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Research Article

The Application of Hypoxanthine Activity as a Quality Indicator of Cold Stored Fish Burgers*

Suhendan METİN, Nuray ERKAN, Candan VARLIK
Istanbul University, Fisheries Faculty, Istanbul-TURKEY

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Abstract: Seafoods are very perishable, and many different methods are used for the determination of their quality. Sensory and hypoxanthine values of cold stored trout burgers, which we prepared, were determined during a 28-day period. Burgers spoiled after the 21st day of storage. The TVB-N values were still very low when the burgers spoiled and the pH values decreased during their storage. Therefore total volatile bases nitrogen and pH analyses were not useful for the determination of the quality of trout burgers. There was a negative correlation between the sensory and the hypoxanthine values during the storage period. Thus, hypoxanthine analysis is recommended as a suitable quality indicator of fish burgers.

Key Words: Hypoxanthine, Fish, Fish burger, Shelf life

Soğukta Depolanmış Balık-Burgerlerin Kalite İndikatörü Olarak Hipoksantin Aktivitesinin Uygulanması

Özet: Su ürünleri çok çabuk bozulabilir olup, bunların kalitelerinin tespiti için birçok farklı metot kullanılmaktadır. Tarafımızca yapılan soğukta depolanan alabalık burgerlerinin 28 gün boyunca duyusal ve hipoksantin değerleri tespit edilmiştir. Burgerler, depolamanın 21. gününden sonra bozulmuşlardır. TVB-N değerleri burgerler bozulduğunda bile çok düşük kalmış, pH değerleri ise depolama süresince düşme göstermiştir. Bundan dolayı toplam uçucu bazik azot ve pH analizleri alabalık burgerlerinin kalitelerinin tespiti için uygun bulunmamıştır. Depolama periyodu süresince duyusal ve hipoksantin değerleri arasında negatif bir korelasyon görülmüştür. Böylece, hipoksantin analizi, balık burgerlerinin kalitelerinin belirlenmesi için uygun bir parametre olarak önerilmiştir.

Anahtar Sözcükler: Hipoksantin, Balık, Balık-burger, Raf ömrü

Introduction

Seafoods are perhaps the most perishable foods and their shelf life is limited by enzymatic and microbiological spoilage. There are many quality control methods for the determination of fish spoilage. The determination of the sensory quality, microbial growth and chemical changes are based on the measurement of postmortem deteriorative changes in the fish. The autolytic deterioration is the cause of the loss of freshness. Bacteriological assessment, and total volatile amine (TVB-N), trimethylamine (TMA-N) and pH analyses do not determine the early postmortem deterioration (1).

Shortly after the death of the fish, Hypoxanthine (Hx) begins to accumulate. However, changes in TVB-N and TMA-N are related to the microbiological activity and they do not increase at the beginning of storage. Thus, nucleotide and Hx measurements have some advantages over TMA and TVB analyses. Spineli (2) reported that the total volatile amine and trimethylamine tests measure the various stages of spoilage caused by bacteria, but the assay of nucleotides and Hx reflects enzymatic spoilage.

The detection of xanthine during advanced spoilage indicates that hypoxanthine is lost through bacterial oxidation (3).

Adenosine triphospate (ATP) is degraded at the postmortem stage by endogenous enzymes in the fish flesh.

$$ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow HxR \rightarrow Hx$$

Hypoxanthine could be broken down by xanthin oxidase:

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$$Hx + O_2 \xrightarrow{xo} xanthine + H_2O$$

$$Xanthine + O_2 \xrightarrow{xo} uric\ acid + H_2O_2 \quad (4,5).$$

Any quality control parameter should accumulate or disappear quickly during spoilage. In addition, it should be absent or present in constant amounts in fresh fish (6).

Hypoxanthine is regarded as the major catabolite of adenosine triphosphate (ATP) and it is a useful freshness indicator because of its gradual accumulation in flatfish. However, extremely rapidly decreasing Hx values precludes its usefulness as a freshness indicator in flathead and Dover sole (7). It is useful to use the IMP/Hx ratio of the muscle for the determination of postmortem age in such cases.

The aim of this study was to investigate the role of Hx in the determination of the shelf life of cold stored trout burgers.

Materials and Methods

The preparation of the samples

The rainbow trout (*Oncorhynchus mykiss*) were gutted, and boiled in water. Their bones, heads and skins were removed. Then the trouts were mixed with spices and shaped in burger form. Salt (2%), semolina (2%), curry (0.2%), flour (10%), black pepper (0.2%), cumin (1%), onion (0.4%), and carbonate (0.2%) were used as additive materials. The burgers were breaded and wrapped in a gas barrier film (O_2 permeability = 6.89 ml/m^2, CO_2 permeability =5.42 ml/m^2, N_2 permeability = 2.48 ml/m^2 at +4ºC, and vapour permeability = 7.86 at 37.8 ± 1ºC, 90% ±RH g/m^2 days.atm). The packages were stored at +4ºC and 3 packages were analyzed at 7-day intervals. Analyses were replicated 3 times. Sensory, pH, TVB-N and hypoxanthine tests were applied until the samples spoiled according to the sensory analyses.

Methods

Five judges assessed the sensory properties and a hedonic scale was used (8). The general appearance, texture, taste and smell were used as criteria for acceptability. The sensory quality of the fresh and cooked burgers was evaluated. The highest number of points given was ten during the sensory evaluation and burgers receiving points lower than four were considered spoiled.

A Metrohm model (632) pH meter was used for the pH measurement. For total volatile base nitrogen (TVB-N) estimation, vapor distillation was used, and the TVB-N value was expressed as mg100g^{-1} fish (9).

The procedure of Jones et al. (10) was employed for the determination of the hypoxanthine levels during storage. Statistical analyses were carried out using Microsoft Excel 2000 program.

Results

Table 1 illustrates the mean sensory, pH, TVB-N and hypoxanthine values for cold stored fish burgers over the 28 days.

Table 1 The sensory pH, TVB-N and hypoxanthine values of cold stored fish burgers

Burgers were of very good quality at the beginning but lost their freshness during the storage period. They were of good quality until the 14^{th} day of storage. After the 21^{st} day they were spoiled. The samples deteriorated in quality over time and a significant ($p \leq 0.05$) difference was determined between the 14^{th} and the 21^{st} days of storage.

pH was measured during the storage period. It was determined to be 6.5 on the first day but this value decreased and was determined to be 5.6 on the last day. There was a significant correlation (0.96261) between the sensory and the pH values during storage.

Total volatile bases nitrogen (TVB –N) values were also measured. The initial value of TVB-N was 10.98 mg/100g fish. This value determined as 20.20 mg/100g fish, and 19.66 mg/100g fish at the 14^{th} and 28^{th} days of

Table 1. The Sensory, pH, TVB-N and Hypoxanthine values of cold stored (+4ºC±1) fish burgers.

Storage days	Sensory	pH	TVB-N mg/100g	Hx (m M/g)
0	9.9	6.5	10.98	2.63
7	9.2	6.5	14.09	2.86
14	8.1	6.1	20.20	3.38
21	4.7	5.8	19.44	4.50
28	1.8	5.6	19.66	4.96

storage respectively. A negative correlation (-0.71131) was determined between the sensory and TVB-N values during the storage of fish burgers.

Hypoxanthine values were determined to be 2.63µM/g, 2.86µM/g, and 3.38µM/g on the first, 7th, and 14th days of storage respectively. Then this value increased and reached 4.50µM/g on the 21st day and 4.96µM/g on the 28st day of storage. There was a significant increase ($p \leq 0.05$) in hypoxanthine values between the 14th and 21st days of storage. A negative and significant correlation (-0.98661) was observed between the sensory and hypoxanthine values during storage.

Discussion

According to the results of sensory analyses (8), trout burgers lost their freshness and were evaluated as spoiled after the 21st day of storage. In another study, Avcı (11) packaged trout burgers aerobically, and found that they were marketable until the 10th day and spoiled on the 12th day of storage. The shelf life of cold stored mackerel burgers was studied by Gokoglu (12). The samples were of good quality until 6 days, marketable on the 8th day and spoiled on the 10th day of storage. Due to the fat content of the fish, the shelf life of mackerel burgers was shorter than that of trout burgers.

pH decreased from 6.5 to 5.6 during the storage period. The decrease in the pH value of fish balls found by Avcı (11) was similar to that of cold stored trout burgers. This could be caused by the fermentation of the potato and bread ingredients of the burgers. Varlik et al. (13) regarded the pH values of 6.8-7.0 as the limit of acceptability for fish. This shows that pH is not a suitable quality indicator for trout burgers.

Total volatile base nitrogen (TVB-N) values increased during the storage period. The initial value of TVB-N was 10.98 mg/100g fish and it was 19.66 mg/100g fish on the last day of storage.

Seafoods were categorized according to the TVB-N values of Ludorff and Meyer (14), and Schormüller (9). They regarded a TVB-N content of 25 mgN/100g as very good, 30 mgN/100g as good, 35 mgN/100g as marketable and the TVB-N values over 35 mgN/100g as spoiled.

The burgers had an off-odor and spoiled on the last day of storage, but the TVB-N values were still very low. It is clear that TVB-N analyses are not useful for the determination of the quality level of trout burgers. Avcı (11) reported a TVB-N value of 14.04mg/100g on the day of spoilage for cold-stored trout burgers.

The hypoxanthine content of the trout burgers was determined to be 2.63 µM/g at the beginning, and reached 4.96 µM/g at the end of storage. There was a negative correlation (-0.98661) between the sensory and hypoxanthine values during storage.

There was a strong and linear relationship between the Hx production and the storage duration of albacore. Because of the linear production of Hx, it was used as a spoilage index of iced albacore (15). Greene and Bernatt-Byrne (16) studied Pacific cod and pollock, and measured the Hx values during storage. Accumulation of Hx in Pacific cod (*Gadus macrocephalus*) fillets was slower than that in Atlantic cod (*Gadus morhua*), but similar to that in North Sea cod (*Gadus callarias*, L.). The Hx levels were negatively correlated with flavor and desirability of the cod and pollock. Jahns et al. (6) reported that the hypoxanthine concentration increased during storage until the values leveled off at 4.7 to 5.0 µM/g, and then the Hx concentration dropped to about 3µM/g. The organoleptic spoilage became obvious at the highest level of hypoxanthine.

Luong et al. (4) studied the hypoxanthine ratio of the cod, salmon and trout. Considerable inosine accumulated and it remained high until 6-7 days of storage. Then the hypoxanthine levels increased significantly and inosine levels decreased.

After 10-18 days of storage, Hx usually reaches its maximum concentration. Spineli (2) studied the degradation of nucleotides in ice-stored halibut and reported that only 50% of the IMP was converted to Hx. Hypoxanthine accumulated slowly and at widely varying rates in halibut. The Hx level was determined to be 3.1 µM/g on the 21st day of storage. In haddock, the concentration of Hx increased rapidly to a peak at 17 days. The hypoxanthine level was approximately 3µM/g on this day. The maximum values of Hx were observed on days 12-14 in lemon sole and day 14 in plaice (3).

Using both CO_2 atmospheres and potassium sorbate-dipping caused decreases in Hx concentrations compared to untreated and air-packaged fish fillets. Hypoxanthine levels were higher in the samples that were aerobically packaged and not dipped in sorbate than in the others.

The use of CO_2 inhibited the formation of Hx and dipping in sorbate resulted in further restricted formation of Hx. (17). Parkin et al., (18) studied the modified atmosphere storage of rockfish fillets. Air-packaged rockfish fillets had maximum levels of Hx between 7 and 14 days of the storage. Bacterial growth and TMA production rates were similar to Hx. Thus, they reported that this test is suitable for the determination of the quality of air-packaged rockfish fillets. However, the Hx values were very erratic in modified atmosphere-stored samples and it is not applicable to samples held under modified atmosphere. Brown et al. (19) stored rockfish and silver salmon under modified atmosphere and included hypoxanthine analyses as a measure of tissue autolysis. They reported that the levels of Hx varied widely and are not very meaningful. They did not determine any particular effect of the modified atmosphere on Hx.

Adenosine triphosphate and its degradation compounds from different parts of whitefish (*Coregonus wartmanni*) were studied. Due to the different physiological conditions of the fish, the concentration of catabolites and Torry-meter readings varied from fish to fish. However, the anatomical location did not affect nucleotide catabolites (20). Some species of flatfish were studied by Greene et al. (7). The gradual accumulation of Hx, which was the major catabolite of ATP in rock and yellowfin sole, made this catabolite a useful freshness indicator. However, extremely rapid or decreasing rates of formation preclude its usefulness as a freshness indicator in flathead and dover sole. The IMP/Hx ratios provide a more accurate estimate of spoilage in such cases. Since the Hx content in fish depends on species, it cannot be used as a freshness indicator alone (21).

Burgers spoiled after the 21st day of storage according to the sensory analyses. There are some criteria for the quality determination of sea foods but many of them are not useful for seafood products. The TVB-N values were still very low when the burgers spoiled and the pH values decreased during storage. It is clear that TVB-N and pH analyses are not useful for the determination of the quality level of trout burgers. There was a negative correlation (-0.98661) between the sensory and hypoxanthine values during storage. The levels of hypoxanthine increased during storage, while the sensory values decreased. It was determined that hypoxanthine analysis was suitable for the determination of the quality of fish burgers.

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