Biochemical changes during bacteriological ensilation of Tilapia

A. A. Zynudheen, K.G. Ramachandran Nair, Jose Joseph
Central Institute of Fisheries Technology [ICAR], Cochin - 682029, India
e-mail: zynu@rediffmail.com

Abstract

Fresh whole tilapia and eviscerated tilapia were fermented after cooking in water with jaggery. On cooling, the starter culture organism Lactobacillus plantarum *@ 5 % (w/v) was added. Changes in biochemical indices like pH, titrable acidity, non-protein nitrogen, alpha amino nitrogen, total volatile basic nitrogen and glucose was assessed during ensilation. The rate of liquefaction and drop in pH was initially fast and on third day the desired pH level of around 4 was attained. The maximum liquefaction was obtained by 13 days of ensilation. The degree of liquefaction was more in case of silage prepared from whole fish when compared to eviscerated fish while the level of total volatile nitrogen was less in eviscerated fish silage.*

Keywords: *Tilapia, Ensilage, Biological, Biochemical changes*

1. Introduction

Fish waste is a potential source of protein for animal nutrition. Industrial processing of fish for human consumption yields around 60 % by-products and only 40 % edible flesh (Raa and Gildberg, 1982). Fish residues can be advantageously upgraded by conversion into fish silage and this approach is more environment-friendly, safer, technologically more flexible and economically more efficient than manufacturing fish meal. Fish silage is an excellent protein product of high biological value for animal feeding which can be produced from, unutilized species such as by catches from marine fishing, fish processing waste and industrial residues, which may cause environmental, health and economic problems. Careful control of the degree of proteolysis and lipid oxidation is required to produce silages of high nutritional value. Fish silage may be defined as a liquid product, made from whole or parts of fish to which no material had been added other than an acid in which liquefaction is carried out by enzymes already present in the fish. The use of lactic acid bacteria [LAB] would have a natural inhibiting activity on the undesirable microflora in wastes, which would result in a natural preservation. Fermentation of fish-carbohydrate mixture by LAB offers scope for the development of a variety of products (Lee, 1989). Lactobacillus plantarum, *one of the highest acid producing bacteria (Steinkraus, 1983) had been used in the preparation of fermented fish by many workers (N. Neethiselvan* et al., *2002; Carl, 1953; Kreuzer, 1953 and James 1966)*

Sustain Fish (2006) B.M. Kurup & K. Ravindran (Eds.), School of Industrial Fisheries, Cochin University of Science & Technology, Cochin-682016, India

The degree of hydrolysis of the protein in fish silage is likely to result in a lower nutritive value for ruminant livestock. The small peptides and amino acids formed during ensilage are more readily available to the ruminal micro flora. (Hall *et al.*, 1985) reported that limited autolysis in fermented fish ensilages may be beneficial in restricting the release of free amino acids which are capable of reacting with lipid oxidation products resulting in the reduction of nutritional value of fermented minced meat based diets. Biological fish silage has been shown to have significantly better nutritional value than the acid fish silage (Kompaing *et al.*, 1980). Fermentation ensiling seems to be a more economical process, compared to acid ensiling as the latter requires expensive acids (Javed Ahmed and Mahendrakar 1996). The present work is carried out to compare the biochemical changes during ensilation of whole and eviscerated tilapia by using lactobacillus.

2. Materials and methods

2.1 Preparation of silage

Tilapia (*Oreochromis mossambicus*) in very fresh condition was obtained from the local fish market and brought to the laboratory. It was washed thoroughly and divided into two lots. One lot (Lot A) was minced as such and used for the preparation of silage and the other lot (Lot B) was minced after removing viscera and gills. Both lots were cooked for 30 min with 30 % by weight of water and 20 % jaggery (W/W). It was cooled and a starter culture of Lactobacillus plantarum was added @ 5 % (W/V). The silage was stored in glass bottles at ambient temperature and stirred daily during the period of experiment. Periodic samples were drawn and analysed for various biochemical parameters to assess the changes during fermentation. The jaggery used for the study was obtained from the local market and the bacterial strain used (*Lactobacillus plantarum*) was obtained from MTCC, Institute of Microbial Technology, Chandigarh.

2.2 Biochemical analysis

Moisture, crude protein, crude fat and ash were determined by standard procedures (AOAC, 2000). Total Volatile Nitrogen (TVN) was determined by the micro diffusion method of Conway (1962). For the determination of pH, direct measurement was carried out using a Cyberscan 510 pH meter. The titrable acidity of the sample was measured by titrating aliquot against standard NaOH using phenolphthalein as indicator and expressed as percent lactic acid. The non-protein nitrogen (NPN) was determined by the micro Kjeldahl method of trichloroacetic acid (10 %) extract. Glucose level was estimated by the method described by (Morales *et al.*, 1973) with anthrone as coloring reagent. The tyrosine was estimated with Folin-ciocalteau reagent by the method of Anson (1938).

Table 1. Proximate composition of whole and eviscerated fish **UNITS++

	Whole fish	Eviscerated fish
Moisture	74.4	72.8
Fat	2.68	2.35
Total nitrogen	2.50	2.81
Crude protein	15.62	17.56
Ash	3.85	4.10
Non protein nitrogen	210	231
TVBN	11	8
Tyrosine	14.1	19.92

3. Results and discussion

Table 2. Biochemical changes during biological ensilation of eviscerated Tilapia

Days of fermentation	PH	Non protein nitrogen	Total volatile basic nitrogen (mg %)	Degree of hydrolysis %
0	6.9	371	10	22.1
1	5.9	588	14	35.0
3	4.3	714	32	42.5
5	4.4	714	52	42.5
7	4.5	735	63	43.8
9	4.5	760	60	45.2
12	4.4	777	-	46.3
14	4.9	763	64	45.4

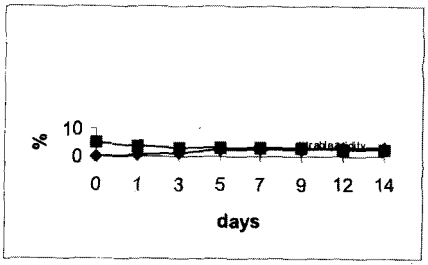

Fig. 1 Changes in titrable acidity and glucose in eviscerated Tilapia during ensilation

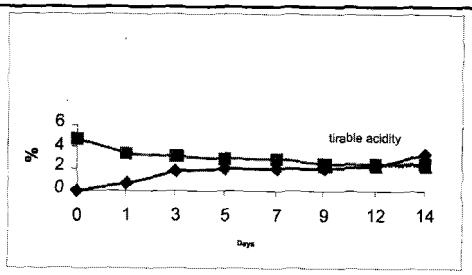

Fig. 2 Change in titrable acidity and and glucose in whole Tilapia during ensilation

The chemical changes in the eviscerated tilapia samples during ensilation are given in Table 2 and Fig. 1. The pH of the samples reached 4.3 by third day indicating sufficient fermentation and acid production. During the same period the titrable acidity was 1.4 % lactic acid. The degree of hydrolysis of the samples during the same

period was 42 %, which has increased to 46 % by the end of fermentation. The total volatile basic nitrogen content has increased from the initial 10 to 52 mg % by 5th day of ensilation and further increase to 64 mg % has been observed by the end of fermentation, which indicates that the breakdown of protein by microbes has intensified after 5 days. The titrable acidity of the sample has increased from 0.65 % to 3.2 % lactic acid by 14th day and a corresponding decrease in the glucose levels was noticed which reduced to 2.54 % by the same period. The tyrosine level, which also indicates the degree of protein hydrolysis, has shown an increasing trend (Fig. 3).

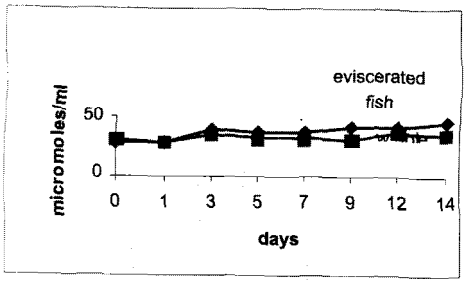

Fig. 3 Changes in tyrosine levels during ensilation

The chemical changes during ensilation of whole tilapia are given in Table 3 and Fig 2. The initial pH of 6.7 reduced to the desired level of 4.3 on 3rd day of fermentation and a marginal increase was noticed during the remaining days. This could be due to the production of various volatile amines. The TVBN value showed a steady increase from an initial value of 14 to 64 mg 100 g^{-1} during the course of fermentation. (Neethiselvan *et al.*, 2002) have observed a TVBN range of 28 - 98 mg % for lactic acid fermented fish silage. The degree of hydrolysis was 43 % by one day fermentation and has increased to 62 % by the end of ensilation. The titrable acidity level has increased with a corresponding decrease in glucose level showing the utilization of glucose by the bacteria.

Table 3. Biochemical changes during biological ensilation of whole Tilapia

Days of fermentation	pH	Non protein nitrogen	Total volatile basic nitrogen (mg %)	Degree of hydrolysis %
0	6.74	380	14	28.5
1	5.64	574	32	43.0
3	4.23	714	41	53.5
5	4.4	756	55	56.6
7	4.4	672	52	50.3
9	4.6	637	60	47.7
12	5.3	784	63	58.7
14	5.3	833	74	62.4

Days of fermentation	Non protein nitrogen Eviscerated	Whole	Degree of hydrolysis % Eviscerated	Whole
1	588	574	35.0	43.0
3	714	714	42.5	53.5
5	714	756	42.5	56.6
7	735	672	43.8	50.3
9	760	637	45.2	47.7
12	777	784	46.3	58.7
14	763	833	45.4	62.4

4. Conclusion

A higher level of hydrolysis during ensilation noticed in the whole fish samples as indicated by the increase in pH. A corresponding increase in the degree of hydrolysis is noticed during the period of ensilation in whole fish samples. Since cooking has already destroyed the enzymes present in both the samples, an increased level of hydrolysis in whole sample could be due to the presence of soft tissues of viscera and gills, which have acted as an easy substrate for the growth of the microbes.

Acknowledgement

The authors are thankful to Dr. K. Devadasan, Director, Central Institute of Fisheries Technology, Cochin for according permission to present the paper.

References

Anson, M.L., 1938. The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. J. Gen. Physiol. 22 79-89.

AOAC, 2000. *Official Methods of Analysis* (17th edn.) Association of Official Analytical Chemists, Gaithersburg. 938.08, 940.25, 948.15.

Carl. L.K., 1953. *Archiv. fur Fischereiuissen Schaft 4, 57*

Conway, E.J., 1962. Determination of Volatile Amines, *Microdiffusion Analysis and Volumetric Error* 5 th edn, Parch Croskey, Sockwood and Son Limited, London. 467.

Hall, G.M., Keeble, D., Ledward D. A., Lawrie, R.A., 1985. J. Food Technol. *20, 1*

James, M.A., 1966. Fish. Technol. 3, 38.

Javed Ahmed., Mahendrakar, N.S., 1996. Autolysis and rancidity development ion tropical fresh water fish viscera during fermentation. Bio Resource Technology 58, 247-250.

Kompaiang, I.P., Arifudin, R., Raa, J., 1980. Nutritional value of ensilaged by-catch fish from Indonesian shrimp trawlers. In: Connell, J.J. (Ed.), Advances in Fish Science and Technology, *Fishing News Books, Surrey. 3 - 9.*

Kreuzer, R., 1953. Archiv. fur Fischereiuissen Schaft 4, 47.

Lee, 1989. A review of processing, post harvest technology, In: Reilly, P.J.A., Perry, R.W.H., Barile, L.E. (Eds.), Preservation and Quality of Fish in South East Asia P.1, *International Foundation for Science, Sweden.*

Morales, M.A., Jabbagy, A.J., Terenizi, H. R., Neurospora News Lett. 1973. 20-24.

Neethiselvan, N., Indira Jasmine, G., Jeyasekeran, G., 2002. Lactic acid fermentation of minced meat of leognatus splendens (Cuvier, 1829) using different bacterial sources. Fish Technol. 39 (2), 129-136.

Raa, J., Gildberg, A., 1982. Fish silage a review: CRC Critical Reviews. Food Science Nutrition 16.383 - 419.

Steinkraus, K.H. (Ed.), 1983. Hand Book in Indigenous Fermented Foods. *Marcel Dekker, Inc, New York. 671.*