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RESEARCH ARTICLE

Effect of Salting and Packaging Treatments on Fresh Rainbow Trout *(Oncorhynchus mykiss)* Fillets During Storage at Refrigerator Temperatures

Pınar OĞUZHAN * Simay ANGİŞ **

* Ardahan University, The Faculty of Engineering, Food Engineering Department, TR-75000 Ardahan - TURKEY
** Ataturk University, The Faculty of Agriculture, Fisheries Department, TR-25240 Erzurum - TURKEY

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Summary

In research was investigated for effect of salting and packaging treatments on the chemical and microbiological properties of rainbow trout fillets stored 25 d at 4±1°C and packaged in vacuum or modified atmosphere (50% CO_2 + 50% N_2) and salted (dry salting and brine salting) with 20% NaCl. Fillets were subjected to microbiological (total aerobic mesophilic bacteria, psychrotrophic bacteria, *Pseudomonas*, lactic acid bacteria, Enterobacteriaceae, yeast and mould) and chemical (pH, thiobarbituric acid reactive substances-TBARS, total volatile base nitrogen-TVB-N) analyzes on certain days (0, 5, 10, 15, 20 and 25th days) of storage. TBARS and TVB-N values were established the highest increase in dry salted samples at the end of storage. Modified atmosphere packaging, in terms investigated of all parameters gave better results than vacuum packaging. According to the results of microbiological analyses, shelf life of rainbow trout fillets was estimated as 10, 15, 20, 25, 15 and 20 days for CV, CM, V1, M1, V2 and M2 respectively

Keywords: *Rainbow trout, Salting, MAP, Vacuum, Shelf life*

Buzdolabı Koşullarında Depolanan Taze Gökkuşağı Alabalığı *(Oncorhynchus mykiss)* Filetoları Üzerine Tuzlama ve Paketleme İşlemlerinin Etkisi

Özet

Araştırmada %20'lik sodyum klorür ile tuzlanan (salamura ve kuru tuzlama) vakum veya modifiye atmosferde (%50 CO_2 + %50 N_2) ambalajlanıp 4±1°C'de 25 gün depolanan gökkuşağı alabalığı *(Oncorhynchus mykiss)* filetolarının kimyasal ve mikrobiyolojik özellikleri üzerine tuzlama ve paketleme işlemlerinin etkisi incelenmiştir. Filetolar depolamanın belirli günlerinde (0, 5, 10, 15 ve 25. gün) mikrobiyolojik (toplam aerobik mezofilik bakteri, psikrotrofik bakteri, *Pseudomonas*, laktik asit bakterileri, Enterobacteriaceae, maya ve küf) ve kimyasal (pH, TBARS, TVB-N) açıdan analiz edilmiştir. Depolama sonunda TVB-N ve TBARS değerleri açısından en yüksek artış kuru tuzlanmış örneklerde tespit edilmiştir. Tüm parametreler incelendiğinde, modifiye atmosfer paketlemenin vakum paketlemeye göre daha iyi sonuç verdiği saptanmıştır. Mikrobiyolojik analiz sonuçlarına göre gökkuşağı alabalığının raf ömrü CV, CM, V1, M1, V2 ve M2 grubu örneklerde sırasıyla 10, 15, 20, 25, 15 ve 20 gün olarak belirlenmiştir.

Anahtar sözcükler: *Gökkuşağı alabalığı, Tuzlama, MAP, Vakum, Raf ömrü*

INTRODUCTION

Microorganisms can reproduce in the muscle tissue of aquatic products because these are not only sensitive but also has reach nutrient content. So, the conservation and handling of aquatic products are very important [1]. One of the conservation processes of aquatic products are salting [2]. Although salting process is itself a preservation technique, for many processing technology (smoking, drying and marinating processes) is used as a preliminary operation. The main purpose of salting the fish meat is a part of the elimination of water. Bacterial activity is largely prevented in high salt concentrations. High salt concentration prevents the development of microflora causing spoilage in fish. In

İletişim (Correspondence)
+90 478 2111244
pinaroguzhan@ardahan.edu.tr

addition, significantly increase the shelf life of fish [3]. NaCl is an essential ingredient in processed meat products for its contribution to the water-holding capacity, prevention of microbial growth, reduction of water activity, facilitating the solubilisation of certain proteins and conferring a typical salty taste by enhancing the flavour of such food products [4]. Vacuum packaging method is a type of passive modified atmosphere. After placing the food into the packaging material suitable for this operation, the package is emptied in the air by vacuum and hermetic (air tight) is made a closure. This method is usually used for preservation of meat products. Vacuum packaging in vacuum, albeit a very small amount will remain at O_2. However, the low percentage of O_2 in the package as soon as possible is used by aerobic microorganisms and CO_2 is produced. These types of products, the proliferation of bacteria and oxidation products is prevented as air is not in the package [5-7]. While abundant data exists on preservation of fish and fishery products using vacuum packaging, including salmon [8-10], ascidia [11], sardine [12,13], trout [14-16]. Modified atmosphere packaging (MAP), is a protecting technique use to extent shelf-life of fish and fish products [17]. Modified atmosphere packaging, elimination of oxygen from inside package and filled with different concentrations of CO_2 and N_2, however, refrigerated storage conditions for aerobic microorganisms, proteolytic bacteria, yeast and mold growth is inhibited [6,18,19]. There are a lot of research related to MAP that extension of the shelf life of fish, including chub mackarel [20], cod [21,22], swordfish [23], rainbow trout [24], salmon [25], bass [26], herring [27], catfish [28]. There is not information in the literature regarding to the effects on chemical and microbiological properties of rainbow trout *(Oncorhynchus mykiss)* fillets prepared with brine method and dry salting method and packaged vacuum or modified atmosphere. The aim of this research is to determine with combined effects of salting (dry salting and brine salting) and packaging (vacuum and MAP) on the shelf-life of refrigerated (4°C) rainbow trout fillets by evaluating certain microbiological and chemical parameters.

MATERIAL and METHODS

Preparing Samples

Fish material, rainbow trout *(O. mykiss)* (250±25 g) were obtained from Ataturk University Agricultural College Fisheries Department's rainbow trout breeding and research center. Fish were carried to laboratory and washed with tap water. The fish (72 samples) were eviscerated, stored until rigor had resolved and then filleted, 144 fillets in total [29]. Fillets were washed again for removing blood and mucous remains. All filleted samples including the control were packaged in obtained from the firm Südpack Verpackungen GmbH+Co (Germany) 15x25 cm PA/PE (Polyethylene/Polyamide) (3-seal bags GB 70) in thickness having an O_2 permeability of 40 cm^3/m^2/day.atm.23°C; N_2 permeability of 24 cm^3/m^2/day.atm.23°C; CO_2 permeability of 145 cm^3/m^2/day.atm.23°C and a water vapour permeability of <3 g/m^2/day.atm.23°C). Fillets, divided into 3 groups. Fresh fish in the first group were evaluated as the control group. The second group were applied at 8°C for 1 h 20% dry salting (NaCl) for each fish. The third group were applied at 8°C for 1 h 20% brine (NaCl) for each fish. Then, fillets were packed by applying vacuum and modified atmosphere.

Treatments included: CV (control samples vacuum packaged) CM (control samples modified atmosphere packaged-50%CO_2 + 50%N_2), V1 (vacuum packaged-dry salted), M1 (modified atmosphere packaged-dry salted), V2 (vacuum packaged-brine) and M2 (modified atmosphere packaged-brine). Each group was included 24 fillets. Rainbow trout fillets stored under refrigeration (4±1°C) and samples were subjected to microbiological (total aerobic mesophilic bacteria, psychrotrophic bacteria, *Pseudomonas*, lactic acid bacteria, Enterobacteriaceae, yeast and mould) and chemical (pH, thiobarbituric acid reactive substances-TBARS, total volatile base nitrogen-TVB-N) analyzes on certain days (0, 5, 10, 15, 20 and 25th days) of storage.

Microbiological Analysis

A sample (25 g) was taken from each trout fillet, transferred aseptically into a stomacher bag containing 225 ml of 0.1% peptone water was added, and the mixture was homogenized for 60 s using a Stomacher (Lab Stomacher Blender 400-BA 7021 Sewardmedical, England) at room temperature. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1% peptonewater) of fish homogenates were spread on petri dish of various agar materials. Total aerobic mesophilic bacteria (TMAB) were enumerated on Plate Count Agar (PCA, Merck 1.05463.0500), and incubated at 30ºC for 2 days. Psychrotrophic bacteria were enumerated on Plate Count Agar (PCA, Merck 1.05463.0500), and incubated at 10ºC for 7 days. *Pseudomonas* were enumerated on cetrimide fusidin cephaloridine agar (CFC, Pseudomonas Agar Base-Oxoid CM0559 + CFC Selective Agar Supplement-Oxoid SR0103) and incubated at 25°C for 2 days. Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe agar (MRS, de Man, Rogosa Sharpe Agar Oxoid CM0361) incubated at 30°C for 2 days. For Enterobacteriaceae (VRBD, Violet Red Bile Dextrose Agar Merck 1.10275.0500) incubated at 30°C for 2 days. Yeast and mould were enumerated on RBC (Rose Bengal Chloramphenicol) Agar (Merck 1.00467.0500) incubated at 25°C for 5 days.

Chemical Analysis

Total volatile basic nitrogen (TVB-N) was determined using the method of Malle and Tao [30]. TVB-N contents were expressed as mg 100/g fish muscle. Thiobarbituric acid reactive substans (TBARS) was determined according to the method of Lemon [31] and Kılıç and Richards [32]. TBARS content was expressed as µmol Malondialdehyde (MDA)/kg fish muscle. pH was determined according to the method of Gökalp et al.[33].

Statistical Analysis

Experiments were replicated twice on two separate occasions with different fish samples. Analyses were run in duplicate for each replicate. All obtained data from this study were subjected to analysis of variance (ANOVA), and followed by Duncan's multiple range test to determine significant differences among means at $\alpha = 0.05$ level, using by SPSS [34].

RESULTS

Microbiological Changes

Changes in TMAB of refrigerated fresh rainbow trout fillets during storage under vacuum and modified atmosphere packaging are shown in *Fig. 1a*. The initial (day 0) TMAB *(Fig. 1a)* of rainbow trout fillets was 3.12 log cfu/g. CV, CM, V1, M1, V2 and M2 rainbow trout fillets exceeded the value of 7 log cfu/g for TMAB, considered as the upper acceptability limit for fresh marine species [35] on days 10, 15, 20, 25, 15 and 20 of storage, respectively. At the end of storage period CV, CM, V1, M1, V2 and M2 respectively, levels of 10.24, 9.94, 8.11, 7.24, 8.53 and 8.51 log cfu/g were reached.

The initial (day 0) Psychrotrophic bacteria *(Fig. 1b)* of rainbow trout fillets was 3.22 log cfu/g. CV, CM, V1, M1, V2 and M2 rainbow trout fillets exceeded the value of 7 log cfu/g for Psychrotrophic bacteria, considered as the upper acceptability limit for fresh marine species [35] on days 10, 15, 20, 25, 15 and 15 of storage, respectively. At the end of storage period CV, CM, V1, M1, V2 and M2 respectively, levels of 10.60, 9.80, 8.24, 7.61, 8.88 and 8.66 log cfu/g were reached. Inhibiting effect of dry salting method was higher than the brine method on the number of total aerobic mesophilic bacteria and psychrotrophic bacteria of fillets during storage.

The initial (day 0) *Pseudomonas (Fig. 1c)* of rainbow trout fillets was 3.50 log cfu/g. At the end of storage period CV, CM, V1, M1, V2 and M2 respectively, levels of 8.33, 8.10, 6.62, 6.16, 7.26 and 7.16 log cfu/g were reached. Initial counts were 2.0 log cfu/g (LAB) *(Fig. 1d)* and 2.0 log cfu/g (Enterobacteriaceae) *(Fig. 1e),* at the end of storage period populations of LAB (8.46, 8.02, 8.52, 8.85, 7.49 and 7.48 log cfu/g) and Enterobacteriaceae (9.55, 9.0, 5.02, 5.33, 5.12 and 5.05 log cfu/g) were recorded for treatments CV, CM, V1, M1, V2 and M2, respectively.

The initial (day 0) yeast and mould *(Fig. 1f)* of rainbow trout fillets was 2.08 log cfu/g. At the end of storage period CV, CM, V1, M1, V2 and M2 respectively, levels of 5.65, 5.40, 4.27, 3.97, 3.96 and 3.84 log cfu/g were reached.

Chemical Changes

- **TVB-Nitrogen:** The amount of TVB-N is an important criteriation in determining freshness of fish and fish products and TVB-N values are increasing in paralel spoilage [36]. The initial (day 0) TVB-N values *(Fig. 2a)* of rainbow trout fillets were 12.42 mg/100 g. CV, CM, V2, M1 and M2 rainbow trout fillets exceeded the value of 25 mg/100 g which an upper acceptable limit TVBN value of 25 mg N/100 for rainbow trout was suggested by [37] on days 15 of storage. V1 rainbow trout fillets exceeded the value of 25 mg/100 g on days 10 of storage.

- **Lipid Oxidation:** Oxidative rancidity may become a problem if higher than normal levels of oxygen are used. Rancidity due to oxidation of polyunsaturated fatty acids (PUFA) in some fish may be a problem in modified atmosphere with O_2 [37-39].

Initial TBARS values *(Fig. 2b)* were 1.95 µmol MDA/kg. At the end of storage period TBARS values 9.19, 8.37, 10.83, 9.99, 9.81 and 9.06 µmol MDA/kg were recorded for treatments CV, CM, V1, M1, V2 and M2, respectively.

- **pH:** pH value of fish meat usually ranges from 5.7-6.6. Fresh fish is close to neutral pH, after the death with be formed the lactic acid firstly falling and then rising again with spoilage [40]. pH values of rainbow trout fillets *(Fig. 2c)* were 6.31. At the end of storage period pH values 6.70, 6.39, 6.29, 6.25, 6.34 and 6.33 were recorded for treatments CV, CM, V1, M1, V2 and M2, respectively.

DISCUSSION

Bacterial growth of modified atmosphere packaged samples are lower than vacuum packaged samples because of CO_2 gas is exist in MAP. The carbon dioxide atmosphere can be considered effectively inhibitory on the total aerobic mesophilic and psychrotrophic flora. Similary results were observed by many other researchers [16,24,41-47]. The application of MAP has been previously reported to extend the shelf life of herring [27], rainbow trout [37], salmon [48], hake [49,50], cod fillets [22,51], Baltic herring fillets [52] and eel [53]. Inhibiting effect of dry salting method was higher than the brine method on the number of total aerobic mesophilic bacteria and psychrotrophic bacteria of fillets during storage.

Similar initial *Pseudomonas* (day 0) were reported for rainbow trout by Frangos et al.[16], Pyrogotou et al.[46] and Mexis et al.[54]. *Pseudomonas* spp. and *Shewanella putrefaciens* were early recognised as putative spoilage inducers in fish muscle and have since then been found in various fish species from fresh- and marine waters as well as in other foods [55-57].

The initial LAB and Enterobacteriaceae numbers 2.0 log cfu/g while this values increased during storage time all groups. Similar findings were found for sardine by Can [47].

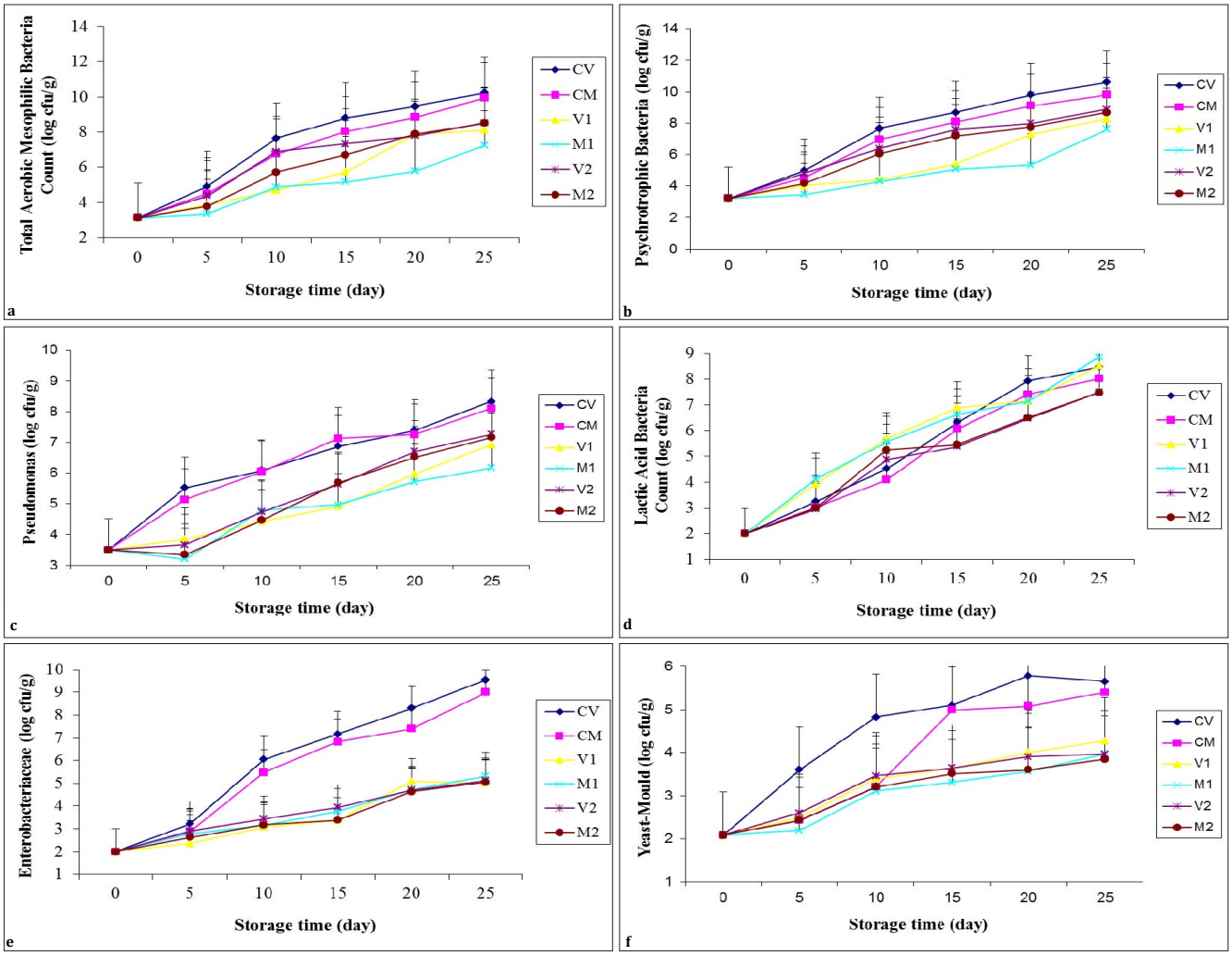

Fig 1. Total aerobic mesophilic bacteria counts (a), psychrotrophic bacteria (b), *Pseudomonas* (c), lactic acid bacteria counts (d), Enterobacteriaceae (e) and yeast and mould (f) changes of salted (brine and dry salting) rainbow trout fillets during cold storage in vacuum and MAP conditions at 4°C

Şekil 1. Soğuk depolama boyunca tuzlanmış (salamura ve kuru tuzlama) alabalık filetolarının 4°C' de vakum ve modifiye atmosfer paketleme şartlarında toplam aerobik mezofilik bakteri (a), psikrotrofik bakteri (b), *Pseudomonas* (c), laktik asit bakterileri (d), Enterobacteriaceae (e), maya ve küf (f)'deki değişiklikler

Similar initial yeast and mould (day 0) were reported for rainbow trout by Can [47] and mud eel by Vishwanath et al.[58].

TVB-N may be considered as a quality index for fish. Its increase related to the activity of spoilage bacterai and endogenous enzymes [59-61]. The initial (day 0) TVB-N numbers 12.42 mg/100 g while this values increased in the duration of storage time all groups. Similarly, TVB-N values have been reported for salmon [41], red mullet [43], trout [62], chub mackarel [63], Atlantic bonito [64] and mahi sefid [65].

In "perfect material" TBA value should be less than 3 mg malonaldehyde/kg, in "good material" TBA value should not be more than 5 mg malonaldehyde/kg and consumption limit for TBA value is between 7 and 8 mg malonaldehyde/kg. TBA values showed the degree of rancidity in the products, and values greater than 3-4 mg malonaldehyde/kg indicated a loss of product quality [24]. Initial TBARS values were 1.95 µmol MDA/kg. Increased TBARS values in the duration of storage time all groups. Similarly, TBARS values have been reported for rainbow trout [24], catfish [28], anchovy [36], mahi sefid [65] and whitefish [66].

The pH in fresh fish flesh in almost neutral. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh. The increase in pH indicates the loss of quality [47]. pH values of rainbow trout fillets were 6.31. Similarly, pH values have been reported for red mullet [43], sardine [47], mahi sefid [65], sea bream [67] and tilapia [68]. pH values of modified atmosphere packaged control group are lower than vacuum packaged samples. This result is occured due to carbonic acid the conversion of carbon dioxide. However pH values of the brine method was higher than the dry salting method. Similary findings were observed by Bilgin et al.[40].

The highest TBARS and TVB-N values were established in the dry salted and vacuum packaged samples while the lowest pH value was determined in the dry salted and modified atmosphere packaged samples at the end of storage.

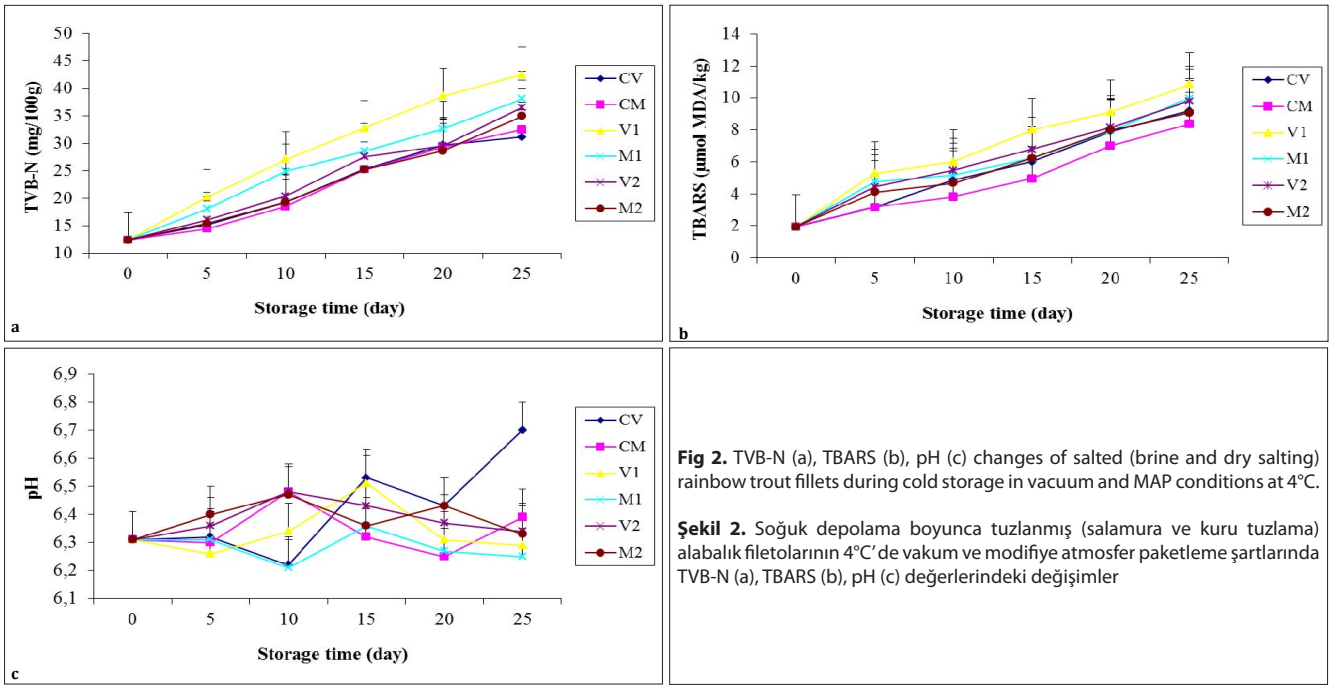

Fig 2. TVB-N (a), TBARS (b), pH (c) changes of salted (brine and dry salting) rainbow trout fillets during cold storage in vacuum and MAP conditions at 4°C.

Şekil 2. Soğuk depolama boyunca tuzlanmış (salamura ve kuru tuzlama) alabalık filetolarının 4°C'de vakum ve modifiye atmosfer paketleme şartlarında TVB-N (a), TBARS (b), pH (c) değerlerindeki değişimler

According to microbiological analyses data, the shelf life of CV, CM, V1, M1, V2 and M2 to 10, 15, 20, 25, 15 and 20 days respectively.

The present study concluded that dry salting and modified atmosphere packaging combination can retard total aerobic mesophilic bacteria, psychrotrophic bacteria, *Pseudomonas* of samples but increase lactic acid bacteria and brine salting and modified atmosphere packaging combination can delay Enterobacteriaceae, yeast and mould of samples during refrigerated storage.

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