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Impact of Sodium Bicarbonate-Enriched Diet on Muscle pH and Quality of Rainbow Trout (*Oncorhynchus mykiss*) During Cold Storage

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ABSTRACT

The effect of sodium bicarbonate (SB) supplementation to fish feed on the quality characteristics of rainbow trout (*Oncorhyncus mykiss*) under post-harvest cold storage conditions was investigated in this study. Rainbow trout fed with 0, 1M, 2M and 3M SB supplemented diets for 21 days before harvesting were stored at 4°C. The highest post-harvest pH level was observed in the group fed with 2M and 3M SB. TVB-N value was found between 14.88-15.68 mg/100g in fish fed with 1M, 2M and 3M SB supplemented diets, while it was determined as 20.14 mg/100g for the control group. At the beginning of storage, similar TBARs values were observed in all groups; however the increase in TBARs in the groups fed with SB supplemented feeds was slower than in the control. Sensory scores of trout fed with SB supplemented diets at the beginning of storage were higher than those of the control group. However, no difference was determined between the groups regarding shelf life (13 days). The best texture scores were observed in the 3M group throughout the storage period. Therefore, it can be concluded that SB addition to pre-harvest rainbow trout feeds can be used as a feed supplements to improve fish flesh quality.

Keywords: Sodium bicarbonate, rainbow trout, pH, cold storage, post-harvest quality

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Sodyum Bikarbonatla Zenginleştirilmiş Diyetin Soğukta Depolama Sırasında Gökkuşağı Alabalığının (*Oncorhyncus mykiss*) Kas pH'ı ve Kalitesi Üzerine Etkisi

Öz: Bu araştırmada balık yemlerine sodyum bikarbonat (SB) ilavesinin gökkuşağı alabalığının (*Oncorhyncus mykiss*) hasat sonrası soğuk depolama koşullarında kimyasal ve duyusal özelliklerine etkisi araştırılmıştır. Hasattan önce 21 gün boyunca 0, 1M, 2M ve 3M SB katkılı yemlerle beslenen gökkuşağı alabalığı 4°C'de depolanmış, raf ömrü sonuna kadar kimyasal ve duyusal değerlendirmeler yapılmıştır. Hasat sonrası en yüksek pH düzeyi 2M ve 3M SB katkılı yemlerle beslenen grupta gözlenmiştir. TVB-N değeri 1M, 2M ve 3M SB katkılı yemlerle beslenen gökkuşağı alabalığında 14,88-15,68 mg/100g arasında bulunurken, kontrol grubunda 20,14 mg/100g olarak belirlenmiştir. Depolamanın başlangıcında tüm gruplarda benzer TBARs değerleri gözlenmiş; ancak SB katkılı yemlerle beslenen gruplarda TBARs artışının kontrole göre daha yavaş olduğu görülmüştür. Depolama başlangıcında SB katkılı yemlerle beslenen alabalıkların görünüm, koku, tekstür, renk ve genel kabul edilebilirlik puanları kontrol grubuna göre daha yüksek bulunmuştur. Ancak raf ömrü (13 gün) açısından gruplar arasında fark gözlenmemiştir. Tüm depolama süresi boyunca en iyi doku skorları 3M grubunda gözlemlenmiştir. Bu nedenle, hasat öncesi gökkuşağı alabalığı yemlerine SB ilavesinin et kalitesini artırmak amacıyla yem katkı maddesi olarak kullanılabileceği sonucuna varılabilir.

Anahtar kelimeler: Sodyum bikarbonat, gökkuşağı alabalığı, pH, soğuk depolama, hasat sonrası kalite

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Introduction

The accumulation of lactic acid due to glycolysis in the post-mortem stage decreases muscle pH in fish. Low pH in the muscle may cause the formation and increase of gaps between muscle clusters (gaping), decreased water holding capacity, denaturation of muscle and sarcoplasmic proteins and lipid oxidation observed in fish fillets. Liu et al., (2020) stated that fish with high muscle pH have higher water content than those with low pH. Consequently, fish tissues with high pH can be described as softer and watery, while others can be described as harder and dry (Chan et al. 2022). Significant correlations were observed between muscle tissue and muscle degradation parameters due to increased cathepsin B+L activity with decreased muscle pH. Bahuaud et al. (2010)

reported that cathepsin B activity is associated with muscle deterioration, and cathepsin L gene expression is associated with muscle deterioration and tissue. However, it was found that there was more metmyoglobin and lipid oxidation in Asian sea bass (*Lates calcarifer*) minced meat at pH 6 than at pH 7 (Thiansilakul et al. 2011). Post-mortem pH value in fish muscle can be affected by many factors such as species, season, size, fishing method, killing method, and starvation status (Özyurt et al. 2007; Li et al. 2017).

Water is an important component of food, and meat products are often lightly salted to increase water holding capacity and improve sensory properties. The functional properties of proteins after processing (e.g. solubility and water binding) are important as they affect the ability of proteins to interact with other components and, therefore, affect the overall product quality. Salting meat products with a combination of sodium chloride (NaCl) and sodium bicarbonate (NaHCO3) has been associated with better texture, aroma, flavor, odor, color and higher water holding capacity. Sodium ions cause swelling of meat proteins. As sodium ions form an ion cloud around the filaments and osmotic pressure increases within the myofibrils, the filament lattice swells and water holding capacity increases (Siró et al. 2009). While water retention in muscle is lowest when the pH of the muscle is close to the isoelectric point (pI) of myofibrillar proteins, displacing the pH away from the isoelectric point improves water retention. Sodium bicarbonate (SB) is an amphoteric compound with good buffering capacity; aqueous solutions become slightly alkaline due to the formation of hydroxide ions (OH-) and carbonic acid (H2CO3), predominantly in the form of bicarbonate (HCO3-) between pH 6.4 and 10.3. It is known that when SB is used in meat products, muscle pH increases and therefore the protein configuration changes (Åsli and Mørkøre 2012; Åsli and Mørkøre 2013; Åsli et al. 2016).

Sodium bicarbonate has been used for many years as a food ingredient, in nutrition and in industrial processes (Balestra and Petracci 2019). It is among the foods generally recognized as safe (GRAS) by the FDA, and the use of sodium bicarbonate as a food additive has been approved by the European Union (EFSA 2011). Adding SB to the water during rearing and post-harvest transportation of fish helps to balance the pH of the water as well as the salt balance of the fish (Martins et al. 2017). There is evidence to support the use of SB in small ruminant production (Jallow and Hsia 2014; Akter 2021; Hassan et al. 2022; Vicente et al. 2023), but the use of SB in aquaculture and changes in post-harvest meat quality have not been focused.

Rainbow trout (*Oncorhynchus mykiss*) is the most common and economical fish species among trout species. The most preferred storage method for fresh trout in retail and industrial applications is cold (2-4 ºC) and ice storage. There are many factors that affect the storage quality of fish during cold storage. Factors such as fish nutrition, pre-harvest handling and ambient conditions after harvest, packaging material used during storage, and processing techniques can be considered among the important factors affecting the quality of cold-stored trout (Aksun and Tokur 2014). This study aimed to investigate the effects of feeding rainbow trout (*Oncorhynchus mykiss*) with different concentrations of sodium bicarbonate added feeds before harvesting on chemical and sensory quality during cold storage. Thus, providing a high pH value to post-mortem fish will improve the meat quality of rainbow trout.

Materials and Methods

Material

In the study, sodium bicarbonate was purchased from a local market. Commercial trout feed was obtained from a private feed company (Abalıoğlu, Denizli, Turkey). A total of 240 rainbow trout (*Oncorhynchus mykiss*) with an average live weight of 250±10 g was used. The content of 6 mm diameter trout feed was 41% crude protein, 23% crude fat, 12% crude ash, 10% moisture, 3% crude cellulose, 1.5% phosphorus and energy content was 4375 kcal/kg. Analytical-grade chemicals and reagents were acquired from Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA).

Preparation of feeds containing sodium bicarbonate, and feeding

The addition of sodium bicarbonate (SB) to the feeds was applied by absorbing method. Solutions containing 1, 2 and 3 M sodium bicarbonate were sprayed on the feeds at a rate of 10% of the feed and mixed at regular intervals until the feeds absorbed the solution. The feeds that absorbed the SB solution well were kept at room temperature and allowed to dry. As a control group, the feeds were treated with pure water without SBC. The prepared feeds were kept in a dry environment at room temperature until the feeding study was carried out.

In the feeding phase of the study, the facilities of a local trout farming enterprise in the region were used. After the fish were adapted to the rectangular concrete ponds for one-week, experimental feeding was carried out twice in the morning and evening at 3% of the fish weight. Fish were fed with control and experimental group feeds for 21 days. Then, no feeding was done for one day, and harvesting was applied on the 23rd. All the experiments were conducted in accordance with the protocols approved by Çukurova University Animal Experiments Local

Ethics Committee (Approval date: 12.12.2023, Decision number:10).

Cold Storage of Rainbow Trout and Quality Control Analyses

The harvested fish were placed in foam boxes on ice and brought to the laboratory. The washed fish were placed on foam plates, covered with stretch film and placed in the refrigerator (2-4 ºC). At least three parallel pH measurements, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARs) analysis and sensory analyses were carried out for each research group on 0^{th}, 3^{nd}, 6^{th}, 9^{th}, 11^{th} and 13^{th} days of the cold storage.

A sample of 5 g of fish meat was stirred in 50 mL of distilled water for 5 min using Ultra-turax. The pH of the fish meat was then measured using a digital pH meter (WTW 315i pH Meter; Weilheim, Germany) (Lima dos Santos et al. 1981). The method of Antonocopoulos et al. (1973) was used to measure rainbow trout's total volatile base-nitrogen (TVB-N) concentration following steam distillation. Based on titration using 0.1 N HCl and a boric acid solution, TVB-N analysis was performed. The results of the analysis were given as mg TVB-N per 100 g of material.

The method of Tarladgis et al. (1960) was used to measure thiobarbituric acid reactive compounds (TBARs) to assess the level of lipid oxidation in the samples. After distillation of 10 g rainbow trout muscle, 5 mL distillate and 5 mL thiobarbituric reagent (0.02 M TBA in 90% glacial acetic acid) were combined and the mixture was incubated in boiling water for 35 min. Absorbance against the blank containing distilled water instead of the sample was measured using a spectrophotometer (Perkin Elmer, Lambda 25) at 538 nm, and the results were presented in mg of malonaldehyde (MA) per kilogram of the sample.

For sensory evaluation, raw fish was evaluated for color, odor, texture, appearance and overall acceptability by 6 panellists using a score of 1-9 (1; disliked at all-9; liked very much) (Paulus et al. 1979). Evaluation continued until the fish became inedible. The study was considered concluded when the panellists acquired the inedibility ratings (4 and below).

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the data, and the Duncan multiple comparison test was used to examine any differences between the groups during storage. Significance level was $p<0.05$.

Results And Discussion

Chemical Changes of Rainbow Trout Fed with SB Supplemented Feed during Cold Storage Period

Muscle pH in live fish is generally around 7, and post-mortem pH is known to vary between 6.0 and 7.0 (Abbas et al. 2008). In the post-mortem period, glycolysis occurs in muscle tissue under anaerobic conditions and lactic acid is formed. Lactic acid formation is known to have a reducing effect on pH. In this study, the pH level of rainbow trout fed with different concentrations of SB supplemented diets is shown in Table 1. At the beginning of storage, the highest pH level was observed in the group fed with 2M and 3M SB supplemented feeds, while the lowest muscle pH was observed in the 1M group ($p<0.05$). Moreover, the highest muscle pH was observed in 2M and 3M groups until the 11th day of cold storage ($p<0.05$). However, on the 13th day, the last day of storage, pH levels were similar in the control group, 1M and 3M groups ($p>0.05$), while the lowest muscle pH was found in trout fed with 1M SB supplemented feeds. Bodas et al. (2007) found that, although the initial pH of sheep fed with sodium bicarbonate supplemented feeds was low, it decreased more slowly in the first 45 minutes after death compared to the control group. However, they determined that the pH decrease rates in muscle after 24 hours were similar in all groups. Ogunwole et al. (2014) stated that the addition of sodium bicarbonate to drinking water had no effect on the muscle pH of broiler chickens. The pH increase observed during storage in this study is an expected result due to the release of alkaline compounds due to endogenous and/or microbial enzymes. Similarly, many researchers reported that the pH value in the muscle increased as the storage process progressed (Abbas et al. 2008; Cao et al. 2020; Lan et al. 2023). Although it is generally stated that the limit value for pH consumption in fish is 6.80-7.00 (Stansby, 1982; Oehlensclager 1992), this value can be affected by factors such as processing and storage conditions and species.

The initial changes that may occur in fish muscle at the post-mortem stage in fish are due to endogenous enzymes that provide proteolysis of muscle proteins and connective tissue. Therefore, it is stated that the chemical changes occurring at the beginning of the post-mortem stage are caused by autolysis rather than microorganism activities (Delbarre-Ladrat et al. 2006). Although there are different views on how the process occurs, many studies suggest that cathepsin, proteasome and especially calpain enzyme systems play a role in postmortem proteolysis and meat softening (Kaur et al. 2021). Proteolytic degradation leading to softening of myofibrils occurs in a case-specific manner, and the activity of enzymes such as calpains is influenced by factors such as pH, temperature and different processing methods (Kaur et al., 2021; Ma and Kim. 2020). Wilhelm et al (2010) reported that low pH values in muscles are consistent with Ca2+ release and increased Ca2+ release leads to increased calpain activity. Generally, the breakdown of proteins and other nitrogenous compounds in fish muscle is measured by an increase in total volatile basic nitrogen (TVB-N). In this study, as shown in Table 2, the TVB-N value determined as 20.14 mg/100g at the beginning of storage in the control group was significantly higher (14.88-15.68 mg/100g) than the rainbow trout fed with 1M, 2M and 3M SB supplemented feeds ($p<0.05$). The lowest TVB-N levels were observed in rainbow trout fed 2M and 3M SB supplemented diets until the 3rd day of storage. On the other hand, the lowest TVB-N content during the whole storage period was found in the group fed with feeds containing the highest level of SB (3M group) ($p<0.05$). The experimental groups (1M, 2M, and 3M) exhibited high muscle pH levels at the start of storage when autolysis was more efficient and microbial activity had not yet increased. The low pH value in the control group might have led to increased enzymatic activity, which would explain why the TVBN value in the control group was higher than in the other groups. Therefore, it can be said that the addition of SB to the feed may cause better initial meat quality. Consistent with the results of this study, there are many studies indicating that the low TVBN value at the beginning of storage increases during storage due to bacterial and internal enzyme activities and reduction of protein and non-protein nitrogenous components (Shokri et al. 2020; Xu et al. 2022; Javadifard et al. 2023). Lang (1983) suggested that the general quality classification of fish and fish products according to TVBN values should be as follows: up to 25 mg/100 g 'high quality'; up to 30 mg/100 g 'good quality'; up to 35 mg/100 g 'limit of acceptability'; and above 35 mg/100 g 'spoiled'. This classification does not always correspond with other quality parameters, but in this study, it was observed to be consistent with the sensory results.

Seafood products are extremely sensitive to oxidation due to their polyunsaturated fatty acid profile. Secondary oxidation products released from lipid oxidation both cause bitter taste in meat and act as precursors for protein oxidation (Hematyar et al. 2019). In addition, it was stated that lipid oxidation products may contribute significantly to the increased risk of cardiovascular and neurological diseases as well as non-infectious chronic diseases such as cancer (Grootveld et al. 2020). TBARs (thiobarbutyric acid reactive substances) analysis is one of the most common methods used to determine the formation of secondary lipid oxidation products in seafood. In this study, TBARs values of rainbow trout fed with different ratios of SB supplemented diets during storage period are shown in Table 3. At the beginning of storage, TBARs values of 2M, 3M groups and control group were similar, while the lowest value was found in 1M group. However, the increase in TBARs in the groups fed with SB-supplemented feeds increased more slowly than in the control group and in general, TBARs values of all groups were lower than the control group during storage. While TBAR values increased with storage time for all samples, they remained below the acceptability limit of 2 mg MA kg-1, as noted by Connell et al. (1990). Prior to this investigation, studies investigating the effect of adding sodium bicarbonate on meat quality generally focused on parameters like meat firmness and water holding capacity rather than lipid quality (Jallow and Hsia 2014; Bodas et al. 2007; Ogunwole et al. 2014; Bodas et al. 2009). Therefore, a comparison could not be made for the results of this study.

Sensory Changes in Rainbow Trout Fed with SB Supplemented Feed during Cold Storage Period

The sensory scores (appearance, color, odor, texture and overall acceptability) of rainbow trout during cold storage are shown in Fig 1. In this study, it was determined that the appearance, color, odor, texture and general acceptability scores in all groups reached the unconsumable limit (4 and below) on the 13th day from the initial good quality values (8-9). Therefore, there was no effect on shelf-life extension of rainbow trout fed with SB supplemented feeds. However, the initial scores of the control group were lower than those of the trout fed diets supplemented with SB in all parameters. Furthermore, it was noted that during the entire storage period, the group fed with feeds supplemented with 3M SB scored higher in texture evaluations than the control group. However, the same trend was not observed for color assessment. In general, the 3M SB supplemented group scored higher in appearance and odor evaluations. Therefore, it can be said that the addition of SB improves the sensory properties of rainbow trout in general, although it has no effect on shelf life extension. Bodas et al (2007) found that although Hue values and yellowness values of sheep fed with SB supplemented feeds were different, whiteness and redness colors were similar to the control. On the other hand, Jallow and Hsia (2014) reported that meat color and texture firmness of sheep fed with SB supplemented feeds were higher than the control group. In this study, similarly, the addition of SB improved texture values but had no significant effect on color values.

Conclusion

It was observed that the muscle pH of rainbow trout fed different levels of SB for 21 days before harvesting was at a higher pH level. However, TVBN and TBARs values, which are indicators of spoilage, were lower and sensory scores were higher in rainbow trout fed with sodium bicarbonate supplemented feeds during cold storage. However, no difference was observed in terms of shelf life. Considering that the addition of SB to pre-harvest feed enriches meat quality, it can be recommended as a potential feed additive in fish feeding.

Storage Days	Control	1M	2M	3M
0	6.70±0.02^{b1}	6.43±0.13 ^{a1}	6.78±0.02 ^{c1}	6.81±0.04^{c1}
3	6.79±0.12^{a12}	6.78±0.06 ^{a2}	6.83±0.02^{b1}	6.85±0.34^{b1}
6	6.87±0.02^{a2}	6.93±0.13^{b2}	7.02±0.12^{c2}	7.09±0.10^{c2}
9	7.12±0.14^{a3}	7.21±0.05 ^{b4}	7.20±0.36^{b3}	7.24±0.12 ^{c23}
11	7.08±0.10^{a3}	7.09±0.06 ^{a3}	7.14±0.02^{b2}	7.16±0.10 ^{b2}
13	7.38±0.02^{b4}	7.36±0.10^{b5}	7.09±0.03^{a2}	7.36±0.09^{b3}

Table 1. Effect of sodium bicarbonate addition to the diet on the pH level of rainbow trout during post-harvest cold storage.

n = 3, ± SD. Letters (a–d) in the same line indicate difference between groups ($p < 0.05$)
Numbers (1-5) in the same column indicate difference according to storage days ($p < 0.05$)

Table 2. Effect of sodium bicarbonate addition to the diet on the TVB-N (mg/100g) level of rainbow trout during post-harvest cold storage.

Storage Days	Control	1M	2M	3M
0	20.14±1.26^{c1}	15.68±1.02^{b1}	14.88±0.42^{a1}	15.26±1.29^{b1}
3	23.47±0.81^{c2}	22.10±0.37^{c2}	19.27±0.38^{b2}	18.34±0.57^{a2}
6	22.56±0.45^{b1}	22.36±0.66^{b2}	26.92±0.44^{c3}	19.75±1.07^{a2}
9	29.30±0.63^{d3}	23.69±0.70^{b2}	25.38±0.40 ^{c3}	21.76±0.78^{a3}
11	34.28±0.47 ^{c4}	32.07±1.37^{c3}	28.64±1.17^{b4}	25.18±0.72^{a4}
13	37.21±0.17^{b5}	35.07±0.37^{b4}	34.94±0.17^{b5}	29.10±0.72^{a5}

n = 3, ± SD. Letters (a–d) in the same line indicate difference between groups ($p < 0.05$)
Numbers (1-5) in the same column indicate difference according to storage days ($p < 0.05$)

Table 3. Effect of sodium bicarbonate addition to the diet on the TBARs (mg MA/kg) level of rainbow trout during post-harvest cold storage.

Storage Days	Control	1M	2M	3M
0	0.44±0.05 ^{b1}	0.40±0.03 ^{a1}	0.46±0.04^{b1}	0.44±0.01^{b1}
3	0.66±0.04^{c12}	0.55±0.00 ^{b2}	0.52±0.01^{b1}	0.48±0.01^{a1}
6	0.84±0.02^{c2}	0.62±0.03^{b2}	0.60±0.02^{b2}	0.54±0.00 ^{a12}
9	0.85±0.04 ^{c2}	0.76±0.01^{b2}	0.62±0.01^{a2}	0.56±0.01^{a12}
11	1.05±0.07 ^{b3}	0.98±0.01 ^{b3}	0.91±0.01 ^{a3}	0.85±0.00 ^{a2}
13	1.65±0.04^{c4}	1.58±0.04^{b4}	1.41±0.01^{b4}	1.05±0.00^{a3}

n = 3, ± SD. Letters (a–d) in the same line indicate difference between groups ($p < 0.05$)
Numbers (1-5) in the same column indicate difference according to storage days ($p < 0.05$)

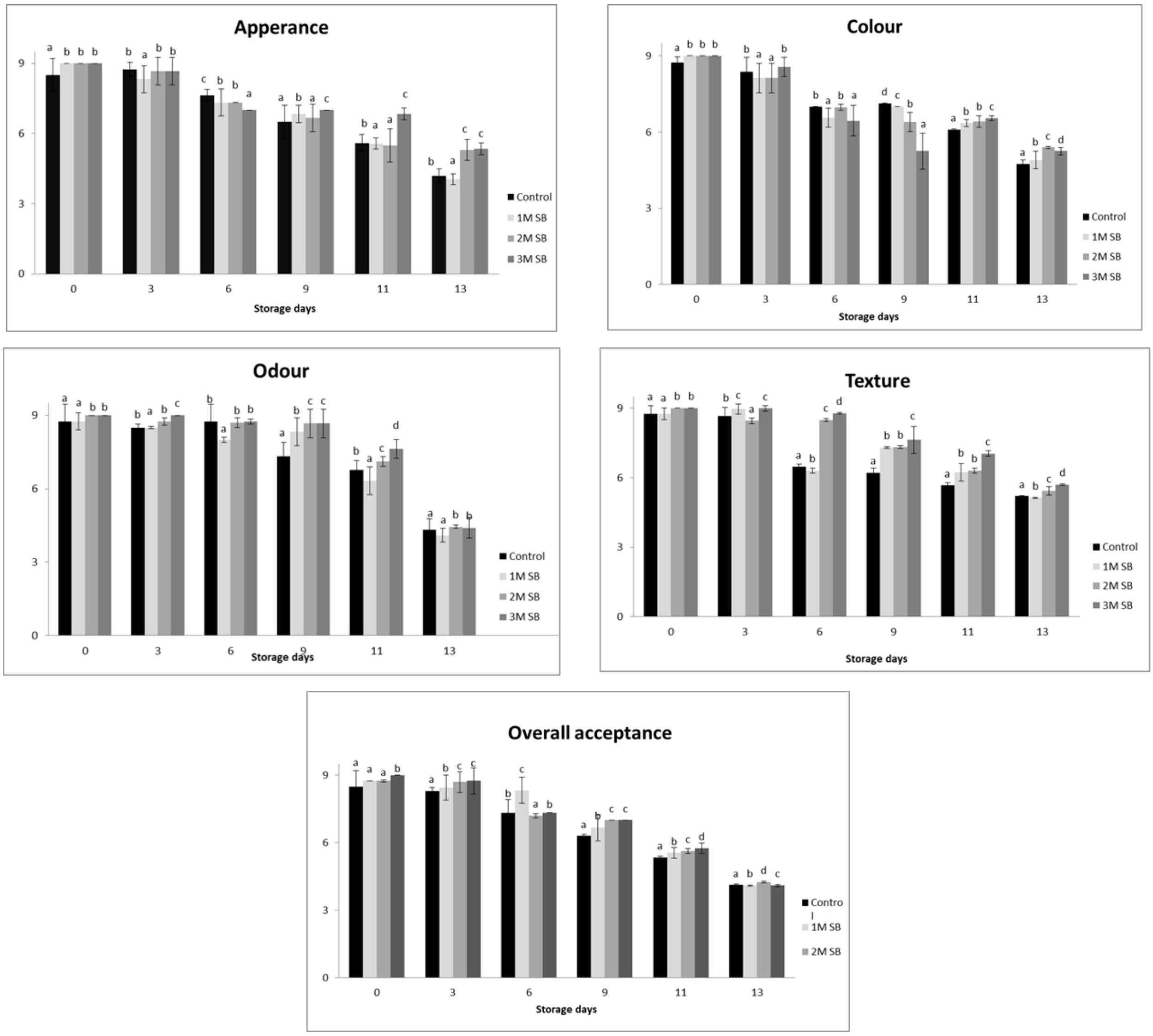

Figure 1. Effect of sodium bicarbonate addition to the diet on the sensory scores of rainbow trout during post-harvest cold storage. Bars with the different letters (a-d) indicate significant differences ($p < 0.05$).

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