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The Effects of Lactic Acid on Chemical Properties of Rainbow Trout (*Oncorynchus mykiss*) Fillets

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Abstract: Fresh rainbow trout (*Oncorynchus mykiss*) fillets were treated with lactic acid at three concentrations [(0 % (control), 2 % and 4 % lactic acid], aerobically packaged and stored at 4 ± 1°C for 9 days. Chemical analysis were done at 0, 1, 3, 5, 7 and 9 days. The storage time and lactic acid treatment caused significant ($p<0.05$) changes in TBARS, TVB-N and pH values. TBARS values in tissue of control group increased the highest acceptable level (25 μ mol kg^{-1}) at day 7. However, that of fillets treated with lactic acid of 2 and 4 % concentrations reached this degree at day 5 during the experimental period. While TVB-N levels reached above 20 mg/100g in control group on the third day, the groups with 2 and 4 % lactic acid reached the same level on the 5th day. Moreover, pH value of all groups did not exceed the limit of acceptability. Considering present data, it was concluded that the used concentrations of lactic acid in this study acted as a pro-oxidant on aerobically packaged rainbow trout fillets. Therefore, it should be taken care using lactic acid as a preservative agent because of its detrimental effect in lipid oxidation of especially aerobically packaged lean fish fillets.

Key words: Lactic acid, fish fillet, lipid oxidation, TVB-N and pH

INTRODUCTION

Preservative agents are required to prevent spoilage in foods Chemical preservatives has been used for this purpose for many years in the food industry. The most common preservative agents are the weak organics acids such as lactic acid. It has been reported that lactic acid retarded the growth of Gram-negative bacteria in fish[1,2]. There is a common belief that the inhibition action of preservatives to be due to the compound crossing the plasma membrane in the undissociated state with low pH. Diffusion of the preservative continues until equilibrium[3]. Many studies have shown the inhibition of growth by weak acid preservatives, for instance, membrane disruption[4], inhibition of essential metabolic reactions [5], stress on intracellular pH homeostasis[6] and accumulation of toxic anions[7].

Although the influence of lactic acid on the microbiological spoilage and shelf life of seafoods has been investigated extensively[1,8-11] the studies on the effects of lactic acid on chemical properties, especially lipid oxidation, are unfortunately scarce in fish products. Therefore, the aim of this study was to test the effect of lactic acid on chemical properties of aerobically packaged rainbow tout fillets at 4°C for 9 days.

MATERIALS AND METHODS

Fish source, treatment and packaging: Fresh water rainbow trout with an average weight of 200 g provided from Research and Extension Center of Fisheries Department in Agricultural Faculty at Atatürk University in Erzurum were transferred to the Meat Processing Laboratory in Food Science Department and decapitated and filleted by hand. The fillets were divided into five groups with three replicates and treated 0 % (control), 2 and 4 lactic acid for 10 min. After dipping they were drained at room temperature, individually wrapped in cling film and stored in a refrigerator at 4 ± 1°C. The fillets groups were subjected to chemical analyzes on the 0, 1, 3, 5, 7 and 9th days of the storage period.

pH measurements: The pH of the triplicate fish muscle samples was measured using a Schott model pH meter (Schott, Lab Star pH), 10 g fish muscle sample was combined with 100 mL^{-1} distilled water and homogenized in an Ultra turrax (IKA Werk Tp 18-10 20.000 rpm) for 30 sec.

Lipid oxidation: Lipid oxidation was determined by the Thiobarbituric Acid Reactive Substances (TBARS) method [12]. The triplicate samples (1 g) were placed in a test tube and homogenized with 6 mL^{-1} Trichloroacetic Acid (TCA) solution (TCA %7.5; EDTA %1; propyl

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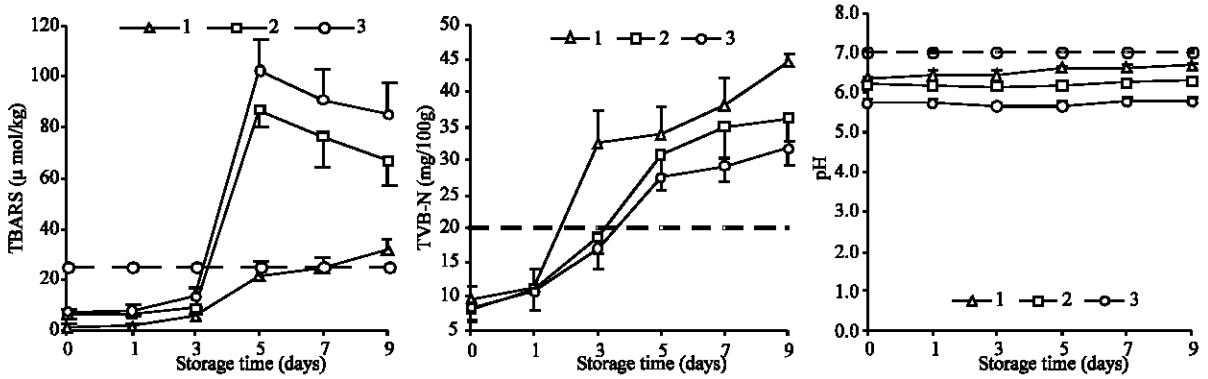

Fig. 1: Effects of the two different concentration of lactic acid on TBARS levels (a), TVB-N levels (b) and pH values (c) of rainbow trout fillets treated with 0 % (control) (1); 2 %; (2) and 4 %; (3) aerobically storaged at 4 ± 1 °C for 9 days. Upper areas of horizantal lines are unacceptable in each Figure.

gallate, solved in 3 mL^{-1} ethanol, %0.1) for 15-30 sec at high speed. The homogenates were filtered through Whatman No.1 paper. The filtrates (1 mL) were transferred to test tubes and 1 mL 0.02 M Thiobarbituric Acid (TBA) solution was added. The mixtures were vortexed and then incubated in a 100°C water bath for 40 min to develop color. After cooling for 5 min under running tap water, the samples were vortexed and centrifuged at 2000 rpm for 5 min at 4°C. The absorbances of the resulting upper layers were read at 532 nm against a blank solution. TBARS values were calculated from a standard curve of Malondialdehyde (MDA) prepared by 1,1,3,3 Tetraoxipropan (TEP) as µ mol kg^{-1} sample(Fig.1a)

Total Volatile Base Nitrogen (TVB-N): The TVB-N concentration is determined by steam distillation method according to Anonymous,[13] . Exactly 10 g of the muscle sample are weighed in a container, mixed with 90 mL (0.1 N) perchloric acid solution, homogenized for two minutes with an Ultra turrax (IKA Werk Tp 18-10 20.000 rpm) and then filtered. Fifty milli liter of the extract was taken and several drops of phenolphthalein, a few drops silicone anti foaming agent and 6.5 mL of sodium hydroxide (20%) solution added. Then the extract was put in an apparatus for steam distillation and steam distillation was started.

The steam distillation was regulated so that around 100 mL of distillate was produced within 10 min. The distillation outflow tube was submerged in a receiver with 100 ml boric acid solution which three to five drops of the indicator solution (2 g Methyl-red and 1 g Methylene-blue are dissolved in 1000 mL 95 % ethanol) were added. After exactly 10 minutes the distillation was ended. The volatile bases contained in the receiver solution were determined by titration with standard hydrochloric solution. The results were expressed as mg TVB-N /100g.

Statistical analysis: Total volatile bases nitrogen (TVB-N) lipid oxidation (TBARS) and pH values of rainbow trout fillets were determined at 4±1°C. Data were checked for normal distributions with normality plots prior to one-way Analysis Of Variance (ANOVA) and followed by Duncan's Multiple Range test to determine significant differences among means at α= 0.05 level.

RESULTS AND DISCUSSION

Before storage, the pH of the samples treated with 0 (control), 2 and 4% lactic acid were measured as 6.3, 6.2 and 5.7, respectively. The value of control group increased and reached up to 6.7 in at the end of storage. However, it decreased in treated samples due to the effect of the lactic acid and as compared to control, their pH values were significantly ($p<0.05$) low. The pH values were about 5.7-6.3 for treated fish fillets and increased during storage (Fig. 1a). The limit of acceptability is usually 6.8-7.0 [14]. Present study neither control group nor the treated with lactic acid groups exceed acceptable limit. Similarly, it was reported that catfish fillets were dipped in lactic acid and their pH values were significantly decreased ($p<0.05$)[1].

TVB-N values of treated samples were lower ($p<0.05$) than control samples after the third day of storage. TVB-N value of control samples were 32.7 mg /100g fish at the third day. However, TVB-N values of treated samples with 2% and 4% lactic acid were 18.8 and 17.2 at the third day, respectively(Fig.1b). Gimenez *et al.*,[15] have proposed 25 mg TVB-N per 100 g fish as the limit of acceptability for fish. Control samples were over the limit of 25 mg/ 100 g fish after the third day of storage and they were spoiled according to maximum acceptable limit. However, treated samples did exceed the limit of 25 mg /100 g fish after the fifth day of storage.

Lactic acid was probably responsible for the elevated TBARS values. It seems that the lipid peroxidation is caused by acidic condition maintaining iron as ferrous ion [16]. Liu [17] reported that iron catalysed oxidation is pH-

sensitive and is most active under acidic conditions. And it has been reported that ferrous ion can induce peroxidation[18]. Gandemer[19] reported that the decomposition of preformed hydroperoxides into peroxyl radicals is catalyzed by ferrous ions which results a removal of a hydrogen radical from the unsaturated fatty acids. Lipid oxidation propagation is increased by decomposition of hydroperoxides since hydrogen is removed at a faster rate by these radicals than initial alkyl radicals.

CONCLUSIONS

present data showed that addition of 2 and 4% lactic acid to rainbow trout fillets aerobically packaged and stored at 4±1°C competed with control in terms of retarding of TVB-N and pH values. However, TBARS values appeared to be negatively affected by the application of the different concentration of lactic acid. The treatment of lactic acid was recommended for chup mackerel fillets for increasing shelf-life and retarding microbial spoilage by Metin *et al.*[8]. However, considering this fact the results of present study suggested that if lactic acid is used in aerobically packaged fish fillets, it can cause the lipid oxidation problems. Therefore, it should be taken care using lactic acid as a preservative agent because of its detrimental effect in lipid oxidation of especially aerobically packaged lean fish fillets.

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