PHYSICO - CHEMICAL CHARACTERISTICS OF SHRIMP FEEDS COMPOUNDED FROM A FEW FERMENTED FEED INGREDIENTS

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BY

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JULY 2000

Dedicated to

my beloved parents

CERTIFICATE

Certified that the dissertation entitled " **Physico-chemical characteristics of shrimp feeds compounded from a few fermented feed ingredients** " is a bonafide record of work done by Mr. Hari, S. under our guidance at the Central Marine Fisheries Research Institute during the tenure of his M. F. Sc. (Mariculture) programme (1998-2000) and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles or for any publication.

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I hereby certify that this thesis entitled **"Physico -chemical characteristics of shrimp feeds compounded from a few fermented feed ingredients"** is based on my own research and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles of recognition.

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सारांश

झींगा खाद्य में अपरंपरागत संघटक के रूप में कैबेज (*ब्रासिका ओलरेशिया* केपिटाटा उपजाति) को सम्मिलित करने के लिए इसके संघटक संयोजकों का विश्लेषण किया गया . इस विश्लेषण में व्यक्त हो गया कि अशोधित प्रोटीन का अंश 15.43% था और अशोधित चर्बी, अशोधित फाईबर और भस्म के अंश क्रमशः 1.50%, 14.57% और 14.46% थे . 52.34% कार्बोहाइड्रेट युक्त यह अपशिष्ट चूर्ण 5, 10 और 15% की मात्रा में झींगा खाद्य में मिलाया गया . इन तीन मात्राओं पर आधारित परीक्षणात्मक खाद्यों को सी डब्लियू -I, सी डब्लियू - II और सी डब्लियू - III से उल्लिखित किया गया और 43.39% प्रोटीन युक्त कैबेज अपशिष्ट रहित नियंत्रक खाद्य में इन तीनों के पोषक मिश्रण तथा अन्य भौतिक - रासायनिक प्राचलों का मूल्यांकन किया गया . 3 से 4 घंटों में इन तीनों खाद्यों के लिए आवश्यक जलस्थायित्व (हाइड्रोस्टेबिलिटी) रिकार्ड किया गया और इन खाद्यों के अशोधित प्रोटीन की मात्रा 41.01 , 38.60 और 37.03% थी. कैबेज अपशिष्ट चूर्ण , जिंजली खली (प्रोटीन की मात्रा 34.41%) और बिनौला खली (कोटनसीड कैक) (प्रोटीन की मात्रा 27.62%) के साथ एक दंडाणु (बैसिलस) (बी. कोगुलन्स)और एक कवक (फंगै) (बिवेरिया जाति) मिलाकर घन अवस्था में किण्वन किया गया. इसके फलस्वरूप किण्वन संपन्न हुए सभी अधःस्तरों में प्रोटीन की उल्लेखनीय बढ़ती के साथ साथ अन्य पोषकों में अनुकूल परिवर्तन दिखाए पडे . इस अध्ययन का परिणाम अत्यंत प्रोत्साहजनक है और इस से यह साबित होता है कि भविष्य में जलजीव खाद्य उद्योग (अक्वाफीड इन्डस्ट्री) के लिए घन अवस्था में किण्वन (सोलिड स्टेट फेरमेन्टेशन) जो सरल है , अत्यंत उचित प्रौद्योगिकी है .

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1. INTRODUCTION

In the recent years, there has been a rapid development in the field of fisheries especially with reference to aquaculture. India has vast fisheries resources with a total EEZ (Exclusive Economic Zone) of 2.02 million square kilometers and a coastline of 8047 kilometers. The total riverine length is above 29,000 square kilometers. The lentic water bodies include lakes, ponds, tanks, reservoirs, bheels, bans, jheels, etc,which are estimated to be nearly 1.29 lakh hectares in area. The rivers upon joining the sea form large areas of brackish water estuaries. The area suitable for brackishwater fish farming is around 2 million hectares. These form a tremendous resource for fisheries in the country. The marine environment produces around 2.6 million tonnes of fish, comprised of different variety of fishes, crustaceans and mollusks. The inland water resources produce around 2.3 million tonne of fish. Eventhough the current inland production lags behind the marine production there has been a tremendous increase in the annual growth rate primarily due to aquaculture.

In the early 90's shrimp farming changed the aquaculture scenario with vast stretches of coastal lands being converted to shrimp farms generating valuable foreign exchange for the country. Simultaneously freshwater prawn farming using the species *Macrobrachium rosenbergii* was also initiated.

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.

Though fish are probably the most efficient feed converters, there exists a dearth in the availability of food supply for cultured fish. Therefore, there is necessity to look for newer or non-conventional feed ingredients to cover this deficit. Fish nutritionists have long tried to use less expensive plant protein sources to partially or totally replace fishmeal as well as shrimp meal. Of all the plant protein ingredients, soybean meal is considered to be the most nutritious and is currently used as the major protein source in many fish diets. However, growth tended to be reduced in fish fed with diets in which soybean meal completely replaced the fishmeal, which could be attributed to the anti-nutritional factors present as well as to the lower assimilation efficiencies of plant protein in comparison to animal proteins. It is therefore, important to identify alternative protein sources to reduce or stabilize the cost of fish production, as ultimately economic factors are the ones, which determine the level of inclusion.

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 feedstuffs can be used as a 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. Example is corn gluten, which is low in lysine and tryptophan but rich in other amino acids. These ingredients are considered to be satisfactory as feed constituents as they usually have the necessary levels of nutrients that are required for a given species. Ex: Herring meal has been known to fully meet the amino acid requirements of trout.

Analysis of most conventional feed ingredients however, indicates that no single ingredient can be used as a complete alternative or substitute for fishmeal. Also there exist some problems with these ingredients. Unconventional ingredients from plant origin have low protein and high carbohydrate and fiber content in addition to anti-nutritional factors and toxins. The commonly used non-conventional feed ingredients include krill meal, single cell proteins, rape seed meal, sunflower meal, poultry byproducts, feather meal, fly larvae, etc.

According to Akiyama (1991) the commercialization of aquaculture is growing, thereby increasing the demand for aquaculture feeds. Traditionally, these feeds have been based on animal protein. However, due to cost and availability considerations, it is inevitable that more plant protein supplements be utilized in feeds. Plant protein supplements are a more cost-effective source of nutrients as compared to animal protein supplements.

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).

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. As gingelly oilcake and cotton seed oilcake are also commonly used in shrimp feeds 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. The present study was therefore proposed with the following main objectives:

1. Proximate composition analysis of cabbage waste, gingelly oilcake and cotton seed oil cake.
2. Fermentation of cabbage waste, gingelly oilcake and cottonseed oilcake individually using both bacteria and fungi.
3. Formulation of feeds with fermented cabbage waste in permutation combination with other conventional feed ingredients.
4. Determination of the physico-chemical characteristics of these feeds using standard methodologies.

2. REVIEW OF LITERATURE

Non- conventional Feed Ingredients

On a purely nutritional basis it has been repeatedly shown that the best food or feed ingredient in terms of nutritional composition palatability, growth and feed conversion efficiency is fishmeal. However, its high cost and competition for use as human food as well as other terrestrial farmed animals makes its availability scarce.

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 aquafeed and make aquaculture sustainable in the long run, intensive research is being focussed on alternative and more sustainable protein sources for use within compounded aquafeeds (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. Lim and Dominy (1989) and Tacon (1993) reviewed the utilization of plant proteins in formulated feeds for warm water fishes. A compendium on the potential use of plant protein in marine fish feeds was given by New (1989). A large number of plant ingredients have been used in animal feeds and fish feeds but relatively few in shrimp feeds. The extent of plant protein utilization is normally influenced by the availability, cost, acceptability, ease of processing, the nutritive value and presence of toxins or anti-nutritional factors (Akiyama,1991).

Tacon (1993) reviewed utilization of oil seeds and their by-products for warmwater fish species. Peanut meal proved beneficial as a source of plant protein for penaeid prawns and its use as oilcake was reported by Ali (1994). Raman *et al.*, (1982) successfully tested artificial feeds prepared from Bengal gram husk, gingelly oilcake, bajra along with fishmeal and prawn waste meal and advocated their use for reducing feed cost. Efficiency of cottonseed meal was examined in the diets of *Macrobrachium rosenbergii* and *Penaeus indicus* (Aquacop, 1976; Ali, 1994).

Keembiyahetty and DeSilva (1993) reported the replacement of 20-33% fishmeal by cow pea and black gram in diets for *Oreochromis niloticus* fingerlings. Wheat flour, wheat gluten and rice bran were recommended for use in feeds of *Penaeus indicus* by Galgani *et al.*, (1989) and Paul Raj (1990).

With pressure mounting to increase the production of aquaculture feeds, tapping of non-conventional and alternate feed resources becomes pertinent. A variety of non-conventional feed resources such as single cell proteins (SCP), leaf meals, aquatic plants like seaweeds, water hyacinth. Coontail, aquatic fern, duckweed, water lettuce were identified for incorporation into fish and shellfish feeds (New, 1976; Tacon, 1990; Tacon, 1993). Cassava or Tapioca meal (*Manihot esculenta*) alfalfa or clover, leaf protein concentrate and coffee were successfully incorporated in feeds of warmwater fishes (Ng& wee, 1989;Viola *et al.*, 1988; Jia *et al.*, 1991 Chow *et al.*, 1983; Ogino *et al.*, 1978 and Christensen 1981). Syslo and Hughes (1981) and Harpaz and Schmalbach (1986) demonstrated the benefits of supplementing dry artificial diets with fresh leaves for lobster and *Macrobrachium rosenbergii* respectively. Catacutan (1993) showed that aquatic macrophyte *Naja graminae* and *Ruppia martima* were consumed by *Penaeus monodon* with high assimilation efficiencies of between 40-76%. Aquatic macrophytes were used as live sources for *Macrobrachium rosenbergii* (Stern *et al.*, 1976). Use of sea-grass for mud crab was evaluated by Raman *et al.*, (1982). These studies showed that live plant organic matter was well utilized by crustaceans.

Solid State Fermentation:

If the aquaculture sector is to reduce its dependence upon fishmeal and farmers are to bring down feed and production costs and improve farm profitability it is right time that modern technology be adopted. Of the wide variety of feed ingredients available in India for production of aquafeeds (New *et al.*, 1993) most are reported to be of too poor quality to produce high quality aquafeeds, especially for shrimp. The proximate composition of various commonly used conventional and non-conventional plant ingredients is given in Table.1.The noticeable variability is due to a number of factors including the method of handling and processing and the nutritional status of the environment in which they were grown, in addition to variations in analytical methodologies.

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).

Some typical substrates used for SSF processes are given in Table 2 but most of these were employed mainly for production of enzyme protein and animal feed. Cassava *(Manihot esculenta Crantz)* has been subjected to SSF for protein enrichment by a wide variety of researches using filamentous fungi using *Aspergillus niger* (Senez 1979; Raimbault *et al.*, 1985) and *Rhizopus* genus (Daubresse *et al.*, 1987; Mitchell *et* *al.*, 1988 and Soccol *et al.*, 1993). The effect of substituting corn with fermented cassava in broiler rations was investigated by Varghese *et al.*, (1996), while Manilal *et al.*, 1987 used *Aspergillus niger* for utilizing starch factory waste by SSF. Protein enrichment of cassava flour and starch factory wastes using the fungus *Trichoderma pseudokongii Rifai* in a solid state fermentation process for incorporation in cattle feeds and broiler rations was developed by Balgalagoplan and Padmaja (1988) and Padmaja and Balagopalan (1990). Biotransformation of a number of crop residues into animal feeds was also achieved by SSF.

Though microbially fermented fish silages and single cell proteins are being used in fish and shellfish diets the advent of solid state fermentation in aquatic diets is comparatively new with very little research having been carried out to date.

The effect of fermentation on the nutritive value of seasame seed meal in the diets for rohu (*Labeo rohita*) fingerlings was reported by Mukhopadhay and Ray (1999). Higher feed effects were recorded in *Moina macrocopa* when cultivated on fermented grass pulps (Yang, 1995). Shimeno *et al.*, (1993) determined the effects on growth, feed conversion and body composition of fermented defatted soyabean meal (SBM) either with *Aspergillus oryzae* or *Eurotium repens* in single moist pellet diet for juvenile yellowtail *Seriola quinqueradiata*. Hossain *et al.*, (1988) reared red sea bream *Chrysophrys major* on highly oxidized scrap meal fermented by a group of microbes and compared their growth and feed efficiency to fish reared on non fermented and defatted scrap meals and white fish meal. Fermented vegetable and kitchen wastes were recommended as feeds for not only zoea but also mysis and upto certain points in the post larval stages of *Penaues monodon,* when diatoms or brine shrimp nauplii were lacking or in short supply.

Sridhar and Chandrashekar (1996) employed SSF technology for fermentation of groundnut oilcake and wheat bran for incorporating into shrimp feeds. The fermented material at varying concentrations was incorporated into feeds and its effect on growth and feed conversion efficiency of *Penaeus indicus* post larvae studied.

The studies carried out so far are scarce but offer immense scope for research to prove beyond doubt, that solid state fermentation, which is simple and economic, is the appropriate technology for the futuristic aquafeed industry. Though not complete in itself the present investigation is also an attempt towards this direction.

Table 1.Proximate composition of some selected feed ingredients of plant origin in India.

Ingredients	Moisture %	Crude Protein %	Crude Fat %	Crude fiber %	Ash %	Nitrogen Free extract %
Rice Polish	12.6	14.5	17.3	7.5	-	-
Rice Polish	10.0	12.2	16.0	9.0	6.0	46.8
Rice Polish	8.4	11.4	15.3	11.0	12.9	41.0
Rice, Broken	10.0	12.0	4.2	5.3	3.1	65.4
Rice Bran	10.1	12.6	11.3	19.3	10.2	36.5
Rice Bran	7.8	7.8	6.1	4.4	20.5	43.4
Rice Bran	8.4	2.9	5.0	18.0	20.3	38.4
Rice Bran	8.7	9.4	4.7	13.5	31.4	32.3
Defatted Rice Bran	7.2	12.1	1.3	15.2	23.8	40.4
Wheat Bran	12.3	15.8	4.3	8.7	-	-
Wheat Bran	10.0	13.5	2.6	12.2	3.0	58.7
Wheat Bran	13.0	8.2	6.6	33.5	4.2	34.5
Wheat Bran	9.3	12.6	7.5	11.9	4.2	54.5
Wheat Broken	9.0	11.5	1.9	4.0	0.2	73.4
Wheat flour	12.6	14.5	3.7	2.7	2.3	64.2
GNOC	7.8	28.6	13.8	7.5	13.4	28.9
GNOC	6.0	37.7	11.5	13.2	7.3	24.3
GNOC	10.0	42.0	7.3	13.0	2.5	25.2
GNOC	8.3	46.6	7.7	6.5	7.7	23.2
GNOC	7.1	35.8	8.5	8.2	10.5	29.9
Groundnut extract	7.0	48.0	2.0	11.2	2.7	29.1
Sunflower Extract	8.0	31.0	2.1	18.4	1.5	39.0
Sunflower Extract	10.2	30.1	2.9	24.7	6.5	25.6
Palm kernel cake	8.9	12.2	4.9	25.6	2.6	45.8
Soyabean meal	11.8	46.3	1.3	5.0	-	-
Soyabean meal	3.0	58.6	1.4	0.4	5.3	31.3
Soyabean meal	10.0	46.0	0.9	7.3	0.6	35.2
Soy sauce waste	12.0	13.5	8.2	5.8	5.3	55.2

Rapeseed cake	11.0	35.9	0.9	13.2	6.9	32.1
Salseed cake	8.6	8.2	2.9	1.7	10.2	68.4
Sesame cake	8.3	41.9	9.2	6.2	14.8	19.6
Sesame cake	10.0	29.0	12.9	18.3	10.0	19.8
Sesame cake	10.0	42.7	6.9	5.7	12.9	21.8
Mustard cake	8.5	30.8	9.3	6.2	10.3	34.9
Mustard cake	9.2	23.6	9.6	6.3	10.4	40.9
Cotton seedcake	7.0	37.0	6.7	13.0	1.0	35.3
Cotton seedcake	8.2	42.7	1.0	12.6	8.2	27.3
Gingelly cake	9.0	34.0	7.8	7.9	3.1	38.2
Gingelly extract	7.0	40.0	2.0	9.7	2.9	38.4
Niger ext.	7.0	35.0	2.0	19.0	3.5	33.5
Copra cake	12.0	22.0	6.5	12.2	5.2	42.1
Copra cake	8.4	20.3	11.4	16.2	6.2	37.5
Copra cake	-	22.0	6.0	12.0	2.1	-
Tobacco seed ext.	7.7	30.6	0.3	-	13.7	47.7
Maize meal	13.5	9.5	4.0	4.0	1.5	67.5
Maize	10.4	4.6	7.8	3.5	1.0	72.7
Sorghum	10.0	9.0	2.8	3.0	0.1	75.1
Spirulina	8.7	50.5	1.0	2.1	11.0	26.7
Tapioca Flour	11.5	3.1	2.3	2.0	2.3	78.8
Tapioca Flour	8.0	1.8	1.3	1.8	0.2	86.9
Coffee pulp	12.3	14.0	1.2	20.8	8.2	43.5
Colocasia meal	5.8	24.6	4.5	8.2	9.9	47.0
Eichornia meal	3.3	19.5	2.3	18.3	9.3	47.3
Pistia meal	4.9	19.5	1.3	11.7	25.6	37.0
Leucaena meal	11.8	33.1	4.7	9.0	7.2	34.2
Mulberry leaf, dry	8.9	27.2	2.4	11.5	8.1	41.4
Salvinia meal	2.6	16.2	1.1	18.5	22.0	39.6

Sources: Michael B.New, Albert G.J. Tacon and Imre Cavas- Farm - made aquafeeds.

Table 2.Some Typical SSF Processes Used For the Production of Protein/Animal Feed

Sl. No	SUBSTRATES	MICROORGANISMS	YEAR
1.	Canola meal	Aspergillus carbonarius	Alachel & Duvnja (1995)
2.	Carob pods	Aspergillus niger	Smail et al. (1995)
3.	Apple pomace	Candida utilis Kloeckera apiculata	Rahmat et al. (1995)
4.	Coffee pulp	Penicillium verrucosum	Roussos et al. (1994)
5.	Sugarcane bagasse	Chaetomium cellulolyticum	Brabo et al. (1994)
6	Sugarbeet pulp & molasses	Fusarium oxysporum Chaetomium cellulolyticum Trichoderma reesei Trichoderma viride Saccharomyces cerevisae	Nigam (1994)
7	Apple pomace	Candida utilis Candida tropicalis T.viride A.niger	Bhalla and Joshi (1994)
8	Orange peels, Grape stalks	Pleurotus ostreatus Agrocybe aegirata Armillarialla mellea	Nicolini et al. (1993)
9	Cassava	Rhizopus arrhizus	Soccal et al. (1993)
10	Oil Palm wastes	C.cinereus P.sajor caju	Kume et al. (1993)
11	Cassava	A.oryzae	Zvauya and Muzondo (1993)
12	Cotton stalks, Perlite	P.ostreatus Ph.chrsosporium	Kerem and Hadar (1993)
13	Wheat straw	P.ostreatus	Tripati and Yadav (1992)
14	Grape pomace, Corn stover	Chaetomium cellulolyticum+ Candida utilis	Girujie et al. (1992)
15	Rice straw, Maize stover	Cyathus stercoreaus Dichotomus squalens Ph.chrysosporium	Karunanandaa (1992)
16	Sugarcane bagasse	Polyporus sps	Nigam (1990)
17	Sugarbeet pulp	T.reesei, Fusarium oxysporum	Nigam et al. (1990)
18	Sugarcane bagasse	Mixed cultures (poluporus, Pleurotus, Trichoderma)	Nigam (1989)
19	Corn cob	A.niger	Singh et al. (1989)

20	Starchy raw materials	A.oryzae, A.niger, A.awamori	Czajkowska et al. (1988)
21	Sugarbeet pulp	T.viride	Durand et al. (1988)
22	Sugarbeet pulp	T.reesei Fusarium oxysporum	Nigam et al. (1988)
23	Wheat straw	Coprinus sps	Yadav (1988)
24	Sugarbeet pulp	Thermophilic fungi	Grajek (1988)
25	Cassava	Rhizopus oryzae	Daubresse et al. (1987)
26	Saccharum munja residues	Pluerotus sps	Gujral et al. (1987)
27	Straw	Candida utilis	Han (1987)
28	Cassava	S.pulverrlentum	Smith et al. (1986)
29	Banana wastes	A.niger	Baldensperger etal(1985)
30	Dried citrus peel	A.niger	Rodriquez et al (1985)
31	Wheat straw	T.reesei Ch.cellulolyticum C.utilis	Abdullah et al. (1985)
32	Sugarcane bagasse	Polyporus sps	Nigam et al. (1989)
33	Wheat straw	T.reesei Endomycopsis fibuliger	Laukevics et al. (1984)
34	Fodder beets	Saccharomyces cerevisae	Gibbon et al. (1984)
35	Pulpmill waste	Ch.cellulolyticum	Tautorus et al. (1983)
36	Soya bean	Rhizopus oligosporus	Rath bun et al.(1980)
37	Cassava	T.reesei+yeast	Opoku et al. (1980)
38	Starch substrates	Various cultures	Senez et al. (1979)
39	Alfaalfa	A.terreus	Bajracharya et al.(1979)
40	Straw, corn stover	Ch.cellulolyticum	Moo-Young et al. (1976)
41	Rye grass	Cellulomonas, A.faecalis T.reesei, A.pullulans	Yu et al. (1976)
42	Rye grass	C.utilis	Han. & Anderson. (1975)
43	Newsprint	Sporotrichum themophile	Barnes (1972)

Source: Uludag, S., Prokop, A. and Tanner R.D (1996).

Plate I: Fresh cabbage waste and cooked, dried and powdered cabbage waste.

3. MATERIALS AND METHODS

Three non-conventional feed ingredients viz. cabbage waste, gingelly oilcake and cottonseed oilcake procured from the local market were analyzed for their proximate composition. Feeds were compounded with varying concentrations of cabbage waste and their physical and physico-chemical characteristics determined. These three ingredients were subjected to Solid State Fermentation (SSF) using both a bacillus (*Bacillus coagulans*) and a fungus (*Beauveria sp.*) and the changes in chemical composition were determined. Feeds of varying concentrations were again formulated with the fermented cabbage waste and the physical and physico-chemical characteristics compared to those of the unfermented material.

Selection of Ingredients

Raw materials for feed formulation i.e. fishmeal, soyabean meal, shrimp meal, wheat flour, groundnut oilcake, gingelly oilcake, cottonseed cake were procured from the local market. They were sorted off extraneous material, oven-dried, powdered in a pulveriser and sieved through a sieve of 250 microns mesh size. Cabbage waste was collected and transported to the laboratory. The leaves were rinsed several times in tap water and finally in double distilled water. After draining for one hour they were dried in an oven at 60 ± 5 ^{o}C and powdered as mentioned earlier (Plate 1). Proximate composition analyses of ingredients was performed for which chemicals were purchased from standard chemical firms of national and international repute.

Feed formulation:

The feed ingredients and other additives like oil, binder, vitamin premix, mineral mix were weighed according to the standard feed formulation given by New, 1987.

Table 3.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° C ± 5° 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 4) except that fish and shrimp meal were equally replaced with 5%, 10% and 15% dried cabbage waste and these feeds were designated as CWI, CWII and CWIII respectively.

Table 4: Percentage composition of the Experimental Feeds prepared using varying concentrations of dried powdered cabbage waste.

INGREDIENTS %	EXPERIMENTAL FEEDS		
	CW I	**CW II**	**CW III**
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 USP 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 CWIV, CWV, CWVI respectively and a set of three feeds each formulated by incorporating 5,10 and 15% cabbage waste fermented using Beauveria sp. and designated as CWVII, CWVIII and CWIX 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.3. The feeds were prepared in the same method as described earlier. They were dried to less than ten- percent moisture content and stored in airtight plastic containers at room temperature till further analysis.

Physical evaluation of the feeds:

The physical appearance of the feeds viz. color, shape, size and pellet diameter were recorded.

Hydrostability tests of the feeds:

Water stability of the control feed was determined by the method of Jayaram and Shetty (1981) with minor modifications. Approximately 5 g of diet was weighed, in triplicate and transferred 4" X4" pouches made of bolting silk. These were immersed in 25 liters of sea water (28ppt salinity and $28 \pm 2°$ C water temperature) in plastic tubs. Pouches were removed from water at 0.5,1,2,3and 4 hours respectively and rinsed with distilled water to remove adhering salts. The contents were transferred to pre-weighed petridishes and dried in an oven at $80 \pm 5°$ C and the resultant loss in dry matter was calculated.

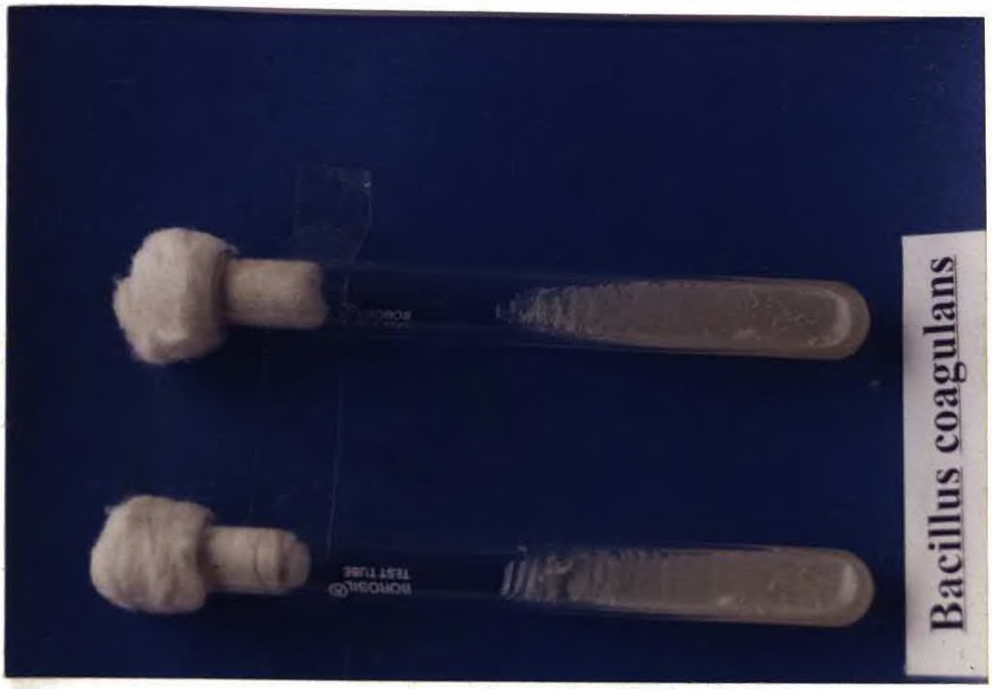


Plate II: Fresh culture of *Bacillus coagulans* used for solid state fermentation of cabbage waste (CW), Cottonseed cake (CSC) and Gingelly oilcake (GOC).

Plate III: Dried and powdered cottonseed cake and gingelly oilcake before inoculation with bacteria and fungi for fermentation.

SOLID STATE FERMENTATION:

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* (Plate II) and fungi *(Beauveria sp.)* 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 along with gingelly oilcake and cottonseed oilcake (Plate III) were dried at 60 ± 5° 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 petriplates along with their contents were autoclaved at 121°C for 60min. and cooled down to room temperature.

Inoculum Preparation:

i)Bacteria:

A loopful of 24 hr old *B.coagulans* culture was first grown in 10ml nutrient broth for 18 hrs at room temperature (28±°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 °C The harvested cells were made up to 10ml using sterile physiological saline (0.85% NaCl) after repeated washings. This was used as inoculum.

ii) Fungi:

20 ml of sterile physiological saline containing 0.1% Tween 80 was added to each fully sporulated (2 week old) slant culture raised on mycological agar by means of sterile pipette. The spores were scrapped using a inoculating needle under strict aseptic conditions and the spore suspension obtained was adjusted to the desired concentration using sterile physiological saline (0.85% NaCl).

Inoculation and incubation:

The prepared inocula were adjusted to a concentration of 2 mg dry cell equivalent in 1ml of cell suspension of bacteria and 2ml of fungal spore suspension per flask (inoculum level selected randomly) for the fungi and added to sterilized moist media in flasks. The contents were mixed and incubated in slanting position at 35 °C and 28 °C for bacteria and fungi respectively with 60-70% relative humidity for 48 to 72 hrs. Petriplates were inoculated and incubated similarly. They were placed in the straight positions. After fermentation the contents were dried to a constant moisture level and analyzed for the various nutrients.

Analytical Methods:

Moisture, ash, crude protein, crude fat and fiber in feed ingredients, fermented substrates and feeds were determined by standard procedures (AOAC 1990) as given below.

Moisture content:

Moisture content in the feed ingredients, substrates and the compounded feeds were determined gravimetrically by oven drying the samples at 80 ° C till consecutive similar readings were obtained. Percentages of moisture in the sample readings were obtained. Percentage of moisture in the samples are calculated as follows:

Moisture content % = weight of fresh sample- weight of dry sample X 100 / weight of fresh sample

Ash content:

Weighed dry samples of the feed ingredients and feed were taken in duplicate silica crucibles, and ashed in a muffle furnace at 600 ° C for 6 hours. Percentage of ash was calculated as follows:

Ash %= weight of ash X 100 / weight of sample

Crude Protein:

Total nitrogen in the feed ingredients, substrates and feeds was determined by the "Kjeldahl's method" and the result was multiplied by a conversion factor of 6.25 to give crude protein %.

Crude Fat:

Lipid content of feed samples was determined by the Soxhlet's Extraction Method using petroleum ether (boiling point 60-80 ° C) as solvent.

Crude Fiber:

Crude Fiber was determined as the fraction remaining after digestion with standard solution of sulfuric acid (0.23N) and Sodium Hydroxide (0.31N) under carefully controlled conditions.

RESULTS

Proximate composition analysis of cabbage waste (CW) was carried out with a view to assess its suitability for incorporation into shrimp feeds as a non-conventional feed ingredient. The proximate composition analysis of two other conventional ingredients viz. cottonseed cake (CSC) and gingelly oilcake (GOC) was also conducted in order to ferment them and evaluate enhancement of their nutritional value by microbial enrichment, for incorporation into shrimp feeds. Feeds were compounded with 5,10 and 15% concentrations of cabbage waste, both before and after fermentation. Pellet characteristics, hydro stability and nutritional composition of these compounded feeds was evaluated.

I. Proximate composition of Cabbage waste (CW) Cottonseed cake (CSC) and Gingelly oilcake (GOC)

Results on the proximate composition of cabbage waste (CW) are recorded in Table 5. Cooked and dried cabbage waste recorded a dry matter content of 95.29% and moisture content of 4.71%. The crude protein content averaged at 15% and the fat content was quite low recording an average value of 1.50%. The crude fibre content of 14.57% was on the higher side and ash content moderate at an average of 11.46%. The carbohydrate content of these waste cabbage leaves was above 50%. The acid insoluble ash content of 0.09% was negligible.

Cotton seedcake recorded 28% protein, while gingelly oilcake recorded a higher value at 34.4% as the crude protein content. Values of 5.22% and 8.83% respectively were obtained for the lipid content of cottonseed cake and gingelly oilcake. A comparatively high value of 16.33% was recorded for the fibre content of cotton seed cake while a very low value of 1.88% was recorded in the case of gingelly oilcake. The ash contents of cottonseed cake and gingelly oilcake obtained in the present study averaged 6.02% for the former and 14.21% for the latter. Values of 43.88% and 39.49% were recorded for the nitrogen free extractives of cottonseed cake and gingelly oilcake respectively. A low AIA value of 0.29 was obtained in the case of cottonseed cake while gingelly oilcake recorded a comparatively higher value of 1.37% as the AIA.

Table 5.Proximate chemical composition of dried unfermented Cabbage waste (CW), Cottonseed cake (CSC) and Gingelly oilcake (GOC).

NUTRIENT	INGREDIENT (%)*		
	CW	CSC	GOC
Dry matter	95.29	99.071	98.824
Moisture	4.71	0.929	1.176
Crude Protein	15.431	27.619	34.414
Ether extract	1.502	5.224	8.833
Crude fiber	14.568	16.333	1.875
Ash	11.455	6.019	14.209
Acid insoluble ash (AIA)	0.094	0.286	1.366
NFE**	52.335	43.876	39.493

* 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)

The proximate composition of fishmeal, shrimp meal, groundnut oilcake, soyabean meal and wheat flour used in compounding the feed was also evaluated and results are recorded in Table 6. Values obtained in this study were in keeping with values already recorded in literature for these conventional feed ingredients.

Plate IV: The control Feed and feeds incorporated with 5, 10 and 15% of dried fermented cabbage waste.

Table.6: Proximate chemical composition of other feed ingredients used in the compounded feed base

NUTRIENT	INGREDIENT (%)*				
	FM	SM	GNOC	SyM	WF
Dry matter	99.271	99. 471	99.169	91.352	94.747
Moisture	0.729	0.529	0.831	8.648	5.253
Crude Protein	61.525	65.091	47.791	47.938	12.188
Ether extract	9.068	3.740	7.071	0.479	1.951
Crude fiber	0.399	3.154	1.714	5.229	1.268
Ash	23.46	17.369	6.520	6.858	1.45
Acid insoluble ash (AIA)	0.517	1.244	1.509	***ND	***ND
NFE**	4.818	10.115	36.264	30.849	77.889

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

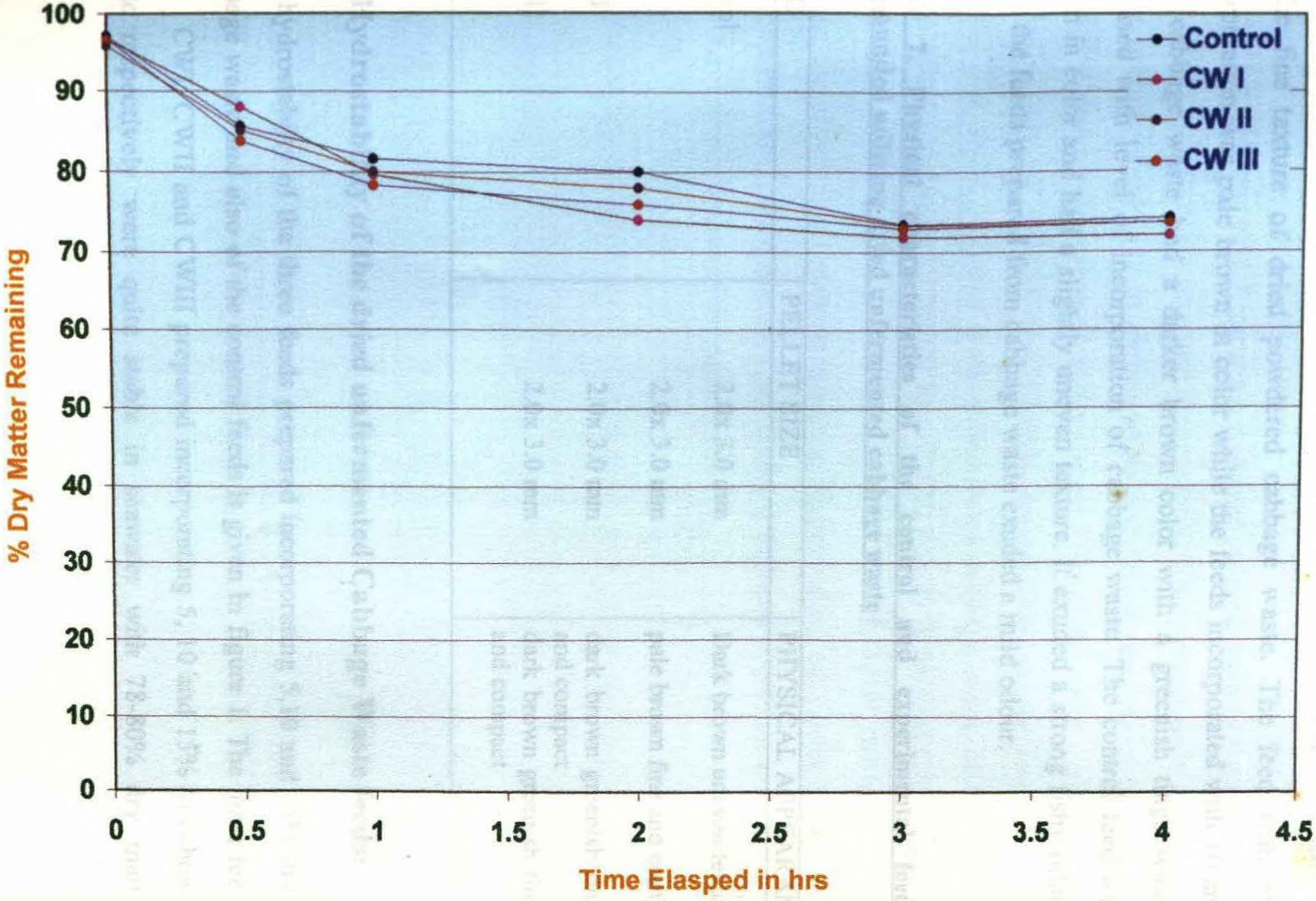
** Nitrogen Free Extractives- calculated as (100-%crude protein +crude fat+ ash + crude fiber+moisture)

***ND – Not Detected.

II. Proximate composition of control and experimental feeds compounded using cooked, dried unfermented cabbage waste:

Three feeds were formulated incorporating 5,10 and 15% of cooked, dried and unfermented cabbage waste in the feed base given in Table 4 (Plate IV) by partly replacing fish meal and shrimp meal. After compounding the feeds were dried to less than 10% moisture and analysed for their physico-chemical characteristics.

Fig: 1 Hydrostability of feeds compounded with unfermented dried cabbage waste (CW)



Physical characteristics of the feeds:

The physical characteristics of the three feeds prepared by incorporating three levels of dried cabbage waste at 5, 10 and 15% and designated as CWI, CWII and CWIII are elaborated in Table 7. A striking characteristic was the uniformity in pellet shape maintained by the three feeds, which may be attributed to the fine texture of dried powdered cabbage waste. The feed with 5% incorporation was pale brown in color while the feeds incorporated with 10 and 15% cabbage waste had a darker brown color with a greenish tinge which increased with level of incorporation of cabbage waste. The control feed was brown in color and had a slightly uneven texture. It exuded a strong fishy odour while the feeds prepared from cabbage waste exuded a mild odour.

Table 7. Physical characteristics of the control and experimental feeds compounded utilizing dried unfermented cabbage waste

FEED	PELLET SIZE	PHYSICAL APPEARANCE
Control	2.0x 3.0 mm	Dark brown uneven texture
CWI	2.0x 3.0 mm	pale brown fine and compact
CWII	2.0x 3.0 mm	dark brown greenish tinge fine and compact
CWIII	2.0x 3.0 mm	dark brown greenish tinge fine and compact

Hydrostability of the dried unfermented Cabbage Waste feeds:

The hydrostabilty of the three feeds prepared incorporating 5,10 and 15% dried cabbage waste and also of the control feeds is given in figure 1. The three feeds viz. CWI, CWII and CWIII prepared incorporating 5, 10 and 15% of cabbage waste respectively were quite stable in seawater with 78-80% dry matter remaining at the end of one hour. This did not decrease any further, so that by the end of 4 hours only a marginal additional loss ranging from 5 to 8% was recorded in the case of these feeds showing them to be highly stable designed specifically for shrimp feeding. The control feed gave similar water stability.

Proximate composition of the unfermented feeds:

Feeds CWI, CWII and CWIII recorded moisture contents ranging between 3.3 to 4.2% (Table.8). Feed CWI prepared incorporating 5% cabbage waste recorded a protein content of 41.01% as compared to 43.39% of the control feed, while feeds CWII and CWIII prepared using 10 and 15% of the dried cabbage waste recorded values of 38.60% and 37.03% respectively as the crude protein contents. The feeds prepared from unfermented cabbage waste had lower fat content ranging between 5.15% for feed CWIII to 5.87% for feed CWI, than the control feed which recorded a fat content of 6.18%. However, the reverse was the case for crude fiber contents wherein, higher values of 2.64%, 3.10% and 3.10% respectively were obtained for feeds, CWII and CWIII as compared to 2.05% crude fiber content of the control feed.

Table.8: Proximate chemical composition of the control and experimental feeds compounded utilizing varying concentrations of dried cabbage waste.

NUTRIENT	FEEDS*			
	Control	CWI	CWII	CWIII
Dry matter	97.048	96.597	95.774	96.704
Moisture	2.95	3.403	4.226	3.296
Crude Protein	43.391	41.011	38.903	37.033
Ether extract	6.182	5.871	5.687	5.152
Crude fiber	2.051	2.637	3.104	3.097
Ash	10.771	10.382	9.958	9.103
Acid insoluble ash (AIA)	0.616	0.508	0.463	0.216
NFE**	34.653	36.696	38.421	42.319

•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)

Ash content decreased slightly in the cabbage waste incorporated feeds with feeds CWI, CWII and CWIII recording 10.38%, 9.96% and 9.10% ash. The ash content of the control feed was 10.77%. A marginal increase was also obtained in the carbohydrate contents of the feeds prepared incorporating 5 to 15% cabbage waste. Here carbohydrate content increased from 34.65% in the control feed to 36.70% upon 5% incorporation of cabbage waste. This increased further from 38.421% upon incorporation of 10% cabbage waste to a higher value of 42.32% upon incorporation of 15% cabbage waste. Acid insoluble ash content decreased significantly from 0.62 % of the control feed to a value of 0.22% in feed CWIII incorporated with 15% cabbage waste.

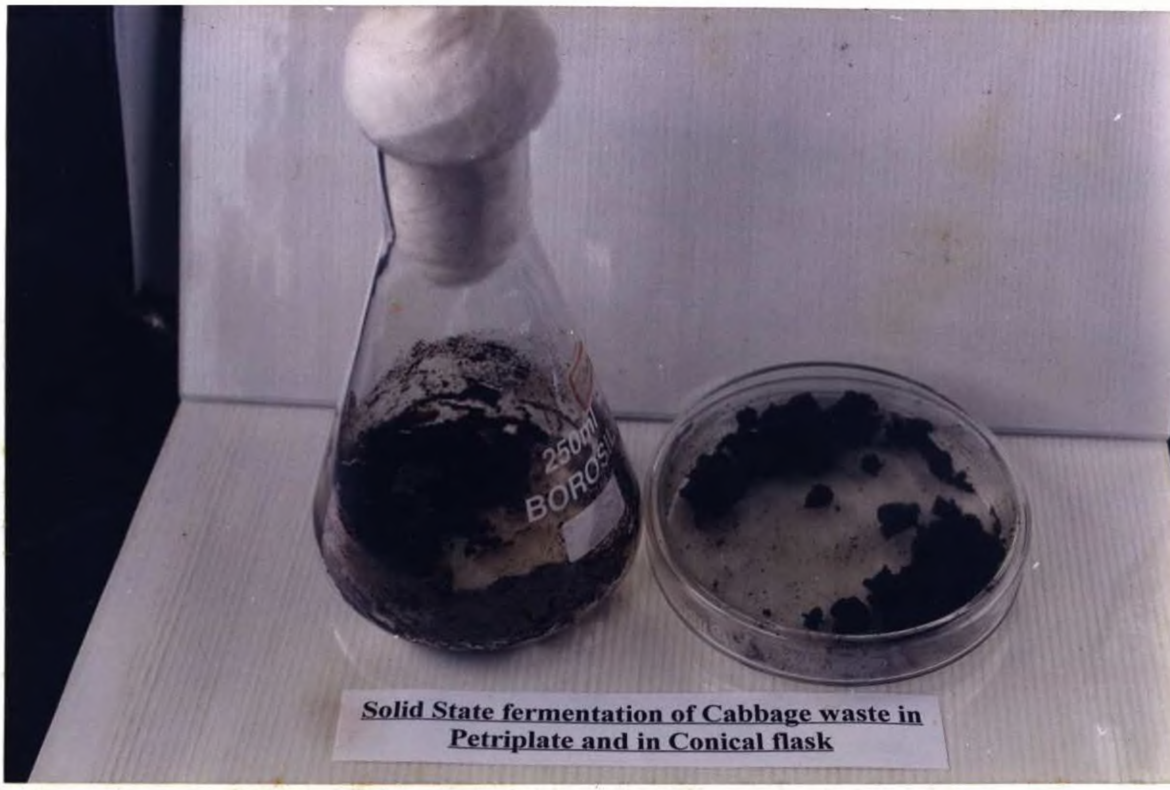


Plate V: Solid State Fermentation of cabbage waste in petri plate and in conical flask with *B. coagulans*.

Plate VI: Solid State Fermentation of Cotton seed cake in petri plate and in conical flask with *B. coagulans*.

Plate VII: Solid State Fermentation of Gingelly oilcake in petri plate and in conical flask with *B. coagulans*.

Plate VIII : Dried cabbage waste after being subjected to fermentation with *B. coagulans*.

Solid State Fermentation of cabbage waste (CW), cotton seed cake (CSC) and gingelly oilcake (GOC):

One non-conventional plant ingredient viz. cabbage waste and two and conventional plant ingredients viz. Cottonseed cake and gingelly were subjected separately to Solid State fermentation using bacteria (*B.coagulans*) and Fungi (*Beauveria sp.*). In the case of fermentation of various substrates carried out employing bacteria, there was no physical change in appearance apart from the fermented odour obtained upon complete fermentation after 48 hrs (Plate V to VII). Dry matter content of dried fermented cabbage waste increased to 99.67% while there was a concomitant decrease in moisture to 0.33%, (Plate VIII, Table 9), A mild increase was obtained in the crude protein with a value of 16.45% being recorded after SSF with *B.coagulans*. Fat content of fermented Cabbage waste showed no change with a value of 1.55% being obtained. A slight decrease was observed in the case of crude fiber and ash contents of *B.coagulans* fermented cabbage waste, which reported values of 11.74% for the former and 13.59% for the later. A value of 56.34% was recorded as the carbohydrate content while acid insoluble ash remained static at 0.09%. In the case of dried fermented cotton seed cake (Plate IX) with 99.28% dry matter and 0.72% moisture, crude protein content recorded a marginal increase recording a value of 29.68%. There was a concomitant decrease in lipid where 4.49% was recorded as the crude fat content (Table 9).

Plate IX : Dried Cotton seed cake after being subjected to fermentation with *B. coagulans*

Plate X : Dried Gingelly oilcake after being subjected to fermentation with *B. coagulans*

Table.9: Proximate chemical composition of cabbage waste, cottonseed cake and gingelly oilcake after fermentation with *B.coagulans*

Nutrient	SUBSTRATE*		
	CW	CSC	GOC
Dry matter	99.668	99.276	99.599
Moisture	0.332	0.724	0.401
Crude Protein	16.453	29.68	39.589
Ether extract	1.548	4.49	8.54
Crude fiber	11.739	17.28	2.93
Ash	13.588	7.707	15.421
Acid insoluble ash (AIA)	0.088	0.237	1.299
NFE**	56.34	40.519	33.451

* 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)

Fermentation of cotton seed cake with *B.coagulans* resulted in a mild increase in both crude fiber (17.28%) and ash contents (7.71%). There was no significant change in the carbohydrate and AIA contents of cotton seed cake fermented with *B.coagulans* as a value of 40.52% was recorded for the former and a value of 0.24% for the later.

Solid State Fermentation of gingelly oilcake recorded a dry matter content of 99.6% and moisture content of 0.4%. Protein increased in this conventional ingredient from the unfermented content of 34.4% to 39.59% (Table 9, Plate X)). Though crude fat, Crude fiber and ash reported mild increases regarding values of 8.54%, 2.9% and 15.42% respectively a decrease was observed in the case of carbohydrate content with a value of 33.45% being recorded as the carbohydrate of gingelly oilcake after fermentation with *B.coagulans*. The acid insoluble ash content however remained unaltered.

Plate XI: Solid state fermentation of cabbage waste, cotton seed cake and gingelly oil cake in petri plates with *Beauveria sp.*.

Plate XII: Solid state fermentation of cabbage waste, in conical flasks with *Beauveria sp.*.

In substrates fermented using *Beauveria sp.* there was a visible change in color and strong mouldy odour predominantly in the case of cottonseed cake and gingelly oilcake, but less so in the case of cabbage waste. Spore formation was visible as early as 48hrs after inoculation, and fermentation was complete within 60 hrs in all the three substrates. (Plates XI to XIII). All the three substrates also recorded an increase in protein content and this increase was higher in comparison to that obtained in the case of the fermentation with bacteria, *B.coagulans*. In cabbage waste protein content increased from an initial value of 15.43% to 20.14% and from 27.62% and 34.41%, the initial values of cottonseed cake and gingelly oilcake to 31.44% and 42.63% respectively (Table 10). Moisture contents recorded were higher at 5.17%, 8.36% and 6.32% respectively for the three substrates viz. cabbage waste, cottonseed cake and gingelly oilcake. Fermentation with *Beauveria sp.* fungi caused a decline in the crude fat, crude fibre and carbohydrate of all the three substrates viz. Cabbage a waste, cottonseed cake and gingelly oilcakes. Values recorded for crude fat were 1.05% for CW, 8.36% for CSC and 6.53% for GOC while corresponding values of crude fiber and carbohydrate recorded in the three substrates were 8.43, 5.33 and 0.99% and 50.95, 42.57 and 26.21% respectively. Acid insoluble ash contents of the three substrates failed to record any significant variations.

Changes in percentage of some biochemical parameters upon solid state fermentation of cabbage waste, cotton seed cake and gingelly oilcake are represented in Table 11.

Table.10: Proximate chemical composition of cabbage waste, cottonseed cake and gingelly oilcake after fermentation with *Beauveria sp.* fungi

Nutrient	SUBSTRATE*		
	CW	CSC	GOC
Dry matter	94.826	91.637	93.686
Moisture	5.174	8.363	6.317
Crude Protein	20.139	31.443	42.63
Ether extract	1.046	2.864	6.532
Crude fiber	8.431	5.326	0.986
Ash	14.261	9.432	17.326
Acid insoluble ash (AIA)	0.068	0.212	1.042
NFE**	50.949	42.572	26.209

* 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)

Table 11. Changes in percentage of some biochemical parameters upon solid state fermentation of cabbage waste, cotton seed cake and gingelly oilcake.

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
	Beauveria sp.	- 0.464	5.044	-0.473	-6.397	3.018	-1.193
Cotton seed cake (CSC)	*B.coagulans*	0.205	2.02	-0.75	0.92	1.688	-3.473
	Beauveria sp.	- 7.434	6.435	-2.148	-10.67	4.217	2.169
Gingelly oilcake (GOC)	*B.coagulans*	0.775	4.925	-0.363	1.02	1.105	-6.377
	Beauveria sp.	-5.141	10.68	-1.965	-0.845	-1.383	-11.987

* 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.

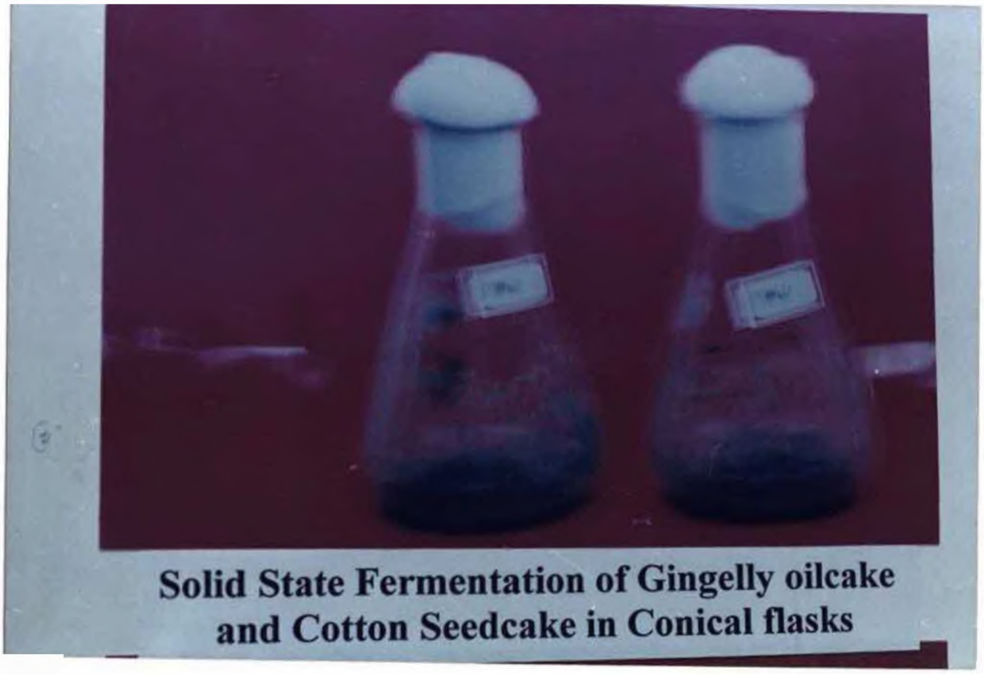


Plate XIII: Solid state fermentation of gingelly oil cake and cotton seed cake in conical flasks.

Plate XIV: The experimental feeds compounded incorporating with 5, 10 and 15 % of dried cabbage waste fermented using *Bacillus coagulans.*

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

Three feeds were formulated incorporating 5, 10 and 15% cabbage waste fermented using *B.coagulans* in a manner similar to that adopted for compounding unfermented cabbage waste feeds. These were dried to less than 10% moisture after compounding and analysed for their physico-chemical characteristics.

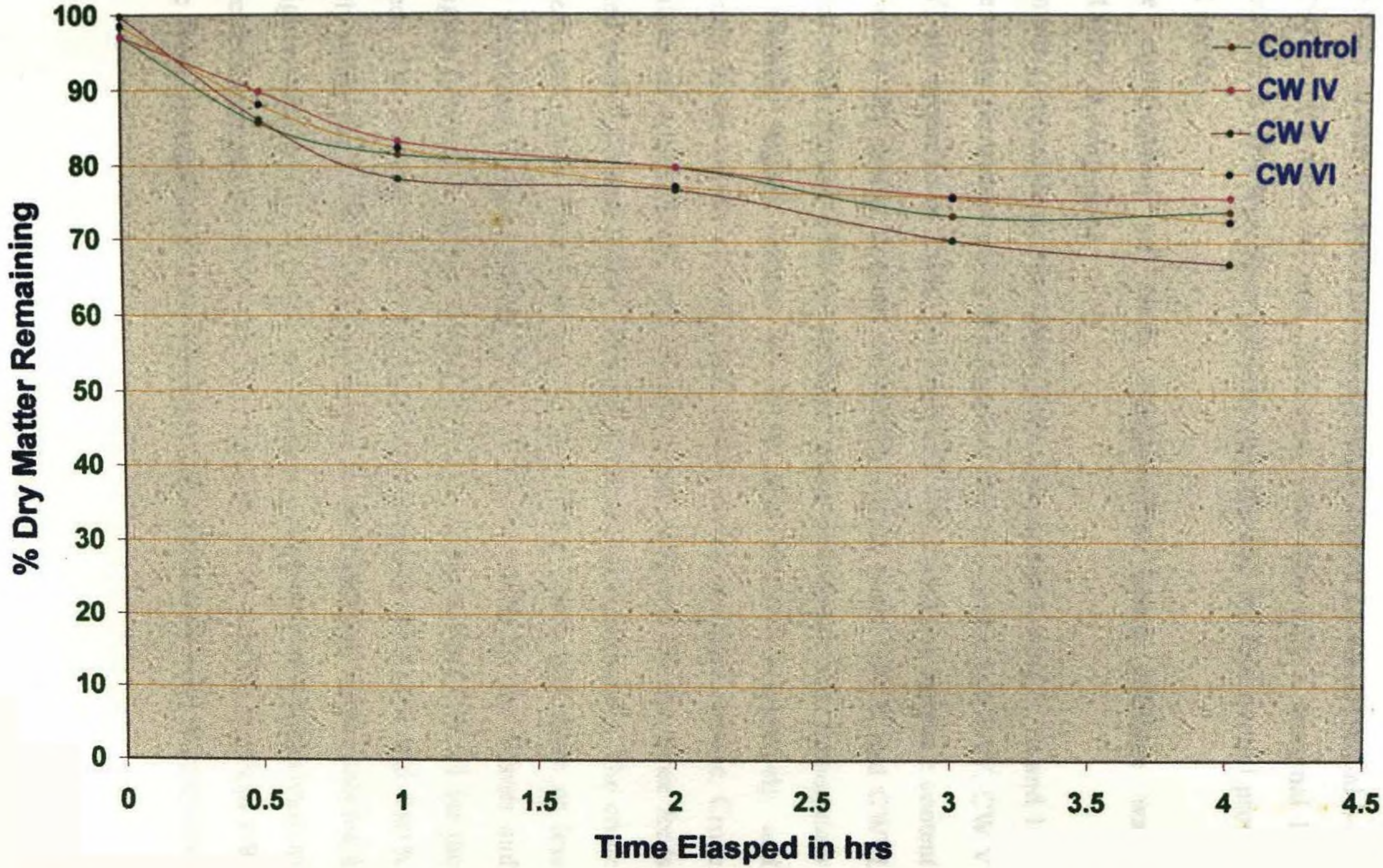
Physical Characteristics of feeds prepared from cabbage waste fermented with *B.coagulans*

The physical characteristics of the three feeds prepared incorporating 5, 10 and 15% levels of cabbage waste fermented with *B.coagulans* and designated as CWIV, CWV and CWVI are elaborated in Table 12 and Plate XIV .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 12. 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

Fig: 2. Hydrostability of feeds compounded with cabbage waste fermented using *B. coagulans*



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

Feeds CW IV, CW V and CW VI 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 CWIV, CW V and CW VI are recorded in Table 13. Feed CW VI recorded a moisture content of 2.94% and a dry matter content of 98.06%, while feeds CW V and CWVI 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 IV to 1.23% in Feed CW VI for the latter. Feeds CW IV and CW VI reported ash contents of 9.11% and 9.01% respectively while feeds CW V 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 VI to 0.548% for Feed CW IV. These feeds incorporated with cabbage waste fermented with *B.coagulans* reported lower carbohydrate contents ranging between 36.25% for feed CW IV (5% CW) to 41.35% for feed CW VI (15% CW).

Table.13: 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)

Proximate composition of experimental feeds compounded with cabbage waste fermented using *Beauvaria sp.*

Three feeds were formulated incorporating 5, 10 and 15% of cabbage waste fermented using *Beauvaria sp.* fungi in the same manner as adopted for compounding unfermented cabbage waste feeds. (Plate 4). These were dried to less than 10% moisture after compounding and analysed for their physico-chemical characteristics.

Physical characteristics of the feeds compounded using cabbage waste fermented with *Beauveria sp.*:

The physical characteristics of the three feeds compounded by incorporating 5,10 and 15% of cabbage waste fermented using *Beauveria sp.* fungi and designated

Fig: 3. Hydrostability of feeds compounded with cabbage waste fermented using *Beauveria sp*

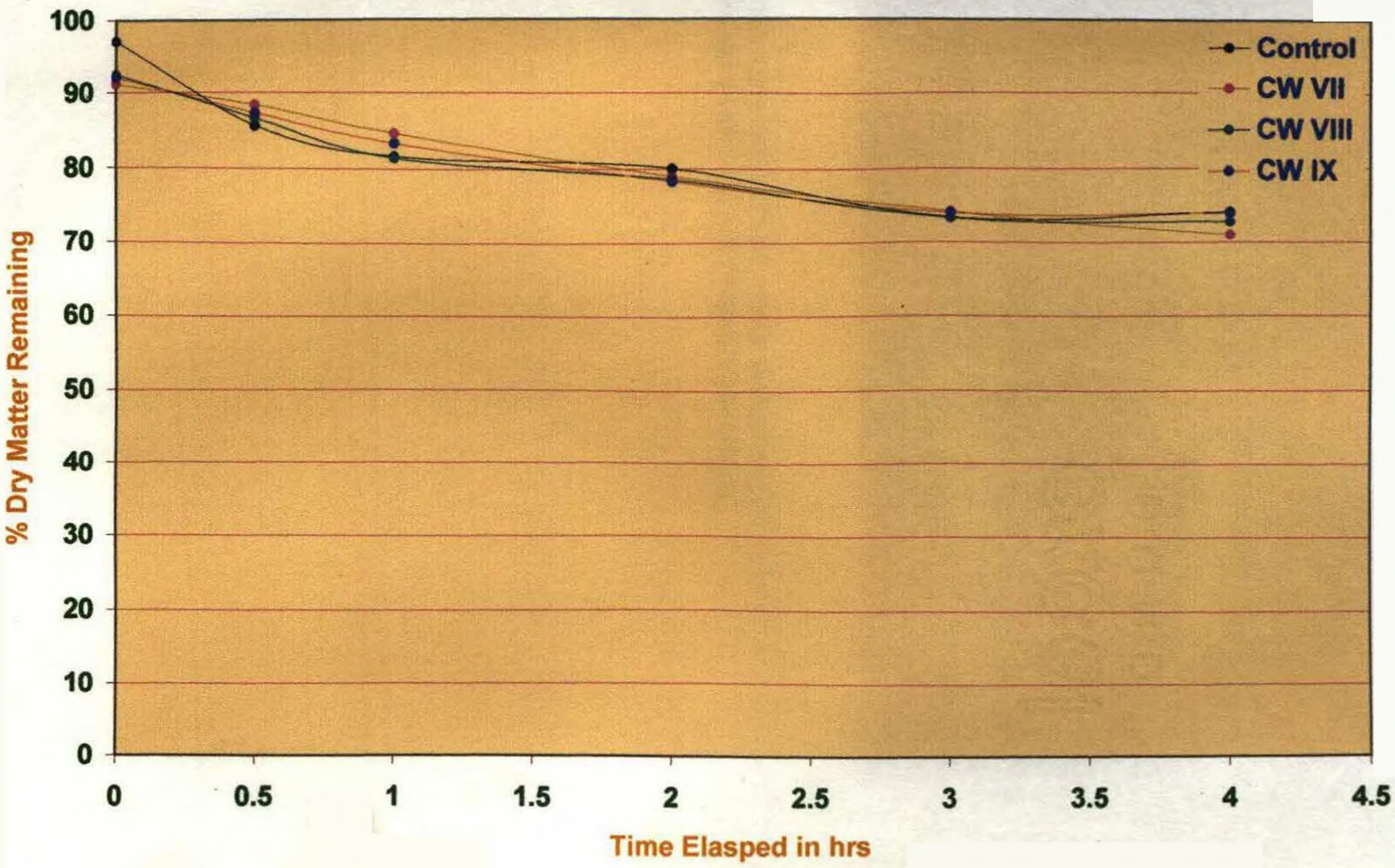


Plate XV: The experimental feeds compounded incorporating with 5, 10 and 15 % of dried cabbage waste fermented using *Beauveria sp.*.

as CWVII, CWVIII and CWIX are elaborated in Table 14 and Plate XV. All the three feeds showed a uniform texture but not as fine as in the case of feeds compounded using cabbage waste fermented with *B.coagulans.* The three feeds had a pale brown color with a whitish tinge on account of the fungus, in comparison to the control feed which had a brown color and an uneven texture. These feeds had a strong fungal odor.

Table 14. Physical characteristics of the control and experimental feeds compounded utilizing cabbage waste fermented with *Beauveria sp.* fungi

FEED	PELLET SIZE	PHYSICAL APPEARANCE
Control	2.0x 3.0 mm	Dark brown uneven texture
CW IV	2.0x 3.0 mm	Brown compact
CW V	2.0x 3.0 mm	Dark brown compact fine texture
CW VI	2.0x 3.0 mm	Dark brown compact fine texture

Hydro stability of the feeds compounded using cabbage waste fermented with *Beauveria sp.*

The hydrostabilty of the three feeds prepared incorporating 5, 10 and 15% of cabbage waste fermented using *Beauveria sp.* is given in Figure 3. These three feeds viz. CWVII, CWVIII and CWIX prepared incorporating 5,10 and 15% of cabbage waste fermented using *Beauveria sp.*were also quite stable in seawater with 81 to 85% dry matter remaining at the end of one hour. After that there was an additional 11-13% loss in dry matter upto 4 hours in seawater. However, the feeds prove to be stable exhibiting a profile very similar to that of the control feeds.

Proximate composition of feeds compounded using cabbage waste fermented with *Beauveria sp.* Fungi:

The proximate composition of the three feeds compounded using 5, 10 and 15% of cabbage waste fermented using *Beauveria sp.* and designated as CWVII, CWVIII and CWIX are recorded in Table 15 (Plate XV).

Table 15: Proximate chemical composition of the control and experimental feeds compounded utilizing cabbage waste fermented using *Beauveria sp.*

Nutrient	Control	Feeds*		
		CWVII	CWVIII	CWIX
Dry matter	97.05	91.13	92.46	92.07
Moisture	2.95	8.87	7.54	7.93
Crude Protein	43.39	45.24	45.39	45.54
Ether Extract	6.18	5.46	5.3	5.28
Crude Fiber	2.05	2.16	2.42	2.61
Ash	10.77	14.46	15.91	15.44
Acid Insoluble Ash (AIA)	0.62	0.23	0.22	0.2
NFE**	34.65	23.81	23.44	23.2

- Values expressed on dry matter basis.
- ** Nitrogen Free Extractives calculated as (100- % crude protein+crude fat+ash+crude fiber +moisture)

These feeds recorded higher moisture levels ranging between 7.5 to 8.9 %. Protein contents of these feeds was higher than the corresponding feeds prepared from unfermented cabbage waste and ranged from 45.24 for feed CWVII incorporated with 5% of the fermented waste to 45.54 for feed CWIX incorporated with 15% of the cabbage waste fermented with *Beauveria sp.* . Fat content of these feeds was lower than that of the control feed and ranged from 5.28 for CWIX (15% incorporation) to 5.46 in the case of feed CWVII (5%

incorporation). Crude fiber values of 2.15, 2.42 and 2.61 % were obtained for feeds CWVII, CWVIII and CW IX respectively and though these were higher than the crude fiber content of the control feed wherein value of 2.05 was recorded, they were comparatively lower to those recorded in the case of feds prepared incorporating 5,10 and 15% of unfermented cabbage waste. The reverse was the case with regard to ash content as values ranging between 14.46% in the case of feed CWVII to 15.91% in the case of CWVIII were recorded which were higher than 10.77% ash of the control feed and also of the three feeds prepared incorporating 5, 10 and 15% of the dried unfermented cabbage waste. Concomitantly, the carbohydrate contents of these three feeds compounded incorporating 5, 10 and 15% of cabbage waste decreased with values of 23.81, 23.44 and 23.20% respectively being recorded. The acid insoluble ash did not vary significantly among these feeds as compared to the feeds prepared utilizing the unfermented cabbage waste but were significantly lower than that of the control feed wherein a value of 0.62% was recorded for the AIA.

DISCUSSION

The present study was designed with the purpose of testing the suitability of cabbage waste (*Brassica olaracea var. capitata)* in shrimp feeds as a non-conventional feed ingredient before and after subjecting to solid sate fermentation. The role of Solid State Fermentation in microbial enrichment and improving the nutritional value of this cabbage waste along with cottonseed cake (CSC) and gingelly oilcake (GOC) also deems to be promising for further research. Aquatic feeds have so far solely depended upon the highly nutritious but at the same time expensive animal proteins, of which the contributions of fishmeal has been most outstanding (New, 1976; Tacon, 1993). However, it is now evident that the future success of the aquaculture industry depends on the development of low-cost nutritious feeds, utilizing more of plant proteins.

Commercial aquaculture feeds for shrimp generally contain 25-45% crude protein and consequently high protein oil seed residues have been used of compounding shrimp feeds (New, 1976). Though feedstuffs of vegetable origin as a whole have been reported to be lower in protein when compared to those of animal origin (Tacon, 1993) numerous studies have shown that live plant organic matter is well utilized by fin fish and crustaceans. (New, 1976; Tacon, 1990; Tacon, 1993; Ng and Wee,Viola 1989; Viola *et al.*, 1988; Jia *et al.* 1991; Chow *et al.*, 1983; Ogino *et al.*, 1978; Christensen, 1981; Syslo and Hughes, 1981; Harpaz and Schmalbach 1986; Catacutan 1993; Stern *et al.*, 1976 and Raman *et al.*, 1982). Further, non-conventional ingredients of plant origin have low protein and high carbohydrate and fiber contents in addition to anti-nutritional factors and toxins. Also variability in nutritional composition (New *et al*, 1993) has been attributed to factors like method of handling and processing, the nutritional status of the environment in which they were grown as well as variations in analytical methodologies.

The protein content of plant protein ingredients used in aquafeeds ranges from as low as 2% to as high as 58.6% in the case of soyabean meal (New et al., 1996). Gopalan *et al.*, (1985) showed the protein content of leafy vegetables to range between 1% to 27% in the dried leaves of rape (*Brassica napus*). In the present study the outer leaves of cabbage discarded as waste recorded a moisture of 4.71% and a moderate protein content of 15.43% making it a non-conventional ingredient worth incorporation in aquafeeds owing to availability round the year. Leafy vegetables have low fat content ranging between 0.1 to 4.8 with low to moderately high carbohydrate contents ranging between 2 to 60.9% in the case of tender tamarind leaves (Gopalan *et al.*, 1985). Cabbage waste recorded a low fat content of 1.5% and a relatively high carbohydrate content of 50%. Though certain leafy vegetables have been reported to contain high ash and fiber contents, cabbage waste recorded ash content of 11.46% and fiber content of 14.57%. There are no reports of the presence of any anti-nutritional factors in cabbage and thus cabbage waste was deemed to be suitable ingredient for incorporation into shrimp feeds.

The needs for better feeds has generated a great amount of practical research work covering several fields including not only nutrition, but also the technology of feed manufacture. As use of feeds in aquaculture increases both production and profits considerably, they should be nutritionally adequate at the same time economical. Recommended protein levels vary from 30 to 57% in various species and sizes of marine shrimp While recommended lipid levels for commercial feeds range between 6% to 7.5% and should not exceed 10% (Akiyama *et al.,* 1991). Carbohydrates are not a dietary essential for shrimp feeds, however their usefulness and cost effectiveness are undeniable. In the present study the three feeds compounded incorporating 5, 10 and 15% cabbage waste were very much within the nutritional standards prescribed for shrimp nutrition.

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 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 aquafeed 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 and gingelly oilcake and cottonseed cake using one strain of bacteria (*B.coagulans*) and one strain of fungi (*Beauveria sp.*) in order to evaluate their suitability for aquafeed formulation.

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). These workers reported a 61.08% increase in protein content of wheat bran and 51.08% increase in protein content in groundnut oilcake upon fermentations with *B.licheniformes*. Fermentation with *Beauveria sp.* of both these substrates led to a protein increase of 30% in wheat bran and 31% in groundnut oilcake. However, the reduction observed by them in the dry matter content of all substrates is in agreement with this study.

In this study protein content increased in all three substrates viz. cabbage waste, cottonseed cake and gingelly oilcake upon fermentation with *B.coagulans* as well as *Beauveria sp.*, 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).

Increases in protein in cassava from an initial value of 1.28 gm / 100 gm dry matter to 14.32 gm /100 gm dry matter was recorded by Balagopalan and Padmaja (1998). Direct fermentation of cassava alone, with *Aspergillus, Neurospora* and *Rhizopus* elevated the protein value by 3% using nitrogenous supplements e.g. 25% pineapple bran increased protein from 4 to 5%, whilst a mixture of 12.5% pineapple bran and 12.5% chicken manure increased the protein content to 7%. Soyabean and groundnut were found to be even better additives to facilitate protein enrichment.

Therefore 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.

The results obtained are very encouraging and clearly indicate that nutritionally inferior substrates can be converted to protein enriched digestible ones for incorporation into aquafeeds.

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.

SUMMARY

1. Cabbage (*Brassica olaracea var. capitata*) waste recorded a moderate protein content of 15% and a low fat content of 1.50%.
2. Dry matter of these cooked, dried and powdered leaves was 95.29% with moisture content of 4.71%.
3. The crude fibre content of 14.57% was high while ash content was moderate at 11.46%.
4. A value of > 50% was obtained as the Nitrogen Free Extractives while acid insoluble ash content was negligible at 0.09%.
5. Cottonseed cake (CSC) recorded 28% protein while gingelly oilcake (GOC) recorded 34.4% protein.
6. Values of 5.22% and 8.83% respectively were obtained for the lipid content of CSC and GOC respectively.
7. CSC had a high fiber content of 16.33% while GOC recorded a low value of 1.88%.
8. CSC and GOC recorded ash content and NFE values of 6.02 and 43.88% for the former and 14.21 % and 39.4% for the later respectively.
9. Feeds compounded with 5, 10 and 15% unfermented cabbage waste showed good texture with a conspicuous green tinge.
10. These three feeds had 78 to 80% dry matter remaining at the end of 1 hr recording an additional 5 to 8% loss at the end of 4 hrs. These results were comparable to the control feed.
11. Feed CW I prepared incorporating 5% cabbage waste recorded a protein content of 41.01%, while feeds CW II and CW III prepared incorporating 10 and 15% dried cabbage waste recorded values of 38.60% and 37.03% crude protein. The control feed had a protein content of 43.39%.
12. These three feeds recorded low fat contents ranging from 5.15% for feed CW III to 5.87% for feed CW I. The control had a fat content of 6.18%.
13. Crude fiber content of these feeds was high ranging from 2.64% for CW I to 3.10% for feed CW III, while the control feed recorded a fiber content of 2.05%.

14. Ash content decreased with increase in level of incorporation of cabbage waste from 10.77% for the control to 9.10% in feed CW III incorporated with 15% unfermented CW.
15. Substrates fermented with *B.coagulans* recorded only a marginal increase in protein content with CW, CSC and GOC recording increases of 0.314, 2.02 and 4.925% respectively.
16. In solid state fermentation with *B.coagulans* an increase in dry matter content was observed (0.205 in CSC to 4.378 in CW) while fat recorded a decrease of 0.023 in CW, 0.75 in CSC and 0.363 in GOC.
17. Favorable changes were also recorded in these three substrates with regard to fiber, ash and NFE.
18. Feeds compounded incorporating 5, 10 and 15% CW fermented with *B.coagulans* recorded uniformity in pellet shape and compactness.
19. Hydrostability of these feeds incorporating 5, 10 and 15% *B.coagulans* fermented CW and designated CW IV, CW V and CW VI recorded a dry matter loss ranging between 10 to 13%, with an additional loss of 6 to 8% within the next 30 min.
20. Feed CW VI recorded a moisture content of 2.94% and a dry matter content of 98.06% while feeds CW V and CW VI incorporated with 10 and 15% cabbage waste fermented with *B.coagulans* recorded values of 99.79 and 99.51% respectively.
21. 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%.
22. Feeds compounded by incorporating 5, 10 and 15% CW fermented using *Beauveria sp.* fungi and designated as CW VII, CW VIII and CW IX had a pale brown colour with a whitish tinge characteristic of fungal fermentations.
23. These three feeds were quite stable in seawater with 81 to 85% dry matter remaining at the end of one hour.
24. Feeds CW VII, CW VIII and CW IX compounded incorporating 5, 10 and 15% CW fermented using *Beauveria sp.* recorded higher moisture levels ranging between 7.5 to 8.9 %.

25. Protein contents of these feeds was higher than the corresponding feeds prepared from unfermented cabbage waste and ranged from 45.24 for feed CW VII to 45.54 for feed CW IX incorporated with 15% CW.
26. Fat content of these feeds was lower than that of the control feed and ranged from 5.28 for CW IX (15%incorporation) to 5.46 for CW VII (5% incorporation).
27. These three feeds recorded values of 2.15, 2.42 and 2.61 % respectively and ash contents of 14.46% in case of feed CW VII to 15.91% in the case of feed CW VIII.
28. Feeds incorporating 5 to 15% of CW fermented with *Beauveria sp.* recorded carbohydrate contents of 23.81, 23.44 and 23.20% respectively which was lower to that recorded in the case of unfermented cabbage waste feeds and feeds prepared incorporating CW fermented with *B.coagulans*.

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