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ENRICHMENT AND IMPROVING THE NUTRITIONAL VALUE OF THIS CABBAGE WASTE USING *Bacillus coagulans*

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Abstract: The present study was carried out to find out the enrichment and nutritional improvement cabbage waste which was used as one of the igradient using *Bacillus coagulans* for aquafeed. CW having 5% cabbage waste in the feeds recorded a protein content of 41.01%, while feeds CW II and CW III prepared incorporating 10 and 15% dried cabbage waste. Crude protein values of 43.07, 41.47 and 38.82% were recorded for the three feeds incorporated with 5, 10 and 15% CW fermented with *B.coagulans*, while lipid content ranged from 5.94% to 5.61%.

Keywords: Cabbage waste, enrichment, nutritional value, solid state of ermentation, *Bacillus coagulans.*

Introduction

In aquaculture more than half the investment comprising 50 to 70 % of the total operating costs goes into feeds as they contribute an essential factor for enhancing fish production. With the intensification of culture activities more emphasis is diverted to fish nutrition and compounded feeds in particular

Commercial aquaculture feeds for shrimp generally contain 25-45% of crude protein because shrimp require such high dietary levels. Consequently only high protein oil seed residues have been used for compounding shrimp feeds (New, 1976). Feedstuffs of vegetable origin as a whole are lower in protein when compared to those of animal origin. Nevertheless among all plant protein sources tested for most crustaceans, soyabean meal has been found to be the most superior on account of its high protein content and essential amino acid profile (Kanazawa, 1995; Akiyama, 1988). In order to reduce the escalating cost of aqua feed and make aquaculture sustainable in the long run intensive research is being focused on alternative and more sustainable protein sources for use within compounded aquafeeds

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(Tacon, 1993). The utility of plant protein as partial replacement for the more expensive animal protein fractions has been examined but results show great variations in the degree of success, which inordinately depend on the species and types of ingredients used.

Non conventional ingredients are ingredients that are capable of partially or completely substituting fishmeal. These have been in use since traditional aquaculture in Asia. It has been found that these feed stuffs can be used as substitute for fishmeal as they are no more abundant than fishmeal but are least expensive. Fishmeal has a well-balanced amino acid profile along with essential fatty acids. All foodstuffs need not have the same amounts of amino acids in fishmeal but they usually exceed the levels found in some amino acids.

The advantages of fermentation have been known over the ages as a means of bioconversion and protein enrichment of food and feed ingredients. It is also increasingly evident that the development of low cost, high quality protein foodstuffs is crucial for the future success of the aquaculture industry. Solid state fermentation is a novel technology by means of which cheap ingredients of lesser nutritive value can effectively be converted into nutritionally rich and easily digestible aquafeeds (Nigam and Singh, 1996).

Of the wide variety of feed ingredients available in India for production of aqua feeds (New *et al.,* 1993) most are reported to be of too poor quality to produce high quality aquafeeds, especially for shrimp substrates, cell substances of the microbes and externalized metabolites (Nigam and Singh, 1996).

Solid State fermentation (SSF) has gained importance in the recent past due to its several advantages over submerged fermentation especially for enrichment of protein of agricultural wastes and sub products. The SSF technology has the advantage of direct utilization of none or very few pretreated solid substrates under aerobic conditions to produce microbial Biomass products (MBP), which contain a mixture of unused substrates, cell substances of the microbes and externalized metabolites (Nigam and Singh, 1996).

Cabbage (*Brassica olaracea var. capitata*) is available in the local vegetable markets throughout the year. The outer green leaves are usually discarded as waste and only the inner compact head is utilized. The waste is available in bulk in most market and its incorporation, as a non-conventional ingredient in shrimp feeds would therefore be a lucrative proposition. It would be most pertinent to carry out further enrichment of these substrates as well along with cabbage waste in order to increase their nutritive value and digestibility.

Materials and Methods

Solid State Fermentation of ingredients was preformed by following the method of Ramesh and Lonsane (1987). Necessary changes were however, made in the methodology and medium composition after optimization of process parameters.

Micro-organisms:

Pure cultures of *Bacillus coagulans* were procured from the Microbiology section of the Department of Biotechnology, Cochin University of Science and Technology. They were maintained as pure cultures by sub-culturing every week on nutrient agar slants for bacteria and mycological agar containing streptomycin for fungi.

Preparation of solid substratum:

Cabbage waste was dried at 60 ± 5^{o} C for 24hrs in an oven. They were powdered in a pulveriser and sieved through a 200-micron sieve to obtain uniform particle size. The pH of each ingredient was determined individually. Moisture content of each substrate was also estimated using 1g of the sample and moisture content of the medium was adjusted to a level varying between 50 and 60%. The solid substrates were dispensed as 5g aliquots in petriplates as also in 250 ml conical flasks and adjusted to the desired level of moisture content with requisite amount of physiological saline (0.85% NaCl), adjusted to the optimum pH (pH 8.0 to 12.0 for bacteria and fungi respectively). The flasks and petri plates along with their contents were autoclaved at 121^{o}Cfor 60min. and cooled down to room temperature.

Inoculum Preparation:

A loopful of 24 hr old *B.coagulans* culture was first grown in 10 ml nutrient broth for 18 hrs at room temperature (28±^{o}2C). 1 ml of the above culture was transferred aseptically to 50 ml nutrient broth and incubated in a rotary shaker at 150rpm for 18hrs at room temperature. Cells were harvested by centrifugation at 10,000 rpm for 15 mins at 4^{o} C The harvested cells were made up to 10ml using sterile physiological saline (0.85% NaCl) after repeated washings. This was used as inoculum.Moisture, ash, crude protein, crude fat and fiber in feed ingredients, fermented substrates and feeds were determined by standard procedures (AOAC 1990).

Table:1 Percentage Composition of the Ingredients used in the Control Feed Formulation

INGREDIENTS	INCORPORATION LEVEL %
Cabbage waste	0.0
Fish meal	15.0
Soya bean meal	36.0
Shrimp Meal	10.0
Wheat flour	18.0
Ground Nut Oil Cake	10.0
Cod liver Oil	2.0
Vegetable oil	2.0
Gelatin	4.0
Vitamin mixture	0.5
Coated vitamin C	0.5
Mineral Mixture	1.0
Di-Calcium Phosphate	1.0
Total	**100.0**

Standard USP XVII mixture procured from SISCO laboratories

Preparation of control feed:

Fish meal, soyabean meal, shrimp meal, wheat flour, groundnut oilcake were weighed individually as per the specifications mentioned in Table 3 and mixed homogeneously. Gelatin was dissolved in sufficient water and heated till it dissolved completely. Wheat flour was then added and gelatinized. The mixture was cooled and the other ingredients and vitamin premix and mineral premix, di-calcium phosphate coated vitamin C and mixed homogeneously to obtain a dough. This dough was palletized into noodles using a kitchen mincer fitted with a 2-mm die. Pellets were initially sundried and then dried in an oven at 60^{o} C $\pm$ 5^{o} C for 12 hrs to less than 10% moisture content. They were manually broken into smaller bits and stored in a plastic container at room temperature. These dried pellets served as the control feed.

Preparation of experimental cabbage waste feeds:

The experimental diets were prepared as per the above formulation (Table 2) except that fish and shrimp meal were equally replaced with 5%, 10% and 15% dried cabbage waste and these feeds were designated as CW1, CW2 and CW3 respectively.

2. Percentage composition of the Experimental Feeds prepared using varying concentrations of dried powdered cabbage waste.

INGREDIENTS %	EXPERIMENTAL FEEDS		
	CW 1	**CW 2**	**CW 3**
Cabbage waste	5.0	10.0	15.0
Fish meal	12.5	10.0	7.5
Soya bean meal	36.0	36.0	36.0
Shrimp Meal	7.5	5.0	2.5
Wheat flour	18.0	18.0	18.0
Ground Nut Oil Cake	10.0	10.0	10.0
Cod liver Oil	2.0	2.0	2.0
Vegetable oil	2.0	2.0	2.0
Gelatin	4.0	4.0	4.0
Vitamin mixture	0.5	0.5	0.5
Coated vitamin C	0.5	0.5	0.5
Mineral Mixture*	1.0	1.0	1.0
Di-Calcium Phosphate	1.0	1.0	1.0
Total	**100.0**	**100.0**	**100.0**

* Standard VSP XVII mixture procured from SISCO laboratories.

Preparation of fermented cabbage waste feeds:

A set of three feeds were compounded by incorporating 5, 10 and 15% of cabbage waste fermented using *B.coagulans* and designated as CWI, CW2, CW3 respectively .The diet devoid of fermented material designated as C served as control. The percentage incorporation of other feed ingredients used in the feed base was as given in Table.2. They were dried to less than ten- percent moisture content and stored in airtight plastic containers at room temperature till further analysis

Table 4. Changes in percentage of some biochemical parameters upon solid state fermentation of cabbage waste

Substrate	Organism	BIOCHEMICAL PARAMETERS*					
		Dry matter	Protein	Fat	Fibre	Ash	NFE
Cabbage waste (CW)	*B.coagulans*	4.378	0.314	-0.023	-3.51	1.613	1.61

* Values expressed on dry matter basis and average of three estimations carried out in triplicate.
** Nitrogen Free Extractives- calculated as (100-%crude protein +crude fat ash + crude fiber+moisture)
- denotes decrease.

Physical Characteristics of feeds prepared from cabbage waste fermented with *B.coagulans*

The physical characteristics of the three feeds prepared incorporating 5, 10 and 15% levels of cabbage waste fermented with *B.coagulans* and designated as CW1, CW2 and CW3 are elaborated in Table 3.The three feeds also exhibited a very uniform texture in comparison to the control feed but comparable to feeds prepared from unfermented cabbage waste. However, these feeds exhibited a darker brown color with no visible green tinge and they also failed to exude any strong odour.

Table 3. Physical characteristics of the control and experimental feeds compounded utilizing cabbage waste fermented with *B.coagulans*

FEED	PELLET SIZE	PHYSICAL APPEARANCE
Control	2.0x 3.0 mm	Dark brown uneven texture
CW VII	2.0x 3.0 mm	Pale brownish fine texture
CW VIII	2.0x 3.0 mm	Pale brownish white texture
CW IX	2.0x 3.0 mm	Pale brownish white fine texture

Hydro-stability of the feeds compounded using cabbage waste fermented with *B.coagulans*

Feeds CW 1, CW 2 and CW 3 compounded incorporating 5, 10 and 15% of CW fermented using *B.coagulans* recorded a loss in dry matter ranging between 10-13%. within 30 min of immersion in seawater (Figure 2.).An additional 6-8% was observed in the next 30 min.

However, after this only a marginal loss ranging between 8-10% was observed after four hrs and the feed overall proved to be quiet stable.

Proximate composition of feeds compounded using cabbage waste fermented with *B.coagulans*

The proximate composition of the three feeds compounded using 5, 10 and 15% of cabbage waste fermented using *B.coagulans* and designated as CW1, CW 2 and CW 3 are recorded in Table 13. Feed CW 3 recorded a moisture content of 2.94% and a dry matter content of 98.06%, while feeds CW 2V and CW3 incorporated with 10 and 15% of cabbage waste fermented with *B.coagulans* recorded slightly higher values of 99.79 and 99.51% respectively and concomitantly lower values of 1.2% and 1.49% as the moisture content. Crude protein values of 43.07, 41.47 and 38.82% were obtained for the three feeds incorporated with 5,10 and 15%of cabbage waste using *B.coagulans.* The crude lipid content and crude fiber contents of these three feeds was more or less consistent recording values ranging from 5.94% to 5.61% for the former and values ranging between 1.69% in feed CW 1 to 1.23% in Feed CW 3VI for the latter. Feeds CW 1 and CW 3 reported ash contents of 9.11% and 9.01% respectively while feeds CW 2 incorporated with 10% cabbage waste recorded a slightly higher value of 9.62% as the ash content. Acid insoluble ash content of these three feeds ranged from 0.31% for Feed CW 3 to 0.548% for Feed CW 1. These feeds incorporated with cabbage waste fermented with *B.coagulans* reported lower carbohydrate contents ranging between 36.25% for feed CW 1 (5% CW) to 41.35% for feed CW 3 (15% CW).

Table.5: Proximate chemical composition of the control and experimental feeds compounded utilizing cabbage waste fermented with *B.coagulans*

NUTRIENT	FEEDS*		
	CW VI	CW V	CW VI
Dry matter	98.064	99.799	99.506
Moisture	2.936	1.201	1.493
Crude Protein	43.074	41.469	38.819
Ether extract	5.936	5.718	5.605
Crude fiber	1.691	1.369	1.229
Ash	9.112	9.622	9.006
Acid insoluble ash (AIA)	0.548	0.436	0.329
NFE**	36.251	40.62	41.352

* Values expressed on dry matter basis and average of three estimations carried out in triplicate.

** Nitrogen Free Extractives- calculated as (100-%crude protein +crude fat+ ash + crude fiber+moisture)

Discussion

The aquatic environment required some degree of water stability of aquatic feeds, especially shrimp feeds. Feed pellets, which disintegrated fast, facilitate faster leaching of nutrients, especially micronutrients, leading to non-availability of animal, pollution of water and economic loss. The feeds compounded in the present study incorporating 5, 10 and 15% of cabbage waste, both before fermentation, and after fermentation with *B. coagulants* accorded the desired water stability of upto 4 hrs in dry matter very much in agreement with the observation of Sanhotra and Pereira (1992).

The use of micro-organisms to convert carbohydrate, ligno-celluloses and other industrial wastes into protein rich food and feed stuffs has been well documented. Cabbage waste on account of its protein content offers great potential in aquafeed formulation especially in the case of freshwater prawns and herbivorous fish species.

Among the processes that can be used to supply proteins, the most important and promising are those based on microbial growth and production of microbial biomass employing solid state fermentation (SSF). SSF technology has the advantage of direct utilization of none or very few pretreated solid substrates under aerobic conditions to produce Microbial Biomass Products (MBP), consisting of a mixture of unused substrates, cell substances of the micro-organisms and externalized metabolites. The reduced reactor volume per unit substrate converted and the direct applicability of the fermented product for feeding purpose makes SSF a very attractive technology (Nigam and Singh, 1996). With the demand for cheaper plant protein sources to supplement the more expensive fishmeal component as well as bring down the cost of aqua feed the use of non-conventional ingredients like cabbage waste and further improvement of nutritive value of conventional ingredients becomes pertinent. In this investigation SSF technology was employed for the production of microbial protein as well as protein enrichment of cabbage waste using (*B.coagulans*)

In the present study, bacterial fermentations recorded mild color change unlike the fungal fermentations, wherein the pronounced spore formation leading to a visible change in color, with a strong moldy odour is in agreement with the results of Sridhar and Chandrasekhar (1996).

In this study protein content increased in cabbage waste, upon fermentation with *B.coagulans* but the increase was higher in the case of fungal fermentations, as compared to the bacterial fermentations, a view contradictory to that observed by Sridhar and Chandrasekhar (1996).

It is evident that apart from the substrate being employed for fermentation, the differences in protein enrichment obtained is also attributable to various strains of bacteria and fungi employed for the SSF process. Further research on the best strain consortia of strains either bacteria or fungi individually or in combination, according the maximum protein enrichment of a substrate would be pertinent. Even in the present study the use of another strain in place of *B.coagulans* or a consortia of bacteria may have yielded better results. Overall, the changes observed with regard to protein enrichment and changes in other nutrients upon SSF proved favorable and are surely indicative of the ability of micro-organisms to carry out bioconversions.

Feeds also showed desirable qualities. SSF resulted in bioconversion and protein enrichment of all substrates. Thus SSF is a novel technology by means of which cheap ingredients of lesser nutritive value can effectively be converted into nutritionally rich and easily digestible aquafeeds. Studies are preliminary and offer immense scope for further research, to prove beyond doubt that SSF, which is simple and economic, is the appropriate technology for the futuristic aquafeed industry.

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