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Shelf-Life Study of Fish Silage Prepared from Freshwater Fish Waste

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ABSTRACT: The present study was undertaken to investigate the suitability for the transformation of fish market waste into silage by using two different solvents; Inorganic (98% sulphuric acid) organic (98% formic acid). Indian aquaculture, which is dominated by carps, is extremely promising and has increased by more than six and a half times in the last two decades, with freshwater aquaculture accounting for more than 95% of total aquaculture production. Only 25-50% of the raw material is used for human consumption, according to estimates. The remaining 50-75% of raw material is processing waste and can be used to make low-valued goods. Fish silage was prepared using 3.5% sulphuric acid and formic acid with added Butylated Hydroxy Tolune (BHT). The storage is always a problem in silage production. The study was conducted for 60days at room temperature. The biochemical parameters (pH, TVB-N, TBARS, AAN) gradually increase during the course of storage but does not exceed the acceptable level. The present study shows that the quality of the fish silage is safe and does not deteriorate during the 60days and utilization of these fish wastes provides more revenue to fish and related sectors.

Keywords: Fish market waste, Fish silage, Biochemical changes.

INTRODUCTION

Aquaculture is a fast growing agribusiness in India. Fishes are good sources of protein; the meat is rich in essential vitamins, minerals and omega-3-fatty acids. Besides its nutritious value it also plays an important part in raising the economy and standards of living (Huisman *et al*., 1989). Aquaculture accounts for about 50% of the world's total fish production (Jayasankar, 2018). India is the 2nd largest producer of fish and the trend is increasing. It accounts for about 5.6% of world's fish production. Fishery accounts for 5% of the total agricultural GDP of India. Majority of fishery in India is from Inland Fisheries predominantly from aquaculture of which freshwater aquaculture accounts for about 95%. Indian aquaculture sector shows a growth rate of 7% (Jayasankar, 2018). Carp is the main culture in Indian freshwater aquaculture, some catfishes and freshwater prawns.

Feed is the key input and fish meal is the main ingredient as a source of valuable animal protein in fish diets (Rangacharyulu *et al*., 2003). Feed cost amounts to about 60% of the production cost. Fish meal is expensive and there is scarcity in supply due to overexploitation. The replacement of fish meal with alternative proteins or alternative processing methods has been developed but the adverse effect has been related to deficiencies of certain essential amino acids, particularly methionine and lysine. To combat this, fish nutritionists have supplemented the diet with crystalline amino acids to improve fish growth and health. On the contrary, animal proteins had an adequate concentration of these amino acids which are essential for normal growth. The advantage of animal protein is the low concentrations of anti-nutritional factors that might reduce the digestibility and assimilation of nutrients, as is the case when fish are fed plant proteins (Abdel-Fattah and El-Sayed, 1999). Thus, studies on the use of other efficient and cheaper sources of protein as substitutes for fish meal are necessary for aquaculture development.

Alternative resources such as meat and bone meal, hydrolyzed feather meal, fleshing-meal and blood meal, dried fish and chicken viscera (Paul *et al*., 1997; Millamena, 2002; Giri *et al*., 2000), poultry silage, crayfish meal and shrimp meal (Middleton *et al*., 2001; Agouz and Tonsy, 2003; Al-Azab, 2005) have been used to replace fish meal either partially or fully. Butthese alternative sources are not sufficient to meet the growing demands of fish raising industry. Fish wastes can be processed into fish feed by fermentation with lactic acid bacteria. Fish waste account for about 30% after the processing which comprises of gills, fins, scales, visceral bones, etc.

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 (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). This procedure is safe, economically advantageous and environment friendly. The pH value of the fish pastes decreases below 4.5 during ensilage and this pH decrease in partly responsible for preservation (Maria *et al*., 2000). Fish silage is generally a product of high biological value presenting practically the same composition as the original raw material (Wassef, 1990, Fagbenro and Jauncey, 1994; Vidotti and Carneiro, 2002). In developing countries like India, fish silage is cheaper to produce, involves simple artisanal technology and possesses good storage properties. It represents an alternative to fish meal in utilizing waste/trash fish (accounted for about 5% of annual farm production). The present study is aim to explore and produce high quality fish silage.

MATERIALS AND METHODS

Freshwater fish processing wastes (viscera, heads, scales, fins, skin and bones) were collected from local fish market. The fish wastes are mainly fromtilapia, catla, rohu, mirgal, amur carp, pangasius. The collected fish wastes are washed and stored at -20^{o}C. For preparation of raw material for fish silage, the frozen wastes were thawed and grinded into paste using a mixer grinder.

A. Production of fish silage using organic acids and inorganic acids

Minced fish waste of 500g each was poured in a six glass container. 3.5% of 98% formic acids and sulphuric acids (Palkar *et al.,* 2018; Mousavi *et al*., 2013) were added into three containers each as a triplicate and 65mg of Butylated Hydroxyl toluene (BHT). The mixture was kept in room temperature and stirred regularly using sterile glass rod and kept 60 days for fermentation. The change in pH was recorded regularly during this period.

B. Biochemical analysis

The proximate composition and pH were measured according to AOAC (2005) official methods. (TVB-N) Total volatile base nitrogen was determined by the Conway microdiffusion method (Conway, 1950). AAN measured according to (Pope and Stevens, 1939), Oxidation stability of the sample was assessed by measuring Thiobarbituricacid (TBA) value (Tarladgis *et al*., 1960).

C. Statistical analysis

The data was analysed using MS-Excel 2010's analysis of variances (ANOVA) tools to see if there was a significant difference. Duncan's multiple range tests (for Post hoc analysis) were used to compare the averages of the parameters evaluated for quality evaluation (p0.05) using statistical analyses (SPSS, version 16.0 for windows).

RESULTS AND DISCUSSION

A. Proximate Composition

The proximate composition of the fish waste is presented in Table 1. The fish waste contained74.82±0.09 % moisture, 16.02±0.14% protein, 4.37±0.22% of lipid and 3.86±0.07% of ash. Similar findings are recorded by Palkar *et al*., (2017) from fish waste, where contained moisture 77.09 ± 0.14 %, crude protein 15.20 ± 0.15 %, fat 4.03 ± 0.07 % and ash 3.30 ± 0.11 %. (Hossain and Alam, 2015) found protein of 14.01±0.68%, lipid of 20.00±1.04%, moisture of 60.62±2.15% and 4.75±0.64% ash from fish viscera. The protein value was in consistent as obtained by (Bechtel, 2003) in fish viscera of 13.0-15.3% protein. Another study conducted by (Tanuja *et al*., 2014), a protein % of 37.7±0.42% on dry weight basis.

Table 1: Proximate composition of fish waste. Values expressed as mean ± SD (n = 3).

	Moisture %	Protein %	Lipid %	Ash %
Fish waste	74.82±0.09	16.02±0.14	4.37±0.22	3.86±0.07

B. pH of the silage during storage period

The changes in pH during the storage period for sulphuric acid and formic acid treated samples are presented in Fig. 1. Maintaining of the acidic pH of the fish silage during the storage period is important to prevent the growth of pathogenic organism and maintain the hygiene of the samples. In the present study, the sulphuric acid treated sample there is a decrease in pH (1.8) at the 3rd day of storage and slowly increases and a constant reading of pH (2.5) was observed from the 36th day till the 60th day of storage. This result is in consistence with (Mousavi *et al*., 2013), observed a stable pH of the sample at 2.58.

A stable pHof 2.66 after the 30th day of storage was observed by (Palkar *et al.,* 2017). The time taken for obtaining a stable pH in the present study have taken more time as compare to studies reported by (Palkar *et al*., 2017: Mousavi *et al*., 2013). This difference could be due to the use of low-fat fish waste mostly from carps, the difference in acids use and concentration and the storage temperature. The formic treated sample, the pH increases slightly during the storage but stable below 4.2 till the 60th day of storage. This is due to the acid impact being neutralised by chemical compounds and reactions with fish waste. Due to rapid chemical changes and accumulation of pH-lowering impact, pH

reduction or stability was occasionally seen in short time intervals. (Mousavi *et al.*, 2013) reported that on day 56, the pH of the sample fixed at 3.88 when 90% formic acid with a weight percentage of 3.5 was employed. This can be ascribed to differences in chemical composition of raw materials, bacterial load, ambient conditions, and the kind and concentration of utilised acid.

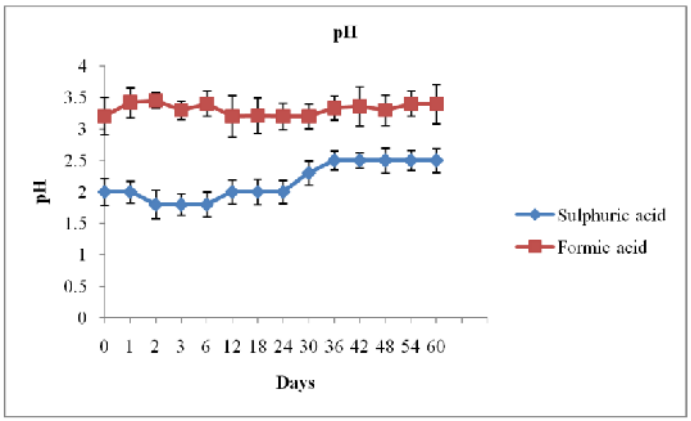

Fig. 1. Changes in pH of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3).

C. TVB-N of the silage during storage period

The changed in the TBV-N value during the storage period of fish silage treated with sulphuric acid and formic acid is plotted in Fig. 2. The acceptable limit of TVB-N for fresh fish is 35-40 mg N100g^{-1}. It is used as a criterion to measure the freshness of raw materials. In the present study, the TVB-N in both the treatment is below the acceptable level during the 60 days of study, though an increasing trend was observed during the 60 days study. The highest TVB-N content recorded till the end of the 60days study was 47.45 7 mgN100g^{-1} for formic acid and 26.21mg N100g^{-1} for sulphuric acid treated sample compared to 17mgN 100g^{-1} and 16mg N100g^{-1} respectively. These findings were in consistent with that reported by (Tanuja *et al*., 2014) of below TVB-N value less than 20 mg N100g^{-1} using freshwater fish waste. But the amount were much lesser as compared to those findings reported by (Kuhlmann *et al*., 2011) level of more than 150mg% in fishmeal, (Haaland and Njaa, 1989) 112 mg/100g in ensilage using 1.4% formic acid. Ali and Sahu (2002) has reported 79.8mg % for acid silage using marine fishes. Ahmed and Mahendrarkar, (1996) using a carp visceral for silage production had reported 9mg % TBV-N. TVB-N consists of ammonia and trimethylamine, where the majority is contributed by trimethyl amine which is absent or found in very limited quantity in freshwater fish. This must be the reason for the low quantity of TVB-N in silage prepared from freshwater fish species waste.9% of total nitrogen as TVBN was reported by Ahmed and Mahendrakar, (1996) infish viscera ensilage. A similar trend was reported by (Nilsson and Rydin, 1963; Zuberi *et al*., 1992; Xavier *et al*., 2017).

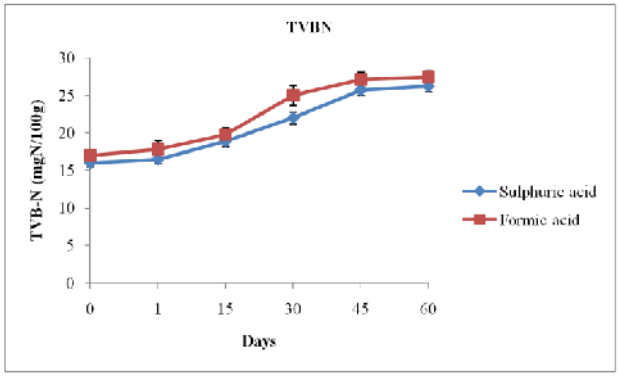

Fig. 2. Changes in TVBN-N content of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3).

D. TBARS of the silage during storage period

The change in TBARS value during the storage period plotted in Fig. 3. TBA is use to measures malanaldehyde formed by the sample during oxidative rancidity. The final TBARS for sulphuric and formic acid treated sample for 60 days are 2.02 mg malonaldehyde/kg and 2.5 mg malonaldehyde/kg respectively. TBARS value increased steadily during

the storage period. These reading are in consistent with the findings made by Tanuja *et al*., 2014. The addition of antioxidants in the silage helps in the slowing down of lipids oxidation (Ahmed and Mahendrarkar, 1996). Sajib *et al*., (2020) reported continuous opening of the storage can for stirring increased the TBARS value, therefore, that limiting the supply of oxygen is required if targeting a high-quality silage production. A four weeks study o carp visceral silage, the TBA value reached 1mg malonaldehyde/ kg oil as reported by Bhaskar and Mahendrarkar (2007).

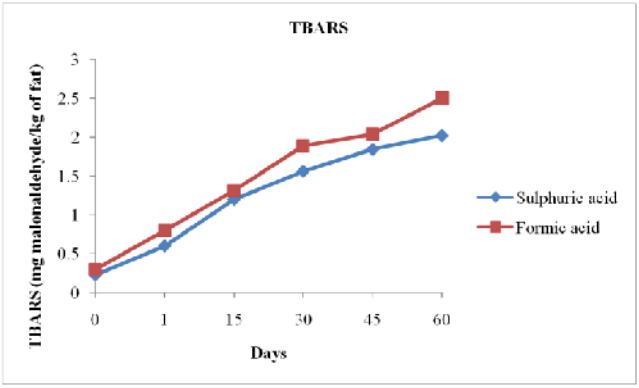

Fig. 3. Changes in TBARS content of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3).

E. Alpha Amino Nitrogen

Alpha Amino Nitrogen is used to measure the protein digestion which is determined by the production and liquefaction of NPN and NH_3 compounds. The AAN compounds of both the treatment are plotted in Fig. 4. There was a steady increase in the AAN content of the fish silage in both the treatment during the 60 days storage. The AAN had increased from 10.04 mg-N100^{-1}g to 44.2 mg-N100 $^{-1}$g in sulphuric acid treated sample and 14.2 mg-N100 $^{-1}$g to 51mg-N100^{-1}g for formic acid treated sample respectively. The rate of liquefaction is different when mineral and organic acids are used for silage production. Proteolysis is inhibited when highacids is used which lowers the pH. The rate of autolysis and yield of soluble materials were lower at pH 3 (Raa and Gildberg, 1982). Stone and Hardy (1986) reported there is no increase in the level of amino nitrogen after 42 days storage of pacific whiting silage using sulphuric acids at 2.45%, which indicates the absence of autolysis. Similar trends were also observed by (Palkar *et al*., 2017) that results in 47.71 mg-N100 $^{-1}$g in sulphuric acid treated sample and52.15 mg-N100 $^{-1}$g in formic acid treated sample after 30days of storage.Endogenous enzymes in the fish viscera operate on the peptide bonds of protein structure during ensilaging. Proteinases catalyse the hydrolysis of peptide bonds, which is a frequent process in nature. Proteinases are multifunctional enzymes that catalyse the hydrolytic breakdown of proteins in aquatic animals. They are mostly produced by the digestive glands (Garcia-Carreno and Hernandez-Cortes, 2000). This will aid in activating the action of the acid or aspartyl proteinases group of endo peptidases (Whitaker, 1994). In the digestive organs of fish, pepsin, gastricsin, trypsin, chymotrypsin, collagenase, elastase, carboxy-peptidase, and carboxyl esterase were discovered (Haard, 1994; Simpson, 2000).

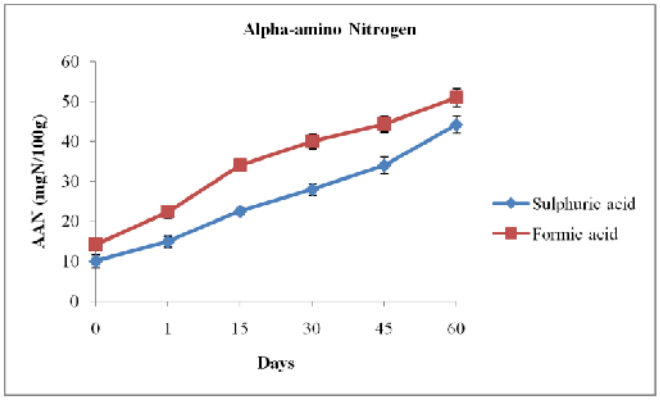

Fig. 4. Changes in AAN content of fish silage treated by Sulphuric acid and Formic acid during storage. Values expressed as mean ± SD (n = 3).

CONCLUSION

Freshwater fish processing generates a large amount of processing waste, the most common of which is visceral waste. Acid ensilation might be a potential option for converting these wastes into valuable byproducts. Only little changes in the dry matter, protein, lipid, and mineral fractions occurred throughout the acid ensiling process, demonstrating the methodology' applicability. In the production of high-quality, nutrient-rich powder fish silage, fish viscera might be a good replacement for expensive fishmeal. In the preparation of fish and animal feed, it will be feasible to partially substitute expensive fish meal. Fish and other animals' growth performance in farm culture conditions should be tested with fish silage. It was necessary to conduct research on the most acceptable packaging for such a product for local marketing. The addition of BHT to acid silages made from carp fish viscera delayed the process of auto oxidation, and the low pH inhibited microbe multiplication. The present study shows that the silage quality is good and does not deteriorate during the 60 days study period and could also be done on a small scale. More income and job possibilities may be produced from the fisheries and allied industries by properly using these wastes.

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Conflict of interest. Nil.

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