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Ensilation of Shrimp Waste by *Lactobacillus fermentum*

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Ensilation of shrimp waste was carried out with of *Lactobacillus fermentum* at room temperature (30±1°C). Formic acid (0.4%) was used to adjust the pH to 5.8 at the start of the experiment. The pH of the medium fell sharply from 5.8 at the beginning to around 4.5 by 2nd day and was more or less stable during the remaining part of ensilation. The protein content of the residue showed a decrease with a proportional increase in chitin content. The ash and crude fat content registered a decrease. The data on biochemical composition of the slurry revealed that the protein content, α amino nitrogen and NPN increased substantially during fermentation. The TVBN value remained constant at about 0.07% on dry weight basis during the period of observation. Protein solubilization of the order of 60% obtained during the present study was independent of initial bacterial density and is within the range of acceptability indicating that *L. fermentum* is an ideal candidate for use in biofermentation of shrimp waste.

Key words: ***L. fermentum,*** **biofermentation, shrimp biowaste.**

Shrimp processing industries in India turn out more than one lakh tonnes of head and shell as industrial waste and is the single largest industrial fish waste in the country causing diverse environmental problems. Shrimp waste can be advantageously upgraded by conversion into ensilage and this approach is ecofriendly, safe, technologically flexible and economically viable. Ensilation can be conducted either by the direct addition of mineral or organic acids (acid silage) or by fermentation with lactate bacteria (biological silage). Various strains of *Lactobacillus* have been used in the production of fish silage which includes *Lactobacillus plantarum* (Viete & Bello, 1992; Ottati & Bello, 1992 a,b; Fagbenro & Jauncey, 1993, 1994,1995; Lassen, 1995b), yoghurt bacteria like *L.bulgaricus* and *Streptococcus thermophilus* (Areche *et.al.*,1992; Yoon *et. al.*, 1997) *Lactobacillus delbruckii* spp. *bulgaricus* and *Streptococcus salivarius* spp. *thermophilus* (Martinez-Valdivieso *et.al.*, 1996). Fermentation of fish waste in the presence of selected strains of *Lactobacillus* results in medium conditioning with lactate and various bacterial proteases. Glucose breakdown by the Lactobacilli results in lactate production in the medium with a concomitant reduction in pH ideal for ensilation through suppression of spoilage micro flora. This approach produces a liquor fraction rich in proteins, minerals and astaxanthin and a solid chitin fraction. Deproteination of the biowaste by liquefaction of proteins occurs mainly by the proteases produced by the added Lactobacilli, by intestinal micro flora of the shrimp or by the in-situ proteases of the biowaste. The liquor fraction could be utilized either as a protein mineral supplement for anthropogenic use or as an ingredient of prawn/animal feed. Improvement of lactate fermentation is generally accomplished by testing one variable at a time. Although, a wealth of information is available on the biological ensilation of fish waste, reports on the fermentation of shrimp waste are scanty. The present study was therefore taken up to assess the efficiency of *L.*

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fermentum at different cell densities to solubilise the proteins from shrimp waste (ensilation).

Materials and Methods

L. fermentum used in this study was obtained from MTCC (Culture No.MTCC B - 903), Institute of Microbial Technology, Chandigarh, India. It was repeatedly sub-cultured in nutrient broth before use to activate the cells as per supplier's instructions. The culture was finally grown in MRS broth and adjusted to known cell densities by plating on MRS agar.

Shrimp processing waste procured from Premier Sea Foods, Cochin, was brought to the laboratory in fly proof plastic containers and gently chopped in a mixer/grinder. pH of the slurry was 8.2. Jaggery obtained from the local market was the carbohydrate source and was finely crushed before addition. The starter was prepared by inoculating a chopped shrimp waste/jaggery/water mixture (50:15:15, w/w/w) with 20% (w/w) of 18-24h old culture of *L. fermentum* in MRS broth adjusted to 2 cell densities viz., 10^6 cfu/ml and 10^8 cfu/ml of *L. fermentum*. Static fermentation of 500 g of the fermenting mixture was conducted at room temperature ($30\pm1^{o}C$) in 1L glass beakers with plastic covers. pH of the fermenting mixture was adjusted to 5.8 with 0.4% formic acid. Six replicates of the mixture were prepared and one among them was sampled on 0, 2, 4, 6, 8 and 12th day. The fermented slurry was filtered through a coarse cloth to separate the liquor from the solid materials containing chitin. The solid fraction was washed with distilled water, drained and analysed for moisture. The oven dried, moisture free samples were used for the determination of protein, chitin, ash and crude fat content. The liquor fraction was analysed for moisture, protein, TVBN, NPN, and α amino nitrogen.

Moisture, protein, chitin, ash and crude fat were determined by standard procedures (AOAC, 1998). pH of the slurry was determined using a combined glass calomel electrode. Non- protein nitrogen (NPN) was determined by the micro Kjeldahl method in trichloroacetic acid (7%) extracts of the liquor. Total volatile basic nitrogen was determined by the micro diffusion method of Conway (1947). All the determinations were made in duplicate.

Results & Discussion

The study was carried out with 2 cell densities viz., 10^6 cfu/ml and 10^8 cfu/ml of *L. fermentum* with an initial pH adjustment to 5.8 before inoculation. The biochemical composition of fermented residue and slurry obtained following ensilation with 24h culture ($3.9X10^8$ cfu/ml) of *L. fermentum* is illustrated in Table 1. The pH of the medium dropped from 5.8 at the start of the experiment to 4.42 by the 2nd day and gradually declined to 4.15 by 12th day. The protein content of the residue decreased from 44.57% to 32.20%, while the chitin content showed an increasing trend. A reduction in crude fat content (5.97% to 2.28%) and ash content (21.39% to 15.69%) was also observed.

Data on biochemical composition of the fermented slurry revealed that the protein content, α amino nitrogen and NPN increased substantially during the fermentation process. The protein level increased from 8.45% at the beginning to 10.82% at the end of 12th day. A concomitant elevation in α amino nitrogen from 0.068% to 0.466% and NPN from 0.473 gm% to 1.223% was also recorded.

. A fall in bacterial density to $4.3X10^6$ cfu/ml in the initial inoculum resulted in more or less similar variations in pH, protein, chitin, crude fat and ash content (Table 2). The protein concentration of the slurry was elevated from 8.02% to 9.85%. The α amino nitrogen and NPN levels also registered a similar increasing trend. Tables 3a & 3b outlines the results of the total protein nitrogen content of the fermented residue and slurry at two initial bacterial densities. It is evident that the solubilization

Table 1. Changes in biochemical composition of fermented residue and slurry during 12 d of fermentation at inoculum level of 3.9 X 10^8 cfu/ml.

Days	O	2	4	6	8	12
A. FERMENTED RESIDUE						
pH	5.80	4.42	4.30	4.29	4.18	4.15
Protein (%, Dry wt.)	44.57	35.76	35.08	33.87	32.80	32.20
TN (%, Dry Weight)	7.13	5.72	5.61	5.42	5.24	5.15
Chitin (%, Dry wt.)	24.77	30.71	34.24	39.63	40.69	44.64
Moisture (%)	71.01	72.87	71.58	72.42	72.38	73.30
Crude Fat (%, Dry wt.)	5.97	5.40	4.12	3.92	2.79	2.28
Ash (%, Dry wt.)	21.39	21.37	19.99	17.98	16.84	15.69
TVBN (%, Dry wt.)	0.08*	Nil	Nil	Nil	Nil	Nil
α amino nitrogen (%, Dry wt)	0.068*	Nil-	Nil	Nil	Nil	Nil
NPN (%, Dry wt.)	0.473*	Nil	Nil	Nil	Nil	Nil
B. FERMENTED SLURRY						
Protein (%, Dry wt.)	Nil	8.45	9.00	9.89	10.44	10.82
TN (%, Dry Weight)	Nil	1.35	1.44	1.58	1.67	1.73
Moisture (%)	Nil	83.30	82.97	82.70	83.02	83.62
TVBN (%, Dry wt.)	Nil	0.08	0.08	0.08	0.08	0.08
α amino nitrogen (%, Dry wt)	Nil	0.153	0.187	0.240	0.344	0.466
NPN (%, Dry wt.)	Nil	0.813	0.948	1.050	1.122	1.223

* Indicate values at the start of ensilation

of protein nitrogen to the slurry was more or less identical at the end of ensilation in both cases and was around 61%.

A reduction in pH from 6.8 to 4.3 during fermentation for a week was reported by Lassen (1995a). Using yogurt bacteria at 40°C, enough acidity could be produced in 48h (Areche *et.al.*, 1992). Further, the time taken in fermented silage to produce a stable and desired pH is usually longer than for acid silages. A pH below 5.0 after 48 h of ensilation is highly advantageous (Yeoh, 1979) and the present results conforming to the above observation.

Deproteination of the biowaste by liquefaction of proteins occurs mainly by the proteases produced by the added Lactobacilli, by intestinal micro flora of the shrimp or by the in-situ proteases of the biowaste. The autolytic activity, which occurs during ensilation, leads to an increase in the concentration of amino acids, amines and peptides. Up to 90% of the organic nitrogen becomes solubilised in acid preserved fish silages (Haard *et.al.*, 1985), whereas, ensilation by biological method yields solubilisation values around 60-70% (Hassan and Heath, 1986; Lindgren & Pleje, 1983). The observed protein solubilization is within the range of acceptability and is indicative that *L. fermentum* can be used in the fermentation of shrimp waste.

Feeding trials performed on several species of monogastric animals show that it might be advantageous to have some predigested food in the diet; but there is a limit over which the animals would have difficulty in using the absorbed protein for synthetic purposes (Espi *et.al.*, 1992). The degree of hydrolysis of proteins in silage is likely to result in lower nutritive value for livestock. The lower degree of hydrolysis in biological silage when compared to acid silages as observed in this study (Table 3), can therefore be regarded as advantageous from the nutritional point of view of animals (Dapkevicius *et.al.*, 1998).

Table 2. Changes in biochemical composition of fermented residue and slurry during 12 d fermentation at inoculum level of 4.3 X 10^6 cfu/ml.)

Days	O	2	4	6	8	12
A. FERMENTED RESIDUE						
pH	5.80	4.75	4.47	4.35	4.22	4.21
Protein (%, Dry wt.)	42.19	32.98	30.84	29.64	28.60	28.26
TN (%, Dry Weight)	6.75	5.28	4.93	4.74	4.58	4.52
Chitin (%, Dry wt.)	25.07	37.95	40.50	42.41	45.35	46.24
Moisture (%)	77.39	81.29	81.16	81.04	80.84	79.65
Crude Fat (%, Dry wt.)	6.62	6.52	6.42	6.01	5.85	5.35
Ash (%, Dry wt.)	25.31	18.92	18.37	17.98	17.48	16.16
TVBN (%, Dry wt.)	0.07*	Nil —	Nil —	Nil —	Nil	Nil
α amino nitrogen (%, Dry wt)	0.062*	Nil	Nil	Nil	Nil	Nil
NPN (%, Dry wt.)	0.391*	Nil	Nil	Nil	Nil	Nil
B. FERMENTED SLURRY						
Protein (%, Dry wt.)	Nil	8.02	8.91	9.35	9.85	9.85
TN (%, Dry Weight)	Nil	1.28	1.43	1.50	1.58	1.58
Moisture (%)	Nil	83.47	82.24	83.28	80.06	80.36
TVBN (%, Dry wt.)	Nil	0.07	0.07	0.07	0.07	0.07
α amino nitrogen (%, Dry wt)	Nil	0.093	0.116	0.173	0.219	0.223
NPN (%, Dry wt.)	Nil	0.575	0.685	0.737	0.804	0.813

* Indicate values at the start of ensilation

Table 3. Effect of initial cell density of *Lactobacillus fermentum* on the properties of ensilage during 12 days of fermentation.

Days	0	2	4	6	8	12
A - Initial Count as 3.9X10^8 cfu/ml						
Wt. of Residue (gm)	Nil	99.40	99.70	99.60	99.20	99.40
Residue (Protein N)	Nil	5.14	5.05	4.87	4.72	4.65
Volume of Slurry (ml)	Nil	399.90	398.70	399.20	399.70	399.10
Slurry (Protein N)	Nil	5.54	5.91	6.48	6.85	7.09
Total Nitrogen	11.76	10 .68	10.96	11.35	11.57	11.72
% Solubilization of N to slurry	Nil	51.80	53.90	57.10	59.20	60.50
B - Initial Count as 4.3.X10^6 cfu/ml						
Wt. of Residue (gm)	Nil	99.30	99.80	99.40	99.50	99.70
Residue (Protein N)	Nil	4.75	4.44	4.26	4.12	4.07
Volume of Slurry (ml)	Nil	399.70	399.60	398.40	399.50	398.20
Slurry (Protein N)	Nil	5.24	5.86	6.15	6.32	6.48
Total Nitrogen	11.13	9.99	10.90	10.41	10.44	10.65
% Solubilization of N to slurry	Nil	52.50	56.80	59.07	60.50	60.80

Shrimp processing waste upon fermentation releases nutrients like carotenoid pigments and n-3 unsaturated fatty acids (Guillou *et.al.*, 1995), which are otherwise difficult to extract even by organic solvents. Almost 70% of the crude fat content of the residue was solubilised following fermentation of biowaste with *L. fermentum*. The observed reduction in crude fat content of the residue indicates that ensilation by *L. fermentum* can effectively release this component into the liquor.

Analysis of biochemical composition of the fermented slurry showed that it is rich in protein and alpha amino nitrogen but low in volatile bases (Tables 1 & 2). TVBN measures the amount of volatile bases formed from the solubilised nitrogen. A constant value of 0.08% and 0.07% on dry weight basis respectively for the cultures recorded during the present study suggests that no spoilage had occurred during the entire period of biowaste fermentation. Since, it leads to much lower formation of TVBN, biological ensilation can be considered as advantageous when compared to acid ensilation (Dapkevicius *et.al.*, 1998). The present results are in confirmation with the above observation.

The acid/alkali preservation of shrimp waste renders the protein component useless, ecologically aggressive and economically non-viable compared to biological ensilation employing *Lactobacillus* sp. (Hall *et. al.*, 1997). The present study indicates that ensilation of shrimp waste by biological methods using *L. fermentum* is a promising technique and that it is independent of bacterial density in the initial inoculum. Further, the combined effect of *Lactobacillus* inoculum and formic acid is an inexpensive and ecofriendly method for fermentation of shrimp waste. Concentration of the fermented slurry or its co-drying with other feed ingredients can help utilization of the protein rich liquor in a cost effective way.

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