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Comparative Study of Fish Silage Prepared from Fish Market Waste by Using Different Techniques

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A B S T R A C T

In the present study an attempt was made for transformation of fish market waste into silage by using three different methods: Inorganic (98% sulphuric acid with weight percentage of 2.5, 3.5 and 4.5%), organic (98% formic acid with weight percentage of 2.5, 3.5 and 4.5%) and biological method (molasses with weight percentage of 5, 10, 15% and curd used as lactic acid bacteria source for fermentation). The chemical, microbiological and nutritional properties of the differently preserved fish silages were estimated during a storage period of 60 days at ambient temperature. The important findings are summarised as the rate of pH, AAN and TVB-N values were gradually increased and then get stable. In case of biological silage 10% and 15 % molasses pH was decreased below 4.5 after 72 hours. The rate of autolysis in sulphuric acid silage was slow compared to formic acid and biological silage. In case of bio-fermented silage (15% molasses) a steady supply of nutrients from molasses showed a steady increase in LAB from 2.24×10^{6} to 3.67×10^{9}cfu/g and then decrease. TPC was decreased from 4.50×10^{6} to 8.20×10^{3}cfu/g. In present study, sulphuric acid 2.5%, formic acid 2.5%, biological silage 5 and 10% silages were corrupted at the end of 24, 24, 12 and 30 days respectively. The appropriate amount of sulphuric acid, formic acid and molasses for preparation of fish silage were determined as 3.5%, 3.5% and 15% respectively.

Introduction

Fish is today considered as the most promising inexpensive alternative source of animal protein consumed by the man and it offers relatively high amount of essential amino acid, minerals and fatty acids as compared to live stock (Huisman *et al.*, 1989). In 2015, total marine fish landing of Maharashtra was 2.65 lakh tonnes (CMFRI, 2015). An average of 30% of the catch was sold locally as a fresh fish, 20-30 % was used in canning industry and the rest was used mainly in fish meal production (Anon, 1999). In the tropical countries, every efforts must be made to preserve fish for human consumption, but seasonal variation in catch, transport difficulties, inadequate processing facilities etc., resulted in the important quantities of fish has been wasted (Disney *et al.*,1977).

During, sea foods processing large amount of fish waste and deteriorated whole fishes are

discarded daily in the fish markets (Faid *et al.,* 1996). Fish waste, includes frames and rests from trimming, guts, skins, fats, viscera, eggs, heads, breasts, scales and deteriorated filets. A fish contain 45 % flesh, 24-27 % head, 12 % skeleton, 3 % skin, 4 % cut off and 12 % viscera including eggs, milts and liver of its total body weight (DOF, 2013). These wastes are a potential source of pollution and contamination of the environment, as they degrade rapidly in warm temperatures. If it is not appropriately stored or managed, it creates aesthetic problems and strong odours due to bacterial decomposition. But on the other hand, they contain relatively high amount of nutrients such as protein, fat and minerals (Djazuli *et al.,* 2007) which is easily available in low cost. So that, there is need for developing new methods for biotransformation of this fishery waste into animal feed to reduce aqua production cost.

Fish meal production is most commonly used method to recover the nutrient loss in discarding the fish processing by products and it was used as animal feed. If there is absence of any fish meal plant in the area due to restriction on fish meal production to avoid fish odours, no transport facility is available towards the nearest fish meal plant and increasing price of fish meal due diminishing fish stock, then one has to look for alternate process

The best alternative way of utilizing fish waste material is the production of fish silage which does not release any off odour during preparation (Pagarkar *et al.,* 2005).The product has a good nutritive quality and can be sufficient for animal feeding. This procedure is safe, cost effective, eco-friendly and has a good nutritive quality which can be adequate for animal feeding (Hanafy and Ibrahim, 2004). Fish silage preparation usually depends on the locally available raw materials and conditions (Hasan, 2003).

Fish silage is defined as a liquid product produced from the whole fish or parts of it, to which acids, enzymes or lactic acid-producing bacteria are added, with the liquefaction of the mass provoked by the action of enzymes from the fish (FAO, 2007). Fish silage is the liquefied product rich in protein and free amino acids (Martin, 1996). The liquefaction of fish mass carried out by enzymes already present in fish (Tatterson and Windsor, 1974). This is obtain by action of the naturally occurring enzymes presence in the whole fish, fish minced or fish offal. The enzymes, mainly from the digestive organ, break down protein into smaller soluble unit and the acid helps to speed up their enzyme activity while preventing bacterial spoilage (Al-Abri *et al.,* 2014).

Depending on the process employed in the preservation of fish waste, fish silage can be categorised in two methods, viz. acid silage and bio-fermented silage (Raa and Gildberg, 1982). Acid silage is produced by mixing fish waste with inorganic (sulphuric acid, hydrochloric acid) and organic acid (formic acid, propionic acid) or mixture of both organic and inorganic acid, while bio-fermented silage is obtain by adding fermentable sugar to fish biomass. Fermentation is carried out by lactic acid bacteria (LAB) which are already present in a fish mass or added externally for successful fermentation. In view of above facts, the present research work was to study different methods of producing high quality of fish silage.

Materials and Methods

Fish market wastes were procured from fish market of Ratnagiri. The fish mainly consisted of heads, tails, gills, fins and visceras of Sardine (*Sardinella fimbriata, Sardinella longiceps*), Mackerel (*Rastrelliger kanagurta*), Tuna (*Euthynnus affinis*, *Auxis*

thazard), Pink perch (*Nemipterus japonicus*), Ribbon fish *(Trichiurus lepturus, Lepturocanthus savala),* Bombay duck *(Harpadon nehereus),* Seer fish *(Scomberomorus guttatus, Scomberomorus commerson),* Mullet *(Mugil cephalus).* The collected fish wastes were washed with potable water and stored at -20 °C until further used.

The fish waste was thawed, washed, and grinded into paste using mixer for preparation of different types of silage.

Silage production using mineral acid (Mousavi *et al.*, 2013)

1.5 kg of minced fish waste was poured in three plastic containers (each container containing 500 gram of fish waste) and 98% sulphuric acid with weight percentages of 2.5, 3.5, and 4.5 % (v/w) and 65 mg of Butylated Hydroxy Toluene (BHT) were added to each sample. The mixture was stirred regularly with sterile glass rod to ensure through mixing and inhibit growth of mould on the surface. Samples were kept at room temperature (28°C to 32°C) for 60 days and stirred every 8 hours. pH changes were measured by pH meter and recorded until it reached a stable level.

Silage production using organic acid (Mousavi *et al.*, 2013)

1.5 kg of minced fish waste was poured in three plastic containers (each container containing 500 gram of fish waste) and 98% formic acid with weight percentages of 2.5, 3.5, and 4.5 % (v/w) and 65 mg of Butylated Hydroxyl Toluene (BHT) were added to each sample. The mixture was stirred regularly with sterile glass rod to ensure through mixing and inhibit growth of mould on the surface. Samples were kept in room temperature (28°C to 32°C) for 60 days and stirred every 8 hours. pH changes were measured by using pH meter and recorded until it reached a stable level.

Silage production by biological method using Curd (Palekar, 2009)

1.5 kg of minced fish waste was poured in three plastic containers (500 grams in each). Then, sugar cane molasses and water was added to each container with weight percentages of 5%, 10%, 15% (v/w) and 30% (v/w) respectively. 65 mg of butylated hydroxytoluene (BHT) were added to each sample. The mixture was stirred using a sterile glass rod to ensure through proper mixing. After these samples were heated in water bath for 15 minutes at 90 °C and then cooled at a room temperature. Then curd (starter culture) with a weight percentage of 10 % (w/w) was added to them. Mix the samples thoroughly and stored in airtight plastic container. pH changes were measured by pH meter and recorded until it reached a stable level.

Preparation of starter culture

Curd was prepared fresh from boiled and cooled milk and kept for overnight. Lactic acid bacterial (LAB) count of curd was estimated by pour plating technique on MRS agar and Lactic Acid Bacteria (LAB) count of 4.20 x 10^6cfu /gram used for preparation of biological silage.

Chemical analysis

Chemical analysis of raw materials and silages were analysed by measuring moisture, protein, fat, ash content and pH according to AOAC official methods (AOAC, 2005). AAN measured according to Benjakul and Morrissey (1997), T-VBN measured according to Beatty and Gibbons (1937) and TPC according to APHA (1992).

Statistical analysis

The data were analysed to test significant difference by applying analysis of variances (ANOVA) tools available in MS-Excel 2010. The significant differences were tested by 5% level of significances and are mentioned as $p < 0.05$ for significances difference (Snedecor and Cocharn, 1967).

Results and Discussion

Biochemical and microbiological analysis of fish market waste

The biochemical and microbiological characteristics of fish waste were analysed during the initial stage of present work. The fish waste used for the present work was having acceptable fishy smell and appearance. Proximate composition of fish waste on wet basis is shown in Table 1. Fish waste contained moisture 77.09 ± 0.14 %, crude protein 15.20 ± 0.15 %, fat 4.03 ± 0.07 % and ash 3.30 ± 0.11 %.On a similar line the biochemical quality of fish market waste contained 79.47 % of moisture, 15.78 % of protein, 2.13% of lipid and 2.32 % of ash in fish waste reported by Palekar (2009). Similar results also given by Abowei and Tawari (2011), Gullu *et al.,* (2015), Tatterson and Windser (1974), Hasan and heath (1987), Ozyurt *et al.,* (2015).The variation noted in proximate composition may be due to the age, sex, body weight, seasonal, feeding aspects of fish material.

The pH, α- amino nitrogen, TVB-N and TPC of fish waste were 6.8 ± 0.49, 10.64 ± 0.13 mg-N100g^{-1}, 18.64 ± 0.09 mg-N100g $^{-1}$ and 5.1× 10^{6} cfu/g respectively. Similar results were depicted by Palekar (2009) had found 7.16 of pH, 18.68 mg-N100g^{-1}of TVB-N, 32.11 mg- N100g^{-1}ofα amino nitrogen (AAN) and 6.7×10^{6}cfu/g TPC in pink perch raw material.

Biochemical and microbiological changes during storage

pH

Sulphuric acid

In present study the pH of different treatment of sulphuric acid silage A1, A2, A3 (sulphuric acid 2.5%, 3.5% and 4.5% respectively) were 2.97, 1.94, and 1.33 initially. In treatment A1 (sulphuric acid 2.5%) pH was reached up to 4.62 at the end of 24th day. Then sample was corrupted. It may be due to quantity of acid used was insufficient to prevent the activity of putrefactive bacteria. In Treatments A2 and A3 (Sulphuric acid 3.5% and 4.5%) pH was stable at 2.66 and 1.92 at the end of 30th day respectively. There was no increased in pH at the end of 60th days of storage. Similar trends were observed by Mousavi *et al.,* (2013) reported that when 98% sulphuric acid with weight percentage 2.5% used sample got musty after 20 days. In sulphuric acid with weight 3.5% and 4.5%, pH of samples reached a stable level at 2.58 and 1.94 respectively after 40 days (Fig. 1).

Formic acid

In present study the initial pH of different treatments of Formic acid silages viz. B1, B2, B3 (Formic acid 2.5%, 3.5% and 4.5%) were 3.43, 3.14, and 2.73 respectively. In, Treatment B1 (Formic acid 2.5%), pH was increased from 3.43 to 4.64 at the end of 24thday. Then sample was corrupted because of amount of acid used was incapable for arrest the action of putrefactive bacteria. pH of treatments B2 and B3 (Formic acid 3.5% and 4.5%) were stable at 3.65 and 3.41 at the end of 24th day respectively. Mousavi *et al.,* (2013) investigated similar trends, when 98% Formic acid with weight percentage 2.5% used sample got musty after 6 days at pH 3.61. Formic acid with weight 3.5% and 4.5%

used pH of sample reached a stable level at 3.88 and 3.61 respectively after 56 and 66 days (Fig. 2)

Biological silage

The initial pH of Biological silage prepared using different percentages of molasses viz. C1, C2 and C3 (Biological silage 5%, 10 % and 15%) were 6.85, 6.56 and 6.75 respectively. During fermentation fall in pH was noticed in all the treatments. In treatment C1 (Biological silage 5%), pH was decreased from 6.85 to 5.24 at the end of 12thday.Then sample was corrupted. It occurs due tofive percent molasses was inadequate to produce good fermentation. Similar observations were reported by Kompiang *et al.*, (1979), they observed Fish: molasses ratio of 100:5 gave silage stable only up to few days. Generally, fish is a poor source of carbohydrates. It needed for to ferment and produce lactic acid in the silage. Production of organic acid helps to reduction of pH value in fermented silage and prevents the growth of spoilage organism. If sufficient carbohydrate is not present in the medium, required levels of acid will not be produced, as results of putrefying bacteria increased. In, treatments C3 (Biological silage 15%) pH were decrease below 4.4 within 72 hours and then increase and stable at 4.30 at the end of 24th days respectively (Fig. 3).

Similar trends were observed by James (1966) that precooked silage showed rapid fermentation and reached a pH of 4.4 within 72 hours. Treatment C2 (Biological silage 10%) got putrefied at the end of 30th day. The last measured pH was 4.65. Similar results were scrutinized by Neethiselvan *et al.*, (2002), Palekar (2009). Recommended pH value for preserved fish silage should be below 4.5 reported by Epse and Lied (1999).The slight fluctuation in pH during the storage period in all silages was probably caused by the dissolving of fish skin, bones and scales (Ozyurt *et al.*,2015). The results indicate that 15 % molasses was sufficient to produce good fermentation.

Alpha Amino Nitrogen (AAN)

Digestion of protein in the silage as determined by liquefaction and production of NPN and NH3.The rapid production of NPN was observed within 30 days of storage.

Sulphuric acid

The AAN content of different treatments of sulphuric acid silage viz. A1, A2 and A3 (sulphuric acid 2.5%, 3.5% and 4.5%) were 25.27, 14.80, 11.89 mg-N100g-^{1}initially and it reached up to 47.71 and 45.24 mg-N100 g-1 at the end of 30th day in treatments A2 and A3 (sulphuric acid 3.5 and 4.5%) respectively (shown in Fig. 4). There was no increase in AAN even after 30thday of storage. Treatment A3 (Sulphuric acid 4.5 %) showed slow rate of autolysis compared to treatments A1 and A2 (sulphuric acid 2.5 and 3.5%).This is occurs due to difference in the pH produced by acids. At high acid concentration of acid shown low pH and inhibitory to proteolysis. When mineral and organic acid are used for production of fish silage the rate of liquefaction of protein is different. At pH 3 the rate of autolysis and yield of soluble matter was less (Raa and Gildberg, 1982).

Raghunath and Mc Curdy (1990) reported that at pH 3 both endopeptidases and exopeptidases were active, the silage quickly breaking down the protein nitrogen to amino nitrogen. But at pH 2 only acid endopeptidase and a weak exopeptidase activity were detected, thus slowing the rate of autolysis. Stone and hardy (1986) reported that acid stabilised silage (sulphur 2.45%) of pacific whiting shown no increase in levels of amino nitrogen even after 42 days of storage indicating the absence of autolysis.

Formic acid

The AAN content of different treatments of Formic acid silage: B1, B2 and B3 (Formic acid 2.5%, 3.5% and 4.5%) were 26.97, 16.31, 14.76 mg-N $100g^{-1}$initially and it reached up to 52.15 and 49.02 mg-N100 g^{-1} at the end 24^{th} day in treatments B2 and B3 (Formic acid 3.5 and 4.5%) respectively shown in Figure 5.

There was no increase in AAN even after 24^{th} day of storage. Babu *et al.,* (2005) reported maximum alpha amino nitrogen value (as % of TN) was lower in 2.5% acid silage (21.21% of TN) than in 3% acid Silage (24.33% of TN) and 2% Acid silage (27.30% of TN). Haaland and Njaa (1989) reported that when acid addition was too low pH increase during storage, resulting in much higher production of ammonia. Palekar (2009) observed AAN of formic acid was 39.76 mg-N $100g^{-1}$ initially which increased up to 272.69 mg-N $100g^{-1}$ at the end of 90^{th} day.

Biological silage

Different treatments of Biological silage viz.C1, C2 and C3 (Biological silage 5%, 10 % and 15%) contained initial AAN were 16.61, 14.28 and 13.34 mg-N $100g^{-1}$ which increased up to 34.92 and 37.35 mg-N 100 g^{-1} at the end 24^{th} day in treatments C2 and C3 (biological 10% and 15 %) respectively.

There was no increase in AAN even after 24^{th}day of storage in treatments C2 and C3 (biological silage 10 % and 15%) respectively. In the present study, the value of protein solubilisation was found to be lower in biological silage compared to acidified silage. Similar observation was detected by Dapkeevicius *et al.,* (1998). Ozyurt *et al.,* (2015) depicted similar results during fermentation of fish silage that AAN was 0.07g/100g initially increased up to 0.69 g/100g at the end of 60 days. Palekar (2009) described AAN in biological silage was increase from 36.67 mg $N100g^{-1}$ to 157.00 mg N100g $^{-1}$ at the end of 90^{th} day of storage (Fig. 6).

Total Volatile base Nitrogen (TVB-N)

In most of the countries, total volatile nitrogen is used as quality criterion for fish silage. TVN consists mainly of trimethylamine (TMA) and ammonia NH_3. TMA originates from bacterial decomposition of trimethylamineoxide (TMAO) and analysis for it may be used as criterion of the freshness of raw materials. The acceptable limit of TVB-N is 35-40 mg/100g (Connel)

Sulphuric acid

TVB-N content of Sulphuric acid treatments A1, A2 and A3 (Sulphuric acid 2.5, 3.5 and 4.5%) were 16.85, 16.99 and 17.17 mg $N100g^{-1}$ initially which increased up to 27.07, 26.29 mg $N100g^{-1}$ in treatments A2 and A3 (Sulphuric acid 3.5 and 4.5%) respectively (shown in Fig. 7).

Formic acid

TVB-N content of Formic acid treatments B1, B2, B3 (Formic acid 2.5, 3.5 and 4.5%) were 19.79, 17.13, 16.99 mg-$N100g^{-1}$ initially which increased up to 27.16, 25.25 mg-$N100g^{-1}$ in treatments B2 and B3 (Formic acid 3.5 and 4.5%) respectively. The similar results were obtained by Haaland and Naaja (1989). According to Ahmed and Mahendrakar (1996a) reported during fermentation of fresh water fish viscera, the TVB-N values increased to 8% of the total nitrogen from initial 1.3%. Tanuja *et al.,* (2014) showed that TVB-N level in both the treatments (with and without antioxidant) were well below the limit of acceptability (35-40 mg %) except on the 90th day of storage (Fig. 8).

Fig.1 pH changes in different treatments of sulphuric acid silage during storage

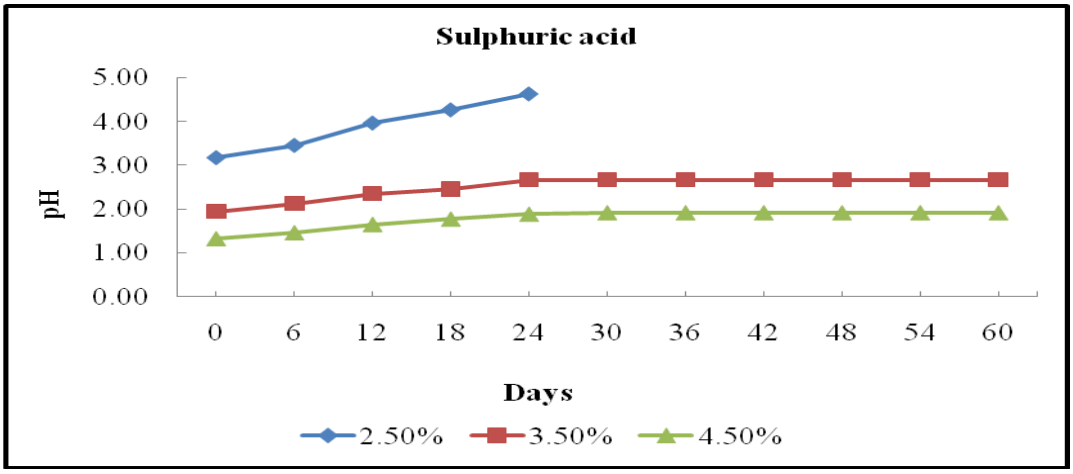

Fig.2 pH Changes in different treatments of formic acid silage during storage

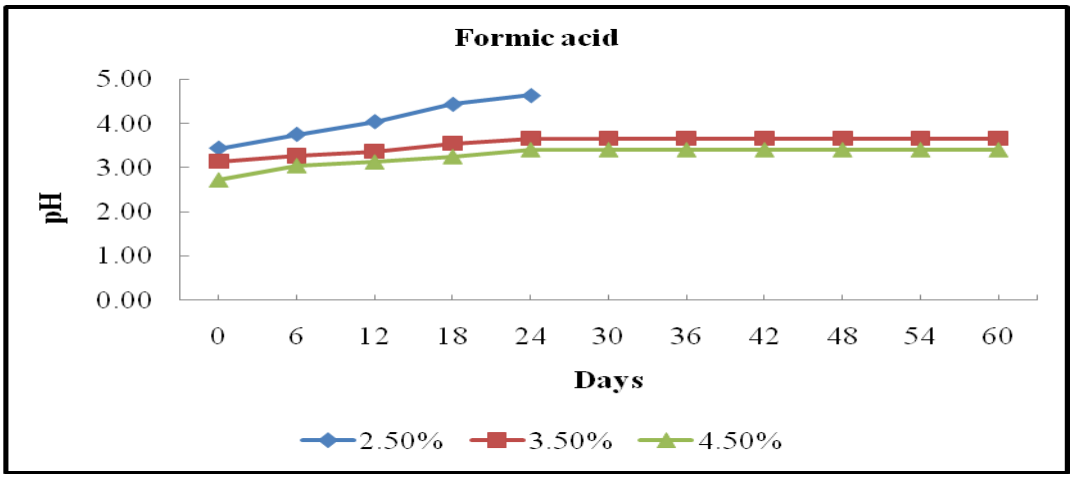

Fig.3 pH Changes in different treatments of Biological silage during storage

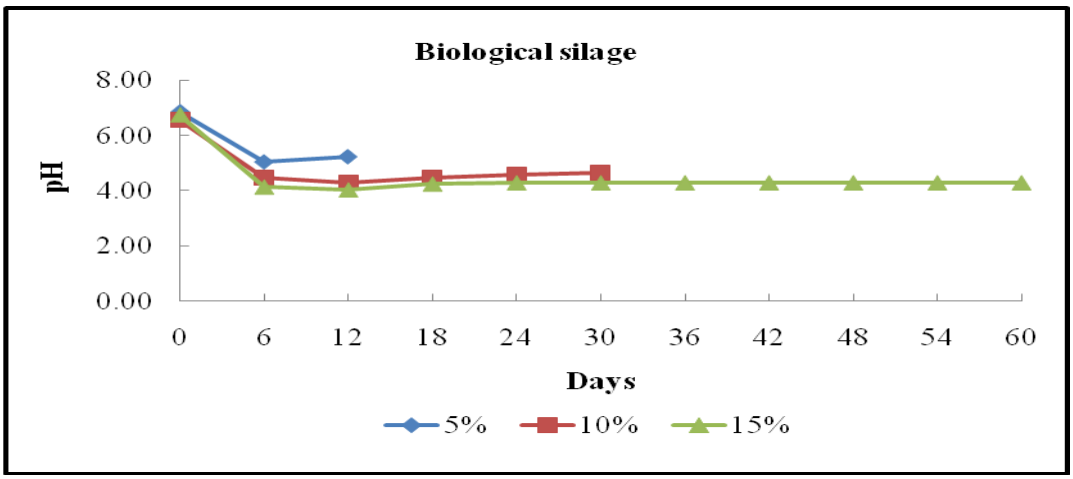

Fig.4 AAN changes in different treatments of sulphuric acid silage during storage

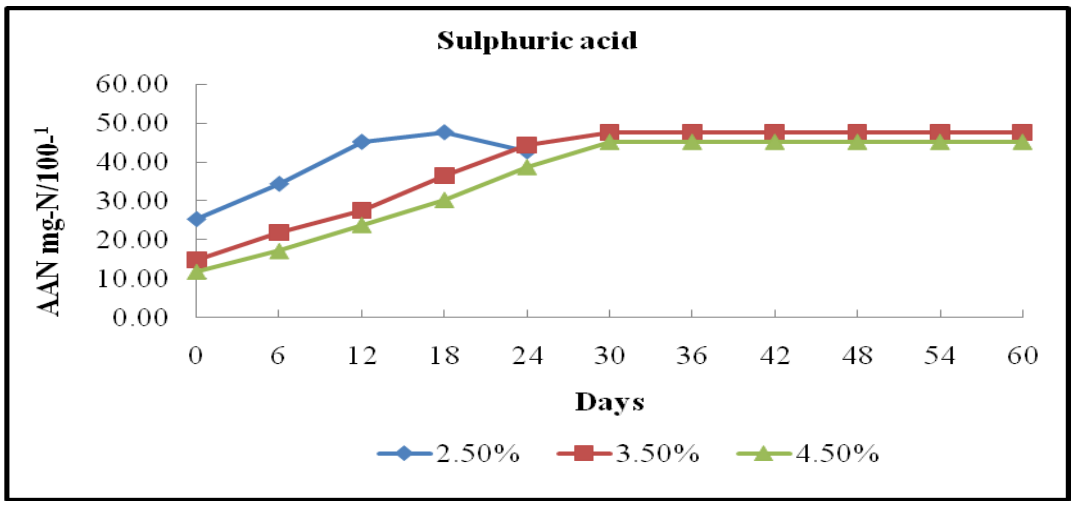

Fig.5 AAN changes in different treatments of formic acid silage during storage

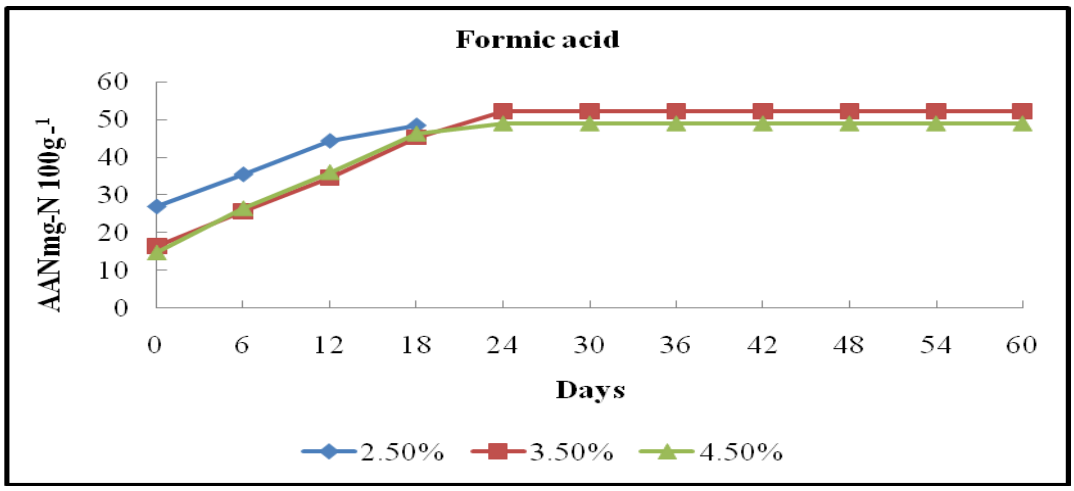

Fig.6 AAN changes in different treatments of biological silage during storage

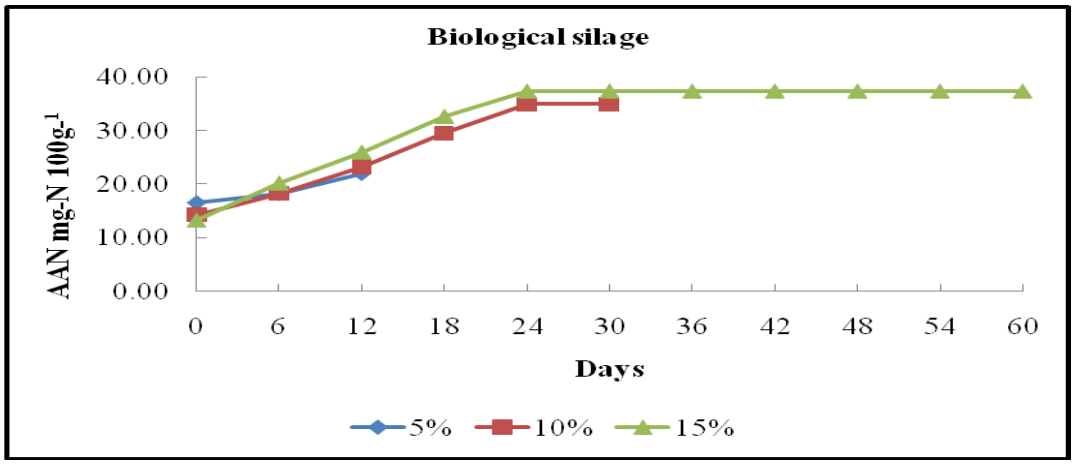

Fig.7 TVB-N changes in different treatments of Sulphuric acid silage during storage

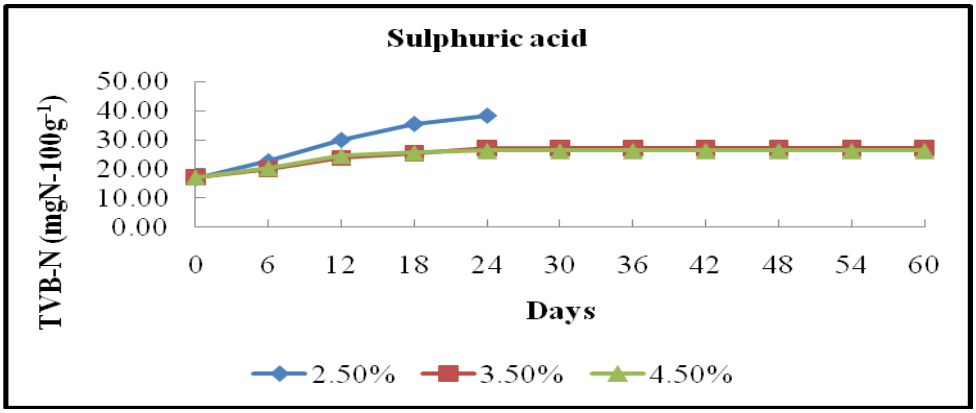

Fig.8 TVB-N changes in different treatments of formic acid silage during

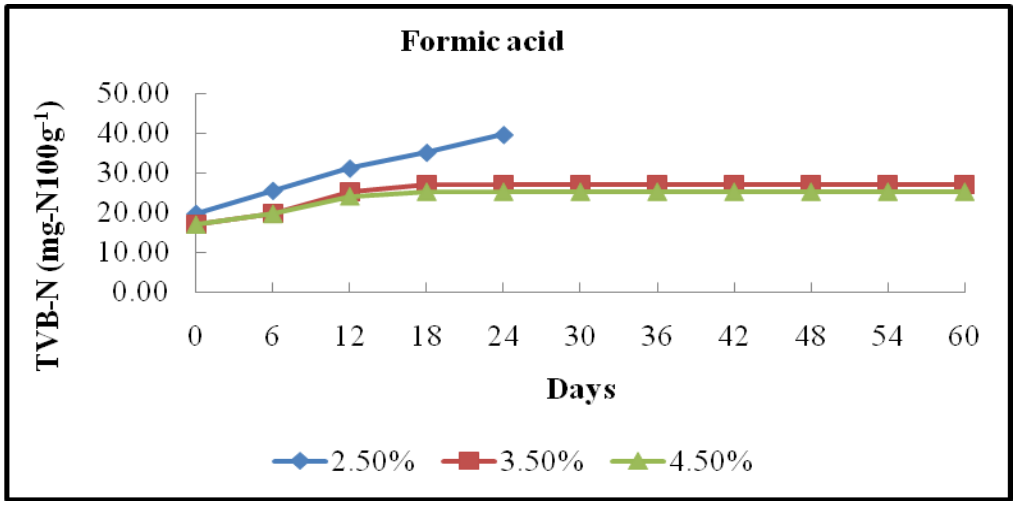

Fig.9 TVB-N changes in different treatments of biological silage during storage

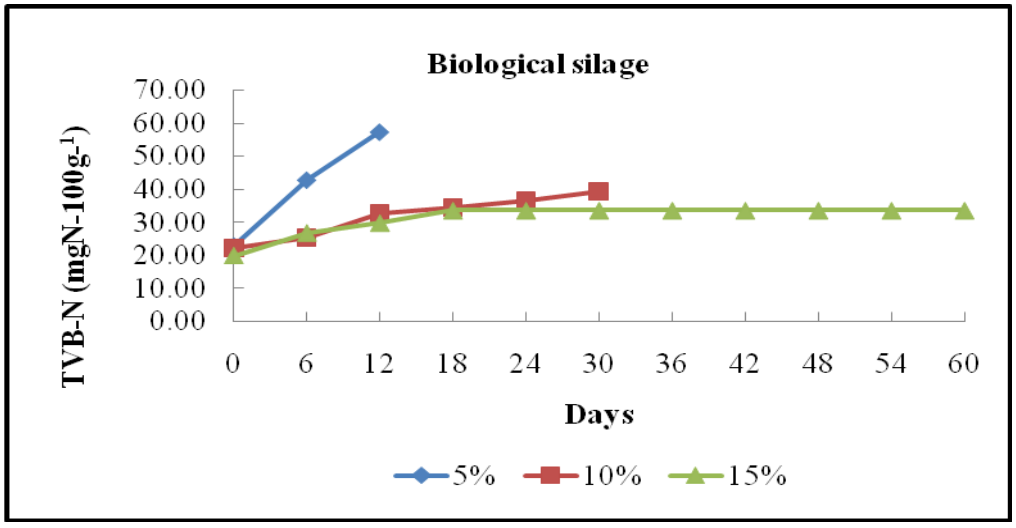

Fig.10 TPC changes in different treatments of biological silage during storage

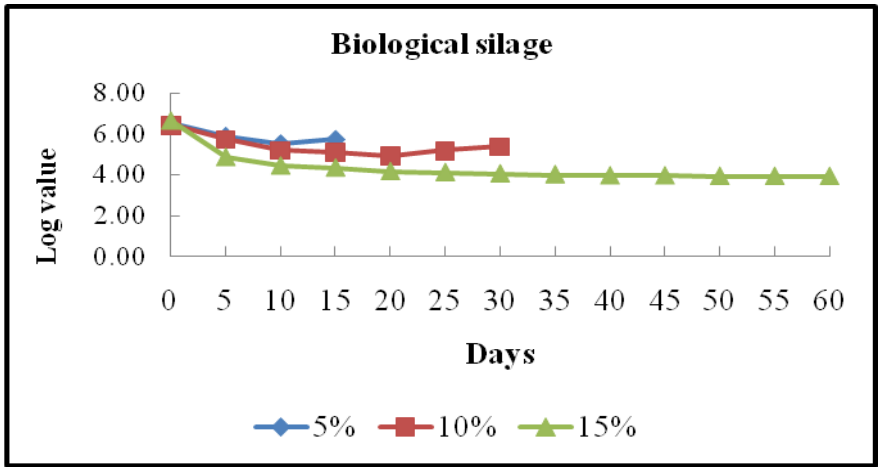

Fig.11 LAB changes in different treatments of biological silage during storage

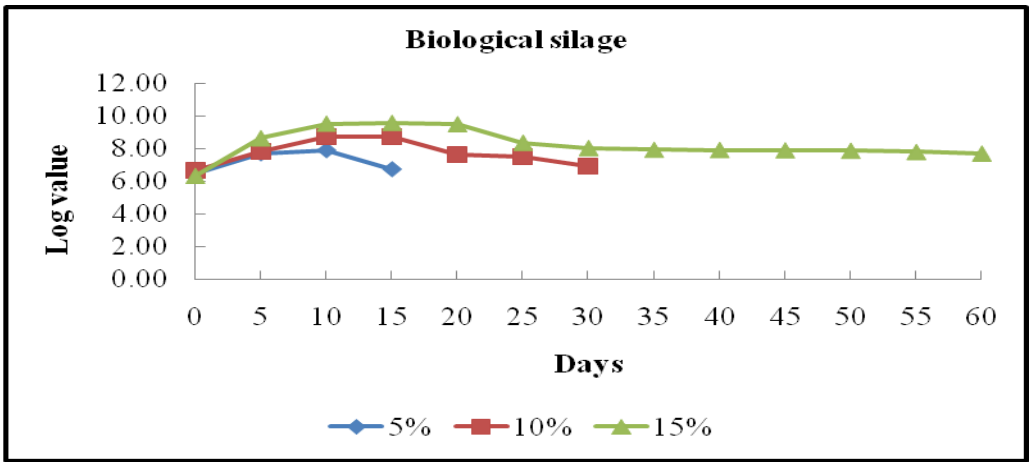

Table.1 Proximate composition of fish market waste

Fish waste	Proximate composition (%)
Moisture	77.09± 0.14
Crude protein	15.20±0.15
Fat	4.03 ±0.07
Ash	3.30 ±0.11

Table.2 Chemical and microbiological analysis of fish market waste

pH	6.5
AAN (mg-N100g^{-1})	10.66
TVB-N (mg-N100g^{-1})	16.55
TPC (cfu/g)	6.2×10^{6}

Palekar (2009) reported similar value of TVB-N in formic acid silage increased from 18.22 mg-N 100 g^{-1} to 57.67 mg-N 100g^{-1} at the end of 90th day of storage.

Biological silage

In case of biological silage TVB-N value of treatments C1, C2 and C3 (Biological silage 5%, 10%, 15%) were 22.73, 22.17, 19.83 mg N100g^{-1} initially and which increased up to 57.35, 39.43, 33.55 mg N100g^{-1} at the end of 12th, 24th, 24th day respectively. In treatment C3 (Biological silage 15 % molasses) there was no changes in TVB-N value at the end of 60th day. Faid *et al.,* (1996) showed that TVN increased in trial 1 from 71.26mg N100g^{-1} to reach 95.03 mg N100g^{-1} after1 day and remain constant around 132 mg N100g^{-1} after 15 days of fermentation at 22º C. The initial TVB-N of CS (Curd silage) was 20.13 mg-N 100g-^{1} which increased up to 133.28 mg -N 100 g^{-1} at the end of 90thday reported by Palekar (2009) (Fig. 9).

Total Plate Count (TPC)

In, biological silage treatments C1, C2, C3 (Biological silage 5, 10, 15%) shown TPC 3.16×10^{6}, 2.74×10^{6}, 4.50×10^{6} cfu/g initially. In the present study TPC were decreased during storage. In Treatment C2 (Biological silage 10%) TPC was decreased and then increased after 24 days. If sufficient carbohydrate is not present in the medium, required levels of acid will not be produced, as results of putrefying bacteria increased. The last measured TPC in C3 (Biological silage 15%) was 8.20 $\times10^{3}$cfu/g at the end of 60th day. Ozyurt *et al.,* (2015) observed slightly similar result. Similar results were depicted by Palekar (2009), Rahmi *et al.,* (2008). Bello *et al.,* (1993) studied bacterial fish silage produced from several fish species mixed with molasses, fruits sorbate (pineapple and papaya) and starter culture of *Lactobacillus plantarum.* It was found that the bacterial silage showed only few aerobic mesophilic organisms due to the low pH values and the development of LAB (Fig. 10).

Lactic Acid Bacteria (LAB)

In biological silage C1, C2 and C3 (Biological silage 5, 10, 15%) initial LAB count were 2.73×10^{6}, 4.70×10^{6}, 2.24×10^{6} cfu/g respectively. A sharp increase in LAB count was observed after fermentation shown in (Table 2 and Fig. 11). The last measured LAB in treatment C3 (Biological silage 15%) was 5.10 $\times10^{7}$cfu/g at the end of 60th day. During storage study there was increase in LAB were observed initially and then gradually decreased up to end of storage. But at a certain stage LAB count was decreased due to depletion in carbohydrate sources. Total LAB counts of silage at maximum bacterial growth in this study were similar to the results reported by Palekar (2009), Zahar *et al.,* (2002) and Ozyurt *et al.,* (2015).

Changes in proximate composition

Protein

In the present study results of protein content in the present experiment showed significantly decreased with respect to increase in concentration of acid as well as increase in experimental duration. During the initial day of the experiment, the average crude protein content recorded was 15.20%. But at the end of the experiment, a fall in protein level was noticed with considerable level of 13.11%, 12.91% in treatments A2, A3 (3.5 and 4.5% sulphuric acid) ensilages, 13.03%, 12.82% in B2 and B3 (3.5 and 4.5% formic acid ensilages) and14.72% in treatment C3 (15 %molasses) respectively at the end of 60 days. Reduction of protein content in the ensilage may be due to break down of protein (FAO, 2007). Vidotti *et al.,*

(2003) observed a reduction in crude protein level in combined (2% each of formic acid and sulphuric acid) fermented silage of tilapia filleting residue when compared to non-fermented tilapia filleting residue. Ramasubburayan *et al.,* (2013) and Palekar (2009) reported similar results were that at end of the experiment, a fall in protein level was noticed.

Fat

The fat content in the present study revealed that at the beginning of the experiment, it was an average 4.03 % in concentrations of sulphuric acid, formic acid, and biological silages. But when the experimental days prolonged, the lipid content increased at the end of the experiment, the increase in lipid content were 5.06 %, 5.24 % in treatments A2 and A3 (3.5 and 4.5 % sulphuric acid silages), 5.02 %, 5.46 % in B2 and B3 (3.5 and 4.5 % formic acid silages) and 4.54 in C3 (biological silage with 15% of molasses) respectively. In the present study the continuous increase in lipid content during storage period may be due to release of fats from raw material Dapkevicius *et al.,* (1998) reported 3.6% increase in lipid content from 11.3 to 14.9% from initial to final stage (15 days) of storage of 3% formic acid ensilage of blue whiting. The lipid content was between 8.08 and 8.27 % in 2, 2.5,3 % concentrations of formic acid silages but at the end of the experiment, the increase in lipid content increased between up to 10.66 and 12.24 respectively in the 2, 2.5, 3 % concentrations of formic acid silages on dry matter basis reported by Ramasubburayan *et al.*,(2013), Palekar (2009).

Moisture

In present study found that significant decrease in moisture content of silages. The initial moisture content recorded was 77.09%. But at the end of the experiment, a significant decrease in moisture level was noticed with considerable level of 75.24%, 75.54% in treatments A2 and A3 (3.5 and 4.5 % sulphuric acid ensilages); 75.32%, 75.49% in B2 and B3 (3.5 and 4.5 % formic acid ensilages) and 73.62 in C3 (biological silage 15 % molasses) respectively. Biological silage shown higher decreased in moisture level compared to acid level. This may be addition of lactic acid bacteria source such as curd might have increased the solid matter and decreased the moisture level. Ozurt *et al.,* (2015) observed moisture content was decreased in all silages (Formic acid, Formic acid + Sulfuric acid, and *streptococcus thermophiles*) except *Lactobacillus plantarum.* Fermented silage showed lesser amount of moisture than acid silage. Palekar (2009) reported moisture content was decreased during storage in formic acid silage, *Lactobacillus plantarum* silage and curd silage after 90 days of storage.

Ash

Result of ash content in the present experiment found that significant decrease in ash content of the silage with respect to increase in acid concentration. During the initial day of the experiment, the average ash content recorded was 3.30 %. But at the end of the experiment, increase in ash level was noticed with considerable level of 5.12, 4.84 % in A2 and A3 (3.5 and 4.5 % sulphuric acid silages); 5.03, 4.72 % in B2 and B3 (3.5 and 4.5 % formic acid silages) and 5.89 % in C3 (biological silage with 15 % molasses) respectively. Ozurt *et al.,* (2015) reported ash content was increased in all silages (Formic acid, Formic acid + Sulfuric acid, *Lactobacillus plantarum* and *streptococcus thermophiles*). Neethiselven *et al.,* (2002) depicted ash in curd, *Lactobacillus plantarum* and FC silages 4.39, 3.37 and 3.35% respectively. Babu *et al.,* (2005), Palekar

(2009) observed that ash content was higher in fermented silage than acid silage

In present study found that fish silage is eco-friendly to the environment, safer, technologically simple and more economical than the manufacture of fish meal. Today, silage technology is most useful for solving the waste problem from industry and fish market. Fish silage would be a good potential source for animals in arid regions as it contained high protein (Al-Abri *et al.,* 2014; Pagarkar *et al.,* 2006; Palekar, 2009). In all three methods of fish silage production (mineral acid, organic acid and biological method), the optimum amount of sulphuric acid, formic acid and molasses were determined 3.5%, 3.5% and 15% respectively.

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