

Effect of the packaging on the quality of *Salmo trutta macrostigma* during chilled storage

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Abstract

This study examined the effect of air (A), vacuum (VP) and modified atmosphere packaging (MAP) on the shelf-life of chilled *Salmo trutta macrostigma*. Fresh fish were stored in air, under vacuum and MAP (40% CO₂:60% N₂) under refrigeration (4 ±1°C) for a period of 12 days. Total volatile basic nitrogen (TVB-N) for fish samples stored in A, under VP and MAP exceeded the limit value of 30 mg N/100 g fish muscle after 8, 10 and 12 days of storage, respectively. Thiobarbituric acid (TBA) values were variable in fish samples, indicative of no specific oxidative rancidity trend. Bacteria grew most quickly in fish samples stored in A, followed by those in VP and the lowest counts were with MAP. The observed shelf life of *S. t. macrostigma* was found to be 6 days in A, 10 days in VP and 12 days in MAP.

Keywords

Salmo trutta macrostigma

Modified atmosphere

packaging

Vacuum packaging

Quality parameters

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Introduction

Salmo trutta macrostigma (Dumeril 1858), is a fish belonging to the Salmonidae family, a native and economically important fish occurring in many parts of Anatolia. This subspecies is important in sportive fishing and locally also in folk medicine (Alp *et al.*, 2003; Geliday and Balik, 2007). Fish contain high quality protein, vitamins and other essential nutrients are an important part of a healthful diet. In fact, a well-balanced diet that includes a variety of fish can contribute to heart health and aid in children's proper growth and development. However, because of its composition (high moisture and protein content) and high pH, fish meat is ideal for growth of spoilage and pathogenic microorganisms (Sivertsvik *et al.*, 2002; Pantazi *et al.*, 2008).

The increasing demand for high quality fresh seafood has intensified the search for technologies that favor fresh fish utilization. Packaging technologies are important to protect products against deteriorative effects, which may include microbial, biochemical, and physical activities from environmental influences. This involves retardation of spoilage, extension of shelf-life, and maintenance of quality in packed food.

Modified atmosphere packaging (MAP) is an effective food preservation method for fish, meat and poultry products that allows retention of their "fresh" character. The principle of MAP is the replacement of packaged air with a different, fixed gas mixture. Such an atmosphere change can affect the microflora of the

product and result in shelf-life extension. Common gases used in MAP are carbon dioxide (to inhibit bacterial growth), oxygen (to prevent anaerobic growth and to retain meat color) and nitrogen (to avoid oxidation of fats and package collapse). These gases can be applied individually or in combination in order to achieve an optimum effect. CO₂-enriched atmospheres are able to suppress aerobic spoilage microorganisms such as *Pseudomonas* spp., which are responsible for off-odor and off-flavor development in fish. The success of MAP in extending seafood shelf life depends on many factors, including good initial product quality, good hygiene during slaughter, correct packing material selection, packing equipment, appropriate gas mixture and gas-to-product volume (g/p) ratio for the product, and maintenance of the process temperatures (Phillips, 1996; Sivertsvik, 2002).

The objectives of this study were to investigate the effect of vacuum and MAP on the shelf-life of refrigerated fresh *Salmo trutta macrostigma* by monitoring microbiological, biochemical and sensory changes in the product as a function of packaging treatment.

Materials and Methods

Sample preparation

130 fresh *S. t. macrostigma* were caught during the spring period (May-June) of 2012 in the

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Munzur Stream in Tunceli located, in the eastern Anatolia region of Turkey. The Munzur Stream is an important water supply in Turkey and also a fishing area for commercial and amateur fishing. The fish were repackaged in insulated styrofoam boxes with ice and delivered to the laboratory in less than 4 h. The average weight and length of the *Salmo trutta macrostigma* were 160 ± 55.94 g and 24.10 ± 4.5 cm, respectively. On the day of processing, the fish were eviscerated and washed with clean water.

The fish were separated into three lots to be packaged in three different atmospheres, including treatments: air (A), vacuum (VP) and MAP (40% CO₂, 60% N₂). Experiments were conducted twice and in each study three replicate samples were analyzed for each treatment, involving 126 fish (3 treatments x 3 replicates x 7 sampling times x 2 experiments). The fish samples in A, VP and MAP were packaged in low-density polyethylene/polyamide (LDPE/PA) barrier pouches (one fish weighing approximately 160 ± 55.94 g/pouch) 75 µm mm in thickness having an oxygen permeability of 52.2 cm³/m²/d/atm at 75% relative humidity (RH), 25°C and a water vapor permeability of 2.4 g/m²/d at 100% RH, 25°C. The VP and the MAP gas mixture was prepared using a Henkelman packaging machine (Boxer 42, Henkelman Ind Co., Netherland). All samples were stored under refrigeration (4±1°C) and analyzed on the 2, 4, 6, 8, 10 and 12th days to determine the changes in product quality.

Proximate composition analyses

The moisture content, protein, fat, and ash of fish samples were measured according to AOAC (2000).

Biochemical analysis

The pH values were measured by immersing a glass-calomel electrode directly into the sample by using a pH meter (Thermo Scientific Orion 3-Star Benchtop, Cambridge, UK). The total volatile basic nitrogen (TVB-N) content was determined according to the method of Antonocopoulos (1973). The value of thiobarbituric acid (TBA) was determined according to Tarladgis *et al* (1960) to evaluate the oxidation stability during storage and the results expressed as mg of malondialdehyde/kg (mg MDA/kg) fish muscle.

Microbiological analysis

A sample of fish (10 g) was diluted with 90 ml sterile 0.1% peptone water and homogenised in a Stomacher (Model 400, Seward, London, UK) at regular speed for 2 min. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, 0.1%

peptone water) of fish homogenates were spread on the surface of dry media. Total viable counts (TVC) were determined using Plate Count Agar (PCA, Merck) after incubation for 48 h at 30°C. Plate count agar was used for psychrotrophic bacteria and incubated at 7°C for 10 days. Results are expressed as a logarithm of colony forming units (log cfu) per gram of sample.

Sensory analysis

The sensory assessment of raw *S. t. macrostigma* stored under refrigeration, was conducted using the Quality Index Method (QIM) developed by Alasalvar *et al.* (2001) with some modifications for gutted and beheaded fish. This sensory assessment approach evaluates freshness by giving demerit points according to certain aspects of general appearances (e.g. skin, slime, eyes, gills, odour). A demerit score, which ranges generally from 0 to a maximum of 3, where 0 represented best quality and higher score (3) indicated poorer quality (see Table 1 below). The total sum of demerit points was 27. Each assessment was carried out by a minimum of seven trained panellists. Panellists were asked to state whether or not the fish were acceptable.

The sensory evaluations of cooked fish were assessed according to Amerina *et al.* (1965). Various

Table 1. Quality index method scheme used for sensory assessment of *Salmo trutta macrostigma* samples

Parameters being assessed	Demerit points			
	0	1	2	3
Appearance	Very bright	Bright	Slightly dull	Dull
Skin	Firm or elastic	Soft		
Slime	Absent	Slightly slimy	Slimy	Very slimy
Stiffness	Pre-rigor	Rigor	Post-rigo	
Eyes				
Clarity	Clear	Slightly cloudy	Cloudy	
Shape	Normal	Flat sunken	Sunken or swollen	
Iris	Visible	Slightly visible	Not visible	
Blood	No blood	Slightly bloody	Bloody	Very bloody
Gills				
Colour	Dark red	Red	Brown	Dark brown or grey
Mucus	Absent	Slight	Moderate	Excessive or sticky
Smell	Neutral	Fishy	Stale	Spoilt

*Sum of score is minimum 0 and maximum 27

sensory characteristics, such as appearance, texture, odour and flavour, were evaluated. A score of 7–9 indicated “very good” quality, a score of 4.0–6.9 “good” quality, a score of 1.0–3.9 denoted as spoiled. Taste panel scoring of the fish was conducted after boiling the dressed fish in 1.5% brine for 10 min.

Statistical analysis

All statistical analyses were analyzed by one-way analysis of variance (ANOVA), using the SPSS statistical package for windows version 16.0 (SPSS Inc., Chicago, IL, USA). Statistical significance level was considered to be $p < 0.05$. All data were expressed as mean \pm SD in each group.

Results and Discussion

Proximate composition

Proximate composition analysis of *S. t. macrostigma* were conducted upon arrival of the fish (day 0). The moisture, protein, lipid and ash contents of fresh fish were found to be 77.68 \pm 0.87%, 18.70 \pm 0.1%, 1.31 \pm 0.40% and 1.33 \pm 0.42%, respectively. The proximate composition of the *S. t. macrostigma* reported in different studies (Bilgin *et al.*, 2007; Duman *et al.*, 2011) showed some differences especially for the lipid content. Such variations in the chemical composition of fish is strongly related to the nutrition, catching season (spawning cycles), sexual variation, fish size, living area, as well as the other environmental conditions (Huss 1988).

Biochemical changes

Changes in pH, TVB-N and TBA for A, VP and MAP fish samples during the 12 days storage period are shown in Table 2. The initial pH of the fish samples was 6.47. After processing, values of pH for A, VP and MAP fish samples were in the range of ca. 6.16–7.17 with statistically significant differences ($p < 0.05$) between packaging treatments, in agreement with results reported for fish in retail packages containing CO₂-enriched atmospheres (Genigeorgis 1985; Arashisar *et al.*, 2004). Lower pH values were recorded for MAP packaged samples. The increase of pH values during the storage period may be attributed to the production of basic compounds such as ammonia, trimethylamine as well as other biogenic amines by fish spoilage bacteria (Ruiz-Capillas and Moral, 2001). The above reported pH values for the VP and MAP samples are in agreement with those reported by Goulas *et al.* (2007) for rainbow trout fillets stored at 1 \pm 1°C.

TVB-N could be used as a quality indicator for

fish products and is associated with the amino acid decarboxylase activity of microorganisms during storage. Various authors have reported that the limit of acceptability for fresh fish was 30 mg TVB-N/100 g of flesh (Connell, 1995; Harpaz *et al.*, 2003). The initial TVB-N in raw fish was 13.02 mg/100 g. The level of TVB-N in freshly caught fish is generally between 5 and 20 mg N per 100 g muscle (Huss, 1988; Connell, 1995). Similar TVB-N values were reported by Bilgin *et al.* (2007). TVB-N values of fish were strongly affected by atmosphere used and increased progressively with time of storage for all lots of packaged products. Ozogul *et al.* (2004) indicated that bacterial catabolism of amino acids in fish muscle results in the accumulation of ammonia and other volatile bases. The TVB-N values of A samples were significantly higher ($p < 0.05$) than all VP and MAP samples throughout the entire storage period. TVB-N values for *S. t. macrostigma* samples stored in air, under VP and MAP exceeded the limit value of 30 mgN/100 g fish muscle after 8, 10 and 12 days of storage, respectively. MAP resulted in the highest preservative effect producing significantly lower ($p < 0.05$). CO₂ inhibits the growth of many spoilage bacteria with the inhibition effect is being increased with increasing CO₂ concentration in the atmosphere (Phillips, 1996; Kaba and Corapcı, 2013). Similar TVB-N values were reported by Goulas and Kontominas (2007).

TBA index is a widely used indicator for the assessment of degree of lipid oxidation (Nishimoto *et al.*, 1985). At the beginning of the storage period TBA values of fish samples were found to be 0.18 mg malonaldehyde (MDA) /kg. As can be seen in Table 2, TBA values in most cases increased gradually up to a certain point during storage. TBA values of fish stored in air, under vacuum and modified atmosphere varied little and in general were between 0.18 and 0.55 mg MDA/kg fish muscle during the entire storage period of 12 days. In our study, TBA values were below the threshold level of 3 mg MDA/kg fish muscle (Connell, 1990). The same differences are obtained between control samples and MAP samples at days 6 and 12 of the storage period. On the other hand, no differences ($p > 0.05$) were observed throughout the storage period between control samples and VP samples. Other authors have reported the same observation in studies where likewise TBA values were low and no rancidity was detected by the panel (Ruiz-Capillas and Moral, 2001; Goulas and Kontominas, 2007).

Microbiological assessment

The changes in toplam viable count and

Table 2. Changes in pH, TVB-N and TBA of *Salmo trutta macrostigma* during chilling storage under various packaging conditions

Parameters	Groups	Storage time (days)						
		0	2	4	6	8	10	12
pH	A	6.47±0.16 ^a	6.79±0.03 ^a	6.76±0.18 ^a	7.14±0.23 ^a	7.17±0.13 ^a	7.01±0.11 ^a	7.15±0.05 ^a
	VP	6.47±0.16 ^a	6.56±0.01 ^b	6.42±0.05 ^b	6.59±0.08 ^b	6.68±0.17 ^b	6.49±0.06 ^b	6.70±0.04 ^b
	MAP	6.47±0.16 ^a	6.39±0.04 ^c	6.16±0.00 ^c	6.44±0.02 ^b	6.45±0.10 ^b	6.59±0.09 ^b	6.45±0.02 ^c
TVB-N (mg/100 g)	A	13.02±0.85 ^a	24.64±0.84 ^a	27.17±1.71 ^a	28.84±1.70 ^a	35.13±1.42 ^a	41.77±1.06 ^a	59.50±2.33 ^a
	VP	13.02±0.85 ^a	20.57±1.59 ^b	23.60±0.87 ^b	25.25±0.33 ^b	27.07±0.68 ^b	29.26±1.18 ^b	37.70±1.25 ^b
	MAP	13.02±0.85 ^a	20.37±0.86 ^b	22.87±0.40 ^b	24.36±1.70 ^b	24.27±3.23 ^b	28.12±1.97 ^b	30.13±1.27 ^c
TBA (mg MDA/kg)	A	0.18±0.04 ^a	0.22±0.09 ^a	0.27±0.05 ^a	0.34±0.30 ^a	0.40±0.21 ^a	0.41±0.01 ^a	0.53±0.12 ^a
	VP	0.18±0.04 ^a	0.13±0.01 ^a	0.31±0.00 ^a	0.32±0.05 ^a	0.39±0.14 ^a	0.45±0.04 ^a	0.55±0.01 ^a
	MAP	0.18±0.04 ^a	0.15±0.02 ^a	0.19±0.00 ^b	0.21±0.00 ^a	0.28±0.03 ^a	0.31±0.00 ^b	0.32±0.06 ^b

The values are expressed as mean ± standard deviation. n=6. Different letters in the same column indicate significant differences ($p<0.05$) during storage periods.

psychrotrophic bacteria counts throughout the storage of refrigerated fish packaged in A, VP, MAP are given in Table 3. Initial toplam viable count of *S. t. macrostigma* of ca. $4.23 \log \text{cfu g}^{-1}$ indicate good fish quality, considering the proposed upper limit for aerobic plate count of $5 \times 10^5 \text{cfu g}^{-1}$ for fresh fish (ICMSF 1986). Although it is widely accepted that the initial microbial load of freshwater fish varies depending on water conditions and temperature, most available literature on different freshwater fish species, rainbow trout reports bacterial counts of 2 and $6 \log \text{cfu g}^{-1}$ (Gonzalez *et al.*, 1999; Chytiri *et al.*, 2004). Toplam viable count reached ca. $7.04 \log \text{cfu g}^{-1}$ for A, $5.85 \log \text{cfu g}^{-1}$ for VP and $5.63 \log \text{cfu g}^{-1}$ for MAP after 8 days of storage. After 12 days of storage toplam viable count of A, VP and MAP samples were 9.89 , 8.82 and $6.78 \log \text{cfu g}^{-1}$, respectively ($p<0.05$). Consequently, for a given sampling day the presence of CO_2 had a significant effect ($p<0.05$) on the growth of mesophiles. Similar MAP effects have been observed for rainbow trout fillets by other researchers (Arashisar *et al.*, 2004; Alak *et al.*, 2010) and for baltic herring fillets (Randell *et al.*, 1997).

The low initial psychrotrophic bacteria count ($4.08 \log \text{cfu g}^{-1}$) indicates good fish quality. In control samples psychrotrophic bacteria counts exceeded the value of $6 \log \text{cfu g}^{-1}$, considered as the upper acceptability limit for marine species (Erkan, 2007) on 6th storage day. On the other hand, in VP and MAP samples psychrotrophic bacteria counts exhibited a growth over the $6 \log \text{cfu g}^{-1}$ on the 8th storage day. The observed shelf life of *S. t. macrostigma*, as

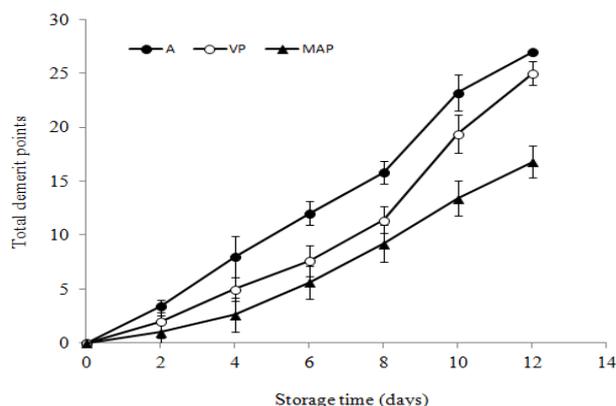


Figure 1. Sensory assessment of *Salmo trutta macrostigma* during chilling storage under different packaging conditions: Air (A), vacuum (VP) and modified atmosphere packaging (MAP). Mean scores of sensory assessment. Standard deviations are indicated by bars

determined by panellists who indicated that the fish were not acceptable, was 6 days in the control group and 12 days for the treated groups (Fig. 1). As with mesophiles, for a given sampling day the presence of CO_2 had a significant effect ($p<0.05$) on the growth of psychrotrophs. These results are in agreement with those reported in similar studies conducted on various fish (Gimenez *et al.*, 2002; Arashisar *et al.*, 2004; Erkan *et al.*, 2007).

Sensory analysis

The sensory quality of raw fish was evaluated same days after being caught and at each sampling time. The total demerit points of raw fish stored under refrigeration are presented in Figure 1. The

Table 3. Changes in the counts of toplam viable count and psychrophilic bacteria of *Salmo trutta macrostigma* during chilling storage under various packaging conditions

Parameters	Groups	Storage time (days)						
		0	2	4	6	8	10	12
Toplam viable count (log cfu/g)	A	4.23±0.97 ^a	3.90±0.21 ^a	4.30±0.70 ^a	5.71±0.14 ^a	7.04±0.90 ^a	7.95±0.45 ^a	9.89±0.55 ^a
	VP	4.23±0.97 ^a	3.74±0.20 ^a	3.76±0.67 ^a	5.23±0.52 ^b	5.85±0.35 ^b	6.24±0.76 ^b	8.82±0.92 ^b
	MAP	4.23±0.97 ^a	3.79±0.36 ^a	3.97±0.22 ^a	5.21±0.10 ^b	5.63±0.78 ^b	5.84±0.47 ^b	6.78±0.39 ^b
Psychrotrophic bacteria (log cfu/g)	A	4.08±0.80 ^a	4.15±1.39 ^a	4.35±0.46 ^a	6.05±0.34 ^a	8.56±0.76 ^a	9.69±0.88 ^a	10.19±0.85 ^a
	VP	4.08±0.80 ^a	3.63±0.47 ^a	3.95±0.56 ^a	5.66±0.58 ^b	6.11±0.41 ^b	6.83±0.77 ^b	9.58±0.84 ^b
	MAP	4.08±0.80 ^a	3.69±0.91 ^a	3.75±0.51 ^a	4.67±0.88 ^b	6.06±0.43 ^b	6.96±0.40 ^b	7.23±0.73 ^b

The values are expressed as mean ± standard deviation. n=6. Different letters in the same column indicate significant differences (p<0.05) during storage periods.

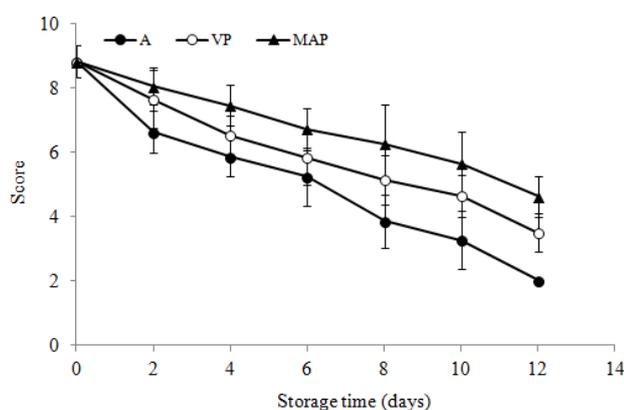


Figure 2. Sensory score of cooked *Salmo trutta macrostigma* during chilling storage under different packaging conditions: Air (A), vacuum (VP) and modified atmosphere packaging (MAP). Mean scores of sensory assessment. Standard deviations are indicated by bars

sensory scores in A, VP and MAP samples declined throughout the 12 days of chilled storage. Using a sensory score of 15 as the limit of acceptability, the observed shelf-life was 8 days in the A samples (demerit score, 15.8), 10 days in the VP samples (demerit score, 19.4) and 12 days for the MAP samples (demerit scores, 16.8). The main aspect related to quality loss was the gill odor. The shelf-life of fish is affected by the initial microbial load of the fish, storage temperature and packaging methods (Church, 1998). Ozogul *et al.* (2000) indicated that sensory evaluation limited the vacuum packages shelf life of herring to 8 days whereas 10 days was obtained with modified atmosphere packages.

Sensory scores of cooked *S. t. macrostigma* were shown in Figure 2. Significant statistical differences were found between the samples (p < 0.05). Acceptability was defined as having a score

of >4. The limit of acceptability was reached after 8 days for the A group (demerit score, 3.83) and 12 days for VP group (demerit scores, 3.48) and MAP group (demerit scores, 4.61) was still acceptable after 12 days. A group received lower scores throughout the storage, especially after day 8 when boiled dish cloth odour, intensive sour off flavor, flakiness of the fillet and dry and fibrous texture developed.

Conclusion

Based primarily on sensory but also on biochemical and microbiological indices determination, MAP was the most effective treatment for the preservation of *S. t. macrostigma*. Based on sensory data the shelf-life of fresh *S. t. macrostigma* was 6 days (under air), 10 days (under vacuum) and 12 days (under modified atmosphere, 40%/60%; CO₂/N₂). The corresponding shelf-life extension for VP and MAP were 4 and 6 days, respectively.

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