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Bacterial Quality of Vacuum Packed Tuna (*Euthynnus affinis*) Chunks Stored Under Abused Refrigerated Temperatures

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Abstract

The effect of abused refrigerated temperatures on the bacteriological quality of tuna (*Euthynnus affinis*) chunks packed in vacuum and air was investigated. Tuna chunks weighing 200 g were divided into two lots. One lot was vacuum packed in high density polyethylene (HDPE) packs of 300 gauge and other the lot was air packed both were stored under abused refrigerated temperatures (10 ± 2°C). The samples were tested for sensory, bacteriological and biochemical quality at periodical intervals. The vacuum packed chunks were sensorially acceptable up to eight days, whereas the air packed tuna was in acceptable condition only for four days. Total bacterial load in fresh chunks was 10^5 cfu•g. Total enterobacteriaceae count was 10^4-10^5 cfu•g in vacuum packed chunks. Total lactics in vacuum packed chunks was almost constant with a value of 10^2 cfu•g and total vibrios load was 10^3 cfu•g. No *Euthynnus coli* was detected on storage under abused refrigerated condition. Similar result was obtained with total anaerobic sulphite reducers in vacuum packed chunks. Histamine formers decreased by a log from 10^2 to 10^1 cfu•g. However, histamine content in vacuum packed tuna gradually increased from a very low level of 0.99 during storage, but within the permissible limit.

Introduction

Fishery products with extended shelf life are gaining popularity, because they are advantageous for marketing and distribution. Daniels (1991) monitored refrigeration temperatures in retail operations and consumers refrigerators and found that many refrigerated meats and seafoods are exposed to temperatures exceeding 10°C. It was observed by van Garde and Woodson (1987) that 20% of home refrigerators are set at temperatures greater than 10°C, which indicates the potential for temperature abuse by the consumers. Temperature abuse of seafood is very critical for the safety of the consumers, because certain strains of pathogenic bacteria are shown to produce toxins even at refrigerated temperatures. Temperature abuse of food products may occur frequently and can pose a biological hazard to the public.

Technologies such as controlled atmosphere packaging, modified atmosphere packaging and vacuum packaging have been shown to extend the shelf life of seafood products (Steir et al. 1981; Genigeorgis 1985; Garcia et al. 1987; Ikawa et al. 1987; Lilly and Kautter 1990; Farber 1991; Gaze 1992). These techniques may change the risks of potential disease outbreaks, because microaerophilic or anaerobic conditions created by these technologies allow bacterial growth and their growth pattern that are different from those normally encountered. Many of these refrigerated and frozen seafoods rely on low temperature as a primary barrier against bacterial growth and toxin production. Lyon and Reddmann (2000) reported the bacteria associated with processed crawfish in vacuum packaged and aerobically packaged conditions. Refrigeration is one of the means of preservation of fish and fishery products. Current vacuum packaging technology enables fishery products to remain acceptable for consumption under refrigeration from 8 to 15 days depending on the species and storage condition. Tuna is a much sought fish in the international market and is mostly sold in the form of chunks and stored at refrigerated temperatures. Hence, in this study an attempt was made to investigate the effect of abused refrigerated temperatures on bacterial quality of vacuum packed tuna (*E. affinis*) chunks.

Materials and Methods

Tuna (*E. affinis*) procured from fish landing center located in Thoothukkudi, India were immediately brought to the laboratory in insulated containers and washed in potable water, beheaded and eviscerated. They were allowed to bleed for 15 min. and washed again in potable water. Tuna chunks each weighing approximately 200 g were cut from the whole tuna of about 10 kg and divided into two lots. One lot was packed in HDPE bags of 300 gauge using µ ALFA - LAVAL (QUICK 2000) vacuum packaging machine under a vacuum pressure of 5 mm Hg. The other lot was packed in polyethylene bags, which served as control. They were then stored under abused refrigerated condition ($10^{o}C \pm 2^{o}C$) until they were sensorially unacceptable. The samples were drawn at periodic intervals for sensory, microbiological and biochemical quality analyzes.

Sensory characteristics and overall acceptability of raw chunks were assessed by a panel of six experienced members of Faculty of Fish Processing Technology on the basis of 10 point scale described by Huss (1988). The fishes were graded as excellent, good, fair, poor and very poor based on the overall acceptability. Microbiological analysis was carried out for the enumeration of total bacterial load (TPC), total enterobacteriaceae, total lactics, *E. coli*, total histamine formers, total vibrios and total anaerobic sulphite reducers (APHA 1976). Chunks were aseptically removed from the package and 25 g was taken in a sterile container, to which, 225 ml of physiological saline was added and homogenized using sterile pestle and mortar. Ten fold dilutions were made using the same diluent for the respective bacterial analyzes. Appropriate dilutions of tuna homogenate were spread plated onto

Trypticase Soya Agar (TSA), Violet Red Bile Agar (VRBA), Tergitol 7 (T_7) and Thiosulphate Citrate Bile salt Sucrose Agar (TCBS) for the enumeration of TPC, total enterobacteriaceae, *E. coli* and total vibrios, respectively. The plates were incubated at 20ºC for 5 days for TPC, at 37ºC for 24 to 48 h for total enterobacteriaceae, *E. coli* and total vibrios and the colonies were counted and expressed as cfu•g. Pour plate technique was followed for the enumeration of total lactics and histamine formers using MRS Lactobacillus Agar and Modified Niven¢s Medium, respectively and the plates were incubated at room temperature (28ºC ± 2ºC) for 48 h for lactics and at 37ºC for 24 to 48 h for histamine formers. The colonies were counted and expressed as cfu•g. The total anaerobic sulphite reducers were enumerated on Differential Reinforced Clostridial Medium (DRCM) by following three tubes MPN technique. About 25 g of the sample was aseptically taken and to which, 225 ml of physiological saline was added and appropriate dilutions were also made using the same diluent. The tubes were incubated in a water bath at 37ºC for 4 days. The tubes exhibiting black precipitate were counted as positive and expressed as MPN counts•g.

Histamine content of the fish was analyzed by the standard fluorometric method (AOAC 1990). Fish flesh (10 g) was homogenized twice with 50 ml of 0.4N perchloric acid and centrifuged at 3000 rpm for 10 min. The volume of the supernatant was adjusted to 100 ml with 0.4 N perchloric acid. From that, 5 ml of filtrate was taken in a separating funnel, made alkaline with 5 ml of 1 N NaOH and to which, 10 ml of distilled water and 2.0 g NaCl were added. The filtrate was extracted four times with successive 25 ml portions of n-butanol. The butanolic phases were again washed with 10 ml of 1 N NaOH saturated with NaCl. Histamine was then extracted five times with 10 ml of 0.1 N HCl and the volume adjusted to 50 ml. The extract was finally derivatized with o-phthaldehyde and the fluorescence intensity was determined using a spectrofluorometer (Model SL-174, ELICO Ltd., India) at an excitation wavelength of 357 nm and emission wavelength of 439 nm.

Results and Discussion

The overall sensory scores of vacuum and air packaged tuna are shown in figure 1. The tuna meat used in the experiment had fresh seaweedy odor, dark brown color and firm texture and was considered good with a score of 7.75. On the second day of storage, the air packed sample exhibited loss of seaweedy odor, change in color to pale brown and slight loss of firm texture, whereas the vacuum packed meat had slight seaweedy odor and no discoloration in the meat. On the 4^{th} day, the air packed tuna exhibited slight ammoniacal odor, loss in texture and loss of color to light brown. On the other hand, the vacuum packed tuna still had the seaweedy odor to a certain extent, but there was a slight loss in the texture. On further storage, air packed tuna became unacceptable with strong ammoniacal and faecal odors and soft texture. This ammoniacal odor associated with fish spoilage was due to the release of metabolites by bacterial action (Hebard et al. 1987,

Lannelongue et al. 1982, Reddy et al. 1992). The meat color became pale brown and slight disintegration of the meat was noticed. However, the vacuum packed tuna still remained good without ammoniacal odor until 8th day of storage. The color of meat turned from dark brown to light brown on this day and the texture became slightly soft. The vacuum packed tuna meat became totally unacceptable on the 10th day with a score of below 4.0. On this day, the ammoniacal odor was very pronounced in tuna meat and the texture became softer with disintegration of meat.

Total bacterial counts of air and vacuum packaged tuna chunks are shown in figure 2. Initially the air packed tuna had total bacterial count of 10^5 cfu•g. The same result has been observed by Jeya Shakila et al. (2002) on various fresh fish samples including tuna. Huss (1995) also reported that

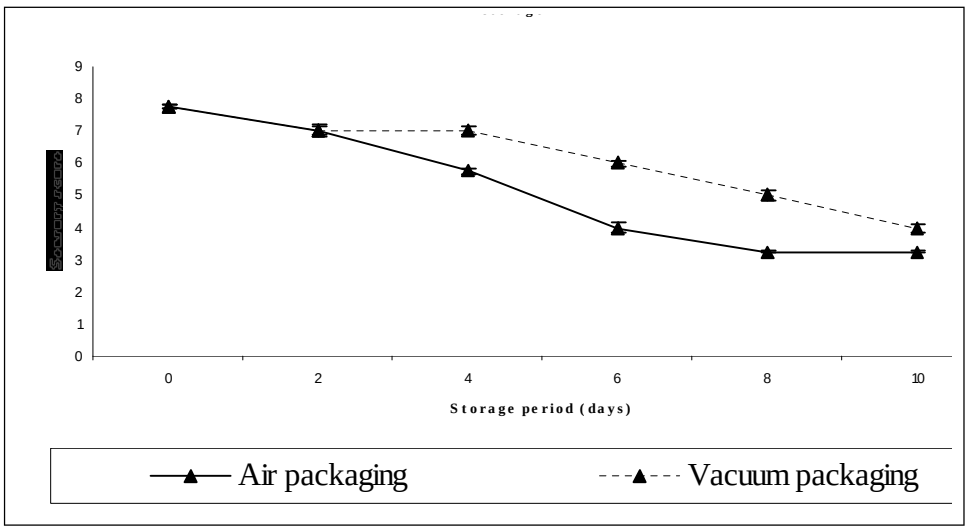

Fig. 1. Changes in sensory scores of air and vacuum packaged tuna chunks during refrigerated storage

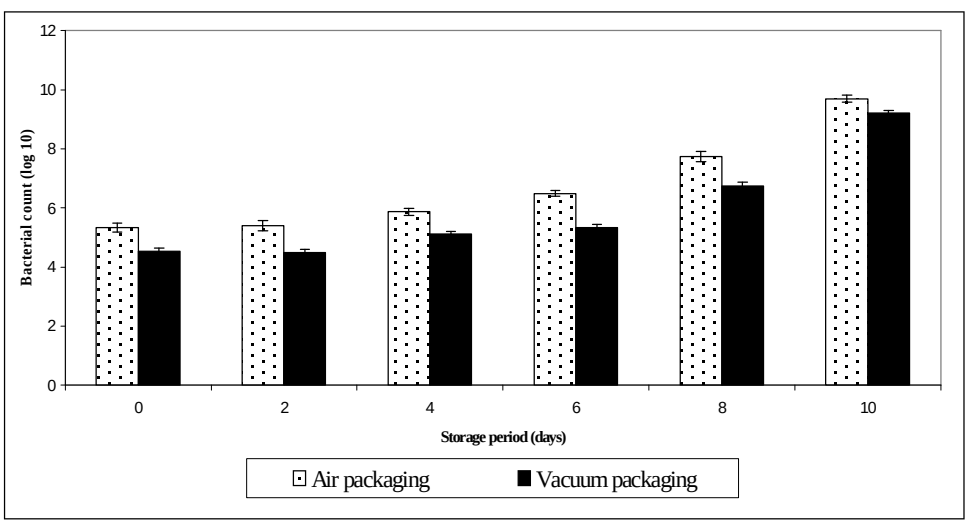

Fig. 2. Changes in total plate count of air and vacuum packaged tuna chunks during refrigerated storage

the tropical fish normally contains such high bacterial population. When vacuum packaged, the counts reduced by one log (10^4 cfu•g). In air packed tuna chunks, the total bacterial load remained more or less constant until 4th day of storage, whereas in vacuum packaged tuna chunks, the initial load (10^4 cfu•g) was maintained until 2nd day and it increased by a log on the 4th day. On further storage, the bacterial load continued to increase sharply in the air packed tuna. This increase coincided well with the sensory scores, as they became unacceptable on the 6th day. But in the vacuum packed tuna, the total bacterial load increased gradually until 8th day and later on the 10th day, it increased progressively to reach a level of 10^9 cfu•g. On this day, the tuna chunks became sensorially unacceptable. Seman et al. (1989) have observed that the growth of recoverable aerobic bacteria was not hindered by low storage temperature, which was in agreement with the results of the present study.

The changes in total enterobacteriaceae counts of air and vacuum packed tuna, held at 10°C are shown in figure 3. The total enterobacteriaceae counts of fresh tuna chunks were 10^5 cfu•g. On storage at 10°C, there was a log reduction in their count in both air and vacuum packed tuna and this decrease was mainly due to the cold shock on enterobacteriaceae. The enterobacteriaceae counts remained at 10^4 cfu•g in both air and vacuum packed tuna chunks until 4th day of storage and later it increased. On the 6th day, the enterobacteriaceae counts increased to 10^5 cfu•g in both the packages and it continued to increase progressively in the air packed tuna on further storage, whereas in vacuum packed tuna, it remained more or less constant until 10th day of storage, indicating that the vacuum condition suppressed their growth to some extent. On the other hand, in air packed tuna, the enterobacteriaceae growth was enhanced from the day when they became organoleptically unacceptable. The changes in

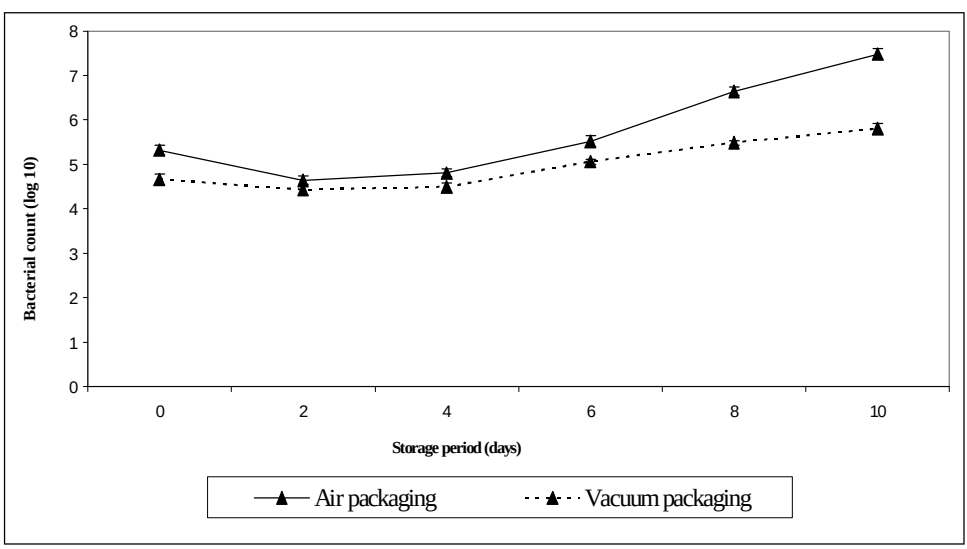

Fig. 3. Changes in the total enterobacteriaceae counts of air and vacuum packaged tuna chunks during refrigerated storage

total enterobacteriaceae counts of air packed tuna do have some correlation with the changes in total bacterial count, with sharp increase in their population once they became sensorially unacceptable. However, in vacuum packed tuna, the growth of enterobacteriaceae was greatly retarded with their counts, more or less constant (10^4 – 10^5 cfu•g) until 10^{th} day, whereas the total bacterial load continued to increase from the 6^{th} day of storage.

The changes in total lactics population of air and vacuum packed tuna chunks are presented in figure 4. Total lactics count of the tuna was very low (10^1 cfu•g), but when the tuna was vacuum packed, their count increased by a log. On the 2^{nd} day of storage, an increase in their counts was observed in both the packages. On further storage, a gradual reduction in their counts was noticed irrespective of air or vacuum packed, until 10^{th} day of storage. However, total lactics count was slightly higher throughout the storage period in the vacuum packed tuna than in the air packed tuna. The microaerophilic condition that prevails in the vacuum package could have enhanced survival of lactic acid bacteria. Pierson et al. (1970) observed that when beef was vacuum packed and stored at 0 – 2°C, the lactobacilli count was 10 to 30% of aerobic plate count. The changes in the sensorial quality did not affect the total lactics count. Similarly there was no correlation between the lactics count and total bacterial load. It has also been observed by Bremner and Statham (1983) that spoilage rates of vacuum packed scallops with or without lactobacilli were similar, which indicates that lactics might not have been involved in the fish spoilage pattern of vacuum packed scallops.

The changes in total vibrios of air and vacuum packed tuna are shown in figure 5. The total vibrios in fresh tuna were quite high (10^4 cfu•g), but when they were vacuum packed the counts was reduced by a log. On the 2^{nd} day of storage, total vibrios count reduced drastically in the air packed tuna, which may be due to the effect of low temperature on the vibrios under air

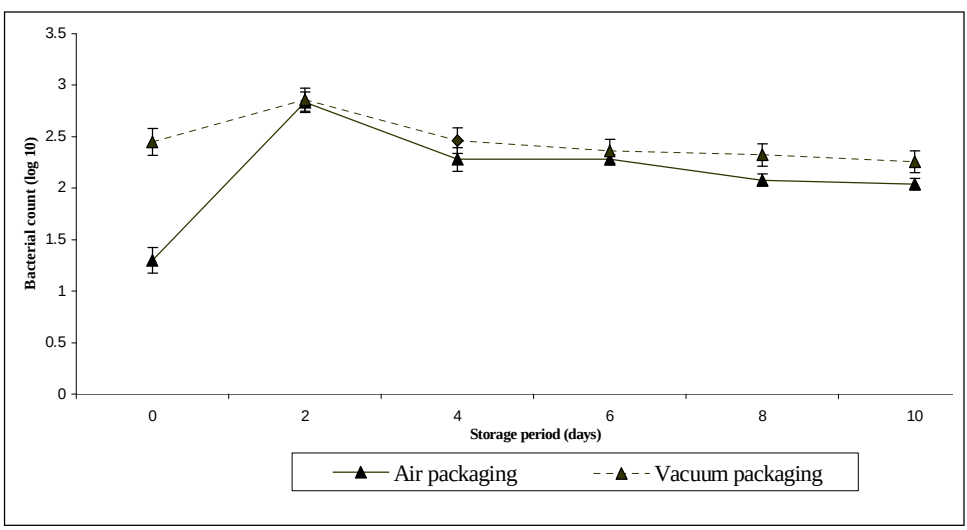

Fig. 4. Changes in total lactics of air and vacuum packed tuna chunks during refrigerated storage

storage. The same effect was also observed in respect of total enterobacteriaceae count in air packed tuna. On further storage, the total vibrios increased very gradually in air packed tuna and reached a maximum level of 10^4 cfu•g on the 10^{th} day, whereas in the vacuum packed tuna, the total vibrios remained more or less same until the end of the storage. This indicates that the vacuum packaging condition did not support the growth of vibrios, but in turn provided an environment to maintain their population. Bremner and Statham (1983) also detected vibrios as the major spoilage flora in vacuum packed scallops stored at 4°C.

The changes in the *E. coli* and total anaerobic sulphite reducing bacteria of air and vacuum packed tuna are given in table 1. The fresh tuna had a total *E. coli* count of 10^3 cfu•g, but when they were vacuum packed, their count reduced by one log. On storage at 10°C, no *E. coli* was detected in both the packages, indicating that their survival was inhibited by the low

Table 1. Changes in total anaerobic sulphite reducers and *E. coli* count of air and vacuum packed tuna chunks during refrigerated storage.

Storage period (days)	Total anaerobic sulphite reducers (MPN/g)		Total *E. coli* (cfu/g)	
	AP	VP	AP	VP
0	1.4*	0.9	2.50×10^3	4.00×10^2
2	ND	ND	ND	ND
4	ND	ND	ND	ND
6	ND	ND	ND	ND
8	ND	ND	ND	ND
10	ND	ND	ND	ND

AP - Air packaging
VP - Vacuum packaging
*ND – Not detected

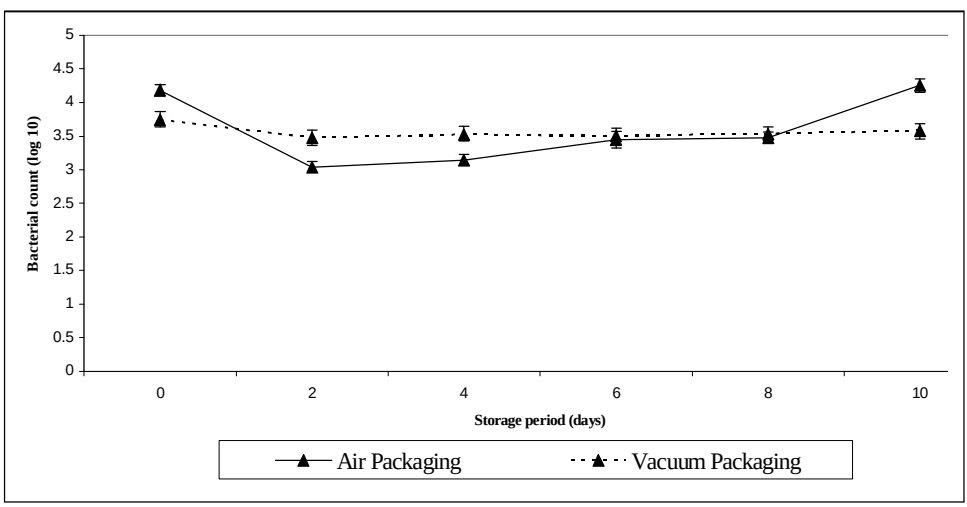

Fig. 5. Changes in total vibrios count of air and vacuum packed tuna chunks during refgirated storage

temperature. The effect of vacuum packaging was not pronounced in respect of *E. coli* at 10°C. Lyon and Reddmann (2000) also recorded no *E.coli* count in vacuum packed craw fish stored at refrigerated temperature of 10ºC. The total anaerobic sulphite reducing bacterial population in fresh tuna was 1.4 MPN counts/g. When the tuna was vacuum packed, their level was reduced to 0.9 MPN counts/g. No anaerobic sulphite reducing bacteria was detected during the storage of tuna chunks at 10°C in both the packages. Vacuum packed tuna initially had very low *E. coli* and anaerobic sulphite reducing bacterial population, but further storage at low temperature completely inhibited their survival. But, Lyon and Reddmann (2000) recorded that the anaerobes plate count were detected till the end of the storage period of both air-permeable and vacuum packaged crawfish. It has also been reported that spoilage occurs in vacuum and modified atmosphere packed (MAP) fish fillets prior to toxin production by anaerobic bacteria, *Clostridium botulinum* type E and hence, it has been recommended to store the vacuum and modified packed seafoods at a storage temperature of 0°C (Eklund 1982; Post et al. 1985).

The changes in total histamine forming bacteria in air and vacuum packed tuna are shown in Fig. 6. In fresh tuna, the total histamine formers were 10^2 cfu•g. On the 2nd day, the count decreased by one log in both the packages due to cold shock. However, no histamine formers were detected in fish stored in ice by few workers (An et al. 2000; Jeya Shakila et al. 2002). The count was more or less constant until 6th day and 8th day in air and vacuum packed tuna, respectively. An increase in their counts was observed in air packed tuna on the 8th day to 10^2 cfu•g and thereafter the counts slightly increased till the end of storage. But, in the vacuum packed tuna, the increase was noticed only on the 10th day when they became sensorially unacceptable. This indicates that vacuum packaging suppressed the growth

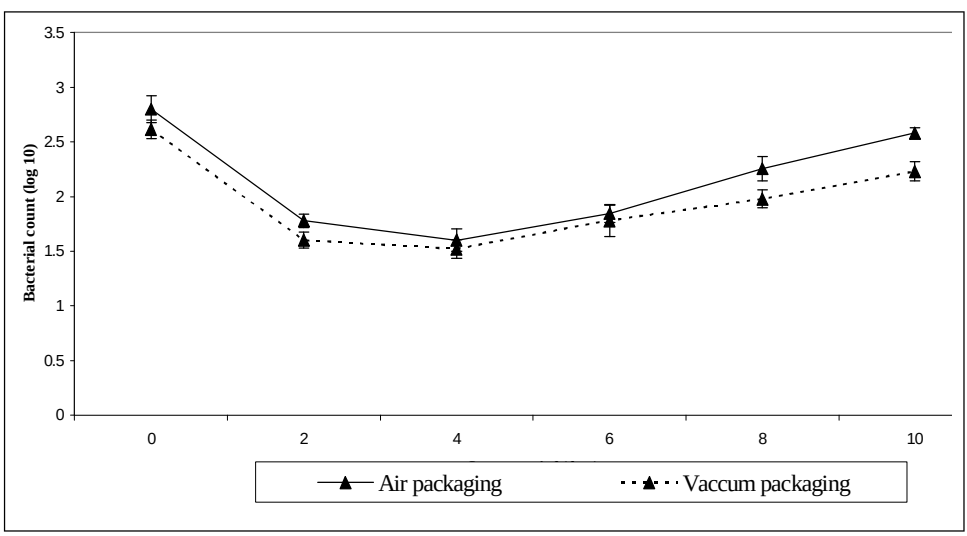

Fig. 6. Changes in total histamine formers of air and vacuum packed tuna chunks during refrigerated storage

of histamine forming bacteria to a greater extent, in relation to their sensorial quality.

Changes in the histamine content of air and vacuum packed tuna during their storage at 10°C are shown in figure 7. Histamine content of vacuum packed tuna was initially very low (0.99 ppm), when compared to air packed tuna (2.99 ppm). On further storage, histamine level slowly increased and attained a maximum level of 21.41 ppm in vacuum packed tuna and 27.81 ppm in air packed tuna. However, there was no significant difference between histamine levels of air and vacuum packed tuna. The histamine content in both the air and vacuum packed tuna did not exceed the maximum permissible level (50 ppm) prescribed for fresh fish by USFDA (FDA 1996) during refrigerated storage.

Conclusion

Results indicate that vacuum packaging completely suppressed the *E. coli* and total anaerobic sulphite reducers, when it was coupled with low temperature storage. However, vacuum packaging did not have any beneficial effect on reducing the total bacterial load, total enterobacteriaceae, total vibrios and total lactics due to abused refrigerated temperatures storage. Hence, it can be concluded that the vacuum packaging of seafoods should be accompanied by proper storage at refrigerated temperatures to protect the safety of the consumers.

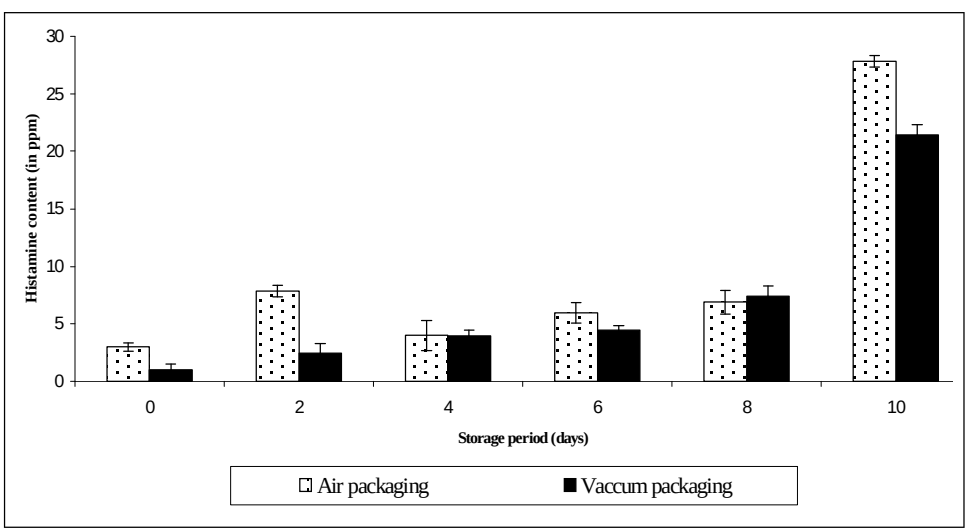

Fig. 7. Changes in histamine content of air and vacuum packaged tuna chunks during refrigerated storage

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