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Effective methods for postharvest intervention in processing of Bombay duck *(Harpodon neherius)*

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ABSTRACT

Predrying chilling and holding of the dressed Bombay duck samples at 0°C registered lower levels of mesophilic, proteolytic and lipolytic bacterial count compared to the samples held at 28°C for 16 hours, and the reduction in the rate of bacterial proliferation and spoilage correlates well with the reduction in temperature of storage. Even though inverse relationship exists between the salt concentration and the microbial proliferation, high concentration of salts results in the products with rancid flavors which correlate well with the decreasing preference. If the material is to be held prior to drying, holding DBD in mixture of 200 IU/g of nisin, 50µg/g of lysozyme and 0.3 mM EDTA (NIS+LYS-28C/16H-DBD) synergistically reduce the spoilage bacteria than each alone. Untreated sundried Bombay duck was unfit for human consumption. Bombay duck treated with the mixture of nisin, lysozyme and EDTA, artificial dried at 45°C were judged superior by sensory evaluation than salted or chilled, artificial dried dressed Bombay duck.

KEYWORDS

Nisin;
Lysozyme;
EDTA;
Artificial drying;
Sun drying;
Salting;
Proteolytic bacteria;
Lipolytic bacteria;
TMAN;
TVBN;
FFA.

INTRODUCTION

Extension of shelf life of dried fish could have a significant effect on the dried fish produced around the world. Initial freshness of the fresh fish has a direct impact on the keeping quality of the dried fish products. At ambient temperature fresh fish becomes unsuitable for human consumption within 12 hours[1] and two hours delay before cooling in ice approximately halves the storage life[2], but cooling fish to -2^{0}C or just above actual freezing temperature, should have a positive effect on extending biochemical life, even in comparison to storage in melting ice[3]. Shelf life of salted seafood depends on the hurdle effect and generally, salted products containing low amounts of salts have highest quality. Fresh fish held with bacteriolytic or bacteriostatic agents like nisin, lysozyme and EDTA, often has an additive, or even a synergetic effect when added together[4].

Bombay duck *(Harpodon neherius)*, is a single species fishery of high magnitude and one of the largest produced and relished dried fish along Gujarat and Maharastrian coast of India. Almost entire catch of

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Bombay duck is dried in sun without salting, as it is unsuitable for use in fresh or frozen form due to high moisture content of 90.98%. Depending upon the distance from the fishing ground to shore it takes more than 4 hours to reach landing centre and drying in sun takes three or many days depending upon the weather condition[5]. Surface skin and visceral parts of Bombay duck have characteristic flora of their own and possess powerful enzymes and bacteria which are responsible for the spoilage[6]. Proteolytic and lipolytic enzymatic activities of microorganisms are the most important cause for food spoilage[7]. Increased trimethylamine levels in tissues are considered to be characteristic of marine fish spoilage[8]. Quality of the dried fish and its keeping quality is influence by the nature of the raw material, predrying delay, unhygienic handling, unpredictable drying condition, improper salting methods, improper drying practice and unprotected storage, and are the main reason for the availability of most of the low quality dried fish along the Tamilnadu and Maharastrian coast[9-11]. Initial freshness of raw material affects the quality of the final dried products[12] and the sun dried fish products were of poorer quality compared to other methods of drying[13].

Fish drying is the first to start with and last to develop in the fish industry. Even today hardly any sophisticated equipments like artificial drier or machinery is employed for the production of dried fish. Most of the drying operations are carried out right along the beaches or roadside pavements making it possible to contaminate by both micro and macro organisms. Drying technique is often considered to produce energy saving as 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.

But even after being single species fishery of high magnitude and one of the largest produced dried fish along Gujarat and Maharastrian coast not much attempt is being made to improve the age old method of sun drying and to study the affect of different methods on the microbiological flora and on the quality of the final dried products during processing. So, this is an attempt to study the effect of temperature, salt, nisin, lysozyme and ETDA on the spoilage bacteria and its impact on the quality of the final dried product.

MATERIALS AND METHODS

Fresh Bombay duck (FBD) and Dressed Bombay duck (DBD)

Fresh Bombay duck (FBD) used for the present studies was obtained from the fishing boats caught using 'dol' net. 'Dol' net is a large conical nylon net bag with rectangular mouth portion and tapering end portion. 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 21-23 cm long; weighing around 80-85 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°C. The dressed Bombay duck (DBD) samples were prepared by splitting open the belly without removing the head, fins or tail under aseptic conditions and washing again under chilled running water system so as to remove blood, slime, dirt etc.

Predrying holding with or without treatment

To study the preprocess spoilage, untreated dressed Bombay duck samples (UT-DBD) were kept at 28°C (UT-28C/16H) or super chilled DBD at 0°C (UT-0C/16H) in thermostatically controlled cooling incubator (Rotek, Cochin) in polythene pouches for various time intervals. To study the effect of nisin and/or lysozyme on the preprocess spoilage, DBD samples were treated at 28 or 0°C with 200 IU/g of Nisin and 0.3 mM EDTA (NIS-28C/16H-DBD or NIS-0C/16H-DBD), 50µg/g of Lysozyme and 0.3 mM EDTA(LYS-28C/16H–DBD or LYS-0C/16H -DBD), or in a combination of 200 IU/g of Nisin, 50µg/g of Lysozyme and 0.3 mM EDTA (NIS+LYS-28C/16H -DBD or NIS+LYS-0C/16H -DBD) for various time intervals. Samples were drawn from NIS-28C/16H-DBD, NIS-0C/16H-DBD, LYS-28C/16H-DBD, LYS-0C/16H –DBD, NIS+LYS-28C/16H -DBD and NIS+LYS-0C/16H –DBD for analysis regularly. Dressed Bombay duck

(DBD) samples were also treated with 10, 20 and 36 % (Saturated) salt solution for studying the effect of sodium chloride on the preprocess spoilage. The ratio of fish to brine was one liter solution for one and a half kilograms of material. In another batch, DBD samples were salted in the ratio of 1:6 salts to fish proportion. During salting utmost care was taken so as to have uniform availability of fine and coarse salt for all the samples. Locally procured common salt having around 99% sodium chloride with the size of 3-5 mm of coarse salt in 2/3 parts and the size of 1-0.5mm fine salt in 1/3 parts were used in 1: 6 proportion in salt to fish ratio. The samples were drawn from DBD treated with 10% sodium chloride solution (SA10-28C/16H -DBD), 20% Sodium chloride solution (SA20-28C/16H -DBD), saturated sodium chloride solution (SA36-28C/16H -DBD) and dry salt (SADS-28C/16H -DBD) during 16 hours of salting or brining at 28 °C for analysis. Prior to drying SA20-28C/16H -DBD samples were dipped in 3.5 % salt solutions and drained.

Sun drying

Untreated sundried dressed Bombay duck (UT-SD24-32-DBD) was prepared by drying DBD in sun, by hanging the fish on 2 cm diameter ropes hung at about 3 meter height and tied on fixed poles at intervals of 4 meters. The DBD samples were hung after interlocking the jaws with heads up and tails down. Around 30-40 fishes were hung per meter. The ambient temperature varied between 24-32°C, air velocity was between 0.053-0.502 meter per second and relative humidity was between 55-68%.

Artificial drying

All other samples processed under various conditions (UT-DBD, UT-28C/16H-DBD, NIS+LYS-28C/16H-DBD and SA20-28C/16H-DBD) were dried at 45°C in a Tory kiln by thermostatically controlled 18 KW electrical heater grids, air velocity of 1.003 meter/second across the trolley driven by a powerful blower which was driven by a motor of 0.75 HP and relative humidity of air was maintained at 60±2% controlled by air inlet and re-circulation damper. 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 on an angle iron trolley. After loading the trolley, it was placed in the drying chamber of the Torry-kiln. The door was closed and the drier was switched on. The temperature, air velocity and humidity was monitored regularly, recorded at regular intervals of time and maintained at constant rate. Samples dried at 45°C (UT-AD45-DBD, UT-28C/16H-AD45-DBD, NIS+LYS-28C/12H-AD45-DBD and SA20-28C/16H-AD45-DBD) are used for further studies.

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 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[14] and expressed as percentage moisture. Salt content of samples were estimated as per[15] and expressed as percentage of salt. The total lipid in the fish was extracted and free fatty acids (FFA) of the lipids were estimated by the method described by[16] and were expressed as percentage of oleic acid on lipid basis. Trichloro acetic acid extract was prepared as per[14] and used for measuring non-protein nitrogenous substances (NPNs) like trimethylamine nitrogen (TMAN) and total volatile bases nitrogen (TVBN). TMAN and TVBN content of the sample was determined by the micro diffusion method as described by[17] and the values were expressed as mg/100g of fish muscle.

Microbiological methods Glassware and prepared media were sterilized using moist heat at 121°C for 15 minutes. Petri dishes, homogenizers, pipettes were sterilized using dry heat at 180°C for 1 hour. 10% skimmed milk, 10% trybutyrin solution were sterilized by Tyndallisation method, where solution was free steamed for one hour on first day and for thirty minutes on the next two successive days. Mesophilic bacterial count (MBC) was determined as per[18] method. MBC was enumerated and expressed as mesophiles per gram of sample on dry weight basis. Proteolytic bacterial count (PBC) was determined using the method of[19]. Proteolytic positive bacterial colony forms clear zone around

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the colony and was expressed as number of proteolytic bacteria per gram of sample on dry weight basis. Lipolytic bacterial count (LBC) was determined by the method explained by[20] using Trybutyrine agar. Hydrolysis of tributyrin results in clearing of medium and formation of a clear zone around the colony, which was enumerated and expressed as lipolytic count per gram of sample on dry weight basis.

Sensory evaluation

Physical characteristics of fresh and dried fish sample were noted in relation to appearance, colour, odor, texture, and flavor. Organoleptic properties were conducted using a ten member panel of trained professionals. The panelists were provided with clean water to rinse their mouth after tasting each sample and samples were already placed in separate booths and each sample was labeled in such a way that the panelist will not be able to identify them. The samples were evaluated using a nine point hedonic scale basis (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely)[21].

Statistical analysis

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

Predrying holding of untreated Bombay duck at 28 or 0°C

When UT-DBD samples were held at 28°C (UT-28C/16H-DBD) for 16 hours, mesophilic bacterial count (MBC) increased by 538.46±0.25 folds, but as the temperature of the storage of the samples reduced to 0°C (UT-0C/16H-DBD) the increase of MBC reduced drastically to 7.89±0.025 folds. UT-28C/16H-DBD registered higher ($p<0.05$) levels of MBC than UT-0C/16H-DBD samples. Proteolytic bacterial count (PBC) increased by 79.57±0.022 folds at 28°C, but only by 15.53±0.021 folds at 0°C. It is instructive to note here that the development of TMAN and TVBN reduced drastically ($p<0.05$) as the storage temperature of the samples reduced to 0^0C(Figure 1). Similarly during 16 hours of holding, lipolytic bacterial count (LBC) increased by 81.5±0.025 folds at 28^0C, but only by 17.2±0.023 folds at 0°C. Accumulation of free fatty

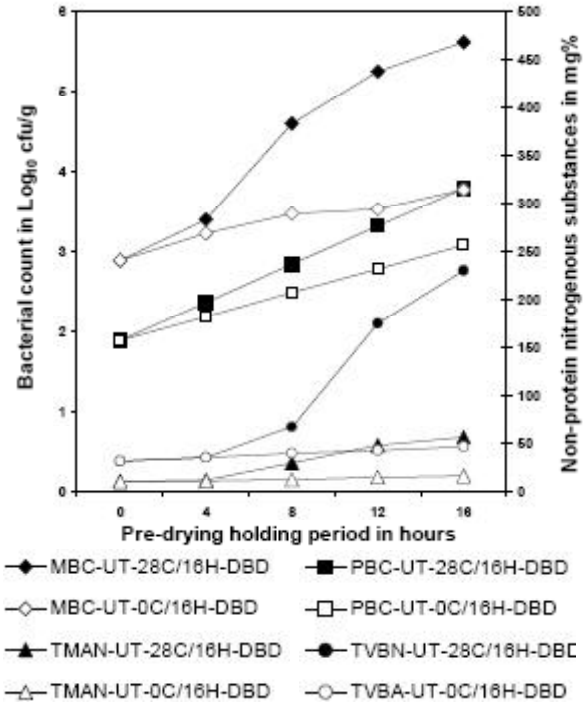

Figure 1 : Changes in MBC, PBC and NPNs in UT-DBD during storage at 0^0C or 28^0C

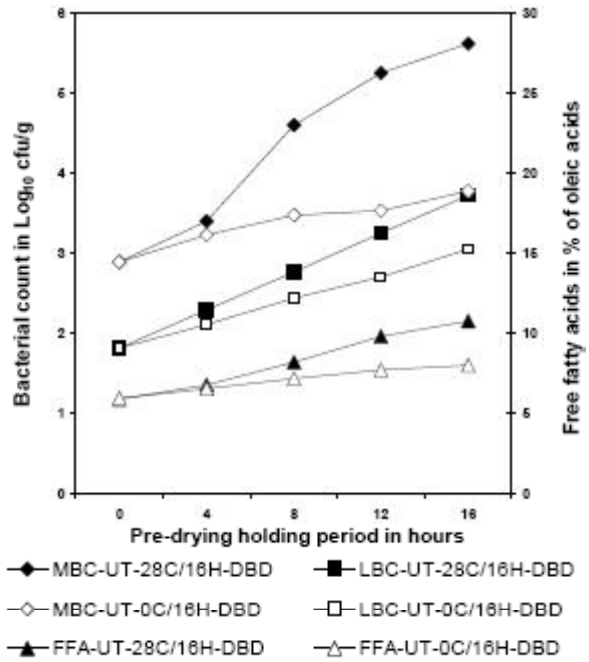

Figure 2 : Changes in MBC, LBC and FFA in UT-DBD during storage at 0^0C or 28^0C

acids (FFA) increased remarkably ($p<0.05$) during the storage of UT-DBD at 28°C, but there was considerable reduction ($p<0.05$) in the development of FFA as recorded in UT-DBD samples held at 0°C (Figure 2). UT-DBD held at 28°C beyond 4 hours were found unacceptable by the sensory panel and scored less than 5 points on Hedonic scale. FFA, TMAN and TVBN registered at this point of storage were 6.76±0.02% of oleic acid, 11.83 mg%, and 35.17±0.15mg% respectively. Freshly caught Bombay duck (FBD) contained semitransparent body, silvery white abdomen and transparent fins, but turned grayish, translucent and found unsuitable for human consumption during 16 hours of holding at ambient temperature without treatment

Predrying holding of dressed Bombay duck in different concentrations of salt at 28°C

During predrying holding of DBD samples at different concentrations of salts at 28°C, MBC increased respectively by 1.4±0.002 and 1.28±0.003 folds in SA10-28C/16H-DBD and SA20-28C/16H-DBD samples, but decreased respectively by 0.32±0.003 and 0.26±0.002 folds in SA36-28C/16H-DBD and SADS-28C/16H-DBD samples. During these period PBC decreased by 0.52±0.002, 0.46±0.001, 0.4±0.003, and 0.40±0.002 folds (Figure 3), and LBC decreased by 0.31±0.003, 0.28±0.002, 0.23±0.001, and 0.2±0.002 folds in SA10-28C/16H-DBD, SA20-28C/16H-DBD, SA36-28C/16H-DBD and SADS-28C/16H-DBD samples respectively (Figure 4). It can be noted from the figure 3 and 4 that FFA, TMAN and TVBN increases gradually ($p<0.05$) with the treatment period, but rate of increase of values decreases ($p<0.05$) with the increase in the concentration of salt. At the end of the salting period moisture content was 86.19±0.035, 81.29±0.04, 70.56±0.03, and 68.02±0.03% in SA10-DBD, SA20-DBD, SA36-DBD and SADS-DBD samples and salt content was 4.11±0.1, 10.89±0.02, 13.01±0.03, 16.66±0.02 and 18.02±0.02% respectively on dry weight basis. Sensory panelists considered SA20-28C/16H-DBD was the best amongst the salted samples and scored highest for flavors (Hedonic scale of 8.6±1.4.)

Predrying holding of dressed Bombay duck with nisin and/or lysozyme at 28 or 0°C

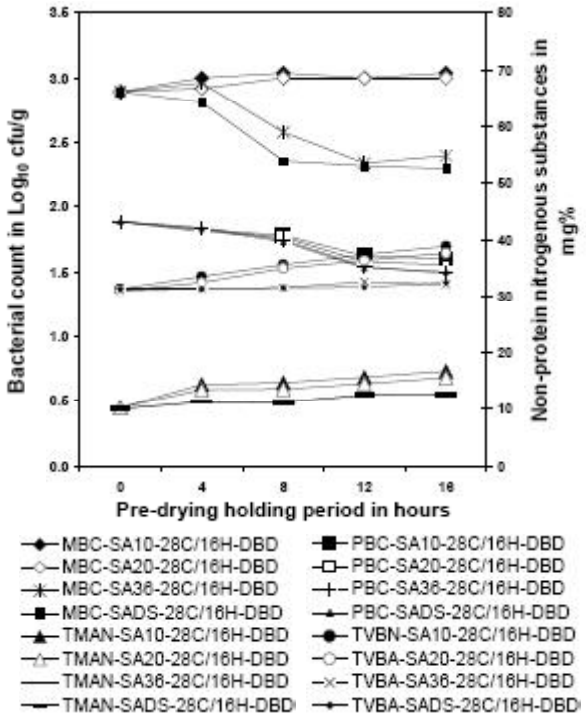

Figure 3: Changes in MBC, PBC and NPNs in SA10-DBD, SA20-DBD, SA36-DBD AND SADS-DBD during storage 28°C

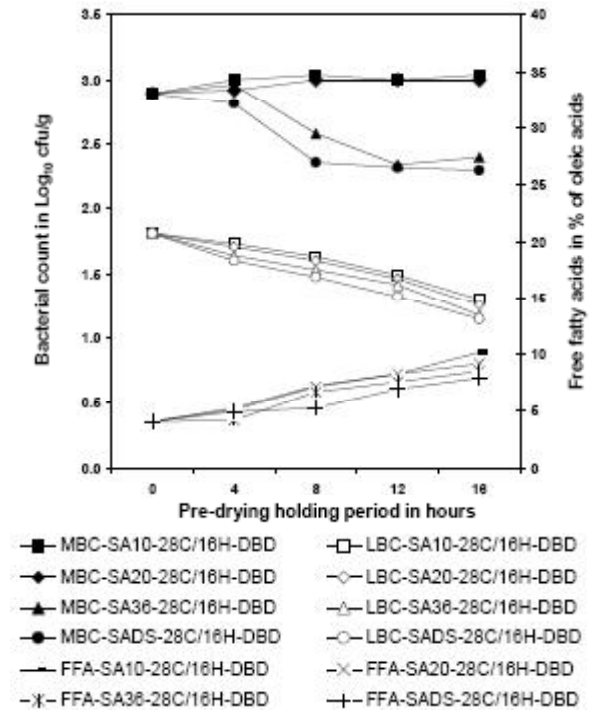

Figure 4 : Changes in MBC, LBC and FFA in SA10-DBD, SA20-DBD, SA36-DBD AND SADS-DBD during storage 28°C

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Six sets of DBD samples were treated either with a mixture of 200IU/g of nisin and 0.3 mM EDTA, 50µg/ml of lysozyme and 0.3 mM EDTA, or 200IU/g of nisin, 50µg/ml of lysozyme and 0.3 mM EDTA for 16 hours at 28°C (NIS-28C/16H-DBD, LYS-28C/16H-DBD, or NIS+LYS-28C/16H-DBD respectively), or 0°C (NIS-0C/16H-DBD, LYS-0C/16H-DBD, or NIS+LYS-0C/16H-DBD respectively).

During the treatment of DBD samples with 200IU/g of nisin and 0.3 mM EDTA at 28°C (NIS-28C/16H-DBD), MBC, PBC and LBC increased by 431±0.19, 76.91±0.029, and 76.91±0.035 folds (Figures 5 and 6), and in untreated samples (UT-28C/16H-DBD), counts increased by 538.46±0.25, 79.57±0.022, and 81.5±0.025 folds by 16 hours respectively (Figures 1, 2, 5 and 6). It is interesting to note here that there was no significant difference ($p>0.05$) in the rate of change of TMAN, TVBN and FFA were registered between untreated (UT-0C/16H-DBD) and treated (NIS-0C/16H-DBD) samples during 16 hours of holding, and NIS-28C/16H-DBD beyond 4 hours was found unacceptable by the sensory panel (Hedonic scale of less than 5).

It can be noted here that during the treatment of DBD samples with 50µg/ml of lysozyme and 0.3 mM EDTA at 28°C for 16 hours (LYS-28C/16H-DBD), MBC, PBC and LBC decreased by 0.535±0.002, 0.662±0.002, and 0.74±0.003 folds(Figures 5 and 6). It is instructive to note here that MBC, PBC and LBC reduced significantly ($p<0.05$) in NIS-28C/16H-DBD samples, and similarly the rate of accumulation of free fatty acids and development of non-protein nitrogenous substances reduced significantly ($p<0.05$). Sensory panel observed that the NIS-28C/16H-DBD samples were unacceptable beyond 8 hours of storage at 28°C (Hedonic scale of less than 5).

It is instructive to note here that when the DBD samples were held at 28°C in combination of 200IU/g of nisin, 50µg/ml of lysozyme and 0.3 mM EDTA for 16 hours (NIS+LYS-28C/16H-DBD), MBC, PBC and LBC decreased by 0.16±0.002, 0.27± 0.002, and 0,17±0.003 folds respectively (Figures 5 and 6). Similarly the rate of accumulation of free fatty acids, oxidation of trimethylamine and development of volatile bases were decreased drastically ($p<0.05$) during holding period and sensory panel observed that the NIS+LYS-28C/16H-DBD samples were acceptable even after 16 hours of storage at 28°C (Hedonic scale of 6.2).

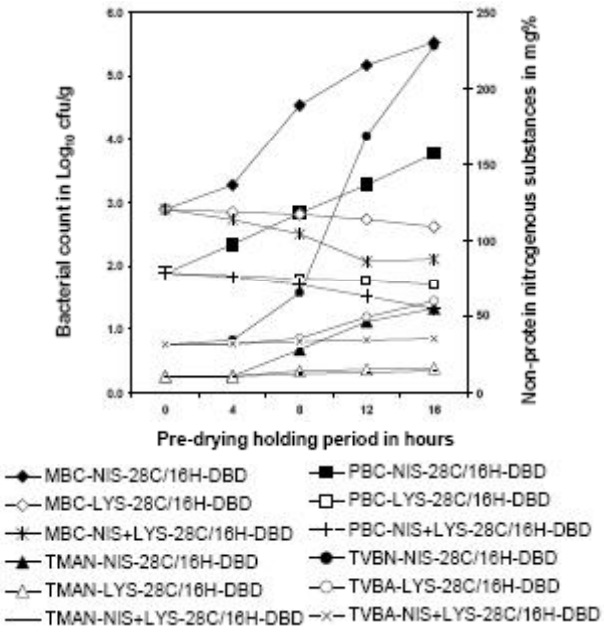

Figure 5: Changes in MBC, PBC and NPNs in NIS-DBD, LYS-DBD or NIS+LYS-DBD during storage 28°C

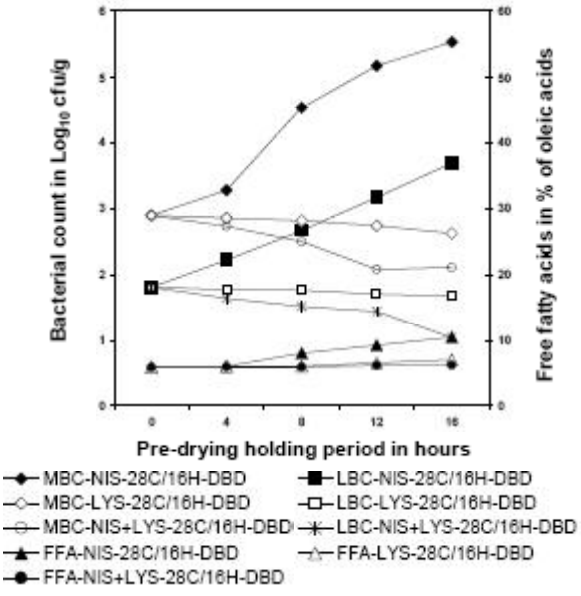

Figure 6: Changes in MBC, LBC and FFA in NIS-DBD, LYS-DBD or NIS+LYS-DBD during storage at 28°C

But it is interesting to note here that there was no significant ($p<0.05$) difference in the rate of increase of proteolytic and lipolytic mesophilic bacteria, accumulation of free fatty acids, oxidation of trimethylamine and development of volatile bases between untreated (UT-28C/16H-DBD) and treated (NIS-28C/16H-

DBD, LYS-28C/16H-DBD or NIS+LYS-28C/16H-DBD) samples (Figures 7 and 8).

Sundrying (SD24-32) or Artificial Drying (AD-45) of DBD

During the sun drying (SD24-32) of UT-DBD samples MBC, PBC and LBC increased respectively by 357±1.23, 233±1.12, and 309±1.32 folds(Figures 9 and 10). It took around 60 hours to reduce water concentration from 90.43±0.21 to 16.16±0.23%, while ambient temperature varied between 24-32°C, air velocity between 0.053-0.502 meter per second and relative humidity between 55-68%. When the temperature of air drying was raised to 45°C with the air speed of 1.003 meter/second and relative humidity of 60%, the period of drying was lower than sundrying. During arti-

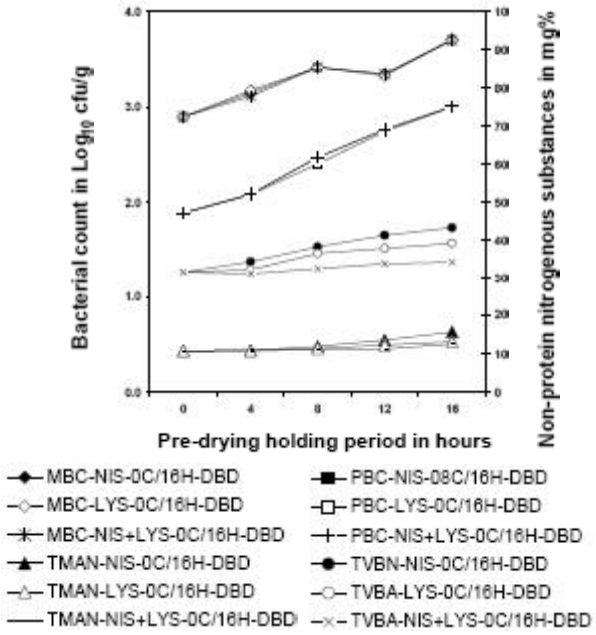

Figure 7 : Changes in MBC, PBC and NPNs in NIS-DBD, LYS-DBD or NIS+LYS-DBD during storage 0°C

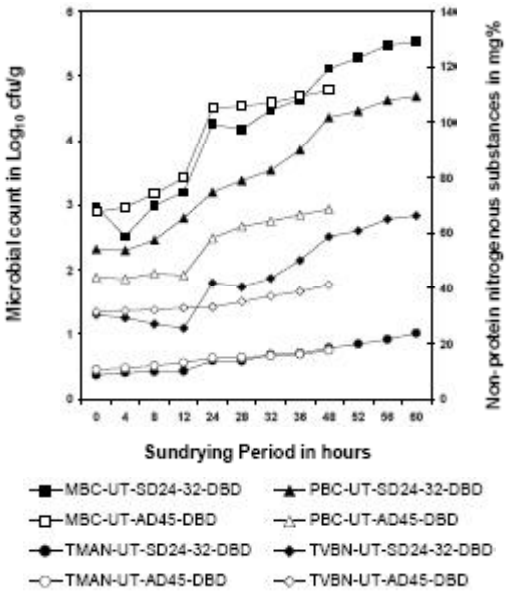

Figure 9 : Changes in MBC, PBC, TMAN and TVBN during sundrying (SD-24-32) or artificial drying (AD-45) of UT-DBD

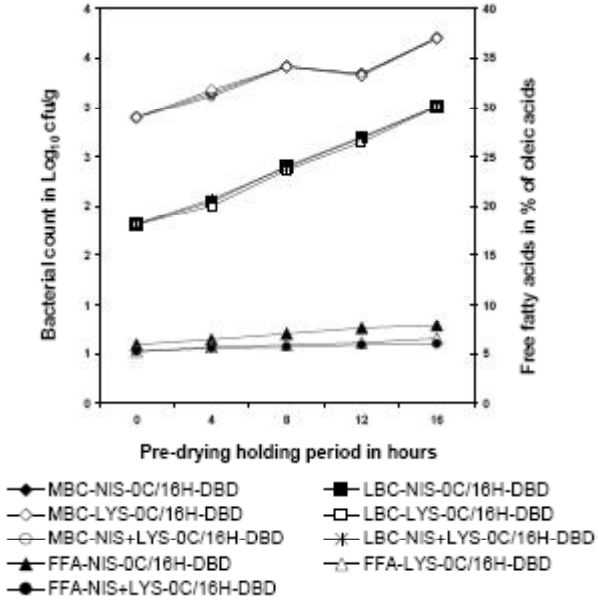

Figure 8 : Changes in MBC, LBC and FFA in NIS-DBD, LYS-DBD or NIS+LYS-DBD during storage at 0°C

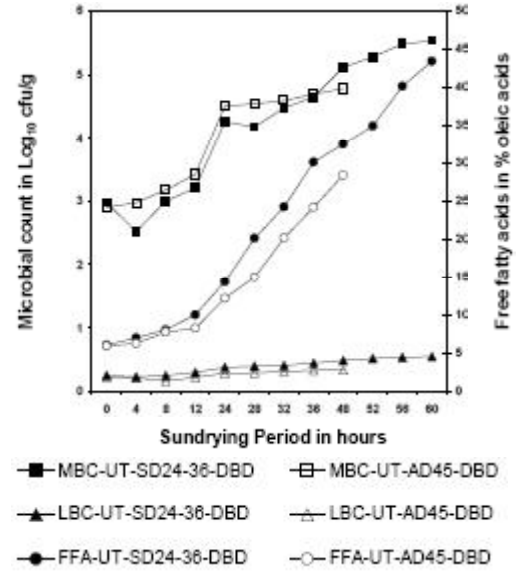

Figure 10 : Changes in MBC, LBCand FFA during sundrying (SD-26-34) or artificial drying (AD-45) of UT-DBD

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ficial drying (AD-45) of UT-DBD samples, MBC, PBC and LBC increased by 75.94±1.55, 11.32±1.23 and 8.3±1.45 folds respectively. It is interesting to note that the TMAN, TVBN and FFA was more ($p<0.05$) in UT-SD24-32-DBD samples, than UT-AD45-DBD, as it took 60 hours for sun drying (SD24-32), but only 40 hours by artificial drying (AD-45). Sensory panelists considered that UT-AD45-DBD samples were better and scored highest for flavors (Hedonic scale of 7.0±1.4), than SD24-32-DBD, which was found unacceptable at the end of the sundrying (Hedonic scale of less than 5 points).

Artificial drying (AD-45) of UT-DBD, UT-28C/16H-DBD, SA20-28C/16H-DBD and NIS+LYS-28C/16H-DBD samples

During artificial drying (AD-45) of UT-AD45-DBD, UT-28C/16H-DBD, SA20-28C/16H-DBD and NIS+LYS-28C/16H-DBD samples MBC, PBC and LBC increased ($p<0.05$) gradually from the initial value (Figures 11 and 12), but there was no significant ($p>0.05$) difference in the rate of increase of bacterial counts were observed in both treated (SA20-28C/16H-DBD or NIS+LYS-28C/16H-DBD)and untreated (UT-AD45-DBD or UT-28C/16H-DBD)samples at any given point of drying period. Even though the rate of increase of MBC, PBC and LBC during artificial drying remained respectively at a rate of 75.85±1.5, 11.51±0.58 and 8.4±0.25 folds, higher ($p<0.05$) levels of MBC, PBC and LBC was registered in UT-28C/16H-AD45-DBD samples, followed by SA20-28C/16H-AD45-DBD, UT-AD-45-DBD, and least count was observed in NIS+LYS-28C/16H-AD45-DBD at the end of the drying period. Similar trend was registered in FFA, TMAN and TVBN values even after having varied initial values. In general terms UT-SD24-32-DBD, UT-AD45-DBD, NIS+LYS-28C/16H-AD45-DBD, SA20-28C/16H -DBD, and UT-28C/16H-AD-45-DBD samples scored 4.2±0.1, 7.0±1.4, 8.4±1.6, 8.2±1.4, and 4.2±1.2 points respectively on Hedonic scale.

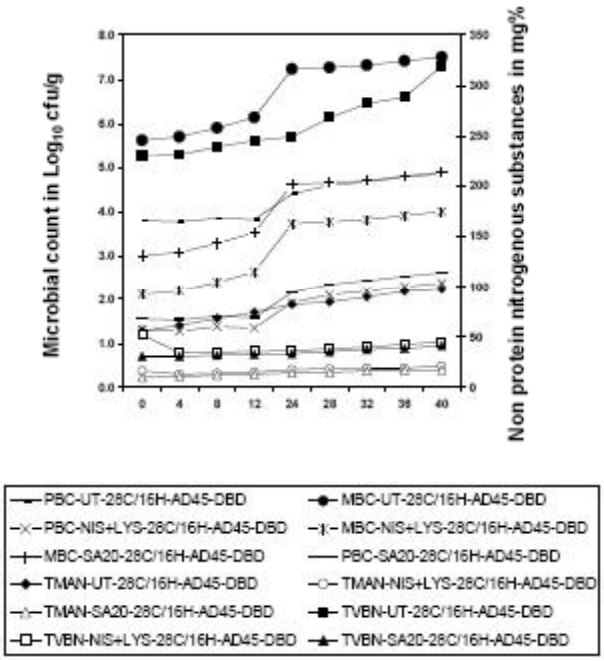

Figure 11 : Changes MBC, PBC, TMAN and TVBN in UT-28C/16H-DBD, NIS+LYS-DBD and SA20-28C/16H-DBD during artificial drying (AD-45)

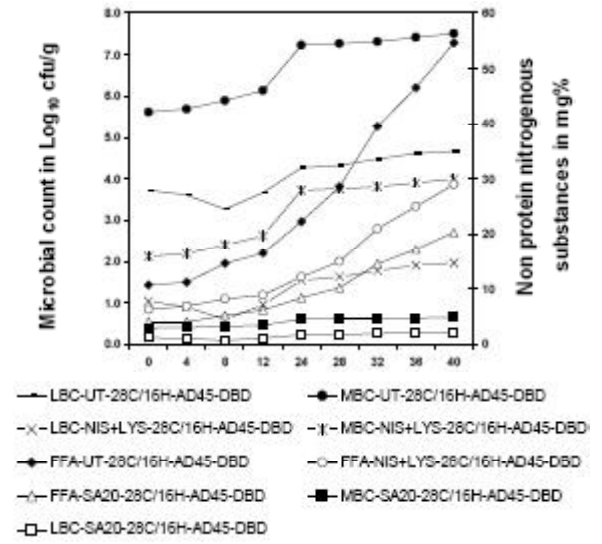

Figure 12 : Changes MBC, LBC and FFA in UT-28C/16H-DBD, NIS+LYSDBD and SA20-28C/16H-DBD during artificial drying (AD-45)

DISCUSSION

When untreated dressed Bombay duck (UT-DBD) was held at ambient temperature of 28°C for 16 hours, mesophilic, proteolytic and lipolytic bacterial count (MBC, PBC and LBC) increased significantly ($p<0.05$), but the microbial proliferation reduced drastically ($p<0.05$) as the temperature of the holding decreased to 0°C. Rate reduction in the proliferation of mesophilic bacteria at reduced temperature may be due to the inhibition of mesophilic bacteria and reduced autolytic

enzymatic activity at low temperature of storage[22,23]. It is important to note here that in tropical and subtropical regions mesophilic gram positive bacterial activity is the primary cause of fish spoilage[24]. In untreated samples (UT-28C/16H-DBD) accumulation of fatty acids, oxidation of trimethylamine and formation of volatile bases correlates ($p<0.05$) well with the proliferation of mesophilic, proteolytic and lipolytic bacteria. Results based on proteolytic and lipolytic bacterial count, protein degradation products, total volatile base and trimethylamine contents, showed that fish stored at ambient temperature deteriorates at higher rate than in ice[25,26]. Even though the rate of proliferation of mesophilic bacteria decreases with the decrease in the temperature of storage, there was a significant ($p<0.05$) increase in proteolytic and lipolytic mesophilic bacteria, accumulation of fatty acids, oxidation of the trimethylamine and formation of volatile bases were registered at 0°C (UT-0C/16H-DBD), which may attribute to the domination and spoilage of proteolytic Pseudomonads over the spoilage flora of aerobically chill stored proteinaceous raw meat effecting quality[27]. The principal spoilage organisms are *Pseudomonas sp* and *Alteromonas putrefaciens* during iced storage, while *Bacillus sp, Pseudomonas sp, Alteromonas putrefaciens* and *Proteus* dominates the spoilage flora at ambient temperature, and TMAN and TVBN proved to be reliable quality indices during ambient storage while their reliability as quality indices during ice storage is questionable[28]. In the present study untreated samples stored at 28°C for 16 hours (UT-28C/16H-DBD) were judged unfit for human consumption by the sensory panel.

When DBD samples were held at 28°C with different concentration of salt (SA10-28C/16H-DBD, SA20-28C/16H-DBD, SA36-28C/16H-DBD and SADS-28C/16H-DBD), MBC, PBC and LBC decreased with the increase in the concentration of salt, which goes well with the findings of Buckle, Souness, Putro, and Wuttijumnong[29]. Inverse relationship exists between salt content and total plate count[30] Decreased rate of accumulation of fatty acids, and formation of volatile bases were registered, as the salt concentration increased, which may attribute to the inhibition of lipolytic activity and proteolytic activity by sodium chloride respectively[31]. Once the salt content of the fresh tissue rises above 9%, the effect of most of the enzymes and bacteria halts[32], but halophilic bacteria grows successfully in the presence of salt[33] The dressed Bombay duck (DBD) samples held at 28°C in 20% salt concentration (SA20-28C/16H-DBD) were judged superior by sensory panelists, but SA36-28C/16H-DBD and SADS-28C/16H-DBD samples were found unacceptable by panelist even after having lower MBC, PBC, LBC, FFA, TVBN, and TMAN due to the high salt content in the flesh, off color and rough texture. Even though increase in concentration of the salt decreases microbial load, it increases the rancidity and order of the rancidity correlates well with decreasing preference[34].

Predrying holding of DBD with 200 IU/g of nisin and 0.3 mM EDTA (NIS-28C/16H-DBD), did not have much ($p>0.05$) effect on the reduction of proteolytic or lipolitic mesophiles, and hence on the accumulation of fatty acids, oxidation of the trimethylamine, and formation of volatile bases at ambient temperature, and these samples were found unacceptable beyond 4 hours of storage. Nisin is primarily active against gram-positive bacteria, but some gram-positive bacteria can become nisin resistant[35]. Nisin sensitivity of Gram-positive bacteria is reported to vary considerably with its efficacy being dependent on the concentration of the nisin and the number of spores or bacteria present. Effects are reported to be enhanced when the bacteria are growing and when nisin is used as part of a multi-preservation system[36]. But lysozyme at 50μg/g and EDTA at 0.3 mM was effective inhibitors of proteolytic and lipolytic mesophilic bacteria and hence accumulation of fatty acids, oxidation of the trimethylamine, and formation of volatile bases in the samples (LYS-28C/16H-DBD) reduced significantly ($p<0.05$). Bacteriostatic and bactericidal properties of lysozyme have been the subject of many studies, and over the last 10 years, several authors have proposed a novel antibacterial mechanism of action for lysozyme that is independent of its 1,4-β-*N*-acetylmuramidase activity. When the samples were held at 28°C in the presence of combinations of 200 IU/g of nisin, 50μg/g of lysozyme and 0.3 mM EDTA for 16 hours (NIS+LYS-28C/16H-DBD), rate of inhibition of mesophilic, proteolytic and lipolytic bacteria was at much higher rate than those recorded in those samples held only with lysozyme

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(LYS-28C/16H-DBD) and hence the rate of accumulation of fatty acids, oxidation of the trimethylamine, and formation of volatile bases reduced drastically ($p<0.05$). It is very interesting to note here that predrying holding of DBD in the presence of 200 IU/g of nisin, 50µg/g of lysozyme and 0.3 mM EDTA was much more effective in reducing the accumulation of fatty acids, oxidation of the trimethylamine, and formation of volatile bases than nisin or lysozyme alone. Nisin in combination with lysozyme are effective in inhibiting spoilage bacteria than each alone[37,38]. Salts of EDTA have long been used as antimicrobial agents, particularly against bacteria[39,40]. They have also been effective as enhancers of other antimicrobial agents, such as lysozyme or nisin[41,42]. EDTA synergistically enhanced the activity of nisin, and lysozyme[43]. But the presence or absence of nisin, or lysine did not have much effect on the proliferation of mesophilic, proteolytic and lipolytic bacteria when the samples were held at 0^0C (UT-0C/16H-DBD, NIS-0C/16H-DBD, LYS-0C/16H-DBD or NIS+LYS-0C/16H-DBD), which may attribute to the reduced enzyme activity at reduced temperature[44].

With the varied ambient temperature, air velocity and relative humidity, it took 60 hours to dry by sun, but when temperature, air velocity and relative humidity of the environment was controlled by artificial means, drying period reduced to 40 hours at 45^0C to reach moisture levels lower than that obtained by sundrying. The rate of increase of microbial count decreases significantly ($p<0.05$) with the increase in temperature using artificial dryer, because higher rate of drying decreases the microbial growth prior to the state of sufficient dryness[45], but lower ($p<0.05$) levels of microbial count was observed during initial period of sun drying which may attribute to the impact of sunrays on microorganisms. Even though no significant difference ($p>0.05$) in the rate of change of both FFA, TMAN and TVBN was observed in UT-SD24-38-DBD and UT-AD45-DBD samples , values were more in former than latter as it took 60 hours by sun, but lesser at higher temperature of artificial drying. Untreated sundried samples (US-SD24-38-DBD) were unacceptable by sensory panelists (with the hedonic scale of less than 5) due to prolonged drying period.

It is interesting to note here that initial microbial count in wet fresh fish (FBD) has a direct bearing on the microbial count of the finished dried product (UT-AD45-DBD and UT-28C/12H-AD45-DBD). For those samples held for greater length of time prior to drying (UT-28C/12H-AD45-DBD), do have higher mesophilic, proteolytic and lipolytic bacterial count compared to freshly dried samples (UT-AD45-DBD) not only in the wet fresh fish but also in the dried fish. In general, salted ones were having lower levels of mesophilic bacteria initially compared to the unsalted ones[46] and this trend continued till the end of the drying. By sensory evaluation, artificial dried samples (UT-AD45-DBD) were better than sundried one (US-SD24-38-DBD), and in artificially dried samples treated DBD with 200 IU/g of nisin, 50µg/g of lysozyme and 0.3 mM EDTA and dried at 45^0C (NIS+LYS-28C/16H-AD45-DBD) were rated superior than salted (SA20-28C/16H-AD45-DBD) or untreated (UT-AD45-DBD or UT-28C/12H-AD45-DBD) samples.

CONCLUSION

Predrying holding of dressed untreated Bombay duck at ambient temperature (UT-28C/16H-DBD) beyond 4 hours of storage was unfit for human consumption (less that 5 points on hedonic scale), but superchilling and reducing the storage temperature of the samples (UT-0C/16H-DBD)to zero degree centigrade drastically ($p<0.05$) reduced the spoilage rate. Direct correlation exists between temperature reduction and rate reduction in proliferation of mesophilic, proteolytic and lipolytic bacteria, and similar correlation was registered between mesophilic, proteolytic or lipolytic bacteria and the formation of trimethylamine, total volatile bases or accumulation free fatty acids respectively. The salting of Bombay duck is better in controlling microbial growth than chilling, especially if the raw fresh Bombay duck is to be held prior to drying. Inverse relationship was recorded between the salt concentration and the microbial proliferation, but direct correlation was established between microbial proliferation and increase in TMAN, TVBN and FFA values during salting. Though lower microbial count is observed in samples treated with saturated sodium chloride solution and dry salt, by sensory evaluation it was judged unsuitable, as increase in the salt concentration of salts correlated well with the development of rancid

flavors and the order of the rancidity correlates well with the decreasing preference. Hence predrying holding of dressed Bombay duck in 20% salt solution (SA20-AD45-DBD) was judged of superior quality by sensory evaluation compared to other salted products. By reducing the temperature or salting prior to drying decreases only the rate of bacterial proliferation but not the actual bacterial load. Lysozyme in combination with nisin and EDTA are effective in inhibiting mesophilic, proteolytic and lipolytic bacteria, and the rate of spoilage than each alone. Sundried products without any holding prior to drying (UT- SD24-32-DBD) are unfit for human consumption at the end of drying, but untreated artificial dried samples (UT- AD45-DBD) were acceptable by panelists. Initial bacterial load in the raw material has a direct impact on the quality of the final dried products and rate of increase of the spoilage bacteria remains constant during drying in all samples even with varied bacterial load. Holding DBD in mixture of 200 IU/g of nisin, 50μg/g of lysozyme and 0.3 mM of EDTA (NIS+LYS-28C/16H-DBD) is better in inhibiting spoilage bacterial load if the material is to be held prior to drying.

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