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Full Length Research Paper

Biotransformation of the fish waste by fermentation

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Fish waste (viscera, backbones, heads and tails) was recovered fish merchants and ground. 50 kg of the ground fish was mixed with 10 kg of molasses and incubated in 200 kg barrels in closed plastic in a room where the ambient temperature varies between 30 and 33°C. Stumps of lactic bacteria and yeasts were used to inoculate the mixture. There was a decrease of pH from the third day which stabilized at 4.2 and a remarkable change of the number of bacteria between the initial and the finished products. The total aerobic mesophilic flora increased from 2.4 x 10^6 to 4.58 x 10^7 cfu and yeasts from 1.30 x 10^3 to 1.59 x 10^5. As for the anaerobic sulfur-reducing (ASR) bacteria, their number reduced from 1.65 x 10^3 to 2 x 10^2, the fecal coliforms were eliminated completely even though their initial number was 1.20 x $10^{3.}$, Also, total coliforms went from 1.9 x 10^4 to zero. At the end this fermentation, the fishy smell was concealed by products of fermentation. There was total absence of nematodes and the useful products (proteins, lipids, mineral elements) can be exploited in the fertilization of soil or used as ingredient to balance the food ration of the animals especially during the periods of drought. The obtained results confirm the efficiency of our isolated inoculum and a fast, simple and less expensive fermentation process.

Key words: Biotransformation, fermentation, nutrition, fish waste, lactic bacteria, yeasts.

INTRODUCTION

Fermented foods were made several hundreds years ago by the use of lactic bacteria. Most of these fermentations were natural or accelerated by the addition of part of the product fermented previously. The acidic ensilage production or fermented is not a recent discovery. In 1920, Virtanen used a sulphuric and hydrochloric acid mixture for the preservation of green fodder. The production of ensilage of the fish waste to an industrial scale in Denmark began in 1948 (Peterson, 1951). A Magazine was published by Hansen (1959) concerning the quality of raw material, packing and experimentations in animal feed. Tatterson and Windsor (1974) described the methods and facilities that they used for the production of tons of ensilage. Such industries can be found in Norway, in Poland, in Argentina, Costa Rica and Sri Lanka.

The fish business creates very quantities of fish waste every day that is collected and dumped in waste sites which sometimes are not controlled. This can sometimes cause health and environmental problems including contamination of the water tables and sickening odours. However, the enormous quantities of fish waste can be used as a source of proteins for all sorts of animals. Previously, the production of fish flour and oil were the only technique adopted for obtaining fish protein. This is based on drying which requires very expensive energy consumption. This has led to research in treatment of enormous quantities of fish waste by biotechnical means by controlled fermentation. This does not require a big investment and by using acidifying microorganisms and molasses (source of carbon), the finished product is without microbial contamination.

This present is aimed at testing the efficiency of the stumps of isolated lactic bacteria and yeasts on the fermentation of fish waste. These same organisms have been used to ferment waste of algae. Our main goal is to obtain complimentary ingredients for the formation of a balanced food ration for animal consumption.

MATERIAL AND METHODS

Isolation of lactic bacteria

For the isolation and purification of the stumps of lactic bacteria, different biotopes were grown on solid MRS (Man, Rogosa and

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Table I. Selection of bacteria acidifying lactic cultures were grown in MRS and incubated at 30°C for 24 h.

Bacteria	Initial pH_i	Final pH_f	Final acidity (%)	Catalase	Gram
BL1	5.70	4.15	0.94	-	+
BL2	5.52	4.05	0.90	-	+
BL3	5.71	3.82	0.96	-	+
BL4	5.70	4.02	0.92	-	+
BL5	5.58	4.35	0.89	-	+
BL6	5.73	3.90	0.93	-	+
BL7	5.63	4.20	0.88	-	+
BL8	5.49	3.88	0.95	-	+
BL9	5.73	4.12	0.93	-	+
BL10	5.75	4.30	0.89	-	+
BL11	5.54	3.72	1.04	-	+
BL12	5.60	4.02	0.95	-	+

Sharpe) media. The cultures of the lactic bacteria were incubated at 30°C for 24 h. The purification involves four successive isolation and growth on solid MRS media.

Antibacterial activity of the selected lactic bacteria

There are several methods for the detection of antibacterial activity of lactic acid producers. Most are based on bacteriocin, a protein secreted by lactic acid bacteria (Kashket, 1987). Bacteriocin detection is based on its inhibitory activity against microorganisms.

Isolation and characterization of the yeasts

The yeast was isolated from sugar-based juice. Their selection is based on their fermentative activity and growth.

Collection of fish waste

Fish waste was collected from fish traders in plastic bags and brought to the laboratory for physical, chemical and microbiological analyses. The sardines represent 50% of treated waste

Fermentation of the fish waste in closed barrels

Anaerobic fermentation of fish is most advisable practice because several studies have shown that fermentation in closed and agitated medium leads to a reduction of the length of fermentation to three days (Laraki, 1999). However, work done in our laboratory has been achieved in half barrels to avoid all obstruction due to a clearing of gas.

The fish waste was used at the rate of about 50 kg/experiment. After drainage, 50 kg of waste are treated in mixture waste and molasses (20%) full in closed barrels. Every barrel was been inoculated by the most effective isolates.

Biochemical assays

The pH of the fermentation was monitored with a pH meter. Acidity is determined by titration against sodium carbonate. Dry matter and ash were determined according to AOAC (1999). The lipid content was been determined after digestion of the sample with 4 N hydrochloric acid followed by soxhlet extraction according to AOAC (1999). The total nitrogen was determined according to Kjeldahl's method (AOAC, 1999). The protein content was estimated by multiplying the total nitrogen by 6.25. Reducing sugars and sucrose after inversion were measured according to the method of the Luff school (French Industrial Standards Authority, 1985). The total mineral matter was also determined.

Microbiological analyses

The initial microbial flora at the beginning of fermentation depends on the fish. The total aerobic mesophilic flora was enumerated on plat count Agar, after incubation at 30°C for 2 to 3 days. The count of salmonellas was done on Salmonella-Schigella media, after incubation at 37°C for 24 h. The identification of the isolates was achieved by the API 20 E gallery (Bio Mérieux). The spores of clostridium were counted on reinforced clostridium agar. The sample was heated to 80°C for 10 min to activate the spores and then incubated at 44°C for 24 h. The enumeration of the staphylococci was done on Chapman media after incubation at 37°C for 24 h. Yeasts were counted on potato dextrose agar, acidified to pH 3.5 after incubation at 30°C for 72 days. Coliforms were enumerated and isolated on MacConkey media after incubation at 40°C for 24 h. Enumeration of the streptococci was on sodium azide media at 30°C for 24 h. The lactic bacteria were counted on middle MRS media at 30°C for 48 h.

RESULTS AND DISCUSSION

Selection of lactic bacteria

The lactic bacteria were isolated from different instant milk, pressed juice and juice mixed with sugar cane. Only two isolates (BL3, BL11) had important antibacterial activity and were used in this investigation. The pH and acidity assays indicate a progressive reduction of pH for all lactic bacteria isolates. The acidity profile is especially remarkable for the original BL11 that reaches a value of 1.04% (Table 1, Figure 1).

Table 2. Selection of saccharolytic and fermentative yeasts. Cultures were grown on semi-synthetic milieu containing 3 g/l of sucrose as a source of carbon at 30°C for 24 h.

Yeast	Initial pH_i	Final pH_f	DOi	DOf	Final acidity (%)
LE1	5.59	4.87	0016	0.45	0.4
LE2	5.58	4.66	0.16	0.36	0.6
LE3	5.32	5.02	0.19	0.14	0.3
LE4	5.59	4.86	0.14	0.89	0.4
LE5	5.62	5.75	0.12	0.33	0.2
LE6	5.08	4.29	0.09	0.32	0.5
LE7	5.05	4.89	0.16	0.82	0.4
LE8	5.56	4.19	0.23	1.178	1.15
LE9	5.50	4.29	0.23	0.94	0.7
LE10	5.09	3.90	0.08	0.85	0.8
LE11	5.06	3.88	0.18	1.121	0.9
LE12	5.52	4.96	0.20	0.56	0.4
LE13	5.50	4.28	0.17	0.97	0.7
LE14	5.41	4.23	0.23	1.05	0.6
LE15	5.33	4.25	0.33	0.75	0.8
LE16	5.41	4.11	0.37	1.3 72	1.2

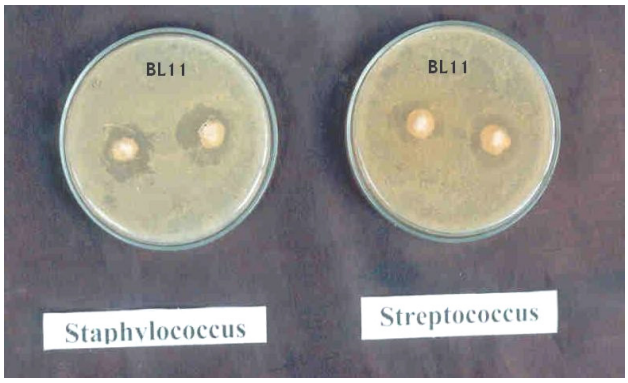

Figure 1. The antibacterial activity of the lactic acid bacteria BL11 against staphylococcus and streptococcus.

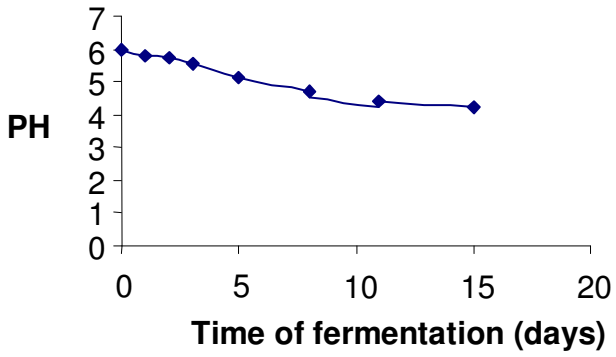

Figure 2. Change in pH during the fermentation of fish waste inoculated with a mixed culture of lactic acid bacteria and yeast (BL10 and LE11).

Isolation and characterization of the yeasts

Among about sixteen isolated yeasts, LE8 and LE16 have the highest acidifying power, biomass and fermentative activity. LE16 was used employed since it was more effective than the LE8 (Table 2).

Fermentation

The variation of the pH in the fish waste inoculated by LE 16 and BL10 is shown in Figure 2. The addition of the inoculum enriched the mixture and adapted the conditions of fermentation. The added inoculum dominated the other microorganisms which explain the quick reduction of pH. The reduction in pH was slow during the first days of fermentation because of adaptation of the lactic flora at the beginning of fermentation. In presence of lactic bacteria and yeast, the pH of the mixture becomes stabilized to between 4.4 and 4.7. The reduction of pH in the fermentation product is evident of good acidification. Besides, the production of antibacterial substances can be useful in the conservation of the products fermented.

The results of the titrable acidity are summarized in Figure 3. The progressive increase of the acidity with time conforms with pH change. With lactic bacteria, only a strong acidifying potential (450 meq/Kg) was obtained. Whereas fermentation of the waste in mixed culture (lactic bacteria and yeast), the acidity reached a value of 600meq/Kg after 15 days of fermentation (Figure 3).

Table 3. The physicochemical analyses of the fish waste before and after fermentation.

Parameter	Initial product	Finished product
pH	6.57	3.8
Ashes (%)	20.80	20.70
Total nitrogen (%)	6.11	6.61
Protein (%)	39.20	42
Nitrogen (%)	2.39	2.85
Sugars (%)	44.1	23.79
Fat (%)	10.4	8.3

Table 4. Microbiological analyses of the fish waste before and after fermentation.

Microorganisms	Unfermentated fish waste (UFC/g)	Finished Product (UFC/g)
Total aerobic mesophilic flora	2.8×10^7	4.16×10^8
Lactic bacteria	50×10^4	35×10^8
Yeasts	1.7×10^4	2×10^5
Total coliforms	2×10^3	0
Fecal coliforms	1.1×10^2	0
Salmonellas	0	0
Fecal streptococci	0	0
Clostridium	0	0
Staphylococci	10	0

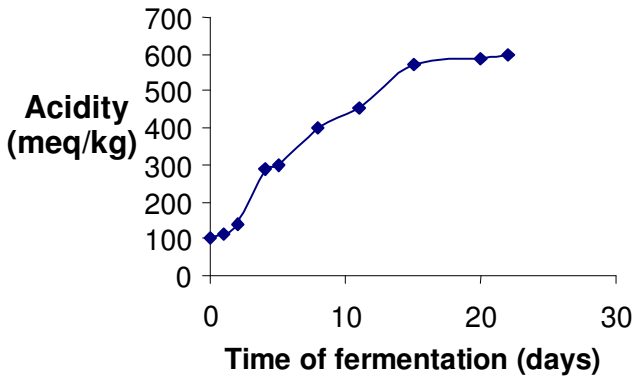

Figure 3. Change in titrable acidity during the fermentation of fish waste inoculated with a mixed culture of lactic acid bacteria and yeast (BL10 and LE11).

Physicochemical analyses

Fermented fish waste is especially rich in minerals (Table 3). There is a reduction in sugars due to its use by the yeasts and the lactic bacteria to produce the alcohol and CO_2, as well as organic acids (lactic acid, acetic acid), respectively. Fish waste proteins undergo a hydrolysis because of proteases present in the fish. There is an increase in total nitrogen at the end of fermentation.

Microbiological analyses

When yeasts find conditions favourable to their growth, they increase quickly and produce alcohol as well as carbon dioxide that can react with water to give carbonic acid:

$$CO_2 + H_2O \rightarrow H_2CO_3$$

The yeasts assure the production of aromatic compounds that is added to those resulting of the involvement of the lactic bacteria. The alcohol and carbon dioxide facilitate the deodorization of the fermentation product (Table 4).

The growth of the total aerobic mesophilic flora is inversely proportional to the evolution of pH. The bacteria contribute to the fermentation by producing organic acids and also inhibitory substances such as bacteriocin. Faid et al. (1995) showed that fish waste fermentation controlled with lactic bacteria can be reinforced by the addition of yeasts. The phenomenon by which the yeasts encourage the lactic bacteria is the topic of several studies in fermentation using these bacteria including the dairy and plant derivatives.

The hygienic flora is of a major importance since it influences the hygienic quality of the product. This flora can be implied in the production of toxins (such as clostridium and botulinum) or the production of bacterial

lipase leading to the oxidization of the fat and resulting in unpalatable end product. Also, proteolysis contributes to the liberation of the peptides, ammonia and amino acids which in excess produces toxic substances. However, the reduction of the total and fecal coli forms from 2 x 10^2 and 1.1 x 10^2 to nil reveals a favourable action of microorganisms employed.

Conclusion

The nutritional value of the fish waste after fermentation depends on the type of fish and the manner of treatment. However, the fermentation of whole fish has a more elevated nutritional value. Like milk, eggs and meat, fish has an important biological value since it contains essential amino acids (Ababouch et al., 1996). Fish is especially rich in vitamins and minerals.

The biotransformation of the fish waste is evident of an efficient, simple and accessible conservation means that improves the hygienic and organoleptic qualities resulting in the reduction of unwanted flora and the disappearance of bad odours. Furthermore, the finished product can substitute other expensive protein ingredients such as fish flour for animal feed.

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