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RESEARCH PAPER

Hydrogen Inclusion in Modified Atmosphere Extends the Shelf Life of Chilled Rainbow Trout Fillets

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Abstract

Different gas formulations with inclusion of hydrogen were evaluated for extension of shelf life of chilled rainbow trout. A control and four treatment samples were as follows: Control (air), MAP1 (50% CO_2 / 50% N_2), MAP2 (60% CO_2 / 40% N_2), RAP1 (50% CO_2 / 46% N_2 / 4% H_2) and RAP2 (60% CO_2 / 36% N_2 / 4% H_2). Samples were stored at +2±1°C for 15 days and periodically analyzed for changes in their quality. TBARS exceeded consumable limits in control after 5 days while treatment samples were under the limit during storage. Consumable limits for TVB-N were exceeded after 7 days in control while treatment samples remained below the limit. The modified atmosphere was significantly effective in retarding protein degradation although a modest difference was observed due to hydrogen inclusion. Gradually increased microbial counts also confirmed quality loss in control while a rather limited change was observed under a modified atmosphere. Microbial loads of treated samples were under consumable limits until the 10th day, while control reached to limits by just the 3rd day. The incorporation of molecular hydrogen in a modified atmosphere may bring benefits to the seafood industry although further research is needed.

Introduction

Consumer demands are increasing towards fresh products with a long shelf life and high quality (Alice et al., 2020). Fresh fish is an extremely perishable food item, especially due to enzymatic reactions, oxidation, and microbial spoilage (Sezer et al., 2022). It has been reported that packaging in a modified atmosphere extends the shelf life of ready-to-eat cod (*Gadus morhua*) fillets from 6-10 days to 18-24 days (Magnússon et al., 2006). The most commonly used gases in modified atmosphere packaging (MAP) are oxygen, nitrogen, and carbon dioxide (Cachaldora et al., 2013). By restricting O_2 and increasing CO_2 levels in MAP, unwanted chemical and enzymatic reactions may be prevented as well as microbial growth (Emborg et al., 2002).

Molecular hydrogen (H_2) is a colorless and odorless gas classified as a food additive with the code E949 (Bulut et al., 2022). Hydrogen gas has many benefits when considered for health and food applications, which can be attributed to its anti-radical, selective antioxidant, anti-apoptosis, anti-inflammatory, anti-stress, anti-cancer, and reducing properties (Köktürk et al., 2022). Hydrogen gas as a reducing and antioxidant agent has been applied in different food products such as butter (Bulut et al., 2022), cold stored fish (Sezer et al., 2022), fresh cheese (Alwazeer et al., 2020), strawberries (Alwazeer and Özkan, 2022), and skim milk gels (Martin et al., 2009) to extend the shelf life and to

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preserve the quality attributes of the product. Recently, reducing the ability of H_2 in the form of biogenic amines was revealed when incorporated into modified atmosphere packaging of seafood (Sezer et al., 2022). Reducing oxidoreduction potential (i.e., ORP, Eh, or redox) of a medium by a reducing agent like molecular hydrogen, oxidation reactions may be limited (Alwazeer, 2020). ORP may also be used as a freshness indicator for seafood showing a relationship between O_2 content and microbial growth (Susanto et al., 2011). Values of ORP are highly associated with the deterioration of fish (Huss and Larsen, 1979). ORP value of seafood depends on different factors including proximate composition, processing method, storage conditions, and concentrations of various redox couples (Susanto et al., 2011). It has also been reported that ORP value may be more sensitive than pH in the assessment of seafood freshness (Agustini et al., 2001). Therefore, it was assumed that a low ORP environment ensured by the inclusion of molecular hydrogen in MAP may extend the shelf life of seafood by suppressing oxidation. Thus, this study was designed to evaluate the effect of molecular hydrogen inclusion in modified atmosphere packaging, which is then called reducing atmosphere packaging (RAP), on the quality and shelf life of rainbow trout during chilled storage.

Materials and Methods

Materials

All chemicals used in the study were of analytical grade and obtained from Sigma Aldrich (Merck, Darmstadt, Germany). Hydrogen, nitrogen, and carbon dioxide gases (99.9%) were obtained from Elite Gaz (Ankara, Türkiye). Rainbow trout samples (each between 250-350 g) were caught from the Euphrates River in December. Samples were immediately brought to the laboratory on ice and washed with cold tap water (about 10°C). Then, fish samples were beheaded, eviscerated, washed with cold tap water again, and dried on paper towel to remove excessive water.

Study Design and Sample Preparation

About 300 g of sample was packaged in polyethylene laminated polystyrene plates using a packaging machine (Lipovak, KV-600, Türkiye). Each package was usually composed of 2 rainbow trouts (beheaded, gutted, skin on, gills and fins off). Different gas formulations were prepared using a semi-automatic gas mixer (Dansensor, MAP Mix 9001 ME, Denmark). One group of the samples was used as control packed under atmospheric air and the other four groups of the samples were packaged under different formulations of modified atmosphere as follows: Control (air), MAP1 (50% CO_2 / 50% N_2), MAP2 (60% CO_2 / 40% N_2), RAP1 (50% CO_2 / 46% N_2 / 4% H_2) and RAP2 (60% CO_2 / 36% N_2 / 4% H_2). Packaged samples were stored in a refrigerator at +2±1°C (regularly checked with an external digital thermometer) for 15 days and analyses were performed initially on cleaned fish and later on packed fish samples on the 1st, 3rd, 5th, 7th, 10th, and 15th days of storage according to the study plan. All analyses were performed at least in duplicate and further replicated when needed.

Chemical Analyses

Chemical quality analyses were carried out according to procedures given by Woyewoda et al. (1986). Briefly, the free fatty acid (FFA) content of homogenized fish samples was determined by titration with sodium hydroxide and calculated as the percentage of oleic acid. The peroxide value (PV) of fish samples was determined by titration with sodium thiosulfate in the presence of potassium iodide and starch solution. The content of thiobarbituric acid reactive substances (TBARS) was determined by absorbance reading at 538 nm after distillation of acid-hydrolyzed fish samples in the presence of thiobarbituric acid. The content of total volatile basic nitrogen (TVB-N) was determined by distillation in the presence of boric acid and titration of the distillate with sulphuric acid. Finally, trimethyl amine nitrogen (TMA-N) in fish samples was determined by absorbance reading at 410 nm in the presence of picric acid (Woyewoda et al., 1986).

Physical Analyses

Gas composition in the package

The CO_2, N_2, and H_2 concentrations in the headspace of polyethylene packets were measured using a stainless steel needle using a gas analyzer (MAT1500, KRÜSS, Germany) (Alasalvar et al., 2005).

pH value

pH values of fish samples were measured with a pH electrode (SP10 R, Consort, Belgium) and a data acquisition multiparameter device (Multiparameter Analyzer C3040, Belgium) by direct immersion of the electrode into the fish tissue after a calibration stage (Martin et al., 2010).

Oxidoreduction potential (Eh)

The *Eh* (mV) values of fish samples were continuously measured by an SP60X electrode (Consort, Belgium) and a data acquisition multiparameter interface (Consort Multiparameter Analyzer C3040, Belgium). The *Eh* electrode, after a cleaning and calibration stage, was inserted directly into fish tissue and kept until a stable *Em* value was read. The *Em* value measured by the electrode was used in the following equation to determine the *Eh* value according to Jacob (1970).

$$Eh = Em + Er$$

Where, *Eh* is the oxidoreduction potential value referred to the normal hydrogen electrode, *Em* is the measured potential value, and *Er* is the oxidoreduction potential of the reference electrode (Ag/AgCl). The Eh7 values were calculated as follows (Caldeo, 2015; Alwazeer et al., 2020).

$$Eh7 = Eh - \alpha(7 - pHm)$$

where α is the pH/*Eh* correlation factor and pHm is the measured pH value of fish samples.

Total color difference (ΔE)

The L* (lightness), a* (red/green), and b* (yellow/blue) values of the inner (meat color) and outer (skin color) parts of fish samples were determined using a color measuring device (CR-410 Konica Minolta Chroma Meter, Japan). Before starting the analysis, the instrument was calibrated with a white calibration plate. The total color difference (ΔE) was calculated by measuring the difference in color parameter at each storage time (final, f) compared to the initial values (initial, 0) as follows:

$$\Delta E = \sqrt{(L_0^* - L_f^*)^2 + (a_0^* - a_f^*)^2 + (b_0^* - b_f^*)^2}$$

Microbiological Analyses

25 g of fish samples were separated from each fish under sterile conditions and then diluted with 225 mL of physiological saline (0.85% NaCl) and homogenized by a Stomacher (Bag Mixer 400, France). The counts of total aerobic mesophilic bacteria (TAMB) and yeast and molds (YM) were determined in triplicate incubated on Plate Count Agar (PCA, Merck, Germany) at +30°C for 3 days and on Potato Dextrose Agar (PDA, Merck, Germany) at +25°C for 5 days, respectively. The counts of total psychrophilic bacteria (TPB) were determined on PCA medium (Merck, Germany) incubated at +4°C for 10 days. The results were reported as the decimal logarithm of colony-forming units per gram (log cfu/g) (Halkman, 2013).

Sensory Analyses

The sensory evaluation of fish samples was carried out by a group of 10 panelists. The evaluated sensory quality notes of the fish samples were the surface appearance, odor and aroma notes, muscle tightness and hardness, and surface stickiness. Considering the previous quality parameters, the general acceptability level was determined using a 5-point hedonic scale (Erdilal, 2008). Sensory analysis was repeatedly performed on the 10th and 15th days of the storage.

Statistical Analyses

Results obtained were designated for statistical analyses and an ANOVA was performed to determine if there was a significant difference between the pairs then Tukey-Kramer test was utilized for the determination of the pairs that were significantly different at a probability level of 95% using JMP 8.0 (SAS, Cary, NC, US).

Results and Discussion

Changes in pH Value

pH is an indicator of time spent after the capture of fish as the pH of live fish is about 7.0-7.3 and pH decreases markedly as glycogen is converted to lactic acid while rigor mortis is set after death (Howgate, 2009). It was determined that the pH value of the trout samples at the end of storage generally changed in a very narrow range and increased in all samples at a non-significant level compared to the first day of storage ($p>0.05$) (Supp. Table 1). On the 1st day of storage, the difference between control, MAP1, and MAP2 was significant ($p<0.05$) while the difference between RAP1 and RAP2 was not. In the first days of storage, the control sample showed significant differences compared with the applications (MAP1, MAP2, RAP2, and RAP2) ($p<0.05$). Change in pH value may occur fast after death and must be followed by frequent measurements right after death, especially in small fish species. In the present study, a dramatic decrease in pH value has not been observed most probably due to the long period spent after death. During preliminary studies and pretreatments, a quick change in pH value was probably over and started to increase again due to the increase in amine concentrations produced from protein deterioration. In addition, pH value cannot be solely considered as a quality index and should be supported by chemical and sensory analyses (Varlık et al., 1993). Susanto et al. (2011) determined the pH range of fish samples between 5.57 and 7.30. pH increase can be attributed to the accumulation of basic compounds such as ammonia and trimethylamine, which are derived from microbial activity during the breakdown of fish muscles (Ruiz-Capillas and Moral, 2005). In the present study, the increase in pH value of the control sample was comparatively higher until the 10th day of storage, therefore, which may be attributed to the limited effect of modified atmosphere packaging.

Changes in Free Fatty Acid Content

Rainbow Trout samples packaged under different gas formulations were followed for their content of free fatty acids during storage at +2±1°C for 15 days (Supp. Table 2). There was a significant increase in free fatty acid content in the control sample ranged from 0.76 to 2.05% while it was limited in treatment samples of MAP

and RAP, indicating the success of both methods in retarding the hydrolysis of oils and the formation of free fatty acids. However, there was an insignificant difference between hydrogen-incorporated RAP1 and RAP2 samples, and conventional MAP1 and MAP2 samples. Additionally, during the first 7 days of storage, FFA values of MAP1, MAP2, and RAP2 did not exceed the level of 1.20%, which was significantly lower compared to that of the control. On the other hand, the FFA value of RAP1 was 1.54% at the end of storage (on the 15th day of storage), which was higher compared with that of MAP1, MAP2, and RAP2 but still lower than that of control. While an increase in the amount of free fatty acids in the control was significant, especially after the first week, there was a limited change in MAP and RAP samples and no significant increase was observed in these samples. It may be concluded that MAP and RAP retards the formation of free fatty acids to some extent at a similar level. Pinheiro et al. (2020) used sardines in MAP packed under different gas formulations (20.8% O_2 / 79.2% N_2, 30% CO_2 / 70% N_2, 30% CO_2 / 70% N_2O and 30% CO_2 / 70% Ar) and stored at 3°C for 12 days. Their results were in good agreement with the results obtained in the present study, concluding that MAP reduced the formation of free fatty acids and not much difference was observed along with different gas formulations studied. Fagan et al. (2004) stored freeze-chilled (-35°C for 2.5 hours and -30°C for 3 days and subsequent storage at +2 to +4°C) salmon (40% CO_2 / 60% N_2) and mackerel (30% N_2 / 40% CO_2 / 30% O_2) fillets under MAP for 7 days. They determined that free fatty acid content did not exceed the level of 1.35% and that the modified atmosphere packaging increased the shelf life of fish.

Trends in PV Levels

Table 1 shows the peroxide value (PV) results of fish samples during chilled storage. The initial PV of the fresh fish sample (before packaging) was 0.60±0.00 meq O_2/kg. The results show that the increase in PV of MAP1 and MAP2 samples was retarded compared to that of the control. In MAP1 samples, PV did not increase significantly until the end of storage (on the 15th day of storage). However, in MAP2, RAP1, and RAP2 samples, a significant increase in PV was observed during the storage. PV reached to the highest values in MAP2 and RAP1 samples at the end of storage. PV of RAP1 and RAP2 increased in parallel, but a significant decrease was observed in RAP2 on the 15th day of storage while PV of RAP1 showed the highest values among all the samples (Table 1). Depending on factors like species, age, gender, and catching season; PV below 4 meq O_2/kg is considered "very good". If PV is from 5 to 10 meq O_2/kg, the quality is "good"; from 10 to 20 meq O_2/kg "edible"; and over 20 meq O_2/kg "inedible". Thus, MAP2, RAP1 and RAP2 samples exceeded the limit of "very good" during the storage but never the edible limit. Rahmatipoor et al. (2017) packaged silver carp using atmospheric air, vacuum, and MAP (55% N_2 / 45% CO_2 / 5% O_2) and stored fish samples at +4°C for 15 days. In that study, the PV of the samples packaged under MAP was lower and in good agreement with the results obtained in the present study. The authors reported that

Table 1. Trends in PV under different gas formulations (meq O_2/kg).

	PV (meq O_2/kg)					
	1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
CONTROL	1.55±0.50^{ABab}	1.05±0.07^{Aa}	0.55±0.07^{Aa}	3.80±0.42^{Cb}	2.25±0.07^{Ba}	2.35±0.21^{Ba}
MAP1	1.35±0.50^{Aab}	1.40±0.14^{Aa}	1.50±0.14^{Ab}	1.10±0.28^{Aa}	1.35±0.07^{Aa}	1.55±0.50^{Aa}
MAP2	0.95±0.21^{Aa}	0.85±0.08^{Aa}	1.10±0.28^{Aab}	1.85±0.64^{Aa}	4.25±0.64^{Bb}	6.70±0.57^{Cb}
RAP1	2.30±0.42^{Aab}	1.65±0.07^{Aa}	1.65±0.21^{Ab}	3.95±0.21^{Bb}	6.45±0.35^{Cc}	7.75±0.07^{Db}
RAP2	2.60±0.00^{Ab}	3.05±0.50^{Ab}	3.55±0.07^{Ac}	3.70±0.42^{Ab}	5.65±0.50^{Bbc}	2.45±0.21^{Aa}

Results are means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between the samples (within the same column) at a significance level of 95%.

Table 2. Trends in TBARS during chilled storage (µmol MDA/kg).

	TBARS (µmol MDA/kg)					
	1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
CONTROL	3.58±0.15^{Aa}	10.83±0.15^{De}	11.86±0.08^{Ee}	27.08±0.15^{Fe}	9.15±0.08^{Cc}	6.34±0.23^{Ba}
MAP1	4.66±0.00^{Ab}	6.23±0.08^{Bb}	8.72±0.08^{Cb}	16.14±0.15^{Dd}	8.99±0.00^{Cc}	6.50±0.00^{Ba}
MAP2	5.42±0.00^{Ac}	7.53±0.08^{Cd}	9.05±0.08^{Ec}	12.62±0.08^{Fc}	7.10±0.08^{Ba}	7.91±0.00^{Db}
RAP1	5.15±0.08^{Ac}	5.63±0.00^{Ba}	9.48±0.08^{Ed}	11.00±0.08^{Fb}	7.48±0.00^{Cb}	8.02±0.00^{Db}
RAP2	9.97±0.00^{Fd}	6.83±0.00^{Bc}	6.50±0.00^{Aa}	9.64±0.00^{Ea}	9.15±0.08^{Dc}	8.45±0.00^{Cc}

Results are means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between the samples (within the same column) at a significance level of 95%.

PV fluctuated between 0.86 and 4.89 meq O_2/kg in all fish samples during the storage and a slight upward trend was observed until the 6th day of storage for all samples, followed by a sharp decrease and then an increase again most probably due to a continuous formation of peroxides and their simultaneous deterioration into secondary oxidation products. In the present study, PV fluctuated between 0.60 and 7.75 meq O_2/kg, and the highest value was shown in the RAP1 sample at the end of storage most probably due to the retarded PV formation. PV of control and MAP1 samples fluctuated within a rather narrow gap because of the further degradation of peroxides into secondary oxidation products as confirmed by the highest TBARS values during the first week of the storage.

Trends in TBARS Content

Table 2 shows the results of thiobarbituric acid reactive substances (TBARS) content in fish samples. The initial TBARS content of trout samples was 1.95±0.00 μmol MDA/kg which gradually increased during the storage. TBARS presents the accumulation of secondary oxidation products that significantly increased in all samples during storage. It reached the highest level at the end of the first week of storage in the control sample but the values for the 10th and 15th days of storage were lower in general. This phenomenon was similar in all samples, which may show further degradation of the relevant substances. However, this increase in TBARS value in treatment samples (MAPs and RAPs) was limited to some extent compared to that of the control. In particular, the lowest TBARS values were observed in the RAP2 sample. The results show that modified atmosphere packaging retards lipid oxidation, where RAP was even more effective in slowing the oxidation compared with MAP (Table 2). When TBARS content reached the peak values at the end of the first week, TBARS values of RAP samples were at lower levels compared to that of the control and MAP samples, indicating the success of H_2 incorporation in retarding the oxidation. At the end of the first week of the storage, a dramatic decrease was observed in TBARS content in all fish samples, which may be due to the further degradation of those compounds reacting with TBA. TBARS values reached their highest on the 7th day of the storage and never exceeded these peak values for all treatment samples as well as the control. Considering overall results, the most dramatic increase was observed in the control while MAP and RAP samples showed a limited change in TBARS content, and a limited increase was observed for RAP samples (Table 2). TBARS content was under consumable limits (~1.5 mg MDA/kg or 21 μmol MDA/kg) during the whole storage for all treatment samples while the consumable limits were exceeded in the control at the end of the first week of storage. Similar results were observed in the study reported by Arashisar et al. (2004). A possible reason for fluctuations in TBARS values may be because of differences in modified atmosphere formulations, which can produce different oxidation products (Doe et al., 1998). Sinnhuber and Yu (1983) reported that TBARS values as low as 4-7 μmol MDA/kg may be associated with poor quality in fish. Therefore, the use of antioxidants such as molecular hydrogen may be needed along with MAP to achieve a longer shelf life in terms of acceptable microbiological and chemical quality features (Masniyom et al., 2002).

Trends in TVB-N Content

The content of total volatile basic nitrogen (TVB-N) indicates the accumulation of hydrolysis and degradation products from proteins, which was initially about 11 mg/100 g sample. Later in the storage, TVB-N values reached almost a level of 30 mg/100 g in the control sample while this increase was limited to a level of 16-17 mg/100 g in other treatment samples (Table 3). The results showed that MAP treatments were effective in retarding the protein degradation regardless of the formulation of MAP and no significant difference was observed between MAP and RAP samples in general. On the other hand, it could be concluded that a modified atmosphere extends the shelf life of fish and keeps TVB-N levels below the consumable limits (about 20 mg TVB-N/100 g). In fact, the consumable limit was exceeded at the end of the 10th day of storage in the control while MAP and RAP samples remained below this limit until the end of the storage. Hisar (2002) studied rainbow trout fillets under air, vacuum and different gas mixtures (100% O_2, 2.5% CO_2/7.5% N_2/90% CO_2, 30% O_2/30% N_2/40% CO_2) stored at 4 and 10°C. They reported that modified atmosphere packaging reduced the formation of TVB-N. In another study conducted by Giménez et al. (2002), it was found that the TVB-N values of trout samples packaged under MAP exhibited similar results

Table 3. Trends in TVB-N during chilled storage under different gas formulations (mg/100 g).

	TVB-N (mg/100 g)					
	1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
CONTROL	13.84±1.02^{Aab}	11.73±1.93^{Aa}	13.82±1.03^{Aab}	14.56±1.98^{Aab}	21.55±1.96^{Bb}	29.93±1,98^{Cb}
MAP1	13.14±0.03^{Aa}	11.72±0.01^{Aa}	12.46±0.99^{Aab}	11.76±0.00^{Aab}	11.76±1.98^{Aa}	17.36±0.00^{Ba}
MAP2	16.66±0.99^{Bb}	11.06±0.99^{Aa}	14.91±0.50^{Bb}	14.87±0.44^{Bb}	15.94±0.01^{Bab}	15.95±0.01^{Ba}
RAP1	10.89±0.75^{ABa}	14.56±0.99^{BCa}	13.13±0.04^{ABCab}	10.36±0.01^{Aa}	13.15±1.97^{ABCa}	16.64±1.00^{Ca}
RAP2	13.86±0.99^{ABab}	13.15±0.01^{Aa}	11.72±0.01^{Aa}	13.15±0.01^{Aab}	13.85±1.01^{ABa}	16.64±1.00^{Ba}

Results are means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between the samples (within the same column) at a significance level of 95%.

and did not exceed 25 mg/100 g during chilled storage. Similarly, the samples packaged with MAP and RAP did not exceed consumable limits. In addition, the results obtained in this study were lower compared to that reported by Goulas and Konrominas (2007) for MAP-treated trout samples.

The formation of TVB-N compounds was generally attributed to the growth of biological activity of spoilage bacteria and the proteolytic action of endogenous enzymes (Bekhit et al., 2021). After the depletion of glucose, bacteria start to use amino acids as an energy source, leading to the development of biogenic amines such as trimethylamine (TMA), Dimethyl Amine (DMA), Ammonia (NH_3), and TVB-N (Prabhakar et al., 2021). It has been also reported that some fish paste-isolated lactic acid bacteria (LAB) could produce biogenic amines (Dapkevicius et al., 2000). Several studies revealed that reducing conditions are not favorable for the growth of some microorganisms including LAB. For example, Ouvry et al. (2002) showed that the growth of *Lactobacillus plantarum* could be slowed under reducing conditions adjusted by hydrogen gas. Furthermore, the lag phase of *Leuconostoc mesenteroides* (LAB strain) increased six fold under reducing conditions adjusted by hydrogen than that of the oxidizing one adjusted by nitrogen (Bourel et al., 2003). Additionally, reducing conditions were found also to be unfavorable for the growth of spoilage bacteria such as *E. coli* (George et al., 1998; Riondet et al., 1999). The previous reports show that the low oxidoreduction potential (Eh) such that adjusted by hydrogen could limit the growth of different microorganisms such as those spoiling seafood. The inclusion of hydrogen in modified atmosphere may lead to a decrease in Eh value that might inhibit the growth of proteolytic enzyme-producing microorganisms. It was reported that formation of biogenic amines in rainbow trout and horse mackerel packaged under hydrogen-included MAP was lower compared to that of samples packaged under conventional MAP (Sezer et al., 2022). Additionally, the restrictive effect of hydrogen-rich water on formation of biogenic amines has been also shown in butter (Bulut et al., 2022).

TMA-N Content

No TMA-N was detected regardless of the samples including treatments and the control. Similar findings were reported in previous studies concluding that TMA-N formation is very low and under detectable limits in pelagic fish species like trout (Pørvik et al., 2000).

Gas Composition in the Packages

Sample packages were analyzed for their gas composition of O_2, CO_2, N_2, and H_2 during storage. The results showed that no oxygen was found in all packages until the end of the storage. Meanwhile, negligible changes in the composition of CO_2, N_2, and H_2 were observed in all packages during the whole storage (data not shown).

Oxidoreduction Potential (Eh)

Oxidoreduction potential (ORP, Eh) can be used as a quality indicator of fish freshness based on the dielectric properties of fish meat, showing the relationship between O_2 formation and the growth of microorganisms (Brown and Finksiger, 1980). It has been previously reported that the measured redox values are pH-dependent (Brown and Finksiger, 1980). Eh may be more sensitive than pH in the assessment of fish freshness (Agustini et al., 2001). In biological environments, where a large number of redox couples are involved in the equilibrium state (Jenness and Patton, 1959), it is more comprehensible to use Eh7 (Eh of a product at pH 7) to overcome the pH dependency (Caldeo, 2015). Therefore, the oxidoreduction potential value (Eh7, mV) of the samples was analyzed and the results were given in Table 4. As can be seen from this Table, Eh7 values showed a dramatic increase during the storage in the control (ranging from +53 mV to -279 mV), while this change was considerably limited in treatment samples except MAP1. Eh7 values decreased in MAP2 and RAP samples within a rather narrow gap, while the Eh7 value of MAP1 changed within a wider gap as in the control. Several studies evaluated the pH and Eh to assess the post-mortem biochemical changes related to fish freshness (Agustini et al., 2001). The Eh values of the present study showed differences from previous reports most probably due to the presence of various redox couples at different levels as well as the differences in chemical composition, processing treatments, and storage conditions (Brown and Finksiger, 1980).

It has been previously reported that the Eh of fish meat would be expected to be positive at the beginning and would turn to be negative when fish spoils during

Table 4. Trends in Eh*7* (mV) during chilled storage under different gas formulations.

	Eh7 (mV)					
	1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
CONTROL	53±18.16^{Bc}	130±36.58^{Bb}	31±5.58^{Bb}	30±52.32^{Bbc}	-259±52.65^{Aa}	-279±138.5^{Aa}
MAP1	21±7.36^{Bc}	106±8.74^{Bb}	35±6.10^{Bb}	-50±139.1^{ABabc}	6.26±29.28^{Bb}	-278±90.94^{Aa}
MAP2	-50±5.74^{Ab}	98±12.60^{Ab}	88±16.70^{Ab}	97±9.70^{Ac}	86±50.07^{Ab}	21±8.19^{Aa}
RAP1	-348±22.21^{Aa}	-269 ±91.95^{Aa}	-336±98.21^{Aa}	-398±1.88^{Aa}	-354±23.49^{Aa}	-298±75.97^{Aa}
RAP2	-393±5.85^{Aa}	-320±2.92^{Aa}	-402±2.82^{Aa}	-288±147.7^{Aab}	-373±18.06^{Aa}	-237±25.09^{Aa}

Results are means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between the samples (within the same column) at a significance level of 95%.

storage (Huss and Larsen, 1979; Agustini, et al., 2001). This phenomenon was related to the presence of redox compounds such as NADH, NADP+, and TMAO at different concentrations in fish, and their disappearance may affect the rates of various oxidoreduction reactions in fish muscle (Ikeda, 1979). Huss and Larsen (1979) showed that Eh was associated with the spoilage of fish and it increased initially and then decreased until negative values were obtained. On the other hand, the initial negative values of Eh observed in RAP samples in the present study may be due to the solubility of hydrogen in fish tissues.

Susanto et al. (2011) found Eh values ranging from +386 to +113 mV for tropical fish species including *Thunnus albacares (*Yellowfin tuna*), Ephinephelus striatus (*Nassau Grouper*), Cyprinus carpio (*Carp*)* and *Osphronemus gourami (*Guramy*)*. They reported that each species showed different Eh and pH values and tropical freshwater fish species showed higher Eh values than tropical marine fish species. In the present study, Eh7 values ranged from +53 to -279 mV in control, from +21 to -278 mV in MAP1, from -50 to +21 mV in MAP2, from -348 to -298 mV in RAP1, from -393 to -237 mV in RAP2 during storage. These differences in Eh value may be due to the parameters such as species, environmental factors, physiological state, microbial growth, catching and harvesting methods, and killing procedures (Oehlenschläger and Rehbein, 2009). Another study reported that fresh tuna samples showed a low rate of increase in Eh value with time and authors suggested Eh value as an indicator of tuna freshness (Agustini et al. 2001).

Total Color Difference (ΔE)

Changes in ΔE values for meat and skin of trout samples are given in Supp. Table 3. The ΔE (meat) values in all trout samples generally changed significantly during storage ($p<0.05$). From the 5th day of storage, the change created a significant difference in all samples ($p<0.05$). When the effect of storage was examined, it was determined that the MAP2 sample showed a higher ΔE (Meat) value than the control but a significantly lower ΔE (Meat) value than the other samples at the end of storage ($p<0.05$) (Supp. Table 3).

The ΔE (Skin) value changes of all trout samples generally changed significantly during storage ($p<0.05$). When the effect of storage was evaluated, the ΔE value of skin for all samples differed significantly ($p<0.05$). At the end of storage, the least color change (ΔE) was observed in RAP1 samples and it was significantly lower than in all samples ($p<0.05$) (Supp. Table 3). According to the results of ΔE; among all applications, MAP2 showed the lowest color change (ΔE) values for fish meat, and the same was for RAP1 in the skin. Accordingly, it was concluded that color preservation of fish skin may be achievable by inhibition of pigment oxidation due to hydrogen incorporation in MAP (Alwazeer and Özkan,2022).

Table 5. Changes in microbial counts (log cfu/g) under different gas formulations.

		Microbial counts (log cfu/g)					
		1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
TAMB	CONTROL	5.42 ± 0.03^{Ab}	6.35 ± 0.07^{Be}	7.47 ± 0.09^{Cc}	8.31 ± 0.02^{Dc}	7.63 ± 0.03^{Cd}	9.36 ± 0.31^{Ed}
	MAP1	6.33 ± 0.04^{Bc}	5.20 ± 0.01^{Ac}	4.48 ± 0.45^{Aab}	5.26 ± 0.02^{Aa}	6.20 ± 0.09^{Ba}	7.35 ± 0.08^{Cab}
	MAP2	4.67 ± 0.03^{Ca}	4.19 ± 0.01^{Ba}	3.72 ± 0.01^{Aa}	4.97 ± 0.04^{Da}	7.10 ± 0.02^{Ec}	7.11 ± 0.03^{Ea}
	RAP1	4.81 ± 0.01^{Ba}	5.85 ± 0.07^{Cd}	4.22 ± 0.12^{Aa}	5.91 ± 0.03^{Cb}	6.32 ± 0.02^{Da}	8.05 ± 0.18^{Eac}
	RAP2	5.52 ± 0.04^{BCb}	4.70 ± 0.04^{Ab}	5.19 ± 0.1^{Bb}	5.93 ± 0.17^{Cb}	6.81 ± 0.09^{Db}	7.99 ± 0.03^{Ebc}
TPB	CONTROL	5.93 ± 0.01^{Aab}	7.00 ± 0.02^{Bc}	8.97 ± 0.03^{Cd}	9.29 ± 0.01^{Dc}	10.77 ± 0.07^{Ec}	10.96 ± 0.04^{Fd}
	MAP1	7.79 ± 0.02^{Dd}	6.46 ± 0.05^{Bbc}	6.01 ± 0.02^{Ac}	5.95 ± 0.01^{Aa}	6.99 ± 0.12^{Cb}	8.04 ± 0.04^{Dbc}
	MAP2	5.81 ± 0.01^{Ba}	5.20 ± 0.07^{Aa}	5.18 ± 0.10^{Aa}	5.95 ± 0.04^{Ba}	6.92 ± 0.01^{Cb}	7.33 ± 0.02^{Da}
	RAP1	6.06 ± 0.04^{ABb}	6.51 ± 0.05^{BCc}	5.41 ± 0.03^{Aa}	7.05 ± 0.45^{Cb}	6.49 ± 0.08^{BCa}	8.29 ± 0.13^{Dc}
	RAP2	6.45 ± 0.07^{BCc}	5.39 ± 0.60^{Aab}	5.74 ± 0.05^{ABb}	5.90 ± 0.06^{ABa}	6.95 ± 0.04^{CDb}	7.81 ± 0.02^{Db}
YM	CONTROL	5.32 ± 0.04^{Aa}	7.84 ± 0.02^{Be}	9.03 ± 0.03^{Cc}	10.15 ± 0.07^{Dc}	10.75 ± 0.06^{Ed}	11.03 ± 0.09^{Fe}
	MAP1	7.23 ± 0.05^{Db}	5.31 ± 0.05^{Ac}	5.99 ± 0.01^{Bb}	6.02 ± 0.07^{Ba}	6.85 ± 0.01^{Ca}	6.59 ± 0.11^{Ca}
	MAP2	5.05 ± 0.07^{ABa}	4.01 ± 0.01^{Aa}	5.03 ± 0.10^{ABa}	5.49 ± 0.73^{Ba}	8.29 ± 0.22^{Cc}	7.43 ± 0.16^{Cb}
	RAP1	5.84 ± 0.9^{ABab}	6.60 ± 0.01^{BCd}	4.38 ± 0.12^{Aa}	7.42 ± 0.07^{CDb}	7.65 ± 0.10^{CDb}	8.62 ± 0.07^{Dd}
	RAP2	6.37 ± 0.04^{BCab}	4.79 ± 0.03^{Ab}	6.25 ± 0.40^{BCb}	6.08 ± 0.02^{Bab}	6.78 ± 0.06^{Ca}	8.09 ± 0.05^{Dbc}

Results are means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between the samples (within the same column) at a significance level of 95%.

Microbial Counts

Trends in microbiological counts of fish samples are given in Table 5. Based on the results, it can be concluded that treatment samples (MAPs and RAPs) were under consumable limits until the 10th day of storage with a few exceptions while the control sample reached the consumable limits until the 3rd day of storage. Tunçtaş (2019) stated that the maximum acceptable value of total mesophilic bacterial load for fish meat is 10^6 cfu/g as well as for total psychrophilic bacteria. In the present study, it was determined that the TAMB exceeded this limit on the 3rd day of storage for the control and the 10th day for the treatment samples, which is most probably due to the presence of oxygen in the control and its absence in treatment samples. Therefore, modified atmosphere showed a retarding effect on TAMB, TPB, and YM. However, no significant difference was observed between treatment samples (MAPs and RAPs) in general. The trends of TPB and YM were similar to that of TAMB (Table 5). Meanwhile, initial TPB and YM counts were a bit higher than that of the control. It has been previously reported that CO_2 has a suppressive effect on the growth of TAMB in fish (Yılmaz, 2004).

Sensory Evaluation

Figure 1 shows the results of the sensory analysis of trout samples before the storage and on the 10th and 15th days of storage. The sensory quality of the treatment samples seems to be preserved to some extent compared with that of the control. However, no significant difference was observed between the different treatment samples (MAPs and RAPs) in general. Sensory quality was preserved at acceptable levels in MAP and RAP samples during the 15 days of chilled storage while the control lost its acceptability on the 10th day of storage. Sensory scores were in good agreement with chemical and microbiological quality indices and showed that modified atmosphere packaging preserved the quality of chilled trout samples until the 10th day of the storage while that of the control was until just the 3rd day of the storage in general (Figure 1).

Conclusions

Considering the chemical quality parameters, it was shown that packaging under a modified atmosphere can suppress fat hydrolysis and oxidation and may prevent microbial growth to some extent. While the control sample exceeded the consumable limit values of different quality parameters, MAP and RAP samples remained below these limit values during the storage. Regarding the microbiological and sensory indices, it may be concluded that the control reached consumable limits on the 3rd day of chilled storage while that of MAP and RAP was extended to the 10th day. In addition, RAP samples showed a better preserving performance according to some chemical parameters of quality although no significant difference was observed

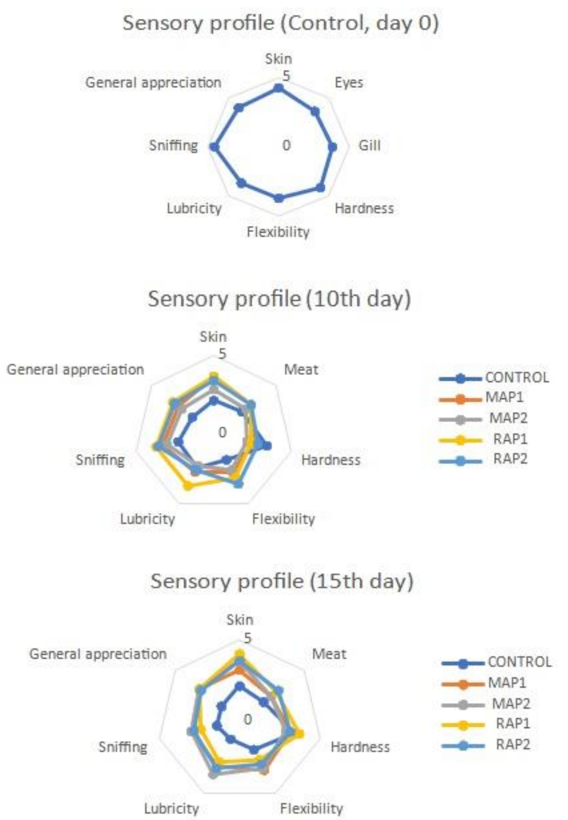

Figure 1. Sensory profile of the samples during chilled storage.

between MAP and RAP samples according to microbiological and sensory quality indices. However, RAP treatment may provide further preservation at different formulations, which was observed in part from some of the chemical quality parameters, especially considering the secondary oxidation of fish oil. Further investigation of other parameters of quality is needed to assess the efficiency of hydrogen incorporation in a modified atmosphere for the extension of the shelf life of fish. The incorporation of MAP with hydrogen gas can bring additional benefits to fish preservation allowing extending its shelf life.

Ethical Statement

No ethical statement is necessary for the study presented in this manuscript to the best of our knowledge.

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Author Contribution

Menekşe Bulut: Data Curation, Formal Analysis, Investigation, Methodology, Writing Original Draft

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Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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References

Agustini, T.W., Suzuki, M., Suzuki, T., Hagiwara, T., Okouchi, S. & Takai, R. (2001). The possibility of using oxidation-reduction potential to evaluate fish freshness. *Fisheries Science*, *67*(3), 547-549. https://doi.org/10.1046/j.1444-2906.2001.00269.x

Alasalvar, C., Al-Farsi, M., Quantick, P.C., Shahidi, F. & Wiktorowicz, R. (2005). Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. *Food Chemistry*, *89*(1), 69-76. https://doi.org/10.1016/j.foodchem.2004.02.013

Alice, E.J., Amanullah, M., Karim, M.A., Hossain, M.A. & Islam, M.T. (2020). Effects of vacuum and modified atmosphere packaging on the biochemical and microbiological quality of sliced goonch fish (*Bagarius bagarius*) stored at refrigerated condition. *Food Research*, *4*(6), 2256-2264. https://doi.org/10.26656/fr.2017.4(6).287

Alwazeer, D. (2020). Importance of consideration of oxidoreduction potential as a critical quality parameter in food industries. *Food Research International*, *132*, 109108. https://doi.org/10.1016/j.foodres.2020.109108

Alwazeer, D. & Özkan, N. (2022). Incorporation of hydrogen into the packaging atmosphere protects the nutritional, textural and sensorial freshness notes of strawberries and extends shelf life. *Journal of Food Science and Technology*, 1-14. https://doi.org/ 10.1007/s13197-022-05427-y

Alwazeer, D., Bulut, M. & Tunçtürk, Y. (2020). Fortification of milk with plant extracts modifies the acidification and reducing capacities of yoghurt bacteria. *International Journal of Dairy Technology*, *73*(1), 117-125. https://doi.org/10.1111/1471-0307.12643

Arashisar, Ş., Hisar, O., Kaya, M. & Yanik, T. (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. *International Journal of Food Microbiology*, *97*:209-214. https://doi.org/10.1016/j.ijfoodmicro.2004.05.024

Bekhit, A.E.A., Giteru, S.G., Holman, B.W.B. & Hopkins, D.L. (2021). Total volatile basic nitrogen and trimethylamine in muscle foods: Potential formation pathways and effects on human health. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 3620–3666. https://doi.org/10.1111/1541-4337.12764

Bourel, G., Henini, S., Diviès, C. & Garmyn, D. (2003). The response of Leuconostoc mesenteroides to low external oxidoreduction potential generated by hydrogen gas. *Journal of Applied Microbiology*, *94*(2), 280–288. https://doi.org/10.1046/j.1365-2672.2003.01832.x

Brown, H.H. & O. Finksiger. (1980). Oxidation reduction potential. *International Microbial Ecology of Foods ,1* (1): 112-115.

Bulut, M., Sezer, Y.Ç., Ceylan, M.M., Alwazeer, D. & Koyuncu, M. (2022). Hydrogen-rich water can reduce the formation of biogenic amines in butter. *Food Chemistry*, *384*, 132613. https://doi.org/10.1016/j.foodchem.2022.132613

Cachaldora, A., García, G., Lorenzo, J.M. & García-Fontán, M.C. (2013). Effect of modified atmosphere and vacuum packaging on some quality characteristics and the shelf-life of "morcilla", a typical cooked blood sausage. *Meat Science*, *93*(2), 220-225. https://doi.org/10.1016/j.meatsci.2012.08.028

Caldeo, V. (2015). *Oxidation-reduction potential and its influence on Cheddar cheese quality* (Doctoral dissertation, University College Cork).

Dapkevicius, M.L.N.E., Nout, M.J.R., Rombouts, F.M., Houben,

J.H. & Wymenga, W. (2000). Biogenic amine formation and degradation by potential fish silage starter microorganisms. *International Journal of Food Microbiology, 57*(1–2), 107–114. https://doi.org/10.1016/S0168-1605(00)00238-5

Doe, P., Olley, J., Haard, N. & Gopakumar, K. (1998). *Methodology for quality measurements. In: Doe, Peter E. (Ed.), Fish Drying and Smoking. Production and Quality.* Technomic Publishing, Lancaster, 117-135.

Emborg, J., Laursen, B.G., Rathjen, T. & Dalgaard, P. (2002). Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2°C. *Journal of Applied Microbiology*, *92*(4), 790-799. https://doi.org/10.1046/j.1365-2672.2002.01588.x

Erdilal, R. (2008). Taze Su Ürünleri İçin Tekstür Profil Analizi. *Ecological Life Sciences*, *3*(3), 424-435. https://doi.org/10.12739/10.12739

George, S.M., Richardson, L.C., Pol, I.E. & Peck, M.W. (1998). Effect of oxygen concentration and redox potential on recovery of sublethally heat-damaged cells of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*. *Journal of Applied Microbiology*, *84*(5), 903–909. https://doi.org/10.1046/j.1365-2672.1998.00424.x

Giménez, B., Roncalés, P. & Beltrán, J.A. (2002). Modified atmosphere packaging of filleted rainbow trout. *Journal of the Science of Food and Agriculture,* 82:1154-1159. https://doi.org/10.1002/jsfa.1136

Goulas, A.E. & Kontominas, M.G. (2007). Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. *Food Chemistry*, 100: 287-296. https://doi.org/ 10.1016/j.foodchem.2005.09.045

Halkman, A.K. (2013). *Ankara Üniversitesi Mühendislik Fakültesi Gıda Mühendisliği Bölümü Gıda Mikrobiyolojisi II Ders Notları*. Ankara, 89.

Hisar, Ş.A. (2002). *Modifiye Atmosferde Ambalajlamanın Gökkuşağı Alabalığı (Oncorynchus mykiss) Filetoların Fiziksel, Kimyasal ve Mikrobiyolojik Özelliklerine Etkisi*. Atatürk University. Institute of Science. Ph.D. Thesis.

Howgate, P. (2009). *Traditional methods. In: Fishery products quality, safety, and authenticity.* Edited by: Harmut Rehbein and Jörg Oehlenschläger. Wiley-Blackwell. A John Wiley & Sons, Ltd, Publication, Chichester, UK. 19-41.

Huss, H. H. (1977). The post mortem changes in the oxidation-reduction potential of fish muscle and internal organs. In *Proceedings of the X International Symposium International Association of Microbial Studies.* (pp. 265-279). Ars Polonia.

Ikeda, S. (1979). Other organic component and inorganic component. In: Connel, J.J. (Ed), Advances in Food Science and Technology. Fishing News Books. Ltd. England. Pp. 111-123.

Jacob, H.E. (1970). *Redox Potential. In Methods in Microbiology*, Norris J.R. & Ribbons D.W. (Eds), Vol. 2, pp. 91-123. New York: Academic.

Köktürk, M., Yıldırım, S., Eser, G., Bulut, M. & Alwazeer, D. (2022). Hydrogen-rich water alleviates the nickel-induced toxic responses (inflammatory responses, oxidative stress, DNA damage) and ameliorates cocoon production in earthworm. *Biological Trace Element Research*, *200*(7), 3442-3452. https://doi.org/10.1007/s12011-021-02908-7.

Magnússon, H., Sveinsdóttir, K., Lauzon, H. L., Thorkelsdóttir, Á. & Martinsdóttir, E. (2006). Keeping quality of desalted cod fillets in consumer packs. *Journal of Food Science*, *71*(2), M69-M76. https://doi.org/10.1111/j.1365-2621.2006.tb08910.x

Martin, F., Cayot, N., Marin, A., Journaux, L., Cayot, P., Gervais, P. & Cachon, R. (2009). Effect of oxidoreduction potential and of gas bubbling on rheological properties and microstructure of acid skim milk gels acidified with glucono-δ-lactone. *Journal of Dairy Science*, *92*(12), 5898-5906. https://doi.org/10.3168/jds.2009-2491

Martin, F., Cayot, N., Vergoignan, C., Journaux, L., Gervais, P. & Cachon, R. (2010). Impact of oxidoreduction potential and of gas bubbling on rheological properties of non-fat yoghurt. *Food Research International*, *43*(1), 218-223. https://doi.org/10.1016/j.foodres.2009.09.032

Oehlenschläger, J. & H. Rehbein. (2009). Basic facts and figures. In: *Fishery products quality, safety, and authenticity*. Edited by: Rehbein, H. & Oehlenschläger, J. Wiley-Blackwell. A John Wiley & Sons, Ltd, Publication, Chichester, UK. 1-18.

Ouvry, A., Wache, Y., Tourdot-Marechal, R., Divies, C. & Cachon, R. (2002). Effects of oxidoreduction potential combined with acetic acid, NaCl and temperature on the growth, acidification, and membrane properties of *Lactobacillus plantarum*. *FEMS Microbiology Letters*, *214*(2), 257–261. https://doi.org/10.1111/j.1574-6968.2002.tb11356.x

Pinheiro, A.C.A.S., Tappi, S., Patrignani, F., Lanciotti, R., Romani, S. & Rocculi, P. (2020). Effects of novel modified atmosphere packaging on lipid quality and stability of sardine (*Sardina pilchardus*) fillets. *International Journal of Food Science & Technology*, *56*(2), 945-953. https://doi.org/10.1111/ijfs.14747

Pørvik, K.A., Steien, S.H., Saltkjelsvik, B. & Thomassen, M.S. (2000). Urea and trimethylamine oxide in diets for seawater farmed rainbow trout: effect on fat belching, skin vesicle, winter ulcer and quality grading. *Aquaculture Nutrition*, *6*: 247-254. https://doi.org/10.1046/j.1365-2095.2000.00143.x

Prabhakar, P. K., Srivastav, P. P., Pathak, S. S. & Das, K. (2021). Mathematical modeling of total volatile basic nitrogen and microbial biomass in stored rohu (*Labeo rohita*) fish. *Frontiers Sustainable Food Systems, 5: 669473.* https://doi.org/10.3389/fsufs.2021.669473

Rahmatipoor, R., Roomiani, L. & Sary, A.A. (2017). Effect of different packaging on the shelf life of silver carp (*Hypophthalmichthys molitrix*) fillets stored at 4°C. *Iranian Journal of Aquatic Animal Health,* 3(2): 22-35. https://doi.org/10.29252/ijaah.3.3.22

Riondet, C., Cachon, R., Waché, Y., Alcaraz, G., & Diviès, C. (1999). Changes in the proton-motive force in *Escherichia coli* in response to external oxidoreduction potential. *European Journal of Biochemistry*, *262*(2), 595–599. https://doi.org/10.1046/j.1432-1327.1999.00429.x

Sezer, Y. Ç., Bulut, M., Boran, G., & Alwazeer, D. (2022). The effects of hydrogen incorporation in modified atmosphere packaging on the formation of biogenic amines in cold stored rainbow trout and horse mackerel. *Journal of Food Composition and Analysis*, 104688. https://doi.org/10.1016/j.jfca.2022.104688

Susanto, E., Agustini, T.W., Ritanto, E.P., Dewi, E.N. & Swastawati, F. (2011). Changes in oxidation and

reduction potential (Eh) and pH of tropical fish during storage. *Journal of Coastal Development*, *14*(3), 223-234.

Tunçtaş, N. (2019). *Gökkuşağı alabalığı (oncorhynchus mykiss) filetolarında modifiye atmosfer ve oksijen emici etiketin ürün kalitesi üzerine etkileri*. (Yüksek Lisans Tezi). Sinop Üniversitesi, Fen Bilimleri Enstitüsü, Sinop.

Varlık, C., Uğur, M., Gökoğlu, N. & Gün, H. (1993). *Su Ürünlerinde Kalite Kontrol İlke ve Yöntemleri*. Gıda Teknolojisi Derneği, 17: 174.

Yılmaz, M. (2004). *Gökkuşağı alabalık (Oncorhynchus mykiss) filetosunda Listeria monocytogenes'in gelişimi üzerine vakum ve modifiye atmosferde ambalajlamanın etkisi.* (Doktora Tezi). Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Erzurum.

Woyewoda, A.D., Shaw, S.J., Ke, P.J. & Burns, B.G. (1986). *Recommended Laboratory Methods for Assessment of Fish Quality*. Canadian Technical Report of Fisheries and Aquatic Sciences, No. 1448, Canada.

Supplementary Table 1. Change in pH in samples stored under different gas formulations.

	1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
CONTROL	6.49±0.01 Ad	6.49±0.03 Ab	6.48±0.05 Aa	6.53±0.02 Ad	6.55±0.04 Ab	6.49±0.04 Aa
MAP1	6.32±0.01 Ab	6.34±0.01 Aa	6.35±0.02 Aa	6.35±0.02 Ab	6.46±0.01 Bab	6.53±0.04 Ca
MAP2	6.25±0.02 Aa	6.43±0.01 Bab	6.39±0.02 ABa	6.48±0.02 Bcd	6.34±0.06 ABa	6.35±0.06 ABa
RAP1	6.4±0.01 Bc	6.42±0.03 Bab	6.33±0.07 ABa	6.24±0.02 Aa	6.41±0.02 Bab	6.43±0.04 Ba
RAP2	6.36±0.01 Abc	6.42±0.04 Aab	6.32±0.03 Aa	6.42±0.03 Abc	6.4±0.04 Aab	6.48±0.07 Aa

Results are given as means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between samples (within the same column) at a significance level of 95%

Supplementary Table 2. Change in free fatty acids (% oleic acid) in the samples.

	First day	1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
CONTROL	0.76±0.04Aa	1.63±0.63ABa	1.46±0.16ABa	1.21±0.12ABa	1.32±0.20ABa	1.88±0.20Bb	2.05±0.12Bc
MAP1	0.76±0.04Aa	1.40±0.32Ba	1.12±0.16ABa	1.15±0.20ABa	1.15±0.04ABa	1.01±0.08ABa	1.15±0.04ABa
MAP2	0.76±0.04Aa	1.29±0.24Aa	1.23±0.24Aa	1.07±0.16Aa	1.32±0.04Aa	0.98±0.12Aa	1.18±0.00Aa
RAP1	0.76±0.04Aa	1.40±0.16CDa	0.95±0.16ABa	1.15±0.04ABCDa	1.21±0.04BCDa	1.04±0.12ABCa	1.54±0.12Db
RAP2	0.76±0.04Aa	1.09±0.12ABa	1.43±0.20Aa	1.09±0.12ABa	0.98±0.12ABa	1.12±0.08ABa	1.04±0.04ABa

Results are means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between the samples (within the same column) at a significance level of 95%.

Supplementary Table 3. Changes in ΔE (Meat and Skin) during cold storage.

		ΔE					
		1st Day	3rd Day	5th Day	7th Day	10th Day	15th Day
Meat	CONTROL	3.67±0.04Bb	3.63±0.04BCb	3.39±0.03CDc	3.35±0.07Dd	1.66±0.20Ee	4.41±0.08Ad
	MAP1	3.78±0.03Db	3.29±0.01Ec	5.66±0.08Cb	3.13±0.18Fe	9.28±0.08Aa	8.11±0.16Ba
	MAP2	3.35±0.07Ec	2.85±0.06Fe	3.91±0.01Cd	3.57±0.04Dc	5.05±0.07 Ac	4.19±0.01Be
	RAP1	4.48±0.03Ca	5.00 ±0.71Ba	1.63±0.10Fe	4.06±0.08Db	2.10±0.14Ed	6.22±0.31Ab
	RAP2	2.48±0.06Fd	3.78±0.06Eb	9.03±0.04Aa	7.67±0.06Ba	5.92±0.01Db	6.08±0.11Cc
Skin	CONTROL	5.03±0.04Ee	8.14±0.05Bb	6.73±0.03Cc	4.39±0.01Fe	6.36±0.03De	8.28±0.01Ac
	MAP1	7.61±0.01Eb	8.69±0.04Ca	9.17±0.01Ba	8.37±0.04Db	9.83±0.04Aa	7.63±0.04Ed
	MAP2	6.95±0.07Ec	7.84±0.01Bc	5.25±0.07Fe	7.66±0.02Cc	7.54±0.03D c	8.66±0.02Ab
	RAP1	8.10±0.14Ca	6.18 ±0.01Ee	8.38±0.02Bb	8.85±0.01Aa	7.74±0.05Db	5.80±0.007Fe
	RAP2	6.72±0.02Cd	7.49±0.01Bd	5.40±0.02Ed	4.71±0.01Fd	6.59±0.02Dd	8.76±0.02Aa

Results are means ± standard deviations. Different uppercase letters indicate significant difference between days (within the same row), different lowercase letters indicate significant difference between the samples (within the same column) at a significance level of 95%.