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Changes in non-protein nitrogenous substances and deteriorative changes in lipids during sundrying or artificial drying of freshly caught Bombay duck (*Harpodon neherius*), and its impact on the final dried product

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ABSTRACT

Effect of fluctuating conditions of sun drying on the spoilage microbes, non protein nitrogenous substances and lipids of the freshly caught Bombay duck during drying and the final dried Bombay duck (*Harpodon neherius*) were studied. Efforts were made to reduce the deteriorative changes during sundrying and storage by artificial drying at 45^{0}C and packing in polyethylene pouches. Rate of increase of free fatty acids, trimethylamaine nitrogen and total volatile bases can be reduced by drying in artificial dryer at 45^{0}C. Even though there was no difference in the rate of increase of peroxide value in either artificial drying or sundrying, formation of peroxides can only be reduced by reducing the drying period. Freshly caught sundried fish was found unfit for human consumption at the end of the sundring and remained so during storage but artificial dried fish was found acceptable till two months of storage.

KEYWORDS

Bombay duck;
Drying;
Non-protein nitrogenous substances;
Free fatty acids;
Peroxide value.

INTRODUCTION

Harpodon neherius, commonly called as Bombay duck, is a single species fishery of high magnitude and one of the largely produced and relished dried fish along Gujarat and Maharashtra coast of India. Almost entire catch of the Bombay duck is consumed in unsalted and sundried form as it is unsuitable to use either in fresh form or in frozen form due to its highest moisture content of 90.98%[1]. Bombay duck is sundried by hanging on scaffoldings and drying in sun which takes about three or more days depending upon the weather condition[2]. As a routine practice they are spread on the beaches or roadsides for further drying making it susceptible to contaminate by micro and macro organisms. Dried Bombay duck is stored in gunny bags or 'cadjan' leaves woven baskets or bamboo strips baskets. Quality of the dried fish and its keeping quality is influenced by the nature of the raw material, predrying delay, unhygienic handling, unpredictable weather condition and unprotected storage, and these are the main reasons for the availability of most of the low quality dried fish along the Kerala, Tamilnadu and Maharashtra coast [3-5].

Changes in the concentration of non protein nitrogenous substance and products of lipid deterioration in fish muscle serves as a measure of freshness or stale-

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ness of seafood[6]. Total volatile bases normally formed by bacterial or tissues hydrolysis through deaminases from free amino acids and nucleotide catabolites[7] and higher levels resulting in unacceptability of product for human consumption[8]. Surface skin and visceral parts of Bombay duck have characteristic flora of their own and processes powerful enzyme and bacteria which are responsible for the spoilage[9]. Total volatile bases from unfrozen seafood consist primarily of ammonia and trimethylamine. Trimethylamine has a characteristic of "fish odour" and is formed due to bacterial trimethylamine oxidase[10]. Free amino acids are some of the precursors for the formation of amines in total volatile base nitrogen, but hydrolysis of the tissue proteins occur in dead fish through endogenous or microbial proteases[11]. Fish lipids are highly unsaturated and easily susceptible for oxidation either due to contact with atmospheric air or endogenous muscular cytochrome oxidase liberating hydroperoxides[12]. Hydroperoxides are odourless, flovourless compounds and not related directly to the actual sensory quality of the product, but may indicate a potential for latter formation of sensorial objectionable compounds of rancification[13]. Lipid hydrolysis in fish is catalysed by lipase which releases free fatty acids from triglycerides and phospholipids[14]. Accumulation of free fatty acids in fish oil is undesirable due to secondary reactions calalysed like increased susceptibility to oxidation and development of off rancid flavours[15] and accumulation of protein denaturation in fish[16]. Spoilage during sundrying can be reduced to greater extent by reducing the period of drying by artificial dryer[12].

Even after being an important fishery of Maharashtra and Gujarat coast not much attempt has been made to improve the age old method of processing and sun drying. Drying technique is often considered to produce durable product of energy saving one compared to frozen products and little or no additional energy is required, and hence considerable scope exists for improving quality of dried fish produced in India to feed the poor people by improving the processing techniques. This is an attempt to study the impact of deteriorative changes taking place during sundrying on the keeping quality of the final dried product, and we have made an effort to reduce the rate of deterioration in Bombay duck by gutting, artificial drying and packing.

MATERIALS AND METHODS

Fish samples

Fresh Bombay duck (FBD) used for the present studies was obtained from the fishing boats caught using 'dol' net. The time lapsed between catching at the fishing ground and landing at 'Sasoon dock', Bombay, may not exceed over four to six hours. The FBD was brought in an insulated container after adequately icing them in the proportion of 1:1 fish to ice, to the laboratory of Central Institute of Fisheries Education (ICAR), Bombay within two hours. The FBD samples belonging to size group of 21 to 23 cm long; weighing around 80 to 85 g were sorted out on a sanitized stainless steel working table. The FBD samples were washed using chilled running water system maintained between 2-4^{0}C. Fresh Bombay duck procured in this manner were used for sundrying or artificial drying.

Effect of fluctuating temperature, air velocity and relative humidity of sundrying (SD24-32) on the quality of the dressed Bombay duck (SD24-32-DBD)

To study the effect of natural conditions of sundrying on the quality of the Bombay duck, freshly caught Bombay duck (FBD) samples were dressed by split opening the belly without removing the head, fins or tail under sanitary conditions, washed under chilled running water system and dried in sun by hanging the fish on 2 cm diameter ropes hung at about 3 meter height and tried on fixed poles at an intervals of 4 meters. Around 30 to 40 fishes were hung per meter. The ambient temperature varied between 24 to 32^{0}C, air velocity was between 0.053 to 0.502 meter per second and relative humidity was between 55 to 68%.

Effect of artificial drying (AD-45) on the deteriorative changes in dressed Bombay duck (AD-45-DBD)

To study the effect of artificial drying on the deteriorative changes in Bombay duck during drying period, dressed Bombay duck (DBD) samples were dried in a Torry kiln (Torry Research Station, UK) at the temperature of 45^{0}C, air velocity of 1.003 meter/second and relative humidity of 60±2% (AD45-DBD). The samples were hung on one meter long iron rods after

interlocking the jaws; the iron rods were mounted on wooden frames, which were placed in the drying chamber of the kiln. The temperature, air velocity and humidity was monitored regularly, recorded at regular intervals of time and maintained at constant rate. Samples were drawn at regular intervals of time for analysis.

Effect of sundrying or artificial drying on the keeping quality of the final dried product during storage

To study the sundrying or artificial drying on the keeping quality of the final dried product during storage, samples dried at fluctuating conditions of sundrying (SD24-32-DBD) and controlled conditions of artificial drying (AD45-DBD) dried samples were kept in bundles of 25 numbers, tied with nylon threads at three equidistant places and kept in 100 gauge sealed polyethylene pouches, properly labeled and storage at normal room temperature of 28-30^{0}C respectively. Samples were drawn at regular intervals for further analysis.

Chemicals

All the chemicals used were of analytical grade and were obtained from Merck Limited (Mumbai, India).

Proximate analysis

The wet fish samples were blended in a homogenizer at 3,000 rpm for 10 minutes and dried fish samples were powdered in a waring blender (Philips, India) at 22,000 rpm for 10 minutes. Analysis of samples at different stages of processing was performed in quadruplicate. Moisture content of the samples were estimated as per FAO[17] and expressed as percentage moisture. The total lipid in the fish was extracted using chloroform methanol phase separation and peroxide value (PV) of the samples were estimated by the method described by Lima and others[18] and expressed as millimoles of oxygen/kg of fat. Free fatty acids (FFA) of the sample were estimated by the method described by IS: 5734[19] and is expressed as percentage of oleic acid on lipid basis. Trichloro acetic acid extract was prepared as per FAO[17] and used for measuring nonprotein nitrogenous substances (NPNs) like trimethylamine nitrogen (TMAN), total volatile bases nitrogen (TVBN) and alpha amino nitrogen (AAN). TMAN and TVBN content of the sample was determined by the micro diffusion method as described by Martin[20] and the values were expressed as mg/100g of fish muscle. The AAN in the samples were estimated by the method described by Pope and Stevens[21] and value is expressed as mg/100g fish muscle.

Microbiological analysis

Sterilization: Glassware and prepared media were sterilized at 121^{0}C for 15 minutes. Petri dishes, homogenizers, pipettes were sterilized at 180^{0}C for 1 hour. 10% skimmed milk, 10% trybutyrin solution were sterilized by tendylisation method, where solution was free steamed for one hour on first day and thirty minutes on next two successive days. Mesophilic bacterial count was determined as per APHA[22] method. Total mould count, proteolytic bacterial and mould count was determined using the method of Leo[23]. Lipolytic bacterial and mould count was determined by the method explained by Collines[24] using Trybutyrine agar.

Statistical analysis

A completely randomized design was performed using quadruplicate samples through the study. One- and two-way ANOVA was performed using Statographics 2.1 (STSC Inc., Rock vile, MD). The difference in means was analyzed using a Turkey HSD test ($p<0.05$).

RESULTS AND DISCUSSION

Deteriorative changes during sundrying and effect of artificial drying on the quality of the Bombay duck

Sundrying took around 60 hours to reduce moisture content from 90±43 to 16.16±0.23, while ambient temperature varied between 24-32^{0}C, air velocity between 0.053-0.502 meter per second, and relative humidity between 55-68% (Figure 1). When the temperature of air was raised and maintained at constant rate of 45^{0}C, with the controlled air speed of 1.003 meters per second, and relative humidity of 60%, the period of drying was 40 hours to reach the moisture level lower than the sundried products (Figure 2). Deterioration of initial freshness of fish is predominantly due to enzymatic, chemical and physical process leading to the accumulation of undesirable characteristics of the fish. Study was conducted on the proteolytic and lipolytic microbial count during Sundrying (SD24-32)

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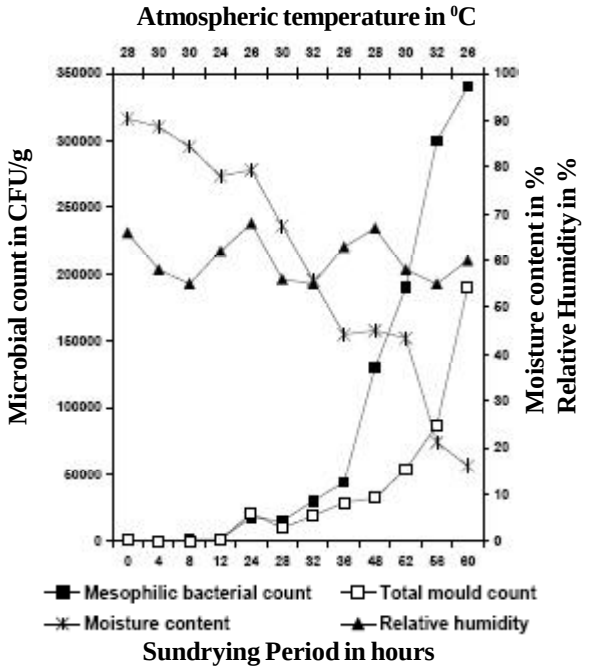

Figure 1: Changes in microbial count of Bombay duck (DBD) during sundrying sundrying (SD24-32)

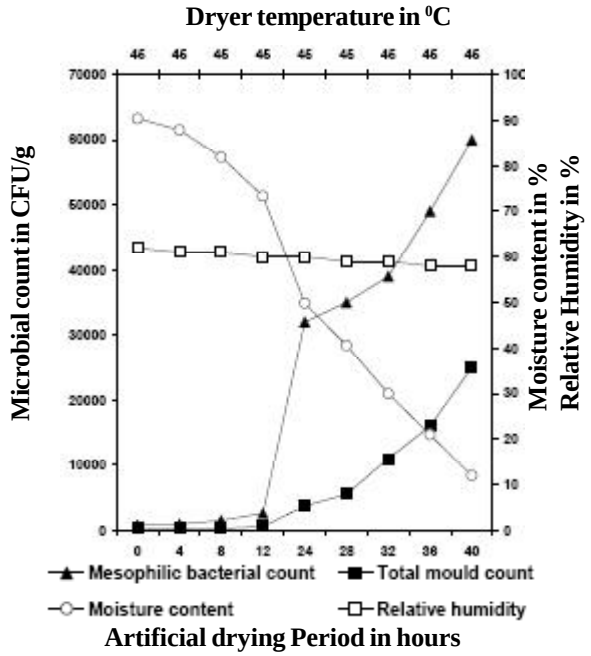

Figure 2: Changes in microbial count of Bombay duck (DBD) during artificial drying (AD-45)

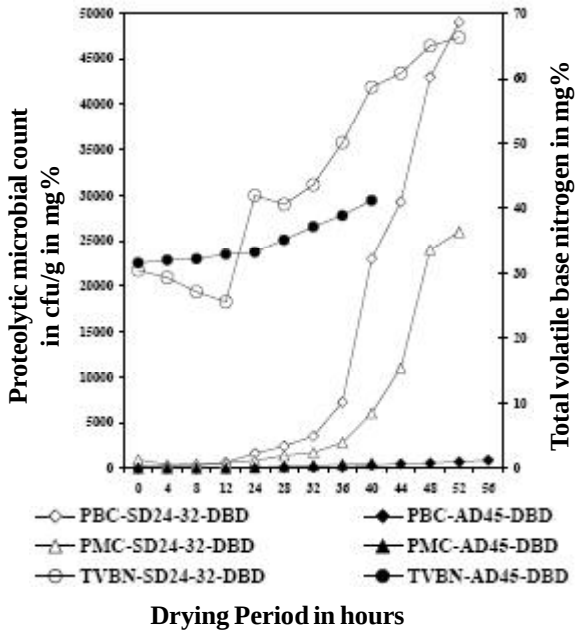

Figure 3: Changes in the PBC, PMC and TVBN during sundrying (SD24-32) or artificial drying (AD-45) of DBD

or Artificial Drying (AD-45) along with the formation of fatty acids, development of peroxides and volatile bases. During Sundrying (SD24-32) of DBD samples PBC and PMC increased by 233±0.01 and 27±0.04 folds, but only 11±0.01 and 18±0.01 folds respectively during artificial drying (AD-45). Increase of TVBN registered was 1.8±0.02 and 1.8±0.01 folds during 40 hours of Sundrying (SD24-32) and artificial Drying (AD-45) respectively, but TVBN was more ($p<0.05$) in SD24-32-DBD samples, than AD45-DBD as it took 60 hours for sun drying (SD24-32), but only 40 hours by artificial drying (AD-45) (Figure 3). Spoilage organisms use non-protein nitrogenous substances present in the fish particularly compounds such as trimethylamine oxide to produce variety of volatile compounds especially trimethylamine which is believed to react with fish fats to produce the typical spoilage odour associated with fish beyond their prime[25]. The concentration of non-protein nitrogenous substances changes due to the activity of endogenous and bacterial enzymes, it can serve to measure either freshness or staleness of fish[10]. Similarly during sundrying (SD24-32) of DBD samples LBC and LMC increased by 309±1.32 and 164±0.02 folds, but only 8±0.02 and 40±0.02 folds respectively during artificial drying (AD-45). Increase of FFA registered was 5±0.02 and 4.8±0.02 folds during 40 hours of Sundrying (SD24-32) and artificial Drying (AD-45) respectively. It is interesting to note that the FFA was more ($p<0.05$) in SD24-32-DBD samples, than AD45-DBD (Figure 4). Accumulation of free fatty acids in fish flesh is undesirable due to secondary reactions leading to the developed of off rancid flavors[26] and acceler-

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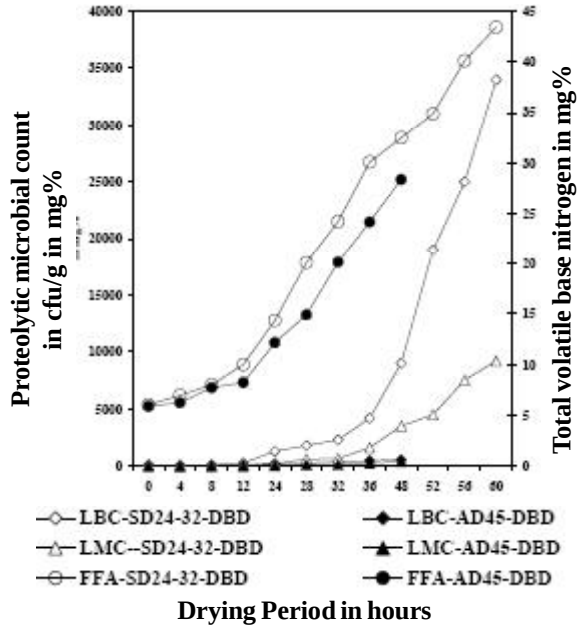

Figure 4: Changes in the LBC, LMC and FFA during sundrying (SD24-32) or artificial drying (AD-45) of US-DBD

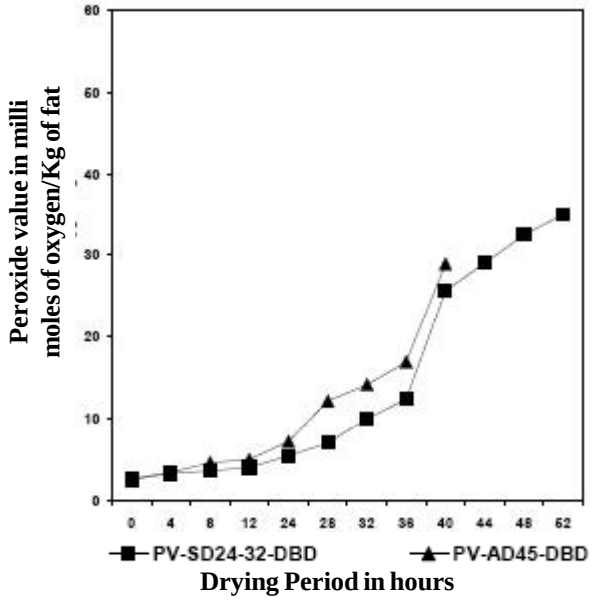

Figure 5: Changes in the PV during sundrying (SD24-32) or artificial drying (AD-45) of DBD

ated protein denaturation in fish[27]. At the end of the sundrying the PV, FFA, TMAN, TVBN and AAN values were 34.99±0.025% oleic acid, 43.42±0.03 millimoles of oxygen/kg of fat, 23.81±0.025 mg%, 66.20±0.03 mg%, and 143.15±0.025 mg%, and for artificial dried products values were 28.96±0.025% oleic acid, 28.96±0.03 millimoles of oxygen/kg of fat, 17.5±0.03 mg%, 41.23±0.03 mg%, and 89.92±0.03

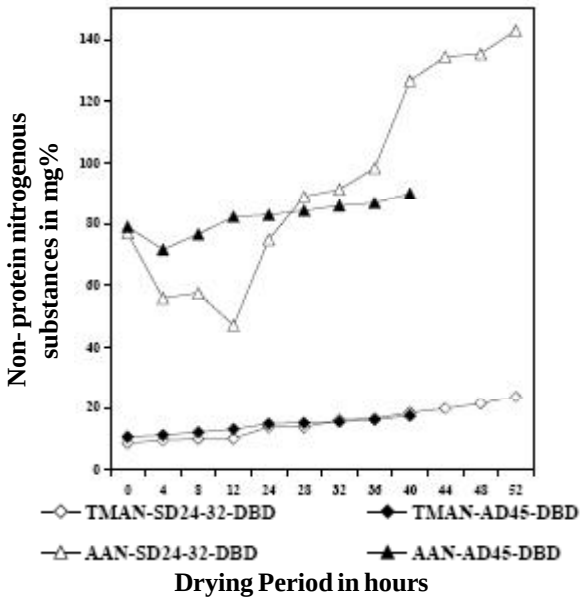

Figure 6: Changes in the TMAN and AAN during sundrying (SD24-32) or artificial drying (AD-45) of DBD

mg% (Figure 3-6). There was not much difference ($p>0.05$) in the rate of change of PV either during sundrying or during artificial drying as both are exposed to aerobic conditions leading to oxidation[28]. Even though hydroperoxides are odorless and flavorless compounds and not related directly to the actual sensory quality of the product, may indicate a potential for a latter formation of sensorial objectionable compounds of rancification and yellowish and brownish discolouration as we have observed in the final dried products[29]. As it took almost 60 hours to dry freshly caught Bombay duck samples by sunshine (SD24-32-DBD) and only 40 hours to dry freshly caught Bombay duck samples by artificial drying (AD45-DBD), FFA, PV, TMAN, TVBN and AAN values were more ($p<0.05$) in former than latter, even though no significant ($p<0.05$) difference in the rate of change of these values observed at any given point of time.

Freshly caught Bombay duck were with reddish semitransparent body, silvery white abdomen and transparent fins, and at the end of sundrying had turned opaque body, with longitudinal shrinkage, faint straw colour, firm texture and possessed pungent odour, while artificial dried samples were grayish white, dry fish odour, very firm with longitudinal shrinkage. In fact direct comparison of sundried products and artificial products may not be hold true as the sundrying was carried out under

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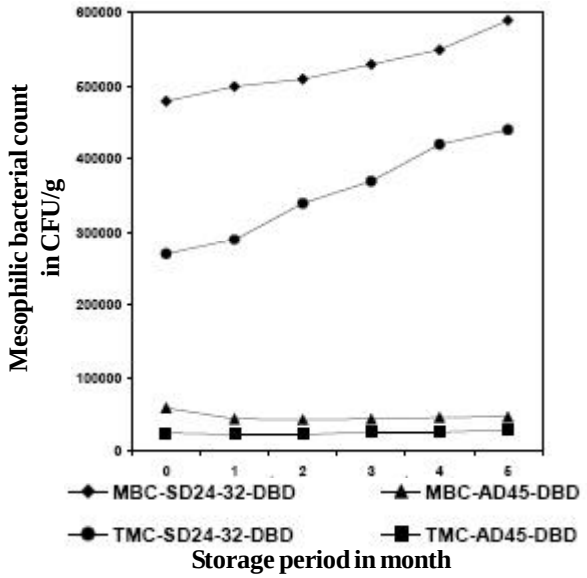

Figure 7: Changes in MBC and TMC during storage of sundried (SD24-32) or artificial dried(AD-45) of DBD kept in sealed polythene pouch

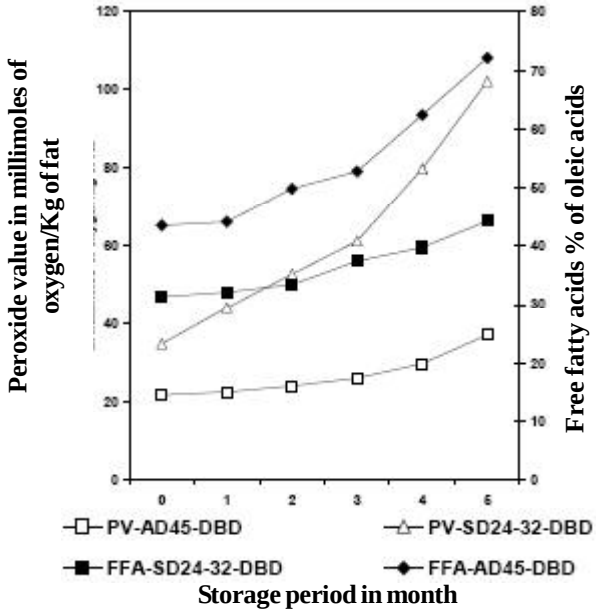

Figure 8: Changes in the FFA and PV during storage of sundried (SD24-32) or artificial dried (AD-45) DBD in sealed polyethylene pouches

natural conditions of temperature, relative humidity and air velocity, but artificial drying was carried out at controlled temperature, relative humidity and air velocity, but the aim of the present work is to compare the deteriorative changes of sundried samples produced under natural conditions with that of the samples dried under controlled artificial conditions.

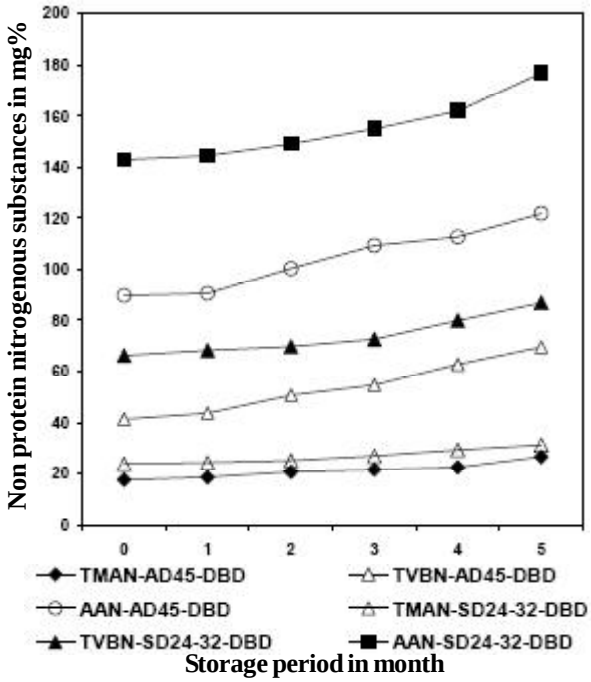

Figure 9: Changes in the TMAN, TVBN and AAN during storage of sundried (SD-24-32) or artificial dried (AD-45) DBD in sealed polyethylene pouches

Effect of drying conditions on the keeping quality of the final dried products: During 5 months of storage in polyethylene pouches at ambient temperature dried fish samples did not show any appreciable change in moisture content as polyethylene film is an effective barrier against vapour. During the storage of sundried and artificial dried products MBC increased by 1.22±0.02 and 1.62±0.03 folds, and TMC increased by 0.79±0.02 and 1.16±0.01 folds respectively (Figure 7). Over 5 months of storage period, PV rose by 2.9±0.03 and 1.3±0.02 folds, while FFA by 1.7±0.01 and 1.3±0.01 folds (Figure 8) respectively in SD24-32-DBD and AD45-DBD samples. TMAN increased by 1.3±0.02 and 1.5±0.02 folds, TVBN increased by 1.3±0.01 and 1.71±0.01 folds, and AAN increased by 1.2±0.01 and 1.4±0.01 (Figure 9) respectively in SD24-32-DBD and AD45-DBD samples. Sundried samples (SD24-32-DBD) were found unfit for human consumption at the end of the drying period and remained so during storage, and artificial (AD45-DBD) samples colour changed to yellow or yellowish brown especially beyond two months and was considered unacceptable beyond 3 months. A PV of 10–15 mg/kg of lipids indicates rancidity[7]. Lipids hydro-peroxides

breakdown with time at a low pH during the storage of a product indicates both an early phase of auto-oxidation and a late stage of a severely oxidized product[30]. It is quite evident that TMAN and TVBN values remained higher ($p<0.05$) during the storage if the initial values were high in the dried fish. Regardless of the type of drying method used there was no significant ($p>0.05$) difference in the rate of increase of TMAN and TVBN at any point of storage period within sealed polyethylene pouches. While in dried fish, spoilage was quite evident, when TMAN values reached 50 mg%[31]. Connell[7] suggested, that TVBN of 100-200 mg% on dry weight basis as the limit beyond which dried fish could be considered as spoiled.

CONCLUSION

Both for economic and quality considerations artificial drying can be adopted as a better option compared to sundrying as the accumulation of free fatty acids and formation of non-protein nitrogenous substances was least with better appealing sensory characteristics. Even though there was no difference in the formation of peroxides either in sundried products or artificial dried products, lower levels were found in artificial dried product as it took only 40 hours to reach a moisture level during 60 hours of sundrying. Sundried product is unfit for human consumption even after being dried without holding and on the other hand artificial drying is ideal to yield a standard consistent product with least batch to batch variation as drying is effected continuously in an efficient manner through controlled air velocity, temperature and relative humidity thought the drying. Spoilage during drying has a direct impact on the quality of the final dried product. Dried samples are advisable to pack in suitable bundles and place them in sealed polyethylene pouches.

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