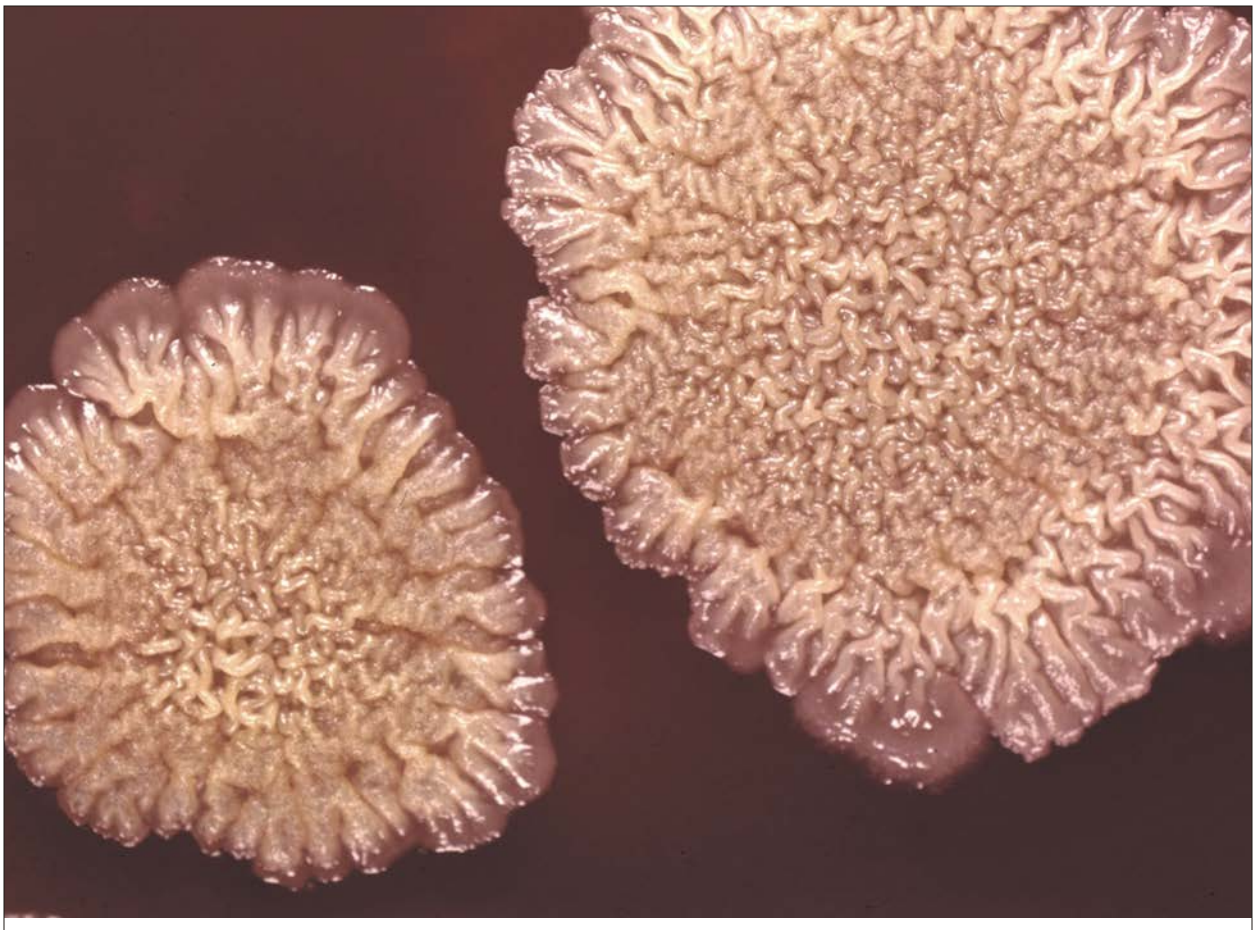

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Effects of Vacuum-packaging on the Microbiological, Chemical, Textural and Sensory Changes of the Solar Rack Dried Sardines During Chill Storage

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ABSTRACT

Solar dried clupeid sardine fishery storage along the Kenyan coast has always been carried out unhygienically using gunny bags kept under ambient conditions. Shelf life changes of the vacuum-packaged solar rack dried sardines during chill storage were therefore examined, to determine the occurring microbiological, chemical, textural and sensory changes in the gunny bag (GBP), control air (CAP), experimental air (EAP) and Experimental Vacuum Packaged (EVP) samples. Pouches (size: 15×22 cm) made of 12 µ-polyester laminated with 300 gauge low-density polyethylene were used for packaging. After packing, all the packs were iced with flake ice in the ratio of 1:1 and stored maintained in an insulated cooler box at 0-2°C. Thereafter, samples were analyzed periodically for chemical, microbiological, textural and sensory characteristics quality changes. *Staphylococcus aureus*, Enterobacteriaceae and Feacal streptococci counts were also determined at the packaging time and at sensory rejection time. Ambient stored Gunny bag and polythene air packaged samples had a shelf life of 14 and 30 days, respectively; chilled polythene air-packaged 45 days and chilled vacuum-packaged 90 days. Thus, vacuum-packaging in combination with chilling was found to be the best in delaying spoilage and thereby significantly extending dried fish products shelf life in tropical environments.

Key words: Fish packaging, chilling, quality, spoilage, shelf life

INTRODUCTION

Fish is perhaps among the most vulnerable world resources that its freshness which is considered a synonym for quality deteriorates rapidly (Ogongo *et al*., 2015; Dewi *et al*., 2011; Khan and Khan, 2001). This spoilage vulnerability coupled with the increasing demand for high quality fresh seafood, has intensified the search for methods and technologies for better fresh fish utilization. One of such major developments in food packaging anticipated for fulfilling fresh fish utilization challenges in stored products is packaging under vacuum or modified atmosphere conditions (Sivertsvik *et al*., 2002). This vacuum-packaging method represents a static form of hypobaric storage widely used in food industries because of its effectiveness in reducing oxidative

reactions in the product at relatively low cost (Gopal *et al*., 1999). It can therefore supplement ice or refrigeration in delaying spoilage, extending shelf life, maintaining higher product quality, assuring product safety and reducing economic loss of stored fish and fishery products.

Texture being a manifestation of the rheological properties (Belton, 2003; Gallegos *et al*., 2004) of a food differs widely among different food types. As a result, fish texture differs widely from that of meat because it contains less connective tissue and the cross-links formed between collagen molecules are weaker thus resulting in a more tender structure. In addition, since many fish species do not have a strong flavour, texture becomes very important for consumer acceptability (Thybo and Martens, 2000; Ashie *et al*., 1996). Imitative tests that attempt to imitate (with instruments) the conditions to which the food is subjected in the mouth or on the plate have therefore been developed for texture measurement analyses. Thus, a more comprehensive description of fish texture is obtained using Texture Profile Analyses (TPA) methods described by Fisher *et al*. (2007), Mezger (2006), Bourne (1978), Breene (1975), Friedman *et al*. (1963) and Johnson *et al*. (1981).

Fresh seafood spoilage is mainly impacted by the Psychrotropic bacteria type of microorganisms (Adams *et al*., 1964) which antimicrobial agents such as sodium acetate have been used to prevent their growths in foods and improve food shelf life under different storage conditions (Kim *et al*., 1995). Sodium acetate is an approved United States Food and Drugs Administration (USFDA) flavouring and pH controlling agent. Zhuang *et al*. (1996) observed that it is effective in controlling natural microbial floral growth on refrigerated catfish fillets. However, temperature abuses on commercial vacuum packaged, or modified atmosphere-packaged fresh fillets, have resulted in rapid growth of *Clostridium botulinum* type E spores during refrigeration storage in between 1-4°C. This is because the organism is non-proteolytic and can grow and produce toxins at a very low temperature of 3.3°C. Although the incidence of botulinum from consumption of refrigerated food is exceedingly low, there have been several reported outbreaks mainly involving type E toxin associated with the consumption of fresh vacuum packed fish products (Huss, 1981).

Therefore, taking into consideration that collective works on various quality aspects including chemical, microbiological, textural and sensory of refrigerated vacuum-packaged stored fish products is scarce within the tropical region, this study undertook to report the effects of vacuum packaging on shelf life of the solar rack dried sardines assessed by chemical, microbiological, textural and sensory parameters during chill storage.

MATERIALS AND METHODS

Packing and storage of the solar rack dried sardines: This packing and storage study was carried out for a period of seven months beginning from August, 2014 to February, 2015 at Kenya Marine and Fisheries Research Institute (KMFRI) Mombasa, Kenya. Fresh sardine fish samples were obtained in bulk from the local Jasini artisanal fishermen in Vanga (S 04°40'16.11", E 039°13'36.96"), Kenya. They were then quickly transferred to large insulated cooler boxes containing crushed ice and transported to the processing sites where the solar rack dryers were installed in Jasini-Vanga next to the landing site. At the processing site, the fish samples were dried for five consecutive days using raised open rack dryers (consisting of an ivory mesh net, raised 0.30 m from the ground to allow for air movement). The dried samples were then divided into four lots. Lot I packed in gunny bag (GBP) and lot II which was polythene air-packed (CAP) and stored in ambient laboratory conditions were the controls. Lots III and IV contained experimental chilled samples for polythene air-packed (EAP) and vacuum-packed (EVP), respectively.

Pouches (size: 15×22 cm), made of 12 µ-polyester laminated with 300 gauge low-density polyethylene, were used for packaging of the dry sardine fish samples. Physical properties of the packaging material, such as heat-seal strength (ASTM., 1972), tensile strength and elongation at break, were determined in the machine direction and in the cross-direction as per, IS 2508 (1984). Oxygen transmission rate (ASTM., 1975) and water vapour transmission rate as per ASTM. (1987) were also determined. Dried sardine fish samples weighing between 200±5 g were placed in each pouch. Lot I samples were sealed by tying the gunny bags using sisal strings wound round their mouths. Lots II and III samples were sealed using an impulse heat-sealing machine whereas Lots IV samples were vacuum-packed at -1 bar pressure. A vacuum sealing machine-Model QS 400 VD supplied by M/s. Sevanna Electrical Appliances Pvt. Ltd., Kerala, India, was used for vacuum packing. Immediately after packing, all the packs were iced with flake ice in the ratio (1:1) fish: ice in an insulated cooler box and transported back to Kenya Marine and Fisheries Research laboratory for storage at chilling temperatures of between 0-2°C. Re-icing was done after draining off the melted ice every day to supplement the melting loss. Samples were thereafter drawn in triplicates from each lot at regular intervals and analyzed mean values for chemical, microbiological, textural and sensory parameters recorded.

Reagents and media: Chemicals used for the experiments were of Sigma brand, Analar grade or guaranteed reagent grade. Dehydrated bacteriological media, such as Plate Count Agar (PCA) (BBL Difco), Baired Parker agar (BP, Himedia), Kenners Faecal (KF) Agar (BBL Difco) and Violet Red Bile Glucose Agar (VRBGA, Oxoid) were used.

Chemical analysis: Proximate composition was determined by AOAC (1998) method. pH was determined according to APHA (1998) using a digital pH meter (Cyber scan 510, UK) after homogenizing 10 g of the fish sample with the same amount of distilled water. Total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) were estimated by micro diffusion method (Conway, 1950). Thiobarbituric acid (TBA) value of the fish sample was estimated spectrophotometrically (Tarladgis *et al*., 1960).

Microbiological analysis: Twenty-five grams of fish were aseptically weighed and homogenized with 225 mL sterile 0.85% normal saline for 1 min in a Stomacher 400 lab blender (Seward medical, London, UK). The homogenized sample was serially diluted using 9 mL sterile saline for bacteriological analysis. Counts of *Staphylococcus aureus* (AOAC., 2002), Faecal Streptococci (USFDA., 1995) and Enterobacteriacea (Koutsoumanis and Nychas, 1999) were determined for fresh fish and for fish at the time of sensory rejection. Total Viable Counts (TVC) were determined in Plate Count Agar by the spread plate method (AOAC., 2002). Fish samples were tested for the presence of *C. botulinum* toxin as per FDA (2001).

Texture analysis: Texture Profile Analysis (TPA) was measured with a universal testing machine (Lloyd instruments LRX plus, UK), as described by Anderson *et al*. (1994), equipped with a load cell of 50 N. Dried sardine fish samples were cooked in 1.5% brine for 10 min, drained well and cooled to room temperature. Thereafter, texture profile analysis was performed on these cooked samples that have been compressed twice by a cylindrical probe of 50 mm diameter at a test speed of 12 mm min^{-1}. The principle behind the usage of the cylindrical probe is that, as the probe is forced into the specimen, a shearing force acts, which causes the sample to deform or rupture.

Table 1: Sensory scores for taste panel studies

Observation (cooked sample) scores	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

This produces a load showing curve resulting from the deformation. Hardness, cohesiveness, springiness and chewiness were calculated as defined in the texture analyzer user manual and TPA results tabulated using Nexygen software.

Sensory evaluation: Sensory analysis is the most used traditional method for fish quality judgments. Thus, various sensory characteristics such as appearance, texture, odour and flavour were evaluated by five trained panelists and scores based on a nine-point hedonic scale as described by Amerine *et al*. (1965). Taste panel scoring of the test fish samples was conducted after boiling the packaging polythene dressed samples in 1.5% brine for 10 min. A sensory score of 4 was taken as the acceptability borderline (Table 1).

RESULTS AND DISCUSSION

Test samples proximate composition and physical properties of the packaging material: Proximate composition of freshly landed test samples showed 75.7% moisture, 3.04% crude fat, 20.3% crude protein and 0.98% ash. The physical property of the used packaging material was also as presented in Table 2.

Chemical assessment

Changes in Thiobarbituric Acid (TBA): Changes in TBA value, which is a measure of oxidative rancidity of the product, were as presented in Fig. 1. The values respectively increased from an initial 0.08-0.5, 0.35, 0.32 and 0.25 mg malonaldehyde kg^{-1} of fish in GBP, CAP, EAP and EVP samples on the 90th day of storage. The results indicated an increasing trend in all of the samples during storage. Similar observations have been previously observed by Huang *et al*. (1994), Josephson *et al*. (1985) and Nolan *et al*. (1989). All polythene air (CAP and EAP) and vacuum packed (EVP) samples exhibited lower TBA values than the gunny bag packed control sample. On the same note, chilled samples exhibited even lower values than the polythene air packed (CAP) sample stored in ambient laboratory conditions. This TBA value reduction during chill storage is in agreement with Rajesh *et al*. (2002) observations. The rate of fat oxidation which has been observed to increase during cold storage was however reduced by vacuum-packing and icing as stated in the works of Baldrati *et al*. (1982) and Varga *et al*. (1980). All of the samples TBA values were also found to be within the acceptability limit range of 1-2 mg malonaldehyde kg^{-1} of fish sample (Lakshmanan, 2000) throughout the storage period.

Changes in pH: Variations in pH values during storage were as depicted in Fig. 2. The initial pH value was 6.5 and on storage, the value increased gradually in GBP. In CAP, EAP and EVP samples, the value decreased initially and then increased. The observed slight decreases in

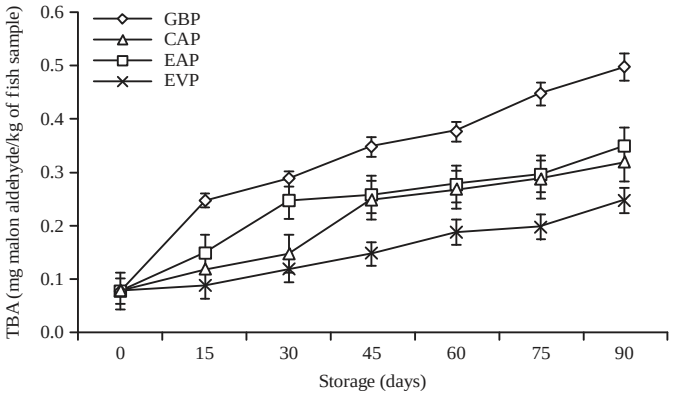

Fig. 1: Changes in TBA values of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

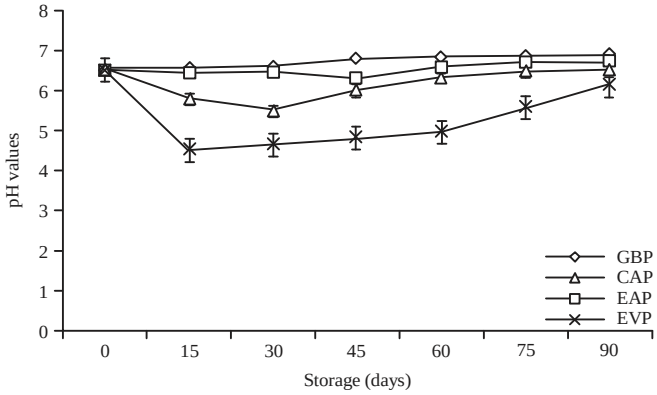

Fig. 2: Changes in pH of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

Table 2: Physical properties of the packaging material

Physical properties	Values
Tensile strength	MD 363 kg cm^{-2}
Tensile strength	CD 349 kg cm^{-2}
Elongation at break	MD 80%
Elongation at break	CD 80%
Heat seal strength	MD 249 kg cm^{-2}
Heat seal strength	CD 194 kg cm^{-2}
Water vapour transmission rate	3.62 g m^{-2}/24 h at 37°C and 90±2% RH
Oxygen transmission rate	65 cc/m^2/atmosphere/24 h at room temperature 28-32°C

MD: Machine direction, CD: Cross-direction

pH values may be attributed to the dissolution of CO_2 in the fish muscle. Similar observations were made by McMeekin *et al*. (1982) who reported a decline in pH of vacuum packed sand flat head fillets stored for 6 days at 4°C. Several authors have also reported decreasing pH values with increases in the concentration of atmospheric CO_2 (Lannelongue *et al*., 1982; Tiffney and Mills, 1982). The increases observed in the pH values may also be attributed to the production of volatile base compounds such as ammonia through spoilage bacterial activity (Cann *et al*., 1983; Reddy *et al*., 1995).

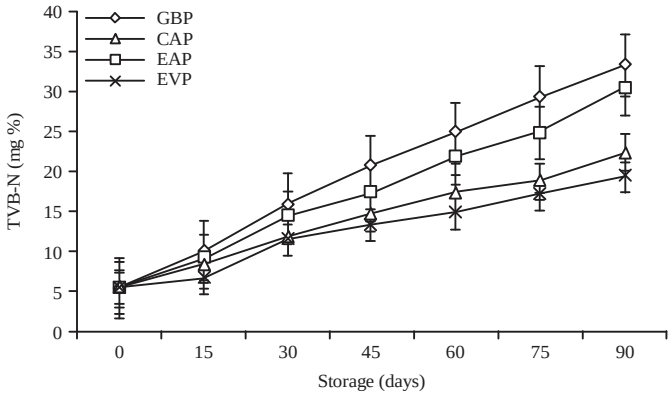

Fig. 3: Changes in TVB-N values of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

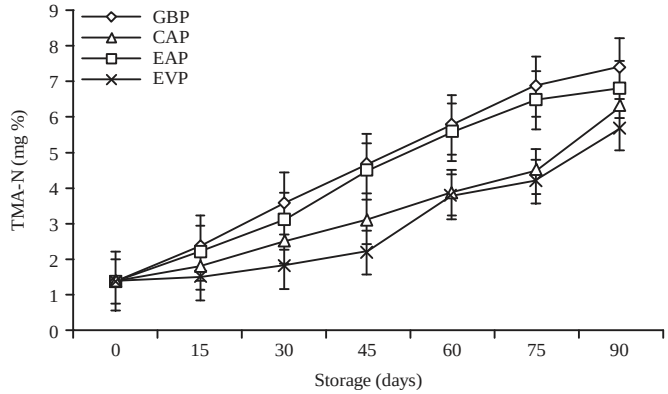

Fig. 4: Changes in TMA-N values of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

Changes in Total Volatile Base Nitrogen (TVB-N): The TVB-N in fish is mainly composed of ammonia, primary, secondary and tertiary amines (Beatty, 1938). A level of 35-40 mg TVB-N 100 g^{-1} of fish muscle is usually regarded as spoilt (Lakshmanan, 2000). Increased TVB-N value change during storage were as shown in Fig. 3 where TVB-N contents increased from an initial value of 5.6-33.5 mg % in GBP, 30.5 mg % in CAP, 22.5 mg % in EAP and 19.5 mg % in EVP on the 90th day of storage. Values were found to be lower in the case of chilled experimental sample packs (EAP and EVP) than in the ambient sample control packs (GBP and CAP). Similar results have been reported by Shalini *et al*. (2000) during refrigerated storage of sodium acetate-treated vacuum-packed *L. lentjan* fillets. Low levels of TVB-N in treated samples were due to either a reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Banks *et al*., 1980). However, even though all samples were within the acceptability limits throughout the storage period, both control samples nearly reached the lower rejection limit target of 35 mg TVB-N 100 g^{-1} of fish muscle.

Changes in trimethylamine nitrogen (TMA-N): TMA-N changes which are also used as fish freshness deciding quality index (Parkin *et al*., 1982) were as shown in Fig. 4. All samples showed

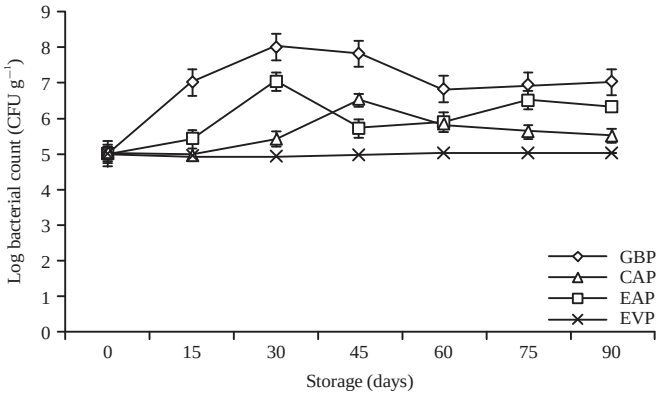

Fig. 5: Changes in total viable counts (20°C) of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

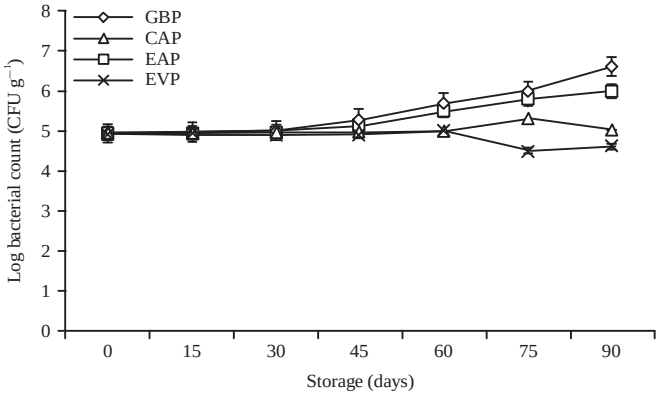

Fig. 6: Changes in total viable counts (37°C) of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

an increasing trend in their TMA-N values with slightly higher increments observed in both the control packs (GBP and CAP). The TMA-N contents increased from an initial 1.4-7.4 mg % in GBP, 6.8 mg % in CAP, 6.3 mg % in EAP and 5.7 mg % in EVP during the whole period of storage. The present study's result are in agreement with those of Ishida *et al*. (1976) and Lakshmanan *et al*. (1996) who both reported that TMA-N formation slows down noticeably in low refrigeration temperature storage conditions above 0°C. This slowing down of TMA-N in reaching 10-15 mg 100 g^{-1} in fish muscle that is considered as the limits of acceptability for human consumption in chilled fish (Connell, 1975), might be attributed to the inhibitory effect of the lower temperatures on bacterial growth. Similar observations had also been previously reported by both Shalini *et al*. (2000) and Rajesh *et al*. (2002).

Microbiological analysis

Changes in Total Viable Counts (TVC): The initial TVC of dried sardines at 20 and 37°C were 4.98 and 4.94 $\log_{10}$ CFU g^{-1}, respectively. Dried sardines' TVC changes at 20 and 37°C during the study were as shown in Fig. 5 and 6, respectively. In the 20°C GBP, CAP and EAP experiment samples, TVC rose continuously and reached about 10^7 CFU g^{-1} on the 14th, 30th and 45th days

of storage when the fishes were, respectively deemed spoilt based on sensory scores. The rise may be attributed to the bacterial growth increases when still using the trapped airs inside the packaging materials before exhaustion. It has also long been known that fish microbiological count determinations at between 20-25°C are always much higher than those determined at 37°C (Castell *et al*., 1948; Liston, 1957) as exhibited by the present study (Fig. 6). The study results also confirmed the earlier findings of Leung *et al*. (1992), Huang *et al*. (1994), Lyon and Reddmann (2000) and Ozogul *et al*. (2000, 2004) that bacteria grows more quickly in fish stored in air than when Vacuum-Packed (VP) at 0°C.

Changes in counts of Enterobacteriaceae, *S. aureus* and feacal streptococci: The initial dried sardines' Enterobacteriaceae counts were 2.81 $\log_{10}$ CFU g^{-1} and by the end of storage, a 0.2 log increase was noticed in EAP and EVP samples whereas 0.4 log increases was observed in CAP samples. This was in agreement with the works on temperate marine fish (Drosinos *et al*., 1997; Koutsoumanis and Nychas, 1999) and temperate fresh water fish work of Savvaidis *et al*. (2002) where *S. aureus* count was 1.2 $\log_{10}$ CFU g^{-1}. All samples registered a 0.2 log reduction counts on the day of sensory rejections which were all within the prescribed limits for fresh dried fish recommended by ICMSF (1986). Thus, the study results indicate good microbiological quality of the dried sardine samples stored at 0-2°C.

***Clostridium botulinum*:** *Clostridium botulinum* toxin was not detected in any of the samples throughout the storage period indicating that there was no temperature abuse during storage. This negative *C. botulinum* toxin result assay is in accordance with the vacuum-packed fish fillets work reported by Lilly Jr. and Kautter (1990) and that for modified atmospheric packed salmon fillets by Reddy *et al*. (1997).

Texture analysis

Changes in Hardness 1 and Hardness 2: Generally, since hardness normally refers to the peak force during the compressive part of the test, hardness 1 therefore, refers to the peak force during first compression whereas, 2 refer to the peak force during the second compression. Hardness 1 and 2 were found decreasing in all the samples during storage with their changes being represented in Fig. 7 and 8, respectively. Hardness 1, decreased respectively from the initial 1.99-1.86, 1.60, 1.54 and 1.4 kgf in GBP, CAP, EAP and EVP samples by the 90th day of storage. The values of both the experimental chilled air (EAP) and vacuum (EVP) packed samples were found to be comparatively lower than those of control ambient gunny bag (GBP) and air packed (CAP) samples. Hardness 2 values for GBP, CAP, EAP and EVP samples decreased respectively from the initial 1.77-1.59, 1.3, 1.24 and 1.09 kgf on the 90th day of storage. In CAP, EAP and EVP samples were also found to have lower Hardness 2 values than GBP samples. These Hardness 1 and 2 value decreases might be attributed to the weakening of connective tissues of the fish muscles during storage.

The results were in agreement with those of Sato *et al*. (1997) who demonstrated that sardine pericellular tissue weakening was correlated with softening. On the other hand, Azam *et al*. (1989) who studied the killing method effect on rainbow trout quality during storage on ice also observed a similar significant softening of both raw and cooked fillets during storage using instrumental measurement (Steven's Compression Response Analyzer, SCRA) method. This was further confirmed by Ando *et al*. (1991) in addition to similar observations being made also by Hatae *et al*. (1985) who reported a softening of the texture in several fish species stored at 4°C using General Foods (GF) texturometer.

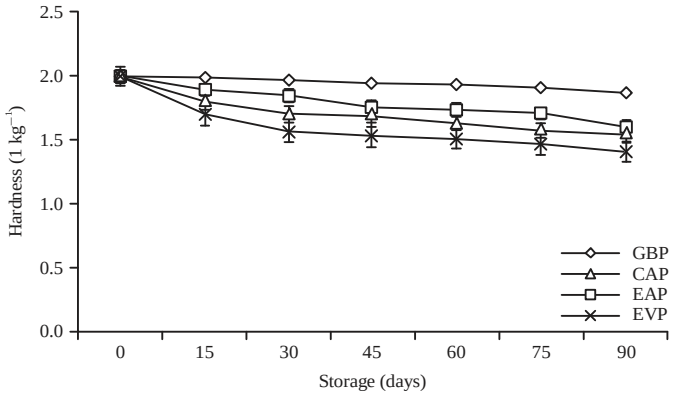

Fig. 7: Changes in hardness 1 values of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

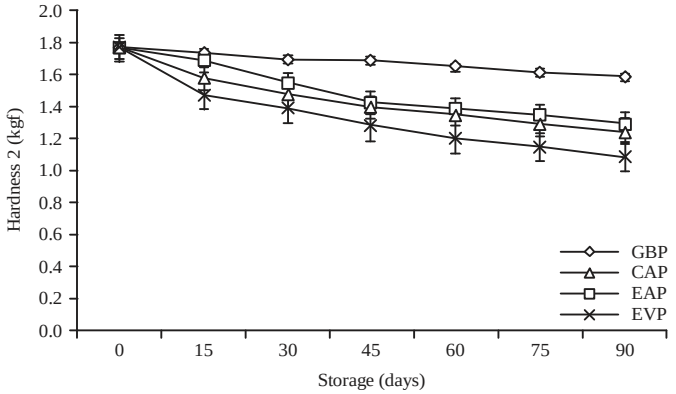

Fig. 8: Changes in hardness 2 values of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

Changes in cohesiveness, springiness and chewiness: Cohesiveness being the ratio of work done during the second compression divided by work done during the first compression is an indication of the materials' viscoelasticity. A value of 1 indicates total materials' elasticity whereas 0 indicates that the sample did not recover at all. The study's cohesiveness value changes were as depicted in Fig. 9 in which respective slight decreases from the initial 0.34-0.22, 0.20, 0.18 and 0.16 were observed in GBP, CAP, EAP and EVP samples at the end of 90 days of storage. This indicated that there was no much change in the internal bonding of the fish muscles during storage.

Springiness (the elastic or recovering property of the fish muscle during compression) changes of the dried sardine samples during the study period were as contained in Fig. 10. In general, a decreasing trend was observed in all test samples during storage. The values respectively decreased from the initial 1.01-0.92, 0.82, 0.72 and 0.70 mm in GBP, CAP, EAP and EVP samples during storage. The values indicated that the fish muscles lost their elasticity during storage.

The values of chewiness that refers to the work done were also as represented in Fig. 11 in which chewiness was found to have decreased in all the samples indicating that the fish muscles became soft during storage.

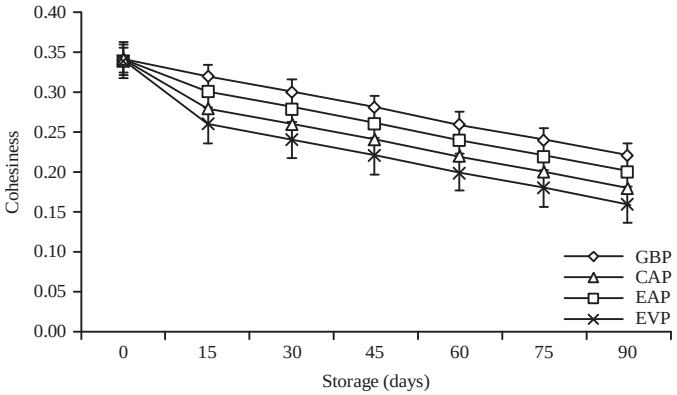

Fig. 9: Changes in cohesiveness of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

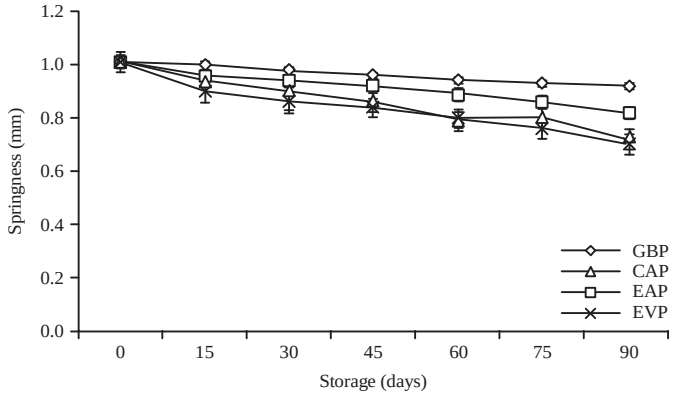

Fig. 10: Changes in springiness values of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

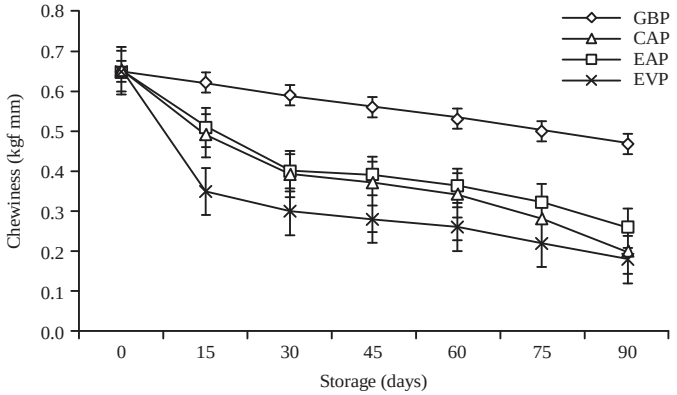

Fig. 11: Changes in chewiness of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

Sensory analysis: Changes in sensory scores were as presented in Fig. 12 in which there were significant declines in the GBP, CAP and EAP sample packs with increasing storage period.

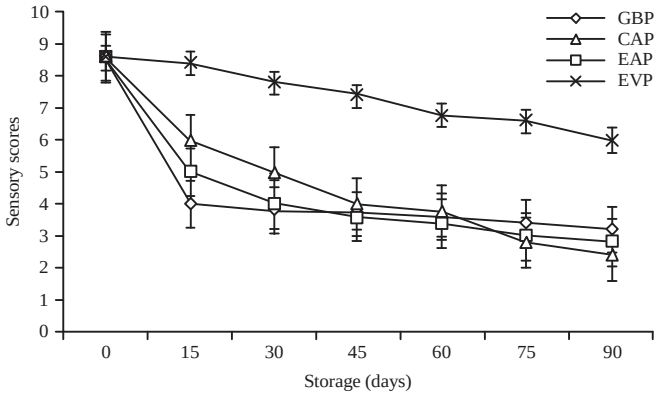

Fig. 12: Changes in overall sensory scores of dried sardines packed differently and stored in a condition of between 0-2°C. Bars represent ±Standard errors

This resulted in fish spoilages giving rise to the subsequent development of strongly fishy, rancid and putrid odours that made the taste panelists to clearly reject the fishes for human consumption by assignment of sensory scores that were lower than 4. Sensory scores in general however declined respectively from the initial pH value of 8.6-3.2, 2.8, 2.4 and 6 in cases of GBP, CAP, EAP and EVP samples. Thus, GBP, CAP, EAP and EVP samples were, respectively acceptable for up to 14, 30 and 45 days whereas, EVP samples remained in good condition up to the 90th day making chilled vacuum-packaging to safely extend the dried sardine samples shelf life for up to 90 days at 0-2°C.

CONCLUSION

The results of the study revealed that polythene air packaging with chilling would not be of much use in prolonging the dried samples shelf life beyond 45 days of storage. Therefore, since several authors have also successfully demonstrated the multiple barrier technology or hurdle concept in which several factors are combined or superimposed at sub-inhibitory concentrations that can effectively control micro organisms in refrigerated seafood's the study results clearly suggest that a combination of different factors such as polythene, vacuum-packaging and storage at refrigerated temperature could be used to prolong the dried sardines' shelf life to a great extent. It should however be emphasized that this chilled polythene vacuum-packaging success is completely dependent on the initial quality of the processed fish and on adequate temperature control throughout the storage period.

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