International Journal of Agriculture and Food Science Technology.
ISSN 2249-3050 Volume 5, Number 2 (2014), pp. 91-98

http://www.ripublication.com

Shelf Life Study of Acid Added Silage Produced from Fresh Water Fish Dressing Waste with and without the Addition of Antioxidants

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Abstract

India accounts for 4.4% of the global fish production. Indian Aquaculture, which is dominated by carps, is highly promising and has grown over six and half fold in the last two decades with freshwater aquaculture contributing over 95% of the total aquaculture production. it is estimated that only 25-50% of the raw material is utilized for human consumption. The remaining 50-75% of the raw material is considered processing waste and can be utilized for low-valued products. Fish silage is a liquid product made from whole fish or parts of fish that are liquefied by the action of natural enzymes in the fish, in the presence of an added acid. It can be used as a feed supplement for fish, livestock and poultry or also as a fertilizer. Acid silage was prepared from the dressing waste of fresh water fishes with (Formic acid+Hydrochloric acid+Butyl Hydroxy Toluene (FHB)) and without (Formic acid +Hydrochloric acid (FH)) the addition of synthetic antioxidant Butyl Hydroxy Toluene (BHT). The Biochemical, microbiological and nutritional quality of the silages were compared. The addition of antioxidant did not significantly ($p>0.05$) alter the proximate composition of the silage (FHB). The addition of BHT significantly reduced the rate of oxidation in FHB. The synthetic antioxidant was not found to significantly reduce the production of volatile bases and also the microbial load.

Keywords: silage, antioxidants, dressing waste, fresh water fish, shelf life

1. Introduction

In India the contribution of aquaculture to the total inland fish production has increased sharply in the last four decades. Fresh water aquaculture contributes around 95% of total aquaculture production in India. (Dehadrai, 2003). Of the total world carp production, 95 per cent of the total world carp production is from Asia. 12% of the total world carp production is from India. Low-income people favor carps because of their low price and good taste. In many areas in Asia, carps are the major source of animal protein for the poor. Majority of the fish markets in India, are dominated by the presence of fresh water fishes. The dressing waste of these fishes usually go as waste. These extremely perishable wastes are high in moisture and range from 30 to 40% crude protein (CP) on a dry matter (DM) basis. Excluding fish discards and skeletons, the largest part of this waste consists of viscera; however, visceral waste has great potential to be used as a protein supplement in animal feeds (Fai et al., 1997). The best alternative solution is to utilize the waste material for the production of by – products. In most cases they are upgraded into fishmeal, but the silage process has been reported to be a feasible, simple and lower-cost alternative (Vidotti et al., 2003). During the last two decades, fish silage has been successfully used as a low cost ingredient in aquaculture diets (Espe et al., 1992).

Fish silage can be defined as a liquid-pasty product generated from fish (dead fish, unused species, marine fishing by-products, commercial fish waste and industrial residues or residual fractions from marine peptone processing) in an acidic medium (Raa and Gildberg, 1982). Formic acid (organic acid) is the best choice for the preparation of chemical silage, the silages made using formic acid are not excessively acidic and therefore do not require neutralization before being used (Oetterer, 2002). Since organic acids are costly, a combination of organic and inorganic acid can be used in the preparation of silage. Acid silages if prepared with only inorganic acids will have a very low pH (around 2) which requires nuetralisation before it can be used in feed (Vizcarra-Magaña et al., 1999). Fish visceral waste are an excellent source of proteolytic enzymes and marine peptones for supporting bacteriocin production (Vázquez et al., 2004a). The low pH created by the addition of the acid help in the activation of these enzymes favouring the liquefaction of the waste. Potassium sorbate and butyl hydroxyl toluene are also added to prevent mould, yeast growth and oxidation development, respectively.

The biochemical, microbiological and nutritional quality of acid added silage prepared from the dressing waste of fresh water fishes with and without the addition of synthetic antioxidant is being investigated here.

2. Materials and Methods

2.1. Preparation of acid silage

Dressing waste of fresh water fishes were collected from a local fish market in Bhubaneswar, Odisha. The waste was washed in potable water, chopped and ground using meat grinder into paste for silage preparation. Acid silage was prepared by acidifying the paste with 1.5% formic acid and 1.5% hydrochloric acid. The silage

was divided into 2 lots. To one lot, FHB, was added 200ppm Butyl Hydroxy Toluene (BHT) and 0.1% Potassium Sorbate. To the other lot, FH, only 0.1% Potassium sorbate was added. Ensilation process was aided by incubating the materials in air tight plastic containers at room temperature (28 ± 2^0 C). The silage was stirred twice daily to ensure the uniform distribution of acid. Once the pH had stabilized, samples of known volume were drawn from both the lots to determine the proximate composition. Samples of known volume were also drawn at 0, 15, 30, 60, 90, 120, 150 and 180 days to study the biochemical and microbiological quality.

2.2. Determination of Proximate composition

Moisture, Crude protein, crude fat and ash content was estimated using the AOAC (2000) procedure. In brief, the dry matter content was determined by drying the homogenate in an oven at 105 ^{0}C until a constant weight was obtained. The crude protein content was calculated by converting the total nitrogen concentration (6.25 x N) determined with the Kjeldahl procedure. Crude fat was determined using the Soxhlet extraction system. Ash content was measured by dry ashing in muffle furnace at 550 ^{0}C for 6 h.

2.3. Biochemical and microbiological analysis for quality assessment

pH of the silage in distilled water (1: 5 W/V) was determined by using a glass electrode digital pH meter (Cyberscan 510, Eutech instruments, Singapore). Total volatile base nitrogen (TVB-N) was estimated by the microdiffusion method (Conway, 1950). Oxidation stability of the sample was assessed by measuring Thiobarbituricacid (TBA) value (Tarladgis et al, 1960). Microbiological analysis of aerobic plate count was determined using standard culture medium. Twenty-five grams of silage was aseptically weighed and homogenized with sterile mortar and pestle with 225 ml sterile Normal saline for 1 min. The homogenized sample was serially diluted using 9 ml sterile normal saline. Further serial dilutions were made and 0.5 ml of each dilution was pipetted onto the surface of the plate count agar (Himedia), in triplicates, after which they were incubated for 48hrs at 37°C.

3. Results and Discussion

3.1. Nutritional quality of acid silages

3.1.1. *Proximate composition:* Proximate composition of the dressing waste of fresh water fishes showed 24.14($\pm$2.63) % dry matter, 37.7($\pm$0.42)% protein, 40.60%($\pm$0.32) crude fat and 4.25% ($\pm$0.44)ash and a pH of 6.67($\pm$0.02). Vidotti et al (2003) has reported that the acid silages prepared from fresh water fishes have a crude protein content of 44.3%. The Table 1 shows the proximate composition of acid added silage prepared from dressing waste of fresh water fishes. The addition of antioxidant does not significantly ($p>0.05$) alter the proximate composition of silage. The silage had a balanced composition in minerals (ash), protein and lipid fractions which make it an interesting product in animal feeding. The results also show that during the ensiling process only slight variations were observed in the dry matter, the protein, lipid and minerals fractions. Vast variation can be found in the proximate

composition of fresh water carp viscera as reported by different authors (Ahmed & Mahendrarkar, 1996. Bhaskar &Mahendrarkar, 2007). The difference in composition could be due to age, sex, body weight, season or feeding aspects (Sikorski and Kolakowski, 2000).

Table 1: Proximate composition of ensiled visceral waste of carps prepared with and without the addition of BHT

Parameters	FH	FHB	t value	P
Dry matter (%)	25.53±0.33[a]	25.39±0.29[a]	0.55	0.6143
Crude protein (%)	38.2±0.88[a]	38.97±1.46[a]	-0.78	0.4794
Crude fat(%)	39.19±0.53[a]	38.75±1.06[a]	0.65	0.5538
Ash(%)	4.44±0.47[a]	4.56±0.23[a]	-0.4	0.7111

3.2. Physico chemical parameters of the acid silages

3.2.1. *Changes in pH.:* Maintenance the acidity in fish silage is important in keeping the product more hygiene and safe by inhibiting the growth of pathogenic organisms. The pH of FH and FHB significantly ($p<0.05$) dropped to 3. 3 and 3.12, respectively on the second day for obvious reasons (Fig 1). Although both the lots showed fluctuations in pH till the 180th day of storage, it never increased above 3.5 which indicate the excellent keeping quality of the silage. The pH at the end of 180th day of storage, 3.44 and 3.41 of FH and FHB respectively are within the acceptable range that should be maintained for silages. The pKa of the preservative acid determines the final pH that silages reach (Raa and Gildberg, 1982). According to Haaland and Njaa (1989) and Vizcarra-Magana *et al.* (1999), deamination reactions probably caused slight changes in pH during storage. The silage at pH 4.5 and above is always susceptible to spoilage caused by *Clostridium botulinum*, *Staphylococcus aureas* and fungus (Anon 1971).

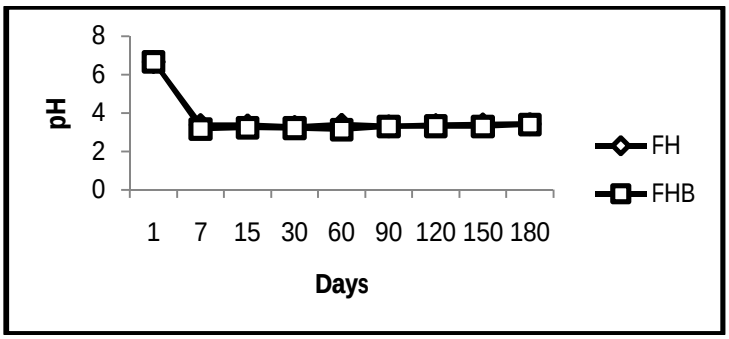

Figure 1 : Changes in pH of ensiled dressing waste of fresh water fishes prepared with and without the addition of BHT stored at 28±2^{0}C

3.2.2. *Changes in Total Volatile Base Nitrogen (TVBN):* The limit of acceptability for TVBN in fresh fish is 34-40mg%. In the present study the TVBN levels in both the treatments were well below the limit of acceptability except on the 90th day of storage, indicating the non spoilage of silage during storage (Fig 2). The maximum TVBN

value reached was 46.73mg/100gm for the lot without synthetic antioxidant. High amount of TVBN to the level>150mg % has been reported in fishmeal, which is the most common animal protein supplement in feeds. (Kuhlmann etal , 2011). Haaland and Njaa (1989) recorded TVBN value in the form of NH_3 as 112 mg/ 100g on the 14th day of ensiling the capelin fish using 1.4% formic acid. Ali and Sahu (2002) has reported a TVBN value of 79.8mg % for acid silage prepared from marine fishes. The major portion of TVBN value is contributed by trimethyl amine which is absent or found in very limited amount in freshwater fishes. This could be another reason for the low TVBN values found in the fresh water fish silage. Ahmed and Mahendrarkar (1996) has also reported a low TVBN value of 9mg % for fermented carp visceral silage. According to Connell (1980) TVBN more than 100 to 200 mg/100gm on dry weight basis of salted dried fish could indicate spoilage. The decrease in TVBN values after 90 days of storage may be due to the escape of ammonia.

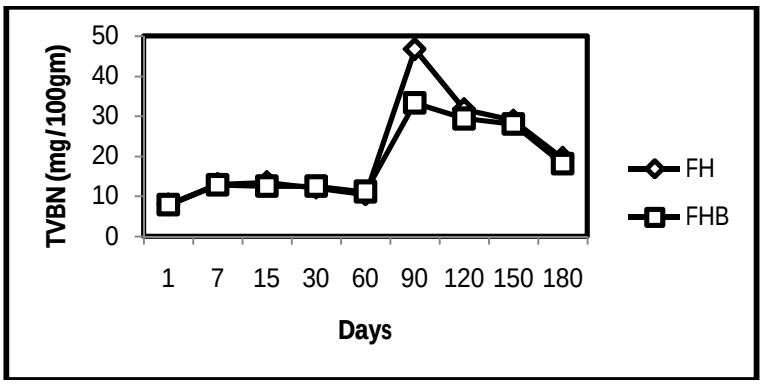

Figure 2 : Changes in TVBN of ensiled dressing waste of fresh water fishes prepared with and without the addition of BHT stored at 28 ± 2^0C

3.2.3. *Changes in Thiobarbituric Acid values (TBA):* Fresh dressing waste of fish had a TBA value of 0.47+0.01mg malonaldehyde/kg. The addition of antioxidant to FHB decreased the TBA value to 0.45 mg malonaldehyde/kg on the 2nd day although the change was insignificant (Fig 3). Delgado etal (2008) has proved the protective effect of antioxidant on the Spanish mackerel silage. Figure 3 depicts that althrough the storage period the fat deterioration by auto oxidation was less in FHB when compared to FH. This asserts the importance of addition of antioxidants to silage especially if made from fatty fish or body parts like viscera. Ahmed and Mahendrarkar (1996) has also reported that addition of antioxidants to silage results in slowing down the auto oxidation of lipids in fish viscera. The maximum TBA values attained at the end of 180 days were 13.57 mgmalonaldehyde/kg and 10.35 mg malonaldehyde/kg in FH and FHB respectively. Bhaskar and Mahendrarkar (2007) has studied that the TBA values in carp visceral silage reached 1mg malonaldehyde/ kg oil by the end of 4 weeks. As per Ke etal (1976), above 10,µmol MDA-equiv per 1 kg fish will probably have rancid flavours.

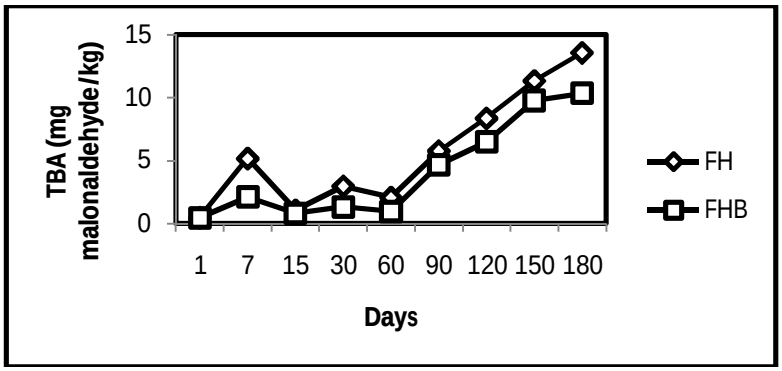

Figure 3 : Changes in TBA of ensiled dressing waste of fresh water fishes prepared with and without the addition of BHT stored at 28±2^{0}C

3.3.Microbiological quality of acid silages

3.3.1. *Total Viable Count (TVC):* Fresh visceral mince had a high bacterial load. Zahar et al (2002) has reported a total bacterial load as high as 4.5x10^5 for fresh sardine waste. Fig 4 depicts the total bacterial load in the FH and FHB till 60 days of storage. In the FH and FHB, there was a highly significant reduction in the total bacterial load on the 2nd day. On the 90th day of storage a 1 log reduction in TVC was recorded. Bhaskar and Mahendrarkar (2007) have also reported a significant reduction in total bacterial count of fish viscera till 4 weeks of storage. Reduction in total bacterial load soon after acidification of fish viscera and during storage of silage has also been observed in earlier studies by Mahendrakar *et al.* (1991). A reduction in TVC from 5 log cfu/gm to 4 log cfu/gm has been reported in marine fishery waste silage stored for 30 days by Ramasubburayan etal (2013).

According to Delgado etal (2008) a reduction of aerobic mesophiles and coliforms was observed in Spanish mackerel silage, due to low pH maintained during the process. The bacterial load in both FH and FHB remained below 3 log cfu/gm upto 6 months storage period. This could be due to the reduction in pH by the acid which induces a bacteriostatic action and also due to the prolonged ensilation period.

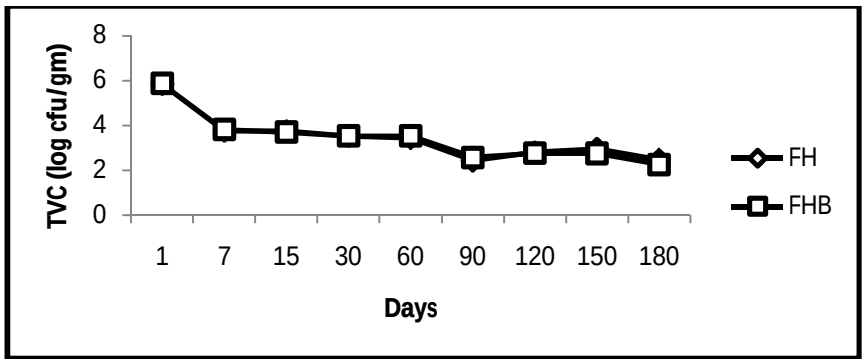

Figure 3 : Changes in TVC of ensiled dressing waste of fresh water fishes prepared with and without the addition of BHT stored at 28±2^{0}C

4. Conclusion

The processing of fresh water fishes results in considerable quantities of processing discards of which visceral waste is the major one. Ensilation using acid could be a viable alternative to convert these wastes into useful by products. During the acid ensiling process only slight variations occurred to the dry matter, the protein, lipid and mineral fractions which prove the applicability of the process. The balanced protein, fat and mineral content of the silages could be made use of in preparation of poultry, fish and livestock feed. The addition of BHT has slowed down the process of auto oxidation in acid silages prepared from carp fish viscera and the low pH has prevented the proliferation of microorganisms.

Acknowledgements

The authors are grateful to the Director of Directorate of Research on Women in Agriculture (DRWA), Bhubaneswar for granting permission to carry out the work and for providing the necessary facilities.

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