extra TBA (10%). Then, 4 ml aliquot was transferred into each test tube and 5 ml TBA (0.02 mol) was added and incubated in 90 °C for an hour. Absorbance at 532 nm was measured using a spectrophotometer (Cecil, Swiss). The results expressed as mg of malondialdehyde per kg of fish. Also, pH was determined for the homogeneous mixtures of fish with distilled water (1:10 w/v); using a digital pH meter (Sartorius, Germany) as described by Benjakul et al. (1997). Water holding capacity (WHC) was measured according to the method of Omana et al. (2010).

### Microbiological analysis

For microbiological counts, blended fish muscle (10 g) was mixed with 90 ml of normal saline and stomached (Interscience, France) for 3 min. Further serial decimal dilutions were made up to 10<sup>-4</sup> by transferring 1 ml dilution to 9 ml buffered peptone water, and then 0.1 ml of each dilution was inoculated onto the surface of nutrient agar (Fluka 70152, Switzerland) plates in duplicate. Two series of cultured agar were made, and the first group was incubated for 7 days at 7 ± 0.5 °C for estimated total psychrophilic bacteria count (TPC). The second group was incubated for 24 h at 35 °C for measuring total mesophilic bacteria count (TMC) according to Kilinc and Cakli (2004).

### Statistical analysis

The results were analyzed by repeated measure ANOVA test using SPSS Inc. software (v. 16.0, Chicago, IL). Means were considered statistically different at 95% confidence levels.

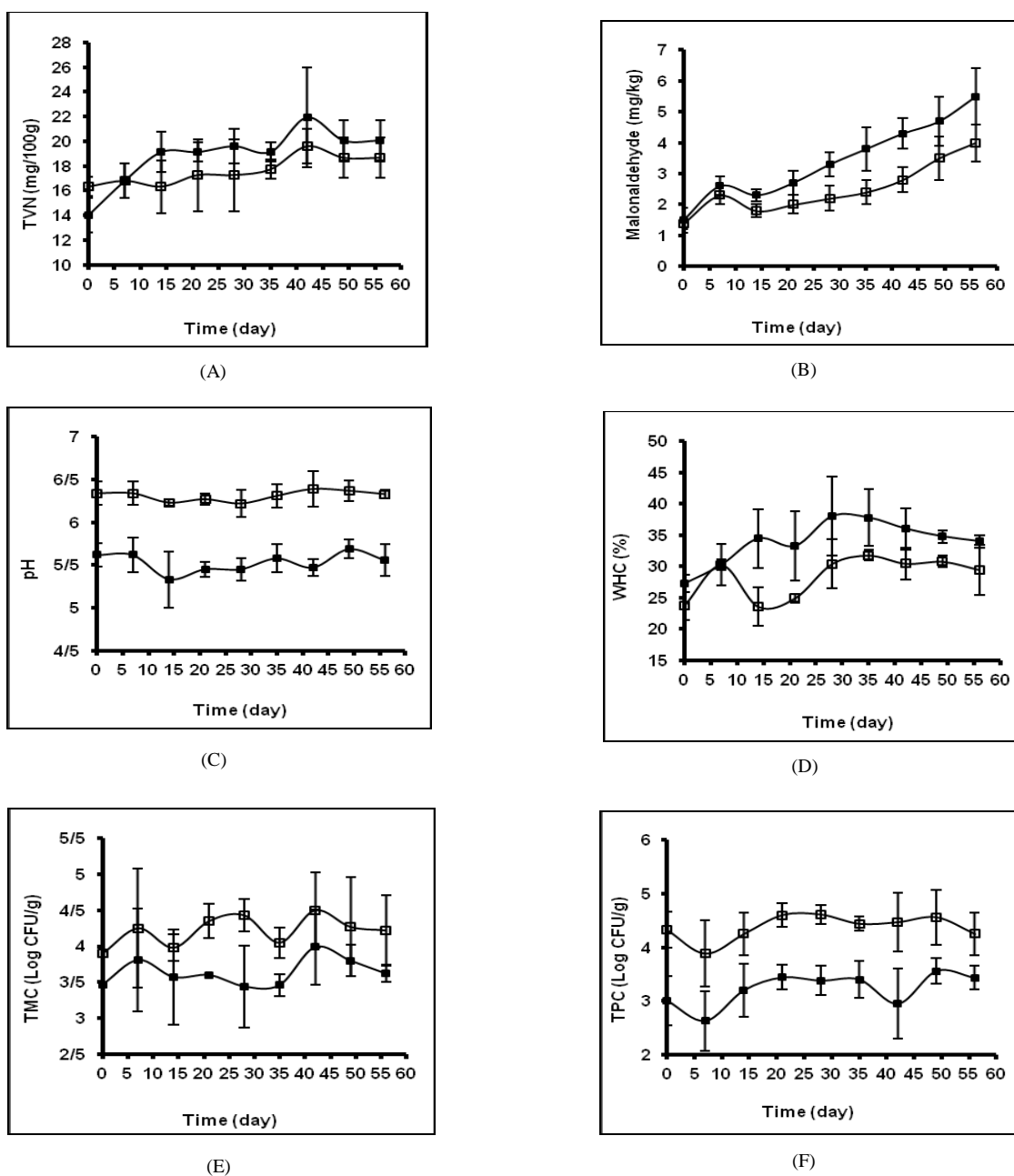
## Results

The TVN value for control and marinated samples are presented in Fig. 1-A. Initial value for marinated samples (14 mg/100 g) was increased during storage. This amount at day 8 was 16.8 and reached to 21.93 mg/100 g at the end of storage. The value for control samples was variable from 16.33 to 19.6 mg/100 g. However, statistical analysis did not show a significant difference ( $p > 0.05$ ) between control and marinated samples. As shown in Fig. 1-B, the changes in TBA value (mg

malonaldehyde/Kg) from days 0 to 56 for control and marinated samples. TBA value in both groups was increased in parallel during storage period between 1.5 mg/kg to 5 mg/kg. Although, the amount for marinated samples was higher than controls, the differences between both groups were not significant ( $p>0.05$ ).

Initial pH of marinated and control fish samples was 5.6 and 6.3, respectively (Fig. 1-C) with a significant difference ( $p<0.05$ ). The changes in WHC value are shown in Fig. 1-D. Although, this amount was increased, the differences between marinated and control samples were not significant ( $p>0.05$ ).

The initial number of the total mesophilic bacteria count in control and marinated fish samples was 3.9 and 3.47 log (CFU/g) and increased to 4.22 and 3.62 log after 56 days storage time, respectively (Fig. 1-E). The number of the mesophilic bacteria in marinated samples always was lower than control samples but it was not significant ( $p>0.05$ ). Contrary to these results, difference between the numbers of the psychrophilic bacteria in control and marinated samples was significant ( $p<0.05$ ), where the total psychrophilic bacteria in control and marinated samples were increased from 4.23 and 3 to 4.25 and 3.43 log, respectively at the end of storage (Fig. 1-F).



**Fig. 1:** Mean values and standard error of three ( $n=3$ ) independent determinations for value of TVN (A), TBA (B), pH (C), WHC (D), TMC (E) and TPC (F), in control and marinated fish samples □: control; ■: marinated

## Discussion

TVN value is a good indicator for determining the spoilage levels in fresh and semi-preserved seafood. In general, the highest acceptable level of TVN in seafood has been determined 35 mg/100 g (Arashisar et al., 2004). The initial level of TVN in range of 10-21 mg/100 g in fresh rainbow trout flesh (Chytiri et al., 2004; Ozogul et al., 2010) and the highest acceptable level in rainbow trout stored in modified atmosphere, 25 mg/100 g has been reported (Gimenez et al., 2002). In a study by Pakawatchai et al. (2009), preservation of minced salmon flesh stored at 4 °C by herb and spice pastes, holy basil and pepper-garlic was determined. They reported that the initial level of TVN in all samples increased from 9 mg/kg to 12-14 mg/100 g over 12 days storage. Surprisingly, the control sample had the lowest TVN value during storage. However, the findings of this study did not support the previous research which mentioned that the level of TVN in marinated samples have been lower than controls (Kilinc and Cakli, 2004; Ozyurt et al., 2012). In our study, although the initial level of the TVN in marinated samples was lower than control but the value in marinated samples was dramatically increased after 5 days and then not significantly remained higher than control samples during storage. The TVN levels in all samples were still under the standard limit (less than 35 mg/100 g). The higher TVN level in marinated samples may be due to a synergistic effect between freezing and some protease enzymes remaining in the ingredients used in the marinade (Pakawatchai et al., 2009).

TBA analysis is widely used for the detection of oxidative rancidity in oil and food samples. In some studies, the maximum TBA value in a good quality of frozen fish, chilled or stored with ice, 5 mg malonaldehyde/kg and for consumable fish flesh, up to level of 8 mg malonaldehyde/kg has been reported (Bremner, 2002). It seems that this value may be variable based on fish type, temperature and storage time. For example 15 mg malonaldehyde/kg has also been proposed as the acceptable TBA value in mackerel fish stored at -3 to 10 °C (Nishimoto et al., 1985). This value for rainbow trout fillet after 6 and 12 days stored in 4 °C has been published 1 and 1.8 mg/kg, respectively, which is much less than mackerel fish (Zolfaghari et al., 2011). In another study, TBA value in vacuum packed rainbow trout fillet after 15 days storage in 4 °C up to 2.3 mg/kg has been calculated (Pezeshk et al., 2012). Our results showed that TBA value for both control and marinated samples was increased during storage. About 2 mg extra malonaldehyde per kg fish flesh was observed in marinated samples compared to control group after 56 days. Although, it may show that the marinating process could be led fish fats to rancidity, statistical analysis showed that this difference was

not significant. The effect of fish marinating on changes of TBA value during storage has been subjected of few studies. For example effects of combining of smoking and marinating by alcohol vinegar and salt on the shelf life of anchovy stored at 4 °C was subject of an investigation. TBA value was significantly increased from 1.9 to 4.25 mg/kg after the storage of 6 months (Ozogul et al., 2010). In another study, the shelf life of pasteurized and no pasteurized sardine marinades with acetic acid, sodium chloride, tomato sauce and spices was investigated. TBA value of pasteurized sardine was increased from 4.33 mg/kg to 8.14 mg/kg and TBA value of no pasteurized sardine increased from 4.47 mg/kg to 8.21 mg/kg at 6 months of storage. The differences between TBA and pH value of both groups were not significant (Kilinc and Cakli, 2005). According to Pakawatchai et al. (2009), rancidity in minced salmon flesh stored at 4 °C was investigated and result was shown that in sample with holy basil paste added, TBA value significantly was higher than the control. The sample with pepper-garlic paste added, TBA value was lower compared with the control after 12 days storage. They concluded that this may be markedly due to the pro-oxidant effect of chlorophyll and other impurities presence in holy basil. Also, presence of antioxidant compounds such as alliin, diallylsulphide, allyl sulphide and propyl sulphide derived from garlic and piperine in black pepper could retard lipid oxidation immediately during mixing and during storage (Pakawatchai et al., 2009). Also, an antioxidant effect by turmeric was observed in vacuum packed rainbow trout fillet during 15 days storage at 4 °C. TBA value in marinated samples was double than control after 10 days storage (Pezeshk et al., 2012). Again, this result was attributed to the antioxidant effect of turmeric. In our work, different materials such as lemon juice, garlic, turmeric, hot chili, etc. with different characteristics were used and so, an overlapping effect could be happened. In another study, TBA value on rainbow trout stored at 4 °C from 0.1 mg/kg in first day increased to 2 mg/kg at day 12 and then reduced to 1.4 at day 18 (Zolfaghari et al., 2011). Similarly, in current study although the samples were stored in freezer during storage, TBA value was firstly increased and then some reduction was observed at day 15. These findings were in agreement with previous studies which mentioned that TBA value maybe reduced several days after storage (Goulas and Kontominas, 2007; Jeevanandam et al., 2001).

Different pH could be useful to evaluate the qualitative changes in fish during storage. The initial pH in fresh fish flesh was in range of neutral but decomposition of nitrogenous compounds lead to an increase in pH during storage. The increase in pH indicated the loss of quality.

In contrast, decrease of pH in stored fish flesh could be observed due to the acid which is a common metabolite from growth of a number of bacteria include lactic acid bacteria, Enterobacteriace and *photobacterium phosphorum* (Hansen et al., 1996). The initial pH value in sardine 5.83 to 6.2 (El Marrakchi et al., 1990; Gokoglu et al., 1998), in anchovy 5.8 (Ozgoul et al., 2010) and in rainbow trout 6.7 (Pezeshk et al., 2012) has been reported. In the present study, the initial pH value of the marinated samples (5.6) was significantly ( $p < 0.05$ ) lower than control samples (6.4) and was almost stable for both groups during storage. This difference could be due to use of acetic acid (lemon juice) for fish marinating. A similar result to our findings was observed by Ozgoul et al. (2010) where the factor was stable in smoked and marinades anchovy after 6 months storage. However, the findings of the current study do not support some previous researches. For example, pH was decreased in marinades rainbow trout in turmeric extract after 20 days storage at 4 °C (Pezeshk et al., 2012) or increased in sardine marinades in tomato sauce according to storage time (Kilinc and Cakli, 2005).

As mentioned before, in the case of WHC value, a non-significant difference was observed between marinated and control samples. Various factors, such as the addition of acid, temperature changes, enzymatic and bacterial degradation could be effective in this regard.

Growth of bacteria is the most important factor in spoilage of fish. In this study, an initial reduction in bacterial viable counts was observed by marinades. As shown in Fig. 1-E and Fig. 1-F, marinating process was more effective on psychrophilic bacteria than mesophilic bacteria and number of psychrophilic bacteria in marinated samples was significantly lower than controls. Surprisingly, there was no extra killing effect on bacteria by marinades and the number of cells in marinated, and control group was almost stable during storage. These findings seemed to be consistent with other research which found that microbial load was decreased just after the shrimps were marinated. Also, it was reported that bacteria were not completely killed by marinating, and live cells were still able to grow in marinated samples. In this condition, they were able to continue their activity more or less rapidly according to their ability to adapt to the medium during storage (Cadun et al., 2008). It can therefore be assumed that concentration of materials in our marinades was not able to prevent bacterial growth during storage. The current data also accords with previous observations, which showed a positive effect on reducing the number of bacteria in the samples stored at different conditions using natural preservatives such as mixture of garlic and black pepper (Pakawatchai et al., 2009), turmeric (Pezeshk et al., 2012), tomato sauce

(Kilinc and Cakli, 2005) and mixture of acetic acid and salt (Kilinc and Cakli, 2004).

## Conclusion

To the best of our knowledge, there is no data regarding the effect of Iranian traditional marinades on the chemical and microbiological changes in fish fillets during frozen storage. The present study was designed to determine the effect of a local marinating on chemical and microbial characteristic of rainbow trout flesh under freezing condition. It is well known that semi-preserved marinades foods like chicken or fish may not allowed be selling after 24 h in ambient temperature, but freeze marinated fish could be have longer shelf life. The most obvious finding was that rainbow trout flesh could be marinated and frozen for at least 56 days with no major unfavorable changes and at the end of the storage, both marinated and control samples were found consumable.

## Conflicts of interest

None declared.

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