

## The Effects of Different Combinations of Eugenol and Acetic Acid on Quality Changes of Marinated Rainbow Trout (*Oncorhynchus mykiss*, W. 1792) Fillets

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### Abstract

In this study, fillets of trout marinated with different concentrations of acetic acid (2% and 4%) and eugenol (0.1% v/w and 0.5% v/w) were preserved at 4 °C. The samples were analyzed in terms of sensory, microbiological and chemical quality. During the preservation, the total mesophilic anaerobe and *Lactobacillus* showed significant changes ( $p<0.05$ ). Eugenol showed no significant effect on the salt and pH ( $p>0.05$ ), whereas thiobarbituric acid (TBA) showed significant changes ( $p<0.05$ ) in both groups and the days of preservation. In sensory analysis, flavor and general acceptability significant differences between the groups ( $p<0.05$ ) during the preservation time were shown. In conclusion, marinades protected their consumed properties for at least 70 days and eugenol had favorable effects on some microbiological and chemical values. The marinades prepared with 0.1 % of eugenol were gained more acclaim in term of sensory.

**Keywords:** Eugenol, marinade, *Oncorhynchus mykiss*, acetic acid, Vacuum package.

## Marine Edilmiş Gökkuşuğu Alabalığı (*Oncorhynchus mykiss*, W. 1792) Filetolarının Kalite Değişimleri Üzerine Farklı Kombinasyonlarda Eugenol ve Asetik Asitin Etkisi

### Özet

Bu çalışmada, farklı konsantrasyonlarda asetik asit (%2 ve %4) ve eugenol (%0.1 v/w ve %0.5 v/w) ile marine edilen alabalık filetoları 4 °C de muhafaza edilmiştir. Örnekler, duysal, mikrobiyolojik ve kimyasal kalite yönünden analiz edilmiştir. Muhafaza süresince, mezofilik ve Laktik asit bakterileri önemli derecede değişim göstermiştir ( $p<0.05$ ). Eugenol muhafaza süresince her iki grupta da, pH ve tuz üzerinde önemli bir etki göstermezken ( $p>0.05$ ), tiyobarbitürik asit (TBA) önemli derecede değişimler göstermiştir ( $p<0.05$ ). Duyusal analizde, lezzet ve genel kabul edilebilirlik muhafaza süresince gruplar arasında önemli farklılıklar göstermiştir. Sonuç olarak, marinatlar 70 gün süresince tüketilebilirlik özelliklerini korumuş ve eugenolün bazı mikrobiyolojik ve kimyasal değerler üzerine olumlu etkileri olmuştur. %0.1 eugenolle hazırlanan marinatlar duysal bakımdan daha fazla beğeni kazanmıştır.

**Anahtar Kelimeler:** Eugenol, marinat, alabalık, asetik asit, Vakum paket.

### 1. Introduction

Marination is one of the fish processing methods and it is the technology used for maturing frozen or salted fish or fish meat with or without the effect of temperature, by treating the product with acetic acid and / or other organic acids and salts. Such products obtained by marination process are called 'marinade'. This technique was developed in Germany and

spread to other countries. Marinades are semi-preserves. Acid is typically acetic acid and when used with salt, it prevents bacteria and enzyme activities for a while and provides the product with unique taste and extended shelf life [1]. Marinade technology is quite advanced; there are three types of marination depending on processing technique and supplementary material added in the product. These are cold marinades, cooked marinades, and fried marinades. There is

no heat treatment on cold marinades and the obtained product's shelf life ranges from 15 days to 6 months. On the other hand, in cooked marinades, fish marinated in brine is cooked by seething in vinegar vapor. Different products can be obtained by adding a variety of sauces or gelatin. In fried marinades, fish are fried between 160 °C and 180 °C and then are subjected to marinating [2, 3]. Essential oils are naturally volatile products obtained from leaves, fruits, husks, and roots of aromatic plants. They have antiseptic, antioxidant, digestive stimulant, antimicrobial, and enzymatic effects. A large part of essential oils are in the GRAS (Generally Recognized as Safe) list; moreover, essential oils are identified as having many positive biological effects on human health, including anticarcinogenic effects. Eugenol is an essential oil obtained from carnations, and it constitutes approximately 70%-80% of carnation extract. It is insoluble in water but easily soluble in alcohol or oil. Besides the use of eugenol as a spice, it is also used as a dental antiseptic [4, 5]. It has been determined to increase the shelf life of foods [6].

This study was conducted to determine effects of different combinations of eugenol and acetic acid on quality changes of marinated vacuum packed rainbow trout (*Oncorhynchus mykiss*, W. 1792) fillets.

## 2. Material and Method

### 2.1. Raw materials

Eugenol oil was purchased from Sigma-Aldrich (E51791). Rainbow trout (*Oncorhynchus mykiss* W.1792), each weighting 200-250 g were obtained from the local fish market in Elazığ, Turkey. Fish were transferred within one hour to the fish processing the laboratory of the Faculty of Fisheries of Firat University, in sealed foam boxes containing ice.

### 2.2. Eugenol addition and Marination process

The fish were beheaded, gutted manually and then washed. Then, the fish were divided into three groups;

The first group: Control group, 5 % (v/w) olive oil was added onto the surface (two sides) of each fillet using a micropipette

The second group : 5 % (v/w) olive oil + 0.1% (v/w) eugenol oil were added onto the surface (two sides) of each fillet using a micropipette

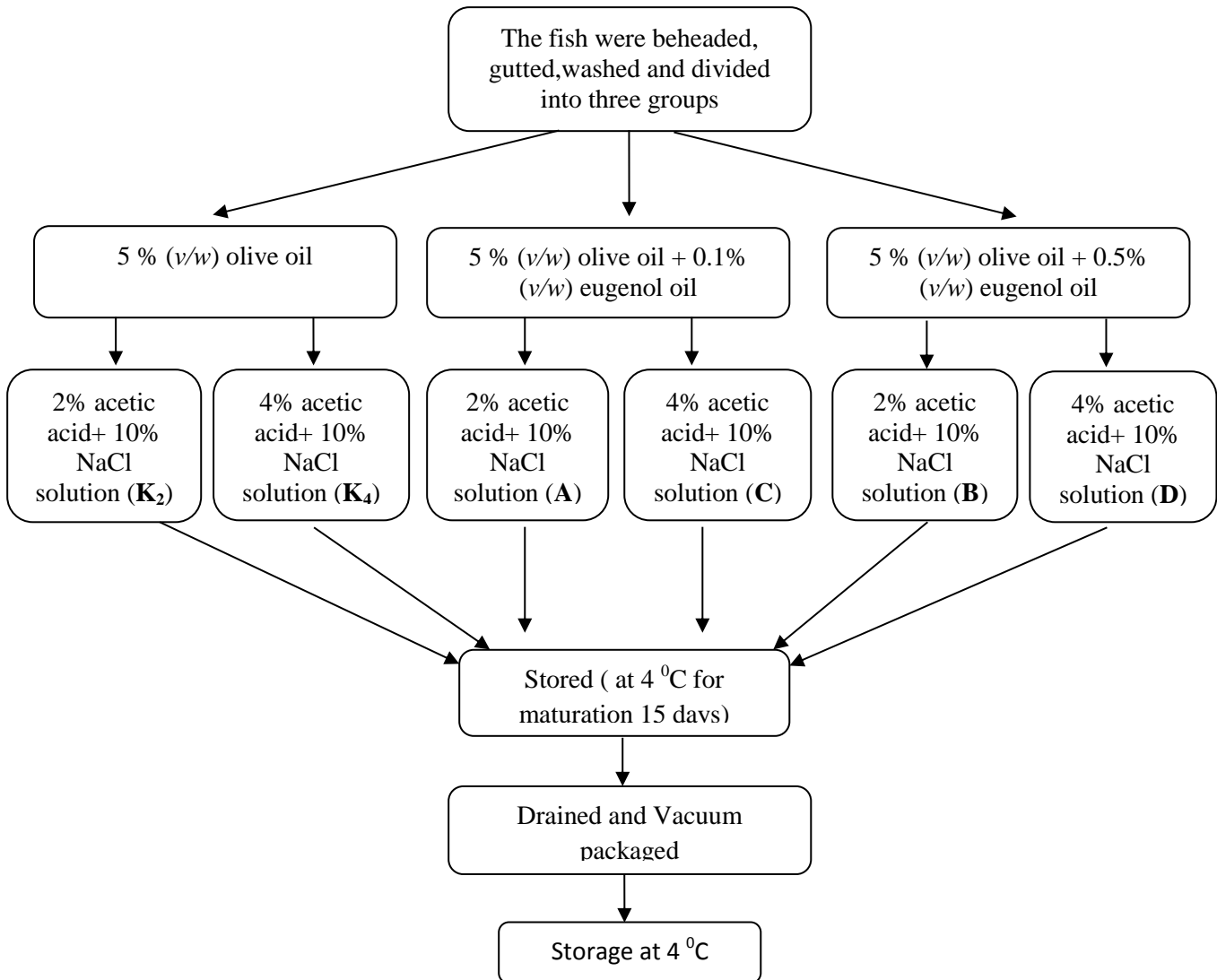
The third group: 5 % (v/w) olive oil + 0.5% (v/w) eugenol oil were added onto the surface (two sides) of each fillet using a micropipette. These groups were re-divided into two group and put on the jars (approximately, 3-L capacity). Then was added in the marinating solution at two different concentrations (2% acetic acid+ 10% NaCl, 4% acetic acid+ 10% NaCl). The jars were stored at 4 °C for maturation 15 days. This solution was drained after maturation. Samples were vacuum-packaged and stored in a refrigerator (4°C) and analyzed as organoleptic, chemical, and microbiological aspects on the 1st, 7th, 14th, 21st, 28th, 42nd, 56th, 70th, 84th, and 98th days of the storage period. The study was carried out in duplicate

### 2.3. Microbiological analyses

A sample of 10 g was taken aseptically from each sample, transferred to a stomacher bag and 90 ml of sterilized peptone water (Buffer Peptone Water, LAB M) were added, and the mixture was homogenized for 2 min with a stomacher (Stomacher 400, Lab. Blender, London, UK). Samples (0.1 ml) of serial dilutions of marinated trout homogenates were spread on the surface of agar plates. Plate count agar was used for psychrotrophic bacteria and incubated at 7 °C for 10 days. Lactic acid bacteria were counted on de Man Rogosa Sharpe agar (MRS, Oxoid, CM361) incubated at 30°C for 5 days. Enterobacteriaceae was determined on Violet Red Bile Glucose Agar (Oxoid CM 485) after incubation at 37°C for 1 day. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g) [7].

### 2.4. Chemical analyses

pH and NaCl content of fish samples were measured by standard methods, following AOAC protocols [8].



**Figure 1.** Experimental design

Thiobarbituric acid value (TBA mg MDA/kg) was determined by a distillation method, according to this method 10 g of sample was macerated with 50 mL water for 2 min after then washed with 47.5 mL water into a distillation flask. 2.5 mL of 4 M HCl was added to bring the pH to 1.5. The flask was heated by means of an electric mantle so that 50 mL distillate was collected in 10 min from the time boiling commences then 5 mL distillate was blended into a glass-stoppered tube with 5 mL TBA reagent. After that it was shaken and heated in boiling water for 35 min. A blank was prepared using 5 mL water with 5 mL reagent. Then the tubes were cooled in water with 10 min and measured the absorbance against the blank at

538 nm using 1 cm cell [9]. TBA number is achieved according to the following formulae that D is absorbance of samples at 538 nm:

$$\text{TBA (mg MDA/kg)} = 7.8 \times D$$

## 2.5. Sensory analysis

Ten experienced panellists (6 female and 4 male, age 28-50), who were members of academic staff and trained in sensory descriptors for marinated trout, were used to evaluate the quality of rainbow trout fillets during storage. Sensory analysis was performed using the methods of Kurtcan and Gonul [10]. Panellists were asked to evaluate sample taste and overall

acceptability on a 5-point hedonic scale ranging from very poor (1) to very good (5).

## 2.6. Statistical analysis

The experiment was replicated three times on different occasions with different marinated fillet samples. Triplicate samples were made per attempt. Data were subjected to analysis of variance (ANOVA) using the General Linear Models procedure of the Statistical Analysis System software of SAS Institute [11]. Differences among the mean values of the various treatments and storage periods were determined by the least significant difference (LSD) test, and the significance was defined either at  $p < 0.05$ .

## 3. Results and Discussion

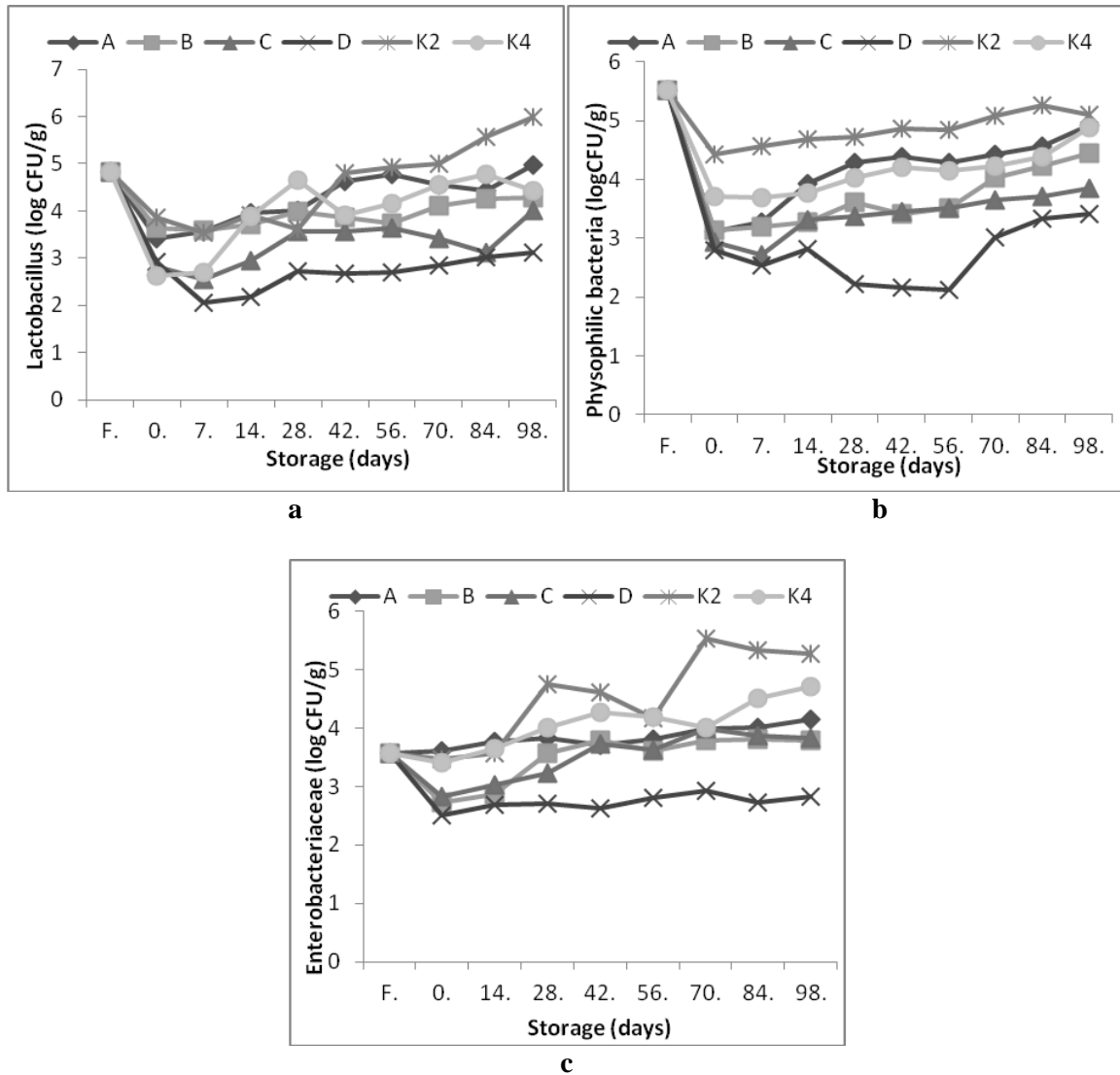
### 3.1. Microbiological changes

Lactobacillus count in fresh rainbow trout fillet which was detected to be 4.82 log<sub>10</sub>cfu/g on average was decreased to 2.63 - 3.85 log<sub>10</sub>cfu/g levels as a result of the marination ( $p < 0.05$ ). Again, it was determined that acetic acid and eugenol were effective on lactobacillus during the storage period ( $p < 0.05$ ). In a related study [12], studied the effects of different essential oils on Lactobacillus and determined that rosemary was the most effective followed by sage, thyme, and clove (Fig. 2. a).

Psychrophilic bacteria count in the fillet was detected to be 5.53 log<sub>10</sub>cfu/g on average. This value decreased in all groups at the beginning of the storage ( $p < 0.05$ ), usually with an increase in the following days reaching to 3.42 - 5.11 log<sub>10</sub>cfu/g level at the end of the storage period (98th day). In a previous study [13], psychrotrophic bacteria count of frozen sardines was determined to be 4.88 log<sub>10</sub>cfu/g in the fresh sample. In the same study, psychrotrophic bacteria counts of pasteurized and unpasteurized marinades were determined to be  $< 10$  cfu/g throughout the storage period. In another study analyzing the antioxidant effect of rosemary extract on deep-water pink shrimp (*Parapenaeus longirostris*) marinades in the storage period [14] psychrotrophic bacteria count of the raw material was 5.25 log cfu/g; it decreased below the

undetectable level ( $< 10$  cfu/g) in the control group and in the samples supplemented with 300 ppm of rosemary extract at the end of marination and throughout the storage period. In a study on marinated warty venus [15], psychrotrophic bacterial count was detected to be  $< 10$  cfu/g until the 22nd day of the storage period. In the same study, psychrotrophic bacteria count increased in the following days of the storage, and was determined as  $2.0 \times 10^2$  at the end of the storage. In our study, the difference between the groups containing essential oils and the control groups was detected to be insignificant in terms of the psychrotrophic bacteria count ( $p > 0.05$ ). Among groups containing eugenol oil, statistically significant differences were determined between B and C groups on the 7th day of the storage; between B, C, and D groups on the 14th day; between C and D groups on the 28th day; and between C and D groups on the 42nd day ( $p < 0.05$ ). Thus, it was concluded that the differences between C and D groups as well as other groups were significant depending on the storage time and a significant difference was detected between C and D groups on the 42nd day of the storage ( $p < 0.05$ ). The findings of this study differ from the data obtained in the relevant literature. This is considered to be due to the fact that the types of essential oils and their concentrations were different from those in the previous studies (Fig. 2. b).

Enterobacteriaceae count in the trout filled was determined to be 3.57 log<sub>10</sub>cfu/g on average. This number was detected to be between 2.51 - 3.61 log<sub>10</sub>cfu/g on day 0 of the storage. Among the groups with and without eugenol, the difference between Group D and other groups was significant on the 28th, 42nd, and 56th days of the storage whereas the difference between the K2 group and other groups was statistically significant on the 70th day; again, the difference between the groups with and without eugenol was significant on the 70th day. The difference between Group D and other groups was determined to be significant on the 84th and 98th days of the storage ( $p < 0.05$ ) (Fig. 2. c).



**Figure 2.** Changes in (a): lactic acid bacteria counts (b): psychrotrophic bacteria counts (c): Enterobacteriaceae counts of marinated *Oncorhynchus mykiss* fillets during storage at 4°. **A:** % 2 acetic acid + % 0.1 eugenol; **B:** % 2 acetic acid + % 0.5 eugenol; **C:** % 4 acetic acid + % 0.1 eugenol **D:** % 4 acetic acid + % 0.5 eugenol; **K2:** % 2 acetic acid **K4:** % 4 acetic acid **F:** Fillet

### 3.2. Chemical Changes

pH is an important intrinsic factor related to fish flesh. During storage, decomposition of nitrogenous compounds leads to a rise in pH in the fish flesh, which may be partly attributed to the production of alkaline compounds. Such increase in the pH indicates the bacterial growth, loss of quality and possible spoilage. Since pH influences the susceptibility to microbial growth, by the addition of acids, is used in the preservation of many foods, including fish products [16]. The pH of the raw rainbow trout fillets used in this study was 5.77. Marination

with both 2% and 3% acetic acid resulted in decrease ( $p < 0.05$ ) in the initial pH by about 1-1.5 units (Fig. 3. a).

The pH of marinated fillets in the present study significantly increased ( $p < 0.05$ ) by the end of the storage period. Significant increase in the pH values with the increase of the storage time has also been recognized marinated anchovies, marinated sardine [13] and marinated Pacific saury [16] during their storage at the refrigerated temperature. On the other hand, eugenol has no effect ( $p > 0.05$ ) on the pH. The present result indicated that marinated fillet samples remained

within the acceptability limits for fish consumption.

Salt levels of marinade samples obtained with addition of sage oil and acetic acid at different amounts ranged between 3.19% and 4.46% throughout the storage period. The differences between groups and various storage durations in terms of salt levels were detected to be insignificant throughout the storage period ( $p>0.05$ )

TBA values is a widely used indicator for the assessment of degree of lipid oxidation. It has been suggested that a maximum TBA value, indicating the good quality of the fish and fish product, is 5 mg MDA/kg, while fish may be consumed up to a TBA value of 8 mg MDA/kg [17]. In this study, the TBA value of fresh raw trout was detected to be 0.67 mg MDA / kg on average. TBA value showed different levels of changes as a result of marination and with respect to the groups throughout the storage period and these changes were statistically significant ( $p<0.05$ ). Similarly, other researcher were reported for pH levels of pasteurized and non-pasteurized sardine marinades in tomato sauce increased from 3.84 to 4.09 and 4.19 respectively at the end of the storage period, sea bream [18] and chub mackerel [19], in which the TBA value increased to the maximal level at a certain period during storage, and thereafter it decreased gradually. In addition, Aksu et al. [20] reported that pH values in anchovy marinated with 2% and 4% acetic acid increased from 4.25, 4.18 to 4.53, 4.31 respectively.

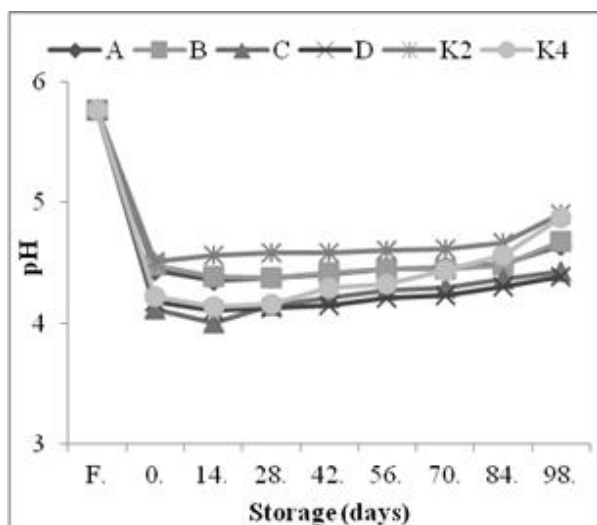
On the other hand, the lowest TBA values were obtained from groups contain eugenol oil (Fig.3a). Some researchers [21, 22], emphasized the inhibitory effect of rosemary, thyme, carnation, and sage on the TBA value and reported that carnation had an antioxidative effect as much strong as BHT and BHA.

### 3.3. Sensorial Evaluation

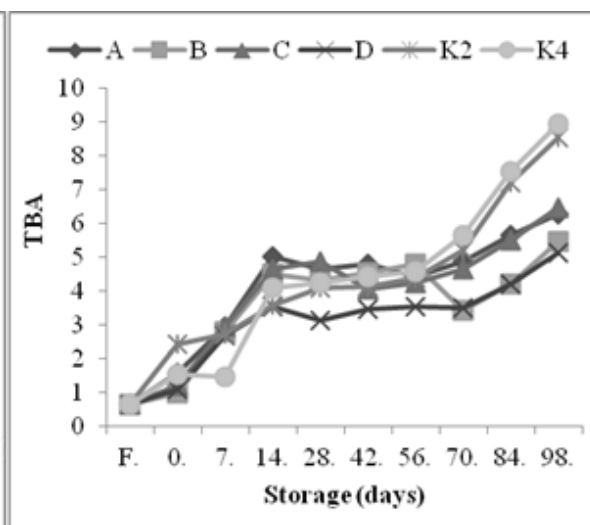
Lipid oxidation and other degradation reactions lead to the formation of low molecular compounds, which contribute to sensory profile. Hydroperoxides and secondary oxidation products can react with protein and amino acids during processing, heat treatment and storage period affecting the flavour, odour and texture of fish products [23]. Sensory characteristics of marined rainbow trout fillets are presented in Fig. 3b-c.

In all groups, the product scored high in terms of taste and general acceptability on the first few days of storage and low in the following days ( $p<0.05$ ). Likewise, it has been reported that the sensory scores of marinated fish significantly decreased with the increase of storage time [16, 24-26].

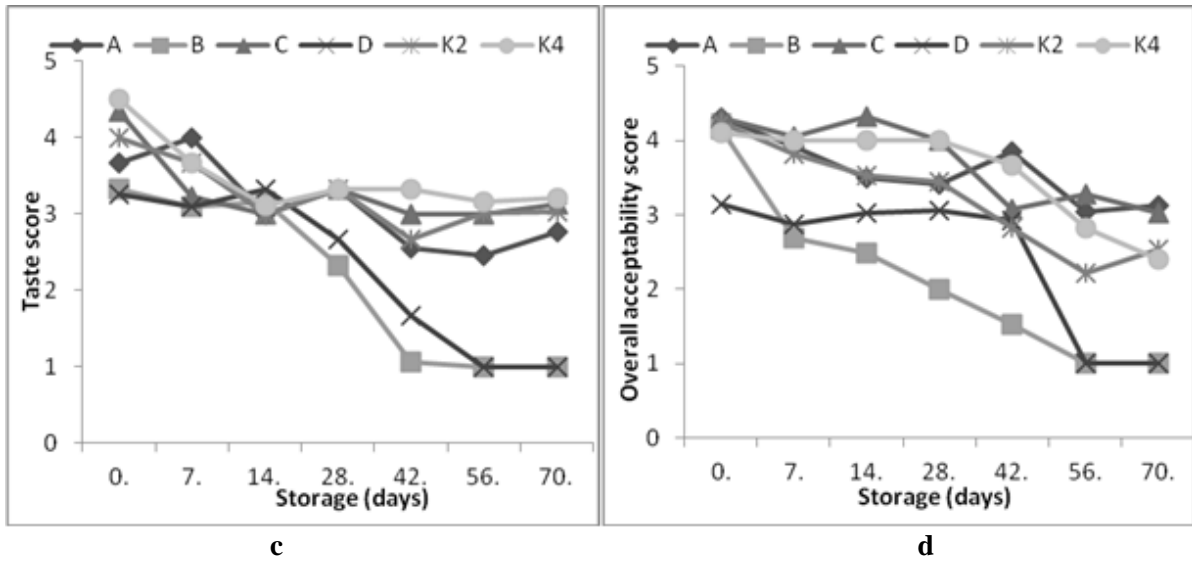
It was observed that groups containing 0.1% and 0.5% eugenol scored the highest in terms of general acceptability ( $p<0.05$ ). On the other hand, no significant difference was determined between the control groups ( $p>0.05$ ). (Fig.3b-c).



a



b



**Figure 3.** Changes in (a): pH (b): TBA (cb): Taste score (d): Overall acceptability of marinated *Oncorhynchus mykiss* fillets during storage at 4°. **A:** % 2 acetic acid + % 0.1 eugenol; **B:** % 2 acetic acid + % 0.5 eugenol; **C:** % 4 acetic acid + % 0.1 eugenol **D:** % 4 acetic acid + % 0.5 eugenol; **K2:** % 2 acetic acid **K4:** % 4 acetic acid **F:** Fillet

#### 4. Conclusion

As a result, it was determined that: marinade samples prepared using by acetic acid and eugenol at different amounts remained consumable for at least 70 days, eugenol had positive impact on certain microbiological and chemical values, however the use of eugenol at high concentrations negatively affected the organoleptic qualities of the product especially in later days of storage, therefore, 0.1% concentration of eugenol is more appropriate, the marinades prepared with this concentration scored higher in terms of organoleptic aspects.

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