ENSILING CUTTLEFISH WASTES FOR USE AS FISH FEED INGREDIENT

By

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DECLARATION

I hereby declare that this thesis entitled "ENSILING CUTTLE FISH WASTES FOR USE AS FISH FEED INGREDIENT" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or any other similar title, of any other university or society.

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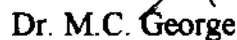

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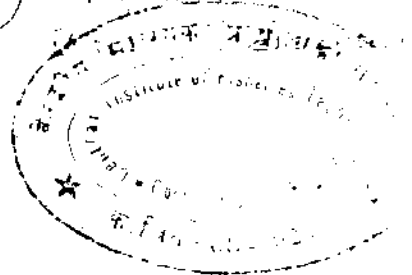

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CONTENTS

	Page No.
I INTRODUCTION	1
II REVIEW OF LITERATURE	2
2.1 History	2
2.2 Resources for Fish silage	3
2.3 Acid preserved fish silage	5
2.3.1 Inorganic acid silage	5
2.3.2 Organic acid silage	6
2.3.3 Quality of acid added silage	10
2.4 Fermented fish silage	16
2.4.1 Quality of fermented silage	22
2.5 Fish silage as feed	24
III MATERIAL AND METHODS	32
3.1 Silage preparation	32
3.1.1 Formic acid fish silage preparation	32
3.1.2 Fermented fish silage preparation	32
3.2 Analyses of the silages	33
3.2.1 Physical analyses	34
3.2.1.1 pH	34
3.2.2 Biochemical analyses	34
3.2.2.1 Proximate composition	34
3.2.2.2. Peroxide value (P.V)	35
3.2.2.3 Non protein nitrogen (NPN) content	35

3.2.2.4 Tri-methylamine (TMA) content 35

3.2.2.5 Total volatile base nitrogen (TVBN) content 35

3.2.3 Microbial analyses 36

3.2.3.1 Total plate count (TPC) 36

3.2.2.1 Most probable number (MPN) of coliforms 37

3.2..3.2 Acid producing bacterial count (APBC) 37

3.3 Preparation of fish feed 38

3.4 Acceptability study 38

IV RESULTS 40

4.1 pH 40

4.2 Total plate count (TPC) 42

4.3 Acid producing bacterial count (APBC) 43

4.4 Most probable number (MPN) of coliforms 44

4.5 Tri-methyl amine nitrogen (TMA-N) and Total volatile base nitrogen (TVB-N) 46

4.6 Non protein nitrogen (NPN) 49

4.7 Peroxide value (PV) 51

4.8 Proximate composition 52

4.9 Acceptability study 53

V DISCUSSION 54

VI SUMMARY 61

VII REFERENCES 63

VIII ABSTRACT 91

LIST OF TABLES

Table 1	pH of various formic acid fish silage preparations	32
Table 2	Quantity and percentage of various ingredients added for preparing fermented fish silage preparations.	33
Table 3	Composition of buffered peptone water.	36
Table 4	Composition of plate count agar (Hi-media)	36
Table 5	Composition of lactose broth (Hi-media)	37
Table 6	Composition of tomato juice agar (Hi-media)	38
Table 7	ANOVA table for pH	41
Table 8	ANOVA table for TPC	43
Table 9	ANOVA table for acid producing bacterial count	44
Table 10	ANOVA table for MPN of coliforms	46
Table 11	ANOVA table for TMA-N content	48
Table 12	ANOVA table for TVB-N content	48
Table 13	ANOVA table for NPN content	50
Table 14	ANOVA table for PV	52
Table 15	Proximate composition of the formic acid fish silage preparation and fermented fish silage preparation at zero day	53
Table 16	Proximate composition of the formic acid fish silage preparation and fermented fish silage preparation at 90th day	53

LIST OF FIGURES

Fig. 1	Variation in pH of formic acid fish silage preparations during storage.	41
Fig. 2	Variation in pH of fermented fish silage preparations during storage.	41
Fig. 3	Variation in TPC of formic acid fish silage preparations during storage.	42
Fig. 4	Variation in APBC of fermented fish silage preparations during storage.	44
Fig. 5	Variation in MPN of coliforms in formic acid fish silage preparations during storage.	45
Fig. 6	Variation in MPN of coliforms in fermented fish silage preparations during storage.	45
Fig. 7	Variation in TMA-N content (—) and TVB-N content (------) of formic acid fish silage preparations during storage.	47
Fig. 8	Variation in TMA-N content (—) and TVB-N content (------) of fermented fish silage preparations during storage.	47
Fig. 9	Variation in NPN content as percentage of total nitrogen in formic acid fish silage preparations during storage.	49
Fig. 10	Variation in NPN content as percentage of total nitrogen in fermented fish silage preparations during storage.	50
Fig. 11	Variation in Peroxide value of formic acid fish silage preparations during storage.	51
Fig. 12	Variation in Peroxide value of fermented fish silage preparations during storage.	51

INTRODUCTION

I INTRODUCTION

The disposal of wastes generated during fish processing is becoming a major problem for the fishing and food industries. In most instances the fish processing waste has no practical value and its disposal may even be a liability to the processor. The fish viscera which is being discarded at present, causing environmental pollution, could be collected and suitably processed to preserve the nutrients for incorporation into animal diets (Ahmed and Mahendrakar, 1995). Gopakumar (1997) considers silage production as one of the best means of preserving agro and animal wastes.

According to MPEDA report (1998), 37,258 tons of cuttle fish was exported from India, during the year 1997-98. The processing waste is around 30 to 40%, which is currently being dumped into the nearby water bodies. No suitable measures are taken to utilise them properly and to prevent environmental pollution. No work seems to have been done for conversion of this material to suitable products.

The objective of this study is to investigate the possibility of producing formic acid silage and fermented silage from cuttle fish processing wastes for incorporation into fish feeds as protein source.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

The traditional use of the word 'silage' has been in conjunction with green forage, preserved either by added acid or by the anaerobic production of lactic acid by bacteria (Raa and Gildberg, 1982).

2.1 History

Silage making has its origins in antiquity.. Kuchler (1926), Kirstein (1963) and Schukking (1976) stated that ensiling was practiced around 3000 years ago. The earliest account of the ensiling process as it is understood today is given by Grieswald in 1842 (Watson and Smith, 1956) in which ensiling of fresh grass in pits is described. Woolford (1984), observes that, in the second and third decades of this century, renewed efforts were made to establish the means of making consistently good quality silage, particularly from grass. In 1920s a Finn, professor A.I.Virtanen (A.I.V), began experiments on the effectiveness of mineral acids as treatments for rapidly reducing the pH and to reduce post harvest losses, particularly of proteins in grass silage. While the principle of the method was by no means new, Virtanen (1933) developed a system which was practicable (Woolford, 1984). The ensiling of animal wastes were prompted by the success of the A.I.V principle. This method was adopted by Edin (1940), to preserve fish waste. He carried out this work further using AIV acid and even suggested a formula based on pH, proteins and ash contents of the materials to calculate the quantity of acid required. Later, Olsson (1942), using Edins formula, worked out a table giving the amount of

acid required for Scandinavian raw materials. The production of silage on an industrial scale started in Denmark in 1948 (Petersen, 1951). Several laboratories around the world carried out extensive research on silage production, storage and application. Fish silages were prepared in Russia (Lagunov *et al.*, 1956), Canada (McBride *et al.*, 1961), The United Kingdom (Tatterson and Windsor,1974) and India (James, 1966). Fish and poultry offal probably form the bulk of animal wastes ensiled (Woolford, 1984).

2.2 Resources for fish silage

The small proportion of the edible fish in the by-catch which realises the highest market price, is usually iced (Young, 1978) or salted (Kompiang *et al.*, 1980), and sold. The remaining mixture of low value fish is the potential raw material for silage. In global terms, it has been estimated that some five million metric tons a year of fish are discarded by countries whose population suffer from protein deficiency (Poulter and Disney, 1981). The use of ice for low value by-catch is hardly viable under most conditions and particularly due to the low price of by-catch and high cost of ice. Conversion of the low value by-catch to silage may probably become an economically viable operation if there is space aboard for silage tanks and outlets for silage close to the landing places for shrimps (Raa and Gildberg, 1982).

To avoid the problem of fishy taint of the carcass, the level of fish oil in the complete diet should not exceed 1% of dry weight (Smith and Adamson,1976). Moreover oxidized oil has been shown to cause both palatability problems and reduced nutritional value of protein (Kompiang *et al.*,

1980). Trawl catches contain a large number of different fish species belonging to more than 40 families (Sinoda *et al.*, 1978; Young, 1978). It seems that Leiognathidae (silver bellies) and Synodontidae (lizard fish) are the only predominant by-catch fish which are so lean that they can be used in significant proportion in diets without de-oiling (Raa and Gildberg, 1982).

Seafood processing wastes potentially are valuable to ensile with crop residue for use as ruminant feed stuffs (Samuel *et al.*, 1991). Only about 10% of the total weight of processing crabs is used as human food, and 50% from fish filleted or dressed (Fontenot *et al.*, 1992). Fishery wastes processed by fermentation to enhance microbial stability would be a practical and economical alternative protein source to traditional fishmeal (Dong *et al.*, 1993). Since astaxanthin is stable in acid silage of shrimp processing waste, ensiling is a realistic way (Torrissen *et al.*, 1981). Prawn carapace silage was prepared by the addition of acid and then fermentation by Sachindra *et al.* (1994). Silage from processing discards were prepared by many workers e.g., acid silage with cod viscera (Backhoff., 1976), scallop viscera (Myer *et al.*, 1990), dogfish offal (Heras *et al.*, 1994).

2.3 Acid preserved fish silage

To preserve green plants with a mixture of hydrochloric acid and sulphuric acid as recommended by A.I. Virtanen in the 1920's, it is normally sufficient to lower the pH to about 4. To preserve fish by the same acids, however the pH has to be below 2 (Edin, 1940). When compared with green forage, fish contains a very low level of free sugars and has a high buffering capacity due to high levels of proteins and minerals present. Therefore, more acid is needed to produce a stable fish silage (Raa and Gildberg, 1982).

Commonly used acid or acid mixtures are formic acid (Hanson and Lovern, 1951), mixture of formic acid and hydrochloric acid (Disney *et al.*, 1977), mixture of sulphuric acid, formic acid and propionic acid (Rattagool *et al.*, 1979 a; Kompiang *et al.*, 1979 a) mixture of formic acid and propionic acid (Kompiang *et al.*, 1979 a).

2.3.1 Inorganic acid silage

Two major disadvantages of using mineral acids are the need to neutralise the silage before use (Petersen, 1953) and the silo corrosion (March, 1962). The former is accomplished by the addition of 2 to 5 kg chalk per 100 kg of silage (Petersen,1953). Stone *et al.* (1989) prepared fish silage of pH 4 by adding sulphuric acid and propionic acid, neutralised with 1.6% $Ca(OH)_2$ to pH 6.5 and dried under vacuum before feeding to rainbow trouts. Mineral acids are considered not to have any specific antimicrobial properties and merely act as acidifying agents (Woolford, 1984). Edin (1940) developed a

formula to calculate the quantity of acid required to lower the pH to 2 in a fish homogenate.

(a x 0.14 + b x 0.9) litres of 14 N acid per 100 kg raw material.

Where 'a' is the percentage crude protein of wet weight and 'b' is the percentage ash of wet weight.

2.3.2 Organic acid silage

The effect of formic acid on silage fermentation has been suggested to be due in part to its acidic nature and in part due to its selective antimicrobial properties, the latter being considered to be linked to the undissociated rather than the dissociated molecule (Craseman, 1941; Richard, 1946; Saue and Breirem, 1969; Papendick and Singh-Verma, 1972). The undissociated organic acid molecule can freely enter into the microbial cell. Inside the cell where the pH is neutral, the organic acid will ionize, gradually leading to a lower pH and accumulation of the anions. These factors contribute to the anti-microbial effects of weak organic acids, (Arnesen *et al.*, 1981; Raa and Gildberg, 1982; Lall, 1991).

More than 50% of the propionic acid (pk_a 4.86) exists in its undissociated antimicrobial form at pH value below 4.86. The pH must be below 3.75 in order to have more than 50% of the formic acid molecules (pk_a 3.75) in the antimicrobial form. The difference in pk_a values of these acids is the main reason why a stable fish silage may be obtained at a higher pH

value of 4.5 with propionic acid (Gildberg and Raa, 1977) than with formic acid where the pH must be below 4 (Olsson, 1942; Tatterson, 1976).

The use of formic acid in silage making was first suggested by Dirks in 1926 (Watson and Nash, 1960), Olsson (1942) indicated that the amount of formic acid required to lower the pH value to 4 is dependent on the composition of the fish and the season. He reported the following two formulae to calculate the amount of acid needed:

0.25 + 0.3% ash = Litres of formic acid (90%) per 100 kg raw material in winter.

0.50 + 0.3% ash = Litres of formic acid (90%) per 100 kg raw material in summer.

Upto 50% of the formic acid content of silage can be lost during ensilage (Henderson and McDonald, 1971).

Unlike mineral acids, organic acids have specific antimicrobial properties. For example, propionic acid is regarded as being distinctly antifungal (Daniel *et al.*, 1970; Gross and Beck, 1970,1972; Gaiger, 1978) and it does have some action against endospore forming bacteria (Woolford, 1975). *Aspergillus flavus* is able to grow in the surface lipid of fish silage (Kompiang *et al.*, 1980) and it may quickly spoil moist mixtures of silage and carbohydrate meals. Strom *et al.* (1980) found that formic acid prevented the growth of *A.flavus* below pH 4 and propionic acid below pH 6. Hoq *et al.* (1995) observed no mould growth in silage during storage and they assumed it to be

due to regular stirring and use of high percentage of formic acid. The antimicrobial properties of the straight chain fatty acid increases with the number of carbon atoms in the compound inspite of there being a corresponding decrease in acidic properties (Woolford, 1975).

Although fish silages produced with formic acid are more expensive than those produced with inorganic acids (Lisac, 1961), liquefaction is faster in formic acid silages and lipids separate more easily from the proteins, and further, formic acid is metabolised in the animal intestine (March, 1962).

To reduce the cost of preservation, different mixtures of inorganic and organic acids were tried by various workers. For example formic acid in mixture with sulphuric acid (Olsson, 1942; Lisac,1961; Disney and Hoffman, 1976; Disney *et al.*, 1977). Sulphuric acid may be replaced by hydrochloric acid (Disney and Hoffmann, 1976; Disney *et al.*, 1977) or phosphoric acid (Jensen and Schmidtsdorff, 1977).

Because of the higher ash content of tropical by-catch fish, more acid is needed to preserve them. About 2.5% formic acid or a mixture of formic and propionic acid being the minimum concentration which ensures preservation of fresh by-catch fish (Kompiang *et al.*, 1980). It is important for the silage process to adopt the most economic combination of acids.

Disney *et al.* (1977) recommended that the fishes should be minced (<3.4 mm particle size) and thoroughly mixed with acids followed by periodic agitation during storage at a temperature of at least 20^0C. The mixing of

mince with the acid may be a significant practical problem, if the fish is fresh, because the muscle components become rubber-like when exposed to acid. The mince, therefore tends to form closed pockets where the acids do not enter quickly enough to prevent spoilage, though if the raw material is not fresh, this is not a major problem (Raa and Gildberg, 1982).

The role of proteases in the liquefaction of fish is undisputed. Fish offal from which the gut has been removed, liquefies alone whereas fillets do not, nor do whole fish or parts of fish which have been heated at 100°C for 15 minutes (Tatterson and Windsor, 1974). The enzymes are mostly pepsin - type stomach proteases and lysosomal enzymes like cathepsin-D, which is the major muscle protease (Gildberg, 1988). The rate of autolysis is determined by the activity of digestive enzymes in the raw material, the physiological condition of the fish at the time it is caught, the pH, the temperature and the preservative acid. The yield of solubilised protein varies, the flesh giving the lowest and viscera the highest (Tatterson and Windsor, 1974; Backhoff, 1976).

Extensive investigations were conducted by Raghunath and McCurdy (1990) on the influence of pH on silage production and reported that the activity of proteases of trout viscera varied with differing pH. Both endo and exopeptidases were active at pH 3.0, the silage quickly breaking down the protein nitrogen to amino nitrogen. But at a pH 2, only acid endopeptidase and a weak exopeptidase activity were detected, thus slowing down the autolysis process. The authors also reported that addition of formic acid limited the increase in pH during autolysis and prevented the increase in amino nitrogen,

indicating that exopeptidases are completely inhibited by formic acid. The critical temperature for commencement of rapid autolysis of an acid silage of fish caught in tropical water is around 30^0C (James, 1966) and for cold water fish is around 20^0C (Raa and Gildberg., 1976). An autolysis temperature of about 30^0C seems suitable for practical purposes (Gildberg and Raa, 1977).Maximum rate of autolysis is obtained at about 50^0C, but at this temperature proteins which dissolve due to autolysis, precipitate again (Raa and Gildberg, 1982). They recorded that there is little difference in the temperature optima of proteases from arctic fish, which spent their entire life below 4^0C and tropical fish, which live at 25^0C to 30^0C. The rate of autolysis of ensiled cod viscera was markedly lower at pH 3 than at 4. Herring silage autolysed better if formic acid was used alone rather than mixed with sulphuric acid or phosphoric acid, because pH was 4.5 in the former case, but 3.1 in the latter (Jensen and Schmidtsdorff, 1977).

2.3.3 Quality of Acid added Silage

About 80% of the protein in an acid fish silage usually becomes solubilised after one week at temperatures around 23-30^0C (Tatterson and Windsor, 1974; Backhoff, 1976; Gildberg and Raa, 1977) A number of workers have reported the presence of a residue resistant to further proteolysis (Tatterson and Windsor, 1974; Backhoff, 1976; Raa and Gildberg, 1976; Gildberg and Raa, 1977; Johnson and Skerede, 1981; Hall *et al.*, 1985 a, b; Raghunath and McCurdy, 1990). The undigested protein in the sludge is seen to be as high as 50% of the total protein in the case of tropical fish. Protein

solubilisation may be reduced by lipid in the fish (Sheikh and Shah, 1974; Raa and Gildberg, 1976). The undigested proteins appear to be peptide aggregates held together by non-covalent forces (Hall *et al.*, 1985 a, b; Raghunath and McCurdy, 1990). The theory of non-availability of suitable amino residues at splitting sites for various process has been discounted by Raa and Gildberg (1976), who found normal or at times even higher concentration of such amino acids as cysteine, and lack of hydroxy proline in the unhydrolysed sediment. Another reason suggested is the accumulation of hydrophobic and aromatic amino acids in the residue (Johnson and Skerede, 1981). The sludge has a high nutritive value (Olley, 1976) but it is not useful for animal feeding because of its high lipid content which causes impaired performance of animals (Kompiang *et al.*, 1980) and leads to carcass tainting (Raa and Gildberg, 1982).

Addition of 10 litre of 37% concentrated formaldehyde solution to a ton of fish silage will stop protein autolysis and lipid oxidation, but it may prove toxic to some animals (Haard *et al.*, 1985). Autolysis of proteins can be minimised by lipid extraction (Hall and Ledward,1986), storing the raw material at -5^{0}C (Stone *et al.*, 1989) or at 2^{0}C (Haaland and Njaa, 1989 a) or at 5^{0}C (Lo *et al.*, 1993) or heating the raw material for 5 minutes at 60^{0}C (Viana *et al.*, 1993).

As for fish meal, raw material with a TVN value higher than 50 mg per 100 g should not be accepted for producing high quality fish silage (Arason, 1994).

Amino acids are very stable in fish silage and only 8% of the amino nitrogen is released as ammonia in a silage of cod viscera stored for 220 days at 27^{0}C (Gildberg and Raa, 1977). A corresponding figure for silage of by-catch fish is 1.3% after 21 days at 30^{0}C (Kompiang *et al.*, 1980). Nevertheless this might imply a significant reduction of the nutritional value of the silage, in case the ammonia is derived from the essential amino acids. Ammonia increases slightly in propionic acid-formic acid silage probably due to deamination reaction catalysed by endogenous enzymes in the raw materials (Gildberg and Raa, 1977). In fact, tryptophan decomposes in an acid silage (Backhoff, 1976; Kompiang *et al.*, 1980) and there are reports claiming that methionine (Atkinson *et al.*, 1974), histidine (Disney *et al.*, 1978) and arginine (Stone and Hardy, 1986) are also unstable. The amide groups of free glutamine are very labile, as NH_3 is detected soon after dissolving it in water (Haaland and Njaa, 1988, 1989 b; Espe *et al.*, 1989). The TVN value will increase in unheated silage due to the ammonia formed from the amide groups of glutamine and asparagine (Arason, 1994). The essential amino acids (EAA) are released more rapidly than the non essential amino acids (NEAA) resulting in a ratio of EAA to NEAA in the free form of 1.38 compared to 0.78 initially in pacific whiting acid silage (Stone *et al.*, 1989). In properly preserved fish silage, the rate of increase of total volatile nitrogen (TVN) and ammonia nitrogen (NH_3N) equals the rate of decrease of amide nitrogen during storage without affecting the aminoacid composition significantly (Haaland and Njaa, 1989 b). Tryptophan is more stable at lower temperature (Backhoff, 1976) in

herring silage and at low pH when present in proteins but degrade when free (Kompiang *et al.*, 1980). It is stable in the heat-treated aqueous phase of a silage of cod or saithe viscera (Strom and Eggum, 1981) and hence enzymes (e.g., tryptophan decarboxylase) may be involved in its degradation (Raa and Gildberg, 1982). In animal diets the tryptophan level can be improved by addition of cereal protein (Johnson and Skerede, 1981; Gildberg and Almas, 1986).

It has been confirmed that the nutritional value of the aqueous soluble phase of a herring silage is limited by tryptophan (Jensen and Schmidtsdorff, 1977). This phase also contains low levels of cystine, tyrosine and phenylalanine because they are retained in the sediment portion (Yanase, 1965; Higashi *et al.*, 1965; Gildberg and Raa, 1977). Tyrosine may disappear from the aqueous phase because it crystallises at the inner surfaces of the storage tank on storing (Raa and Gildberg, 1982).

Methionine is the growth limiting amino acid in fish silage (Jensen and Schmidtsdorff, 1977) and is stable in acid fish silage (Wignall and Tatterson, 1976; Strom and Eggum, 1981). Histidine may be the limiting amino acid in fish silage prepared from partly spoiled fish (Disney *et al.*, 1978) and enzymes are responsible for slow ammonia production and histidine decomposition in acid silage (Raa and Gildberg, 1982). Other workers (Backhoff, 1976; Johnson and Skerede, 1981; Jackson *et al.*, 1984 ; Austreng and Asgard, 1986) have reported that tryptophan may be the limiting amino acid in silage. Ninhydrin reactive substance increased in a formic acid capelin

silage during the first month and stabilized at higher levels at the two higher temperature viz., 20^0C and 37^0C than at the lower of 2^0C (Haaland and Njaa, 1989 a).

Thiaminase activity has been demonstrated in fresh water fish (Gnaedinger and Krzeczkowski, 1966), in anchovy (Ishihara *et al*., 1972) and in carps (Evans, 1975). The thiaminase can be inactivated by heating at 82^0C for five minutes. (Gnaedinger and Krzeczkowski, 1966). Activity of lipases is enhanced by raising the temperature of the silage particularly during the early stages of the process (Tatterson and Windsor, 1974; Tatterson, 1976; Wignall and Tatterson, 1976; Gildberg and Raa, 1977). Rates of oil hydrolysis in silage produced from different fish species vary (Reece, 1981).

Lipids may hinder diffusion of enzymes and thus reduce the rate of autolysis, especially at temperatures below their melting point (Raa and Gildberg, 1982). Liberated fatty acids from lipid hydrolysis cause solubilisation of proteins (King *et al*., 1962; Anderson *et al*., 1963). Formation of lipid hydroperoxides depends on many factors including temperature of storage, available oxygen, the presence of pro and antioxidants and reactivity of the lipids (Raa and Gildberg, 1982).

Disney *et al.* (1978) showed that hydroperoxides are formed in relatively high levels and are very stable in a silage/carbohydrate feed and do not decrease during one month of storage at tropical temperatures. Secondary products of lipid oxidation may react with proteins and cause reduction of the nutritional value (Gardner, 1978; Lall, 1991). The peroxide value may be used

as an index of nutritional value of silage (Dugan, 1975; Barlow and Pike, 1977). Peroxide value in excess of 100 per kg diet causes loss of appetite, decreased ability to gain weight, resulting in even death. Bentsen (1985) was awarded a patent on the use of antioxidants and preservatives in fish silages used for animal feeding.

Wood *et al.* (1985) reported an unexpected low peroxide and anisidine value since the formic acid fish silage, when allowed to stand, had a surface film of unsaturated oil available for rapid oxidation. Oxidised fish oil may cause less carcass taint than oil protected from oxidation by antioxidants possibly because, the tainting ability is associated with the uptake of unsaturated fatty acids (Opstvedt *et al.*, 1971).

Feeds, including oxidised lipids, are accepted by fish, but may cause reduction of feed intake through reduced palatability resulting from off-flavours (Koshio *et al.*, 1994).

Infectious pancreatic necrosis virus (IPNV) would be inactivated by heating the silage to a temperature of 60^0C for 5 hours or by the addition of the virucidal agent Virkon powder at a concentration of 1/100 w/v (Smail *et al.*, 1990). In silages subsequently treated with 40 Kgy of cobalt 60 irradiation prior to inoculation, Rohovec and Whipple (1990) found that *Aeromonas salmonicida* survived for less than 3 minutes. *Mycobacterium chelonei* survived less than 2 hours in either sulphuric acid silage or lactic acid bacterial silage. *Renibacterium salmoninarum* survived less than 30 minutes in lactic acid bacterial silage. Infectious hematopoietic necrosis virus (IHNV) survived

30 seconds in the irradiated silage, but IPNV survived for more than 7 days. Although more heat resistant, IPNV and *R.salmoninarum* should also be killed when incorporated into fish silage and heated to 82^0C for 5 minutes after a 15 minutes period of heating at 65^0C (Whipple and Rohovec, 1994).

2.4 Fermented fish silage

Fish with its high content of protein and fat and its low carbohydrate content, can be a good substrate for ensiling provided it is mixed with a carbohydrate source (Nilsson and Rydin, 1963). The concept of microbial fermentation of fish waste was started in the year 1953 by Tarr *et al.* who used cultures of *Lactobacillus plantarum*. Very convincing results on the storage life and nutritional value of lactic acid fermented silage has been reported (Nilsson and Rydin, 1963; Roa, 1965; Krishnaswamy *et al.*, 1965; James *et al.*, 1977; Stanton and Yeoh, 1977; Lindgren and Pleje, 1983; Adams *et al.*, 1987; Twiddy *et al.*, 1987; Zuberi *et al.*, 1992). The ensiled product is preserved by the anaerobic production of lactic acid by micro-organisms (Rydin, 1961).

According to Langston and Bouma (1960 b), the dominant organisms in the formation of lactate silage are *Lactobacillus brevis, Lactobacillus plantarum* and *Pediococcus* sp. Lactic acid bacteria (LAB) are gram positive, microaerophilic, asporogenous, usually nonmotile and are capable of fermenting sugars (Orla-Jensen, 1919) while spoilage bacteria utilize amino acids as a source of energy and thus produce ammonia, LAB have limited ability to decompose amino acids, except for arginine if glucose is absent (Johnsson,1979). Glucose, in particular, represses the production of

deaminating enzymes in competing spoilage bacteria (Raa and Gildberg, 1982).

Wirahadikusumah *et al.* (1972) found that by the first day, when the pH had reached 5.5, the oval, cocci, *Streptococcus lactis* and *Leuconostoc mesenteroides* dominated.Thereafter it declined to be replaced by round cocci (mostly *Streptococcus faecalis*) reaching a maximum by the fourth day, while the rod group (heterofermentative lactobacilli) increased slowly to a maximum by the third day. This change of population occurred when the pH had changed from 5.5 to 4.5. By the fifth day the round cocci and rods gave way to new rods (homofermentative lactobacilli).

Members of the genera, *Lactobacillus*, *Leuconostoc* and *Pediococcus* are non-pathogenic whereas some members of the genus *Streptococcus* cause serious diseases in both man and animals (Woolford, 1984).

Homofermentative LAB anaerobically produces two moles of lactic acid while heterofermentative LAB anaerobically produces one mole of lactic acid, one mole of carbon dioxide and one mole of ethanol per mole of glucose fermented as reported by Wood, 1961. He has also mentioned that homofermentative LAB catabolises the hexoses by Emden-Mayerha - parnas pathway and the heterofermentative LAB by the phosphoketolase pathway.

Heteroformentative bacteria causes intensive gas production, which can be prevented by adding 5% sodium chloride (Stanton and Yeoh, 1977). Gas production is a practical problem and further, only less acid is produced

per mole of glucose (Raa and Gildberg, 1982). They have suggested addition of small quantities of organic acids and boiling the fish prior to inoculating with a suitable starter culture to control gas production. Ahmed and Mahendrakar (1995) recommended, increasing the quantity of molasses to suppress the gas producing heterofermentative bacteria.

LAB are tolerant to high concentration of carbon dioxide (Ingram, 1975) while other bacteria are substantially less tolerant (ICMSF, 1980). Hence the early production of carbon dioxide could be a factor in the rapid suppression of spoilage and food poisoning bacteria (Owens and Mendoza, 1985). According to them factors that influence LAB growth include availability of fermentable carbohydrate and organic growth factors, concentration of sodium chloride, organic acids and carbon dioxide, temperature, anaerobiosis, pH value, production of inhibitory compounds, buffering capacity, and initial number of LAB and competing microbes.

A temperature range of 20^{0}C to 40^{0}C with a optimum of 30^{0}C to 32^{0}C (Pederson, 1971) and a moisture content of 40% (Knight, 1976) are required by the LAB to grow well. The pH has to be lowered to $\leq$ 4.5 within 1 to 2 days to retard growth of putrefactive bacteria (Disney *et al.*, 1977; Jangaard, 1987) and to provide an optimal pH for endogenous digestive proteases (Lall, 1987).

The preservative action of LAB may be due to lowering of pH (Langston and Bouma, 1960), production of hydrogen peroxide (Dahiya and Speck, 1968; Gilliland and Speck, 1975), production of antibiotics (Baribo and Foster, 1951; Deklerk and Coetzee, 1961; Upreti and Hinsdill, 1973; Lindgren

and Clevstrom, 1978), changes in the oxidation - reduction potential of the environment (Barber and Deibel, 1972) and production of bacteriocins (Graham and McKey, 1985).

Lactic acid have a pK_a of 3.87 which corresponds to a pH value of below about 4.9 (ICMSF,1980). The production of lactic acid in ensilage brings down the pH to a level of 3.9 to 4.8 (Langston *et al.*, 1958). This pH suppresses the growth of undesirable microbial pathogens (Langston and Bouma, 1960 a).

Proteinaceous foods with its high buffering capacity requires greater amount of acid to bring down the pH value. If the buffering capacity at pH values of 5 to 6 is high, it is likely that considerable spoilage can occur before sufficient acid is produced to lower the pH (Owens and Mendoza, 1985).

Lactic acid is not effective against fungi because the latter can utilize the former as its carbon source (Woolford, 1984).

Anaerobic condition, heat production and the antagonistic effects of fatty acids (Khan and Katamay, 1969; Chung and Geopfert, 1970) along with the production of acid, its toxic effect etc., suppress the undesirable micro organisms (McCaskey and Anthony, 1979)

Clostridium botulinum Type E and its toxin is eliminated in silage when the pH reached 5.2 except when there is high number of spores or toxin (Wirahadikusumah, 1968). Some lactobacilli produce sufficient H_2O_2 to inhibit salmonellae and staphylococci (Dahiya and Speck, 1968). The most

known antibiotic from LAB is nisin which is produced by strains of *Streptococcus lactis* (Wirahadikusumah *et al.*, 1971). Antibiotic-like substances, lactolin, produced by certain *Lactobacillus* has an inhibitory effect on coliforms, *Bacillus* spp., gram negative bacilli, *Staphylococcus aureus* and *Clostridium botulinum* (Wirahadikusumah, 1971, a, b). Common food pathogens like *Staphylococcus aureus, Salmonella typhimurium, Clostridium sporogenes* and *Escherichia coli* rapidly disappear during fermentation. (Twiddy *et al.*, 1987).

Starch is hydrolysed to maltose by amylase, which is then converted to glucose by maltase which, in turn is catabolised to lactic acid by LAB (Wirahadikusumah, 1968). Nilsson and Rydin (1965) proposed that fish be fermented along with cereal meals as starch source and malt meal as amylase source. Wirahadikusumah *et al.* (1972) noted that some strains of LAB had the ability to hydrolyse starch. *L.amylophilus, L.amylovorus* and *L.acidophylus* can hydrolyse starch (Kandler and Weiss, 1986).

Stability of the silage depends upon the fish to starch ratio (Stanton and Yeoh, 1977). In silages derived from mixture of animal products and cereal meal, any influence on fermentation will largely be borne by the cereal meal (Woolford, 1984). The use of molasses or tapioca is ideally suited to tropical countries as they are abundantly available (Gopakumar, 1997). Fish: molasses ratio of 100:5 gave a silage stable only upto a few days and ratios of 100:10 and above gave stable silages having a typical acid smell (Kompiang *et al.*, 1979 b). Silages made from mixtures containing a total carbohydrate

content of 10% are the best to prepare stable silage (Hercules and Heydenrych, 1985; Zuberi *et al*., 1992). Silage preparation depends upon the quality of molasses (Zuberi *et al*., 1992). They recommend the usage of 5% molasses along with 5% sucrose or glucose to prepare stable silage. To attain a pH of less than or equal to 4.5 in ensiled salmon viscera containing 1% LAB and 1% Calcium propionate, Dong *et al.* (1993) found that a minimum of either 5% molasses or 3% dextrose is required as fermentation sources. Sachindra *et al.* (1994) biologically ensiled shell-free prawn carapace pulp by initially bringing down the pH below 6.0 with the addition of 1 to 2% acetic acid solution and then adding 15% molasses. Cisse *et al.* (1995) ensiled a combination of 65% (w/w) cooked and ground small sardines, 25% (w/w) corn flour, 5% (w/w) sugar and 5% (w/w) fermented cassava. Peat extract is a satisfactory sources of carbohydrates for the fermentation by LAB in cod offal silage (Martin and Bemister, 1994) Inclusion of salt will suppress volume rise but restrict the utility of fermented silage product as an ingredient in animal diets (Ahmed and Mahendrakar, 1995).

The quality of ensiled material is improved by the addition of different additives and the reduction of the amount of sugar added (Svensson and Tveit, 1964). The level of the carbohydrate in the silage prior to the drying process has to be low to minimise browning reaction; and the silage has to contain the majority of the nitrogen fraction as intact protein or peptides rather than as free amino acids (Dong *et al*., 1993).

Live Fish has a very low population of LAB and Clostridia but high population of aerobic bacteria and coliforms (Wirahadikusumah, 1968; Smith and Adamson, 1976; Schroder *et al*, 1980). LAB can be inoculated by the addition of pure culture (Nilsson and Rydin, 1963) produced commercially or by mixing with a 'sauerkraut' liquid (Stanton and Yeoh, 1977; Yeoh, 1980) or by reinoculation from a pervious silage (Twiddy *et al.*, 1987).

Inoculum of *L. plantarum* was used to successfully ensile minced herring (Roa, 1965) with 10% molasses, abalone viscera (Olley and James, 1972) with 10% maltose, and silver bellies, jew fish and sole fish (James *et al.*, 1977) with 15% molasses. Successful preservation has been achieved by favouring growth of LAB naturally present (Olley, 1976; Rattagool *et al.*, 1979 a; Kompiang *et al.*, 1980; George, 1990). Addition of pure culture is not found necessary or advantageous (Wee *et al.*, 1986; Fraizer, 1988). Starter culture must be used when the fish is boiled prior to processing (Raa and Gildberg, 1982).

Zuberi *et al.* (1992) observed not much difference in the fermentation behaviour between pure culture of *L. plantarum* and sauerkraut as innoculum. Whittenbury (1961) has laid down the criteria required by a culture to be considered as having potential as an inoculum for silage.

2.4.1 Quality of fermented silage

Maximum degree of protein digestion in fermented silage is significantly lower than that in acid silage (James *et al.*, 1977; Kompiang *et al.*,

1980). Zuberi *et al.* (1992) observed no noticeable difference in the extent of hydrolysis between fermented and acid silages. According to James (1966) uncooked silage shows more degradation of protein by autolysis than precooked silage. Formalin at the rate of 5ml/kg fermented silage is a good proteolytic inhibitor (Fagbenro and Jauncey, 1994). The quality of fermented fish offal and poultry waste can be determined by estimating the changes in alanine concentration, lactic acid production and redox potential (Lassen, 1995).

Oil is trapped in the polysaccharide added, but this is not a significant problem since the lactic acid fermentation process seems to stabilize the oil and even improve its acceptability in the animal ration (Raa and Gildberg, 1982). Formation of high levels of thiobarbituric acid-reactive substance in freeze-dried silage is inhibited by the addition of 0.025% (w/w) ethoxyquin to the silage prior to drying (Dong *et al.*, 1993). Ginger extract at the rate of 5ml/kg proved effective as an antioxidant in fermented tilapia silage (Fagbenro and Jauncy, 1994).

The production of tri-methyl amine nitrogen (TMA-N), volatile base nitrogen (VB-N) and ammonia (NH_3) in microbial silage are much higher, during storage compared to acid silage (Zuberi *et al.*, 1992) especially during the first two days of storage when the pH is decreasing. As much as 12% of the nitrogen is released as ammonia during 4 weeks of storage of fermented silage of Baltic herring at 28^0C (Nilsson and Rydin, 1963). Corresponding

figures for acid-preserved silages are 3% only as observed by Gildberg and Raa (1977) or 1.5 to 2% as observed by (Kompiang *et al.*, 1980).

While LAB normally require fermentable carbohydrate for growth some are able to generate energy from L-arginine (Bauchop and Elsden, 1960; Jonsson *et al.*, 1983).

Several strains of *Streptococcus faecalis* decarboxylated tyrosine to tyramine (Gale, 1940). Certain *Lactobacillus sp.*, although not commonly found in silage, decarboxylated histidine, lysine and ornithine (Rodwell, 1953; Recsei, 1972).

According to Nilsson and Rydin (1965), good quality silage is characterised by low ammonia content of 12% of total nitrogen and furthermore, butyric acid must only occur in insignificant quantities.

Microbial fermentation adds flavour to the product and may prevent rancidification and other chemical reactions which cause quality reduction (Roa, 1965; Wirahadikusumah, 1968; James *et al.*, 1977). The odour of microbial silage is more acceptable than acid silage throughout the storage (Krishnaswamy *et al.*, 1965; Zuberi *et al.*, 1992). James *et al.* (1977) has shown that little change took place when fermented silage is stored over a period of 12 months.

2.5 Fish silage as Feed

Several workers have successfully utilised acid-preserved silage obtained from different raw materials in diets for different animal species.

Some reports show that the silage is a good source of protein and that its nutritional value is comparable with that of fishmeal (Stormo and Strom, 1978; Skerede, 1981; Strom and Eggum, 1981; Raa and Gildberg, 1982; Jackson *et al.*, 1984; Krogdahl, 1985).

A survey of literature on the use of fish silage as poultry feed reveals that results are very conflicting and contradictory. Nutritional value is comparable with that of fishmeal in diets for chicken (Poulter *et al.*, 1979) but a slight (Disney *et al.*, 1978) and significantly lower value (Kompiang *et al.*, 1980; Disney and Hoffman, 1976, Rattagool *et al.*, 1979 a and b) has also been found, depending on the level of inclusion.

A very poor growth of chicken occurs when silage makes up to more than 20% of the dry weight of the diet (Disney and Hoffman 1976; Kompiang *et al.*, 1980). Chicks grew fast on conventional broiler diets to which 1% formic acid (Disney and Hoffman, 1976) or 1% formic acid - propionic acid mixture (Rattagool *et al.*, 1979 a;Kompiang *et al.*, 1980) has been added. Therefore the growth depressing effect cannot be due to the residual organic acids.

Silage prepared from spoiled fish caused poor growth of chicks, but, silage produced from a mixture of spoiled fish and fresh fish (1:1) gave good growth and no mortality (Disney *et al.*, 1978). Nutritional quality of silage is improved by boiling at the beginning or end of ensiling (Kompiang *et al.*, 1980). Microbial fish silage is a satisfactory replacement for fishmeal at level upto 8% of the diet (dry fish silage basis) for chicks (Komping *et al.*, 1980).

The better performance of fermented silage, compared to acid silage, is likely due to the protective action by the fermentation process on fish lipids (Raa and Gildberg, 1982).

No side effects were noted and the taste test conducted using the chicken fed with the fermented silage diets showed no difference when compared to other chicken commercially available (Guevara *et al.*, 1992). On the contrary feeds containing fermented silage gave a lower weight gain, less appetite and chicken with leg weakness and thinner plumage when compared to the commercial feeds (Bigueras - Benitez and Nacorda, 1992).

Feed conversion efficiency and daily live weight gain of pig fed on a cereal diet with fish silage had been shown to equal that of fish meal (Cameron, 1962; Rangkuti *et al.*, 1979) and is significantly better than a diet based on Soya protein (Batterham and Gorman, 1980). On the other hand, there are reports that fish silage as pig feed is inferior nutritionally at 5 to 10% inclusion level (Smith and Adamson, 1976) or at 25% (Whittermore and Taylor, 1976) of the dry matter. If the oil content is high, it is safest to use the silage exclusively for breeder stock (Raa and Gildberg, 1982). Satisfactory results were obtained when pigs were fed with fermented fish silage (Avdalov *et al.*, 1992). Ottati and Bello (1992) recorded that fermented fish silage can partially substitute Soya flour in pig diets during the fattening period.

A major goal in fish nutrition is to formulate diets which give fast growth and optimal fish health and product quality at the lowest possible cost (Lie *et al.*, 1988). Such feeds are becoming increasingly important as an

alternative to live feeds in the aquaculture industry (Coutteau and Sorgeloss, 1992; Curatalo *et al*., 1993).

In diets for carps fish silage has been shown to be equally good as a source of protein and even better if the fish is boiled prior to ensiling (Djajasewaka and Djajadiredja, 1979).

Feeds acidified with formic or sulphuric acid at 1.25 % inclusion level or greater may depress trout growth whereas hydrochloric acid had no apparent effect on growth or proteolytic activities (Rungruangsak and Utne, 1981).

Crampton *et al.* (1982) observed that salmon in sea cages had significantly faster growth rates when fed on a 25% silage diets than when they were fed on a commercial dry pellet diet. Srinivasan *et al.* (1985) reported that *Cyprinus carpio* and *Cirrhinus mrigala* fed on feed containing fish silage gained double the weight of those fed on the control diets containing mustard oil cake and rice bran.

Wood *et al.* (1985) observed poorer growth response of common carp using silage based diets compared to a fish meal based diet. Eels lost appetite and growth when 40% silage was included in the ration without previous neutrilisation (pH 5.2) (Affandi, 1985).

Silage preserved with sulphuric acid and proponic acid are shown to have lower acceptability to Atlantic salmon than that preserved with formic acid (Austreng and Asgard, 1986). Goncalves *et al.* (1989) also reported that incorporation of silage in the diet resulted in an increase in the specific growth

rate (SGR), food conversion ratio (FCR), and protein efficiency ratio (PER) of eel compared to a control diet containing fish and meat meal.

Feeding trials with Atlantic salmon (Lall, 1991) and rainbow trout (Arason, 1994) on diets containing 60% silage preserved with hydrochloric acid observed growth equivalent to that on a diet of fresh fish. Rainbow trout showed no aversion to feed nor growth depression when fed with diets containing propionic acid preserved fish silage (Lall, 1991). Silage produced with propionic acid, either alone or in combination with formic or sulphuric acid, is not palatable to Atlantic salmon (Arason, 1994).

The utilisation of silage from non-economic fish or fish offal as a source of protein has been justified for salmonids by Asgard and Austreng (1981); Hardy *et al.* (1984) and Jackson *et al.* (1984) and and for major carps by Ali *et al.* (1994).

Free amino acids and hydrolysed proteins are poorly utilized by carp particularly if the feed is acidic (Aoe *et al.*, 1970; Nose *et al.*, 1974).

Rainbow trout had higher weight gains, PER (Hardy *et al.*, 1983), net protein utilization (NPU), and apparent digestibility coefficient (ADC) (Hardy *et al.*, 1984) when fed with silages in which autolysis was terminated after 3-7 days or restricted by temperature and pH (Hardy *et al.*, 1983; Stone and Hardy, 1986) than when autolysis was permitted to continue until high levels of free amino acids appeared in the silage.

Plasma level of essential amino acids remain at elevated levels for longer periods when trouts (Yamada *et al.*, 1981) or carp (Plakas *et al.*, 1980; Plakas and Katayama, 1981) are fed on diets containing intact protein (casein) rather than an equivalent diet containing free aminoacid as the protein sources.

Rainbow trouts, when fed with a properly balanced crystalline aminoacid diet grew nearly as well as when fed with a high quality protein diet (Aoe *et al.*, 1970). Digestion and absorption of amino acids from fishmeal begins in the stomach, reaches a maximum rate in the pyloric caeca and continues in the middle and posterior intestine (Ash, 1980; Dabrowski and Dabrowski, 1981). Gradual liberation of aminoacids from fishmeal by intestinal enzymes ensures that amino acids are available over a prolonged time period, whereas fish silage contains high levels of essential aminoacids in the free form which are available for immediate absorption (Stone *et al.*, 1989). The essential aminoacids, if pre-maturely absorbed, may be irreversibly metabolised further so that they will not be available for protein synthesis (Geiger, 1947). Diets made with silages contained more intact proteins if the ingredients are stored at -5^{0}C prior to ensiling (Stone *et al.*, 1989).

Digestibility of astaxnthin in shrimp waste increased from about 45% to about 71% as a result of ensiling and the rate of accumulation of the pigment in the rainbow trout muscle is markedly higher in fish fed on silage diet than those given fresh or dried shrimp waste (Torrissen *et al.*, 1981). Guillou *et al.*, (1995) reported that the content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of shrimp waste (24.7% and 20.3% respectively)

increased upon ensiling (43.9% and 45.5%, respectively). Astaxanthin is protected against oxidation in shrimp waste silage, but a slow conversion of its diester to the corresponding monoester is observed when the shrimp waste silage is stored at 4 - 5^0C for 21 days (Torrissen *et al.*, 1981). Shrimp waste silage could be incorporated into moist (Torrissen *et al.*, 1981; Tidemann *et al.*, 1984; Ouellet *et al.*, 1992) and dry feeds to provide a natural source of dietary astaxanthin to salmonids (Guillou *et al.*, 1995). Formalin-treated tilapia silage diet gave significantly reduced digestibility of dry matter, nitrogen or lipid compared to the control (Fagbenro and Jauncey, 1994).

Fermented fish silage co-dried with soyabean meal, poultry by-product meal, hydrolysed feather meal or meat and bone meal, is a suitable supplement in dry fish diets for tilapia (Fagbenro, 1994). Protein efficiency ratio is high in tilapia fed with fishmeal and fish silage mixture (1:1) (Lapie and Bigueras - Benitez, 1992). Flavour evaluation by taste panel indicated no significant difference between fillets of Atlantic salmon fed on silage feed and other diets (Parrish *et al.*, 1991). Silage fed turbot (*Scophthalmus maximus*) showed poorer feed conversion efficiency and growth when compared to a control feed (Calcedo Juanes, 1989),while Sea bass fingerling (*Lates calcarifer*) fed with fish silage showed the lowest growth rate when compared to other diets (Khamchai Lawonyawut, 1987).

The use of fermented feed products in animal feeding, e.g., pigs and poultry, has attracted the interest of both scientists and commercial companies. Of special interest is the positive effect of such feed on problems concerning

diseases. Those that contribute to an improved health situation for the animal are called 'probiotics', which are controlling factors of the intestinal flora of the animals (Strom and Raa, 1992). In addition to varying the composition of the feed for manipulating the microflora, research has shown that unwanted coliforms can be controlled by feeding lactobacilli to the animals (Sissons, 1989). He attributed the mode of action of LAB to several factors such as; competitive attachment, bacterial activity, enterotoxin neutralisation, prevention of toxic amine synthesis and enhanced immunity. According to Feighner and Dashkevicz (1987) the antibiotic growth effect is due to the prevention of bacteria that are synthesising enzymes which catalyse the hydrolysis of bile salt. Such hydrolysis otherwise, will result in reduced ability to digest lipids. The observation that intraperitoneal injection with lactobacilli raised the activities of macrophages and lymphocytes in mice, indicates the role of lactobacilli in the unspecific immune system of animals (Sisson, 1989).

MATERIAL AND METHODS

III MATERIAL AND METHODS

3.1 Silage preparation

Fresh cuttle fish processing waste was brought from Integrated Rubiyan Exports Ltd., Aroor, Cochin, to the laboratory in iced condition. The waste was minced thoroughly in a mincer and was used for making acid and fermented silages.

3.1.1 Formic acid fish silage preparation

Formic acid (98%) was added to 2.5 kg of the minced waste and mixed thoroughly to obtain the required pH as given in Table 1.

Table 1. pH of various formic acid fish silage preparations

Preparation No.	pH
I	3.5
II	3.8
III	4.0
IV	4.5

The formic acid fish silage preparations was distributed in clean plastic containers, closed tightly and stored at room temperature which varied from 28-32^{0}C, for a period of 90 days. The samples were swirled twice daily during the storage period.

3.1.2 Fermented fish silage preparation

Fermented fish silage preparations were made by the addition of carbohydrate sources such as molasses, tapioca powder and rice bran. These ingredients were added to the minced waste at varying proportions as shown in Table 2.

Table 2. Quantity and percentage of various ingredients added for fermented fish silage preparations.

Fermented fish Silage preparation No.	Cuttle fish waste		Molasses		Tapioca powder		Ricebran		Total	
	Qty. in Kg	%	Qty. in Kg	%	Qty. in Kg	%	Qty. in Kg	%	Qty. in Kg	%
A	1.75	70	0.375	15	0.125	5	0.25	10	2.5	100
B	1.75	70	0.375	15	0.25	10	0.125	5	2.5	100
C	1.75	70	0.25	10	0.25	10	0.25	10	2.5	100
D	1.75	70	0.25	10	0.375	15	0.125	5	2.5	100
E	1.75	70	0.25	10	0.125	5	0.375	15	2.5	100

The fermented fish silage preparations was distributed in clean plastic containers, closed tightly and stored at room temperature, which varied from 28-32^{0}C, for a period of 90 days. The samples were swirled twice daily during the storage period.

3.2 Analyses of the silages

Physical, biochemical and microbial analyses were carried out. The proximate analyses were done only on the initial and final day (90th day) of storage. Comparison of the initial and final proximate composition was done statistically using paired t-test. Various biochemical analyses, viz., peroxide value (PV), total volatile base nitrogen (TVBN), tri-methyl amine nitrogen (TMAN), non protein nitrogen (NPN), total nitrogen (TN) and pH, were carried out after 0,2,3,5,7,14,30,60 and 90 days of storage. Analyses of variance were carried out for comparing the various biochemical parameters of different fish silage preparations.

Microbial analyses, viz., total plate count (TPC) and most probable number (MPN) of coliforms were carried out for formic acid fish silage preparations, whereas for fermented fish silage preparations, acid producing bacterial count (APBC) and MPN of coliforms were determined. Analyses were done after 0,2,3,5,7,14,30,60 and 90 days of storage. Microbial parameters of different fish silage preparations were compared statistically by analyses of variance technique.

3.2.1 Physical analyses

3.2.1.1 pH

The pH was measured using Elico digital pH meter. The instrument was set using standard buffers of pH 4 and 9.2.

3.2.2 Biochemical analyses

3.2.2.1 Proximate composition

Boyd's (1979) method was used to estimate the moisture content. Pre weighed samples were heated at 105^0C for 30 minutes and then dried at 65^0C till a constant weight was obtained. Ensiled materials with molasses were dried in hot air oven at a temperature of 70^0C till a constant weight was obtained. Crude protein content was estimated by Microkjeldahls' method (AOAC, 1983). The nitrogen content was multiplied by the factor 6.25 to get the protein content. To estimate the crude fat content the AOAC (1984) method involving solvent extraction using petroleum ether (40-60^0C) in a soxhlet extraction apparatus was followed. The crude fibre content was estimated by the method of Pearson (1976), and ash content was determined by

burning the sample at $550^0C \pm 10^0C$ for 6 hrs, in a muffle furnace. The carbohydrate content (nitrogen free extract, NFE) was determined by Hastings (1976) difference method on a dry weight basis.

NFE = 100 - (% crude protein +% crude fat + % crude fibre + % ash)

3.2.2.2 Peroxide value (P.V)

Connell's (1975) method was used for the determination of P.V and expressed in milliequivalent per 1000 g fat.

3.2.2.3 Non protein nitrogen (NPN) content

NPN content was determined by homogenising samples for 1 minute with 10 volumes of 10% trichloro acetic acid (TCA) and measuring the total Kjeldahl nitrogen (TKN) of the filtrate by the AOAC (1984) method.

3.2.2.4 Tri- methyl amine (TMA) content

To an aliquot of the sample, an equal volume of 10% T.C.A was added, allowed to stand for 15 minute and filtered through a Whatman No.1 filter paper. TCA extract thus obtained was used to determine TMA Content with Conways microdiffusion apparatus by the method of Conway (1947).

3.2.2.5 Total volatile base nitrogen (TVBN) content

The TCA extract, as prepared in 3.2.2.4., was used to determine TVBN content by the method of Conway (1947).

All analyses were done in triplicate.

3.2.3 Microbial analyses

3.2.3.1 Total plate count (TPC)

The diluent used was buffered peptone water. The medium used for plating was Plate Count Agar (Hi - media, Table 4). Serial decimal dilutions were made. One ml of each dilution was pipetted out into sterile petridishes in duplicate. The molten medium (kept at 45 ± 1^0 C in a water bath) was poured into the petridishes, mixed, solidified and incubated at room temperature for 48 hours. Plates that gave colonies between 30 and 300 were used for counting. Average counts were reported as log number per gram sample.

Table 3. Composition of buffered peptone water.

Ingredients	Gram/Litre
Peptone	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate	9.0
Potasium di hydrogen phosphate	1.5
Distilled water	1000 ml
pH	7.0

Table 4. Composition of plate count agar (Hi-media)

Ingredients	Gram / Litre
Casein enzymic hydrolysate	5.0
Yeast extract	2.5
Dextrose	1.0
Agar	15.0
Distilled water	1000 ml
pH	7.0±2

3.2.2.1 Most probable number (MPN) of coliforms

Each sample was serially diluted in lactose broth (Table 5) containing 1 in 10, 1 in 100 and 1 in 1000 dilutions. Each dilution was transferred to a set of five tubes, at the rate of 10 ml per tube with a Durham's tube each in it. The tubes were incubated at a temperature of 37^0C for 48 hrs. The number of tubes that showed gas formation in each dilution were noticed. MPN of coliforms per gram sample was determined using Mac Crady's tables.

Table 5. Composition of lactose broth (Hi-media)

Ingredients	Gram / Litre
Beef extract	3.0
Peptone	5.0
Lactose	5.0
Distilled water	1000 ml
pH	6.8

3.2.3.2 Acid producing bacterial count (APBC)

The method followed was the pour plate technique as for TPC, but using tomato juice agar (Table 6) with 0.75% $CaC0_3$ as the medium. Incubation was done at a temperature of 32^0C for 48 hours. Colonies that showed halo zone around them were considered as those of acid producers. Counts were reported as log number per gram sample.

Table 6. Composition of tomato juice agar (Hi-media).

Ingredients	Gram / Litre
Tomato juice	20.0
Casein enzymic hydralysate	10.0
Peptonised milk	10.0
Agar	11.0
Distilled water	1000 ml
pH	6.1 ± 0.2

3.3 Preparation of fish feed

The formic acid silages and fermented silages which preserved well were further mixed with tapioca powder and rice bran as filler to prepare the fish feed. The resultant dough was steam cooked for 30 minutes, then extruded through a pelletiser and dried at 50^0C in a hot air oven. The feeds were packed individually in sealed plastic containers and stored at room temperature.

3.4 Acceptability study

Common carp (*Cyprinous carpio*) fingerlings of uniform size were procured from a local farm and starved for 24 hours. They were weighed and dispersed at the rate of 10 fingerlings per tank in 21 circular, flat bottomed fiber glass tanks each of 100 litre capacity, and filled to $3/4^{th}$ of the capacity with water. Each silage based diet was fed twice daily at the rate of 3% of the body weight per day as according to Aoe *et al.* (1970). Feeds were placed in petridishes and immersed in the water. Water exchange was done in the morning and in the evening prior to feeding. Water in all the tanks were kept

aerated continously except during the period of feeding. The dishes were observed after 15 minutes to find out whether the feed had been consumed or not.

RESULTS

IV RESULTS

The results of the experiment can be considered conveniently under the following heads.

1. pH.
2. Total plate count (TPC).
3. Acid producing bacterial count (APBC).
4. Most probable number (MPN) of coliforms.
5. Tri - methylamine nitrogen (TMA-N) and Total volatile base nitrogen (TVB-N).
6. Non-protein nitrogen (NPN).
7. Peroxide value (PV).
8. Proximate composition.
9. Acceptability study.

4.1 pH

Figure 1 shows the change in pH of the formic acid fish silage preparations (I, II and III). From the figure it can be understood that there is a gradual increase in pH of all the three formic acid fish silage preparations, from the 0 - day to the 90th day. Figure 2 shows the change in pH of the fermented fish silage preparations (A, B, C, D and E). The pH decreased to the level below 4.2 by the third day in the case of fermented fish silage preparations A,B and E, and by the fifth day in the case of fermented fish silage preparations C and D. Thereafter till the 90th day there was no remarkable change in the pH.

The result of the comparison of the pH between the treatments by ANOVA technique is shown in the Table 7.

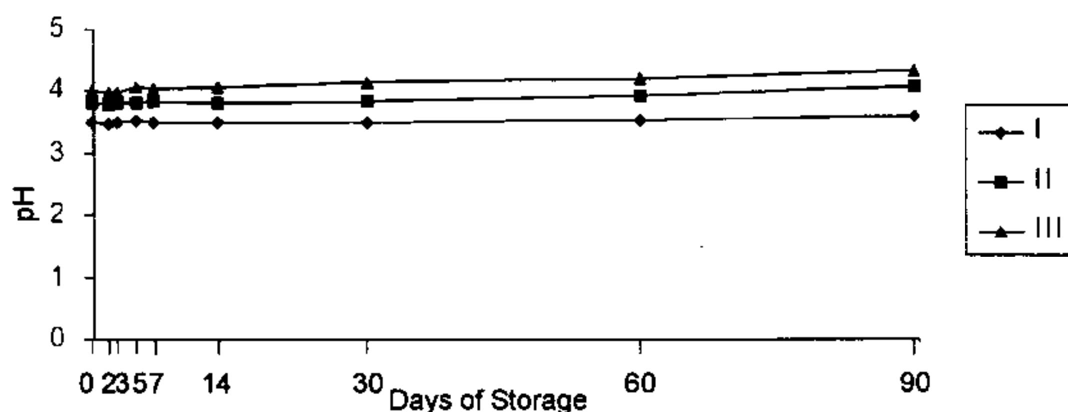

Fig. 1.Variation in pH of formic acid fish silage preparations during storage

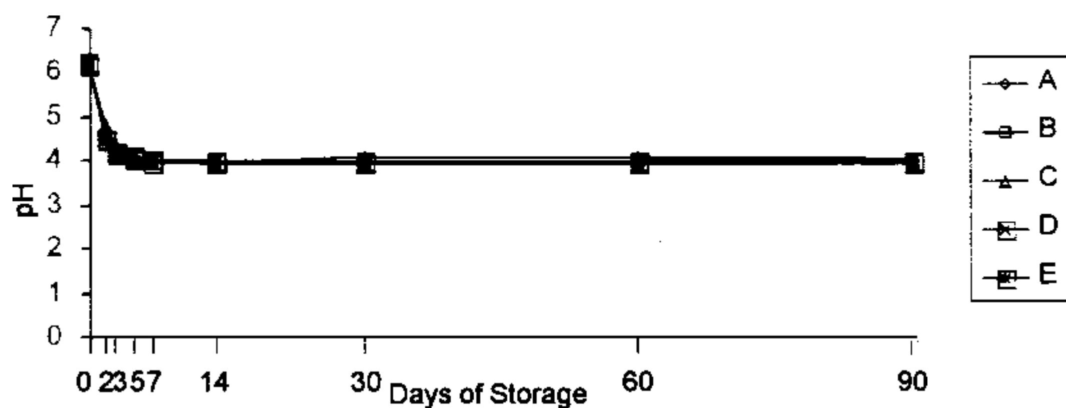

Fig.2. Variation in pH of fermented fish silage preparations during storage

Table 7. ANOVA table for pH

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares.	F
Treatment	7	6.09	0.871	5.56*
Error	56	8.77	0.157	
Total	71	27.46		

*Significant difference at 5% level

The critical difference value at 5% level for the pair wise comparison was calculated to be 0.374.

Pair wise comparison of pH values.

Silage	I	II	III	B	E	A	D	C
Treatment mean	3.516	3.854	4.092	4.273	4.311	4.320	4.349	4.423

The treatments that are not significantly different are connected with horizontal lines.

4.2 Total plate count (TPC)

Figure 3 shows the TPC of the formic acid fish silage preparations (I, II and III). The result of the comparison of the TPC between the formic acid fish silage preparations by ANOVA techniques is shown in Table 8.

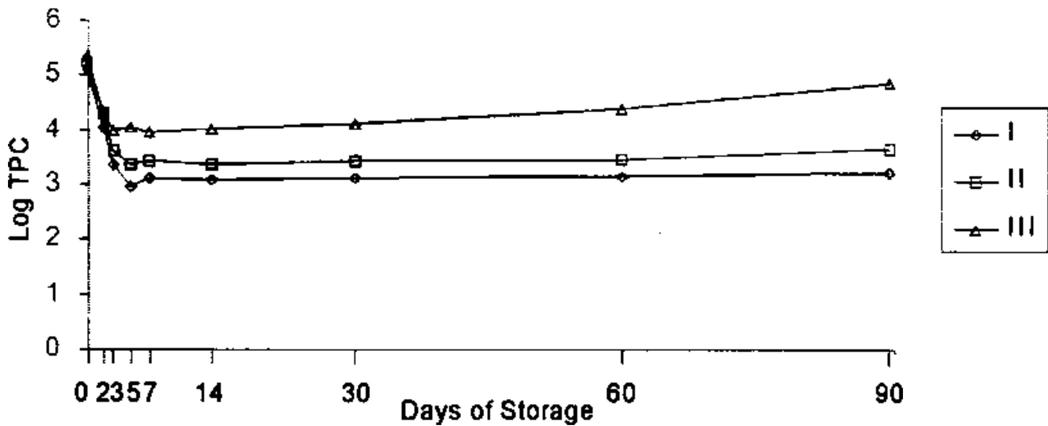

Fig.3. Variation in TPC of formic acid fish silage preparations during storage

Table 8. ANOVA table for TPC

Source of variation	Degrees of freedom	Sum of squares	Mean Sum of squares	F
Treatment	2	3.54	1.771	29.77*
Error	16	0.95	0.059	
Total	26	12.30		

*Significant difference at 5% level.

The critical difference value at 5% level for the pair wise comparison was calculated to be 0.2427.

Pair wise comparison of TPC

Silages	I	II	III
Treatment mean	3.468	3.758	4.339

The treatments that are significantly different are separated with horizontal line.

4.3 Acid producing bacterial count (APBC)

Figure 4 shows the APBC of the fermented fish silage preparations (A.B.C.D and E). The result of the comparison of the APBC between the fermented fish silage preparations by ANOVA techniques is shown in Table 9.

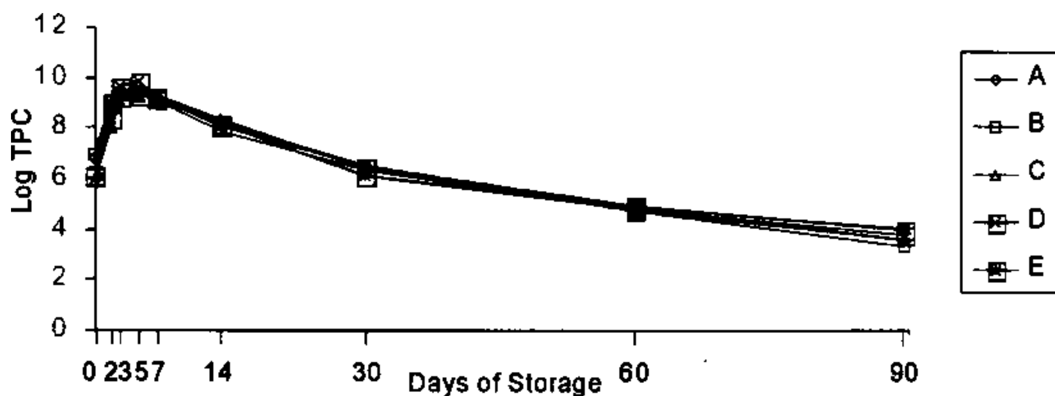

Fig.4. Variation in APBC of fermented fish silage preparations during storage

Table 9. ANOVA table for acid producing bacterial count.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares.	F
Treatment	4	0.23	0.057	0.67
Error	32	2.71	0.085	
Total	44	180.71		

Since the F-value is lesser than the table value (2.0332) at 5% level, it can be inferred that all the treatments are similar in terms of acid producing bacterial count.

4.4 Most probable number (MPN) of coliforms

The MPN of coliforms of the formic acid fish silage preparations (I,II and III) is depicted in the Figure 5. By the 7th day coliforms were not detected. Figure 6 shows the MPN of coliforms of the fermented fish silage preparations. By the 14th day coliforms were not detected.

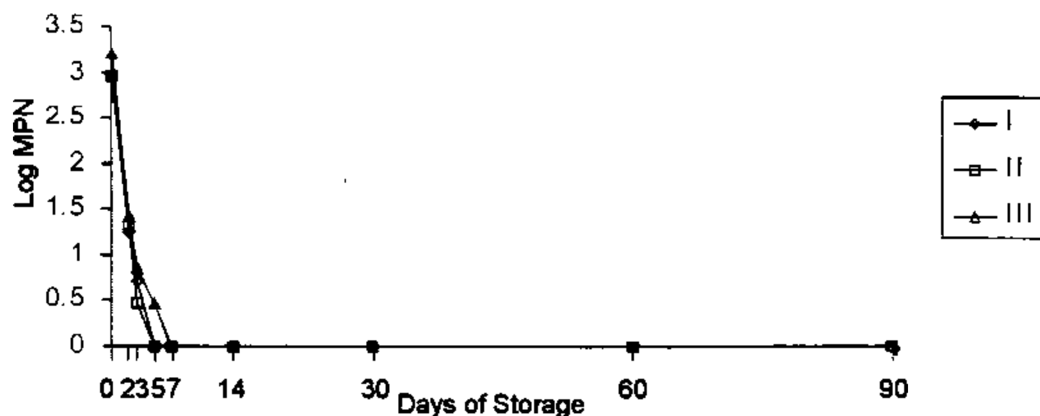

Fig.5. Variation in MPN of coliforms in formic acid fish silage preparations during storage

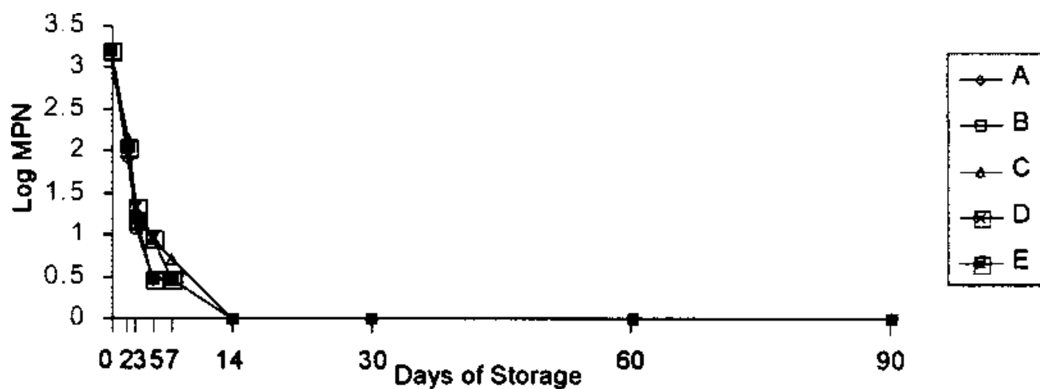

Fig.6. Variation in MPN of coliforms in fermented fish silage preparations during storage

Log (x+1) transformation was made to MPN of coliforms. The result of the comparison of the MPN of coliforms between treatments by ANOVA techniques is shown in the Table 10.

Table 10. ANOVA table for MPN of coliforms

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares.	F
Treatment	7	1.42	0.202	6.53*
Error	56	1.73	0.031	
Total	71	77.95		

*Significant difference at 5% level.

The critical difference value at 5% level for the pair wise comparison was calculated to be 0.166.

Pair wise comparison of MPN values.

Silage	II	I	III	A	B	E	D	C
Treatment mean	0.529	0.547	0.662	0.804	0.809	0.820	0.891	0.918

The treatments that are not significantly different are connected with horizontal lines.

4.5 Tri - methyl amine nitrogen (TMA-N) and Total volatile base nitrogen (TVB-N)

Figure 7 represents the change in TMA-N content and TVB-N content of the formic acid fish silage preparations (I,II and III). The figure shows that the TMA-N content increased to reach a maximum value, within 5-14 days in the case of silages I and II and by 90th day in the case of silage III. The TVB-N content increased in all the three formic acid fish silage preparations. It increased to reach a value of 198.27 mg% in the case of silage III, whereas for silage I it was 73.58 mg% and for silage II, 85.69 mg%. The changes in TMA-

N content and TVB-N content of the fermented fish silage preparations (A, B, C, D and E) is shown in the Fig. 8. The figure reveals that, the TMA-N content increased to a maximum value by the 14th day in all the fermented fish silage preparations. As in the case of the formic acid fish silage preparations, there was a decrease in the TMA-N content from the 14th day to the 90th day. The TVB-N content increased in all the fermented fish silage preparations during the period of study.

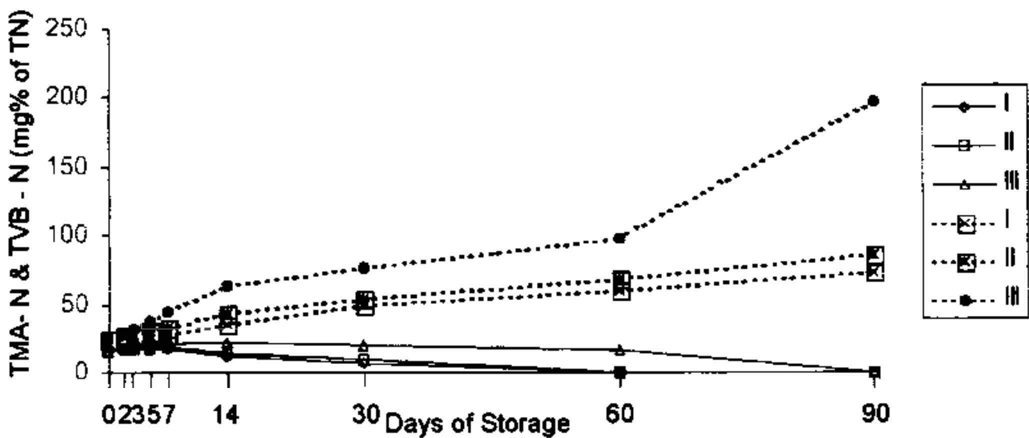

Fig.7. Variation in TMA-N content (——) and TVB-N content (-----) of formic acid fish silage preparations during storage.

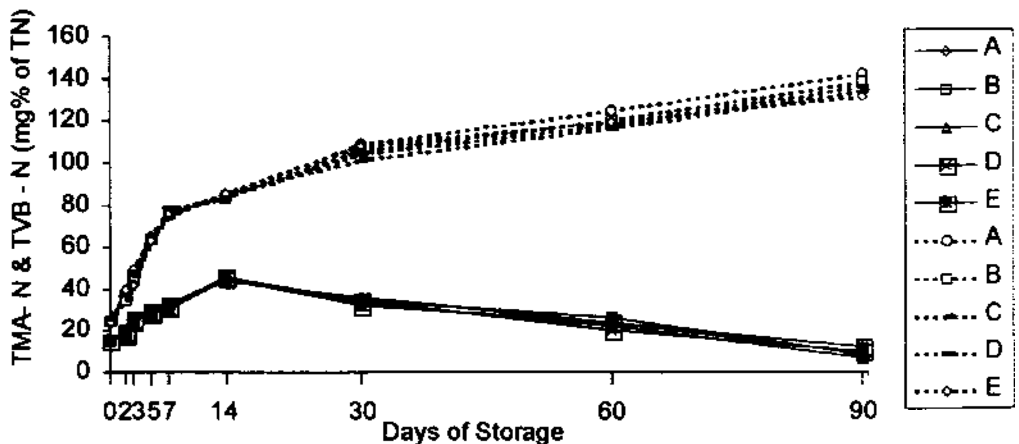

Fig.8. Variation in TMA-N content (——) and TVB-N content (-----) of fermented fish silage preparations during storage.

Log (X+1) transformation was made to the TMA-N content values. Table 11 depicts the comparison of the TMA-N content between the treatments by ANOVA technique.

Table 11. ANOVA table for TMA-N content.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares.	F
Treatment	7	2.756	0.392	7.16*
Error	56	3.07	0.055	
Total	71	10.55		

*Significant difference at 5% level.

The critical difference value at 5% level for the pairwise comparison was calculated to be 0.2215.

Pair wise comparison of TMA-N values.

Silage	I	II	III	C	D	B	A	E
Treatment mean	0.923	0.949	1.154	1.376	1.387	1.391	1.397	1.400

The treatments that are not significantly different are connected with horizontal lines.

Table 12 depicts the comparison of the TVB-N content between the treatments by ANOVA technique

Table 12. ANOVA table for TVB-N content.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares.	F
Treatment	7	16294.07	2327.724	11.30*
Error	56	11536.66	206.012	
Total	71	105465.21		

* Significant difference at 5% level

The critical difference value at 5% level for the pairwise comparison was calculated to be 13.558.

Pair wise comparison of TVB-N content values.

Silage	I	II	III	C	E	B	D	A
Treatment mean	39.188	44.030	67.124	75.507	76.648	76.907	77.312	78.807

The treatments that are not significantly different are connected with horizontal lines

4.6 Non protein nitrogen (NPN)

NPN content expressed as percentage with respect to total nitrogen (TN) is shown in Fig.9 for the formic acid fish silage preparations (I,II and III) and in Fig.10 for the fermented fish silage preparations (A,B,C,D and E). It can be seen from the Fig.9 that about 50% of the protein is hydrolysed by the 14th day in the case of all the formic acid fish silage preparations. In the case of the fermented fish silage preparations (Fig. 10) the rate of hydrolysis is slow and about 50% of the protein is hydrolysed by the 90th day.

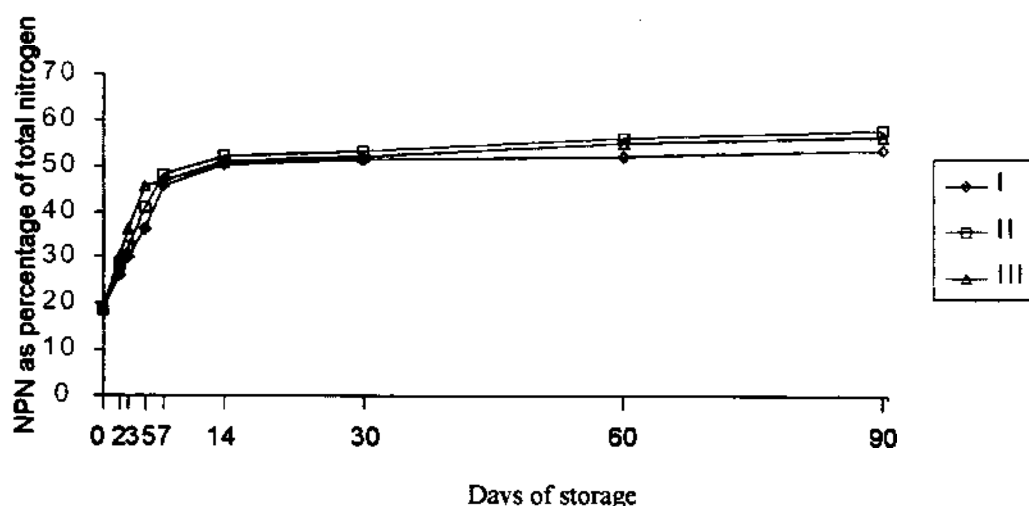

Fig.9. Variation in NPN content as percentage of total nitrogen in formic acid fish silage preparations during storage.

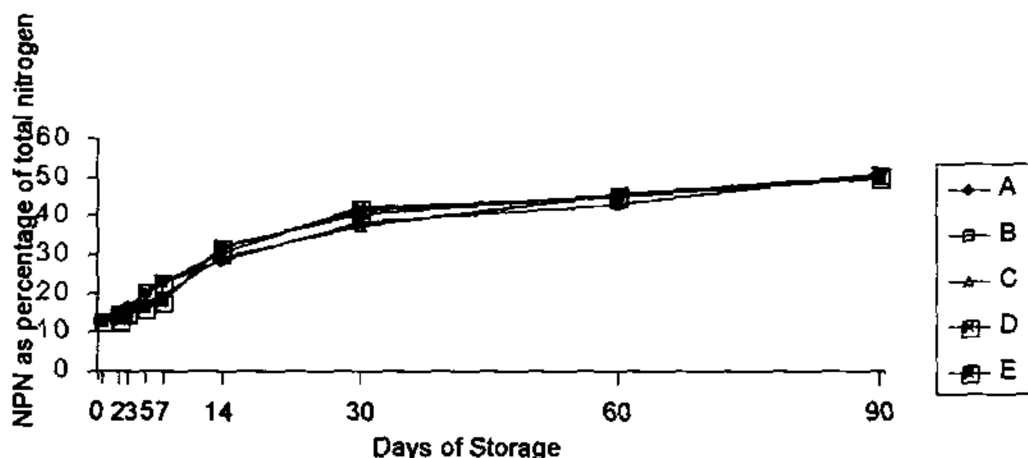

Fig. 10. Variation in NPN content as percentage of total nitrogen in fermented fish silage preparations during storage.

The ANOVA worked out for the different treatment with respect to NPN content is shown in Table 13.

Table 13. ANOVA table for NPN content.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares.	F
Treatment	7	1628.46217	232.63745	18.934*
Error	56	688.0315	12.2863	
Total	71	16426.76913		

*Significant difference at 5% level.

The critical difference value at 5% level for the pair wise comparison was calculated to be 3.31.

Pair wise comparison of NPN content values.

Silage	I	II	III	C	E	B	A	D
Treatment mean	40.42	43.07	43.59	26.95	26.95	27.11	27.63	28.18

The treatments that are not significantly different are connected with horizontal lines.

171625

4.7 Peroxide value (PV)

The PV of formic acid fish silage preparations (I,II and III) are shown in Fig.11 and that of fermented fish silage preparations (A,B,C,D and E) in Fig.12. Fig. 11 and 12 show that the peroxide value increased in all the silages and reached maximum values by the 30th day followed by a decrease. It can be noticed that the peroxide values are higher in the case of formic acid fish silage preparations when compared to fermented fish silage preparations.

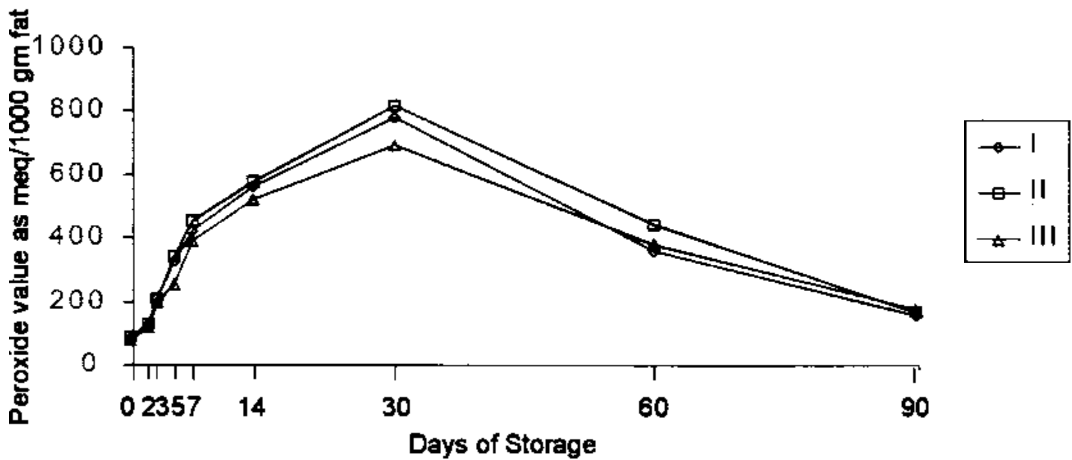

Fig. 11.Variation in Peroxide value of formic acid fish silage preparations during storage.

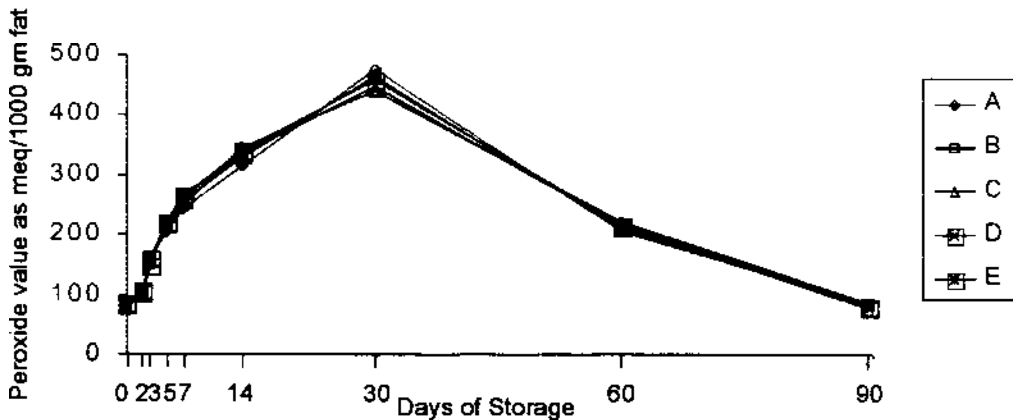

Fig.12. Variation in Peroxide value of fermented fish silage preparations during storage.

The results of the comparison of the PV between the treatments are represented in Table 14.

Table 14. ANOVA table for PV.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares.	F
Treatment	7	274258.79	39179.827	13.34*
Error	56	164507.35	2937.631	
Total	71	2097967.28		

*Significant difference at 5% level.

The critical difference value at 5% level for the pair wise comparison was calculated to be 51.202.

Pair wise comparison of PV.

Silage	A	C	D	B	E	III	I	II
Treatment mean	208.112	210.451	211.031	211.278	214.528	312.419	336.067	359.926

The treatments that are not significantly different are connected with horizontal lines.

4.8 Proximate composition

When the proximate composition of the formic acid fish silage preparations (I,II and III) and fermented fish silage preparations at the zero day was compared with the respective proximate composition at the 90th day by the paired t-test there was no significant difference at 5% level.

Table 15. Proximate composition of the formic acid fish silage preparations and fermented fish silage preparations at zero day

Percentage	I	II	III	A	B	C	D	E
Moisture	77.34	77.14	77.08	69.18	69.64	68.23	68.46	68.84
Protein	14.23	14.34	14.46	12.24	11.67	12.33	11.78	14.64
Carbohydrate	0.32	0.20	0.33	9.00	10.30	9.85	11.34	5.74
Fat	6.76	6.58	6.24	6.53	5.69	6.53	5.73	7.33
Fibre	0.11	0.36	0.47	0.98	0.86	1.03	0.90	1.19
Mineral	1.24	1.38	1.42	2.07	1.84	2.03	1.79	2.26

Table 16. Proximate composition of the formic acid fish silage preparations and fermented fish silage preparations at the 90th day

Percentage	I	II	III	A	B	C	D	E
Moisture	77.86	77.36	76.93	69.38	69.76	68.48	68.53	69.23
Protein	13.91	14.07	14.76	12.36	11.82	12.41	11.92	14.48
Carbohydrate	0.25	0.28	0.10	8.89	9.91	9.33	11.08	5.51
Fat	6.62	6.64	6.40	6.61	5.74	6.64	5.91	7.41
Fibre	0.09	0.29	0.45	0.64	0.88	1.06	0.84	1.21
Mineral	1.27	1.36	1.36	2.12	1.89	2.08	1.72	2.16

4.9 Acceptability study

Silage III was not used for the preparation of feed, since it produced off odour. It was observed that all the silage-based diets were consumed by the common carp fingerlings within 15 minutes of feeding.

DISCUSSION

V DISCUSSION

Pairwise comparison of pH values showed that there is no significant difference between formic acid fish silage preparations I and II and between formic acid fish silage preparations II and III. All the five fermented fish silage preparations did not show any significant difference between each other and were similar to formic acid fish silage preparation III. But the formic acid fish silage preparation III produced foul smell by the 90th day of storage, where as the fermented fish silage preparations preserved well. The preservation of fermented fish silage preparations at higher pH value may be attributed to the other known preservative action of LAB such as production of hydrogen peroxide (Dahiya and Speck, 1968; Gilliland and Speck, 1975; Lindgren and Clevstrom, 1978), changes in the oxidation- reduction potential of the environment (Barber and Deibel, 1972) and Production of bacteriocins (Graham and McKey,1985). From the Fig.1 it can be understood that there is an increase in pH of the formic acid fish silage preparations by 90 days of storage. Wood *et al.* (1985) reported a similar increase in pH from 3.2 to 3.9 in formic acid fish silage when stored for six months. Henderson and McDonald (1971) had recorded that upto 50% of the formic acid content of silages can be lost during ensilage. Hence the increase in pH may be also due to the loss of formic acid during ensilage in addition to the action of putrefactive bacteria.

The formic acid fish silage preparations I, II and III were significantly different from one another with respect to TPC. The TPC of the formic acid fish silage preparations I and II decreased to a minimum of log 2.98 and log

3.36 (Fig.3) respectively in five days and remained more or less stable till the 30th day. On further storage upto the 90th day, there was a slight increase in TPC. In the case of formic acid fish silage preparation III the lowest TPC of log 3.96 (Fig.3) was observed on seventh day and there after till the 90th day, an increase in TPC was observed. This increase in TPC in the case of formic acid fish silage preparations is in agreement with the findings of Wood *et al.* (1985) and Hoq *et al.* (1995). This increase is probably on account of the increase in pH values observed as mentioned above. Figure 5 shows that by the seventh day the coliforms became undetectable in the formic acid fish silage preparations.

The TMA-N content of the formic acid fish silage preparations I and II increased to a maximum of 18.08 mg % and 18.86 mg % in five and seven days respectively. There after the TMA content decreased gradually in both the silages to finally reach a value of zero by the 60th day. In the case of silage III the maximum TMA content of 22.64 mg % was observed on the 14th day of storage, which on further storage decreased to zero by the 90th day. Haaland and Njaa (1988) also observed a similar result in formic acid silage ($pH<4.2$) of Capelin and Mackerel. They have recorded that bacterial decomposition of tri-methyl amine oxide (TMAO) produces TMA and that bacterial activity in properly produced silage is negligible, thus there will not be any further decomposition of TMAO after ensiling. But in their silage also there was an increase in TMA-N content from 5 mg/g total nitrogen to 8 mg/g total nitrogen by the 18th day and then fell to 6 mg/g total nitrogen by the 30th day and again

increased to 8 mg/g total nitrogen by 60th day and there after it fell to zero by 120th day. Quiet contrary is the result obtained by Zuberi *et al.* (1992) with their formic acid silage (pH 4.0) of trash fish (mainly sardine). They observed that TMA-N content increased throughout the storage period of 365 days, even though there was a reduction in the TPC. Thus, in addition to bacterial decomposition of TMAO, there may be mechanisms for the break down of TMAO to TMA. The decrease in the TMA content may be due to the further break down of TMA and its escape due to its volatile nature.

Raa and Gildberg (1982) recommended a pH of 4.2 as microbiologically safe for fermented fish silage preparations. In the present study this pH was reached in 3-5 days in all fermented fish silage preparations (Fig. 2). The APBC was not significantly different between the fermented fish silage preparations. It increased to a maximum by the fifth day of storage. The average APBC on the fifth day was log 9.45, which decreased to log 3.71 by the 90th day. Even though the initial number of LAB was above 10^6 (Fig. 4), as recommended by Gross (1969), the rate of fall in pH (Fig.2) was slightly lower. This may be on account of the growth of heterofermentative LAB, which are known to produce less lactic acid when compared to the homofermentative LAB. Wood (1961) has recorded that homofermentative LAB anaerobically produces two moles of lactic acid while heterofermentative LAB anaerobically produces one mole of lactic acid, one mole of carbon dioxide and one mole of ethanol per mole of glucose fermented. Even though the lowering of pH to a level below 4.2 took 3 to 5 days, spoilage did not occur. This may be due to the

other known preservative mechanisms of LAB mentioned above. Owens and Mendoza (1985) had reported that the carbondioxide produced by heterofermentative bacteria can suppress the growth of spoilage bacteria.

The Fig. 6 shows that by the 14th day there were no coliforms to be detected. George (1990) recorded that the total coliform count of all the fermented catfish waste were between log 4.0 to 6.68 on the initial day, which gradually reduced to log 1.0 by the 15th day.

Pairwise comparison of TMA-N values of fermented fish silage preparations shows that there is no significant difference between each other. Like the formic acid fish silage preparations, there was a decreasing trend in the case of fermented fish silage preparations also (Fig.8) , but the TMA-N content did not drop to zero by the 90th day in any of the fermented fish silage preparations. The TMA-N content of the fermented fish silage preparations was significantly higher than that of the formic acid fish silage preparations. Similar observation was made by Zuberi *et al* (1992) also. This may be due to the action of TMAO reducing bacteria.

Unlike TMA content, TVBN content increased from zero day to the 90th day of storage in both formic acid fish silage preparations (Fig. 7) and fermented fish silage preparations (Fig. 8). Formic acid fish silage preparations I and II are similar with respect to TVBN content and the formic acid fish silage preparation III and all other fermented fish silage preparations had TVBN content markedly greater than formic acid fish silage preparations I and II. This is in agreement with Kompiang *et al.* (1979 b) and Zuberi *et al.*

(1992). By 90th day the silage III attained the highest TVBN content, which also developed off odour and increased bacterial activity. Brady (1966), McDonald and Whittenbury (1973) has reported that amino acids are one possible source of energy for LAB. Zuberi *et al.* (1992) recorded that high ammonia (NH_3) content in microbial silage might be due to deamination of aminoacid by LAB and attributed the higher values of TVBN content in fermented silages to the production of excessive amounts of NH_3. The increase in TVBN content may also be on account of the action of other putrefactive bacteria.

There is no significant difference between the rate of autolysis among the fermented fish silage preparations, but were significantly different from the formic acid fish silage preparations. The autolytic changes of the formic acid fish silage preparations were at a higher rate than that of the fermented fish silage preparations. This is in agreement with the findings of Kompiang *et al.* (1979 b) and Zuberi *et al.* (1992). Autolysis, in terms of NPN formation reached 50% by the 14th day in the case of formic acid fish silage preparations (Fig. 9) and further increase in autolysis is at a very low rate. In the case of fermented fish silage preparations (Fig. 10) the autolysis has reached around 50% only by 90th day. Hence the rate of autolysis is lower in the case of fermented fish silage preparations. This phenomenon as assumed by Raa and Gildberg (1982) may be due to the attachment of enzymes and protein substrates to the polysaccharides thus preventing their interaction. Gildberg and Raa (1977) have observed that degree of autolysis and protein

solubilisation in silage varied with the nature of raw materials and is 40-45% in the case of tropical fishes. Gopakumar (1997) reported that undigested proteins of the silage are seen to be as high as 50% of the total protein in case of tropical fishes. Takahashi (1960) had concluded that for autolysis of cuttle fish (*Ommastrephes sloani pacificus*) viscera the desirable pH is about 5 and temperature is about 50^0C. Hence the low rate of autolysis and low NPN fraction may be related to the characters specific to the species, and the lack of optimum pH and temperature for ensiling.

Figure 11 and 12 show that in all the silages the peroxide value increased to a maximum by the 30th day, thereafter it decreased. This is possibly because of their further break down to more stable secondary products (Dugan, 1975). All the formic acid fish silage preparations had peroxide value significantly higher than the fermented fish silage preparations. The peroxide value of the oil in moist fermented silage is low (Wirahadikusumah, 1968) and still lower values have been found in a dried fish silage preserved by acid addition (Disney *et al.*, 1978). Raa and Gildberg (1982) reported that lactic acid fermentation stabilise the oil in addition to it being efficiently trapped in microbial silage because of the high binding ability of the carbohydrate additive and the slow autolysis of fish proteins. This may be the reason for the lower peroxide value of all fermented fish silage preparations in the present study.

The results show that the formic acid fish silage preparations I and II and all fermented fish silage preparations preserved well when stored for 90 days.

The proximate composition values of the silages did not significantly differ from the zero day and the 90th day. Twiddy *et al.* (1987) had also reported a similar observation. The crude protein content was lower in fermented fish silage preparations than in acid silages due to the addition of carbohydrates in the former for facilitating fermentation.

Feeding trials where conducted using common carp fingerlings in order to test for the acceptability. All the silage based diets were consumed by the common carp fingerlings within 15 minutes of feeding.

SUMMARY

VI SUMMARY

1. The disposal of cuttle fish processing waste is becoming a major problem for the seafood processing industries.
2. The cuttle fish processing waste contains nutrients, which could be used for incorporating in animal diets, if suitably processed.
3. Ensiling is considered to be a cheap and efficient method of preservation of fish wastes.
4. The cuttle fish waste was used for preparing formic acid added silages and fermented silages.
5. The silages were stored for 90 days, during which storage studies were conducted based on physical, biochemical and microbiological parameters.
6. Based on the results of the storage studies it was inferred that formic acid fish silage preparations of pH 3.5 and pH 3.8 preserved well, whereas the formic acid fish silage preparation of pH 4.0, produced off odour by the 90th day of storage and the formic acid fish silage preparation of pH 4.5 spoiled by the 14th day. Hence formic acid fish silage preparation of pH 3.8 can be considered to be the ideal pH for preserving cuttle fish waste for at least 90 days.
7. All the five combination of fermented fish silage preparations studied preserved well.
8. The formic acid fish silage preparations and the fermented fish silage preparations, which preserved well, were mixed with tapioca powder and rice bran at varying proportion, before preparing dry pellets.

9. The feeds thus prepared were fed to common carp fingerlings twice daily at the rate of 3% of the body weight per day.
10. All the silage-based diets were consumed by the common carp fingerlings within 15 minutes of feeding.

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VII REFERENCES

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ENSILING CUTTLEFISH WASTES FOR USE AS FISH FEED INGREDIENT

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ABSTRACT OF THE THESIS

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VIII ABSTRACT

The disposal of cuttle fish processing waste is becoming a major problem for the seafood processing industries in India. Formic acid fish silages and fermented fish silages were prepared using the waste. The silage preparations were stored for 90 days. Samples were drawn on 0,2,3,5,7,14,30,60 and 90th days of storage. The pH and various biochemical parameters like, total nitrogen (TN), non protein nitrogen (NPN), total volatile base nitrogen (TVBN), tri methyl amine (TMA) and peroxide value (PV) were carried out. *Microbial analyses, viz., total plate count (TPC) and most* probable number (M.P.N) of coliforms were carried out for formic acid fish silage preparations, whereas for fermented fish silage preparations, acid producing bacterial count (APBC) and M.P.N of coliforms were determined.

All the combinations of fermented fish silage preparations studied, preserved well. A pH of not more than 3.8 was necessary for the formic acid fish silage to be preserved for 90 days. All the silages, which preserved well, were used for making fish feed. The pelletised feeds were fed to common carp (*Cyprinus carpio*) fingerlings. The feeds were consumed by the fishes within 15 minutes of feeding.