ENSILED PRODUCT FROM FISH BY MICROBIAL FERMENTATION

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Fish ensilage for animal feed stuff was prepared from Jew fish *(Pseudosciaena spp.)* and Silver bellies *(Leiognathus spp.)* by fermentation with pure culture of *Lactobacillus plantarum* NCIB 6105. The precooked ensiled product gave better product of fish silage (high content of lactic acid, about 5 per cent). Protiein Nitrogen content ranged between 1.76 to 1.94 per cent. During storage for one year, the Protein Nitrogen loss was not significant. The material can be used as a supplemental animal ration.

INTRODUCTION :

The possibility of preserving surplus fish and fish offal in a liquid form for animal feed has been suggested by many workers (Petersen 1951; Hanson *et al.*, 1951; Freeman *et al.*, 1956; and Sperling *et al.*, 1959). Ensilage or liquid preservation was achieved by directly using mineral acids or by producing the required acid indirectly making use of microorganisms. The ensiled product prepared by fermentation is considered better as the rancid odour is not developed (Nilsson *et al.*, 1961). It is generally in the form of a slurry and can be transported easily in bulk and used as such in supplemental animal rations.

The practical applicability of this novel method had been demonstrated conclusively by Kreuzer (1954). Nilsson *et al.* (1961) studied the effect of malt enzymes with starch substrate for the production of ensiled product. The nutritional value of ensiled product from fresh water species has been reported by Krishnaswami *et al.*, (1965). However no concerted effort has been made in India to examine the possibility of converting miscellaneous fish into ensiled product for animal feed. With the rapid development of fishing operations by mechanised boats the amount of miscellaneous fish caught along with prawns has been increasing. This poses the problem of better and more effective utilization of the trash fish. So the work was carried out in this connection on the amenability of conversion of these fishes into ensiled product.

Materials And Methods

Jew fish *(Pseudosciaena spp.)*and Silver bellies *(Leiognathus spp.)* were obtained fresh from fishing vessels operating at Cochin. The whole fresh fish after cleaning was minced well, mixed with 10 per cent molasses and made into a slurry with 30% by weight of water. This material was kept in bitumen-coated wooden barrels. A pure culture (18 to 22 hours old) of *Lactobacillus plantarum* NCIB 6105, an active homofermentor producing lactic acid from molasses was added to the homogenised slurry and stirred well. The product was allowed to ferment at room temperature.

The relative effect of precooking the fish material before subjecting it to the action of *L. plantarum* was carried out. This slurry was cooked for 20 minutes and cooled to room temperature. This was allowed to ferment with the culture. By this precooking procedure it was possible to prepare silage within 72 hours, when the pH reached 4.4 and even lesser in some cases, while uncooked ensiled product showed a pH of 5.0 only during that period. Experiments were also carried out by adding each time the actively fermenting silage (between 24 hours to 48 hours old) instead of the pure culture, at a level of 10 per cent to the homogenized material in successive lots.

Dry matter content of fish silage was estimated by drying at 105°C (± 1°C) for 16 hours in air oven. Ash was determined by ignition of dried samples in an oven for 18 hours at 540°C. The Total Nitrogen (TN) and Water Soluble Nitrogen (WSN) were determined by Kjeldahl method, the latter after extraction of the sample with distilled water. For the determination of non-protein Nitrogen (NPN), the water soluble extracts were adjusted to a final concentration of 10% (w/v) with trichloro acetic acid, chilled at 2°C for 24 hours and filtered. The filtrate was used for the determination of N. P. N. Steam Volatile Nitrogen (SVN) was determined from NPN extract in the presence of borate buffer. ∝-amino Nitrogen was estimated by Pope and Stevens method (1938). Lactic acid was estimated by A.O.A.C. method (1960)- The vitamins were determined by microbiological methods (Kavanagh, 1963).

Results And Disscussion

The results obtained with fish ensilage prepared from Jew fish and Silver bellies indicated that they were good source of proteins, minerals and vitamins. The mineral matter was of the order 2.2 to 2.5% (Table I). The data also showed that there was no apparent difference in the levels of the above constituents between silage prepared from cooked meat and that prepared from fresh fish. However, WSN, NPN and ∝- amino Nitrogen were higher in uncooked ensiled product while cooked one showed comparatively low values (Table II).

It was noticed that there was some amount of degradation of protein particularly in the initial stages of fermentation until enough lactic acid was produced in the system. The degree of degradation was higher in the uncooked product than in the cooked product. The amount of SVN in the former was about 11% of TN in the course of first two days while in the latter it was about 5%. Further loss in the following days of fermentation was less than 1% in the cooked product while in the case of uncooked it was over 3% TN. The higher SVN in the uncooked product might probably have been due to the mixed microbial development in the initial stages of fermentation. The precooking procedure allowed the lactic acid bacteria to grow rapidly with the result enough lactic acid bacteria was produced in the

minimum possible time to preserve the protein. The good quality of fish ensilage was indicated by the high content of lactic acid and low pH. The lactic acid content was higher in the eooked ensiled product. The pH reached 4.4 within three days while the uncooked ensiled one showed pH 5.0 during that time. Nilsson *et al.* (1961) considered that if the Ammonia Nitrogen content exceeds more than 20% of TN the silage product could not be utilized as animal feed.

The product was slightly brownish in colour and gave fermented odour. Storage studies for one year indicated that the material was in good condition. (Table IV). The nitrogen loss was not significant and the pH was not altered. Bacteriological studies indicated that Salmonella and other pathogenic anaerobes were not present in the product. The vitamin content of the ensiled product were estimated by the microbiological methods (Table V). Nicotinic acid content showed between 0.69 to 0.75 mg%. The other vitamins, thiamine, riboflavin, pantothenic acid and vitamin B_{12} values were 18.20, 19.14, 17.26, 17,94, 12.44, 11.92 μg% and 29.23, 23.46 mμg% respectively. The results indicate that fish ensilage with its high nutritive values can be used to suppliment poultry and cattle feeds.

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TABLE I DRY MATTER AND LACTIC ACID CONTENT OF COOKED AND UNCOOKED ENSILED PRODUCT

Sample	Dry matter %	Ash content %	Lactic acid %	pH
JEW FISH (Pseudosciaena spp.)				
Cooked ensiled	22.65	2.47	4.98	4 4
Uncooked ensiled	22.96	2.38	3.13	5.0
SILVER BELLIES (Leiognathus spp.)				
Cooked ensiled	23.14	2.32	5.02	4.4
Uncooked ensiled	23.08	2.19	3.47	5.0

TABLE II COMPARISON OF NITROGEN DISTRIBUTION IN COOKED AND UNCOOKED FISH ENSILAGE

(Sampled after 4 weeks)

Sample	Total Nitrogen mg %	Water soluble Nitrogen mg %	Non-protein Nitrogen mg %	∝-amino Nitrogen mg %	Steam Volatile Nitrogen % of TN
JEW FISH					
Cooked ensiled	1940	420.20	180.30	62.29	7.87
Uncooked ensiled	1920	1020.00	460.10	170.41	18.63
SILVER BELLIES					
Cooke ensiled	1760	540 10	201.30	79.73	8.26
Uncooked ensiled	1790	990.40	390.60	129.20	17.68

TABLE III EFFECT OF PRECOOKING THE FISH ON THE REDUCTION OF STEAM VOLATILE NITROGEN DURING FERMENTATION

Number of days of fermentation	JEW FISH*		SILVER BELLIES*	
	Cooked Ensiled	Uncooked Ensiled	Cooked Ensiled	Uncooked Ensiled
Initial	3.12	3.53	2.97	3.24
1.	6.49	10.34	5.14	11.27
2.	7.97	14.12	7.08	15.43
3.	8.13	16.44	7.36	16.11
4.	8.24	17.27	7.72	16.43
6.	8.29	17.52	7.81	16.72
8.	8.35	17.57	7.88	16.87

* SVN percentage of of TN

TABLE IV STORAGE STUDIES OF COOKED FISH ENSILAGE

	One month		3 months		6 months		9 months		12 months	
	J. f*	S. b*	J. f	S. b	J. f	S. b	J. f	S. b	J. f	S. b.
TN mg%	1952	1744	1948	1723	1956	1729	1949	1742	1945	1728
WNS mg%	429.2	516.9	435.3	529.3	431.8	521.4	436.4	532.2	440.3	541.4
SVN % of TN	7.68	8.14	8.23	8.64	8.22	8.92	8.37	8.81	8.46	9.02

* Jf: = Jew fish

* Sb: = Silver bellies

TABLE V COMPOSITION OF VITAMINS IN COOKED PRODUCT

(Sampled after 4 weeks)

Vitamin	Jew fish ensilage	Silver bellies ensilage
Thiamine μg%	18.20	19.14
Riboflavin μg%	17.26	17.94
Panthothenic acid μg%	12.44	11.92
Nicotinic acid mg%	0.69	0.75
Vitamtn B_{12} m μg%	29.23	23.46

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