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Research Article

Degradation Products of Adenine Nucleotide in Rainbow Trout (*Oncorhynchus mykiss*) Stored in Ice and in Modified Atmosphere Packaging

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Abstract: The breakdown products of adenosine triphosphate (ATP) were separated using a rapid HPLC method. The K-value, Ki-value and H-value were also determined as a means of evaluating the quality of rainbow trout held in ice and modified atmosphere packaging comparing with sensory and microbiological analysis in terms of fresh fish quality. Results from the present research indicated that modified atmosphere did not extend the shelf life of trout but inhibited microbial growth compared to ice storage. However, there was no significant difference ($P>0.05$) between the two conditions. K and Ki-values provided a useful freshness index of fish for both storage conditions. However, H values of trout in ice and MAP increased very slowly although the fish continued to deteriorate at later stages of the storage period.

Key Words: Rainbow trout, ATP degradation products, K-value, MAP

Buzda ve Atmosferi Değiştirilmiş Paketlerde Saklanan Gökkuşağı Alabalıkları'nda (*Oncorhynchus mykiss*) Adenin Nükleotid Yıkılma Ürünleri

Özet: ATP'nin yıkılma ürünleri HPLC kullanılarak ayrıştırıldı. Buzda ve atmosferi değiştirilmiş naylon paketlerde tutulan alabalığın kalitesini belirlemede kullanılan K, Ki ve H değerleri ayrıca belirlendi ve bu değerler duyusal ve mikrobiyolojik analiz sonuçları ile karşılaştırıldı. Bu araştırmadan elde edilen sonuçlar, atmosferi değiştirilmiş paketlerin gökkuşağı alabalığının raf ömrünün uzatmadığı fakat buz ortamı ile karşılaştırıldığında mikrobiyolojik büyümeyi azaltığı bulunmuştur. Bununla beraber, önemli bir fark bulunmamıştır ($P>0.05$). K ve Ki değerleri, iki ortamdada baliğın tazeliğinde kullanılan yararlı indeksler olduğu bulunmuştur. Bununla beraber, buzda ve atmosferi değiştirilmiş paketlerde saklanan alabalıklardan elde edilen H değerleri, balık bozulduğu halde çok yavaş olarak yükselmiştir.

Anahtar Sözcükler: gökkuşağı alabalığı, ATP yıkılma ürünleri, K değeri, MAP

Introduction

Adenine nucleotides and their degradation products have been used as chemical indices of freshness in fresh fish. Adenine nucleotides are degraded by endogenous enzymes in fish during early stages of the storage period (1). The microbial metabolism also contributes to degradation towards the later stages of storage shelflife. In post-mortem fish muscle, degradation of adenosine triphosphate (ATP) takes place according to the following sequence:

ATP → ADP → AMP → IMP → HxR → Hx*

IMP is known to contribute to the pleasant flavour of fresh fish whereas Hx is involved in the progressive loss of desirable fresh fish flavour (2). Concentration of hypoxanthine has been proposed as an index of fresh fish quality but it did not correlate with sensory analysis (3). On the other hand, the K value proposed by Satio et al. (4) includes intermediate breakdown products. The K value is used extensively as a commercial index for estimating fish freshness in Japan (5). The K value is defined as the ratio of the sum of inosine (HxR) and hypoxanthine (Hx) to the sum of ATP and related catabolites (ADP, AMP, IMP, HxR and Hx) expressed as a

* ADP = adenosine disphosphate, AMP = adenosine monophosphate, IMP = inosine monophosphate, HxR = inosine, Hx = hypoxanthine

percentage. Since ATP is completely degraded to IMP within 1 day, it has been replaced by the Ki value (HxR + Hx)/ (IMP+ HxR + Hx), which does not require determination of ATP, ADP or AMP concentrations. The Ki value reduces variability compared to using hypoxanthine concentration (6). In addition, H-values have been described by Luong et al. (7) as an index of freshness quality. The usefulness of these freshness indices depends on the fish species being examined.

Modified atmosphere packaging extends the shelflife of refrigerated fresh fish, especially under carbon dioxide atmospheres (1, 8-10) where microbial growth is reduced (11). ATP and its breakdown products have been suggested as a freshness index of modified atmosphere packaged fresh fish.

Therefore, the objective of the present study was to compare the quality of rainbow trout stored in ice and modified atmosphere pack (MAP) (40% CO_2, 30% O_2, 30% N_2) in terms of adenosine breakdown products, and sensory and microbiological analysis.

Materials and Methods

Storing and sampling of fish

Rainbow trout of 250-300 g were obtained from a fish farm and brought to the laboratory. They were immediately gutted and divided into two lots. One lot was stored in ice and the other was placed in pouches in lots of 3 fish. The pouches were evacuated, flushed and sealed using a Multivac model A 300 vacuum-packaging machine (Bury, Lancs., UK). The gas ratio was 40% CO_2, 30% O_2 and 30% N_2 and the gas/fish ratio was 2:1 for MAP storage conditions. Both lots were stored at chill temperature (2ºC).

Duplicate samples were taken in order to analyse the ATP and its breakdown products and the total viable count from each of three different fish held under both storage conditions.

Adenosine Nucleotide Breakdown Product Analysis

ATP and its breakdown products were determined using a rapid HPLC method (12). The extraction procedure was based on that of Ryder (13). The K-value, Ki- value and H-value were expressed as a percentage and the formulas used are as follows:

{K-value(%)=[(Hx+HxR)/(ATP+ADP+AMP+Hx+HxR)] x 100}

{Ki-value(%)=[(Hx+HxR)/(IMP+Hx+HxR)] x 100}

{H-value(%)=[(Hx)/(IMP+Hx+HxR) x 100}

Sensory evaluation

The Tasmanian Food Research Unit sensory assessment scheme was used with minor modification for trout. Two fish from each storage condition were assessed by 6 trained people. The attributes (skin, slime, scales, eyes, gills, belly, etc.) were assessed and the appropriate demerit point score was recorded against the days of storage (14). Each attribute was given one of four simple descriptors, from 0 to a maximum of 3, where 0 represented good quality or very fresh fish and a higher score indicated deteriorating fish or progressively poorer quality. The scores for the separate characteristics were summed to give an overall sensory score.

Microbiological Analysis

Ten grams of fish muscle were homogenised with 90 ml of ringer solution. Further decimal dilutions were made and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar plates in triplicate. They were then incubated for 2 days at 30ºC.

Results and Discussion

Figures 1 and 2 show the mean values of ATP and its breakdown products obtained from trout stored both in ice and MAP respectively. The initial concentrations of ATP, ADP and AMP were low and decreased gradually under the two conditions. IMP decreased more slowly in MAP than in ice. Hypoxanthine values for aerobically held trout samples were higher than those for trout held in carbon dioxide modified atmosphere throughout the storage period, which shows that the presence of CO_2 may have an effect on the hypoxanthine level.

Figure 3 shows the increase in the pattern of K, Ki and H values for trout stored in both ice and MAP. There was no significant difference between the two storage conditions ($P>0.05$). The values of K, Ki and H were slightly lower in MAP than in ice. There was a good agreement between the rise in K values and the progressive loss of fish freshness. Huynh et al. (2) found the same results with sockeye salmon and herring. In the present study, K values paralleled Ki values throughout

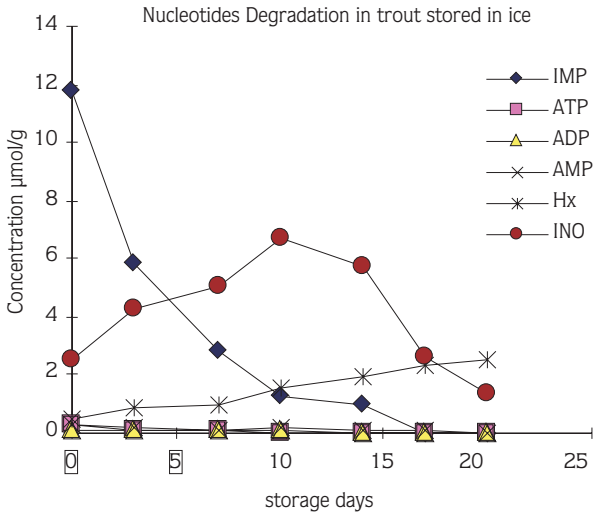

Figure 1. Nucleotide degradation in trout stored in ice.

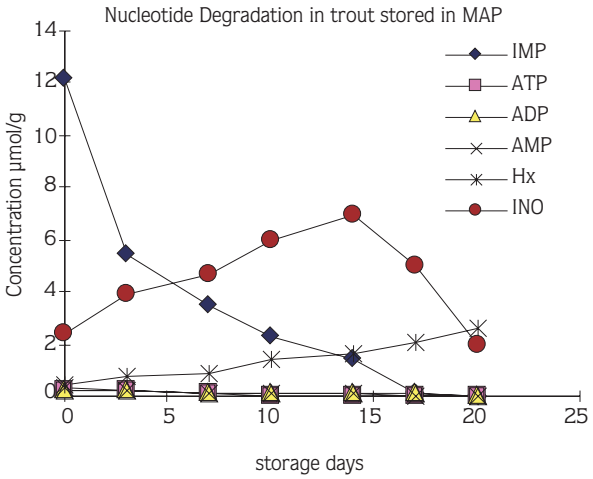

Figure 2. Nucleotide degradation in trout stored in MAP.

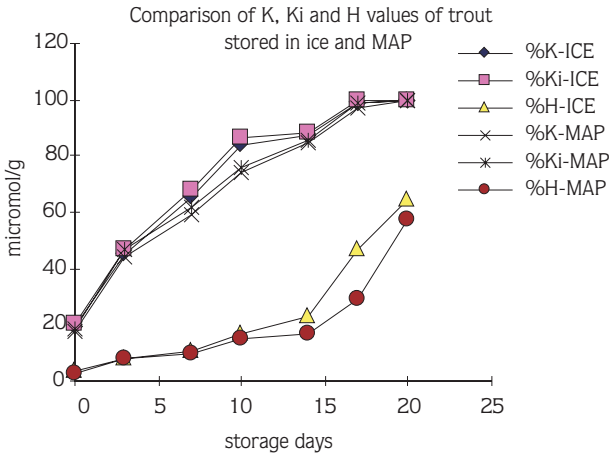

Figure 3. Comparison of K, Ki and H values of trout stored in ice and MAP.

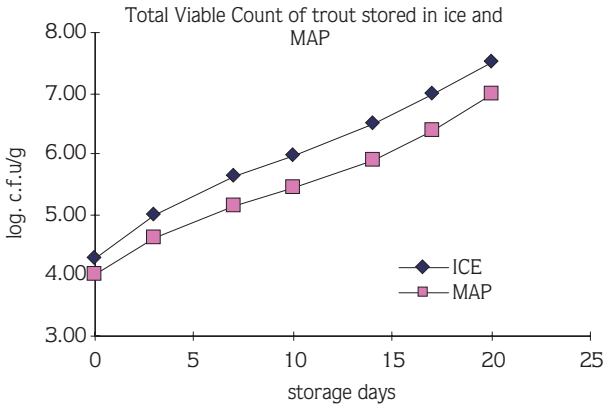

Figure 4. Total viable count in trout stored in ice and MAP.

the storage period under the two conditions. However, H values of trout in ice and MAP were found to increase slowly during the storage period compared to K and Ki values. The CO_2 concentration did not seem to affect the K , Ki and H values of trout. Randell et al. (15) studied the effect of the gas/product ratio and CO_2 concentration on the shelf life of MA packed fish. They also found that CO_2 concentration did not affect the K values of rainbow trout.

The achievable shelf life of MAP depends on the species, fat content, initial microbial load, gas mixture, gas/product ratio and storage temperature (15-17). In the present study, bacteria in rainbow trout grew quickly in ice and slowly in gas packages (Figure 4), although there were no significant differences ($P>0.05$) in total viable counts. MAP storage slightly inhibited the microbial growth of trout (14 days) but it did not extend the shelf life of trout compared to trout in ice (13 days).

Unacceptable sensory quality of modified atmosphere packaged trout occurred at 11 days for MAP and 12 days in ice, at which time K values were greater than 73% (Figure 5). The shelf life of trout was shorter in MAP than in ice in terms of sensory analysis because the odour of the package lowered the odour scores. In addition,

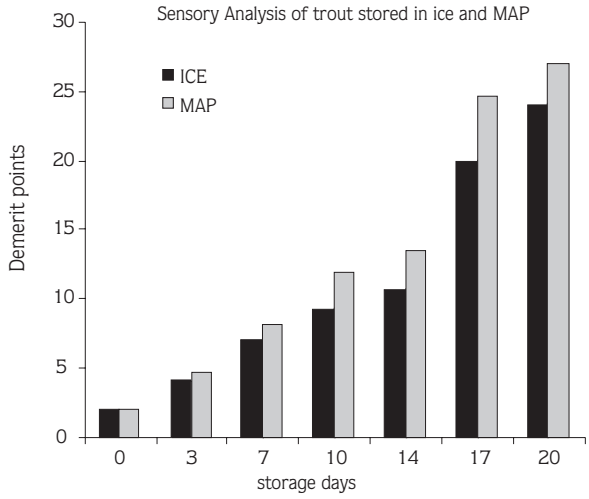

Figure 5. Sensory analysis of trout storedin ice and MAP.

trout drip that formed in gas packages impaired the sensory quality. The appearance of trout was poorer in MAP than in ice due to the excessive drip. These results are in agreement with those of Randel et al. (16).

In conclusion, the results from rainbow trout indicated that K and Ki values correlated with the perceived loss of freshness, with the exception of H values. However, these values did not reflect product acceptability. The use of modified atmosphere packing did not have an effect on K and Ki values. However, carbon dioxide reduced the rate of hypoxanthine formation. Although the trout in MAP showed shorter a shelf life in terms of sensory analysis, there was an increase in K and Ki values with an increase in storage time, which indicated that K and Ki values provide useful indicators of freshness in trout stored in ice and MAP.

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