**Lactic acid fermentation of shrimp waste**

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LACTIC ACID FERMENTATION

OF

SHRIMP WASTE

By

L.L.S.S.K. DE SILVA

A Master's Thesis
Submitted in partial fulfilment of the requirements
for the award of:
Master of Philosophy of the Loughborough University of Technology.

March 1989
Loughborough University of Technology
Dedicated to my parents
ACKNOWLEDGEMENTS

I also wish to acknowledge the guidance of my supervisor, Dr G M Hall in carrying out this research project. Gratitude is also extended to Dr Roy Protheroe for editing a part of the thesis and also Mr D J Smith for technical assistance provided in carrying out the project.

The technical advice offered by the staff of Overseas Development Natural Resources Institute (ODNRI) and the assistance in providing literature is also acknowledged.

This project was carried under the technical assistance programme of the Government of United Kingdom. I wish to acknowledge the assistance provided by the Overseas Development Agency in arrangements with the University of Technology, Loughborough and the British Council in coordinating the fellowship and making travel arrangements.

I also wish to acknowledge the Council of the Sri Lanka Standards Institution for granting study leave for me to undertake this project.
ABSTRACT

Key words: Lactic Fermentation, Shrimp Waste, Chitin, Astaxanthin, Shrimp Feed, Cassava.

The enzymic hydrolysis of shrimp waste, stabilised using an acid fermentation technique, was observed to develop a mechanism to separate and isolate useful products, such as proteins, chitin and astaxanthin. Initially the effectiveness of the fermentation technique was studied with added carbohydrate (lactose) with an inoculum (Stabisil, comprising of *Streptococcus faecium* strains M74, *Lactobacillus plantarum* and *Pediococcus acidilacti*).

With 20:2 (% w/w of waste) lactose and Stabisil the pH dropped below 4.0 after 5 days and remained at this level for 21 days, with 57.5% protein nitrogen separating in the liquor: 68.8% chitin remained in the residue. The astaxanthin remained in the residue and was readily soluble in chloroform.

Cassava was successful as a natural carbohydrate source for shrimp waste fermentation with the pH dropping to 4.2 after 5 days, in a 1:1 w/w combination with shrimp waste, no further drop of pH was noted in a 1:1 combination with pre-fermented cassava.

Shrimp waste: cassava (2:1) decomposed after 3 days while with pre-fermented cassava the pH dropped to 4.4 after 7 days and to 4.1 after 14 days and remained below 5.0 after 21 days.
With 1:1 pre-fermented cassava 63.6% of protein nitrogen was reported in the liquor and all chitin was left in the residue. The highest increase in the liquor level of 315% was observed with 1:1 shrimp waste pre-fermented cassava.

A combination of fresh or pre-fermented cassava and shrimp waste (1:1) with 2\% (w/w) stabisil, gave a pH of 4.1 and 4.4 after 7 days respectively.

Fresh or pre-fermented cassava (2:1) with 2\% (w/w) stabisil added gave a pH of 4.4 and 4.5 respectively after 7 days.

Shrimp waste with boiled cassava (with 2\% w/w stabisil added) in a 1:1 or 2:1 mixture gave a pH of 4.4 after 7 days in both cases.
ACKNOWLEDGEMENTS.

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1. INTRODUCTION

The demand for fish and fishery products is on the increase as fish products are preferred over other meat products partly due to health reasons. The technology has improved to increase the catch and regulations drafted to protect fishing grounds. Improved processing techniques introduced and stringent measures introduced to reduce spoilage and to improve the quality of processed fish.

It is estimated that approximately 25 per cent of fish caught in the tropics may be lost before consumption (ILO, 1982) due to many reasons. Spoilage due to inadequate facilities for preservation is observed to be one of the main factors especially in the tropical countries.

Accurate statistics on fish discarded as waste are not available and fishery by-products from waste and by-catch represent almost one-third of the world catch (Windsor and Barlow, 1981). Recovery of useful products from waste is drawing increased attention as these provide an additional supply of proteins both for human and animal consumption. Also many industries such as pharmaceutical depend on the products recovered from the fish waste for raw materials.

Utilisation of Antarctic Krill (Euphausia superba) is also drawing attention and is considered the largest source of proteins from the sea (Suzuki, 1981).

Among the fish consumed as a source of protein the demand for shellfish had increased throughout the world and shrimp processing in many
developing countries and investments on increased catching processing and also farming are encouraged due to high income and also the employment generated in many stages of the industry.

World landings of shrimp in 1981 were around 1.7 million tons and the supply from developing countries amounted to about 66 per cent of the total world production. The production of farmed shrimps in 1983 was in the range 80,000 to 100,000 tons annually (ITC, 1983). Many new exporting countries such as China, Vietnam and Gambia have entered the shrimp trade on a broader scale. Shrimp farms are established in many countries and countries such as Taiwan, Equador and Panama are heavily dependent on farmed shrimps to exceed the supply from the sea.

The utilisation of shrimp waste is receiving attention of many countries as a considerable quantity of waste is produced from the expanded shrimp processing industry. The waste consists of proteins and chitin among other recoverable products, especially in the head waste. This waste is not properly utilised in many countries and even has caused an environmental problem (Meyers, 1986). The carapace represents nearly 70 per cent of the shrimp (Meyers and Benjamin, 1987), and in tropical shrimps the head consists of 34 to 45 per cent of the shrimp by weight and further 10 to 15 per cent in tail and swimmeretts (Barratt and Montano, 1986). Chitin, astaxanthin and proteins in the shrimp waste are basically used for fish meal but a large quantity is discarded.
Due to increased competition the international trading projects directed towards utilisation of the waste are considered favourably as these will strengthen the economic viability and the competitiveness of the industry.

The fermentation method for the conservation of fish waste has a number of features which make it attractive for operation in tropical developing countries. Among the factors which favour the adoption of fermentation are: the low cost, the possibility of using locally available carbohydrates and also the low technology needed compared with that for other preservation techniques such as refrigeration and canning.

With the demand for fish meal increasing, waste from fish processing operations and underutilised species was evaluated as alternative sources of protein for animal feed. The use of fermentation or acid addition to produce fish silage was studied as a way of producing fish protein for use in animal feed (Van et al., 1983).

The biological fermentation referred to as lactic acid fermentation is widely practised in South-East Asian countries. The use of local sources of carbohydrates such as tapioca starch as an energy source was studied and a successful fish silage was established using 1:1 fish/starch ratio and with lactic acid starter cultures (Peterson, 1953; March, 1962 and Stanton and Yeoh, 1977).

The advantages reported with the use of fish silage include improving the quality of eggs and broiler meat with improved calcium uptake.
(Wirahadikusumah, 1969) and Norwegian research on the effect of the fish silage on egg production in hens (Windsor, 1974) drew increased attention to the development of fermentation of fish waste.

The ensilation of shrimp waste reported in Norway enabled the recovery of many industrially useful products such as chitin, chitosan and astaxanthin and the process was found to be economical (Raa, Gildberg and Strom, 1983).

The use of ensilation in developing countries was considered less favourably due to technical hazards involved with the corrosive acids used and also the costs involved. Whereas biological fermentation technology was practised in many developing countries specially in the South-East Asian countries.

Areas needing further research to develop fermented fish products include starch utilisation with the possibility of using cheaper sources such as cassava to reduce the production costs. Work on the role of salt with the possibility of reducing or replacing its usage should be considered in detail for improving the palatability as well as improving the effectiveness of the autolysis.

Further, the information on the characteristics of suitable strains of lactic acid bacteria for inoculation into fish/starch mixtures, the factors affecting fermentation i.e. development of lactic acid bacteria, could assist in the use of local starter cultures. Studies on spoilage bacteria and food poisoning
organisms and their effect on the organoleptic properties are essential in improving the production and acceptability of these products. Methods for maintaining the organoleptic quality are considered important as a uniform product quality needs to be maintained to undertake commercial scale production of these products. This research could enable the application of fermentation technology to improve the economic status in many developing countries.

Among the fermented fish products those of shrimps are consumed in the South-East Asian region and the fermentation of small varieties of whole shrimps are commonly observed. The application of lactic acid fermentation for shrimp waste were made with varying success (Putro, 1980). The studies on shrimp waste were more directed for isolating chitin and associated products and most work in this field is associated with the use of acids (Rama Chandran Naik et al., 1986).

Information on the potential for utilisation of protein matter found in the shrimp head-waste is limited, and difficulties were observed in the initial stages of the fermentation process and shrimp waste is generally considered as a raw material less suitable for the application of lactic acid fermentation (Bawa H & Mon Kano, 1986).

This project was undertaken to develop an intermediate level low cost technology to utilise shrimp waste in developing countries and this needs to be followed up with further research to recover and industrial scale production.
2. UTILISATION OF FISH AND FISH BY-PRODUCTS:

Unexploited fish resources which could enormously contribute to the world protein supply such as Antarctic Krill alone constitute a quantity equal to the whole of the rest of the Accurate statistic on the total potential of the world's fish supply from the ocean are lacking and it is estimated that around 100 - 150 million tonnes with a further 30 - 80 million tonnes as a possible supply. The potential contribution from the fish farms are also expected to play a significant role in increasing the world fish supply situation (FAO, 1987).

Even where fishing is developed for human consumption, fishery by-products have a large role to play as the maximum yield from fish will only be about 50 per cent edible material in the form of fillet of fish flesh. The remainder such as the frame and head is almost as high in protein content as the fillet but is not normally eaten. The waste from the canning industry such as off cuts could be utilised for by-products manufacture.

A major proportion of the fish caught is consumed in the fresh form, although the quantity consumed in this form is decreasing. In 1973 consumption of fresh fish amounted to nearly 43 per cent of the total consumed and in 1986 the total fresh fish consumed was 20.0 per cent of the total catch of 91,457 tonnes (FAO, 1987). The consumption of frozen fish has increased from 10 per cent in 1960 to 25 per cent in 1973 and remained static around 23 per cent in 1986 (Table 1). However, the production of frozen fish has not increased markedly in the developing world. A remarkable increase in the consumption of frozen fish has been reported in a number of developing countries and this trend is expected
to continue in the developing world both for exports as well as domestic markets.

Canned fish production has shown a steady increase and in 1973, it has accounted for 15 per cent of fish for food as compared to 12 per cent in 1960. This remained stable through 80's and amounted to 13.4 in 1986. Although the cured fish production (salted, dried and smoked) has remained relatively stable over the period under examination, the proportion of the catch of fresh fish processed in these ways has declined from 24 per cent in 1960 to 17 per cent in 1973 and decreased to 13.4 in 1986.

The share of the world's catch for reduction to fish meal and oil had reached a maximum of about 40 per cent in the late 1960's but has fallen to around 30 per cent in 1973-74 and this trend continued to 1986. Accurate statistics on other products such as those fermented are not available.

To provide the whole of the world's population with a consumption per capita equivalent to that at present in the developed countries, would require between 280 and 300 million tons in the year 2000.

It is obvious that vast improvements in production, handling, processing and distribution will be required to fulfill these demands.

The fish reserves not exploited due to lack of catching technology or not utilised for processing traditional products and those considered less suitable has an enormous potential in increasing the world fish supply. The production of fishmeal and oil only contribute
indirectly to protein nutrition and has limited effect in improving the nutritional standards.

Table 1. Pattern of fish utilisation (per centages);

<table>
<thead>
<tr>
<th></th>
<th>81</th>
<th>82</th>
<th>83</th>
<th>84</th>
<th>85</th>
<th>86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total world catch</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Marketing fresh</td>
<td>20.1</td>
<td>18.3</td>
<td>18.3</td>
<td>18.3</td>
<td>18.3</td>
<td>20.0</td>
</tr>
<tr>
<td>Freezing</td>
<td>23.0</td>
<td>24.2</td>
<td>24.4</td>
<td>24.1</td>
<td>23.8</td>
<td>23.4</td>
</tr>
<tr>
<td>Curing</td>
<td>15.8</td>
<td>15.5</td>
<td>15.7</td>
<td>14.7</td>
<td>15.2</td>
<td>14.7</td>
</tr>
<tr>
<td>Canning</td>
<td>14.7</td>
<td>13.9</td>
<td>14.3</td>
<td>13.9</td>
<td>13.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Reductions*</td>
<td>25.4</td>
<td>27.1</td>
<td>26.3</td>
<td>28.0</td>
<td>28.1</td>
<td>28.4</td>
</tr>
<tr>
<td>Miscellaneous purposes</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Source FAO (1987)

*Only those destined for the manufacture of oils and meals is included. Raw material destined for marketing fresh, freezing, curing, canning and miscellaneous purposes is excluded, such waste quantities are included under the other disposition channels.

The production of fishmeal and oil showed a decline and is generally considered uneconomical. However it is expected that the demand for fishmeal production is expected to be high as long as the competition of fishmeal in the market for animal feeding remain strong. The trend is expected to be that, the conventional resources of small pelagic fish,
presently converted into fishmeal, will be released for direct human consumption and unconventional resources will be considered for fishmeal production.

Among the steps required to effectively utilise fish catch, it is important that traditional industries are developed in addition to modern processing industries.

In addition to increasing the catch, the reduction of post-harvest losses and recovery from waste could boost the world protein supply from fish. The post-harvest losses is mainly due to poor handling techniques and this could amount to about 25 per cent of the total catch.

The waste which results from the processing activities is increasingly utilised as large quantities of waste is produced from filleting, canning and freezing industries. As stated, in the filleting industry the waste could be about 50 per cent and in shrimp processing industry 40 to 70 per cent of the shrimp is considered as waste.

The main processes where the waste is utilised are:

a) Fish meal - animal and fish feed,
b) Fish oil,
c) Fish silage,
d) Hydrolysed fish products,
e) Fish protein concentrate (FPC)
f) and other fishery by-product industries such as Pet foods, Insulin, Pearl, Leather, Gum and Gelatin, Pharmaceutical products, Chitin, Paints, Lubricants, Grease, and Water repellents.
2.1 Fermented fish products.

In fermented fish products, the proteins are broken down into their constituent peptides and amino acids, and the addition of salt in the traditional products allows the development of characteristic odours and flavours. These products are popular in South-East Asian countries and some of the common sauces and pastes produced are given in Table 2.

The fish is packed with layers of dry salt and left for long periods, liquification of the cellular tissues commences and a 'pickle' is formed. By leaving the fish in contact, the active proteolytic enzymes of the fish liquify the tissues further to produce a fish sauce. Fish sauce can be produced if the liquid is drawn off at intervals, or the period of contact is kept short. By reducing the period of contact to a much shorter time and if the amount of salt used is limited; salted fish which has undergone some softening will be produced.

Many attempts were made to classify the different types of fermented fish products, Subba Rao (1967) divided fermented fish into three groups according to the final appearance. These three groups are;

i) products in which the fish retain substantially their original form or in which large chunks are preserved (eg. pedahsiam (Thailand), makassar (Indonesia) and buro (Philippines);

ii) products in which the fish are reduced to a paste (eg. ngapi (Burma), pra-hoc (Cambodia), belachan/trassi (Malaysia/Indonesia) and Bagoong (Philippines);
<table>
<thead>
<tr>
<th>Country</th>
<th>Product</th>
<th>Fish species used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burma</td>
<td>Nagpi</td>
<td>Anchoviella.</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Ketjap-Ikan</td>
<td>Stolephorus, Clupea, Leiagnathus.</td>
</tr>
<tr>
<td></td>
<td>Trassi (paste)</td>
<td>shrimps.</td>
</tr>
<tr>
<td>Kampuchea</td>
<td>Nuoc-mam-gau-ca</td>
<td>Thynnithys, Rasbora,</td>
</tr>
<tr>
<td></td>
<td>Prahoc</td>
<td>Ophiocephalus, Clarius,</td>
</tr>
<tr>
<td></td>
<td>(paste)</td>
<td></td>
</tr>
<tr>
<td>Laos</td>
<td>Nam-pla</td>
<td>Thynnethys, Rasbora,</td>
</tr>
<tr>
<td></td>
<td>Padec (paste)</td>
<td>Ophiocephalus.</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Budu</td>
<td>Stelophorus.</td>
</tr>
<tr>
<td></td>
<td>Balachan (paste)</td>
<td>shrimps.</td>
</tr>
<tr>
<td>Philippines</td>
<td>Patis</td>
<td>Stelophorus (Commersoni, indicus)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clupeoideslile, Sardinella,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decapterus, Leiognathus.</td>
</tr>
<tr>
<td></td>
<td>Bolbokwa</td>
<td>small shrimps and fish.</td>
</tr>
<tr>
<td></td>
<td>Bagoong</td>
<td>fish and shrimps.</td>
</tr>
<tr>
<td>Thailand</td>
<td>Nampla</td>
<td>Stelophorus, Ristrelliger.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cirrhinus, Clupea.</td>
</tr>
<tr>
<td></td>
<td>Kapi (paste)</td>
<td>small fish and crustaceans.</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Nuoc-nam</td>
<td>Stelophorus, Ristrelliger,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Engraulis, Decapterus, Dorsoma.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clupea.</td>
</tr>
<tr>
<td></td>
<td>mam-tom (paste)</td>
<td>shrimps.</td>
</tr>
<tr>
<td>Khymer-Rebulic</td>
<td>Mom-rot</td>
<td>Fresh water shrimps.</td>
</tr>
<tr>
<td>Country</td>
<td>Species</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>shrimps.</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Shotsuru, Astroscopus japonicus (sand fish), Clupea pilchardus (sardine), Onastrephis sloani (squid), Omnastrephis (squid), and planktonic shrimps.</td>
<td></td>
</tr>
<tr>
<td>India and</td>
<td>Columbo-cure, Rastrelliger, Cybium, Clupea.</td>
<td></td>
</tr>
<tr>
<td>Pakistan</td>
<td>Sardinella, Jelio, Carangidae, Engraulis pupapa, Teuthis.</td>
<td></td>
</tr>
<tr>
<td>Hong-Kong</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>Garos, Scomber colias.</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>Pissala, Aphya pellucida Gobius, Engraulis, Atherina, Meletta.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anchovy, Engraulis encrasichlus.</td>
<td></td>
</tr>
</tbody>
</table>

(iii) Products in which the fish are reduced to a liquid (eg. budu (Malaysia), patis (Philippines), nuoc-nam (Vietnam) and nampla (Thailand).

Amano (1962), in his review classified the fermented products according to the presumed main characteristics. The three groups were;

(i) traditional products mainly fermented by the action of enzymes normally present in fish flesh and entrails with added salt;

(ii) traditional products fermented by the combined effects of fish enzyme supplied in the form of starter cultures on fish flesh and entrails with added salt;

(iii) non-traditional products manufactured by accelerated fermentation, acid ensilage and chemical hydrolysis.

In the recent classification of Adams, Cooke and Pongpen (1985), the traditional fermented fish products in South-East Asia are grouped according to the substrates used during the fermentation.

(i) Those produced from mixtures of fish/salt. e.g. fish sauces, (Table 3).

(ii) Those produced from mixtures of fish/salt/carbohydrates. Eg. pla-ra (Thailand) and burong isda (Philippines). (Table 4).
TABLE 3. Fermented Fish/salt products in Thailand.

<table>
<thead>
<tr>
<th>Product</th>
<th>Type of fish used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoi-dong</td>
<td>Molluscs.</td>
</tr>
<tr>
<td>Kapi</td>
<td>small fish and crustaceans.</td>
</tr>
<tr>
<td>Nam-pla</td>
<td>small fish.</td>
</tr>
<tr>
<td>Nam-budu</td>
<td>small fish.</td>
</tr>
<tr>
<td>Nam-khoei</td>
<td>crustaceans.</td>
</tr>
<tr>
<td>Pla-thu-khem</td>
<td>chum mackerel.</td>
</tr>
<tr>
<td>Tai-pla</td>
<td>variety of fish.</td>
</tr>
</tbody>
</table>

*Adams et al., 1985.*
### TABLE 4. Fish/salt/carbohydrate products of Asia.

<table>
<thead>
<tr>
<th>Country</th>
<th>Product</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>paak or mam-chao</td>
<td>fish/salt/cooked rice. (Rao, 1967).</td>
</tr>
<tr>
<td>Indonesia</td>
<td>makassar</td>
<td>fish/salt/boiled rice. red rice/spice. (Rao, 1967).</td>
</tr>
<tr>
<td>Japan</td>
<td>funa-sushi</td>
<td>fish/salt/cooked rice. (Matsushita, 1937).</td>
</tr>
</tbody>
</table>

Adams et al., 1985.
The reviews of Van Veen (1965) and Orejana (1983) describe specially those products in group one, but a distinct difference between these two groups was hardly observed. The second group of products where lactic acid fermentation occurs contribute to the shelflife of the products.

Owens and Mendoza (1985) in their review have classified the fermented fish products on the basis of the conditions under which enzymic hydrolysis occurs and the involvement or non-involvement of microbial fermentation.

The reviews of Westenberg (1951), Hess (1953), Amano (1962), Veen (1965), Mackie et al., (1971), Tanikawa (1971), Orejana (1983), and Steinkraus et al., (1983), cover different products made in South-East Asia and Japan from aquatic animals.

The consumption of fermented fish products can be traced back to Roman era in Europe (Badam, 1984) and today, these popular foods supplement the protein deficiencies in the staple diet (Amano, 1962).

Orejana (1983) summerised the fermentation methods and these methods were grouped into four groups. These groups are:

(i) Those containing high concentrates of salt, with 15 to 20 per cent in the final product.
(eg. nampla (Thailand), budu (Malaysia), patis (Philippines), and nuoc-nam (Vietnam).

(ii) Those produced by employing a process of fermentation with the generation of organic acids or other processing materials such as added carbohydrate sources to conserve the product.

In this group of traditional products the fish enzymes and microbial enzymes act together in the presence of added salt. (eg. funazushi (Japan), burong-dalag (Philippines).

(iii) Those produced by employing preservatives or mineral acids to eliminate microflora.

(eg. forage crops).

(iv) Those produced by employing an initial dosage of organic acids or other preservative agents instead of mineral acids.

Saisithi (1987) separated the traditional fermented products into three groups as;

(i) Those fermented by the combined action of fish enzymes and lactic acid bacterial enzymes normally present in fish/salt mixture. eg. bagoong and patis (Philippines), balachan (Malaysia), budu, nam-pla and pla-ra (Thailand), shotsuru (Japan).
(ii) Those fermented mostly by lactic acid bacterial enzymes which are normally present in the fish/salt/carbohydrate mixture. eg. balao-balao and burong-isda (Philippines), pla-chom, pla-som, pla-paeng-daeng and som-fug (Thailand).

(iii) Those fermented by lactic acid bacteria but the carbohydrate added is fermented by yeasts and moulds added in the form of starter culture. eg. pla-chao (Thailand).

The chemistry, biochemistry and microbiology of fermented fish products are well documented. (Amano, 1962; Van Veen, 1965; Mackie et al., 1971 and Batra and Milner, 1976). Proximate analysis of fish and shellfish used in the products of South-East Asian countries are available and the differences in the proximate composition of the raw material is correlated with the variation in nutritive and organoleptic qualities of the different fish sauces (Meinke, 1974; Orejana, 1973; Raa and Gildberg, 1980).

The practise of using salt in many traditional fermented products has been analysed and its role in inhibiting the growth of spoilage bacteria is recognised (Prescott and Dunn, 1959). The level of salt used is of importance as high salt concentrations could effect the enzymic activity (Amano, 1962; Voskresensky, 1965) and on the other hand the bacteria of fresh fish such as Micrococcus is considered to offer high resistance at low concentrations of salt (Dussault, 1957).

Most of the proteolytic activity in fish fermentation was observed during the first two months (Rose, 1918; Uyenco et al., 1953 and Orejana and
Liston, 1979) and the soluble protein/polypeptides ratio was relatively constant after one month indicating that proteolytic activity is high during the early stages of fermentation. Further evidence of the high proteolytic activity during the early stages emerged from the studies of Beddows et al., (1979) with budu and Guevarra et al., (1978) with patis from anchovies.

The fermented fish products are of significant importance due to economic and nutritional value and were considered important than fish preserved by other methods such as salting and drying (Mackie et al., 1971). The importance of fermented fish products in improving the nutritional status in South-East Asian countries are discussed in detail by various authors. (Amano, 1962; Van Veen, 1965; Saisithi et al., 1966; Areekul et al., 1974; Saisithi, 1981). Amano (1962) reported that protein supplement from fish sauce is as high as 7.5 per cent of an individual's nitrogen intake and Jensen and Howe (1964) indicated that the high value of lysine in fish sauce could compensate for low levels of lysine in rice. The fermented fish sauces are considered a good source of essential amino acids and Vietnamese generally receive 1.2 to 2.4 g nitrogen from these products (Geiger and Borgstrom, 1962). Further the amino acids are well preserved in these products (Amano, 1962; Beddows et al., 1976; Orejana, 1978), and have a higher lysine but a lower tryptophane content than meat with a relatively high methionine content (Mackie et al., 1971).

Dougan and Howard (1975) described three major factors contributing to aroma of Thai fish Sauce. These are (a) ammoniacal aroma due to ammonia
and trimethylamine; (b) cheesey aroma due to low molecular weight volatile fatty acids; and (c) meaty aroma due to a large number of volatiles such as ketones and keto acids.

Reports on the flavour and aroma are also made by Boez and Guillermin (1930), Troung-van-Chom (1963), Alm (1965), Van Veen (1965), Saisithi et al., (1966), and Orejana and Liston (1981).

Another important chemical change during fermentation is associated with the breaking down of the lipids which leads to the development of characteristic flavours and aromas and accompany the browning of the sauce during fermentation (Dougan Howard, 1975; and Saisithi, 1967).

The production of flavour and aroma is also related to the growth of different types of bacteria present and Saisithi (1967) attributed the typical aroma of Thai fish sauce to *Pediococcus halophilus* which was isolated. The bacteria and yeasts occurring naturally in fish, those associated with the environment and sea water, and organisms associated with additives such as salt are expected to have an effect on the final microbial composition of the system (Avery, 1950; Nagoa and Kimura, 1951; Velankar, 1957; Sands and Crisan, 1974; Orejana and Liston, 1979).

The proteolytic enzymes found in the fish such as pepsin (acid protease), trypsin and chymotrypsin (serine protease), cathepsin and thiol proteinases and carboxypeptidases and aminopeptidases will show varying activity due to the different pH required for maximum activity, and that found during
fermentation. Trypsin activity at the initial stages of patis were reported by Orejana (1978).

In the traditional products the difficulties in maintaining a uniformity in the quality is mainly due to the diverse types of raw material used and also due to the number of establishments involved in production (Subba Rao, 1967). The poor hygienic conditions and the quality of water used in fermentation of fish are some areas which require consideration. The necessity to establish standards for minimum levels of amino acids and total nitrogen were also discussed (Mackie et al., 1971).

The possible health hazards which could reflect the lack of quality control measures which could even result in food poisoning are not completely eliminated. Botulism has been reported from a variety of fermented and cured fish products, particularly involving the consumption of 'izushi' in Japan (Nakamura et al., 1956 Yamamoto, 1960).

Regulations for the quality of patis and nuoc-nam are already introduced and more effective programme to establish effective standards and enforcement is considered essential to protect the consumer from health hazards due to the poor quality of fermented fish products.
2.2 Added acid (fish silage).

Acids both organic and inorganic, individually or in mixtures are often used in fish silage. Application of acids to preserve fish is commonly practised in countries such as Denmark and Norway. Nearly 60,000 tons of fish is preserved annually in Denmark using acid silage and commercial scale preservation using acids are reported in Poland and Norway (Raa et al., 1983).

In the preparation of animal feed, the use of organic acids is preferred as fish could be preserved at a moderately high pH and such fish silage could be used in animal feed without neutralisation. Stable silage was reported at a pH of 2 with inorganic acids (Edin, 1940) and organic acids such as formic at 3.5 to 4.0 (Tatterson, 1976) and with propionic acid at 4.5 (Gildberg and Raa, 1976).

The antimicrobial activity of organic acids is found to be an added advantage in establishing a fish silage (Strom et al., 1979; Raa et al., 1983). The antimicrobial activity was related to the size of the acid molecules and the antimicrobial activity of propionic acid enabled a stable silage at a higher pH level than with formic acid (Tatterson, 1976 and Gildberg and Raa, 1977).

The quantity of inorganic acid required to lower the pH in the silage to a level of 2 was related to the concentration of proteins and ash (minerals) present in the raw material to be ensiled. Bony fish with low oil content usually require 6.3 kg (3.4 lit.) to 2.8 kg (1.5 Lit.)...
of sulphuric acid per 100 kg of fish and 9 to 14 litres of 14N inorganic acids (Raa et al., 1983).

The hazards involved in the use of inorganic acids and practical problems in neutralizing (and also the high salt level resulting from neutralization) have undesirable effects on nutrition specially in animal feeds and this coupled with the problems of corrosion had restricted the use of these acids.

The organic acids though expensive than inorganic acids, in considering the other savings of high efficiency, and also these could be used without neutralisation with added antimicrobial functions, have advantages over the use of inorganic acids.

Different combinations of organic and inorganic acids identified depending on the type of fish to be used to minimise the costs involved. (Olsson, 1942; Disney and Hoffman, 1976; Disney, Tatterson & Olley, 1977; Jensen and Schmidtsdorff, 1977). With tropical by-catch more acid is needed due to the higher ash content (Kompiang, Arifudin and Raa, 1980).

James, Iyer and Nair (1977) reports that the liquid silage products prepared from jew fish (Pseudocsiaena spp.), silver bellies (Leiognathus spp.) and sole fish (Cynoglossus semifasciatus) by both microbial fermentation and formic acid silage were highly stable and did not require neutralization prior to animal feeding. The formic acid products
gave a pungent odours while the others gave agreeable odours. The protein digestion in the biological method was lower than with the formic acid silage. In both methods, the free amino acids decreases after six months of storage. The pH of the medium did not change appreciably from 4 to 4.5 during storage.

Disney et al., (1977) in the review on fish silage suggest that despite the disadvantages of the use of acids in rural communities in tropical countries offer the most promise. On the other hand, biological fermentation possibly offers the most likely means of producing human food from waste fish.

It may be necessary to evaluate the present situation in considering the recent research and economic feasibility in undertaking fish silage work in the developing countries. A survey on the fish fermentation work has already been intiated by FAO/Infofish and the final observations are awaited.
2.3 Fish meal.

The waste from fish filleting operations are utilised as the main source of supply in the fish meal and fish oil manufacture and large fish meal plants are in operation in countries such as Peru, Norway, South Africa, USSR, Denmark, USA, Chile and Japan.

Virtually any fish or shellfish is used in the fish meal manufacture but those produced from whole shellfish or shellfish waste are low in proteins and high in ash content due to the presence of shell. Chitin which is found in fishmeal produced from shellfish is not considered to be of any nutritive value and is in lesser demand as an animal food with the exception of feeding farmed salmonoids. Here the carotenoid pigment in shellfish such as shrimps naturally contains the red colouring pigment which enhances the red colouration in salmon.

The production of fish meal in 1977 were nearly 4,000,000 tons (FAO, 1978) and a major part is used for feeding pigs and poultry, although there is a relatively small specialised market for fish meal in feeding fish, such as salmon, trout, catfish and eels, mink and pets and also for young pre-ruminant animals, such as calves.

Virtually any fish or shellfish can be used to make fish meal and the nutritional value of proteins from vertebrate fish differs very little from one species to another. The protein content of meals made from whole shellfish or shellfish waste will be lower compared to meals from other types of fish and high in ash content because of the presence of the shell (section 4.1).
Although in general the demand for meals with low proteins are less, those from shellfish will have the added advantage of having chitin which is of value in feeding of farmed salmonoid (sections 4.2 and 4.3).

The production of fish meal utilising the by-catch fishery and also non-edible fish is of importance in developing fisheries. Considering the large quantities of fish available, production of fish meal enables transport to places of need and reduces loss due to spoilage.

Processing of fish meal consists of a partial separation of the three main constituents; solids, oil and water (Figure 1). The water content is reduced from about 70 - 80% to about 10% so as to ensure cessation of all types of decomposition. The oil must be reduced to less than 15% in the final meal so as to improve the stability of the product and also to reduce the likelihood of fishy taint being produced in the animal being fed and to yield a valuable separate product. The composition of the material at each stage in the process is shown in Figure 2.

The production process involves cooking, where the fish is heated to about 100°C and pressing to separate the liquor. The press liquors are concentrated by a process of centrifuging. The stickwater is concentrated in multi-effect evaporators where stickwater is passed over a series of heated plates.

Environmental problems caused by the effluents and also the health hazards due to contamination with salmonella had caused manufacturing difficulties thereby escalating the production costs.
Fig 1  Fish meal processing shown in diagrammatic form.

Taken from Windsor and Barlow (1981)
Fig 2 Composition of fish material during the process.

<table>
<thead>
<tr>
<th>Material</th>
<th>Water %</th>
<th>Solids %</th>
<th>Fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>raw fish</td>
<td>70</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>press cake</td>
<td>53</td>
<td>44</td>
<td>3</td>
</tr>
<tr>
<td>press liquor</td>
<td>78</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>dilute stickwater</td>
<td>95</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>concentrated stickwater</td>
<td>65</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>fish meal</td>
<td>9</td>
<td>85</td>
<td>6</td>
</tr>
</tbody>
</table>

Taken from Windsor and Barlow (1981)
2.4 Fish protein concentrate (FPC).

In the production of fish protein concentrates, as the name implies, protein is more concentrated than in the original fish and these are intended for human consumption and the final product has no resemblance to the original fish species used.

There are two main types of FPC: Type A, which is a virtually odourless and tasteless powder having a maximum fat content of 0.75% and Type B, a powder having no specific limits as to odour or flavour but with a definite fishy flavour.

The production of FPC Type A involves initial washing and mincing and a series of extractions and alternating centrifuging followed by a vacuum drying and grinding and packing (Figure 3).

The process of FPC Type B is based upon the production of ordinary fish meal, but materials of construction and design and lay-out of plant are reorganised to meet food standards of hygiene.

The possibility of using FPC Type A in a number of products such as bread, pasta, soups and rice dishes, milk shakes, biscuits, infant milk foods, breakfast cereals are established more on experimental conditions. Nutritive studies have demonstrated the beneficial effect to growing children and pregnant or nursing mothers. The amino acid pattern of the supplemented diet is improved where less than 5% is added (Windsor and Barlow, 1981).
Due to the high cost of production the future of FPG Type A depends on governmental nutritional policies except for free distribution through aid programmes, and the use as a relatively expensive health food or additives in higher priced markets rather than in solving the nutritional problems.

FPC Type B with a composition similar to fish meal is distributed under many aid programmes and often helped in improving the nutritional status in some countries such as Senegal, Mali, Liberia, Philippines and Sri Lanka. FAO reports indicate that almost 19,000 people consumed FPC on a regular basis and reactions varied widely both between countries, within different groups in the same country and with different individuals in the same group.

However, a product with improved organoleptic properties and with some fish texture needs to be developed with improved packing methods to protect from insect infestation.
Figure 3 Diagram of a fish protein concentrate Type A production plant.

Taken from Windsor and Barlow (1981)
2.5 fish oil.

Most fish oil available in the market is fish body oil which results from the pressing of whole pelagic fish. The production of liver oils is a smaller scale operation with oil from cod, coalfish and haddock as the major types.

Fish liver oil is used in medicinal or veterinary purposes and most fish oils are valued due to the vitamin A and D and most contracts specify the minimum quantity of these two vitamins.

Fish oil is becoming important due to the current concern in Western society regarding coronary thrombosis and its association with cholesterol and the high level of unsaturated fatty acids in fish oil is known to reduce blood cholesterol levels. Fish oil in the diet also leads to the production of prostaglandins, which are important lipids and powerful metabolic and physiolocal regulators playing an important role in preventing excessive fat deposition in the arteries.

The EPA or 20:5n-3 (eicosapentaenoic acid) and DHA or 22:6n-3 (docosahexaenoic acid) in fish oil are of importance as either nutritional supplements, or as therapeutic agents inhibiting a variety of pathological conditions in man and are popularly known as the omega-3 fatty acids. EPA and DHA naturally make up at most only about 25 per cent of fish and fatty acids. (Ackman, 1988).
Most marine fish oils from northern latitudes contain very low levels of omega-6 fatty acids, typically 1-2 per cent of linoleic acid (18:2n-6) and 0.5 - 1.0 per cent of arachidonic acid (20:3n-6).

The acid ensilation could be used in the autolysis to yield liquid-free muscle pieces and pure oil floating on a liquid of dissolved skin, intestines and belly line. The skin and other collagen rich tissues dissolve faster in an acid than in a neutral solution, whereas the opposite is true for muscle. This basis is used in silage for de-skinning of fish and extrusion of oil. (Raa and Gildberg, 1976; Gildberg and Raa, 1979; Eide, et al., 1979).

This method was successfully applied to tropical sardines and had the advantage that no complex and expensive equipment was needed to produce a food quality oil (Raa, 1980).

3.0 FERMENTED SHRIMP PRODUCTS
Fermented shrimp products are common specially in South-East Asian countries and the procedures used in their manufacture are similar to those for fermented fish products. The shrimp processing industry, which does not differentiate between shrimps and prawns to any great extent, uses the larger species in freezing and canning which are high value products and are usually supplied to sophisticated markets. Small varieties which are not suitable for freezing and canning, which involves deheading and/or peeling and in instances cooking, are used in the fermentation.
Some of the shrimps used are, *Acetes* or small planktonic shrimps living mainly in the estuarine and coastal waters of the sub-tropical regions, in bagoonalamang (Philippines), *Mysid* and *Decapod* and *Acetes* species in belachan (Malaysia), very small shrimp and planktonic shrimp called rebon (*Schizopodes*, *Mysis*, etc) in trassi udang (Indonesia), atya species in patis (Philippines) *Peneus indicus* or small species of *Macrobrachium* in balao-balao (Philippines).

Although the shrimps used in fermentation are in a minor crustacean group, represented by a few species, it supports a subsistence fishing industry of considerable importance in countries such as Malaysia. They appear in very large swarms in the shallow in-shore coastal waters, which are brackish with a salinity of 0.3 per cent or less during certain seasons of the year (Pathansali, 1966).

3.1 sauces and pastes.
Accurate production figures of shrimp based sauces and pastes are lacking and many of these are produced on house hold level with a few commercial scale productions in operation.

Similar to fish products many fermented shrimp products are made using high salt concentrations. Salt concentrations used in some of the products are given in Table 5.
TABLE 5. Fermented shrimp product (sauces and pastes);

<table>
<thead>
<tr>
<th>Product</th>
<th>% salt used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balao-balao</td>
<td>upto 20 w/w shrimp</td>
<td>Arreyo et al., 1977</td>
</tr>
<tr>
<td>Bagoong</td>
<td>1:3 v/v shrimp</td>
<td>Beans-Artega, 1977a</td>
</tr>
<tr>
<td>Belachan</td>
<td>1:20 w/w shrimp</td>
<td>Merican, 1977a</td>
</tr>
<tr>
<td>Trassi</td>
<td>1:10 w/w</td>
<td>Van Veen, 1965</td>
</tr>
<tr>
<td>Shrimp Jeot</td>
<td>20 - 25 w/w shrimp</td>
<td>Lee et al, 1977</td>
</tr>
<tr>
<td>Nuoc-nam</td>
<td>1:3 to 1:2 w/w</td>
<td>Truong, 1951</td>
</tr>
</tbody>
</table>


As shrimps contain less fermentable carbohydrates compared to fish, bacterial growth is less favoured and the fermentation is largely enzymatic with or without bacterial assistance in proteolysis. Bacterial action is assumed to play a role in flavour development.

Some of the microorganisms found are given below:

Belachan        Bacillus, Pediococcus, Lactobacillus, Micrococcus, Sarcina, Staphylococcus, Clostridium, Breviabacterum-like and Corynebacterium-like bacteria.

Shrimp jeot     Halobacterium, Pediococcus, Sarcina, Saccharomyces, and Torulopsis.

Source: Saisithi, 1987
Commonly consumed shrimp pastes are balao-balao, which is produced using whole raw shrimps with a rice and solar salt mixture (Arrayo et al., 1977) and burong hippon which is similar to burong dalag, a fermented fish/shrimp/rice coloured with 'angkak' (Orillo and Pederson, 1968 (Philippines), belachan (Malaysia), and sauces such as nampla (Thailand), nuoc-nam and mam (Vietnam), budu (Malaysia) and trassi (Indonesia). Angkak is rice with a red colour due to the growth of red mould Monascus purpureus during fermentation or the addition of food dyes such as erythrosine.

A detailed study on fermented shrimp sauces and pastes were made by Steinkraus (1983), and information on production aspects are well documented (Uyenco et al., 1953; Richard, 1959, van Veen, 1965; Bean-al ega, 1977a; Merican, 1977a; Ismail, 1977; and Beddows and Ardeshir, 1979).

These are commonly produced on a household scale in most countries in South-East Asia and a few commercial scale operations are also established. For understanding the techniques involved it is useful to study the production procedure followed in one of the most popular products using shrimp as the raw material.

Balao-balao is prepared by mixing rice cooked to a pasty consistency and mixed with fresh raw shrimps and salt (3.85% w/w) and allowing the mixture to ferment for 3 days at room temperature. Balao-balao is generally marketed in 300 g plastic packets and refrigerated if necessary.
The shrimps used in balao-balao are *P.indicus*, locally called suahe, which is a salt water shrimp or fresh-water tiny species of *Macrobrachium*, locally known as tagunton, caught in the Laguna lake. The fresh-water species are sometimes considered to be the species of *Palaemon* (Davidson, 1976).

For using in the production of balao-balao, the shrimp needs to be of high quality and usually the shrimps used are purchased alive and salted immediately. The percentage composition (wet weight basis) of tagunton shrimps: moisture 74.4; proteins 20.2; fat 1.9; ash 3.5; and calcium 2351 mg; phosphorus 382 mg; iron 15.2 mg in 100g with vitamin A 2851 u; thiamin 0.02 mg; riboflavin 0.20 mg and niacin 2.7 mg per 100 gms.

Rice with intermediate amylase content (eg grade C4) is boiled with water (1:2.36 rice: water w/w) until somewhat pasty and mixed with solar salt (92.25 per cent NaCl) prepared by evaporating sea water.

The production process involves two different methods. First the whole live shrimps are washed and packed in salt (20% weight of shrimps) in brine water and drained after 2 hours. The drained shrimps are blended with 3 - 12 per cent solar salt and rice in a ratio of 1:4.8 w/w shrimps: rice, and allowed to ferment in glass jars at tropical room temperature.

In the alternative method, shrimps are washed, trimmed and blended with rice 10, 15 or 20 per cent salt and allowed to ferment. In both these processes the fermentation is brought about by the microorganisms present in the substrate and no inocula is added.
It is observed that high salt concentrations require longer fermentation periods and an organoleptically acceptable product containing 4.82 per cent NaCl requires 7 - 10 days to complete fermentation. For developing a product with satisfactory flavour it is recommended that balao-balao with at least 3% salt and a pH of 4.0 be maintained during fermentation.

Bagoong, which is a fish or shrimp paste, uses either dilis (a fish, Stelophorus), alamang (Shrimp, Acestes indicus), and sisi (oyster, Plitula sp). In the production process shrimp is crushed using the fingers with solar salt (3 : 1 v/v of solar salt) and allowed to ferment in covered jars, with occasional stirring. The fermentation usually is completed in 3 days. In some instances bagoong is either cooked or a preservative is added to improve the keeping quality before storage.

4. UTILISATION OF SHRIMP WASTE

The waste produced during processing of shrimps contains industrially useful products and research on the recovery of those products is carried out in many countries. The recovery of proteins, chitin and astaxanthin, enhance the economic viability of shrimp processing industry. Shrimp processing in many countries has become important due to the economy and employment generated and large investments in shrimp farming made during the last decade (ITC, 1983). Barratt and Montano (1986) reported that in Equador frozen shrimp production had increased by 200 per cent in 1983 with approximately 20,000 tons of shrimp waste.
This increase in shrimp production in the five year period without a parallel development of technology of utilising shrimp heads had resulted in a waste collection problem and pollution.

The quantity of shrimp waste accumulated in India is estimated to be about 60,000 tons per annum, from which approximately 2,000 tons of chitin could be prepared.

In tropical shrimps the head consists of 34 to 45 per cent of the weight of the whole shrimp, and the tail and swimmeretts contributing a further 10 - 15 per cent (Barratt and Montano, 1986). The carapace (exoskeleton) could even amount to 70 per cent of the whole shrimp (Meyers and Benjamin, 1987). Waste from shrimp aquaculture which is mainly the head material represents a significant portion of the weight of the whole shrimp, varying with different cultured species.

The composition of the shrimp waste is found to vary significantly depending on the processing techniques (Meyers, 1986). Other components such as astaxanthin also vary depending on the source of the raw material (Meyers, 1977). In machine peeling operations, a considerable quantity of shrimp meat is lost in the shelling, fluming and washing operations.
4.1 shrimp meal.

Shrimp meal produced by different methods usually contain proteins ranging from 22 to 53% and chitin from about 9 to 54%. The protein and chitin contents also depend on the type of waste used in producing shrimp meal. Protein and chitin levels of shrimp meal produced by different processes and from various raw material are given in Tables 6 and 7.

TABLE 6. Protein and chitin (percentage) in shrimp meal

<table>
<thead>
<tr>
<th>Protein (crude)</th>
<th>Chitin</th>
<th>Protein (corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-hydrated</td>
<td>37.3</td>
<td>20.6</td>
</tr>
<tr>
<td>Sun-dried</td>
<td>51.7</td>
<td>9.0</td>
</tr>
<tr>
<td>Shrimp heads</td>
<td>58.2</td>
<td>11.1</td>
</tr>
<tr>
<td>Shrimps hulls</td>
<td>45.9</td>
<td>54.2</td>
</tr>
</tbody>
</table>


Significant differences in the carotenoid content are recorded in shrimp meals due to varying processing techniques.

The shrimp silage from species in the Gulf of Mexico was reported to contain at least 5 times more astaxanthin than that present in machine dried meal, i.e. 33 ug astaxanthin/g waste compared with 6 ug/g waste,
respectively (Benjamin, 1982). Ensiled product from far Northern shrimp, such as Pandalus borealus, contain significantly greater pigment concentrations due to the high initial levels present.

Uncontrolled heat during the production of shrimp meal could affect the level of astaxanthin as carotenoids are susceptible to excessive heat thereby lowering the value of shrimp meals. Acid ensilation is adopted in Norway as a source of astaxanthin to be utilised for salmon farming.

<table>
<thead>
<tr>
<th>TABLE 7. Percentage protein chitin levels of two types of shrimp meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (crude)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Mechanically processed</td>
</tr>
<tr>
<td>Shrimp hulls</td>
</tr>
<tr>
<td>Shrimp heads</td>
</tr>
</tbody>
</table>

The usefulness of processes based on recovering different products from shrimp waste can be understood when the chemical composition of the waste is analysed. The shrimp head and shells contain 53.5% and 22.8% protein respectively. The analysis of shrimp heads and shells is given in Table 8. Differences reflect the proportion of flesh and body tissues found in the heads correspond with the shells.
Shrimp head meal is considered a good source of proteins, carotenoid pigments such as astaxanthin (4.3), fatty acids and chitin or fibre. Analysis of shrimp heads and shell are given in Table 8.

Astaxanthin found in shrimp waste serves as a natural source of carotenoid pigmentation in cultured trout and salmon and this pigment is also helpful in the pigmentation of broilers and improves the colour of egg yolk (Meyers, 1986). Ramachandran Nair et al., (1986) state that the recoverable chitin from shrimp waste in India amounts to about 2,000 tons annually and could produce an additional 20,000 tons of chicken by using chitin in poultry feed. The reduction in food consumption together with the increase in weight in chicken could result in increasing the profits by 70 per cent to the farmer (4.2).

TABLE 8. Analysis of shrimp heads and shells (per cent)

<table>
<thead>
<tr>
<th>Protein (corrected)</th>
<th>Fat</th>
<th>Chitin</th>
<th>Ash</th>
<th>Calcium</th>
<th>Phosphours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrimp heads</td>
<td>53.5</td>
<td>8.9</td>
<td>11.1</td>
<td>22.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Shrimp shells</td>
<td>22.8</td>
<td>0.4</td>
<td>27.2</td>
<td>31.7</td>
<td>11.1</td>
</tr>
</tbody>
</table>


The shrimp feed used in United States and Far East, formulated with shrimp meal comprising of as much as 30% of the final formulation,
has been used successfully (Meyers, 1986). (A detailed account of the uses of chitin and astaxanthin are given in sections 4.1 and 4.2).

In addition to using chitin and astaxanthin, other products from shrimp ensilage such as amino acids present in aqueous extracts have shown to exhibit feeding stimulatory properties to a variety of fish species (Mackie, 1982). The lipids and fatty acids in shrimp meal also vary depending on the manufacturing process. The fatty acid components of shrimp meal produced by sun-drying and vacuum drying are given in Table 9. The lipids and fatty acids present in shrimp diet are important due to specific requirements of shrimps for certain fatty acids (Meyer, 1986). Processed shrimp heads are considered a good source of fatty acids for growth of the fresh water prawn *Macrobrachium rosenbergii* (Sandifer and Joseph, 1976). The analytical data will enable the formulation of diets with the required ingredients.

Meyer (1986) reported, that the amino acid profile of well processed shrimp meal is comparable to that of soya bean or fish meal (Table 10) and can be used as an alternative form of protein supply in areas where protein ingredients are needed.

The quality of shrimp meal is affected by high microbial and enzymatic activity in the head tissues. The resulting decomposition is accelerated by high ambient temperatures. Lack of water, ice and cooling facilities and also poor transport facilities contribute to this situation. The limitations in commercial drying
### TABLE 9. Major fatty acids of two shrimp meals:
(Per cent by weight)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Sun dried (3.5% liquid Penaeid)</th>
<th>Vacuum dried (9.2% liquid pandalid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>16:0</td>
<td>20.8</td>
<td>10.1</td>
</tr>
<tr>
<td>18:0</td>
<td>8.7</td>
<td>2.0</td>
</tr>
<tr>
<td>20:0</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>22:0</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>16:1</td>
<td>10.9</td>
<td>9.0</td>
</tr>
<tr>
<td>18:1</td>
<td>21.4</td>
<td>17.9</td>
</tr>
<tr>
<td>20:1</td>
<td>3.3</td>
<td>13.4</td>
</tr>
<tr>
<td>22:1</td>
<td>1.5</td>
<td>18.4</td>
</tr>
<tr>
<td>18:2w6</td>
<td>2.9</td>
<td>1.4</td>
</tr>
<tr>
<td>20:2w6</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>20:4w6</td>
<td>1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>18:3w3</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>18:4w3</td>
<td>0.2</td>
<td>1.1</td>
</tr>
<tr>
<td>20:5w3</td>
<td>2.1</td>
<td>7.6</td>
</tr>
<tr>
<td>22:6w3</td>
<td>2.2</td>
<td>9.0</td>
</tr>
</tbody>
</table>

1 Notation indicates the number of C atoms in the molecule: the number of double bonds and specifies the position of the double bond nearest the terminal methyl group. (Source: Meyer5, 1986).
### TABLE 10. Amino acid composition of shrimp fish meal:

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Fish meal (Menhaden)</th>
<th>shrimp meal (sun dried)</th>
<th>shrimp head meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>5.97</td>
<td>5.29</td>
<td>7.56</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.98</td>
<td>6.31</td>
<td>6.70</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8.76</td>
<td>10.74</td>
<td>9.07</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.70</td>
<td>1.59*</td>
<td>2.35</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>12.20</td>
<td>15.46</td>
<td>13.61</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.58</td>
<td>4.29</td>
<td>6.57</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.52</td>
<td>1.90</td>
<td>2.23</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.28</td>
<td>3.26</td>
<td>6.20</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.10</td>
<td>7.57</td>
<td>6.72</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.66</td>
<td>6.17</td>
<td>9.20</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.89</td>
<td>2.84</td>
<td>1.65</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.92</td>
<td>4.56</td>
<td>4.60</td>
</tr>
<tr>
<td>Proline</td>
<td>4.52</td>
<td>3.44</td>
<td>3.31</td>
</tr>
<tr>
<td>Serine</td>
<td>3.55</td>
<td>4.53</td>
<td>3.49</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.85</td>
<td>4.28</td>
<td>4.22</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.16</td>
<td>1.26</td>
<td>0.63</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.25</td>
<td>3.64</td>
<td>3.64</td>
</tr>
<tr>
<td>Valine</td>
<td>4.98</td>
<td>4.42</td>
<td>6.77</td>
</tr>
</tbody>
</table>

* 1/2 cystine

facilities close to shrimp farming regions, as well as main supply points for sea prawns, limit the effective utilisation of shrimp head waste.

Ensilage of shrimps using acids carried out by Benjanim (1982), produced a microbiologically and a pH-stable product and confirmed the presence of significant indigenous proteolytic activity in the shrimp head material. The proteolytic activity of several bacteria isolated from the raw shrimp head was comparable with that of the supplemental enzyme used in the project, i.e. papain (1%), in the production of the stable silage. Amino acid composition of the shrimp head silage was comparable with that of the dried shrimp head meal and compared favourably with herring silage (Table 11.)

The use of chitin from shrimp waste has been drawing attention within the industry (Williams, 1959; Nagasave et al., 1970; Radhakrishnan, and Prabhu, 1971; Madhavan and Ramachandran Nair, 1974; Brezeski, 1987). The use of chitin specially in the pharmaceutical industry and astaxanthin as a colouring pigment in fish feed is outlined in sections 4.2 and 4.3.
### TABLE 11. Amino acid composition of shrimp head meal and silage compared with herring silage (g/100g protein):

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Shrimp head silage</th>
<th>Shrimp head meal&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Herring silage&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAU</td>
<td>2.5</td>
<td>1.6</td>
<td>not avail.</td>
</tr>
<tr>
<td>ASP</td>
<td>10.9</td>
<td>10.0</td>
<td>7.0</td>
</tr>
<tr>
<td>THR</td>
<td>2.9</td>
<td>3.9</td>
<td>3.0</td>
</tr>
<tr>
<td>SER</td>
<td>2.4</td>
<td>2.9</td>
<td>3.2</td>
</tr>
<tr>
<td>GLU</td>
<td>10.8</td>
<td>9.5</td>
<td>9.8</td>
</tr>
<tr>
<td>PRO</td>
<td>3.5</td>
<td>4.3</td>
<td>not avail.</td>
</tr>
<tr>
<td>GLY</td>
<td>5.5</td>
<td>4.4</td>
<td>6.4</td>
</tr>
<tr>
<td>ALA</td>
<td>4.9</td>
<td>4.9</td>
<td>5.2</td>
</tr>
<tr>
<td>VAL</td>
<td>4.4</td>
<td>3.8</td>
<td>3.3</td>
</tr>
<tr>
<td>MET</td>
<td>1.9</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>ILE</td>
<td>3.9</td>
<td>3.0</td>
<td>2.8</td>
</tr>
<tr>
<td>LEU</td>
<td>5.8</td>
<td>5.0</td>
<td>5.2</td>
</tr>
<tr>
<td>TYR</td>
<td>3.6</td>
<td>2.6</td>
<td>1.6</td>
</tr>
<tr>
<td>PHE</td>
<td>3.2</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>HIS</td>
<td>3.4</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>LYS</td>
<td>6.0</td>
<td>5.2</td>
<td>6.2</td>
</tr>
<tr>
<td>ARG</td>
<td>6.2</td>
<td>5.4</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Source: 1 Meyers (undated); 2 Whittmore and Taylor, 1976.
4.2 chitin/chitosan.

Chitin is a macro-molecular, linear polymer of anhydro-N-acetyl-D-glucosamine (N-acetyl-2-amino-2-deoxy-D-glucose). This is the second most abundant natural polymer after cellulose (Figure 4a). Similarly to cellulose, this is a polysaccharide, a compound formed by many identical simple sugar molecules. The nitrogen per cent in chitin is theoretically 6.89 against 1.25 or less for artificially substituted cellulose.

Chitin is immiscible in water and in many commercial solvents. In pure form, it has limited applications but the commercially treated form of chitin has many industrial uses. Chitosan the main derivative of chitin is a deacetylated form and is of commercial importance.

Chitin is found in the skeleton material of crustacean shells, cuttlefish and squid as well as fungi and the cuticle of insects. Processing of these crustaceans into different types of food, results in a large quantity of waste containing approximately 10 to 60 per cent chitin on a dry basis, depending on the processing method (Brezeski, 1987). Squilla, a by-catch from shrimp trawling is found to contain high quality chitin and chitosan.

The annual production of chitosan is estimated to be around 1,600 tons, with Japan and United States being the major producers. The production of chitin in India, Italy and Poland is much less and projects are planned in countries such as Brazil, Cuba, Ireland, Norway, Uruguay, and the Soviet Union.
Figure 4(a) Structure of Chitin

Figure 4(b) Structure of Astaxanthin
The most economical sources of chitin at present is considered to be shrimp waste and also the shell of lobsters, and crabs are excellent sources (Ramachandran et al., 1966).

4.2.1 chitosan.
Unlike chitin, chitosan is readily soluble in various acidic solvents forming a viscous solution. The preparation of chitosan involves fusion with potassium hydroxide in an innert atmosphere (Horowitz et al., 1967).

The many industrial uses of chitin and chitosan has been reported, over 200 by Brezeski (1987), in industry, biotechnology, food processing, agriculture, pharmacy, medicine and natural environment preservation.

Chitosan has been found to be particularly effective in aiding the coagulation of protein material from fish, vegetable, egg breaking, meat, poultry and other food processing plant waste. As chitosan is biodegradable, any material remaining after use is harmless and non-toxic.

The importance of shrimp waste as shrimp meal is now well recognised (4.1). The use of chitin in animal feed is found to promote the growth of broiler chicks. Incorporation of chitin in poultry feed at a level of 0.5 per cent was found to decrease the food consumption ratio and increase the carcass weight by 12 per cent (Ramachandran Nair et al., 1986). This situation had resulted in an average increase of 70 per cent
profit to the farmers in India. Similar growth increases were reported with chicks fed with chitin products in USA and Japan. Krill chitin was used in Poland in insemination of boars for separating mobile and non-mobile spermatozoa.

Treatment with chitosan had been developed in the USA to help protect crops against damage by a soil fungus.

In the food industry, chitin due to its superior thickening qualities is used as a stabilising agent. Because of its emulsifying properties chitin in the microcrystalline form is used as a source of dietary fibre in the baked foods with added improvements, in flavour, colour and shelflife compared to other sources of fibre.

Other major uses of chitosan are in water purification, chromatography, in medical, ecological and pharmaceutical sciences.

Uses in the manufacture of adhesives, formulation of cosmetics and in textile industry to impart antistatic and soil repellent properties are reported.

The production of improved quality paper, in photography and in many other industrial purposes has made chitin and chitosan useful industrial products (Brezeski, 1987).

A process of separating all of the by-products from crustacean shell waste at a reasonable cost has been developed by Food Chemical and Research Laboratories Ltd. 4900, 9th Avenue, NW, Seattle, in 1971. (Figure 5a).
Fig. 5(a) Flowsheet of the pilot plant for the production of chitosan. (1) shell, (2) protein, (3) chitin, (4) deproteinized shell, (5) chitosan. (Courtesy of Food, Chemicals & Research Laboratories, Ltd., Seattle.)

Taken from Muzzarali (1973)
4.3 astaxanthin.

Astaxanthin is found in many crustaceans and, as this is the natural colouring pigment in salmonoids, is in demand in the fish feed industry. This is found among the pigments such as B-carotene, echineone, canthaxanthin, 3 - 3' - dihydroxy - E carotene and the minor pigment dihydroxypiradixanthin. These are concentrated in the carapace portion with other pigments such as phoemicoxanthin, lutein, zeaxanthin (Katayama, Katama and Chichester, 1971). The structure of astaxanthin is given in Figure 4(b).

The proposed metabolic pathway from B-carotene to astaxanthin involves the conversion of B-carotene to echineone, canthaxanthin, phoemicoxanthin and then to astaxanthin.

The usefulness of astaxanthin in commercial fish feed is restricted due to a preference for canthaxanthin due to convenience in its usage as a dry powder. (Schmidt and Baker, 1969; Ugelten, 1974; Torrissen, 1978).

Simpson and Haard (1985) described using the enzyme trypsin and a chelating agent for extracting a carotenoprotein complex from shrimp processing wastes. This process enabled the recovery of about 80 per cent of the protein and carotenoid pigments present in the shrimp offal and was devoid of chitin with a reduced ash content.

Astaxanthin produced during acid ensilation has circumvented some of these drawbacks as the product from semimoist silage is found to be stable and either used on the fish farms as an additive to moist feed
for salmonids, at a level of 5 per cent, or transported to feed compounding factories to be incorporated in dry rations. A modified acid silage using phosphoric acid, propionic acid and antioxidants, both liquid and water-soluble with sulphuric acid was outlined by Raa et al., (1983). The extraction of astaxanthin directly from shrimp processing waste in the production of chitosan from chitin is already established and is patented (Raa and Hanson). With the proposed ban on the usage of canthaxanthin in fish feeds (Anon, 1981) the production of astaxanthin is considered to be economically attractive (Figure 5b).

The astaxanthin from shrimp processing waste is found to be stable in acid silage, but for a slow conversion of its di-ester to the corresponding mono-ester (Torrissen et al., 1982). The accumulation by rainbow trout of the astaxanthin present in the waste material was improved by ensiling, to about 71 per cent as compared to 45 per cent in the corresponding fresh or dried material. Also the rate of accumulation of the pigment in the fish muscle was markedly higher in fish fed with the silage diet than those given fresh or dried shrimp waste.
Figure 5(b) Ensiling and processing of shrimp waste

Taken from Raa et al., (1983)
The usefulness of shrimp by-products as potential flavour components of processed seafood products as well as livestock feed formulations especially in shrimp farms has been recognised. The importance of feeding stimulants such as amino acids and nucleotides which enhance the value of the diet considerably in improving the colour of aquatic species has been reported (Piedad-Pascual and Destajo, 1979; Hardy et al., 1984; Tidemann et al., 1984; Meyers, 1986).

Recovery of the nucleotides and amino acids from shrimp cannery effluents (Feedstuffs, 1974) and the extraction of flavour components from the shrimp head waste and using as flavour additives for certain foods have been proposed (Marine News Letter, 1975). The possibility of using shrimp heads in certain pet foods was also considered.

In addition to the recovery of proteins, chitin and astaxanthin; D-glucosamine hydrochloride (GAH), which can be prepared from shrimp and shrimp waste, is used in the pharmeceutical industry for biochemical research and as a starting material for the preparation of valuable intermediates such as D-arabinose and D-arabonic acid recommended in the manufacture of riboflavin and L-alanine required for the production of vitamin B6 (Research and Industry 18:54, 1973).

The production of prawn crackers using the head powder (Narkviroj and Buckle, 1987) and sausages using the shrimp meat discarded from the canneries were considered as other possibilities. In addition to sausages, other shrimp meat-based products, such as shrimp flavoured loaves, stuffing and chips, using comminution and fabrication techniques common to the food industry are reported (Meyer, 1987).
5. LACTIC ACID FERMENTATION

The use of lactic acid fermentation technology for preservation of forage crops, which constitutes the major component of the winter feed for some animal, has its applications in fish preservation. The advanced technology in cheese making and other fermentation industries has contributed to the development of its application to other products such as fish and vegetable crops.

During ensilation, which is the process by which silage is produced, lactic acid is generated by the action of lactic acid bacteria (LAB). Sugars present in the raw material are converted to lactic acid and acetic acid thereby reducing the pH to a level at which the growth of clostridia are prevented.

During the growth of clostridia (secondary fermentation) the lactic acid produced during lactic fermentation (primary fermentation), sugars, proteins and amino acids are converted to butyric and higher fatty acids, amines, amides and ammonia.

The success of the ensilation is based on the high ratio of the primary fermentation to the secondary fermentation. The dry matter (DM) content of the material to be ensiled is also considered in establishing a satisfactory silage. With high DM, a stable silage has been established at a high pH value (Woolford, 1985). The practises to be followed in establishing a silage both for plant and animal products are already available (Wignall and Tatterson, 1976 and Anon, 1977).
McDonald (1981) and Kalac and Woolford (1982) outlined the nutritional aspects, chemical and physical, and chemical changes accompanying ensilation which are harmful for both human and livestock, and also the systems employed to evaluate the silage quality.

To develop fermentation technology and study its application to different types of raw materials, it is necessary to analyse the microbial changes taking place in the system and also the physical and chemical changes associated with the fermentation process.

5.1 microbiology of the silage.

The microflora initially present in the silage undergo a series of changes, both in qualitative and quantitative terms. The Gram-negative heterophylic aerobic chromogenic bacteria which predominate in the initial stages of the silage are progressively replaced by facultative and strictly anaerobic Gram-positive types. Coliforms which are Gram-negative such as E.coli and Klebsiella, and members of the genera Streptococcus, Leuconostocs, Lactobacillus, Pediococcus, Bacillus and Clostridium become significant. (Kroulik et al., 1955a, 1955b; Gibson et al., 1958, 1961; Langston et al., 1958, 1962, LangstonBouma, 1960 a,b,c, Gouet and Chevalier, 1966; Dickinson et al., 1975).

The yeasts detected in the silage are from the environment and not the fresh crop and are confined to a few genera such as, the top-growing types: Candida, Hansenula and Pichia and bottom-growing types

The change in microflora during fermentation always follows a regular sequence. Initially coliforms multiply and usually after about the 7th day begin to decrease in number being progressively replaced by LAB, such as Streptococci, Leuconostocs and pediococci, which in turn are suppressed by slower growing but highly acid forming Lactobacilli. The rate of reduction of coliforms is being regarded as a good yardstick of silage acidification and a guide to whether the silage will be established or not (Weise, 1968). The change in sequence of microflora observed in baloa-baloa, which is fermented shrimp and rice product was found to agree with the sequence reported with vegetable products. (Solidum and Acedevo, 1983).

The changes of microflora which occur in the silage are due to the powers of survival and not to one group growing more rapidly than the other. This power of survival refers to acid tolerance and acidification potential of the genera concerned (Woolford, 1980). One possible reason for the qualitative change within the lactic microflora in silage is antagonism by these organisms towards other ones likely to thrive in the silage. As the fermentation proceeds the proportion of the lactic organisms antagonistic to other silage microorganisms increases (Nilsson and Nilsson, 1956; Wirahadikusumah et al., 1972).
During the latter stages, homofermentative Lactobacillus species such as *L. plantarum* and *L. curvatus* tend to predominate in the well preserved silage. These are invariably replaced by heterofermentative varieties such as *L. brevis* and *L. buchneri* in the terminal stages of the silage. (Woolford, 1985).

Initiation of the secondary fermentation depends on physical and chemical aspects which are present in the system and cannot be predicted accurately. DM content together with other factors contribute to this situation. Clostridia are strict anaerobes which survive in the early stages of the silage, as endospores. Once the conditions become favourable, these germinate and the possibility of constituents in silage having a stimulatory role in this event is propounded (Gibson, 1965). Saccharolytic varieties of clostridia such as *C. butyricum* or *C. tyrobutyricum* which are more acid tolerant than their proteolytic relatives (e.g. *C. sporogenes*) initiate secondary fermentation.

The breaking down of lactate to butyrate is considered the main cause for spoilage of the silage. The role of clostridia in the fermentation of lactate is considered more significant compared to that of LAB.

Woolford (1975a, 1976, 1977) and Burmeister et al., (1976) reported that yeasts do not play a role in the spoilage, and they even improve the stability of the silage by directly inhibiting moulds.
The role of the initial level of LAB necessary to establish a silage has been studied (Langston et al., 1958; Kempton and San Clemente, 1959; Langston and Bouma, 1960c; Ohyama et al., 1973). Good quality silage has resulted from crops with relatively low populations of LAB and vice versa. The populations of LAB in both good and bad quality silage can be similar and in some instances can apply to strict anaerobes as well. However, silage which spoils will usually sustain a large increase in clostridia and most of these will be of the saccharolytic type. Better quality silage results if the early silage flora is comprised of cocci, although no explanation is forthcoming for the apparent preomiance of low acid-producing lactobacilli in poor quality silage. In general a high population of lactobacilli is found to be associated with good silage. The numbers of clostridia are closely related to the chemical composition particularly of butyrate (Beck, 1972).

During exposure of the silage when spread for feeding-out, the loss of fermentation acids and residual sugars, stimulates the growth of yeasts and to a certain extent moulds, thereby contributing to the aerobic deterioration process (Beck and Grcs, 1964; Daniel et al., 1970; Beck, 1975; Moon et al., 1980; Ohyama et al., 1980; Woolford et al., 1982).
5.2 chemistry of silage.

The basic chemical compounds of forage crops which undergo change were reported by McDonald et al., (1973) to be water soluble carbohydrates, organic acids and nitrogenous compounds.

The water soluble carbohydrates present in the forage crops include fructose, glucose, sucrose, fructosans which are hydrolysed through the Embden-Meyerhoff-Parness (EMP) glycolytic pathway where 1 mol of fructose or glucose is converted to 2 mol of lactate (Wood, 1961). In the heterolytic mechanism mannitol and ethanol are produced in addition to lactate in accordance with the pentose monophosphate scheme as indicated by Wood (1961). The organic acids mainly citric and malic, present in the raw material (forage crops) together with the salts are reported to influence the fall in pH and Playne and McDonald, (1966) reported that 80 per cent of the buffering properties of the forage were due to the organic acids and 10–20 per cent from proteins.

The magnitude of this buffering capacity is defined as mg lactic acid require to lower the pH of 1 g herbage DM to 4.0 (McDonald and Henderson, 1962).

The lactic acid fermentation in contrast to other fermentation-based industrial processes such as antibiotic production or brewing, depends on the LAB gaining dominance over the remainder of the microflora. In other fermentation-based industrial processes the medium is freed of indigenous microorganisms by sterilisation and inoculation with specific organisms.
The lactic fermentation can be controlled to some extent by the use of additives which needs consideration due to additional cost involved. Those factors which affect the fermentation process and also the final products expected, determine the type of additives used in the fermentation process.

The additives used are generally classified into four groups. These are acids, fermentation inhibitors, fermentation stimulants and specific agents. (Woolford, 1985). Some of the common acids used are mineral acids such as sulphuric, hydrochloric, orthophosphoric and organic acids such as formic acid make the environment unsuitable for Clostridia. (Woolford, 1975b and 1978b, Wilson et al., 1979). Formaldehyde and some sulphates are commonly used to inhibit the generation of coliforms in general and thereby control the fermentation (Woolford, 1975). Some stimulants are used to encourage rapid proliferation of LAB or establish dominance of the enzymes. Antimicrobial are used for controlling fermentation and generally discourage the growth of Clostridia present in the system.
5.3 Conditions affecting lactic acid fermentation.

For improving the organoleptic quality and the shelflife of traditional products, reducing microbiological risks and to accelerate the process, it is necessary to study the key parameters involved in fermentation.

Effective fermentation depends upon the rapid growth and acid production by lactic acid bacteria and suppression of competing microbes by low pH values, weak organic acid anions and in some cases other antimicrobial factors in the system.

The review of Owens and Mendoza (1985) outlines the factors that influence the growth of LAB and the rate at which the pH value of the ferment declines and the competitors are suppressed. The factors outlines are:

- availability of fermentable carbohydrates;
- availability of organic growth factors;
- anaerobiosis;
- temperature;
- sodium chloride concentration;
- concentration of organic acids and pH value;
- carbon dioxide concentration;
- production of other inhibitory compounds;
- buffering capacity of the substrate;
- initial numbers of lactic acid bacteria and;
- initial numbers of competing microbes.
5.3.1 carbohydrates.
Because available free sugars to initiate fermentation are low in fish flesh the production of fermented fishery products require an added source of sugar. Some of the common sources of sugar are rice, molasses and other cereals. In most South-East Asian products rice is used in different forms. (An account of the publications on the different sources of sugars is given in the section 2.1).

5.3.2 organic growth factors.
The vitamins, amino acids and other organic growth factors are usually derived from the fish tissues.

5.3.3 anaerobic conditions.
As the prevention of the growth of spoilage organisms is important in the fermentation process the establishment of an anaerobic condition at the initial stage of the silage. The effect of the nature of the gas in the atmosphere during incubation had a little effect on the fermentation itself (Adams et al., 1987).

5.3.4 temperature.
A change in the temperature has a considerable effect on the rate of fermentation thereby influencing the microbial population and the final flavours of the products (Pederson, 1971; Stanton and Yeoh, 1977; and Hassan and Heath, 1986).
5.3.5 Salt concentration.
Salt is often used in the fermentation of many products and the usefulness of salt is basically due to the withdrawal of water and nutrients from the fish flesh thereby aiding the lactic acid bacteria to compete with spoilage organisms.

However the salt appears to have an effect on the activity of enzymes and increased salt levels tend to reduce the rate of fermentation. (Hassan and Heath, 1986).

5.3.6 pH.
A low pH value in the system tends to encourage the growth of lactic acid bacteria and these are found to be exceptionally tolerant to the low pH. It was observed that the addition of acids such as lactic, acetic or citric, at the initial stages did not assist the lactic fermentation (Adams et al., 1987).

5.3.7 Carbon dioxide.
As lactic acid bacteria are tolerant towards high concentrations of carbon dioxide compared to the majority of other bacteria early production of carbon dioxide in the system is considered to assist fermentation (Ingram, 1975; ICMSF, 1980).

5.3.8 Other inhibitory compounds.
The production of other inhibitory compounds such as nisin, are reported to be antagonistic towards the other competing organisms (Hurst, 1981).
5.3.9 buffering action.
A buffering action in fermentation due to proteins, salts and water is observed with most fish varieties. Vegetable products which have a low buffering action require less acid compared to fish silage (Nilson and Rydin, 1965 and Pederson, 1971).

5.3.10 initial LAB content.
The initial number of lactic acid bacteria present plays a significant role in the fermentation and a low number of spoilage organisms assist the fermentation process as the competition at the initial stage of the fermentation is reduced.

Studies on a fish; salt and glucose system by Adams et al., (1987) indicated that the fermentation rate increased in the range of 0-5 per cent w/w (of fish mince) of glucose or sucrose, whereas increases in salt concentration from 0-6 per cent slowed the rate of pH decrease. The lowering of pH was reduced at lower temperatures, but little variation was observed in the LAB/spoiler ratio during a 7 day incubation period.

Work on fish/salt cassava mixture by Adams et al., (1987) indicated that a cheap carbohydrate source could be used to produce a stable product.
5.4 ensiling of fish by lactic acid fermentation.

Preservation of fish or production of waste products by fermentation requires added sources of sugars, preferably with starter cultures for the initiation of proliferation of LAB (Raa et al., 1983). The LAB required for the fermentation is usually found to be indigenous in fish, but in low numbers (Schroder et al., 1980, Knochel, 1981).

Studies on the addition of sugars in the fermentation of fish have been carried out (Kompiang et al., 1963; Nilsson and Rydin, 1963 and Roa, 1965). Work has already been carried out to study the sequence of the change of microflora in the silage (Solidum, 1985). Studies on the reduction of coliforms present in the silage and the possible reasons were also outlined (Durairaj et al., 1976; Wirahadikusumah, 1968; James and Nair, 1977; Lindgren and Clestrom, 1978; and Schroder et al., 1980).

It is well established that many fish sauces are primarily produced by autolysis (Amano, 1962). Orejana and Liston (1979) showed that microorganisms play no essential role in the production of Philippine fish sauces. Other products clearly do depend upon the microbial acid production for fermentation (Orillo and Pederson, 1968).

The mechanism of autolysis and information on the enzymes which are responsible for autolysis are outlined in publications on this aspect (Hayashi and Nagai, 1973; Tatterson and Windsor, 1974;

The traditionally preserved fish products depend on the visceral enzymes of which pepsin from stomach and trypsin from pyloric caeca are recognised (Mackie et al., 1971).

In the traditional process of fish preservation by fermentation, the activity of the spoilage bacteria is inhibited by raising the osmotic pressure by adding salt or sugar or by lowering the pH by the addition of acids, or by adopting both together. The use of high concentrations of salt or sugar, to control bacterial spoilage hinders its use as either animal or human food due to palatability and associated technical difficulties as salt is known to inhibit the activity of proteolytic enzymes.

The growth of the spoilage bacteria is also prevented by maintaining the temperature above the survival temperature and hydrolysis is effected by selecting enzymes which are active at these relatively high temperatures.

Lowering of pH by acid was first demonstrated by A.I. Virtanen (Petersen, 1953) in Finland, and is now commonly used in Denmark, Poland, Norway and UK (Wignall and Tatterson, 1976). In fish preservation pH is usually lowered to about 4.0.
5.5 health hazards associated with preserved fishery products;

The incidence of health hazards, caused possibly by fish including fermented fish products, are outlined in Eitenmiller, Orr and Wallis, 1962; Zaitsev et al., 1969; Bartl, 1971; Healy and Juranek, 1979; Sakaguchi, 1979.

The control of *C. botulinum* is considered in detail although there is no direct evidence to establish any health hazards caused by the fermented fish products. (Zaitsev et al., 1969; Lerke, 1973 and Sakaguchi, 1979).
6. OBJECTIVES.

The study was designed with the following objectives:

(a) To use lactic acid fermentation for the stabilisation of shrimp waste.

(b) To apply different commercial innocula and energy sources (carbohydrate) to effect the lactic acid. Stabisil which is a commercially prepared inoculum and lactose was selected for fermentation.

(c) To perform lactic acid fermentation using innocula and energy sources available in tropical countries such as Sri Lanka. Cassava was selected as an energy source for initiation of lactic acid fermentation as it is readily available as a cheap energy source. Cassava when fermented has the benefit of improved nutritional value with improved acceptability and cassava powder is used in the Thai fermented fish product som-fak.

(d) To develop product streams from shrimp waste for chitin, astaxanthin and a protein product. The commercial applications for chitin and astaxanthin is established and the potential use of protein as an animal food is accepted.
7.0 MATERIALS AND METHODS:

7.1 materials:

7.1.1 shrimp waste:
Frozen head-on shrimps (*Penaeus monodon*) packed by c.v. Indonesia Seafood Corporation, Tropodo 1/27, Waru, Sidoarjo, Indonesia (Sea Master Brand) were deheaded and minced using an industrial mincer and frozen at -18 degrees C. This was defrosted prior to use in the fermentation. At the time of use these shrimps were nearly six months old.

7.1.2 starter culture:
Stabisil, a commercial bacterial aid to the fermentation of offal intended for silage usually applied in the form of liquid supplied by Nutrimix, Boundary Road, Lytham, Lancs.

The active ingredients present in Stabisil were *Streptococcus fecium* strains M74, *Lactobacillus plantarum* and *Pediococcus acidilactici*.

A solution of Stabisil was prepared using the minimum quantity of water possible and the solution was added to provide a predetermined quantity of Stabisil to the system.

7.1.3 carbohydrate sources:

7.1.3.1 lactose:
Lactose SLR grade supplied by Fisons Scientific Equipment was used as a source of carbohydrates in some experiments.
7.1.3.2 cassava:
Cassava tubers purchased from the market in Leicester, were peeled and chopped into medium size pieces, frozen at -18 degrees C and defrosted and ground using a domestic grinder prior to use in the fermentation.
7.2 methods:

7.2.1 fermentation:

Shrimp waste 50 g in glass sample bottles with tightly fitting caps was mixed thoroughly with the predetermined quantities of carbohydrates (lactose or cassava) as designated for the experiment using a domestic mixer. When required Stabisil solution was added and mixed further. Special care was taken to prevent air bubbles in the mixture as this could lead to spoilage.

The bottles with the contents were maintained at 25 degrees C in a water bath and the temperature was maintained by constant stirring. Duplicate samples were used in all experiments.

The temperature of 25 degrees C were preferred for the fermentation although the optimum temperature for fish was considered to be around 37 Degrees C. (Stanton & Yeoh, 1977; Hassan and Heath, 1986). At 25 degrees C, though the efficiency of the system is expected to be low, the cost of energy will be reduced by working at this temperature as this is close to ambient temperature in the tropics.

Samples were drawn at intervals and tested for pH, titrable acidity (TTA), and for microbiological examinations. Care was taken to minimise any possible contamination while sampling by using sterilised equipment and containers. Standard plate count of lactic acid bacteria (Lactobacilli and Pediococi) and Lactobacilli were done.
The increase in liquor level, separation of total nitrogen (protein and chitin) into the liquor and residue, chitin nitrogen in the residue, non-protein nitrogen in the liquor were reported at the termination of the fermentation. Amino acids present in the liquor were also reported.

7.2.2. physical measurements:
For separation of liquor and seidment the mixture was centrifuged at 2971 g for 15 minutes.

7.2.3. chemical analysis:

7.2.3.1. pH and titrable acidity (TTA):
A sample of 10 g were diluted with 10 ml of distilled water and pH and TTA were measured.

The pH was recorded using a PYE UNICAM pw 9418 pH meter with CORNING (Cat. No. 0031120 7H) electrodes.

The TTA was estimated by titrating against 0.1N sodium hydroxide to a final pH 8. The percentage lactic acid was calculated by multiplying the volume of alkali used by a factor of 0.09, assuming that all acid present in the sample is lactic acid (Adams et al., 1987).

7.2.3.2. analysis for nitrogen:
Estimation of nitrogen was made using a KJELTEC system and expressed as percentage of sample (d.w.b.)
7.2.3.3. non protein nitrogen:
Non protein nitrogen in the liquor was determined after homogenising with 20 per cent tri-chloroacetic acid (Blackhoff, 1976). and expressed as percentage of total liquid nitrogen.

7.2.3.4. chitin and chitin nitrogen:
Chitin nitrogen were estimated by acid and alkali digestion, and subsequent estimation of nitrogen using KJELTEC system (Black and Schwartz, 1950) and expressed as percentage of sample. Chitin was calculated by multiplying chitin nitrogen by 6.89.14.5

7.2.3.5. amino acids:
Amino acid composition was recorded using Chromakon 500 automatic amino acid analyser.

7.2.3.6. estimation of fat:
The mixture was centrifuged at 2761 g for 15 minutes and the liquor was separated by decanting. Fat content was determined on dry basis by Soxhlet distillation with 16 hours acetone (AOAC, 1984. 18.046) and expressed as percentage of liquor.

7.2.4. bacteriological analysis:
7.2.4.1. total plate count:
Total plate count was estimated, using Nutrient Agar, after 5 days at 37 degrees C.
7.2.4.2. lactic acid bacteria (LAB):

Medium used for the estimation of *Lactobacilli*, *Pediococci* and *Leuconostocs* is given below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Lemco</td>
<td>10 g</td>
</tr>
<tr>
<td>Pepton (Evans)</td>
<td>10 g</td>
</tr>
<tr>
<td>Yeast extract (difco)</td>
<td>5 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 g</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Triammonium citrate</td>
<td>2 g</td>
</tr>
<tr>
<td>Salts solution</td>
<td>5 ml</td>
</tr>
<tr>
<td>Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

The following salt solution was prepared and added to the above solution.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salts solution</td>
<td></td>
</tr>
<tr>
<td>Mg. SO₄·7H₂O</td>
<td>8.0 g</td>
</tr>
<tr>
<td>Mn. SO₄·4H₂O</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

(Keddie, 1951 modified)

pH of the medium was adjusted to 7.0. This is known as GYLA medium. This was incubated for 5 days at 37 degrees C.
5.2.4.2. Lactobacilli:

For estimating Lactobacilli (GYLA) was made selective by the addition of a sterile solution of thallus acetate.

(Gibson, Stirling, Keddie & Rosenberger, 1958 modified).

- Lab Lemco: 10 g
- Peptone (Evans): 10 g
- Yeats extract (Difco): 5 g
- Glucose: 5 g
- Agar: 20 g
- Distilled Water: 1000 ml
- Thallus acetate: 1 g

pH was adjusted to 7.0

Colonies were counted after 5 days at 37 degrees C.
8. RESULTS AND OBSERVATIONS:

8.1 pH and TTA changes;

The LAB generate lactic acid and acetic acid from the fermentable sugars present in the system and as a consequence the pH is reduced to a level at which spoilage is prevented. Due to the growth of Clostridia remaining in the silage and utilising lactic acid, sugars, proteins and amino acids and producing butyric and higher fatty acids, amines, amides and ammonia the pH of the system tends to increase. The pH is considered a more reliable indicator than counts of microflora on the state of preservation of the system.

The increased acidity during fermentation indicates the growth of different types of lactic acid bacteria. Initial L. plantarum and L. curvatus are gradually replaced by high acid forming L. brevis and L. buchneri in the terminal stages of the silage. A secondary growth of Pediococci could also increase during the latter stages of the silage.

The sequence of change reported is Streptococci and Leuconostocs predominating around 5, the Lactobacilli around 4.5 and Pediococci around a pH of 4.

The reduction in pH prevents spoilage as the growth of coliforms is prevented at this pH level and is satisfactorily used in preservation of fish. Storage of fish is carried out at a pH around 4.0 and liquifaction of fish takes place due to the enzymes present.
The traditional preservation techniques depending on the visceral enzyme such as pepsin and cathepsin are well known. The addition of acid or the production of lactic acid leads to a pH at which the enzyme is possible.

The effectiveness of the fermentation is monitored by the rate of pH decrease and the balance between LAB counts and total spoiler counts.

As the pH is considered to be the better indicator for the effectiveness of a silage and is easy to carry out, this parameter is often observed in silage systems.

For studying the effectiveness of the fermentation of different shrimp waste models pH and titrable acidity are used as the indicators. The buffering capacity of shrimp waste requires higher acid production to reduce the pH of the system to the desired levels compared with fish.

8.1.1 shrimp waste with lactose and Stabisil:

(Tables 12 and 13, Figures 6-9)

In this system the most rapid lowering of pH within the first 48 hours was observed with 15 per cent lactose and 4 per cent Stabisil, where the pH was reduced to 4.7 and then to 3.5 in 5 days from the initial level of 7.3. The increase of lactose level to 20 per cent did not improve the fermentation rate.

With 2 per cent Stabisil an increase of lactose from 10 to 20 per cent produced a similar pH change during the first 48 hours which after 5 days resulted in reducing the pH to 4.2 and this level was maintained until the 23rd day.
The comparison of the pH levels of 20:2 and 20:4 lactose and Stabisil combinations show hardly any improvement possibly indicating that the carbohydrate content was not limiting the fermentation.

The lactic acid produced interpreted as TTA, indicates a greater production of acid initially with 4 per cent Stabisil but, after 8 days, the TTA level in the 20:2 lactose: Stabisil model resulted was 4.1 per cent compared to 3.4, 3.0 and 3.3 in the 10:4, 15:4 and 20:4 models and this trend is continued till 23 days.

The TTA levels confirm the most preferred combinations observed in pH measurements.

Due to high buffering capacity of shrimp waste an acid level over 2 per cent was necessary to reduce the pH below 4.5 and around 4 per cent to maintain at pH 4.4, the lowest level observed in the whole system.

8.1.2. cooked shrimps, lactose and Stabisil:

(Tables 14 and 15, Figures 10-15)

In this model the initial pH of 8.3 was reduced to 6.5 even with 10 per cent lactose and 1 per cent Stabisil indicating the reduced competition to lactic acid bacteria from other organisms. During the first 48 hours the biggest drop to 6.3 was observed with 15:2 combination. And the drop of pH to 4.2 on the 6th day confirmed the lower carbohydrate level needed to effectively reduce the pH compared to 20 per cent lactose level with fresh shrimp waste (8.1.1.).
TABLE 12. pH in the mixture of shrimp waste with variable lactose and Stabisil (% w/w) at 25 degrees C.

<table>
<thead>
<tr>
<th>Comb.</th>
<th>days</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>19</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>lac: stab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:2</td>
<td></td>
<td>6.7</td>
<td>4.3</td>
<td>3.8</td>
<td>4.4</td>
<td>4.5</td>
<td>4.4</td>
<td>4.6</td>
</tr>
<tr>
<td>15:2</td>
<td></td>
<td>6.8</td>
<td>4.3</td>
<td>4.1</td>
<td>4.3</td>
<td>4.5</td>
<td>4.4</td>
<td>4.6</td>
</tr>
<tr>
<td>20:2</td>
<td></td>
<td>6.6</td>
<td>3.8</td>
<td>4.2</td>
<td>4.2</td>
<td>4.3</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>10:4</td>
<td></td>
<td>5.4</td>
<td>3.6</td>
<td>4.0</td>
<td>4.0</td>
<td>4.1</td>
<td>4.0</td>
<td>4.3</td>
</tr>
<tr>
<td>15:4</td>
<td></td>
<td>4.7</td>
<td>3.5</td>
<td>3.9</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>20:4</td>
<td></td>
<td>5.6</td>
<td>3.6</td>
<td>3.9</td>
<td>4.0</td>
<td>4.1</td>
<td>4.0</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Note: Initial pH of the mixture was 7.3

TABLE 13. TTA (%) in the mixture of shrimp waste with variable lactose and Stabisil (% w/w) at 25 degrees C.

<table>
<thead>
<tr>
<th>Comb.</th>
<th>days</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>19</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>lact: stab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:2</td>
<td></td>
<td>0.68</td>
<td>2.39</td>
<td>4.10</td>
<td>4.19</td>
<td>4.46</td>
<td>4.77</td>
<td>4.23</td>
</tr>
<tr>
<td>15:2</td>
<td></td>
<td>0.54</td>
<td>2.52</td>
<td>4.10</td>
<td>4.23</td>
<td>4.37</td>
<td>4.77</td>
<td>4.32</td>
</tr>
<tr>
<td>20:2</td>
<td></td>
<td>0.72</td>
<td>3.02</td>
<td>4.10</td>
<td>4.28</td>
<td>4.28</td>
<td>4.82</td>
<td>4.36</td>
</tr>
<tr>
<td>10:4</td>
<td></td>
<td>0.86</td>
<td>3.02</td>
<td>3.42</td>
<td>3.92</td>
<td>3.60</td>
<td>4.14</td>
<td>3.78</td>
</tr>
<tr>
<td>15:4</td>
<td></td>
<td>1.04</td>
<td>3.46</td>
<td>3.02</td>
<td>3.96</td>
<td>3.56</td>
<td>4.19</td>
<td>3.83</td>
</tr>
<tr>
<td>20:4</td>
<td></td>
<td>1.04</td>
<td>3.52</td>
<td>3.33</td>
<td>3.38</td>
<td>3.51</td>
<td>3.83</td>
<td>3.60</td>
</tr>
</tbody>
</table>
Figure 7.
PpH in the mixture of shrimp waste with variable lactose (% w/w) and 4% (w/w) Stabisil at 25°C
Figure 6.
P\(H\) in the mixture of shrimp waste with variable lactose \%(w/w)\) and 2\%(w/w) Stabisil at 25°C.
Figure 8.
TTA in the mixture of shrimp waste with variable lactose (% w/w) and 2% (w/w) Stabisil at 25°C

Legend
- lactose 10
- lactose 15
- lactose 20
Figure 9.
TTA in the mixture of shrimp waste with variable lactose (% w/w) and 4% (w/w) Stabisil at 25°C
Figure 10.

pH in the mixture of cooked shrimp waste with variable lactose (\%w/w) and 1\%(w/w) Stabisol at 25\(^\circ\)C
Figure 11.

pH in the mixture of cooked shrimp waste with variable lactose (% w/w) and 2% (w/w) Stabisil at 25°C

Legend
- lactose 10
- lactose 15
- lactose 20
Figure 12.
PH in the mixture of cooked shrimp waste with variable lactose (\%/w/w) and 4\% (w/w) Stabisil at 25\°C

Legend
- lactose 15
- lactose 20
Figure 13.

pH in the mixture of cooked shrimp waste with variable lactose (% w/w) and 1 % (w/w) Stabisil (powder) at 25 °C.

Legend:
- ■ lactose 10
- □ lactose 15
Figure 14.
P\(\text{H}\) in the mixture of cooked shrimp waste with variable lactose (%w/w) and 2%(w/w) Stabisil at 25°C.
Figure 15.
P.H in the mixture of cooked shrimp waste with 20\% (w/w) lactose and 4\% (w/w) Stabisil (powder) at 25°C.
The increase of Stabisil level to 2 per cent enabled the pH decrease to 4.2 with 15 per cent carbohydrate on the 6th day. This trend was not maintained when the lactose level was increased to 20 per cent as the pH was maintained at 4.5.

The use of 1 per cent Stabisil powder failed to drop the pH below 5.3 either with 10 and 15 per cent lactose and any increase of the level of Stabisil used did not increase the drop in pH (Tables 15 and Figures 13-15).

8.1.3. Fresh shrimp waste and cassava (fresh or prefermented): (Tables 16 and 19, Figures 16-19).

The initial pH of 7.7 dropped to 4.4 with 1:1 shrimp and fresh cassava combination after 6 days indicating carbohydrate and initial LAB levels for effective fermentation. With 1:1 combination a pH level of 4.5 was achieved within 3 days whereas, with 2:1 cassava the lowest pH level achieved in 3 days were 5.3 and the sample was decomposed (Table 6). The pH of fermentation of cassava alone was 3.4 after 3 days fermentation.

With 1:1 prefermented cassava a lower pH level of 4.7 in 3 days compared to 5.0 with 50% (2:1) cassava was observed (Table 18, Figure 18) but little difference in the pH level was noted as the fermentation progressed and after 21 days, the pH with 1:1 prefermented cassava was 4.2 compared with 4.6 with 2:1 prefermented cassava.
<table>
<thead>
<tr>
<th>Comb. lac:stab.</th>
<th>(days)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>10</th>
<th>11</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:1</td>
<td></td>
<td>6.5</td>
<td>6.7</td>
<td>6.1</td>
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<td>5.0</td>
<td>4.8</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>15:1</td>
<td></td>
<td>6.6</td>
<td>6.7</td>
<td>6.7</td>
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<td>5.6</td>
<td>5.1</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>10:2</td>
<td></td>
<td>6.1</td>
<td>5.9</td>
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<td>4.9</td>
<td>4.8</td>
<td>4.7</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:2</td>
<td></td>
<td>6.3</td>
<td>6.5</td>
<td>5.3</td>
<td>4.9</td>
<td>-</td>
<td>4.2</td>
<td>4.6</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>20:2</td>
<td></td>
<td>6.2</td>
<td>6.3</td>
<td>5.4</td>
<td>4.9</td>
<td>-</td>
<td>4.5</td>
<td>4.7</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>15:4</td>
<td></td>
<td>5.7</td>
<td>5.3</td>
<td>5.3</td>
<td>5.0</td>
<td>-</td>
<td>4.7</td>
<td>4.9</td>
<td>4.8</td>
<td>4.7</td>
<td>4.4</td>
</tr>
<tr>
<td>20:4</td>
<td></td>
<td>5.8</td>
<td>5.8</td>
<td>5.2</td>
<td>4.9</td>
<td>-</td>
<td>4.5</td>
<td>4.7</td>
<td>4.6</td>
<td>4.5</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Note: Initial pH of the mixture was 8.3.
TABLE 15. pH in the mixture of cooked shrimp waste and variable lactose and (powder) Stabisil (% w/w) at 25 degrees C.

<table>
<thead>
<tr>
<th>Comb. lac:stab.</th>
<th>days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:1</td>
<td></td>
<td>6.8</td>
<td>6.2</td>
<td>5.6</td>
<td>5.3</td>
<td>5.4</td>
</tr>
<tr>
<td>15:1</td>
<td></td>
<td>6.4</td>
<td>6.5</td>
<td>6.1</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>10:2</td>
<td></td>
<td>7.5</td>
<td>6.4</td>
<td>6.2</td>
<td>5.6</td>
<td>5.8</td>
</tr>
<tr>
<td>15:2</td>
<td></td>
<td>7.8</td>
<td>6.3</td>
<td>6.3</td>
<td>6.4</td>
<td>6.2</td>
</tr>
<tr>
<td>20:4</td>
<td></td>
<td>6.2</td>
<td>6.7</td>
<td>6.4</td>
<td>6.4</td>
<td>6.2</td>
</tr>
</tbody>
</table>

**Note:** initial pH 8.2
The use of pre-fermented cassava gave an improvement over the use of fresh cassava as after 6 days the observed pH was between 4.4 for both fresh and pre-fermented cassava although twice the amount of fresh cassava was needed (2:1 not 1:1 mixture).

More acid was produced with pre-fermented cassava, as expected, both at 2:1 and 1:1 level compared with fresh cassava, although the 1:1 fresh cassava had produced more or less the same level of acid after 6 days.

8.1.4 Shrimp waste, cassava (fresh, boiled and pre-fermented) with Stabisil. (Tables 20 and 21, Figures 20-23).

Stabisil was maintained at 2% based on the experience from shrimp waste, lactose and Stabisil system (8.1.1). The lowering of the pH with both fresh and pre-fermented cassava was similar in the presence of a 2% inoculum with 1:1 level, pH reducing to around 4.5 within 3 days and around 5 at 2:1 level during the same period. This trend was continued even after 13 days.

The presence of an inoculum with boiled cassava failed to bring about an improvement in the rate of drop of pH, as at both 1:1 and 2:1 level pH was around 4.5 after 3 days and after 14 days this was between 4 and 4.5.

A similar rate of production of acid was observed with both fresh, boiled and pre-fermented cassava at both levels.
TABLE 16. pH in the mixture of shrimp waste with variable cassava (fresh) (w/w) at 25 degrees C.

<table>
<thead>
<tr>
<th>ratio</th>
<th>days</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>shrimp/cassava</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td></td>
<td>7.8</td>
<td>6.7</td>
<td>6.0</td>
<td>5.3</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>7.7</td>
<td>5.6</td>
<td>4.9</td>
<td>4.5</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>cassava (alone):</td>
<td></td>
<td>5.9</td>
<td>3.6</td>
<td>3.4</td>
<td>3.4</td>
<td>3.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>

* sample was decomposed.

TABLE 17. TTA in the mixture of shrimp waste with variable fresh cassava (w/w) at 25 degrees C.

<table>
<thead>
<tr>
<th>ratio</th>
<th>days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>shrimp/cassava</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td></td>
<td>0.30</td>
<td>0.70</td>
<td>0.80</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>0.30</td>
<td>0.80</td>
<td>1.40</td>
<td>2.30</td>
<td>2.30</td>
</tr>
<tr>
<td>cassava (alone):</td>
<td></td>
<td>0.20</td>
<td>0.72</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>ratio</td>
<td>days</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>shrimp/cassava</td>
<td>2:1</td>
<td>7.8</td>
<td>6.3</td>
<td>5.5</td>
<td>5.0</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
<td>7.7</td>
<td>5.8</td>
<td>4.8</td>
<td>4.7</td>
<td>4.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ratio</th>
<th>days</th>
<th>0.04</th>
<th>0.61</th>
<th>1.21</th>
<th>1.28</th>
<th>2.51</th>
<th>3.33</th>
<th>3.36</th>
</tr>
</thead>
<tbody>
<tr>
<td>shrimp/cassava</td>
<td>2:1</td>
<td>0.05</td>
<td>0.70</td>
<td>1.35</td>
<td>1.49</td>
<td>2.66</td>
<td>3.68</td>
<td>3.66</td>
</tr>
</tbody>
</table>
Figure 16.
PH in the mixture of shrimp waste with variable cassava % (w/w) and cassava (alone) at 25°C

Legend
- cassava 50%
- cassava 100%
- cassava (alone)
Figure 17.
TTA in the mixture of shrimp waste with variable cassava and cassava(alone) at 25°C.

Legend
- cassava 50%
- cassava 100%
- cassava (alone)
Figure 18.

pH in the mixture of shrimp waste with variable pre-fermented cassava (% w/w) at 25°C

Legend
- ■ cassava 50%
- □ cassava 100%
Figure 19.
TTA in the mixture of shrimp waste with variable pre-fermented cassava at 25°C
TABLE 20. pH in the mixture of shrimp waste with variable cassava (fresh, boiled and pre-fermented) (w/w) and 2% Stabisil at 25 degrees C.

<table>
<thead>
<tr>
<th>ratio shrimp/cassava</th>
<th>days</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>13</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Cassava:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td></td>
<td>7.9</td>
<td>5.3</td>
<td>5.0</td>
<td>4.6</td>
<td>4.5</td>
<td>4.4</td>
<td>4.3</td>
<td>-</td>
<td>4.2</td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>7.9</td>
<td>5.1</td>
<td>4.8</td>
<td>4.4</td>
<td>4.2</td>
<td>4.1</td>
<td>4.1</td>
<td>-</td>
<td>4.1</td>
</tr>
<tr>
<td>Boiled Cassava:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td></td>
<td>7.9</td>
<td>6.3</td>
<td>5.0</td>
<td>4.7</td>
<td>4.5</td>
<td>4.4</td>
<td>-</td>
<td>4.4</td>
<td>4.3</td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>7.9</td>
<td>5.9</td>
<td>4.7</td>
<td>4.5</td>
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<td>4.4</td>
<td>-</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Pre-fermented Cassava:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td></td>
<td>7.8</td>
<td>5.4</td>
<td>5.0</td>
<td>5.0</td>
<td>4.8</td>
<td>4.5</td>
<td>4.4</td>
<td>-</td>
<td>4.3</td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>7.8</td>
<td>5.2</td>
<td>5.0</td>
<td>4.8</td>
<td>4.7</td>
<td>4.4</td>
<td>4.2</td>
<td>-</td>
<td>4.1</td>
</tr>
<tr>
<td>ratio shrimp/cassava</td>
<td>days</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>13</td>
<td>14</td>
<td>21</td>
</tr>
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<td>----</td>
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<tr>
<td>Fresh Cassava:</td>
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</tr>
<tr>
<td>2:1</td>
<td>-</td>
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<td>0.68</td>
<td>1.12</td>
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<td>-</td>
<td>1.40</td>
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<tr>
<td>1:1</td>
<td>-</td>
<td>0.40</td>
<td>0.59</td>
<td>0.79</td>
<td>1.00</td>
<td>1.22</td>
<td>1.34</td>
<td>-</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>Boiled Cassava:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td>0.01</td>
<td>0.23</td>
<td>0.58</td>
<td>0.92</td>
<td>1.02</td>
<td>1.23</td>
<td>-</td>
<td>1.41</td>
<td>1.54</td>
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</tr>
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<td>0.01</td>
<td>0.23</td>
<td>0.64</td>
<td>0.85</td>
<td>0.99</td>
<td>1.08</td>
<td>-</td>
<td>1.19</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>Pre-fermented Cassava:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2:1</td>
<td>0.01</td>
<td>0.39</td>
<td>0.58</td>
<td>0.64</td>
<td>0.76</td>
<td>1.11</td>
<td>1.30</td>
<td>-</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>0.01</td>
<td>0.43</td>
<td>0.56</td>
<td>0.61</td>
<td>0.79</td>
<td>1.06</td>
<td>1.36</td>
<td>-</td>
<td>1.58</td>
<td></td>
</tr>
</tbody>
</table>
Figure 20.

pH in the mixture of shrimp waste with 50% (w/w) cassava [fresh, boiled and prefermented] with 2% (w/w) Stabisil at 25°C
Figure 21.
P\(\text{H}\) in the mixture of shrimp waste with 100\%(w/w) cassava[fresh, boiled and pre-fermented] and 2\%(w/w) Stabisil at 25°C.
Figure 22.
TTA in the mixture of shrimp waste with 50% (w/w) cassava [fresh, boiled and pre-fermented] with 2% (w/w) Stabisil at 25°C
Figure 23.
TTA in the mixture of shrimp waste with 100% (w/w) cassava [fresh, boiled and pre-fermented] with 2% (w/w) Stabisil at 25°C
8.2 increase in liquid level:

8.2.1. shrimp waste and lactose with Stabisil. (Table 22).
A higher release of liquid was noted with 2% Stabisil compared to 4% irrespective of the lactose level. With 2% Stabisil around 125% of liquor level was observed compared to 68 to 81.8% with 4% Stabisil.

8.2.2. shrimp waste with cassava (Table 23).
A greater amount of liquid was released at 1:1 level compared with 2:1 addition for both fresh and prefermented cassava, and with prefermented cassava the release was higher than with fresh cassava. The results take into account the water added with the prefermented cassava.

8.2.3. shrimp waste with cassava (fresh, boiled and prefermented and Stabisil (Table 24).
In the presence of Stabisil the increase in liquid was more of less the same with fresh, boiled and prefermented cassava and this was independant of the quantity of cassava used, although a lesser release was observed with fresh cassava at 50% level (2:1) and boiled cassava at 100% (1:1) addition.

8.3 release of nitrogen:
The proteins during the fermentation change into amino nitrogen and soluble proteins (Rose, 1918; Uyenco et al, 1953; Orejana and Liston, 1979).
The estimation of nitrogen in the different fractions of the silage is an indication of the proteolysis in the silage. In many fermented fish products reports on nitrogen analysis are available. Uyenco et al., (1953) reported an increase in amino nitrogen in nuoc-nam. Similar observations are made by Guevarra et al.,(1972) with patis from anchovies.
TABLE 22. Increase of liqour in the mixture of shrimp waste with lactose and Stabisil (after centrifugation);

<table>
<thead>
<tr>
<th>Lact:Innoc. (ratio)</th>
<th>% liqour (initially)</th>
<th>% liqour (after 23 days)</th>
<th>increase</th>
<th>% increase (over initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:2</td>
<td>28.6</td>
<td>64.1</td>
<td>35.3</td>
<td>123.4</td>
</tr>
<tr>
<td>15:2</td>
<td>28.6</td>
<td>64.2</td>
<td>35.6</td>
<td>124.5</td>
</tr>
<tr>
<td>20:2</td>
<td>28.6</td>
<td>64.5</td>
<td>35.9</td>
<td>125.5</td>
</tr>
<tr>
<td>10:4</td>
<td>44.4</td>
<td>74.6</td>
<td>30.2</td>
<td>68.0</td>
</tr>
<tr>
<td>15:4</td>
<td>44.4</td>
<td>80.7</td>
<td>36.3</td>
<td>81.8</td>
</tr>
<tr>
<td>20:4</td>
<td>44.4</td>
<td>79.3</td>
<td>34.9</td>
<td>78.6</td>
</tr>
</tbody>
</table>
TABLE 23. Increase of liquour in the mixture of shrimp waste and cassava (after centrifugation).

a). Fresh cassava:

<table>
<thead>
<tr>
<th>Combination</th>
<th>% liquor (initially)</th>
<th>% liquor (6 days)</th>
<th>Increase</th>
<th>% Increase (over initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>4.8</td>
<td>12.7</td>
<td>7.9</td>
<td>164.6</td>
</tr>
<tr>
<td>2:1</td>
<td>6.4</td>
<td>12.5</td>
<td>6.1</td>
<td>95.3</td>
</tr>
</tbody>
</table>

b). Pre-fermented cassava:

<table>
<thead>
<tr>
<th>Combination</th>
<th>% liquor (after 21 days)</th>
<th>% liquor (after 28 days)</th>
<th>Increase</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>27.7</td>
<td>115.0</td>
<td>87.3</td>
<td>315</td>
</tr>
<tr>
<td>2:1</td>
<td>18.5</td>
<td>51.4</td>
<td>32.9</td>
<td>178</td>
</tr>
</tbody>
</table>
TABLE 24. Increase in the liquor of shrimp waste, cassava (fresh, boiled and pre-fermented) with 2% (w/w) Stabisil (after centrifugation).

<table>
<thead>
<tr>
<th>Combination</th>
<th>% liquor (initially)</th>
<th>% liquor (after 28 days)</th>
<th>increase</th>
<th>% increase (over initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a). Shrimp waste: Fresh cassava.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>16.7</td>
<td>61.7</td>
<td>45.0</td>
<td>269.5</td>
</tr>
<tr>
<td>2:1</td>
<td>21.1</td>
<td>43.6</td>
<td>22.5</td>
<td>106.6</td>
</tr>
<tr>
<td>b). Shrimp waste: Boiled cassava.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>16.7</td>
<td>35.5</td>
<td>19.0</td>
<td>113.8</td>
</tr>
<tr>
<td>2:1</td>
<td>21.1</td>
<td>64.4</td>
<td>43.3</td>
<td>205.7</td>
</tr>
<tr>
<td>c). Shrimp waste: Pre-fermented cassava.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>20.0</td>
<td>62.7</td>
<td>42.7</td>
<td>213.5</td>
</tr>
<tr>
<td>2:1</td>
<td>23.7</td>
<td>71.6</td>
<td>47.9</td>
<td>202.1</td>
</tr>
</tbody>
</table>
Backhoff (1976) reported the pattern of increase of non-protein nitrogen in the silage produced from different parts of cod with acid. An increased free nitrogen in cod silage (with the gut as a component) was used as an indicator of the activity of gut enzymes.

Hassan and Heath (1986) estimated the soluble nitrogen to study the effect of pre-heating white perch in lactic fermentation studies and reported that pre-heating reduced the initial amount of soluble nitrogen substances and substantially reduced the amount released during fermentation.

With shrimp waste, in addition to protein nitrogen the distribution of chitin nitrogen requires consideration as any use of shrimp silage depends on the availability of chitin for use either as food or any other industrial purpose in addition to the liquification of proteins during fermentation.

8.3.1. shrimp waste with lactose and Stabisil:

(Table 25 and 26).

Most combination of lactose and Stabisil gave around 50% of the total nitrogen released into the liquor and about 55% of protein nitrogen except for 20:2 combination.

Most of the chitin was left in the residue particularly so for the 4% Stabisil combinations.

The accuracy of the method used to detect the chitin nitrogen account for about 85% of the chitin present. A more accurate method using chromatographic methods is available (Holan et al, 1980).
TABLE 25. Percentage distribution Nitrogen, Protein Nitrogen and Chitin Nitrogen (% Dry weight basis) in the liquor and residue of shrimp waste, lactose and Stabisil (after centrifugation).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10:2</td>
<td>5.7</td>
<td>5.68</td>
<td>4.53</td>
<td>6.71</td>
<td>1.67</td>
<td>5.87</td>
</tr>
<tr>
<td>15:2</td>
<td>6.6</td>
<td>6.31</td>
<td>5.43</td>
<td>6.21</td>
<td>1.49</td>
<td>4.67</td>
</tr>
<tr>
<td>20:2</td>
<td>5.32</td>
<td>4.26</td>
<td>4.15</td>
<td>5.70</td>
<td>1.61</td>
<td>4.09</td>
</tr>
<tr>
<td>10:4</td>
<td>5.95</td>
<td>5.74</td>
<td>4.78</td>
<td>6.33</td>
<td>2.12</td>
<td>4.21</td>
</tr>
<tr>
<td>15:4</td>
<td>5.31</td>
<td>5.09</td>
<td>4.14</td>
<td>6.14</td>
<td>2.01</td>
<td>4.13</td>
</tr>
<tr>
<td>20:4</td>
<td>5.00</td>
<td>4.80</td>
<td>3.83</td>
<td>5.82</td>
<td>1.78</td>
<td>4.04</td>
</tr>
</tbody>
</table>
TABLE 26. Percentage distribution of Nitrogen, Protein Nitrogen and Chitin Nitrogen in the liquor and residue in the mixture of shrimp waste, lactose and Stabisil (calculated from Table 25.).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Com.tn</td>
<td>(silage)</td>
<td>(silage)</td>
<td>(silage)</td>
<td>(silage)</td>
<td>(silage)</td>
</tr>
<tr>
<td>10:2</td>
<td>49.8</td>
<td>52.3</td>
<td>47.7</td>
<td>71.4</td>
<td>28.6</td>
</tr>
<tr>
<td>15:2</td>
<td>47.8</td>
<td>50.2</td>
<td>49.8</td>
<td>63.7</td>
<td>36.3</td>
</tr>
<tr>
<td>20:2</td>
<td>40.03</td>
<td>42.5</td>
<td>57.5</td>
<td>68.8</td>
<td>31.2</td>
</tr>
<tr>
<td>10:4</td>
<td>48.24</td>
<td>57.7</td>
<td>42.3</td>
<td>90.6</td>
<td>9.4</td>
</tr>
<tr>
<td>15:4</td>
<td>47.93</td>
<td>57.5</td>
<td>42.5</td>
<td>85.9</td>
<td>14.1</td>
</tr>
<tr>
<td>20:4</td>
<td>48.00</td>
<td>55.4</td>
<td>44.6</td>
<td>76.1</td>
<td>23.9</td>
</tr>
</tbody>
</table>
Non protein nitrogen measured in the liqour was about 50 to 56% of the total nitrogen in the liqour except for the 10:2 combination gave 41% NPN in the liqour (Table 27).

8.3.2. shrimp waste, cassava with Stabisil:

(Tables 28 and 19).

With cassava (1:1) and Stabisil (2%) the release of protein nitrogen was increased to over 60%.

Nearly all the chitin appeared to be left in the residue and no chitin nitrogen was detected in the liqour.

Non-protein in the liqour for cassava (1:1) and Stabisil (2%) was 90% of the total nitrogen and as no chitin nitrogen was found in the liqour, this should be true non-protein nitrogen (see footnote in Table 27).

8.4 Microbiological changes.

8.4.1. shrimp waste and lactose with Stabisil (Table 30).

The initial TPC count of $10^5$ was reduced to $10^3$ after 5 days and again increased to $10^6$ to $10^7$ after 23 days.

The initial LAB count of $10^5$ was increased to $10^8$ during the period of 23 days with lactobacillus increasing from $10^5$ to $10^9$ during the period.

8.4.2. shrimp waste and cassava (Table 31).

The initial bacterial count of $10^9$ TPC was increased to $10^{10}$ during the first day of the fermentation and reduced to $10^8$ on the second day.

This continued to increase and on the 5th day the TPC was $10^{14}$. 
TABLE 27. Non Protein nitrogen in the liquor of the mixture of shrimp waste and variable lactose and Stabisil (on dry weight basis):

<table>
<thead>
<tr>
<th>Lact:Stab</th>
<th>% NPN</th>
<th>% N(liq)</th>
<th>%NPN/N(liq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:2</td>
<td>2.31</td>
<td>5.68</td>
<td>40.67</td>
</tr>
<tr>
<td>15:2</td>
<td>3.23</td>
<td>6.31</td>
<td>51.19</td>
</tr>
<tr>
<td>20:2</td>
<td>2.37</td>
<td>4.26</td>
<td>55.63</td>
</tr>
<tr>
<td>10:4</td>
<td>3.24</td>
<td>5.74</td>
<td>56.45</td>
</tr>
<tr>
<td>15:4</td>
<td>2.78</td>
<td>5.09</td>
<td>54.62</td>
</tr>
<tr>
<td>20:4</td>
<td>2.52</td>
<td>4.80</td>
<td>52.08</td>
</tr>
</tbody>
</table>

Note: non-protein nitrogen in liquor of the mixture of shrimp waste and 100% (1:1) fresh cassava (w/w) after 21 days:

\[
\frac{\% \text{NPN}}{\% \text{N(liq)}} = 1.26, \quad \frac{\% \text{N(liq)}}{\% \text{N(liq)}} = 1.40, \quad \frac{\% \text{NPN}}{\% \text{N(liq)}} = 90.0
\]
TABLE 28. Percentage distribution of Nitrogen, Protein Nitrogen and Chitin Nitrogen (dry weight basis) in the mixture of shrimp waste, fresh cassava (100% w/w) and Stabisil (2% w/w) (after centrifugation).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tot.N (silg.)</td>
<td>1.41</td>
<td>1.40</td>
<td>1.40</td>
<td>1.43</td>
<td>0.31</td>
<td>0.29</td>
<td>1.14</td>
</tr>
<tr>
<td>Nin (liq.)</td>
<td>1.36</td>
<td>1.42</td>
<td>1.42</td>
<td>1.34</td>
<td>0.26</td>
<td>0.23</td>
<td>1.11</td>
</tr>
</tbody>
</table>

*note: Chitin N was not detected in the liquor fraction.*
TABLE 29. Percentage distribution of Nitrogen, Protein Nitrogen and Chitin Nitrogen in the liquor and residue in the mixture of shrimp waste, fresh cassava (100% w/w) and Stabisil (2% w/w) after centrifugation (calculated from Table 28.).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49.6</td>
<td>63.6</td>
<td>36.4</td>
<td>nil</td>
<td>93.5</td>
</tr>
<tr>
<td>2</td>
<td>51.1</td>
<td>61.2</td>
<td>36.9</td>
<td>nil</td>
<td>88.5</td>
</tr>
</tbody>
</table>
TABLE 30. Microbiological analysis after 0,5 and 23 days with 20% lactose and 2% Stabisil (inoculum) (orgs/ml silage).

<table>
<thead>
<tr>
<th>Days</th>
<th>TPC</th>
<th>GYLA</th>
<th>GYLA with Thallus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$10^5$</td>
<td>$10^5$</td>
<td>$2.2\times10^5$</td>
</tr>
<tr>
<td>5</td>
<td>$10^3$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>$10^6-10^7$</td>
<td>$10^8$</td>
<td>$1.2\times10^9$</td>
</tr>
</tbody>
</table>
The LAB counts which was around $10^6$ initially, increased to $10^{16}$ after 2 days and gradually decreased to $10^9$ on the 11th day. A similar trend was observed with Lactobacillus with the initial level of $10^{10}$ increasing to $10^{13}$ after one day and remained at the same level even after 11 days.

8.4.3. shrimp waste with cassava (fresh, cooked and prefermented) with 2% Stabisil. (Table 32).

With fresh cassava the initial TPC level of $10^6$ remained low around $10^2$ to $10^3$ until the 14th day and then increased to $10^4$ after 21 days.

The initial LAB level of $10^4$ increased to $10^8$ on the 21st day. A similar trend for Lactobacillus was seen with the initial level of $10^3$ increasing to $10^7$ on the 21st day. A similar trend was observed with boiled cassava although at a lower level.

A faster rate of increase for LAB and Lactobacillus observed with prefermented cassava and these were higher without Stabisil.

8.5 sensory evaluation:

8.5.1. shrimp waste with lactose and Stabisil (Table 33).

As fermentation proceeds the liquid level tended to increase and to be darker, specially with lower levels of lactose. In all the six combinations studied there was no indication of decomposition except with 10:2 combination where a slight smell of ammonia was observed on the 8th day.
TABLE 31. Microbiological analysis of the mixture of shrimp waste and fresh cassava (100% w/w) (cfu/ml silage).

<table>
<thead>
<tr>
<th>Days</th>
<th>TPC</th>
<th>GYLA</th>
<th>GYLA (with Thallus acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$8.3 \times 10^9$</td>
<td>$1.2 \times 10^6$</td>
<td>$1.6 \times 10^{10}$</td>
</tr>
<tr>
<td>1</td>
<td>$1.4 \times 10^{10}$</td>
<td>$6.1 \times 10^{11}$</td>
<td>$2.1 \times 10^{13}$</td>
</tr>
<tr>
<td>2</td>
<td>$9.1 \times 10^8$</td>
<td>$2.4 \times 10^{16}$</td>
<td>$1.4 \times 10^9$</td>
</tr>
<tr>
<td>5</td>
<td>$2.1 \times 10^{14}$</td>
<td>$9.2 \times 10^{12}$</td>
<td>$5.1 \times 10^{13}$</td>
</tr>
<tr>
<td>8</td>
<td>$3.8 \times 10^{10}$</td>
<td>$2.5 \times 10^{10}$</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>$1.8 \times 10^9$</td>
<td>$1.5 \times 10^{13}$</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 32. Microbiological analysis of the mixture of shrimp waste, cassava (100% w/w) with 2% Stabisil (w/w). (cFw/ml silage).

<table>
<thead>
<tr>
<th>Days</th>
<th>TPC</th>
<th>GYLA</th>
<th>GYLA (with Thallus acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cFw/ml silage</td>
</tr>
<tr>
<td>0</td>
<td>5.28x10^6</td>
<td>6.6x10^4</td>
<td>2.8x10^3</td>
</tr>
<tr>
<td>4</td>
<td>1.5x10^2</td>
<td>1. x10^5</td>
<td>1.6x10^4</td>
</tr>
<tr>
<td>7</td>
<td>5.8x10^4</td>
<td>1.5x10^9</td>
<td>6.0x10^8</td>
</tr>
<tr>
<td>21</td>
<td>3.5x10^4</td>
<td>1.3x10^8</td>
<td>1.4x10^7</td>
</tr>
</tbody>
</table>

b. Boiled Cassava:

<table>
<thead>
<tr>
<th>Days</th>
<th>TPC</th>
<th>GYLA</th>
<th>GYLA (with Thallus acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.34x10^4</td>
<td>1.6x10^4</td>
<td>3.1x10^3</td>
</tr>
<tr>
<td>4</td>
<td>1.6x10^2</td>
<td>5.0x10^5</td>
<td>1.6x10^4</td>
</tr>
<tr>
<td>7</td>
<td>1.48x10^3</td>
<td>3.2x10^9</td>
<td>7.8x10^8</td>
</tr>
<tr>
<td>14</td>
<td>2.3x10^3</td>
<td>1.2x10^8</td>
<td>8.1x10^7</td>
</tr>
<tr>
<td>21</td>
<td>2.1x10^4</td>
<td>3.1x10^8</td>
<td>3.5x10^8</td>
</tr>
</tbody>
</table>
c. Pre-fermented Cassava:

<table>
<thead>
<tr>
<th>Days</th>
<th>TPC</th>
<th>GYLA</th>
<th>GYLA (with Thallus acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$1.3 \times 10^8$</td>
<td>$1.46 \times 10^4$</td>
<td>$1.3 \times 10^4$</td>
</tr>
<tr>
<td>4</td>
<td>$1.4 \times 10^2$</td>
<td>$2.6 \times 10^6$</td>
<td>$1.4 \times 10^5$</td>
</tr>
<tr>
<td>7</td>
<td>$2.7 \times 10^2$</td>
<td>$3.9 \times 10^9$</td>
<td>$2.1 \times 10^8$</td>
</tr>
<tr>
<td>21</td>
<td>$2.6 \times 10^4$</td>
<td>$1.2 \times 10^9$</td>
<td>$1.4 \times 10^7$</td>
</tr>
</tbody>
</table>
With the increase in lactose level fat was observed to separate at the top of the liquor fraction.

Astaxanthin remained with chitin in the residue as a pink sediment indication that this is stable in the system without undergoing any change such as 'melonosis'.

8.5.2. shrimp waste with fresh cassava (Table 34).
The observation with fresh cassava indicate that with lower levels of cassava the system could not be stabilised as the 2:1 combination was decomposed on the 3rd day.

Astaxanthin was stabilised in the sediment of the 1:1 mixture which had a pleasant smell of cassava.

8.5.3. shrimp waste with cassava (fresh, boiled and prefermented) and 2% Stabisil (Table 35).
The fresh cassava and Stabisil combinations were decomposed on the 14th day of the fermentation whereas with boiled cassava slight decomposition was observed from the 7th day.

The fermentation of prefermented cassava and Stabisil combination did not show any indication of decomposition even after 21 days.
TABLE 33. Sensory evaluation of the mixture of shrimp waste with variable lactose and Stabisil at 25 degrees C.

<table>
<thead>
<tr>
<th>Combn. of lact:Stab</th>
<th>2 days</th>
<th>8 days</th>
<th>16 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:2</td>
<td>Greenish yellow liquid with brownish sediment.</td>
<td>Brownish liquid with buff coloured oil separation with pink sediment.</td>
<td>Pleasant smell.</td>
</tr>
<tr>
<td>15:2</td>
<td>Similar to 10:2</td>
<td>More liquid</td>
<td>Pleasant smell.</td>
</tr>
<tr>
<td>20:2</td>
<td>Similar to 10:2</td>
<td>Similar to 15:2</td>
<td>Liquid becoming dark.</td>
</tr>
<tr>
<td>10:4</td>
<td>Similar</td>
<td>Lighter coloured liquid.</td>
<td>Darker liquid.</td>
</tr>
<tr>
<td>15:4</td>
<td>Similar</td>
<td>Similar</td>
<td>Darker liquid.</td>
</tr>
</tbody>
</table>
of fat.
Pleasant smell.

<table>
<thead>
<tr>
<th></th>
<th>Similar</th>
<th>Similar</th>
<th>no change.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:4</td>
<td></td>
<td></td>
<td>no change.</td>
</tr>
<tr>
<td>20:4</td>
<td></td>
<td></td>
<td>no change.</td>
</tr>
</tbody>
</table>
TABLE 34. Sensory evaluation of the mixture of shrimp waste with fresh cassava at 25 degrees C.: 

<table>
<thead>
<tr>
<th>Combination</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 B</td>
<td>Similar to A but lighter in colour.</td>
<td>Similar to A but lighter in colour, with a pleasant smell and generation.</td>
<td>More liquid than A, with a pleasant smell.</td>
<td>Pinkish (mostly shell) with a greenish liquid. Pleasant smell. (close to smell of cassava).</td>
<td>No change.</td>
</tr>
</tbody>
</table>

* Fermentation was discontinued.
TABLE 35. Sensory evaluation of shrimp waste with cassava (fresh, boiled and pre-fermented) and Stabisil 2% (w/w) at 25 degrees C.

<table>
<thead>
<tr>
<th>Shrimp waste with fresh cassava:</th>
</tr>
</thead>
<tbody>
<tr>
<td>combination.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Day 2</td>
</tr>
<tr>
<td>Day 3</td>
</tr>
<tr>
<td>Day 7</td>
</tr>
</tbody>
</table>
Shrimp waste with pre-fermented cassava:

<table>
<thead>
<tr>
<th>Combination</th>
<th>1:1</th>
<th>2:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>B</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Greyish mixture with a pleasant smell. (smell of cassava)</th>
<th>Similar to A. With less gas. Gaseous.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>Pinkish (due to shell) with brownish sediment and greenish liquid separating. Pleasant smell.</td>
<td>Similar to A. Less gas.</td>
</tr>
<tr>
<td>Day 3</td>
<td>no change</td>
<td>no change.</td>
</tr>
<tr>
<td>Day 7</td>
<td>no change</td>
<td>no change.</td>
</tr>
<tr>
<td>Day 14</td>
<td>no change</td>
<td>no change.</td>
</tr>
<tr>
<td>Day 21</td>
<td>no change</td>
<td>no change.</td>
</tr>
</tbody>
</table>
Shrimp waste with boiled cassava:

<table>
<thead>
<tr>
<th>combination</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 A</td>
<td>Greyish brown mixture, pleasant smell.</td>
<td>Pinkish-brown, greenish liquid and a pleasant smell.</td>
<td>no change</td>
<td>slight decomposition</td>
<td>no change</td>
<td>less pleasant smell, with a slight ammonical smell.</td>
</tr>
<tr>
<td>2:1 B</td>
<td>Similar to A but lighter colour.</td>
<td>Pinkish-brown, greenish liquid and a pleasant smell.</td>
<td>slight decomposition</td>
<td>more decomposition</td>
<td>decomposition, with less liquid.</td>
<td></td>
</tr>
</tbody>
</table>
8.6. amino acid analysis of the liqour of the shrimp waste, lactose and 4% Stabisil mixture (Table 36).

The change in the lactose level apparently had no effect on the amino acid profile of the liqour as similar profiles were observed with three combinations of lactose with 4% Stabisil.

The lysine levels were slightly lower than that with shrimp head meal as reported by Meyers (1986). (See Table 10).

The observations are comparable with that of shrimp head silage established with acid (Meyers and Benjamin. (See Table 11).

8.7. fat analysis of the liqour of the mixture of shrimp waste with lactose and Stabisil (Table 37):

With the increase in lactose level a better separation of fat was observed with the maximum level of 34.58% with a 20:4 lactose and stabisil combination.

8.8. separation of astaxanthin:

The astaxanthin in the sediment of the silage produced with shrimp waste lactose and cassava were found to be soluble in chloroform more than acetone ethyl alcohol or petroleum either.

A pink solution resulted when the sediment was shaken with chloroform and chitin was left in the bottom as white flaky residue.
TABLE 36. Amino acid composition of the liquor in the mixture of shrimp waste, lactose and 4% (w/w) Stabisil:

<table>
<thead>
<tr>
<th>Lactose (%w/w)</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>(g/100 g protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic</td>
<td>9.81</td>
<td>9.12</td>
<td>9.07</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.55</td>
<td>2.90</td>
<td>2.20</td>
</tr>
<tr>
<td>Serine</td>
<td>1.62</td>
<td>1.64</td>
<td>1.64</td>
</tr>
<tr>
<td>Glutamic</td>
<td>14.78</td>
<td>12.12</td>
<td>13.27</td>
</tr>
<tr>
<td>Proline</td>
<td>4.88</td>
<td>7.11</td>
<td>5.12</td>
</tr>
<tr>
<td>Glycine</td>
<td>9.90</td>
<td>9.40</td>
<td>8.57</td>
</tr>
<tr>
<td>Alanine</td>
<td>10.43</td>
<td>1.38</td>
<td>10.49</td>
</tr>
<tr>
<td>Cysteine</td>
<td>5.96</td>
<td>6.74</td>
<td>6.90</td>
</tr>
<tr>
<td>Valine</td>
<td>7.11</td>
<td>7.59</td>
<td>7.67</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.81</td>
<td>2.74</td>
<td>2.97</td>
</tr>
<tr>
<td>iso-Leucine</td>
<td>5.92</td>
<td>4.94</td>
<td>6.45</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.76</td>
<td>8.66</td>
<td>8.22</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.46</td>
<td>1.73</td>
<td>1.75</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.40</td>
<td>4.42</td>
<td>3.53</td>
</tr>
<tr>
<td>Ammonia</td>
<td>2.01</td>
<td>2.02</td>
<td>1.62</td>
</tr>
<tr>
<td>Lysine</td>
<td>3.56</td>
<td>3.71</td>
<td>3.47</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.65</td>
<td>3.38</td>
<td>2.95</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.29</td>
<td>1.41</td>
<td>2.42</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>negligible</td>
<td>negligible</td>
<td>1.71</td>
</tr>
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</table>
TABLE 37. Fat content in the liquor of the mixture of shrimp waste, lactose and Stabisil at 25 degrees C.

<table>
<thead>
<tr>
<th>Combination lac:stab.</th>
<th>% Fat (liq)</th>
</tr>
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<tbody>
<tr>
<td>10:2</td>
<td>4.50</td>
</tr>
<tr>
<td>15:2</td>
<td>8.60</td>
</tr>
<tr>
<td>20:2</td>
<td>22.86</td>
</tr>
<tr>
<td>10:4</td>
<td>18.65</td>
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<tr>
<td>15:4</td>
<td>28.92</td>
</tr>
<tr>
<td>20:4</td>
<td>34.58</td>
</tr>
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</table>
9.0 DISUSSION:
Shrimp waste is generally considered as a less suitable medium for lactic acid fermentation (Putro, 1980) and most of the studies carried out with shrimp waste were based on acid silage and directed to isolate chitin and associated products. Information on the potential for utilising proteins in the shrimp waste were basically for the production of shrimp meal.

It was required to conduct preliminary work to study the possibility of establishing a biological silage with shrimp waste. The potential for developing pastes/sauces, based on fish/carbohydrate combinations is recognised (Cooke et al., 1987). Utilising proteins in the shrimp waste for similar products either for human or animal feed using the fermentation technology is still to be examined.

In reviewing the potential for fermented fish product by Cooke et al., (1987) indicated that most of the studies were directed towards improving the products of fish/salt/carbohydrate type mixtures and these were expected to offer considerable scope for new products from under-utilised species. The possibility of a shorter time of production compared to fish/salt products and a lower salt content should allow the advantage of using these products as a main course rather than as condiment as observed with high-salt fish sauces and products.

In fermentation studies, the rate of pH decrease and the balance between LAB counts and total spoiler (bacterial) counts are used to monitor the efficiency of fermentation (Cooke et al., 1987). A rapid drop in pH to a level below 4.5 within first 48 hours is considered a measure of effective fermentation.
This study was designed to investigate the possibility of stabilising shrimp waste by ensilation and to identify the possible innocula and natural sources of energy. To observe fermentation a simple combination of lactose and Stabisil was used.

These preliminary studies on the feasibility of conducting a lactic acid fermentation with shrimp waste of tropical shrimps were successful. These studies indicated that the pH of the shrimp waste could be lowered with lactose as a carbohydrate source and an innoculum such as Stabisil.

The preliminary studies also indicated that the raw material quality needs to be considered as the pH was not reduced with some of the shrimps processed in Thailand as these were decomposed. The literature review indicated that the initial microorganisms present in the raw material determine the production of a biological silage. The spoilage organisms could dominate the LAB present in the raw material in the competition for the limited sugars available in raw shrimp waste.

The survey on the microorganisms present in the tropical shrimps indicate a comparatively high level (Cann, 1977) and this may be a limitation in the selection of raw material for lactic acid fermentation.

Lactose was used in these studies as it is readily fermentable by the organisms and the study would not be complicated by a more complex carbohydrate for the preliminary studies.
The addition of carbohydrate is considered necessary to promote a satisfactory fermentation since fish flesh is considered very low in free carbohydrate (Cooke et al., 1987). Shrimp was generally considered a poor raw material for lactic acid fermentation due to the high buffering action observed due to the shell components. In addition there is a general requirement for LAB for fermentation but these are available in limited numbers in fish (Knochel, 1981). So that an innoculum to act as a starter was considered necessary.

This study was carried out at 25 degrees C as the fermentation was close to optimal at this level and had the added advantage that this is close to the ambient temperature in the tropics where any development work of fermentation of shrimp waste could be carried out.

The initial stage of the study was concentrated to identify the most suitable combination of shrimp waste/lactose and Stabisil and six combinations of shrimp waste/lactose/Stabisil were fermented.

This preliminary study indicated that with lactose and Stabisil fermentation the waste could be stabilised and around 50% of the proteins could be separated from the chitin. The increase in liquid level in the mixture indicated the effectiveness of autolysis.

The separation of fat from chitin, which was mostly left in the sediment was effective. The astaxanthin was left with the chitin and the possibility of extracting with an organic solvent was recognised.
Attempts to ferment with Stabisil powder did not meet with success possibly indicating that the initial LAB is less active with solid Stabisil.

To study the factors controlling the shrimp waste fermentation a comparison with some results of fermentation studies with fish could be useful.

The studies carried out with perch (Hassan and Heath, 1986) with lower levels of lactose such as 5% had produced as much as 5.22% acid in lowering the pH to 4.44 in 2 days and 6.68% acid in lowering the pH to 4.41 after 7 days. These TTA levels are higher than observed with shrimp waste in this study. As such, buffering action alone in shrimp waste will not affect the production of acid.

In comparison observations with minced whiting/glucose/salt combinations with lactic starters (L.plantarum commercially referred to as "Lactostart" and P.pentosaceus commercially referred as "Pediostart") indicated that the rate of pH drop increased with glucose or sucrose content up to about 5% (Adams et al., 1987). The pH observed with 4:1.05% (w/w) combination of glucose, sodium chloride and Lactostart in minced whiting was below 5.5 after 2 days and around 4.3 after 7 days.

Observations with shrimp waste: lactose and Stabisil failed to indicate any significant difference in the fermentation with the different combinations of lactose and Stabisil, although the 15:4 combination appear to give the most rapid drop in pH (see Table 12 and Figure 7).
The next stage of the study was devoted to study the possibility of replacing lactose with a cheaper and readily available energy source in the tropical countries. The usefulness of cassava as an excellent substrate for LAB growth is recognised by Cooke et al., (1987) and indicates the possibility of using it as a source of carbohydrate for fermentation studies.

The study of shrimp waste: fresh cassava shows that a ratio of 1:1 shrimp waste: cassava is required to establish a good silage as the results were in agreement with the criteria of a successful lactic acid fermentation specified by Adams et al., (1987). The quantity of cassava used is of importance as with 2:1 shrimp waste/cassava, even after 3 days the pH was above 5 and the samples were decomposed.

The observations with rice as a carbohydrate source in shrimp/rice/salt combinations of balao-balao, more acid was required to lower the pH. But no significant difference was observed after the 5th day (Solidum et al., 1983). However the shrimp used in balao-balao studies were P.indicus, which is a smaller variety with a higher percentage of flesh. Increased buffer action with shrimp waste could make fermentation difficult compared to balao-balao.

The use of cassava instead of lactose had improved the proteolysis as high as 90% of the protein in the liqour non-protein N compared to about 50% with lactose and Stabisil (Table 27). In using cassava the liquid had increased indicating improved liquification.
A lower pH was observed with cassava fermented alone associated with low TTA levels indicating the higher buffering capacity in the shrimp waste/cassava combination due to shrimp waste.

Lactic acid production with cassava:shrimp waste was lower than with shrimp waste/lactose and Stabisil combinations and therefore the level of cassava required to reduce the pH is high perhaps due to a lower level of variable fermentable sugars.

The results are comparable with those reported with 1:1 combination of fish:cassava (fresh) combination, where haddock (Gadus aeglefinus) was used (Twiddy et al., 1987), where the pH was reduced to around 4.5 with 100 (w/w) cassava.

Pre-fermentation of cassava improved the rate of pH reduction such that the quantity of cassava added could be less (2:1 combination) than with fresh cassava. Pre-fermentation had no effect in the 1:1 combination compared with fresh cassava.

With haddock and pre-fermented cassava lesser quantity of cassava (4:1) was needed for a similar pH reduction (Twiddy et al., 1987) indicating the higher buffering action with shrimp waste.

Prefermentation of cassava could become useful if smaller quantities of cassava is to be used to ferment shrimp waste and this could depend on the end use of the products, ie. whether for animal feed or human food to be developed.
The production of lactic acid is in agreement with the drop in pH in these combinations.

In comparison of shrimp fermentation with a similar natural carbohydrate source such as rice, the balao-balao studies where whole shrimp/rice/salt combinations are used (Solidum et al., 1983), cassava appears to be more an effective natural carbohydrate source.

The increased liquid levels in shrimp waste fermentations does not seem to provide a clear indication of the effectiveness of fermentation.

The third stage of the study was used to observe the improvements of fermentation using an added inoculum with cassava.

The addition of inoculum (Stabisil) to improve the fermentation has no significant effect in the reduction of pH with pre-fermented cassava either in 1:1 or 2:1 combinations.

Fermentation of 1:1 and 2:1 combinations in both fresh cassava and pre-fermented cassava were similar indicating the dominating effect of LAB from the inoculum.

The fermentation of shrimp waste/boiled cassava and Stabisil was slower than the other two combinations indicating the lower LAB activity but achieved a satisfactory pH after 7 days.
The addition of innoculum did not improve the fermentation in the 1:1 combination with fresh cassava but the pH in the 2:1 combination was reduced to below 5 after 2 days compared to pH 6 in the similar combinations without Stabisil.

These results indicate the addition of Stabisil could improve the fermentation at lower levels of addition of both pre-fermented and fresh cassava.

The initial TPC counts in the shrimp waste: lactose and Stabisil combinations (Table 30) indicate possibly the initial microbial load in the shrimp waste. Considering reports of the microbial population of tropical shrimps (Cann, 1977) and also the situation where over 50% of the total microrganisms are found in the head, these figures are low and perhaps this will be an indication of the raw material quality necessary for fermentation.

On the 5th day of fermentation, TPC reduced to $10^3$ indicating the reduction of spoilage organisms in the silage, due to the reduction in pH, which agrees with the general trend observed during fermentation. After the 23rd day TPC begins to increase accompanied by an increase in pH indicating the termination of the fermentation, possibly as all the sugars were used up, and the spoilage is initiated.

The LAB counts of $10^5$ initially in the silage could be due to the contribution of Streptococcus, Lactobacillus and Pediococcus from Stabisil. In comparison with the LAB reported in fish which is around
$10^2$ these figures are high and possibly leads to a stable silage (Knochel, 1981).

During the latter part of the fermentation, Lactobacilli tend to predominate as observed around $10^9$, with TTA around 4.0%.

With cassava (1:1), the initial TPC count was around $10^9$, possibly due to the combined microbial content of shrimp waste and cassava, and was reduced to $10^8$ on the second day with the commencement of fermentation and with a drop in pH to 4.9 TPC begins to increase after the 5th day with increase in pH (Table 31).

The LAB count of $10^6$ is comparable with that of the shrimp waste: lactose combination. On this basis the shrimp waste: cassava model is expected to follow a similar fermentation pattern provided the sugars available in this model are similar.

The LAB counts which were initially around $10^6$ increased to $10^9$ which is reflected in the increased percentage TTA from .3 to .8. The high Lactobacillus level in the latter stages of fermentation account for the rapid increase of percentage TTA after 3 days.

In the shrimp: cassava model with added Stabisil, the initial LAB count of $10^4$ is increased to $10^9$ on the 7th day and this is accompanied by the pH and TTA levels of 4.1 and 1.22 respectively (Table 32).
With boiled cassava the TPC level was low as expected due to the destruction of microflora during boiling.

The LAB levels were similar in fresh, boiled and prefermented cassava with Stabisil, indicating that LAB from Stabisil predominates in the systems.

The nitrogen analysis in the liquor and the residue of shrimp waste and cassava 1:1 combination indicates a more effective proteolysis as over 60% protein nitrogen was detected in the liquor. The residue contained almost all the chitin and suggests that this could be a useful method for the separation of chitin from the proteins in the waste.

This study showed that a successful fermentation could be carried out with shrimp waste in the presence of initially a fermentable sugar, and an added inoculum.

This also showed that a natural source of carbohydrate could be used as a source of energy and a source like cassava could be used in the fermentation without an added inoculum.

For limiting the use of cassava depending on the end use of the products from fermentation the use of an added inoculum could become useful.

The separation of chitin during fermentation was effective and this situation will enable the use of required quantities of chitin and protein to be combined in a feed formulation.
Astaxanthin was stabilised without any change and this will improve the nutritional value of the final products by improving the protein uptake and also could be separated and used as a colouring pigment in fish feed.

The separation of fats will be an added advantage as this will improve the protein uptake and of astaxanthin associated with the fat.

Further studies involving the use of local innocula to improve the fermentation and also the pre treatment of cassava for use in fermentation are required. The optimal levels of cassava to be used depending on the requirements of the process needs to be investigated.

The separation of chitin and astaxanthin and purification aspects are to be followed up. The use of proteins as a fish feed formulation or animal feed will enhance the economic viability of the project as would the development of prawn flavours.
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Addendum

