PRODUCTION OF FISH POWDER BY ACID HYDROLYSIS

by

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DEDICATION

To: Ninka, ba Musoke, Kalema,
"Zibiri" and the Liscotts
who, through their actions,
have always reminded me
what friends are for.

ABSTRACT

Haplochromis spp. is an underutilized fish stock abundant in L. Victoria and occurring in many African lakes. Its small size and boniness make it unpopular as human food.

A process for producing fish powder for human consumption from whole *Haplochromis* by partial acid hydrolysis was developed. Whole fish was minced and mixed with hydrochloric acid in the ratio 30:100 v/w. The mixture was continuously stirred for 30 minutes, neutralised with NaOH to the original pH of the mince, homogenized and drum dried or spray dried. The effect of varying temperature, acid concentration and using cooked or uncooked fish as raw material on hydrolysis were investigated by determining changes in TCA-soluble N of the hydrolysate over the 30 minute period. Seven temperatures between 25 and 84°C and four acid concentrations (2.5M, 5M, 7.5M and 11.3M) were investigated.

The overall extent of hydrolysis for each 'run' was determined by calculating the nett increase in TCA-soluble N expressed as % of total N, and it ranged between 0.8% and 10.4% and 6.7% and 34.1% for cooked and uncooked fish respectively. The extent of hydrolysis was greater in uncooked than cooked fish due to the synergistic effect of the endogenous proteolytic enzymes in uncooked fish and its absence in cooked fish. Cooking had destroyed the enzymes.

Eight different products were produced. Data for crude protein, true protein, amino acid profiles, total lipid, ash, NaCl, a_w, *in vitro* digestibility, colour, particle size distribution and sensory evaluation of the products were obtained. The data indicated the products had a high nutritional value and would be microbiologically stable for several months but susceptible to oxidative rancidity unless antioxidant

were added. Using the data a process in which uncooked fish would be partially hydrolysed with 2.5M HCl at 48^{o}C (approximately) for 30 minutes and the hydrolysate spray dried was proposed.

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CONTENTS

I. INTRODUCTION

A considerable proportion of the world's fish catch is regarded as "industrial fish", a term used to describe those species of fish which for various reasons are considered only suitable for reduction to fish meal and oil. Statistics show that about 30% of the total world catch is converted to fish meal and oil (FAO, 1982).

There are a number of reasons why certain fish stocks are regarded as only suitable for fish meal production. Small size and high oil content renders certain fish species such as anchovy and menhaden difficult to process by conventional methods for direct human consumption. In some cases the apparent "unpalatability to human tastes" of particular fish species makes them unsaleable (at least economically) as human food, so they are best reduced to fish meal. Examples of such fish are the sand eel *(Ammodytes marinus)* and Norway pout *(Trisopterus esmarki)* (Barlow and Burton, 1974). In other instances fish such as herring, winter sprat and mackerel, which could otherwise be utilized for human food are reduced to fish meal due to lack of "human consumption outlets" (Bailey, 1976).

There is no doubt that fish meal plays an important role in alleviating protein shortage in human nutrition. Meal is fed to poultry, pigs or fish (in fish farming) which convert it into protein eaten by humans. However, to produce 1 kg of edible chicken protein 3 kg of fish meal protein is required, giving a conversion figure of about 33% (Barlow and Burton, 1974). A lower conversion figure of about 17% is given for other domestic animals (Finch, 1970). It would therefore seem that there is considerable wastage along the line. Perhaps this apparent wastage would not matter so much in developed countries where there is adequate supply of protein. However, in developing countries, particularly in Africa, where protein deficiencies are chronic (FAO, 1982) every protein source should primarily be utilized for human food.

Since the supply of food fish is not likely to increase significantly in the near future research efforts should be concentrated in developing fishery products for human consumption from among the fish stocks that are presently either reduced to fish meal or under-utilized. The most effective way of utilizing fish protein could be achieved by using the whole fish for the production of concentrated protein for human consumption (Barlow and Burton, 1974).

This thesis is concerned with an acid hydrolysis method for producing fish powder from whole fish *(Haplochromis spp.)* for direct human consumption.

2. LITERATURE SURVEY

2.1 Underutilized Fish Species

The realisation that traditional fish species were fully exploited, and in some cases over-exploited (Bailey, 1976) led many countries to start looking for new marine protein sources not used before. Studies have been conducted on the large stocks of sardines in Japan (Nakamura, Fujii and Ishikawa, 1978; Connell, 1982), anchoveta in Peru, Patagonian hake *(Merluccius merluccius (L))* in Argentina and Uruguay (Lupin *et al*, 1980) and rattail *(Macrourus spp.)* and others in North America (Kremsdorf *et al*, 1979; Connell, 1982). Blue whiting *(Micromesistus poutassou)*, greater silver smelt *(Argentina silus)*, lesser silver smelt *(Argentina sphyraena)* and Norway pout *(Trisopterus esmarki)* have been investigated in Britain and other European countries (Bailey, 1976). Other species include Baltic sprats *(Clupea sprattus (L))* in Poland (Sikorski and Naczk, 1982) and oil-sardines *(Sardinella longiceps* Valenciennes), Bombay duck *(Harpodon nehereus)*, sole *(Cynoglossus semifasciatus)*, croaker *(Micropogon undulatus (L))* and silver-bellies *(Leiognathus sp.)* in India (Sripathy, 1982).

The by-catch from shrimp trawlers off the coasts of South America, Africa and Asia has also attracted a lot of attention in recent years (Disney, 1977; Stanley, 1981; Young and Tableros, 1981; Tableros and Young, 1982; Tan Sen Min *et al*, 1982; Young, 1982a and 1982b). The freshwater *Haplochormis* species complex, occurring in many African lakes, is another underutilized resource which has been the subject of much study (Dhatemwa, 1981; Ssali, 1981; Mlay and Mkwizu, 1982).

Most of the underutilized species are difficult to process by conventional methods due to their small size, the dark colour of the flesh or boniness. However a considerable degree of success has been

achieved using flesh/bone separators to obtain minces which are subsequently used to make products such as fish sticks and portions (King, 1976), dried/salted fish minces (Dhatemwa, 1981; Young *et al*, 1981; Sripathy, 1982), roller dried minces (Jensen, *et al*, 1982) surimi and kamaboko (Ishii and Amano, 1974; Ishikawa, *et al*, 1979; Connell, 1982).

The role of underutilized fish species in human nutrition is likely to increase particularly in communities which are chronically short of protein in their diets. Considering that protein-deficient communities are predominantly found in developing countries, where facilities for preserving fish in fresh or frozen form are lacking and distribution systems are inadequate, emphasis should be placed on developing dehydrated products which have a long shelf life.

2.2 Processing of Underutilized Species

The very nature of the so called underutilized species (their small size or unattractive appearance or boniness, etc) presents one immediate problem; they would not be readily accepted by the consumer without changing their natural form (as fillets or whole fish). Therefore the processing methods which have been developed to render them more acceptable to the consumer have invariably involved converting the fish into minces, powders or liquids. The different methods and products that have been developed are discussed in more detail below.

2.2.1 Fish Meal

Although fish had been used to feed livestock (and as a fertilizer) for centuries (March, 1962) industrial fish meal production was not developed until the beginning of the 20th century (Kreuzer, 1974). The development enabled full utilization of all those species of fish

that would otherwise not be accepted as human food fish and also waste from filleting operations.

Details of fish meal manufacture are well covered by FAO (1975). Briefly, however, the process involves feeding the fish into a hopper (large fish being chopped coarsely first while smaller fish are fed in directly) from where they are taken to a steam cooker. The cooked fish are strained and then pressed into a cake which is subsequently dried and milled. The press liquor is fractionated into oil, stickwater and fine sludge by centrifugation. The oil is purified and sold separately. The stickwater and sludge are concentrated and finally added to the presscake prior to drying.

The fish meal manufacturing process outlined above is continuously being improved, as shown by a number of patents that have been applied for or granted in the field. In a Norwegian Patent Application (Tronstand, 1977) a process is described where the time for drying fish meal is shortened, helping to prevent denaturation of digestible protein, destruction of vitamins and development of an unpleasant colour. Shimose and Tanaka (1981) describe a batch process, that does not pollute the environment, for making fish meal products. Bladh (1982), on the other hand, describes a process where fish meal and oil are obtained by heating a finely divided fish mass using a heat exchanger and subsequently drying the fish in such a way that the resulting fish meal has the desired low water and oil content.

While the developments described above have been based on sophisticated technology in developed countries other efforts have also been made to produce fish meal at village level, in developing countries. Mlay and Mkwizu (1982), for example, developed a method of producing fish meal from *Haplochromis* using simple materials which are easily available locally. The meal produced by such a process was reported to compare well with other meals produced by conventional methods.

Every year about 30% of the world's fish catch is converted into fish meal and oil (FAO, 1982). Most of this meal would subsequently be converted by animals into products suitable for direct human consumption. However, a considerable amount of the nutrients contained in the fish meal would be lost for human consumption as a result of moving one trophic level along the food chain (Finch, 1970; Barlow and Burton, 1974; Valand, 1979). This apparent waste of resources becomes more pronounced when one considers the fact that a large part of fish meal production takes place in developing countries (Table 1) where the supply of animal protein is scarce. The meal is then exported to developed countries (mainly Europe and the United States) where the supply of animal protein is abundant (Valand, 1979).

It is in the light of this discrepancy that during the last few years (from the mid-seventies) serious endeavours have been made to produce "fish meal" suitable for direct human consumption. This aspect is discussed in more detail in Section 2.2.3.

2.2.2 Minces

The development of flesh/bone separators for the fishery industry was a major achievement in the processing of underutilized species. The machines were developed in Japan for the production of bone-free minced fish for subsequent use in preparation of various Japanese fish products (Spinelli and Dassow, 1982). Keay (1979) gives a brief but informative description of fish mince production while a more detailed discussion on the development of flesh/bone separators is given by Drews (1976).

During the deboning process fish (whole, fillets, trimmings or frames) are fed from a hopper to pass between a moving thick elastic conveyor belt and the outside of a revolving perforated stainless

TABLE 1: Fish Meals and Solubles: Production and Exports[1]

	1978-79 average	1979	1980	1976-78 average	1979	1980	1976-78	1979	1980
	PRODUCTION thousand tonnes			EXPORTS thousand tonnes			EXPORTS AS A PERCENTAGE OF PRODUCTION		
World total	4655	4817	4692	2085	2462	2337	44.8	51.1	49.8
Developing countries	1429	1702	1569	980	1364	1227	68.6	80.1	78.2
Latin America	1169	1445	1298	808	1153	1019	69.1	79.8	78.5
Africa	46	38	54	26	24	23	56.5	63.1	42.6
Far East	174	200	198	115	164	162	66.1	82.0	81.8
Developed countries	3226	3115	3123	1105	1098	1110	34.2	35.2	35.5
North America	480	530	515	69	40	107	14.4	7.5	20.8
Western Europe	1122	1010	1014	946	949	922	84.3	94.0	90.9
Eastern Europe and USSR	657	593	637	18	20	22	2.7	3.4	3.4

(1) Food and Agriculture Organisation (1982)

steel drum. The flesh is forced through perforations into the interior of the drum. A stationary screw discharges the mince from the end of the drum. Skin and bone are retained on the outside of the drum where they are continuously scraped off by a blade.

Mincing offers three main advantages (Ravichander and Keay, 1976). Firstly, it facilitates the utilization of fish species which are too difficult to fillet economically due to their small size or awkward shape. Secondly, it increases the yield of (almost) bone-free flesh compared with filleting alone; up to twice as much can be recovered by separating flesh directly from headless, gutless fish. If the fish are filleted first an additional 8-12% flesh can be separated from the filleting waste. Thirdly mincing provides an opportunity for greater control over product flavour, appearance, texture and storage properties. Minces can easily be mixed with additives such as antioxidants, spices, etc.

However, mincing also has its disadvantages. In the first instance it changes the original texture of the fish, particularly when a drum with smaller perforations is used and/or the tension on the belt is increased. The flavour and sometimes the colour of the mince may change depending on whether it is from fillets, trimmings, frames or whole fish (Whittle, 1982). While mince from trimmings is of high quality, with properties similar to that directly from fillet, mince from frame is of lower quality; discoloured with blood and deteriorates more quickly in the cold store. It is thought that the activity of tissue enzymes, which increases when muscle tissue structure is disrupted during deboning, contribute to the textural, colour and flavour changes (Svensson, 1980). Minced flesh particularly from fatty fish such as herring (lipid content can be as high as 20%) may be susceptible to oxidative rancidity during cold storage although this can be controlled (Cole and Keay, 1976).

It has been established that to produce good quality mince the fish must be gutted, headed and washed and the section of backbone immediately above the belly cavity should be removed first (Keay, 1979). However, it is sometimes more practical to mince the whole fish first and wash the mince afterwards. This is particularly so in the case of very small fish such as *Haplochromis* (Dhatemwa, 1981).

In the following sections the different uses to which minced fish can be put are discussed.

2.2.2.1 Frozen blocks of minces

Minced fish can be frozen into blocks which may be processed into fish cakes and patties, fish burgers, fish portions (Blackwood, 1974) fish fingers (Ravichander and Keay, 1976) or other "frozen, battered-breaded, ready-to-fry products" (Sripathy, 1982). The acceptability of products made from minced fish may depend on factors such as species (Ravichander and Keay, 1976) or the actual quality of the mince, in the first place (Whittle, 1982).

Frozen mince tends to deteriorate in quality during cold storage. This is usually attributed to biochemical reactions taking place in the mince. In gadoid species (such as cod and haddock), for example, trimethylamine oxide (TMAO) is a natural component of the muscle. In the kidney and liver tissues and pyloric caeca of these fish is an enzyme system that can catalyse the breakdown of TMAO to formaldehyde (FA) and dimethylamine (DMA) (Svensson, 1980). It is the FA and DMA so produced that partly causes the deterioration in quality of mince from gadoid fish during cold storage. Experiments have shown that elimination of the kidney and liver tissue and/or thermal inactivation of the TMAO-degrading enzymes prior to deboning can increase the storage life of frozen minces (Svensson, 1980).

2.2.2.2 "Surimi" and "Kamaboko"

"Surimi" is the Japanese name for specially prepared minced fish. It is the raw material for making "Kamaboko" which is a preparation of fish jelly. Surimi and Kamaboko constitute the largest single seafood product consumed in Japan (Slavin, 1981). In 1978 production of Surimi was estimated at 365,000 tonnes (FAO, 1979). A detailed description of varieties and methods of Kamaboko production is given by Suzuki (1981).

2.2.2.3 Salted and dried fish mince

The production of salted and dried cakes from minced fish has been adopted as an effective way of processing underutilized species in tropical developing countries (Disney and Poulter, 1977; Young *et al*, 1979; Dhatemwa, 1981; Stanley, 1981; Young, 1982a and 1982b). The method has been found to be more appropriate for a number of reasons. First, dried products constitute the majority of cured fish products in the tropics (Wood, 1981) so the consumers can easily identify with salted/dried cakes. Secondly, refrigeration facilities are inadequate, if not lacking, in most tropical countries and drying facilities prolong shelf life of fishery products. Dried products can easily be transported to areas distant from the fishing ports. Thirdly, salted/dried minces offer a relatively cheap source of valuable proteins, an important factor in developing countries.

Del Valle (1974) described a "quick-salting process for fish" intended to produce a good quality but cheap product with a long shelf life at ambient temperature. The fish flesh (mince or fillet) is ground and mixed with salt. The salt dehydrates the fish flesh and the salt-extracted water is removed by pressing. The cakes are finally dried either in the sun or artificially. The high salt content which can vary from 22% to 100% of the weight of wet flesh, depending on the species, prevents microorganisms and vermin from attacking the

fish cakes (Del Valle, 1974). In preparation for consumption the cakes are desalted by immersing once or twice in boiling water.

As mentioned in Section 2.1 the by-catch from shrimp trawling operations in tropical waters has attracted considerable attention from fish technologists. During shrimp trawling an average catch may contain between 10 and 30% shrimp, the rest being a mixture of up to 100 species of fish. They vary in size and chemical composition (Meinke, 1974), some may even be poisonous (Stanley, 1981), and would present handling problems on board. From the operators' point of view it is more economical to keep the shrimp and dump the fish back into the sea (Meinke, 1974). The total amount of fish that is wasted in such a manner is estimated to be between 4 and 6 million tonnes (Meinke, 1974; Disney and Poulter, 1977) and is probably higher (Stanley, 1981).

Young *et al* (1979; 1981) have successfully produced dried salt/fish cakes from Mexican shrimp by-catch which could be competitively marketed at prices economical to the fishermen (Young, 1982b). Similar work has been conducted in Guyana (Stanley, 1981). Dhatemwa (1981) produced salted/dried cakes from whole ungutted *Haplochromis* spp. Washing the mince three times with chilled water removed the gut material and reduced the microbial load. A yield of 39.4% of whole fish was obtained, after washing. The mince contained nematode worms (13-39/kg) which could not be removed by washing. The optimum salt concentration was 40% (w/w) and there was some protein loss, up to 5.9%, in the salt water. The cakes were microbiologically stable at 20-25°C for more than 14 weeks.

2.2.2.4 Roller dried mince

In Denmark experiments have been carried out to produce roller-dried fish protein from minced fish (Herborg, *et al*, 1974). Jensen, *et al*

(1982) produced roller-dried *Haplochromis* mince mixed with 20% (w/w) cassava or maize flour. The products were found to be acceptable at "two informal presentations" in Tanzania. The production costs were calculated at 22 Tsh/kg (2.30 US $/kg).

While minced fish technology has made a considerable contribution towards the processing of underutilized fish resources there are some limitations to its application in developing countries. In most tropical countries only salted/dried fish minces have a potential, considering the lack of cold storage facilities, distribution systems and the low purchasing power of the majority of the people. Yet a certain proportion of communities in these countries are not accustomed to salted/dried fish. Therefore some carefully planned marketing strategies would have to be developed. The by-products of the flesh/bone separation process (bones, scales, viscera etc) would not be easily utilized for human food. A separate process would therefore be needed to convert them into animal feed or fertilizer to facilitate effective utilization of the resources.

2.2.3 Fish Protein Concentrate (FPC)

Fish protein concentrate is any stable fish preparation, suitable for human consumption in which the protein is more concentrated than in the original fish (Windsor, 1977). Although the idea of producing FPC dates back to the days of Pompeii, (Windsor, 1977) the first modern attempt to produce the product on a commercial scale was made in the 1890s in Norway. In the 1930s many patents were granted in Germany for processes for manufacturing "albumen from fish" (Wallerstein and Pariser, 1978). Since then research into the production of FPC has been carried out in a number of countries including Canada, USA, Chile, Morocco, Sweden (Wellerstein and Pariser, 1978), Iceland (Hannesson, 1961), India (Ismail, *et al*, 1968), South Africa (Dreosti, 1961; 1972). Fish protein concentrate technology is one of the most documented subjects as reviewed by Tannenbaum, *et al* (1974); Pariser, *et al* (1978) and Sikorski and Naczk (1981).

There are basically two types of FPC. Type A which is a virtually odourless, tasteless powder with total fat content less than 1% and protein content above 75% (Windsor, 1977; Pariser and Wallerstein, 1980) and Type B which has a fishy odour and contains some fat.

2.2.3.1 FPC Type A (FPC A)

Several methods of FPC A manufacture have been developed and described usually involving one or more solvent extraction steps, acid or alkali treatments and other miscellaneous treatments (Pariser, 1971). However, all essentially involve the removal of most of the water and almost all the fat from the fish using chemical solvents. The solvents most successfully used are ethanol and propanol. Acetone (Fougere, 1961), 1,2-dichloroethane (Pariser, 1971), hexane (Hannesson, 1961), heptane and isobutyl alcohol (Dreosti, 1961) have also been used but with less success. In a typical FPC A plant clean, fresh mince is fed to a series of extractors (usually three) in which most of the water is removed and the fat content reduced to about 1%.

The FPC produced by extraction is a highly nutritious but bland product with no functional properties. Although FPC was meant to make a major contribution towards alleviating malnutrition in developing countries it never lived to its expectations (Pariser, *et al*, 1978; Wallerstein and Pariser, 1978; Pariser and Wallerstein, 1980). The product failed to find a market among the people for whom it was intended. Several reasons may account for the failure. For example, the product was developed in industrialised countries without due regard to the social, cultural and eating habits of the groups for which it was intended. In fact FPC A was produced to American, Canadian or European standards (hence odourless, colourless, tasteless) without realising that the majority of Asian and African populations would prefer a product with a fish smell and taste. Secondly FPC A production required massive inputs of capital and

high technology; resources which are scarce in developing countries. According to Wallerstein and Pariser (1978)

> *"... FPC technology was developed because the idea, the technical capacity and the will to create the product existed ... Technologists became so involved in the process (and its intermediate goals) that their work became an objective in itself, a technological 'Mt Everest' to be climbed because it was there ..."*

2.2.3.2 FPC Type B (FPC B)

As a result of failure of marketing FPC A attention was focused on FPC B which has a distinct fishy taste and smell and contains up to 10% fat. It is very similar to fish meal except that the raw material for FPC B has to be food grade and higher standards of hygiene and quality control are observed (Valand, 1979). FPC B is principally produced in Norway where it is reported that there is a growing interest in the product among commercial food companies and international food aid organisations (Wray, 1982). Norwegian FPC B is called Norse Fish Powder (NFP). There are three different types made from blue whiting, sprats or capelin.

Compared with FPC A production of NFP is a relatively simple process. Freshly caught whole fish (which must be less than 24 hours old from catching) is washed and heated for 25 minutes (Wray, 1982) after which it is pressed and centrifuged to remove part of the lipid. The resulting press cake has a low fat and high protein content. The press liquid contains oil and water-soluble proteins and vitamins. The oil is separated and the fat-free liquid is concentrated and added to the press cake. The cake is finally dried and milled into NFP.

In Norway NFP has successfully been incorporated into fish soups, fish crisps, noodles, fish balls, pizzas and fish sauces. However

only small amounts of NFP are used this way. The bulk of the product is used in relief and rehabilitation programmes in developing countries (Valand, 1979; Wray, 1982). In an extensive survey carried out by FAO in Africa, Asia, the Carribean and Latin America it was found that FPC B was generally acceptable in limited amounts in food-aid programmes in a number of countries (Valand, 1979; FAO, 1980). FAO (1980), however, recommended that

> *"... product development should be continued, both as regards modifications of the present product and of food products using FPC B as an ingredient ..."*

2.2.3.3 Modified FPC

In addition to FPC B increasing efforts have also been put into producing an FPC with functional properties. It has been realised that most processes for producing FPC A involve conditions which cause denaturation of many of the proteins from the native conformation to the form of random coil or aggregations (Sikorski and Naczk, 1981). In the final product a large proportion of hydrophobic groups is exposed to the solvent resulting in the proteins showing an extremely low water affinity. Consequently recent investigations have been centred around finding methods of improving the functional properties of FPC and extending its use in the food industry. Hyder (1972) for example, described a process for preparing FPC with rehydration and emulsifying properties. The process involved comminution of the fish with 5% (w/w) sodium chloride, adjusting the pH to 2.5 with 1N HCl, extraction of the lipids with a 1:1 mixture of 95% ethanol and hexane by refluxing at 70^{o}C for 30 minutes, removal of the solvents by filtration or centrifugation and finally drying the FPC below 70^{o}C in air or vacuum. The FPC produced had an amino acid profile similar to fish muscle, was bacteriologically sterile and had a water retention capacity at least twice that of the original fish muscle. The product also could

be used as binder protein in sausage-like products.

Boyarkina, *et al* (1981) described a process for manufacturing FPC suitable for use in meat emulsions. The product was reported to have a soluble protein content of 15% with all the essential amino acids, and was light in colour. It was free of fish flavour and had a swelling capacity of 500%. Soft sausages and frankfurters could be made using this FPC and costs were reduced without impairment of quality or nutritional value.

In Peru a food processor developed a "refined fish protein" (RFP) that was said to be suitable for inclusion in a wide range of processed food products including baking mixtures, cheeses, breakfast cereals, milk products, soups and plant protein products (Hannigan, 1982). RFP is made from the edible portions of various species of fish which are gently extracted using hexane and ethanol.

Although "modified FPC" may find increasing uses in various food processing industries it is only likely to be used in developed countries (Connell, 1982) and only in relatively small quantities. It is unlikely to replace FPC A as a "panacea for human nutrition". FPC B, however, may have a better chance of acceptance in developing countries.

2.2.4 Fish Protein Hydrolysates

For the purposes of this discussion the term "fish protein hydrolysates" covers those products derived from fish tissue by the action of enzymes, alkalis or acids either singly or in combination. Fish protein hydrolysates can be prepared as dry powders or liquors and are finding increasing use as animal or human food ingredients.

2.2.4.1 Enzyme hydrolysates

Fish hydrolysates may be produced by the action of the natural proteolytic enzymes of the fish (autolysis) (McBride *et al*, 1961) or the action of commercial enzymes deliberately introduced for the purpose (Mackie, 1982). Fish sauces, fish silage and other hydrolysed fish proteins are produced in this manner.

2.2.4.1.1 Fish sauce

Fish sauces for human consumption have been produced in South-East Asia for a long time. Fish sauce is known under different names in various countries of the region; for example nuoc-mam in Kampuchea (formerly Cambodia) and Vietnam; nam-pla in Thailand and Laos; patis in the Philippines; ketjap-ikan in Indonesia and ngapi, in Burma. Although there are many different types of fish sauce they are basically produced by the same principle; fish is preserved by salt and protein is hydrolysed by the endogenous enzymes of the fish (Van Veen, 1965; Saisithi *et al*, 1966). Fish sauces are used mainly as condiments for flavouring rice dishes. Amano (1961) gives a good account of the different fish sauces and their methods of manufacture. Nuoc-mam is one of the fish sauces which have been extensively studied.

Recent developments in fish sauce manufacture have included attempts to shorten the fermentation period from several months to a few weeks. The use of commercial proteolytic enzymes (Howard and Dougan, 1974; Beddows and Ardeshir, 1979a; Ooshiro *et al*, 1981), raising the temperature (Beddows and Ardeshir, 1979a) or adding acid (Beddows and Ardeshir, 1979b) have been investigated for this purpose.

Fish sauce contains a considerable amount of salt and concentrations between 25 and 32% NaCl have been reported (Chayovan *et al*, 1983).

However, there has been increasing concern about the connection between high dietary sodium and hypertension (Institute of Food Technologists, 1980). As a result some research has been directed at reducing the sodium content of fish sauce without affecting the sensory qualities of the product. Chayovan *et al* (1983) reported finding that a mixture of sodium chloride and potassium chloride (NaCl:KCl - 50:50) could be used as a substitute for NaCl without affecting the quality of the sauce.

2.2.4.1.2 Fish silage

Fish silage is presently solely used for supplementing animal feeds. Its production is similar to fish sauce production in that the natural proteolytic enzymes break down the fish tissue. The major difference is in the method of preservation. During fish sauce manufacture salt is added to prevent the growth of spoilage bacteria. However, during fish silage production low pH is used to preserve the silage. Fish silage production has been reviewed by Disney, Tatterson and Olley (1977) and Raa and Gildberg (1982).

The proximate composition of fish silage is very similar to that of the raw material from which it is made (Tatterson and Windsor, 1976; Disney, Tatterson and Olley, 1977) and a number of studies have been conducted on its nutritional value (Whittemore and Taylor, 1976; Disney, Tatterson and Olley, 1977; Kompiang, Arifudin and Raa, 1980; Raa and Gildberg, 1982; Hall, 1983). Fish silage can satisfactorily be used for feeding pigs particularly if it has a low lipid content (McBride, Idler and Macleod, 1961; Whittemore and Taylor, 1976). Some studies have shown, however, that some toxic substances may be formed in the silage during processing, probably as a result of interaction between oxidized lipids, amino acids and proteins (Hall, 1983).

2.2.4.1.3 Other enzyme hydrolysates

A more recent development in the preparation of fish protein hydrolysates is the employment of commercial enzymes to accelerate the hydrolysis of fish tissue. The raw material (which may be whole fish, fillets or fish offal) is comminuted, proteolytic enzymes added and the mixture digested at 40-60^{o}C for about 30-60 minutes. The suspension is sterilised and the enzymes inactivated by heating for 10 minutes at 100^{o}C. Bones and undigested material are removed by sieving after which the hydrolysate is concentrated or dried (Mackie, 1973; Ritchie and Mackie, 1982). Enzyme hydrolysates exhibit desirable functional properties such as solubility, unlike conventional fish meal and solvent-extracted FPC. One of the disadvantages of the enzyme hydrolysates, however, is the bitter flavour believed to be due to low molecular weight peptides produced during hydrolysis. Research has shown, however, that by keeping the duration of hydrolysis to a minimum (Mackie, 1973) and using enzymes with known specificity (Hill, 1965) the problem can be avoided. The applications of enzyme-produced protein hydrolysates and the mechanism of hydrolysis are reviewed by Mohr (1977) and Mackie (1982).

2.2.4.2 Alkaline Hydrolysates

Alkaline hydrolysis has not found wide application as a means of producing fish hydrolysates because the method results in the destruction of a number of amino acids. For example serine decomposes to glycine and alanine; threonine yields glycine, alanine and α-amino butyric acid; arginine gives rise to ornithine, citrulline and ammonia while cysteine and cystine yield alanine, hydrogen sulphide, ammonia and pyruvic acid (Hill, 1965). The resulting products have very unpleasant flavours (Prendergast, 1974). Not surprisingly other studies have demonstrated that alkali-treated proteins are perhaps of lower nutritional quality than untreated ones.

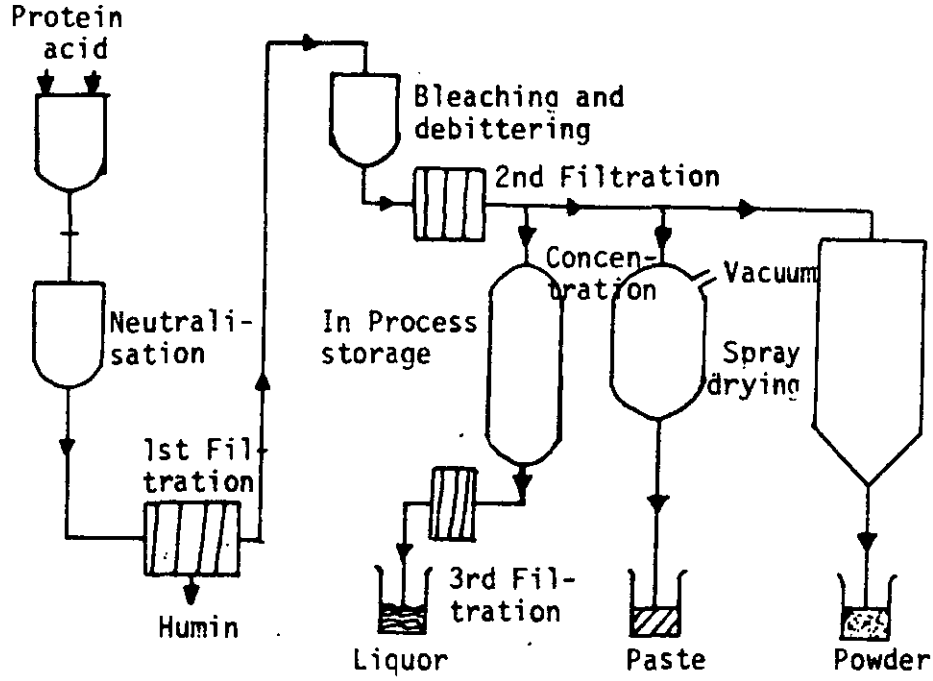
Robbins and Ballew (1982), for example, demonstrated that severe alkaline treatment of soy proteins reduced total sulphur amino acid bioavailability by 71% and histidine bioavailability by 80%. Patents, however, have been granted for processes reported to produce bland flavoured protein hydrolysates by alkali extraction (Gasser and Huster, 1973; Bosund, Bengtsson and Ostman, 1978). Alkaline hydrolysis is used for hydrolysing protein during the determination of tryptophan and also employed in the commercial production of the amino acid for nutritional purposes.

2.2.4.3 Acid Hydrolysates

Acid hydrolysis has classically been used for the hydrolysis of proteins, prior to amino acid analysis (Moore and Stein, 1963) and for commercial production of protein hydrolysates (Prendergast, 1974). For small quantities of proteins, particularly for amino acid analysis, the proteins are heated in sealed tubes *in vacuo* at 110^{o}C in 6N HCl for 24 hours. In large scale hydrolysis, however, the methods are more elaborate.

The main steps for the production of commercial hydrolysates are outlined in Figure 1 (Prendergast, 1974). Protein and hydrochloric acid are cooked in a reaction vessel at elevated temperature until hydrolysis is complete. The mixture is pumped into a second vessel where it is neutralised with sodium hydroxide or sodium carbonate to pH 4.8 to 6.0. During hydrolysis an insoluble black material called humin is formed. Humin which is thought to be the product of a condensation reaction between tryptophan and aldehydic components, is filtered off and discarded. The filtrate is then pumped into another vessel containing activated carbon which absorbs the bitter tasting amino acid phenylalanine, colouring substances and tyrosine. The decolourised liquor is filtered a second time and either stored, concentrated to a paste in vacuum pans or dried.

FIGURE 1: Commercial hydrolysate production[1]



(1) Prendergast (1974)

There are more elaborate versions where acid is recovered and reused in a batch or continuous process (Hampton, 1977).

In a Canadian patent Moshy and Whaley (1963) disclose a method for the production of a deodorized FPC from fish having a high oil content. The fish is comminuted with water to form a slurry which is acidified to "about the mean isoelectric point of the proteins contained therein" (pH 4.0-5.5). Antioxidant is then added to the slurry which is then passed through a 16-20 mesh screen to separate out bones and scales thus lowering the ash content to about 5% or less on a dry weight basis. The filtrate is heated to 80°C for about 20 minutes and then filtered to produce a semi-solid wet cake. The cake is mixed with a bland solvent such as isopropanol to extract the lipid. The solid is separated from the solvent by filtration. The residual solvent and fish odour and aroma are removed from the wet cake by a humidification process. The solid is subsequently vacuum dried.

In another patent (Roels, 1972; Roels, 1974) a protein hydrolysate is recovered from whole fish or fish products by acid hydrolysis. Finely comminuted fish is digested with hydrochloric acid or sulphuric acid at a pH of about 1 and a temperature of 120°C, and elevated pressure, for about 15 minutes. The mixture is cooled and centrifuged or filtered to remove insoluble material. The acidic filtrate is neutralised by the addition of a base or passed through a suitable ion exchange medium to the desired pH. The neutralised liquid is finally spray dried. The powder is pale yellow or brown in colour but may be further decolorized before drying by passing the aqueous protein solution through a charcoal adsorption or ion exchange column. An oil and mineral product may be obtained as by-products of the process.

Acid hydrolysis is a relatively cheap and rapid way of preparing hydrolysates which have good flavours (Prendergast, 1974). Although sulphuric acid and hydrochloric acid are equally good in effecting hydrolysis commercially, hydrochloric acid is preferred because after neutralisation the resulting salt (sodium or potassium chloride) need not be removed since a certain amount of salt is used in most foods.

2.3 Fish Chemistry

2.3.1 Fish Proteins

Excluding water protein is the main component of the flesh of most fish species. By proximate analysis total nitrogen (which is a measure of crude protein) ranges from 2.1 to 3.0% of the fresh muscle weight, with clupeids and elasmobranchs having the lower values (Dyer and Dingle, 1961). The percentage crude protein in whole fish is in general slightly lower (1-2%) than that of the fish flesh (Murray and Burt, 1969). Some of this nitrogen is classified as non-protein nitrogen (NPN) and varies from 9 to 18% of the total nitrogen in teleost fishes and from 33 to 39% in elasmobranchs (Simidu, 1961). The NPN fraction comprises: volatile bases (ammonia, mono-, di- and trimethyl-amines), trimethylammonium bases (trimethylamine oxide and betaines), guanidine derivatives (creatine and arginine), imidazole or glyoxaline derivatives (histidine, carnosine and anserine) and other varied nitrogenous compounds (urea, amino acids and purine derivatives). Trimethylamine oxide occurs only in marine fish and urea occurs only in elasmobranch fish. The NPN fraction is soluble in trichloroacetic acid (TCA) and the remaining TCA-insoluble fraction constitutes the "true-protein nitrogen" (TPN).

The skeletal musculature of fish is divided into a number of segments called myotomes which are separated from one another by thin sheets of connective tissue called mycommata (Dyer and Dingle, 1961). Each myotome is made up of a large number of parallel muscle fibres (myofibres) ending at the boundary myocommata. Under the microscope the muscle fibres appear to be striated similar to skeletal muscle fibres of higher animals. Each myofibre is composed of a number of myofibrils and these are in turn made up of filaments which are the contractile units. The spaces between the myofibrils are filled with an intracellular fluid, the sarcoplasm, which contains enzymes, nucleotides, amino acids and peptides. There are many detailed descriptions of the structure and functioning of skeletal muscle such as Huxley (1972) and Lawrie (1979).

There are three types of proteins in muscle classified according to their solubility (Jebsen, 1961; Suzuki, 1981):

1. Sarcoplasmic proteins, which are soluble in water or dilute salt solutions, include enzymes and myoglobin and constitute 26-30% of the protein in fish muscle, varying between species. It is generally a higher proportion in pelagic fish such as sardine and mackerel and lower in demersal fish like plaice and snapper (Suzuki, 1981).

2. Myofibrillar proteins which are soluble in salt solutions of high ionic strength (>0.5) are composed of tropomyosin, actin, myosin and actomyosin which give the muscle its power of contraction. These proteins account for 66-77% of the muscle proteins of fishes (compared with only 40% of muscle proteins in mammals).

3. Stroma proteins form the connective tissue of the muscle. They are insoluble even in strong salt solutions and amount only to

3% in teleost muscle but 10% in elasmobranchs (and 17% in mammals). Stroma proteins consist mainly of collagen which, on boiling with water, gives rise to gelatin, a mixture of peptides. The amount of stroma protein relates to tenderness.

Prolonged cold storage and dehydration of fish results in denaturation of myofibrillar proteins. The various biochemical changes that take place have been studied in great detail by a number of workers and have been reviewed by Connell (1968); Olley (1980); Sikorski (1980); Suzuki (1981). Various methods are used to measure protein denaturation and these include protein extractability (Dyer and Morton, 1956; Dyer *et al*, 1956; Cowie and Little, 1966), reactions between proteins and lipids (Buttkus, 1967), lipid oxidation (Kolodziejska and Sikorski, 1981) and sensory evaluation (Connell, 1957).

2.3.2 Fish Lipids

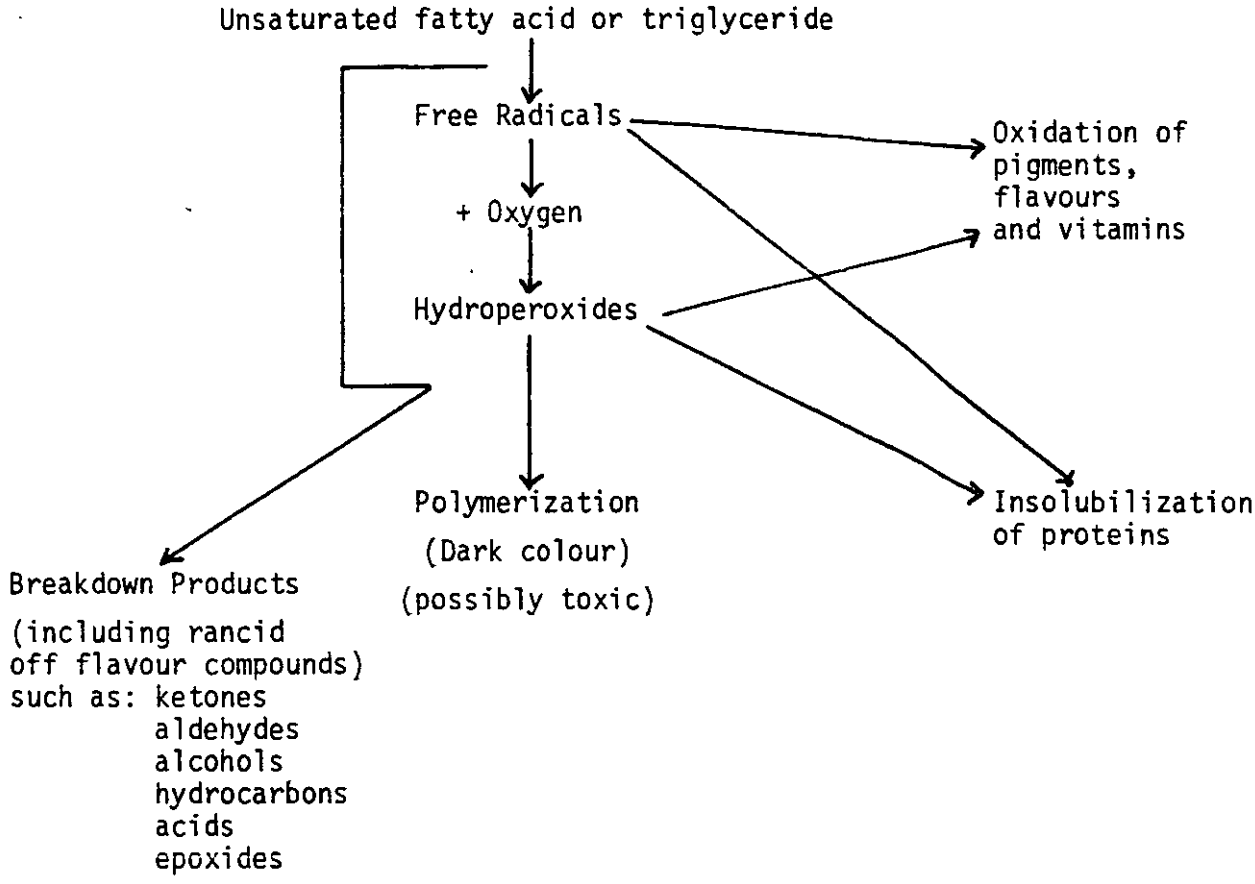
Fish lipids are complex mixtures of triglycerides, phospholipids, diacyl glyceryl ethers, hydrocarbons, sterols, carotenoids, vitamins and wax esters (Malins, 1967). The total lipid content of fish varies widely from species to species and, especially with fatty fish, from one individual of the same species to another. Typical values for lean fish such as cod, plaice and *Tilapia* are about 1% in the flesh, whole *Haplochromis* spp. about 6% and the fatty species such as mackerel and herring can vary between 2 and 20%. The variation is caused by a number of factors such as the season of the year when the fish are caught, the fishing ground, the age, sex, size and nutritional status of the fish (Stansby and Olcott, 1963; Love, 1970; Hardy and Keay, 1972). In all fish species, lipid content is subject to much wider variation than the water, protein or mineral content (Stansby and Olcott, 1963). There is an inverse relationship between lipid content and water content in fatty fish,

but the sum total of the two components is fairly constant at about 80% (Murray and Burt, 1969).

From a nutritional point of view fish lipids provide energy (MAFF, 1976), are a source of essential fatty acids (Aaes-Jørgensen, 1967), have a cholesterol-lowering effect in man and laboratory animals (hypocholesterolenic agents) and are a source of vitamins A, D and E (Ackman, 1974). On the other hand fish lipids present a problem from the processing and storage point of view.

Fish lipids contain a significant amount of highly unsaturated fatty acids which are highly susceptible to autoxidation resulting in the development of rancid off-flavours. The mechanism of lipid oxidation in foods is very complicated and although a considerable amount of research has been conducted on the subject (Labuza, 1971; Ackman, 1980; Hardy, 1980) some aspects of the process are not fully understood. However, it is believed that autoxidation involves three mechanisms: initiation, propagation and termination. The initiation step involves the production of free radicals. The primary initiation reaction involves oxygen, an initiator such as ultraviolet light and a "sensitizer" such as myoglobin and haemoglobin (Tappel, 1955), heavy metals and their salts. Free radicals are highly reactive so, in the presence of oxygen, they readily form peroxides or hydroperoxides (Figure 2). Hydroperoxides are unstable and can break down to produce more free radicals, thus initiating a chain reaction. This is the propagation stage. The break down of the hydroperoxides into products such as aldehydes and ketones is the principal cause of the rancid off-flavours whilst reactions, particularly with proteins reduce the shelf life and nutritional value of the fish products. The propagation process is terminated when radicals interact with each other. The theory and mechanisms of lipid autoxidation are comprehensively reviewed by Labuza (1971) and Hardy (1980).

FIGURE 2: Overall Mechanism of Lipid Oxidation[1]



(1) Labuza (1971)

Lipid oxidation can be controlled or prevented generally in four ways; storage at low temperature, prevention of the initiation reaction, exclusion of oxygen and removal of free radicals. The most common form of control is storage at low temperatures for every $10^{o}C$ reduction the oxidation rate falls by a factor of 2 to 3 (Hardy, 1980). However, during prolonged cold storage the fish is susceptible to dehydration which accelerates oxidation. The effect can be minimized by glazing or overwrapping with waxed paper, aluminium foil or plastic films; and the best effect can be achieved by freezing in water. The water prevents dehydration and also tends to remove occluded air between the fish and also acts as a barrier to oxygen. Vacuum packaging where fish are sealed in flexible bags made of materials that have a low oxygen permeability has been successfully used as a method of control (Shevchenko and Antonov, 1976). Chemical means may be used to reduce or prevent initiation reactions and the removal or lowering of the concentration of free radicals. Compounds such as the antioxidants Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA), ascorbic acid, riboflavin derivatives (Hardy, 1980) have been found to be effective provided they are applied to the site of reaction. This can be done effectively with minced fish because intimate mixing is possible (Moledina *et al*, 1977). Anti-oxidants have been used to minimize lipid oxidation during the production of fish protein hydrolysates from fatty fish (Ritchie and Mackie, 1982).

2.3.3 Water and Other Chemical Components

Water is the main constituent of fish flesh, accounting for about 80% of the weight of a fresh lean fish fillet. Fatty fish contain less water, averaging around 70% (Murray and Burt, 1969) (see Section 2.3.2). Some of the water is known as "free" and the rest "bound". "Bound water" in fresh fish muscle is tightly bound to the

proteins in the structure and its loss during prolonged chilled or frozen storage is associated with reduced quality of the fish flesh.

The presence of a relatively large proportion of water makes fish very susceptible to microbial and chemical deterioration since it has been demonstrated that bacteria and moulds thrive at high concentrations of water. Spoilage can be delayed or prevented by drying, salting or employing other means which reduce the water activity (a_w) of the fish (Troller and Christian, 1978). The preservative effect of a_w is discussed in more detail in Section 2.6.

Approximately 1% of the fillet of lean fish is comprised of "ash" a general term used to include all non-volatile inorganic constituents (minerals) of the fish. Most analytical work on the mineral content of fish has been done on fillets therefore few figures are available for whole fish (Causeret, 1962). Table 2asummarises the mineral content of some fish species. Whole fish has a much higher phosphorus and calcium content than the meat alone. The richness in the two elements is due to the skeleton and scales both of which contain tri-calcium phosphate (apatite) and calcium carbonate. In small sized species the scales particularly contain a relatively considerable amount of calcium. Da Costa and Stern (1956), for example, found that Portuguese sardines preserved in oil contain 580, 490 or 54 mg of calcium per 100g respectively, depending on whether they are intact, boneless or both skinless and boneless. The corresponding amounts of phosphorus are 624, 545 and 320 mg per 100g.

Fish also contain other chemical components such as the B vitamins (Murray and Burt, 1969), organic acids which arise from the tricarboxylic acid cycle, nucleotides and volatile compounds arising

TABLE 2a Ash and Mineral Content of Some Fish and Fish Products

Species	Portion Analysed	% Ash	mg per 100g Na	K	Ca	Mg	P	Fe	Cu	Zn	S	Cl	I
Cod(1)	Fillet	1.2	77	320	16	23	170	0.3	0.06	0.4	200	110	0.15
Herring(1)	Fillet	1	67	340	33	29	210	0.8	0.12	0.5	190	76	0.05
Salmon(1)	Fillet	1	98	310	27	26	280	0.7	0.2	0.8	170	59	-
Portuguese sardines (2)	Whole fish (canned)	-	-	-	580	-	624	-	-	-	-	-	-
Various West African species (2)	Whole fish	-	-	-	888	-	350	-	-	-	-	-	-
White fish meal (3)	-	20.0	-	900	8000	-	4800	30	0.7	1.0	-	-	-
Peruvian anchovy meal (3)	-	15.4	870	650	3950	250	2600	24.6	1.1	11	-	-	-
Haplochromis spp (4)	Whole fish	5.6	-	-	480	-	-	-	-	-	-	-	-
	Head	8.7	-	-	747	-	-	-	-	-	-	-	-
	Gut	1.6	-	-	18	-	-	-	-	-	-	-	-
	Fillet	1.1	-	-	23	-	-	-	-	-	-	-	-

(1) Paul and Southgate (1978); (2) Causeret (1962); (3) Windsor and Barlow (1981); (4) Ssali (1981).

from heat processing of fish and oxidation of oil during storage and heating. The minor organic and inorganic components of fish are reviewed by Ikeda (1980).

2.4 Fish Microbiology

The microbiology of fish and fishery products is particularly important for two reasons; the predominant role of bacteria in fish spoilage and the public health significance of some micro-organisms associated with fish.

When fish is newly caught the flesh is sterile. However, the skin, gills and intestines may carry heavy loads of bacteria and estimates of 10^2-10^6 bacteria/cm^3 skin surface and similar or higher counts for the gills have been quoted (Liston, 1980). Qualitatively and quantitatively the microflora is a function of the environment. Fish caught from tropical and subtropical waters have been found to show greater counts than those caught in temperate waters (Kriss, 1971). Research has also shown that fish caught from temperate waters are dominated by large numbers of psychrophilic Gram-negative genera (*Pseudomonas, Alteromonas, Moraxella, Acinetobacter, Flavobacterium/Cytophaga* and *Vibrio)* while fish from warmer waters are predominated by the more mesophilic Gram-positive genera such as the *Micrococcus, coryneforms* and *Bacillus* (Shewan, 1977). Spoilage bacteria mainly belong to the *Pseudomonas* and *Alteromonas* groups (Herbert *et al*, 1971).

In addition to spoilage microorganisms fish are occasionally associated with certain microorganisms of public health significance. There are two species of pathogenic bacteria which are reported to occur naturally on fish. These are *Clostridium botulinum* type E and *Vibrio parahaemolyticus* (Hobbs and Hodgkiss, 1982). *C. botulinum* type E causes botulism and has been known to occur in marine and lake

sediments and in fish intestines. It is unusual in being able to grow and produce toxin at low temperatures (3-5^{o}C). It is not normally present in freshly caught fish in large numbers and if growth and production of toxin occur in fishery products it is usually a result of mishandling and processing at some stage. Other potentially pathogenic bacteria occasionally associated with fish include *C. perfringens, Staphylococcus, Erysipelothrix, Edwardsiella, Salmonella, Shigella, Franciscella, Vibrio cholerae* and other vibrios (Liston, 1980). All these organisms are most probably introduced by cross-contamination from terrestrial sources.

Salting and drying are two of the oldest preservative methods still widely used in many parts of the world, particularly in the tropics. Preservation is effected through the lowering of water activity, although NaCl itself in higher concentrations may be lethal for some bacteria and yeasts due to osmotic effects (Liston, 1980). Since most bacteria require a_w values above 0.90 for growth these organisms play no role in the spoilage of dried foods. Scott (1957) has related a_w levels to the probability of spoilage as follows. At a_w values between 0.80 and 0.85 spoilage caused by fungi occurs within 1-2 weeks. At an a_w value of 0.75 spoilage is delayed with fewer types of organisms in those products that spoil. At an a_w of 0.70 spoilage is greatly delayed and may not occur during prolonged storage. At an a_w of 0.65 very few organisms are known to grow, and spoilage is very unlikely to occur even up to 2 years.

The lowering of pH is another effective method of inhibiting microbial growth and fish silage production is based on this principle. Most microorganisms do not grow below pH 4.0.

2.5 Acid Hydrolysis of Fish Proteins

The literature concerning acid hydrolysis of fish proteins is apparently scanty. Almost all the published material on the subject describes processes for production of fish hydrolysates without discussing the mechanism and kinetics of the process (see Section 2.2.4.3). A considerable amount of information, however, can be derived from amino acid sequence studies. Much of the earlier literature on hydrolysis can be obtained from reviews such as that of Synge (1943) and the later aspects of hydrolysis are reviewed by Sanger (1952) and Hill (1965), among others.

A number of physical and chemical methods are used to follow the course of protein hydrolysis. Each method depends upon the measurement of the disappearance of protein or the appearance of hydrolytic products during the course of the reaction, using techniques such as spectrophotometry and turbidimetry, titrimetry and colorimetry (Colowick and Kaplan, 1957). The choice of a suitable method depends upon specific factors which are unique to each experimental situation and information required.

Data from partial hydrolysis studies has shown that each protein with its specific and unique sequence of amino acids presents a wide variety of peptide bonds. Thus partial hydrolysis of a protein involves cleavage of a variety of bonds which gives rise to a complex mixture of products. Gordon *et al*,(1941) studied the partial hydrolysis of wool, edestin and gelatin using excess 10N HCl at 37^{o}C for several days. Throughout the course of hydrolysis aliquots of the reaction mixtures were analysed for total free amino acids, total number of peptides, average length of peptide chains and the rates of liberation of cysteine, ammonia (amide nitrogen) and the amino groups of hydroxyamino acids. The results showed that free amino acids were liberated right from the start of hydrolysis, and after about a week 37% of total residues were free amino acids. The

remainder of the residues were mostly dipeptides. Ammonia was liberated rapidly and was almost completely released within 48 hours. Cysteine was liberated at a rate not significantly different from the average rate of liberation of other amino acids. The bonds involving the amino-groups of serine and threonine were more labile to acid hydrolysis than bonds formed by other amino acids. This finding was studied in more detail and confirmed by other workers (Hill, 1965). These results indicated that the course of partial acid hydrolysis is not a random process but that it exhibits a certain degree of specificity.

The rapid and complete release of ammonia during the early stages of hydrolysis is the result of cleavage of the amide groups of glutamine and asparagine (Chibnall *et al*, 1958). Under conditions of complete hydrolysis the yield of ammonia is about 10% higher than that expected (Gordon *et al*, 1941) and this is attributed to the liberation of the α-amino nitrogen of serine and threonine as ammonia (Smyth *et al*, 1962). The resistance of dipeptides to acid hydrolysis seems to be due to the inhibitory effects of the positively charged ammonium group adjacent to the susceptible bond. The positive charge tends to repel the H^+ ions and thus the dipeptide bond is more resistant to hydrolysis than a similar type of bond at a greater distance from the amino terminus. Bonds which resist hydrolysis need not possess a greater thermodynamic stability than bonds which are hydrolysed. Once a dipeptide bond is formed it is more kinetically stable than an analogous bond in a polypeptide.

Although the studies of Gordon *et al* (1941) did not reveal which peptide bonds were most resistant to acid hydrolysis later studies by Synge (1944) and Christensen (1943; 1944) showed that dipeptides containing amino terminal valine and leucine (Hill, 1965) were not cleaved completely on prolonged hydrolysis. Synge attributed the stability of these peptides to the steric limitation imposed by the isopropyl and isobutyl side chains of valine and leucine on the approach of H^+ ions to the peptide bond.

The bonds formed by aspartyl residues are very susceptible to hydrolysis in dilute acid. Under appropriate conditions, therefore, dilute acids can be used to specifically break those bonds formed by aspartic acid thus degrading high molecular weight polypeptides into smaller peptides. Sanger (1949) explained the lability of the aspartyl bond by suggesting that the negatively charged carboxyl groups of aspartic acid would attract H^+ ions in dilute solutions and thereby increase the lability of the neighbouring peptide bonds. However certain linkages such as that of valylaspartic acid may be sufficiently resistant to prevent complete liberation of aspartic acid (Hill, 1965).

Under special conditions partial acid hydrolysis can be used to obtain peptides which contain disulphide linkages in the form in which they occur in the intact protein. This involves preventing disulphide interchange reactions which allow random cleavage and reformation among the disulphide bonds. This can be achieved by using mixtures of equal amount of 20N sulphuric acid and glacial acetic acid at 37°C in the presence of thiol compounds (Ryle *et al*, 1955). It is significant to note that partial acid hydrolysis of proteins under conditions favouring the disulphide interchange reaction forms the basis of a method for determination of cysteine and cystine content of proteins (Glazer and Smith 1961). Other side reactions which occur between and within peptides during partial hydrolysis have been reviewed by Hill (1965).

Complete acid hydrolysis of proteins is the basis for amino acid analysis. Total acid hydrolysis can be achieved by treatment of protein with 6N HCl at 110°C *in vacuo* for 24 hours (Moore and Stein, 1963). Lucas and Sotelo (1982), however, claim that similar results can be obtained by using 6N HCl at 145 °C *in vacuo* for 4 hours only. Generally the composition of hydrolysates prepared in the manner described reflects the amounts of amino acids in the protein before hydrolysis. The exceptions are cases where certain amino acids are

destroyed by acid. Glutamine and asparagine are destroyed extensively in acid hydrolysis. Glutamine is converted quantitatively to glutamic acid and asparagine to aspartic acid with the concomitant release of stoichiometric amounts of ammonia as a result of hydrolysis of the amide groups. The ammonia which is produced within the first few minutes of hydrolysis is derived solely from these amides (Hill, 1965). Tryptophan in proteins is also destroyed by acid although considerable amounts often can be detected if oxygen and reducing substances are strictly excluded. Olcott and Fraenkel-Conrat (1947) showed that tryptophan is not destroyed extensively when heated at 100°-125°C in 6-7N HCl (or sulphuric acid) *in vacuo*. When tryptophan is heated under similar conditions in the presence of air, zinc, serine, pyruvic acid, cysteine (or cystine) and a number of other substances, large losses are observed with the formation of humin. Tryptophan is therefore best estimated by either direct analysis of the intact protein (Bencze and Schmid, 1957) or analysis of enzymatic hydrolysates (Tower *et al*, 1962) or by alkaline hydrolysis (Miller, 1967).

Other amino acids are destroyed to a lesser extent as shown by decreasing yields as a function of time of hydrolysis. Rees (1946) showed that 5-10% of the serine and threonine are destroyed, the exact amount depending on the time of hydrolysis. Other amino acids such as cysteine, aspartic acid, glutamic acid, lysine, arginine, tyrosine and proline have also been reported to be destroyed (Hill, 1965). Low yields of cysteine and cystine are often observed in acid hydrolysates particularly when carbohydrates are present. More accurate results can be obtained by determining these amino acids as cysteic acid (Moore, 1963). Methionine may be determined as methionine sulphoxide after alkylation of the thio-ether sulphur with iodoacetate and determination of the sulphoxide as the sulphone after oxidation with performic acid (Moore and Stein, 1963).

The stability of peptide bonds formed by valine, isoleucine and leucine often leads to low yields of these amino acids in total hydrolysates.

Usually maximal yields are obtained after hydrolysis for 70 hours (Noltmann *et al*, 1962).

2.6 Dehydration of Fish Hydrolysates

The removal of water from food material is one of the oldest methods of food preservation. The methods of removing water range from the simplest sun drying to the relatively sophisticated freeze drying. Desrosier and Desrosier (1977) restrict the term "food drying" to the natural sun drying, where there is limited control over the process, and the term "food dehydration" to describe the process of artificial drying where there is complete control over climatic conditions within a chamber or microenvironment control. Brennan *et al* (1979), however, use the terms interchangeably to describe "the unit of operation in which nearly all the water normally present in a food stuff is removed by evaporation or sublimation, as a result of the application of heat under controlled conditions". For the purpose of this discussion only the drying of fish hydrolysates by artificial means will be considered.

2.6.1 General Theory of Dehydration

Foodstuffs may be dried in various media such as air, superheated steam, vacuum, inert gas and by direct application of heat. The most widely used medium, however, is air because it is abundant, convenient and overheating of the food can be controlled. Air conveys heat to the food, causing the water to vapourize, and subsequently removes the water vapour from the dehydrating food (Desrosier and Desrosier, 1977). Food dehydration is based on the principles of heat- and mass-transfer and these are well discussed in a number of references such as Van Arsdel (1973); Karel (1975); Lund (1975) and Brennan *et al* (1979).

The overall effect of dehydration is to lower the water activity (a_w) of the food. Water activity is defined by the equation

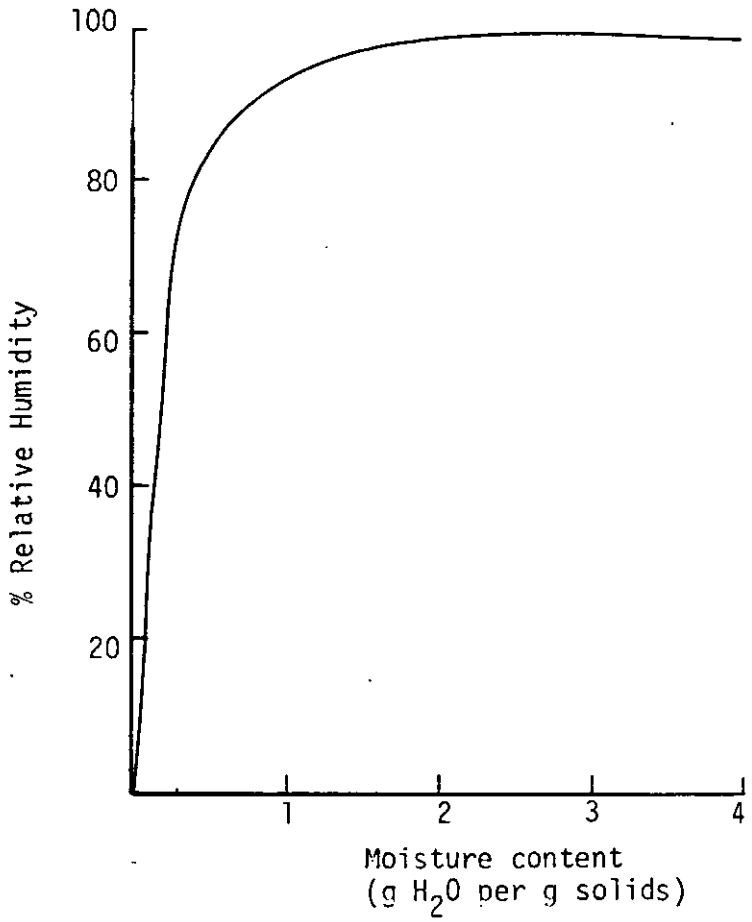
$$a_w = \frac{P}{P^o} = \frac{E.R.H.}{100}$$

where a_w = water activity
P = partial pressure of water in food
P^o = vapour pressure of pure water at a given temperature
E.R.H. = equilibrium relative humidity (%)

The water activity of most tissue foods is very close to that of pure water since their moisture contents range from 60 to 95% on wet basis (Figure 3). This makes them most susceptible to microbiological attack. Below about 50% moisture content water activity falls rapidly due to various physical and chemical factors. In this region water activity relationships can be represented by Figure 4 (Labuza, 1968; Labuza, Tannenbaum and Karel, 1970). The curve is called the water sorption isotherm and can be obtained by methods described by Taylor (1961). The isotherm can be divided into a number of sections depending on the state of the water present. Region A represents a monolayer region which contains 5-10% water. Region B corresponds to adsorption of additional layers of water over the monolayer and region C is a capillary condensation region in which water condenses in the porous structure of the food and acts as a solvent for various solutes. Most dehydrated foods fall in the lower portion of the curve including the monolayer and multilayer regions.

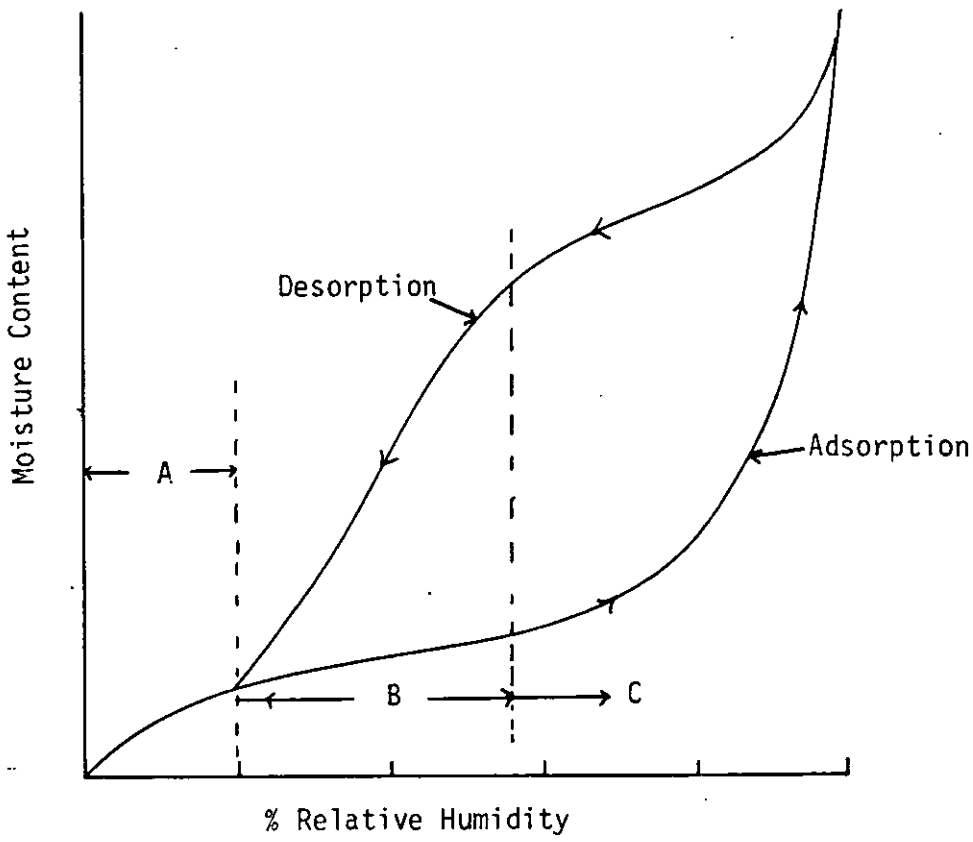
Figure 5 is a graphic illustration of the effect of water activity on the stability of foods. It can be seen that at high water activity levels bacterial spoilage plays the dominant role. As a_w decreases yeast and mould growth replace bacteria as spoilage agents. The water

FIGURE 3: Humidity-moisture relationship for foods(1)



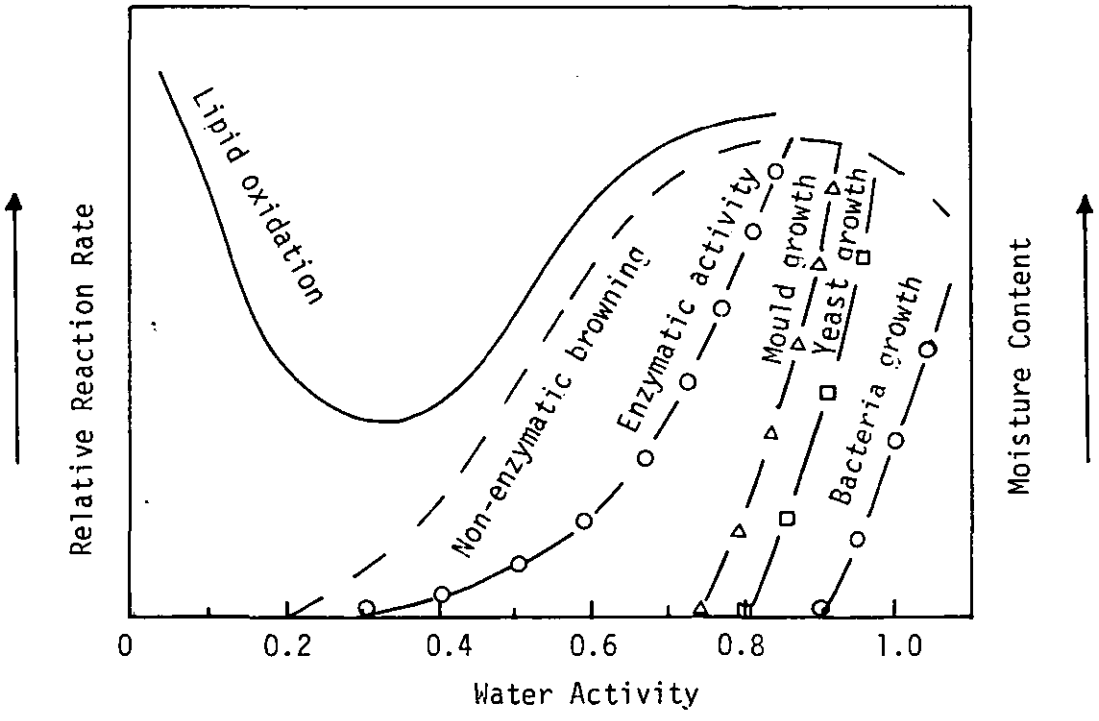
(1) Labuza (1968)

FIGURE 4: General Sorption Isotherm[1]



(1) Labuza (1968)

FIGURE 5:- Stability of foods as a function of water activity(1)



(1) Labuza (1971)

relations of microorganisms are discussed in more detail by Scott (1957).

At low water activity levels, in the absence of microbial spoilage, foods become more susceptible to chemical deterioration. At very low a_w levels and in the presence of oxygen fishery products in particular become highly susceptible to oxidative rancidity. This is due to their relatively high content of unsaturated fatty acids (see Section 2.3.2). The properties of water that are pertinent to lipid oxidation are:

1. Water acts as a solvent in which reactants are able to dissolve, be transported and react.

2. Water can interact either chemically or by hydrogen bonding with other species.

A more detailed discussion of the two roles played by water in lipid oxidation is given by Labuza, Tannenbaum and Karel (1970).

Research has shown that there is an optimum moisture level for dehydrated foods at which the rate of rancidity development is reduced, if not prevented (Labuza, 1971). It has also been shown that the basic protective function that water exhibits when the moisture content increases from the absolute dry state (Figure 4) could be accounted for by two factors:

1. Water interacted with metal catalysts rendering them less effective in lipid oxidation.

2. Water hydrogen bonded with hydroperoxides so that they are no longer available for decomposition through initiation reactions (see Section 2.3.2).

The two factors slow the rate of initiation. Another theory of how water interacts to slow lipid oxidation was proposed by Salwin (1959), who suggested that water is attached to sites on the food surface preventing oxygen from reacting with unsaturated fatty acids. The suggestion was based on the finding that there is an optimum moisture content, close to the monolayer coverage of water on the food. Below this level oxidation occurred and above it other chemical reactions occurred. Ideally foods should be dehydrated to a water activity level where physical, chemical or microbial deterioration are minimal.

2.6.2 Dehydration Methods and Equipment

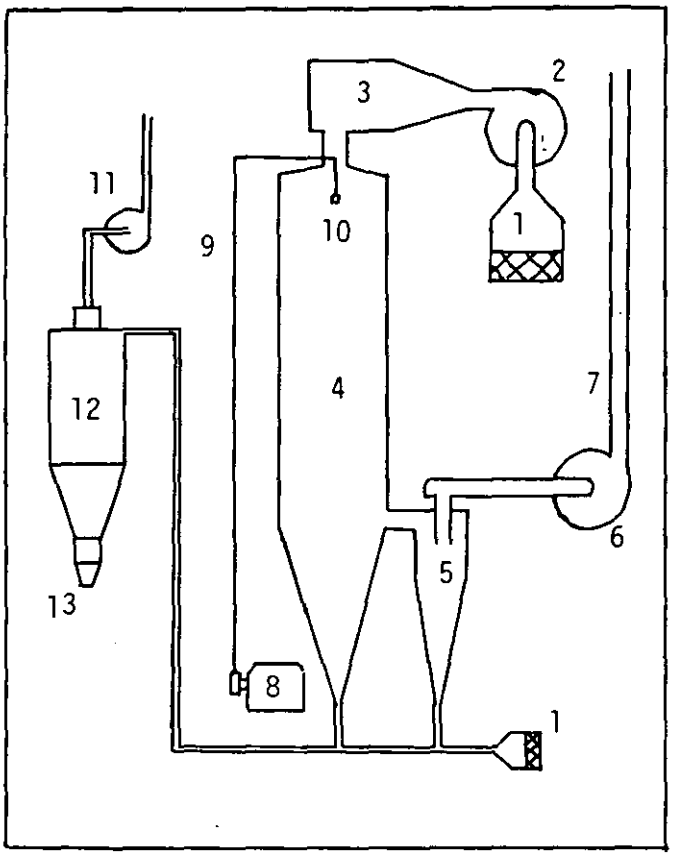
There are several possible methods and equipment for dehydration of fish hydrolysates. However, the nature of the product limits the choice to only a few. Fish hydrolysates are predominantly composed of protein which can easily be denatured on prolonged subjection to high temperatures. The presence of unsaturated fatty acids also warrants that the hydrolysates be dried as quickly as possible to minimize possible lipid oxidation. In addition a large amount of water has got to be removed from the hydrolysate in a relatively short time. When all the different permutations are taken into account only spray drying and drum drying remain as the most appropriate methods for drying fish hydrolysates.

2.6.2.1 Spray drying

Spray drying is used extensively in the food industry to dry solutions and slurries. The process essentially consists of four elements (Figure 6):

A. an air heating and circulating system, (1-3)
B. a spray-forming device (10)
C. a drying chamber (4)
D. a product recovery system (5, 11-13).

FIGURE 6: Outline of a spray drying process(1)



1. Air filter
2. Inlet air fan
3. Gas burner
4. Drying chamber
5. Cyclone
6. Exhaust air fan
7. Exhaust stack
8. High pressure pump
9. Feed line
10. Nozzle atomiser
11. Powder conveying fan
12. Powder silo
13. Powder valve

(1) Spray Processes Ltd.

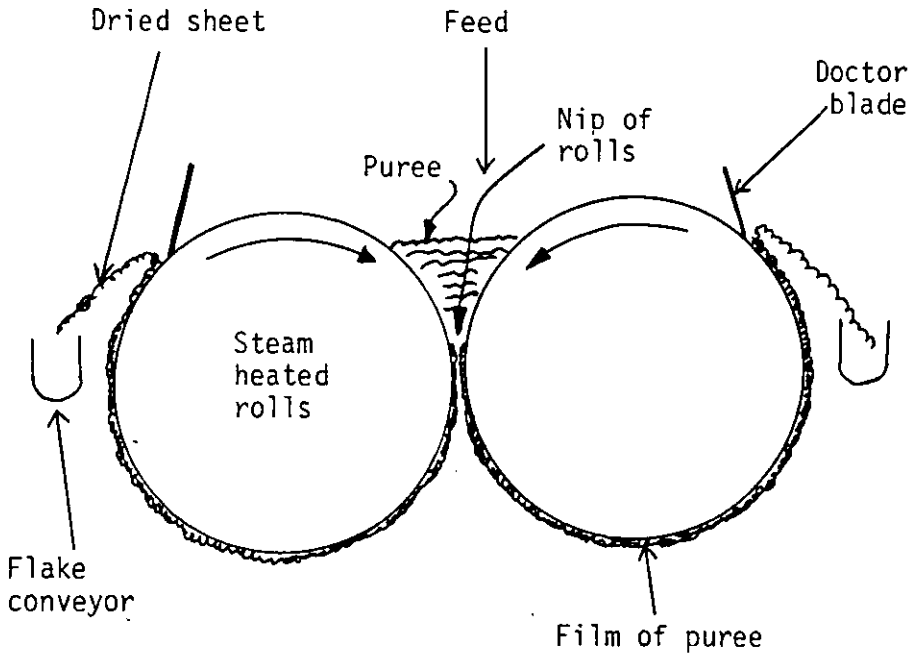
The food material is atomized into the drying chamber where it is put into direct contact with a stream of heated air and rapidly dried into a powder. The dry particles, suspended in the air stream, flow into separation equipment where they are removed from the air, collected and packaged. Each of the steps described is actually a complicated and delicately balanced engineering operation and there are a number of references on the operation and applications of spray driers such as Seltzer and Settelmeyer (1949) and Masters (1972).

A wide range of products have been successfully spray dried including fish concentrates, coffee, egg, milk, blood, vegetable protein (Karel, 1975). The process is the preferred choice in many instances due to the high quality of the product. The very short drying times (1-10 seconds) and the relatively low product temperatures make the methods particularly suitable for drying heat sensitive foods (Brown *et al*, 1973; Brennan *et al*, 1979).

2.6.2.2 Drum drying

Drum dryers consist of one or more drums which are hollow and heated internally usually by steam (Figure 7). The drums are mounted to rotate about the symmetrical axis and customarily driven with a variable speed drive. A thin uniform layer of the material to be dried is applied to the revolving drum. Heat is transferred by conduction through the metal wall to the thin layer of material from which water is vapourized during a partial revolution of a drum. The dried material is scraped from the rotating drum surface by a stationary knife, or doctor blade, which is depressed against the drum at the appropriate location. There are various types of dryers the details of which are discussed in references such as Spadaro, Wadsworth and Vix (1966); Brown *et al* (1973) and Karel (1975). Drum dryers have been used to dry cereals, potatoes, fish meal, soup stocks and milk, among other things. Although drum dryers,

FIGURE 7: Double drum dryer[1]



(1) Spadaro, Wadsworth and Vix (1966)

particularly operating at atmospheric conditions, have low operating costs compared with spray dryers, for example, their product is of a lower quality than that of spray dryers (Spadaro, Wadsworth and Vix, 1966).

2.7 AIMS OF THE PRESENT WORK

There have been several attempts to develop alternative processing methods for effective utilization of the underutilized stocks of *Haplochromis* in Lake Victoria. Most of these attempts have proved unsatisfactory (Dhatemwa, 1982). Although a plant has been built to reduce the fish to fish meal for animal feeds in the Tanzanian sector of Lake Victoria (CIFA, 1982), and there are plans to build a similar factory in Uganda (Uganda Ministry of Animal Industry, 1983), it would be more beneficial to use the resource for direct human consumption. Dhatemwa, (1981) showed that salted fish cakes could be produced from deboned *Haplochromis*. However the process resulted in low yields and some protein losses. The acceptability of the products in the Ugandan market was also unknown.

This study aimed at the following:

A. To utilize whole fish to produce a powder with which Ugandan consumers could identify.

B. To produce a relatively cheap powder, fit for human consumption and with a high nutritional value.

C. To produce a product which is microbiologically and chemically stable for long periods of time at tropical ambient conditions.

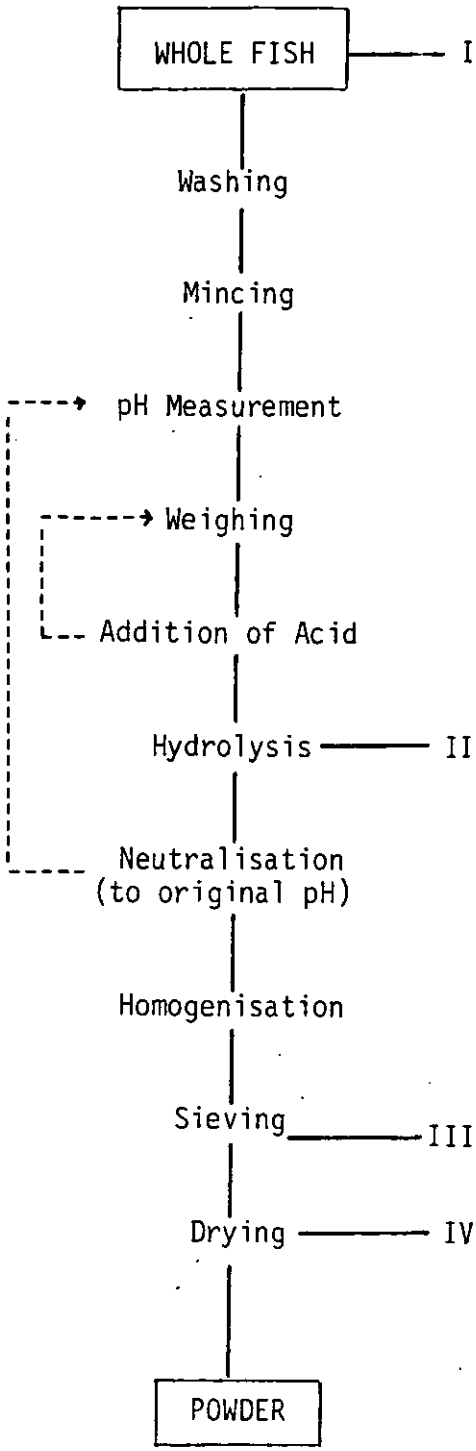
2.8 EXPERIMENTAL RATIONALE

Figure 8 outlines the experimental procedure for attaining the above mentioned objectives. The use of acid facilitated the utilisation of whole fish (including bones, skin and scales), minimising wastage. Acid also offered a relatively cheap "hydrolysing agent" which would cause the least undesirable effects to proteins, compared with alkali

and enzymes. Hydrochloric acid was chosen because after neutralisation common salt (NaCl) would be produced as a by-product and its removal from the product would not be necessary since it is usually added to food during cooking. The process was also designed to involve the least number of necessary, but simple, operations, giving rise to a powdered product similar to what Ugandan consumers were already accustomed to.

The main unknown parameters envisaged were: the effect of using cooked or uncooked fish as raw material, optimum acid concentration and reaction temperature, minimum duration of hydrolysis, pH changes during hydrolysis and the overall effect of the process on the nutritionally important constituents of the fish and some organoleptic and storage properties of the product. Only those aspects considered to be most relevant in assessing the proposed technology were considered.

FIGURE 8: OUTLINE OF THE EXPERIMENTAL PROCEDURE FOR PRODUCTION OF FISH POWDER BY ACID HYDROLYSIS



Notes to Figure 8:

I: Variation was in using cooked or uncooked fish as raw material (See 3.3.1).

II: Variations were in using different acid concentrations and different temperatures (See 3.3.2.1 and 3.3.2.2).

III: Variation was in bleaching (or not) of the hydrolysate (see 3.3.5).

IV: Variation was in drum drying or spray drying (See 3.3.6).

3. EXPERIMENTAL

3.1 MATERIALS

3.1.1 Fish

Haplochromis were caught by bottom trawling (depth 25-45 metres) from Kitubulu-Nsazi, an inshore fishing ground of Lake Victoria, Uganda. The batches used were caught during October 1982 and June 1983.

The fish were frozen, packed in an insulated box and flown to the United Kingdom. They were kept in a deep freezer (at -20°C) until processed.

3.1.2 Chemicals

Unless otherwise stated the chemicals used were of Analytical Grade. Single distilled water was used throughout.

Unless otherwise stated all determinations were done in duplicate.

3.2 METHODS

3.2.1 Raw Material Evaluation

3.2.1.1 Total nitrogen was determined by Kjeldahl using the Tecator Kjeltec System and the results expressed as percentage of the sample.

3.2.1.2 Trichloroacetic acid (TCA) - insoluble nitrogen

Approximately 2g of minced whole fish was ground with 10% TCA (10 ml) and filtered. The residue was washed with three 10 ml aliquots of

TCA and after draining the nitrogen content of the residue was determined as in 3.2.1.1. The results were expressed as percentage of the sample.

TCA-soluble nitrogen was calculated by difference between total nitrogen and TCA-insoluble nitrogen and expressed as percentage of total nitrogen.

3.2.1.3 Amino acids

Samples were hydrolysed with 6N HCl according to the method of Moore and Stein (1963) and analysis was performed on a Chromakon 500 amino acid analyser connected to an integrator for computation of the results. Proline and tryptophan were not determined. Hydroxyproline was determined according to the method of Stegemann and Stalder (1967).

3.2.1.4 Total lipid

This was determined by a modified Bligh and Dyer (1959) method. Approximately 10g of the mince was accurately weighed in a plastic container (chloroform and methanol resistant). Water (8 ml), methanol (40 ml) and chloroform (20 ml) were added in that order. The mixture was homogenised at constant speed for 60 seconds using a Silverson Laboratory homogeniser, Model L2R fitted with a tubular (17 mm) head. Chloroform (20 ml) was added and the mixture homogenised for 30 seconds. Finally water (20 ml) was added to the mixture and homogenised for a further 30 seconds. The homogenate was transferred to centrifuge tubes and centrifuged at 2000 rpm for 20 minutes. The top aqueous layer was discarded. The total volume of the (bottom) chloroform layer was measured and 20 ml pipetted into a dry tared beaker. The solvent was evaporated off over a steam bath and finally in an oven at 105°C for 15 minutes. After cooling in a desiccator the beaker was weighed and the lipid content determined by difference.

3.2.1.5 Total ash

Was determined by igniting the sample at 500°C in a muffle furnace (Pearson, 1981).

3.2.1.6 Sodium chloride

Was determined by the titrimetric method of Mohr (Pearson, 1981).

3.2.1.7 Moisture

Was determined by drying the samples in an air oven at 105°C to constant weight (European Communities Commission Directorate-General for Agriculture, 1979).

3.2.1.8 Standard plate count

Aerobic mesophilic microorganisms were estimated in the raw material only by the Pour Plate method in Agar and incubating the samples at 30 ± 1°C for 48 hours (ICMSF, 1978).

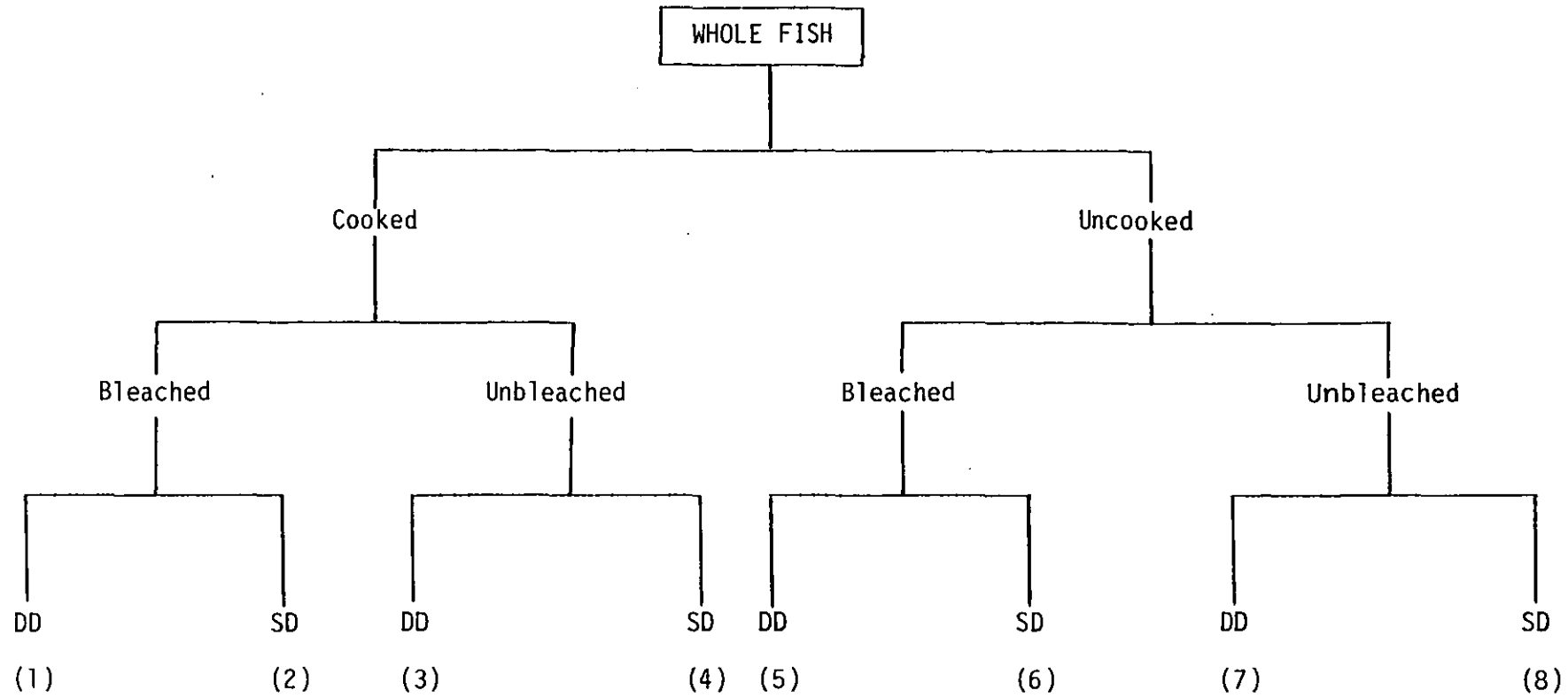
3.3 EXPERIMENTAL PROCEDURE

The sequence of the individual operations in the experimental procedure are outlined in Figure 8. Figures 9 and 10 show the overall experimental scheme detailing the variable parameters and the envisaged final products. The apparatus set up for hydrolysis is shown in Figure 11.

3.3.1 Fish Preparation

Prior to processing the fish were thawed at 4°C, washed and allowed to drain. Some fish were cooked prior to processing and others were processed uncooked (Figure 8 Note I and Figures 9 and 10). "Cooking" was attained by steaming the fish in a pressure cooker for 5 minutes.

FIGURE 9: SCHEME FOR PRODUCING DIFFERENTLY PROCESSED FISH PRODUCTS



Notes:
i) DD = drum dried
ii) SD = spray dried
iii) Final products 2, 6 and 8 were not produced
iv) Final product 4 corresponds to product C11.3M in Figure 10

FIGURE 10: FURTHER DEVELOPMENT OF THE SCHEME OUTLINED IN FIGURE 9 TO PRODUCE SELECTED FISH POWDERS

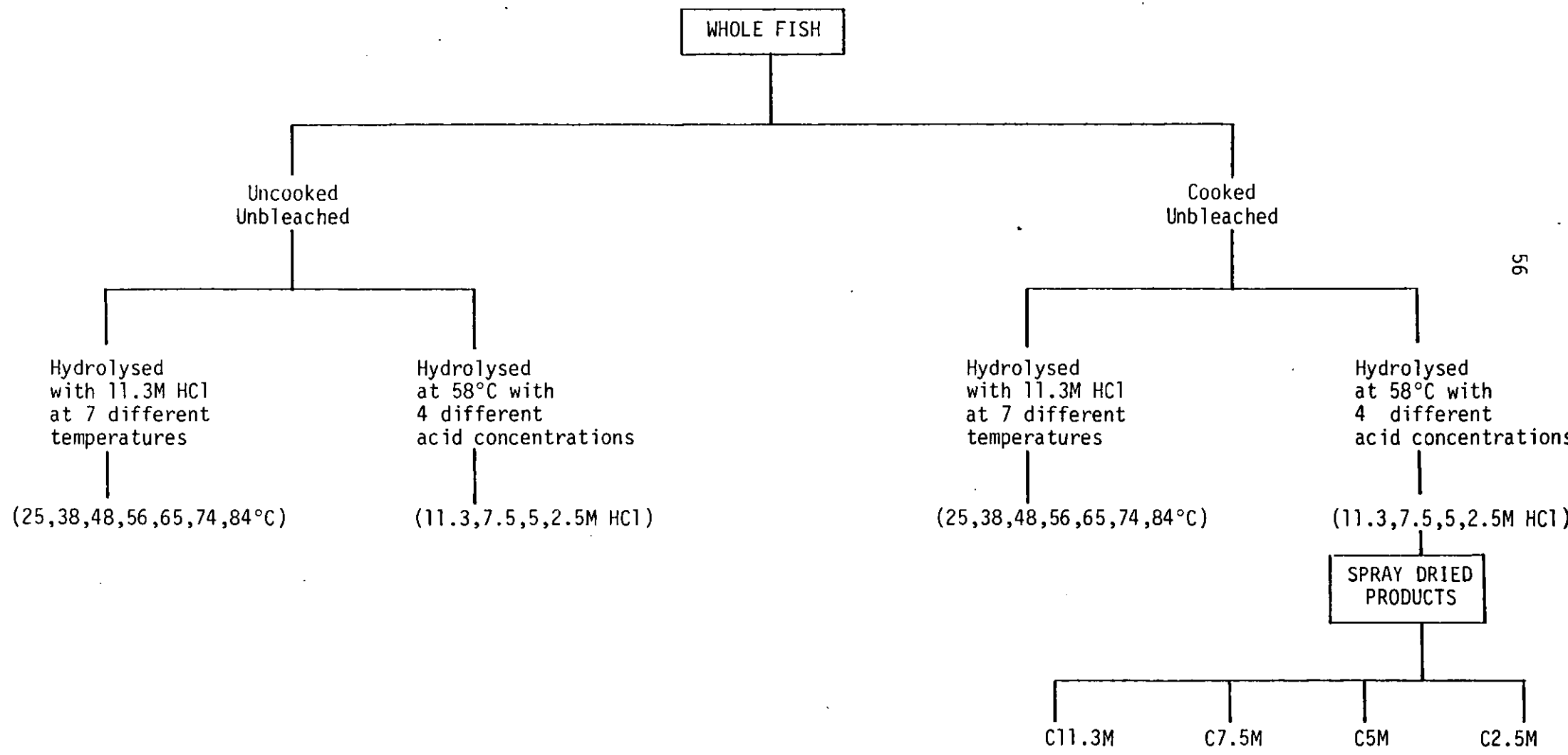


FIGURE 11: Apparatus for acid hydrolysis of Haplochromis

The fish (cooked or uncooked) were minced using a Hobart mincer model E4522. The pH of the mince was determined on a small sample according to the method recommended by Pearson (1981).

3.3.2 Hydrolysis

A sample of the mince was weighed in the reaction vessel and hydrochloric acid added in the ratio 3:10 (v/w) and the mixture continuously stirred with a mechanical stirrer. Hydrolysis was assumed to have started when the acid-mince mixture was homogeneous and was continued for 30 minutes for each "run". At intervals, during the course of hydrolysis aliquots (4 ml approximately) of the reaction mixture, hereafter referred to as "hydrolysate", were pipetted into test tubes containing 10% TCA (3 ml) and the mixture vigorously shaken. The tubes were subsequently centrifuged at 3000 rpm for 30 minutes and the nitrogen content of the supernatant and residue determined separately by Kjeldahl to give TCA-soluble and TCA-insoluble nitrogen respectively. The determination of TCA-soluble (and TCA-insoluble) nitrogen was a measure of protein hydrolysis by the acid. The effects of varying acid concentration and temperature on

a) TCA-soluble nitrogen of the hydrolysate
b) pH
c) proximate chemical composition of the intermediates and final product

were studied as detailed below (3.4).

3.3.2.1 Acid concentration

Hydrolysis at constant temperature (58°C approximately) was carried out using four different acid concentrations; 11.3M, 7.5M, 5M and 2.5M approximately (see Figure 8 and Note II and Figure 10).

3.3.2.2 Temperature

For the 11.3M acid concentration hydrolysis was done at seven different temperatures: 25, 38, 48, 56, 65, 74 and 84°C approximately (see Figure 8 Note II and Figure 10).

3.3.3 Neutralisation

After hydrolysing for 30 minutes the acid was neutralised with sodium hydroxide solution (10M, 7.5M, 5M and 2.5M corresponding to the acid concentration used) to the original pH of the mince (see 3.3.1).

3.3.4 Homogenisation

The neutralised hydrolysate was homogenised, using a Silverson Laboratory homogeniser Model L2R fitted with a square hole high shear screen, at constant speed for ten minutes. The homogenate was filtered through a metal sieve Mesh No. 22, (aperture 710 microns BS 410:1962) prior to drying.

3.3.5 Bleaching

In a variation in the process the hydrolysate was bleached after the homogenising and sieving operations (see Figure 8 Note III). The

hydrolysate was divided into two equal parts. One part was left unbleached while the other was bleached with a solution of hydrogen peroxide according to the method of Interox Chemicals (1979). For every 100g of the hydrolysate a solution of anhydrous sodium carbonate (0.4% w/v) was added and the mixture stirred for 5 minutes. Hydrogen peroxide (6 ml, 0.75%, w/w) was added to the mixture and the pH adjusted to pH 10.5 by addition of sodium hydroxide. The mixture was stirred for a further 15 minutes and centrifuged at 3000 rpm for 30 minutes and the supernatant discarded. Water was added to the residue and homogenised at constant speed until a homogeneous slurry was formed. The hydrolysate was centrifuged again, as above, after which another volume of water was added to the residue and homogenised as before. The volume of water added was calculated so as to make the final solids content of the slurry 15% approximately. The hydrolysate was finally drum dried on a 15 by 15 cm laboratory drum drier at 124^{o}C producing final products 1, 3, 5 and 7 in Figure 9.

The unbleached portion was centrifuged once, the supernatant discarded and the residue homogenised in water, as outlined above, prior to drum drying.

3.3.6 Spray Drying

After homogenisation and sieving (Figure 8) the hydrolysate was spray dried in a Becker Dryer (air inlet temperature 185^{o}C, exhaust temperature 87^{o}C). Final product No. 4 in Figure 9 and products C11.3M, C7.5M, C5M and C2.5M in Figure 10 were produced in this manner. The only variation to spray drying was the drum drying of the bleached and unbleached products mentioned in 3.3.5 (see also Figure 8 note IV and Figure 9).

3.4 PRODUCT AND PROCESS EVALUATION

The overall effect of the process was evaluated by determining the amounts of the nutritionally important constituents of the intermediates and the final products (1, 3, 4, 5 and 7 in Figure 9 and C11.3M, C7.5M, C5M and C2.5M in Figure 10), with particular emphasis on protein.

3.4.1 Protein

3.4.1.1 Total, TCA-soluble and TCA-insoluble nitrogen

were determined (as outlined in 3.2.1.1 and 3.2.1.2) in the product, after bleaching (see 3.3.5) and after drying.

3.4.1.2 Amino acids

The amino acid composition of the final products was determined as described in 3.2.1.3.

3.4.1.3 Protein digestibility

A multienzyme *in vitro* method described by Hsu, *et al* (1977) was used to determine protein digestibility. The enzymes used were Porcine pancreatic trypsin (No. T-0134) Type IX, activity 15,000 BAEE units per mg protein; Bovine pancreatic α-chymotrypsin (No. C-4129) Type II, activity 51 units per mg protein and Porcine intestinal mucosa peptidase (No. P-7500), activity 100 units per g solid.

The protein substrate (i.e. the final product) was prepared as an aqueous suspension (6.25 mg protein/ml) adjusted to pH 8.0 with 0.1N HCl and/or NaOH while stirring in a 37°C water bath. The multienzyme solution (1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase/ml) was freshly prepared and maintained in an ice batch and adjusted to

pH 8.0 with 0.1 N HCl and/or NaOH. Five ml of the enzyme solution were added to 50 ml of the protein suspension which was being stirred at 37°C and the change in pH recorded automatically over a 10 minute period using a recording pH meter.

The procedure was repeated using casein substrate as reference material.

3.4.2-3.4.4 Total lipid, ash and salt (NaCl)

were determined as in 3.2.1.4, 3.2.1.5 and 3.2.1.6 respectively.

3.4.5 Moisture

was determined as water activity (a_w) using a Navasina EEJA-3 water activity meter.

3.4.6 Colour

The colour of the powder was determined using the Erichsen Colorimeter 511/E with a white tile as a reference.

3.4.7 Organoleptic testing

Sensory evaluation of the fish powder was conducted with the aid of an expert panel comprised of staff and students at the Fisheries Training Institute, Entebbe. The fish powder was incorporated into groundnut sauce (a common East African dish) and the panel asked to assess (i) odour, (ii) salt content, (iii) rancidity, (iv) fish taste and give scores ranging from 1 to 5. The mid-point score (3) was regarded as satisfactory for parameters (i), (ii) and (iv) and for rancidity a score of 0 was ideal and 5 was undesirable. The panel was also asked to record any comments they cared to make. A sample of the score sheet used is shown in Appendix I.

The samples for sensory evaluation were prepared as follows:

Sample 0: Only groundnut sauce - without fish powder.

Sample 1: 1 tablespoonful (13g approximately) of fish powder was added to 100 ml cold water and mixed after which it was added to already cooked groundnut sauce, mixed thoroughly and brought to the boil and served.

Sample 2: Two tablespoonsful (25g approximately) of fish powder were added to 100 ml cold water and the procedure for Sample 1 repeated.

Sample 3: Three tablespoonful (39g approximately) of fish powder were added to 100 ml cold water and treated as Sample 1.

3.4.8 Particle Size Analysis

was carried out by two methods: using a Malvern Laser diffractometer and by sieve analysis.

4. RESULTS AND DISCUSSION

4.1 RAW MATERIAL EVALUATION

4.1.1 Total Nitrogen

The total nitrogen of both cooked and uncooked *Haplochromis* was determined to assess the crude protein content of the raw material and the results are shown in Table 2b. Uncooked fish contained 9.1% and cooked fish contained 9.7% nitrogen, corresponding to 57.1 and 60.6% crude protein respectively. The apparent difference between the two results is not significant when the respective standard deviations are taken into account. The two results are also in agreement with those reported by Ssali (1981) for whole (uncooked) *Haplochromis* (60%) caught during November 1980. The fish used for the present study were caught during October 1982 and June 1983. It would therefore appear that there is no periodic variation in raw material composition as far as crude protein is concerned. This is desirable if product consistency is to be maintained.

4.1.2 True-Protein and Non-Protein Nitrogen (NPN)

The true-protein content of cooked and uncooked fish is shown in Table 2b. Non-protein nitrogen constituted approximately 17% of total nitrogen of uncooked fish and 15% of total nitrogen of cooked fish. These findings are consistent with those obtained from earlier chemical composition studies on *Haplochromis* (Ssali, 1981) in which NPN ranged between 15.2 and 21.1%, averaging at 17%. True-protein nitrogen was determined as TCA-insoluble nitrogen which excludes low molecular weight nitrogenous compounds such as nucleotides, amino acids and peptides, (Ikeda, 1980). A high amount of NPN in fish tissue is not desirable because it renders the fish more susceptible to rapid bacterial spoilage because low molecular weight components such as amino acids are easily utilised for food by bacteria. The presence

of a large proportion of NPN in fish also somewhat lowers its nutritional value.

The NPN content of *Haplochromis* compares with that of other teleost fishes in which it is reported to account for from 9.2 to 18.3% total nitrogen (Simidu, 1961).

4.1.3 Total Lipid

The total lipid content of uncooked and cooked fish was 24.3 and 25.3% respectively (Table 2b). The apparent difference between the two figures is not significant when the respective standard deviations are taken into account. The values, however, are about 19% higher than those obtained for fish caught during November 1980 (20.1%) (Ssali, 1981). Lipid plays an important role during fish processing and storage due to the high susceptibility to oxidation of the polyunsaturated fatty acids present in fish. It would therefore be desirable to have the lipid content of the final product as low as possible. The finding that the total lipid content of the fish caught in November 1982 was higher than that caught in November 1980 emphasises the need to carry out regular proximate composition analyses on the raw material to ensure product consistency.

4.1.4 Total Ash

The ash content of *Haplochromis* is shown in Table 2b (19.3%) and the figure is very similar to that reported by Ssali (1981) (19%). The relatively high proportion of ash in the fish is attributed to the presence of bones and scales which are rich in tri-calcium phosphate (apatite) and calcium carbonate (Causeret, 1962).

TABLE 2b Proximate composition of cooked and uncooked *Haplochromis* (raw material)

Sample	Per Cent[1]				
	Total Nitrogen (TN)	Crude Protein (TNx6·25)	"True Protein" (TCA-insoluble Nx6·25)	Total Lipid	Ash
Uncooked fish	9.1 (0.5) n=11	57.1 (3.4) n=11	47.5 (0.3) n=3	24.3 (0.8) n=4	19.3 (0.1) n=4
Cooked fish	9.7 (0.4) n=7	60.6 (2.3) n=7	51.5 (0.2) n=3	25.3 (1.3) n=4	19.3 (0.1) n=4

Notes: 1. Dry weight basis
2. Standard deviation in parentheses.
3. n = number of samples analysed

TABLE 3: Standard plate count of cooked and uncooked *Haplochromis* (raw material)

Sample	Standard Plate Count[1] per g	Standard Deviation
Uncooked fish	1.47×10^6	0.27
Cooked fish	2.72×10^2	0.27

1. Quadruplicate determinations.

4.1.5 Standard Plate Count (SPC)

Table 3 shows the results of SPC for the uncooked and cooked fish. As expected cooking considerably reduced the bacterial load from 1.5×10^6/g in the uncooked fish to 2.7×10^2/g in the cooked fish. The bacterial count for the uncooked fish was high but not unusual considering that the sample was whole fish containing skin, gills and gut material. Dhatemwa (1981) reported TVC (at 25^oC) of 1.2×10^5 and 6.2×10^5 for two batches of (unwashed) *Haplochromis* mince. Bacterial loads of 10^3- 10^5, 10^6 and 10^4- 10^6 have been reported for skin (per cm^2), gills (per g) and guts (per g or ml) respectively of fish from tropical and subtropical waters (Shewan, 1977).

Enumerating bacteria is not an ideal means of determining fish quality as it is expensive and time consuming and the numbers are not always directly related to sensory properties and cannot give useful information in the early stages of spoilage (Connell, 1982).

Standard plate counts also provide very little information regarding the type of organisms present. Fish caught in polluted waters, for instance, are likely to carry organisms of faecal origin such as *E.coli*. Specific tests have got to be conducted to detect such organisms (ICMSF, 1978).

4.2 HYDROLYSIS

4.2.1 Effect of Using Cooked or Uncooked Fish as Raw Material

The effect of precooking the fish on the course of hydrolysis was determined by measuring the amount of TCA-soluble nitrogen of the hydrolysate, at intervals, over a thirty minute period. Thirty minutes was selected as the duration of hydrolysis for two practical reasons. Firstly preliminary studies showed that when fish was

hydrolysed at the highest temperature (84 ± 4°C) the hydrolysate darkened after about 35 minutes due to the formation of humin. The formation of humin in the final product would have made the product unattractive and probably reduced its nutritional value. At 30 minutes, however, there was no sign of darkening of the hydrolysate even at the highest temperature. Secondly preliminary studies also showed that 30 minutes was the minimum amount of time required for the acid to soften all the fish tissues at the lowest temperature (25°C). For a fixed acid concentration (11.3M HCl) the studies were conducted at 7 different temperatures (25, 38, 48, 56, 65, 74, 85°C approximately) and at a fixed temperature (58°C approximately) hydrolysis was carried out using 4 different acid concentrations (11.3M, 7.5M, 5M and 2.5M HCl). Similar studies were conducted on uncooked fish and the results, expressed as TCA-soluble nitrogen as percentage of total nitrogen of the whole contents of the reaction vessel, are shown in Figures 12-21 and Appendices 2-21.

Table 4 shows the nett increase in the amount of TCA-soluble nitrogen of both cooked and uncooked fish at the 7 different temperatures and Table 5 shows the increase with respect to the 4 acid concentrations.

It can be seen that invariably at all temperatures and acid concentrations more hydrolysis took place in uncooked, than cooked fish. The difference may be accounted for by the endogenous enzymes of the fish acting synergistically with the acid to break down the fish protein. It is highly likely that uncooked fish contained active proteolytic enzymes such as cathepsins (Mycek, 1970) and pepsin (Merret *et al*, 1969 ; Ryle, 1970) which are known to be active at acid pH. Cathepsins and pepsin have been demonstrated to occur in the peritoneal cavities of freshly killed carp or trout (Tarr, 1972) and pepsins from dogfish *(Mustelus canis)* have been described by Merrett *et al* (1969). It is therefore reasonable to assume that the same enzymes exist in *Haplochromis*. Considering the very low pH in the reaction vessel (which could be as low as pH 0.1) the most likely enzyme to have been active at such low pH is pepsin.

Pepsin exists intracellularly as the zymogen pepsinogen which is secreted into the stomach in this form where it is converted to the active enzyme pepsin by acid pH and pepsin itself (Dixon and Webb, 1958; Lehninger, 1975). Pepsin has very broad specificity but preferentially attacks peptide bonds involving residues of aromatic amino acids as well as leucine (Lehninger, 1975). In this study mincing whole fish (Figure 8) facilitated the dispersion of pepsinogen (and other proteolytic enzymes) throughout the fish tissue. When acid was added to the mince the pepsinogen was converted into pepsin. The pH-activity profile of pepsin (Appendix 22) shows that the optimum pH of the enzyme is between pH1 and 2.0. It is therefore feasible that under the conditions of *Haplochromis* hydrolysis the enzyme retained some of its activity. It is also probable that at any one time in the reaction vessel, particularly during the initial period of hydrolysis, there may have existed "pockets" of fish tissue where the pH was optimum for pepsin activity.

4.2.2 Effect of Temperature

The effect of temperature on acid hydrolysis of cooked and uncooked *Haplochromis* was determined by hydrolysing the fish at seven different temperatures (25, 37, 46, 56, 64, 74 and 84°C approximately for cooked fish and 25, 38, 48, 56, 65, 74 and 84°C approximately for uncooked fish). The course of hydrolysis was assessed as described in 4.2.1.

Figure 22 shows the effect of temperature on hydrolysis of cooked fish (see also Figures 12-18). Overall there was an increase in the amount of TCA-soluble nitrogen with temperature corresponding to increased hydrolysis. Table 4 shows the nett increase in TCA-soluble nitrogen of both cooked and uncooked fish for the seven different temperatures. As Table 4 and Figure 12 show for cooked fish at 25°C there was hardly any hydrolysis at room temperature. At 37°C, however, there was a nett increase in TCA-soluble nitrogen of cooked fish (4.9%) and the increase was slightly higher than that observed for 46°C (4.1%) and 64°C (4.7%).

FIGURE 12: Acid hydrolysis of cooked and uncooked *Haplochromis* at 25 ± 1 and 25 ± 3°C respectively (11.3M HCl)

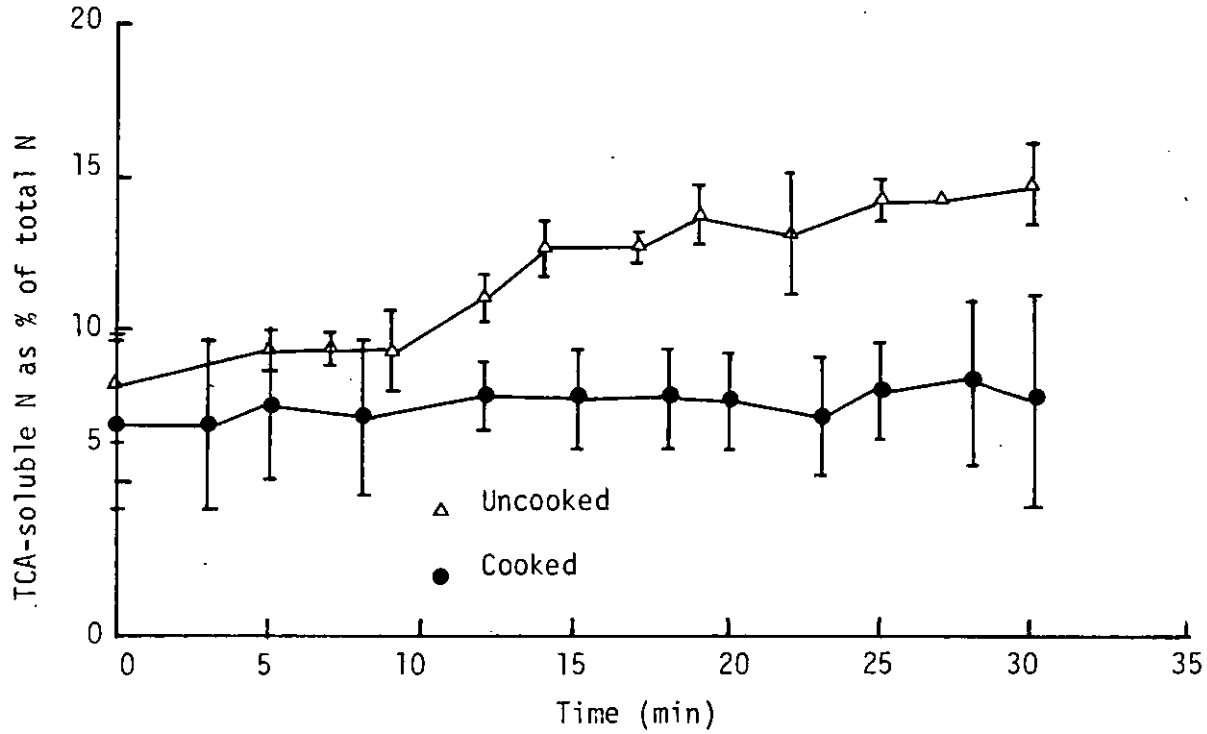


FIGURE 13: Acid hydrolysis of cooked and uncooked *Haplochromis* at 37 ± 3 and 38 ± 2° respectively (11.3M HCl)

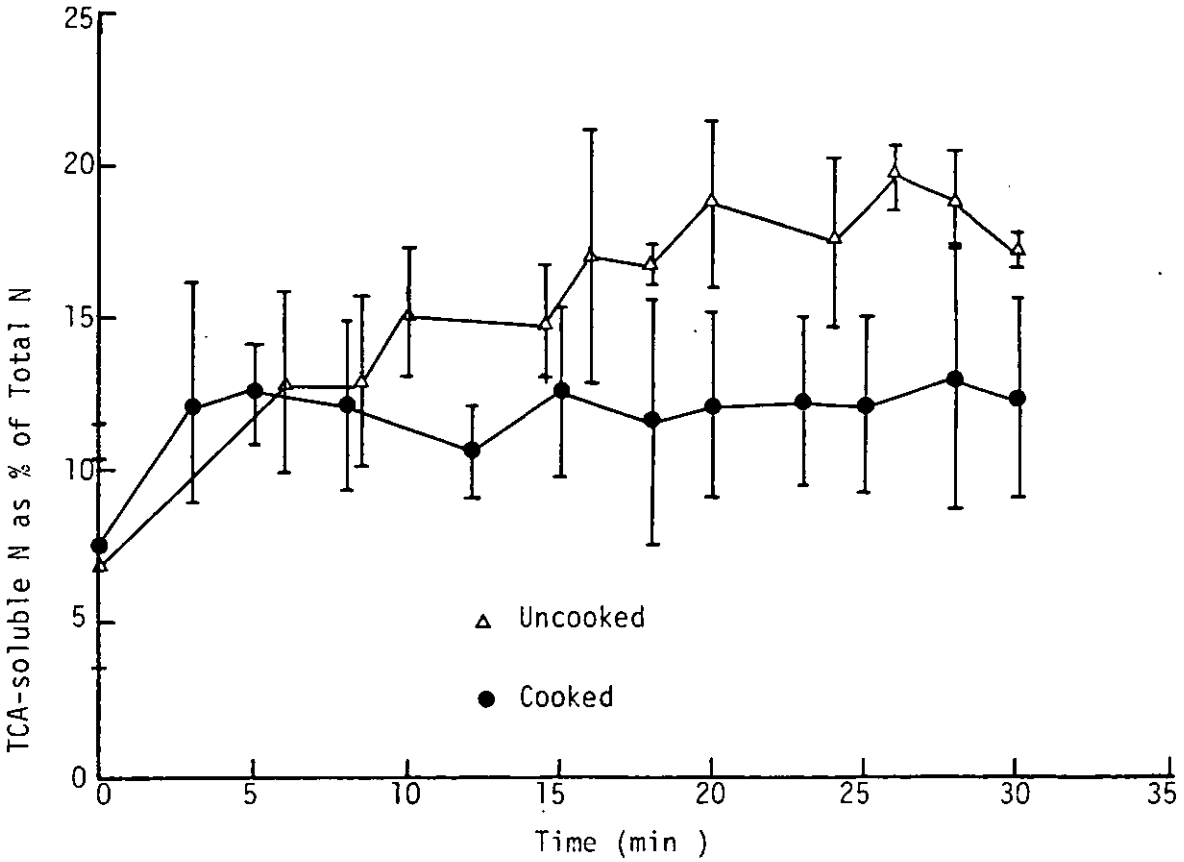


FIGURE 14: Acid hydrolysis of cooked and uncooked *Haplochromis* at 46 ± 2 and 48 ± 3°C respectively (11.3M HCl)

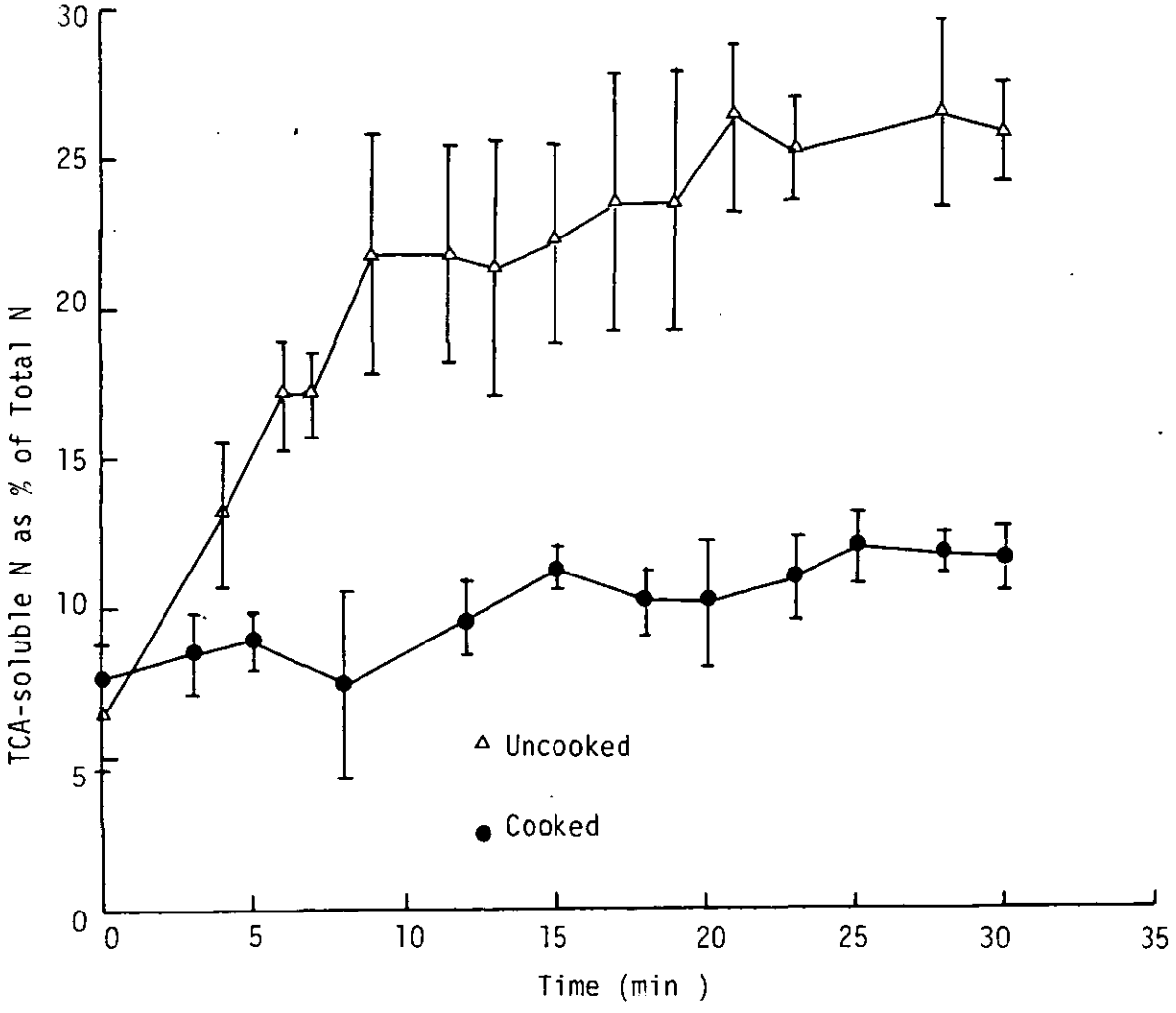


FIGURE 15; Acid hydrolysis of cooked and uncooked *Haplochromis* at 56 ± 2 and 56 ± 4°C respectively (11.3M HCl)

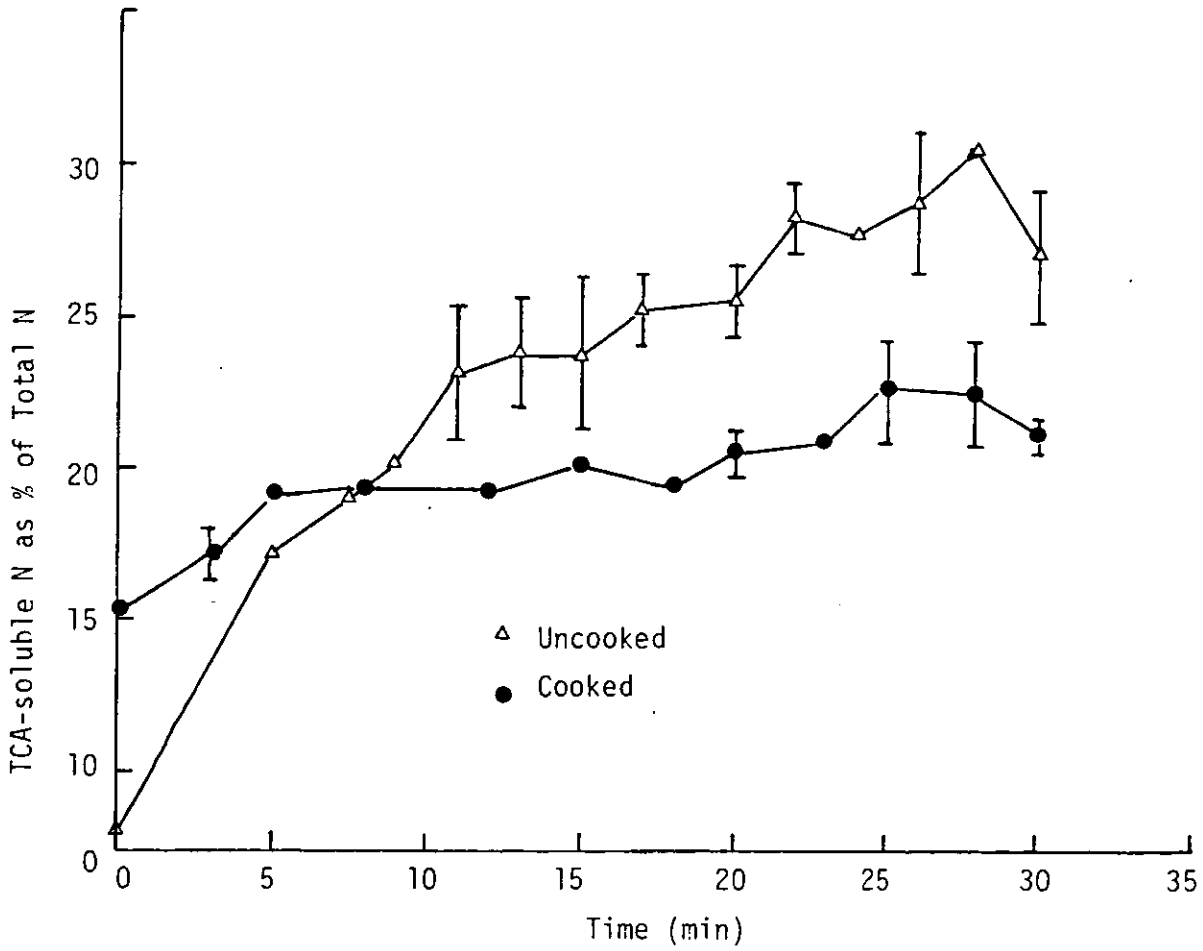


FIGURE 16: Acid hydrolysis of cooked and uncooked *Haplochromis* at 64 ± 4 and 65 ± 4°C respectively (11.3M HCl)

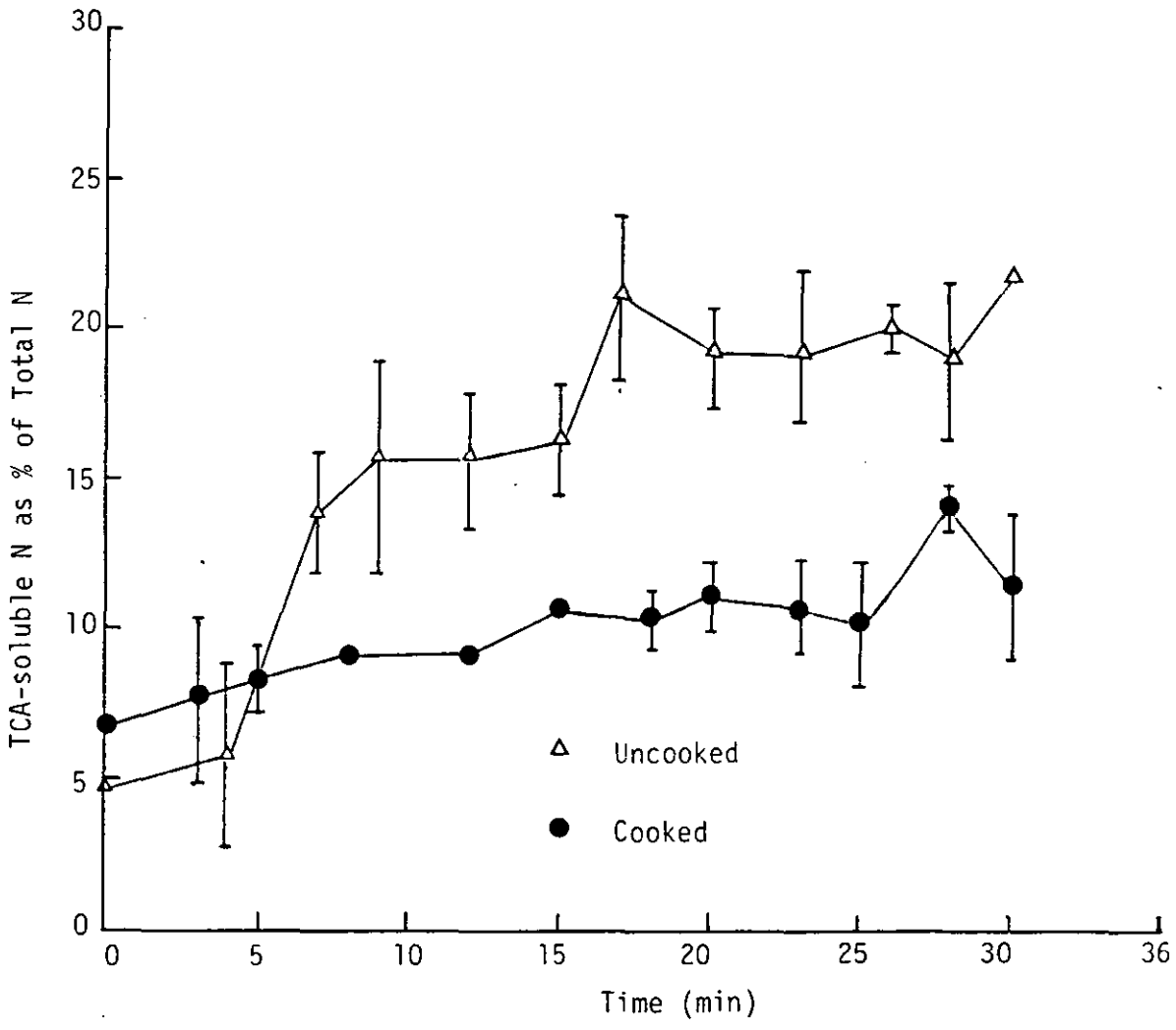


FIGURE 17: Acid hydrolysis of cooked and uncooked *Haplochromis* at 74 ± 4 and 74 ± 6°C respectively (11.3M HCl)

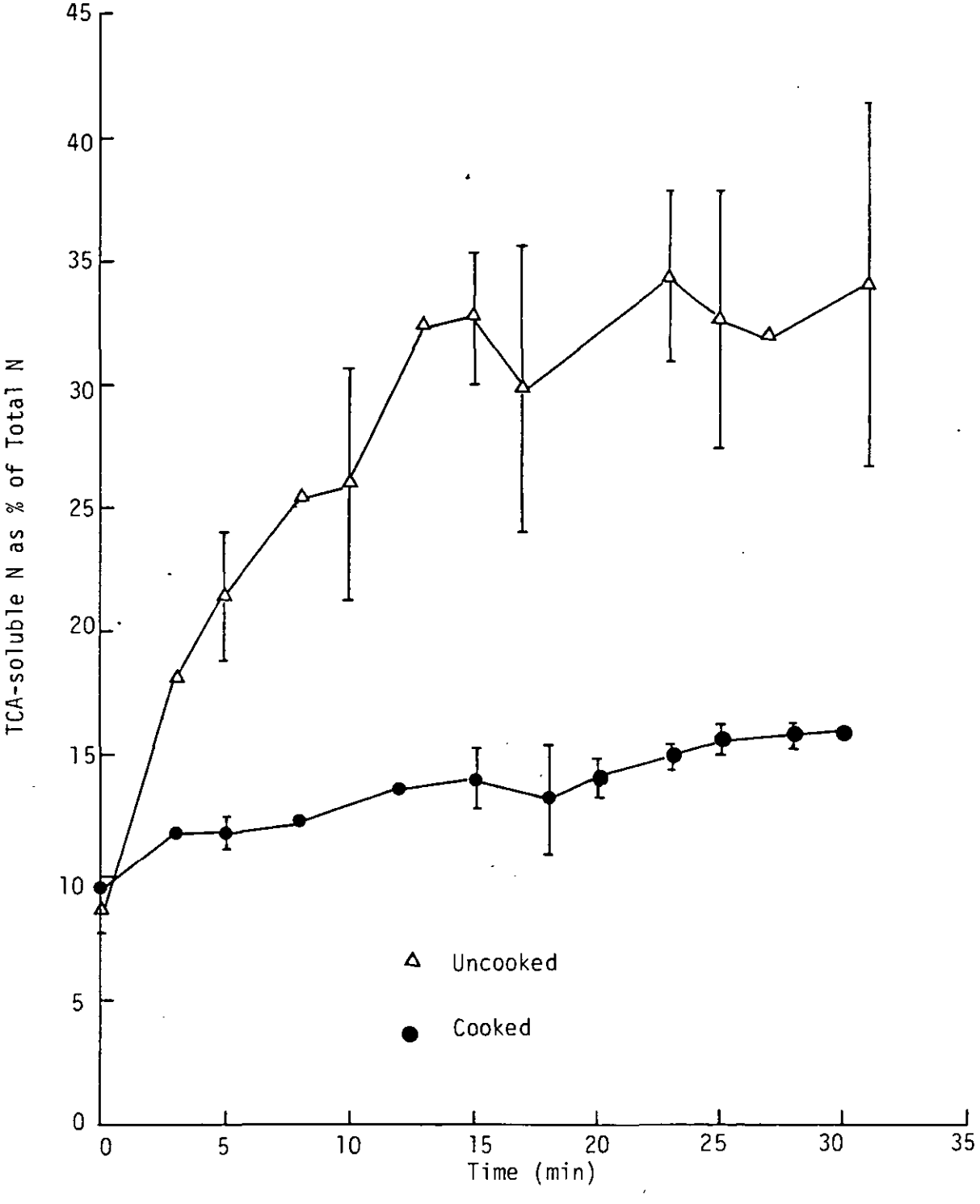


FIGURE 18: Acid hydrolysis of cooked and uncooked *Haplochromis* at 84 ± 4°C respectively (11.3M HCl)

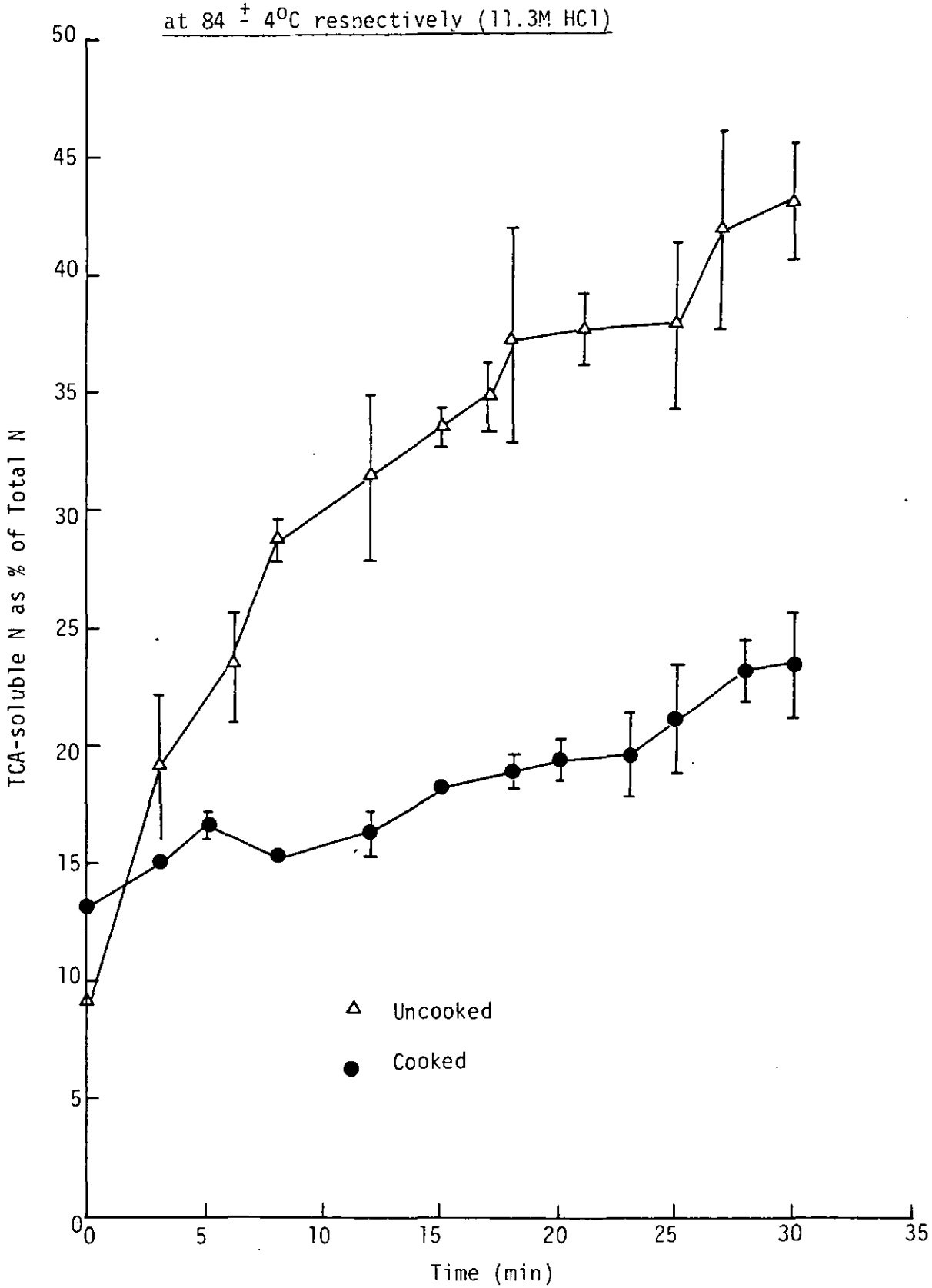


FIGURE 19: Acid hydrolysis of cooked and uncooked *Haplochromis* at 58 ± 4°C respectively (2.5M HCl)

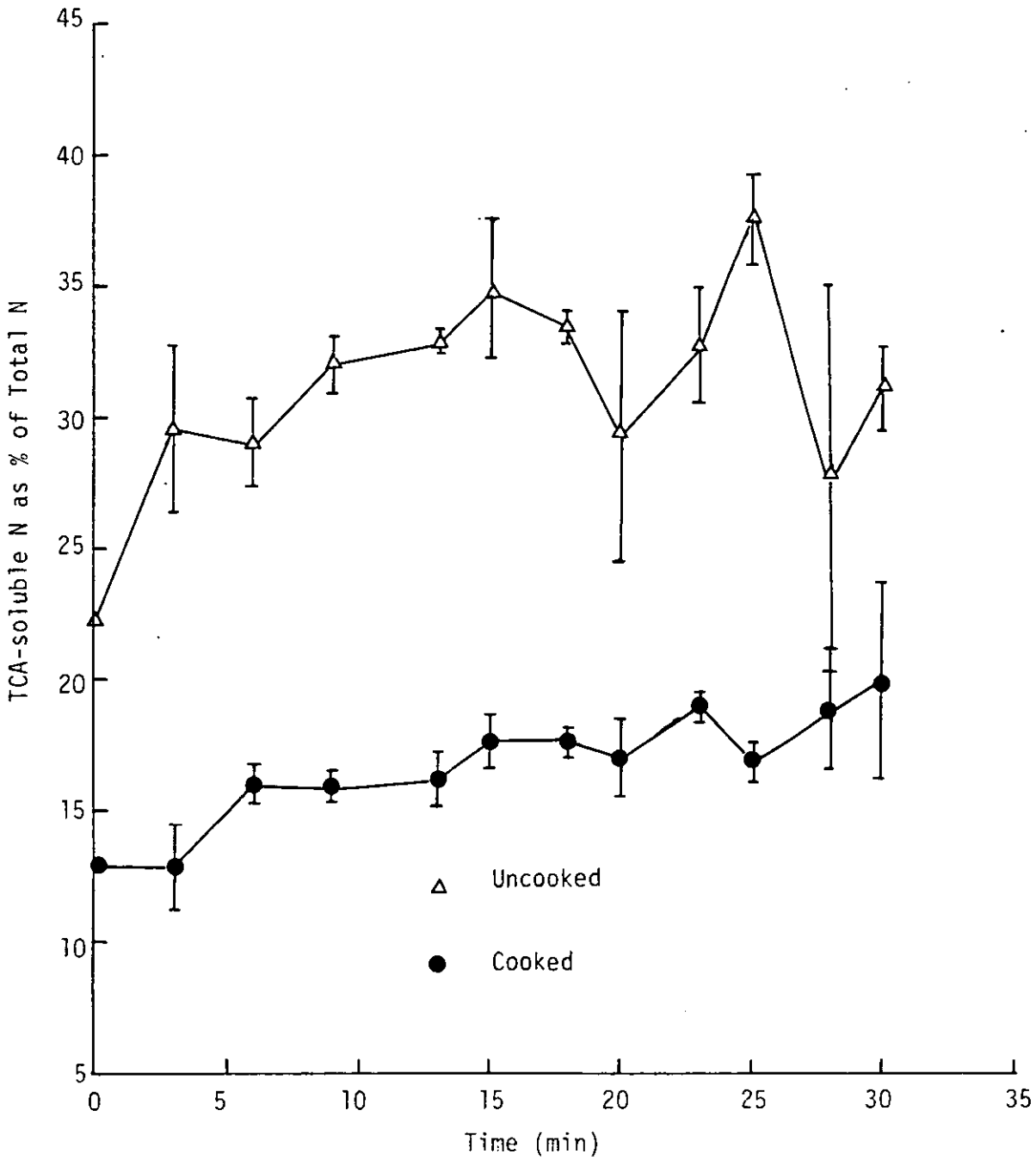


FIGURE 20: Acid hydrolysis of cooked and uncooked *Haplochromis* at 59 ± 4 and 59 ± 3°C respectively (5M HCl)

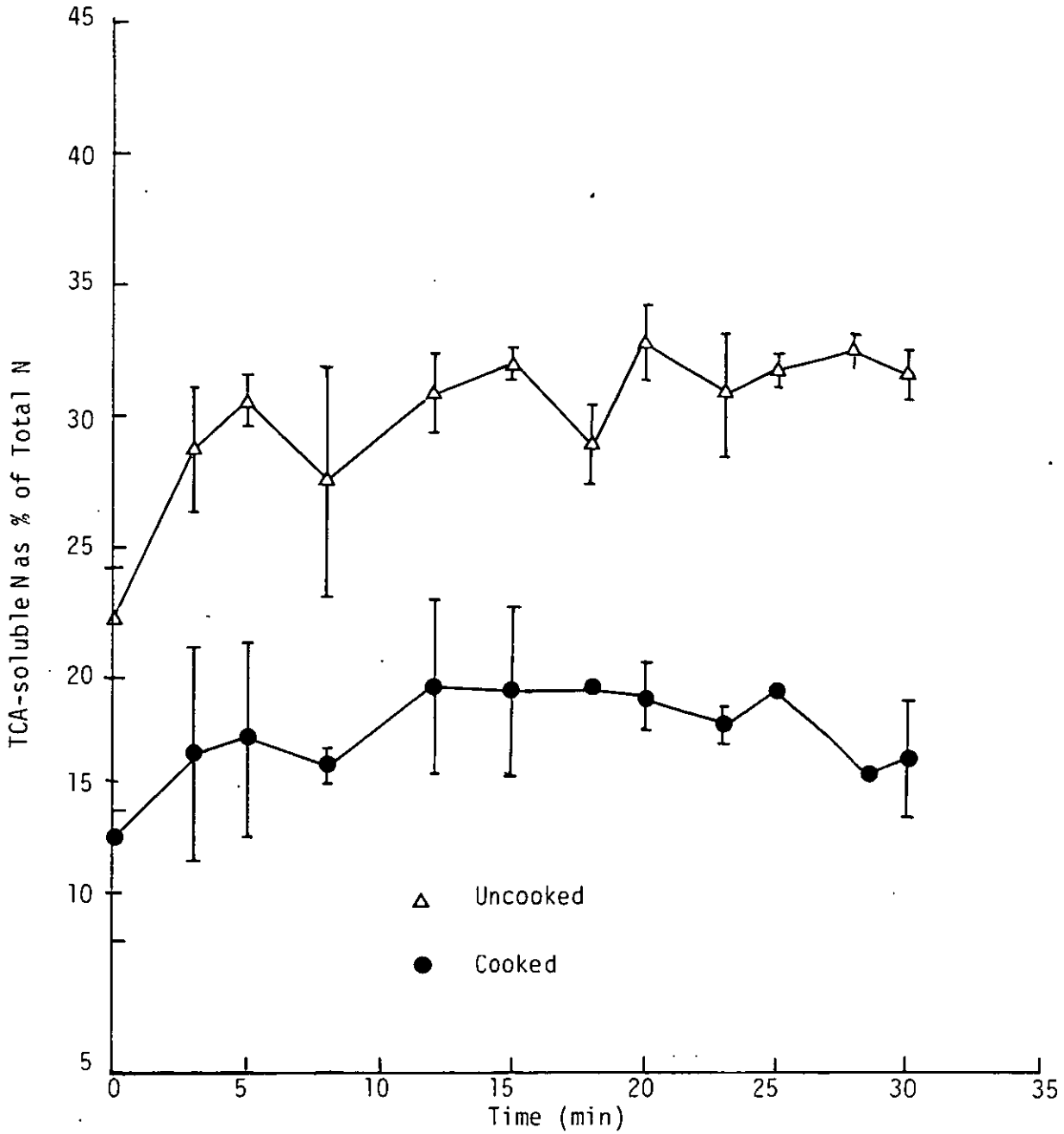


FIGURE 21: Acid hydrolysis of cooked and uncooked *Haplochromis* at 58 ± 2 and 60 ± 4°C respectively (7.5M HCl)

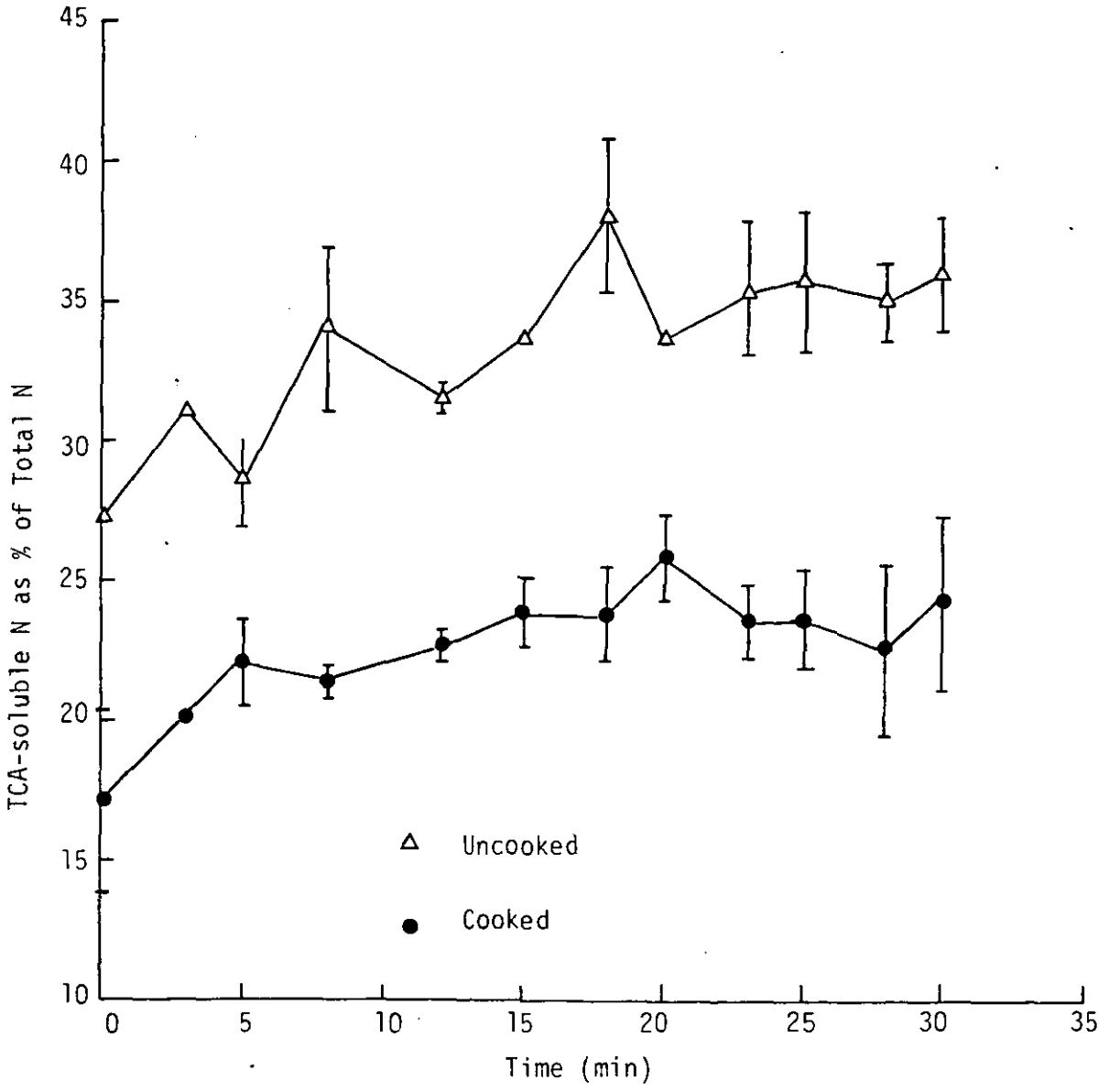


TABLE 4: Nett increase in TCA-soluble nitrogen of hydrolysates of cooked and uncooked *Haplochromis*

(Acid concentration 11.3M HCl)

Temperature[1] (°C)	Nett Increase TCA-soluble N (%)	
	Cooked	Uncooked
84 ± 4	10.4	34.1
74 ± 4 (74 ± 6)	6.4	25.5
64 ± 4 (65 ± 4)	4.7	17.1
56 ± 2 (56 ± 4)	5.8	19.0
46 ± 2 (48 ± 3)	4.1	19.9
37 ± 3 (38 ± 2)	4.9	10.3
25 ± 1 (25 ± 3)	0.8	6.7

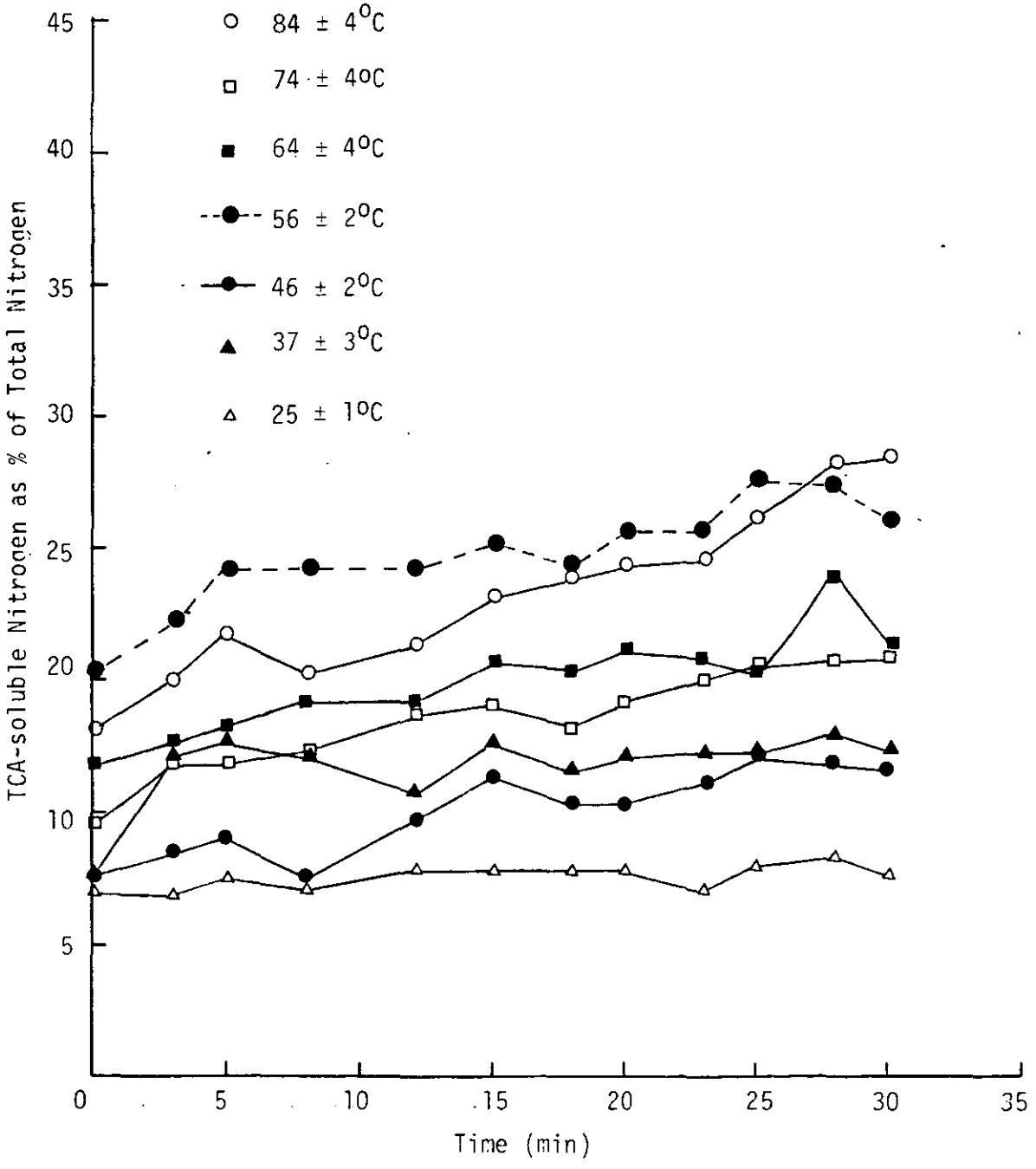
1. Temperature values in parentheses refer to uncooked fish, where different from those for cooked fish.

TABLE 5: Nett increase in TCA-soluble nitrogen of hydrolysates of cooked and uncooked *Haplochromis* over a 30-minute period (acid concentrations 11.3M, 7.5M, 5M and 2.5M)

Acid Concentration (M)	Temperature[1] (°C)	Nett Increase in TCA-soluble N (%)	
		Cooked	Uncooked
11.3	56 ± 2 (56 ± 4)	5.8	19.0
7.5	58 ± 2 (60 ± 4)	7.2	8.7
5.0	59 ± 4 (59 ± 3)	3.1	9.5
2.5	58 ± 4	7.1	9.0

1. Temperature values in parentheses, refer to uncooked fish, where different from those for cooked fish.

FIGURE 22: The effect of temperature on acid hydrolysis of cooked *Haplochromis* (11.3M HCl)



In fact the observed order of nett increase of TCA-soluble nitrogen for cooked fish was 84 > 74 > 56 > 37 > 64 > 46 > 25^{o} (Table 4). Yet according to the principles of chemical reaction kinetics the extent of hydrolysis over the seven-temperature range should have been expected to be in the order of increasing temperature up to the point at which thermal denaturation occurs.

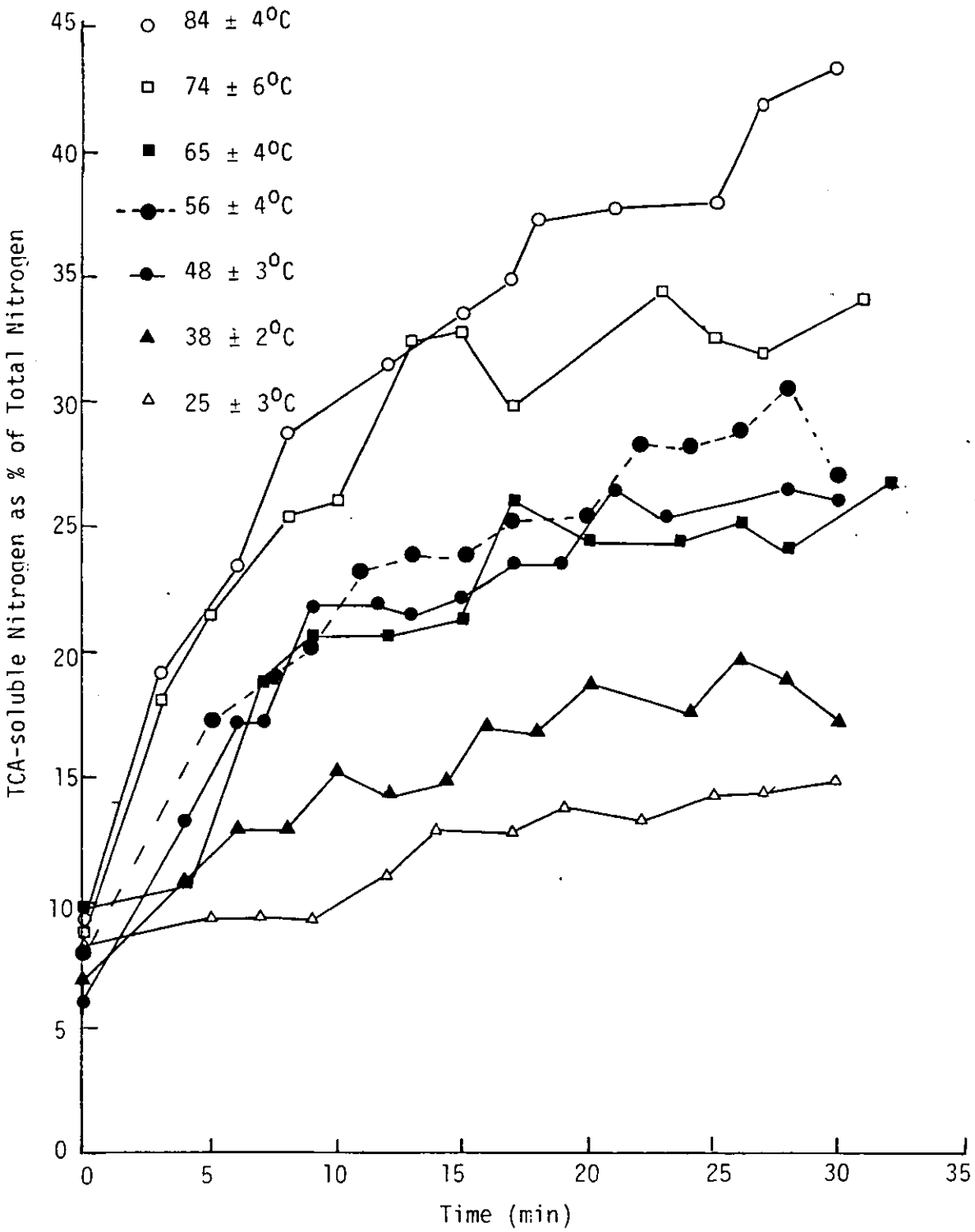
When evaluating these results it is important to realise that hydrolysis proceeded for only 30 minutes, therefore only partial hydrolysis was achieved. As Hill (1965) pointed out partial hydrolysis of a protein involves cleavage of a variety of bonds leading to a complex mixture of products. It should be realised that determining hydrolysis by measurement of TCA-soluble nitrogen has its limitations; only those nitrogenous molecules below a certain molecular weight are soluble in TCA. Therefore although peptide bonds may have been broken, unless the resulting molecules were small enough to dissolve in TCA, such hydrolysis would not be detected by the method. In this study the situation was further complicated by the presence of not only various types of proteins (sarcoplasmic, myofibrillar and stroma proteins, see 2.3.1) but also lipids, inorganic salts (both endogenous and those resulting from the action of acid on the bones and scales) and other substances discussed in 2.3.3. The interaction of the various substances mentioned under the experimental conditions in this study is not known and would be very difficult to predict. There is a possibility that due to the presence of such a multitude of compounds new peptide bonds may be formed while others are being attacked by the acid. (For example, the possibility of rearrangement or ring formation among peptides during hydrolysis of proteins by enzymes and hot dilute acids was mentioned by Gordon *et al* (1941). The result would be little or no increase in TCA-soluble nitrogen. Although acids have been used extensively for hydrolysis of proteins there is little available information on the parameters which control the extent and specificity of acid hydrolysis (Hill, 1965).

Another complicating factor in this study is the fact that the fish used had been previously frozen for several weeks. As discussed in 2.3.1 prolonged cold storage results in denaturation of fish proteins. The effect of this denaturation on protein hydrolysis is not known.

Figure 23 (also Figures 12-18) show the effect of temperature on acid hydrolysis of uncooked *Haplochromis*. As in the case of cooked fish the amount of TCA-soluble nitrogen increased with temperature, therefore least hydrolysis took place at 25° and the most at 84°C. While there was no effective increase in TCA-soluble nitrogen in the case of cooked fish at 25°C the nett increase for uncooked fish at the same temperature was 6.7% (Table 4). Similarly at the highest temperature (84°C) there was a substantial nett increase in TCA-soluble nitrogen (34.1%) (Table 4). This compares with the results from cooked fish where the nett increase in TCA-soluble nitrogen was only 10.4% (Table 4). As discussed in 4.2.1 the substantial difference between the two could be attributed to the presence of proteolytic enzymes in uncooked fish and their absence in cooked fish.

It is significant to note that the nett increase in TCA-soluble nitrogen of uncooked fish with respect to the seven temperatures was in the order 84 > 74 > 48 > 56 > 64 > 38 > 25°. More protein was hydrolysed at 48° than 64° and 56°C contrary to what might have been expected. Work on fish silage production (see 2.2.4.1.2) has shown that the rate of autolysis increases with temperature but not with a constant Q_{10} value (Raa and Gildberg, 1982). The rate of autolysis is reported to increase very markedly within a rather narrow temperature interval and this may be the temperature at which the collagen in the connective tissues becomes heat denatured and thus more susceptible to proteolytic degradation. This process may in turn lead to exposure of other tissue components to digestive enzymes. From the results in Table 4 it is possible that the *Haplochromis* collagen was denatured around 48°C and thus the observed increased hydrolysis.

FIGURE 23: The effect of temperature on acid hydrolysis of uncooked *Haplochromis* (11.3M HCl)



The nett increase in TCA-soluble nitrogen of uncooked fish at 74 and 84°C was 25.5% and 34.1% respectively (Table 4). The corresponding values for cooked fish were 6.4% and 10.4% indicating that there was still some enzyme activity in the uncooked fish even at such relatively high temperatures. This was in turn an indication of high thermostability of *Haplochromis* proteolytic enzymes. As suggested in 4.2.1 the enzymes acted synergistically with the acid to bring about the increased hydrolysis observed in uncooked fish.

The results discussed in this section have important implications from the processing point of view. Since this study was not aimed at producing low-molecular weight fish hydrolysates it would be unnecessary to aim for processing conditions which bring about maximum hydrolysis. Rather the aim should be to soften all the fish tissues sufficiently to be turned into powder with the minimum inputs. However, bearing in mind the fact that before the final product is consumed it has to be rehydrated (see 3.4.7) some degree of solubility is desirable. Therefore from the results discussed so far (Table 4) and considering the desire to minimise production costs the optimum processing conditions (so far) would be to hydrolyse uncooked fish at about 48°C.

4.2.3 Effect of Acid Concentrations

Four different acid concentrations (2.5M, 5M, 7.5M and 11.3M HCl) were used to hydrolyse cooked and uncooked fish. Changes in TCA-soluble nitrogen and pH over a 30-minute period were determined. The object of experimenting with the four acid concentrations was to find the lowest concentration which could soften the fish tissues within 30 minutes sufficiently for them to be turned into powder and on neutralisation with alkali (Figure 8) would form enough salt (NaCl) to preserve the final product against microbial attack.

Figure 24 illustrates the changes in TCA-soluble nitrogen during acid hydrolysis of cooked fish at constant temperature (approximately) using different concentrations of acid. Figure 25 shows corresponding changes in uncooked fish. Table 5 shows the nett increase in TCA-soluble nitrogen of cooked and uncooked fish for the four acid concentrations. In cooked fish the greatest increase occurred with the 7.5M concentration (7.2%) closely followed by the 2.5M concentration (7.1%). The least increase occurred with the 5M concentration (3.1%). The nett increase in TCA-soluble nitrogen corresponding to the highest acid concentration (11.3M) was 5.8%. In uncooked fish, however, the highest nett increase in TCA-soluble nitrogen was with the 11.3M concentration (19%) and the least with the 7.5M concentration (8.7%). The nett increase for the 7.5M concentration was less than half of that for the 11.3M concentration. Although the 2.5M concentration is exactly half of 5M the corresponding nett increase in TCA-soluble nitrogen was only 5% lower than the increase corresponding to the 5M concentration. This indicates that the nett amount of hydrolysis was not necessarily directly proportional to the acid concentration.

Table 5 further shows that more protein was hydrolysed in uncooked fish than cooked fish irrespective of the acid concentration. As discussed in 4.2.1 and 4.2.2 the difference was due to the activity of enzymes in uncooked fish and their absence in cooked fish. It is interesting to note that for the 11.3M and 5M acid concentrations the nett increase in TCA-soluble nitrogen for uncooked fish was about 3 times that in cooked fish. Yet for the 7.5M concentration nett TCA-soluble nitrogen was only 1.2 times higher in the uncooked than cooked fish; and for the 2.5M concentration nett TCA-soluble nitrogen was about 1.3 times higher in the uncooked than cooked fish.

The results reported above are not conclusive enough to draw definite conclusions regarding the effect of acid concentration on acid hydrolysis of *Haplochromis*. In addition, as pointed out in 4.2.2, hydrolysis

FIGURE 24: The effect of acid concentration on acid hydrolysis of cooked *Haplochromis*

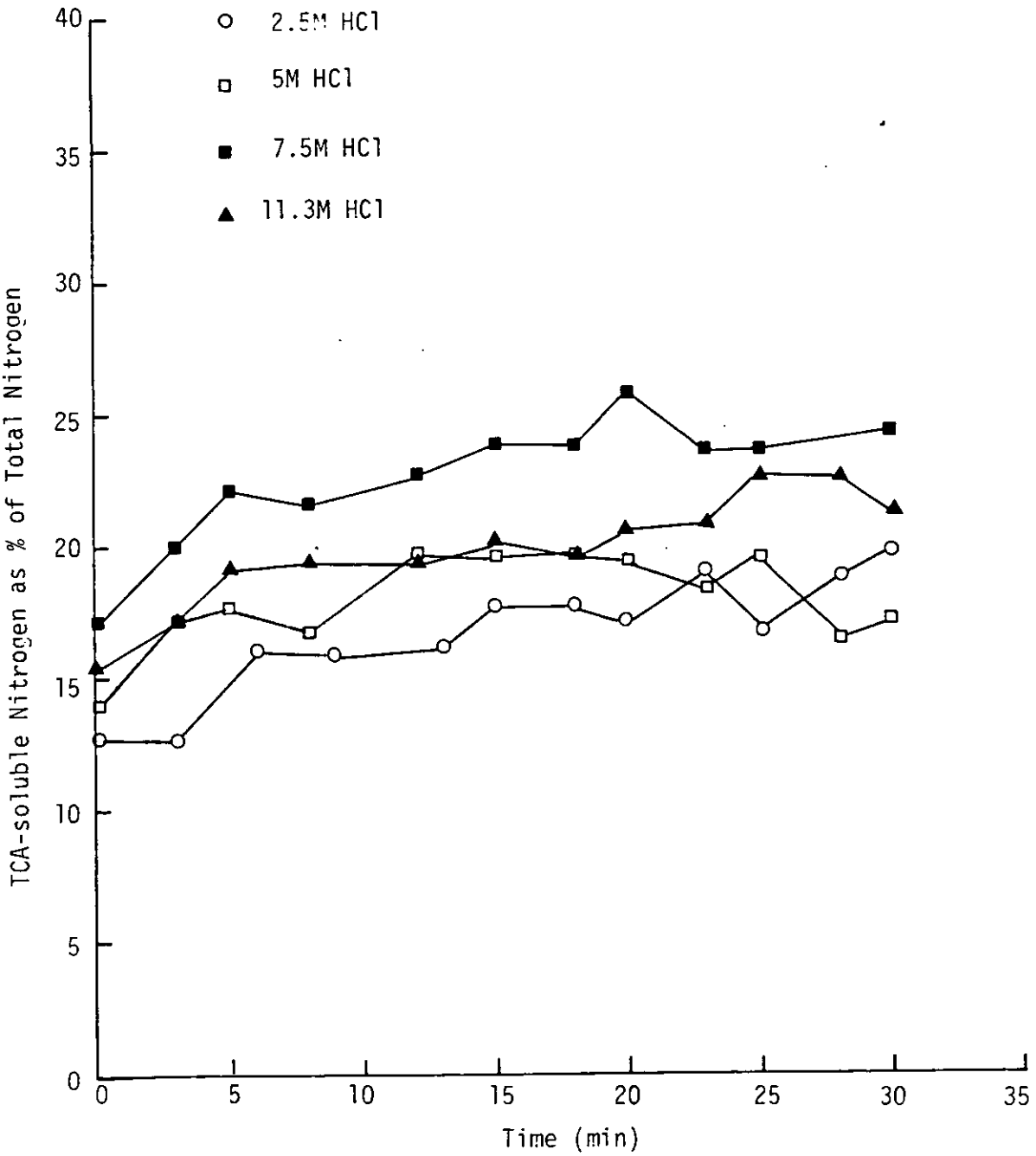
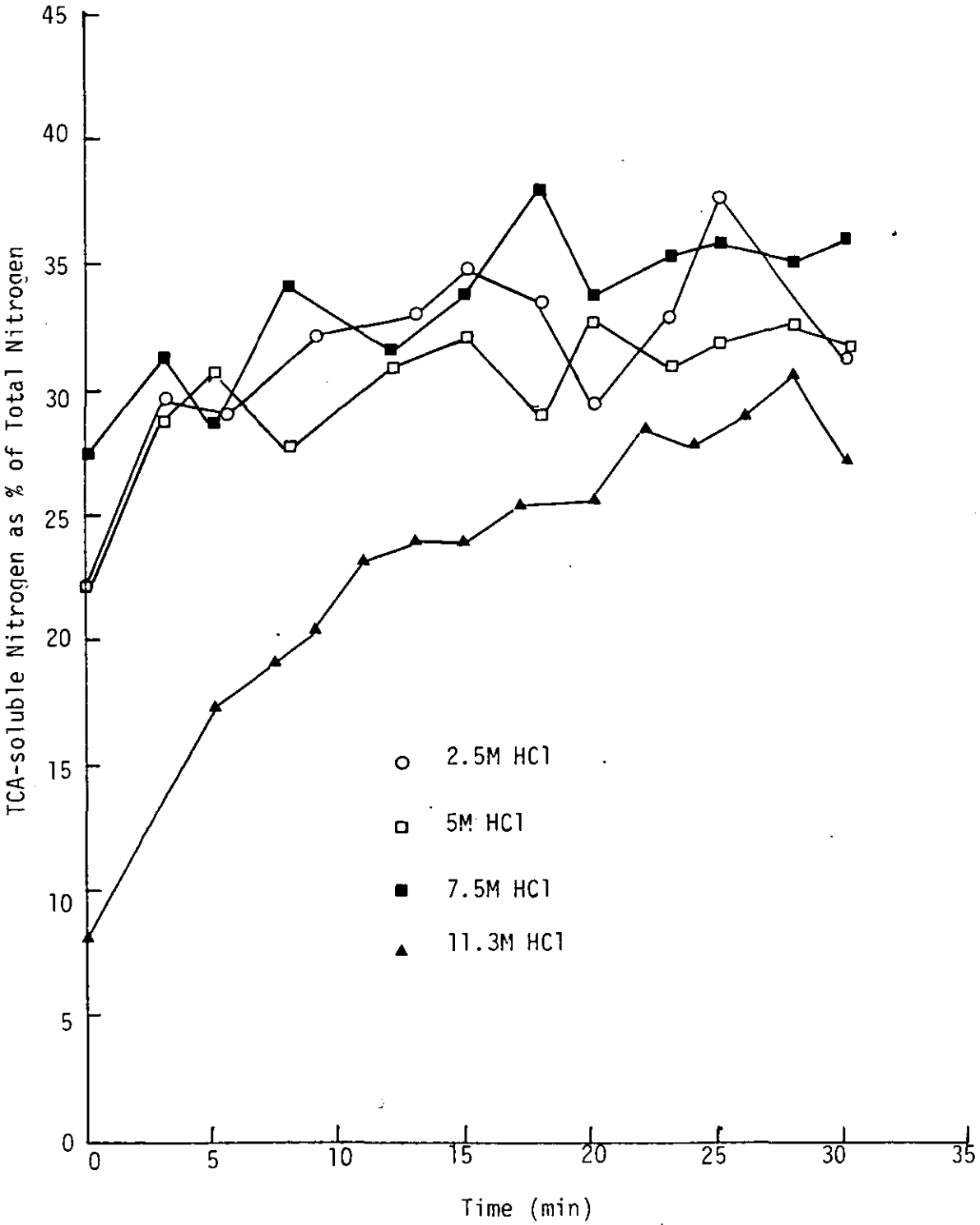


TABLE 11: Proximate composition of raw material and various products[1] resulting from acid hydrolysis of *Haplochromis*

Sample	Per cent[2]						Water[3] Activity (a_w)
	Total Nitrogen (TN)	Crude Protein (TNx6.25)	"True Protein" (TCA-insoluble Nx6.25)	Total Lipid	Ash	Salt (NaCl)	
Uncooked fish U	9.1 (0.5) n = 11	57.1 (3.4) n = 11	47.5 (0.3) n = 3	24.3 (0.8) n = 4	19.3 (0.1) n = 4	0.2 (0.01) n = 4	-
Cooked fish C	9.7 (0.4) n = 7	60.6 (2.3) n = 7	51.5 (0.2) n = 3	25.3 (1.3) n = 4	19.3 (0.1) n = 4	0.1 (0.05) n = 4	-
C2.5M	7.7 (0.05) n = 3	48.3 (0.2) n = 3	64.9 (0.5) n = 3	14.7 (1.1)	28.6 (0.2) n = 4	4.9 (1.9) n = 4	0.50
C5M	7.3 (0.1) n = 3	45.6 n = 3	61.9 (1.8) n = 3	13.6 (1.8)	37.3 (5.4) n = 4	10.2 (6.0) n = 4	0.59
C7.5M	6.6 (0.1) n = 3	41.4 (0.5) n = 3	60.8 (1.9) n = 3	14.5 (0.6)	38.5 (0.2) n = 4	16.1 (5.0) n = 4	0.47
C11.3M	5.6 (0.2) n = 4	34.9 (1.1) n = 4	60.0 (8.1) n = 4	9.5 (0.4) n = 4	49.9 (1.5) n = 4	38.0 (2.0) n = 4	0.36
UA	7.0 (0) n = 2	43.7 (0) n = 2	56.5 (2.4) n = 2	12.6 (1.2) n = 2	36.1 (0) n = 2	11.4 (0.1) n = 2	0.33
UB	7.3 (0.1) n = 2	45.6 (0.6) n = 2	67.2 (0.6) n = 2	11.2 (1.3) n = 2	31.1 (0.2) n = 2	0.6 (0.05) n = 2	0.34
CA	6.8 (0) n = 2	42.2 (0) n = 2	63.6 (1.3) n = 2	9.5 (0.6) n = 2	40.9 (0.1) n = 2	12.9 (0.25) n = 2	0.32
CB	6.6 (0.2) n = 2	41.2 (1.1) n = 2	58.3 (1.7) n = 2	9.0 (0.4) n = 2	40.9 (0.5) n = 2	1.8 (0.3) n = 2	0.34

Notes: (1) See Appendix 23 for explanation of sample codes. (2) Dry weight basis. (3) Single determination.
(4) Standard/mean deviation in parentheses.

FIGURE 25: The effect of acid concentration on acid hydrolysis of uncooked *Haplochromis*



proceeded for 30 minutes only. Yet in the few parallel studies that have been reported in the literature protein hydrolysis proceeds for several hours. Gordon *et al* (1941), for example, reported that during the partial acid hydrolysis of wool, edestin and gelatin using 10N HCl at 37^{o} only 22.9% amino nitrogen (expressed as % of total N) of gelatin was evolved after 19 hours of hydrolysis. Other similar work reviewed by Hill (1965) confirms that it takes several hours to effect appreciable amount of hydrolysis using acid. In a patent specification described by Hampton (1977) hydrolysis of protein by HCl is carried out at atmospheric pressure and/or at near the boiling point for 10 to 24 hours. It is therefore not surprising that in the case of *Haplochromis* relatively little hydrolysis took place. However, as pointed out in 4.2.2 the objective of this study was not to achieve maximum hydrolysis. The acid was primarily used to effect softening of the fish tissues for subsequent production of powder. Therefore from the results shown in Table 5 and other observations during the course of this study, any of the four acid concentrations could soften the fish tissues adequately. Uncooked fish would be a better choice of raw material than cooked fish.

4.2.4 Changes in pH During Hydrolysis

Figure 26 illustrates the changes in pH during hydrolysis of cooked fish at different temperatures. Figure 27 shows similar changes in uncooked fish and Table 6 summarises the nett changes in pH for both cooked and uncooked fish. It can be seen from both Figure 26 and Table 6 that in the case of cooked fish there was a nett pH drop between time 0 and 30 minutes. However the pH drop is neither gradual nor related to temperature of hydrolysis nor to the nett increase in TCA-soluble nitrogen (Table 4). It can also be seen in Figure 27 and Table 6 that except for the highest (84^{o}) and lowest (25^{o}) temperatures there was a nett pH drop for uncooked fish. As in the case of cooked fish there is no correlation between the nett pH drop and nett change in TCA-soluble nitrogen or temperature of

FIGURE 26: Changes in pH during acid hydrolysis of cooked *Haplochromis* at different temperatures (Acid concentration 11.3M HCl)

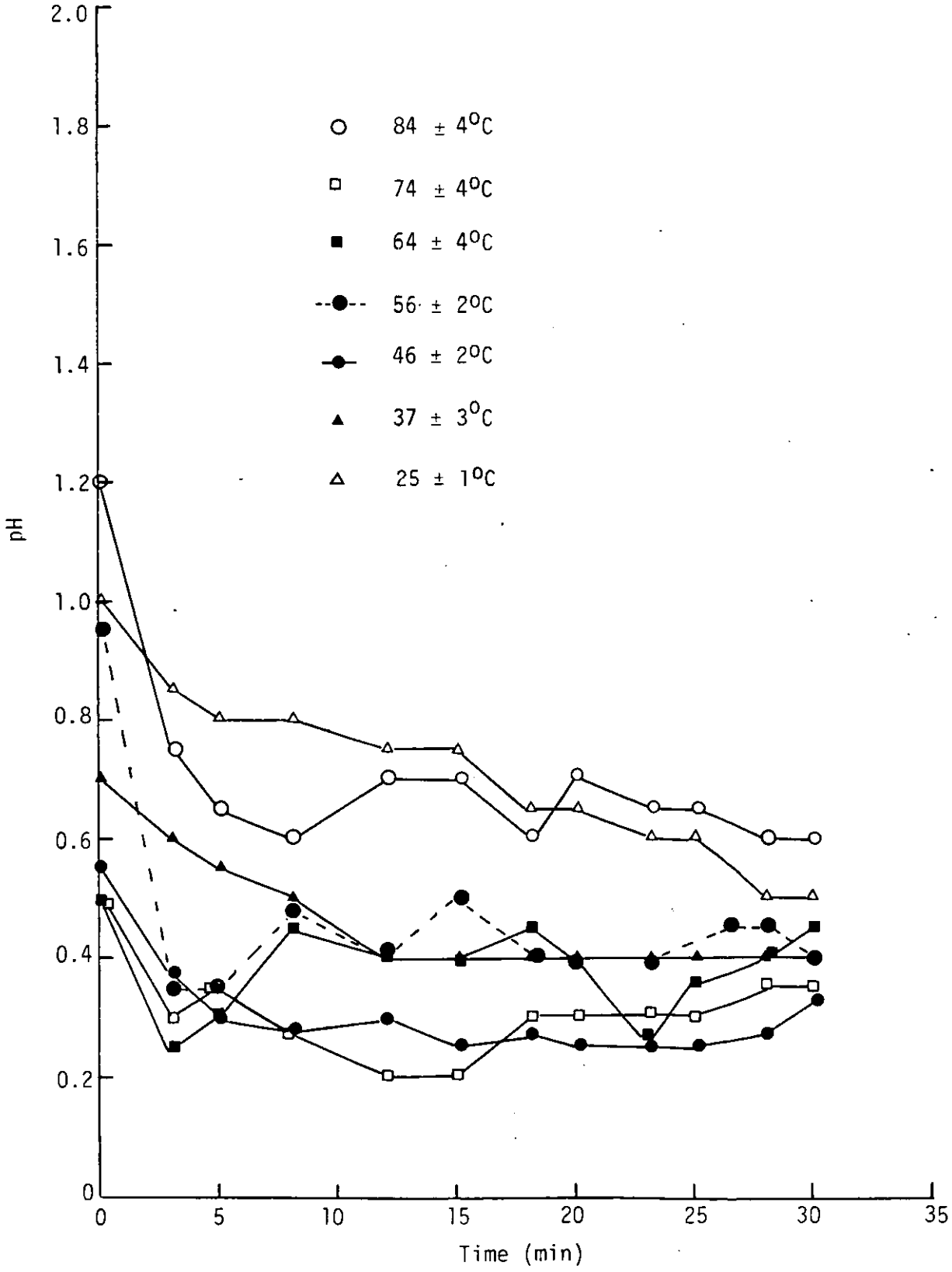


FIGURE 27: Changes in pH during acid hydrolysis of uncooked *Haplochromis* at different temperatures (Acid concentration 11.3M HCl)

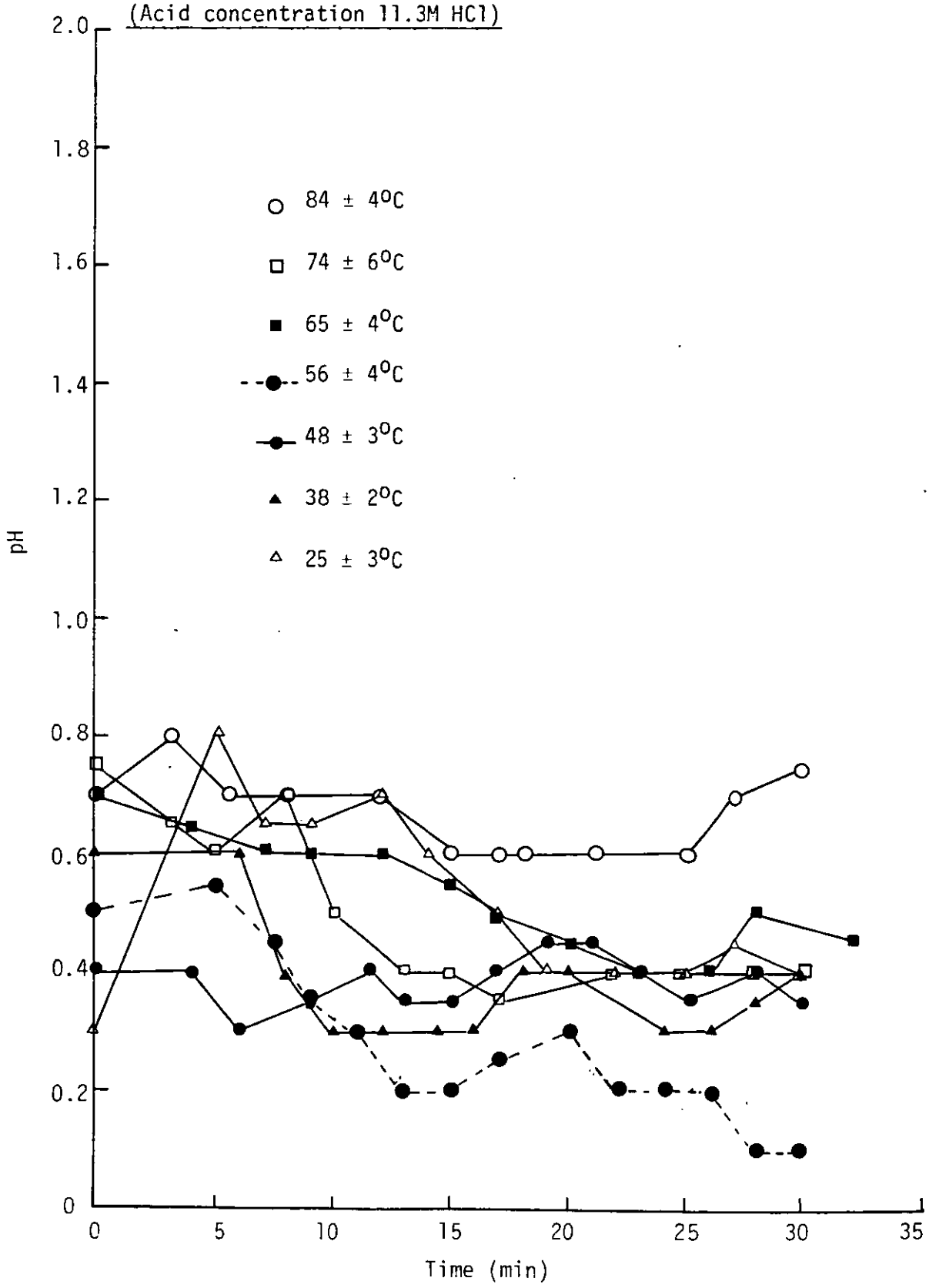


TABLE 6: Nett changes in pH after acid hydrolysis of cooked and uncooked *Haplochromis* at different temperatures (acid concentration 11.3M HCl)

Temperature[1] (°C)	Initial pH of Mince		Nett Change in pH	
	Cooked	Uncooked	Cooked	Uncooked
84 ± 4	7.2	6.9	-0.60	0.05
74 ± 4 (74 ± 6)	7.2	7.0	-0.15	-0.35
64 ± 4 (65 ± 4)	7.1	6.8	-0.05	-0.25
56 ± 2 (56 ± 4)	7.4	6.9	-0.55	-0.40
46 ± 2 (48 ± 3)	6.9	6.9	-0.23	-0.05
37 ± 3 (38 ± 2)	7.4	7.0	-0.30	-0.20
25 ± 1 (25 ± 3)	7.4	7.1	-0.50	0.10

(1) Temperature values in parentheses refer to uncooked fish, where different from those of cooked fish.

hydrolysis. A pH drop as observed is a characteristic feature of protein hydrolysis reactions. The decline in pH is caused by the freeing of amino acid carboxyl groups from the protein chain by the hydrolytic agent (acid or enzyme). In fact pH drop during proteolysis has been adapted into an *in vitro* method for determining protein digestibility (Hsu *et al*, 1977). The apparent irregularities in the pH-time curves can be attributed to a number of factors. The main one is the fact that there is a mixture of molecular species and ions in the reaction vessel which are likely to exhibit buffering tendencies. Therefore in the light of the complexity of the situation it is more meaningful to look at the general trends and overall picture (of the pH changes) rather than the details.

Figures 28 and 29 respectively show the changes in pH during hydrolysis of cooked and uncooked fish using different acid concentrations. Table 7 shows the nett changes in pH after hydrolysis. It is significant to note that in both cooked and uncooked fish, except for the highest acid concentration (11.3M) the pH increased rather than decreased over the 30 minute period. It is also of interest to note that the increase in pH was inversely proportional to the acid concentration. This observation could also be attributed to the buffering capacity of fish tissues. Studies on fish silage production have shown that the quantity of acid required to lower the pH to 2, for example, depends on the concentration of protein and ash in the raw material according to the following empirical formula (Raa and Gildberg, 1982)

$$(a \times 0.14 + b \times 0.9)\ell \text{ of } 14N \text{ acid per } 100 \text{ kg raw material}$$

where a = % crude protein of wet weight and b = % ash of wet weight. (The formula was based on work with a mixture of sulphuric and hydrochloric acid with a normality of 14). In this study a constant volume of acid was added to the fish mince, irrespective of acid concentration (see 3.3.2). Since the % protein and ash were constant it can be seen

from the empirical formula that the lower the acid concentration the less effect it had on lowering the pH. Due to the buffering capacity of the fish tissues the quantity of acid needed to lower the pH to a given value would be inversely proportional to the acid concentration.

FIGURE 28: Changes in pH during hydrolysis of cooked *Haplochromis* using different acid concentrations

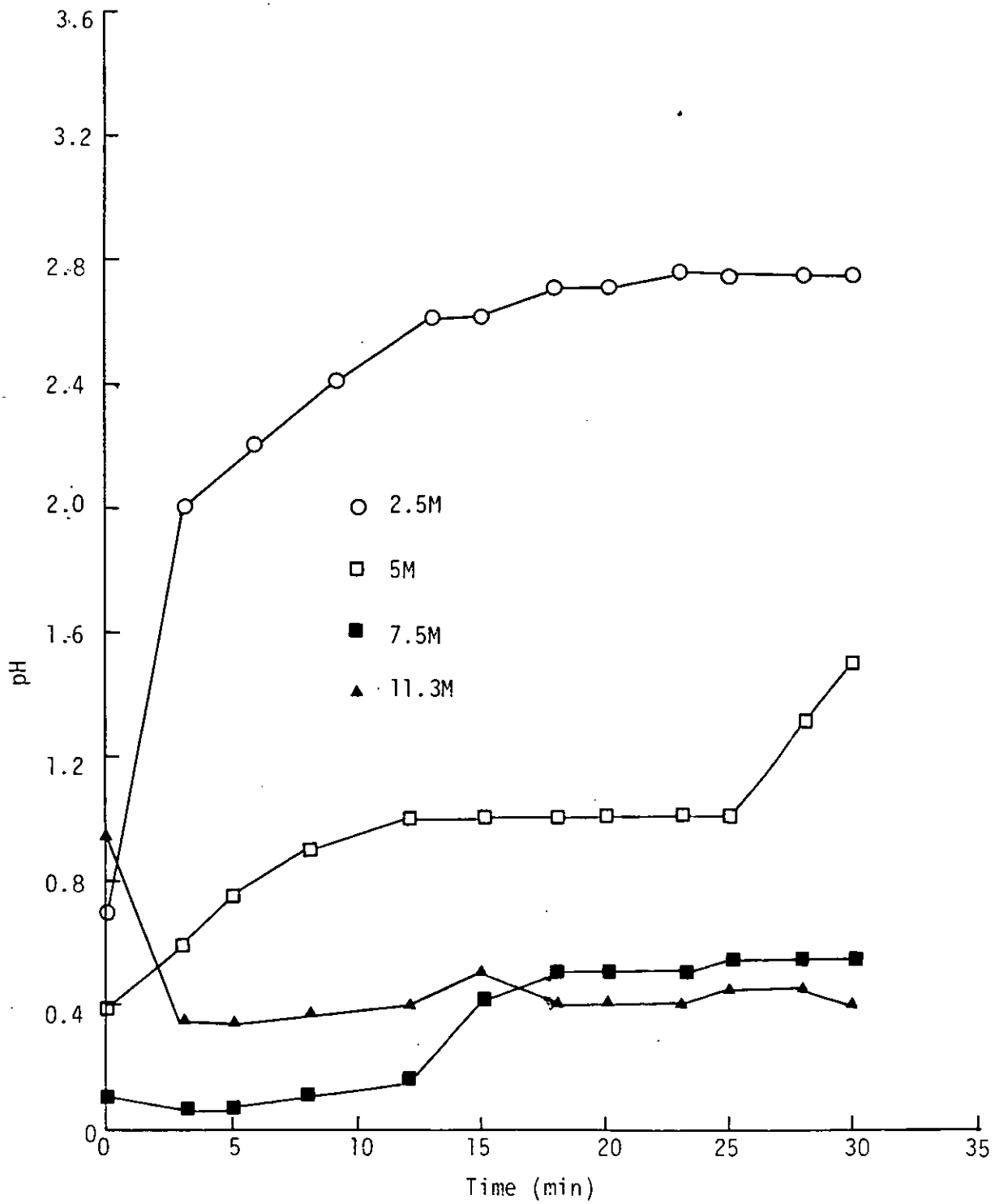


FIGURE 29: Changes in pH during hydrolysis of uncooked *Haplochromis* using different acid concentrations

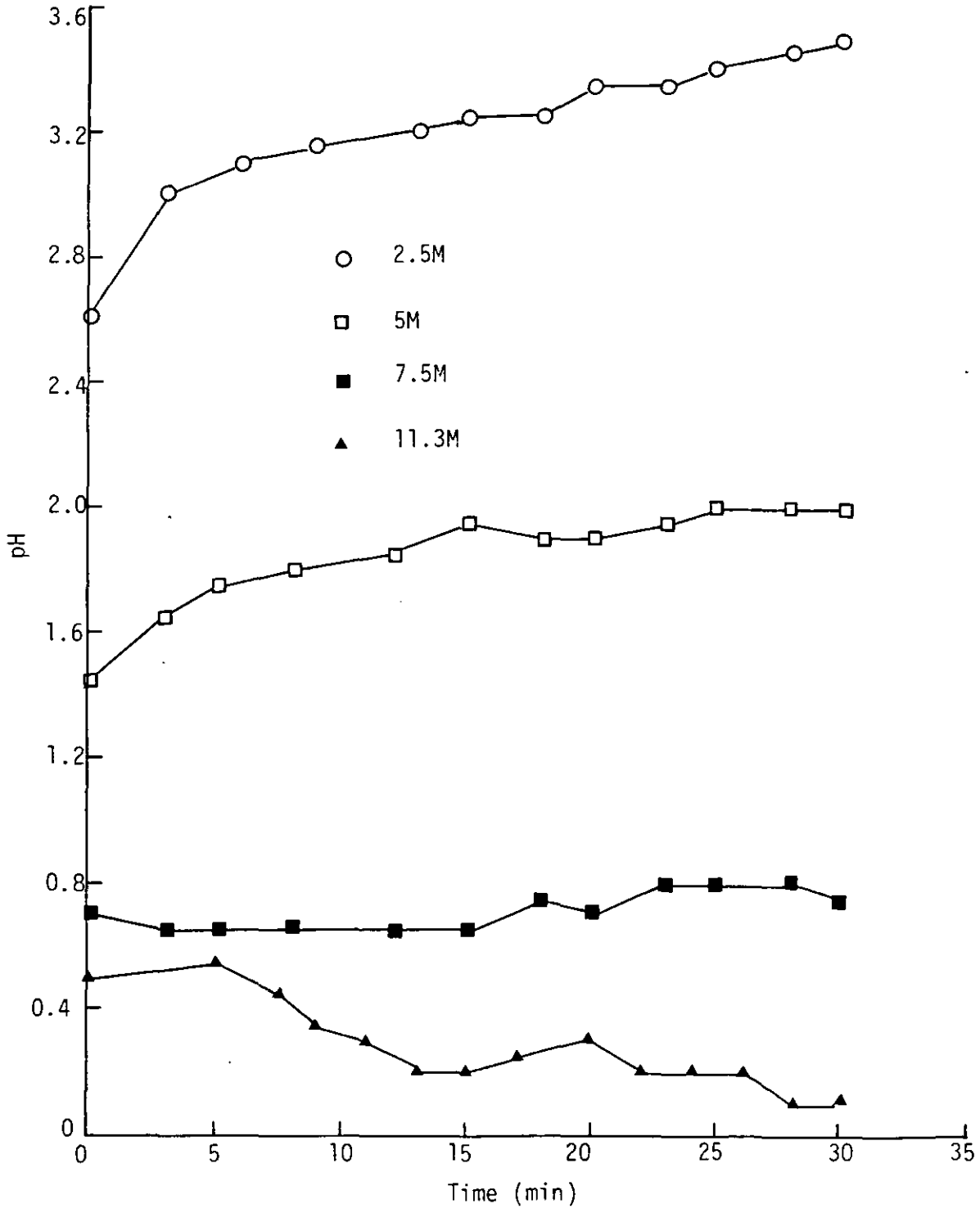


TABLE 7: Nett changes in pH after hydrolysis of cooked and uncooked *Haplochromis* using different acid concentrations

Acid Concentration (M)	Initial pH of Mince		Nett Change in pH	
	Cooked	Uncooked	Cooked	Uncooked
11.3	7.4	6.9	-0.55	-0.40
7.5	6.85	7.0	+0.45	+0.05
5	6.75	6.85	+1.10	+0.55
2.5	6.9	6.95	+2.05	+0.90

4.3 NEUTRALISATION

At the end of each hydrolysis "run" the acidic hydrolysate was neutralised with sodium hydroxide solution (10M, 7.5M, 5M and 2.5M depending on the acid concentration used for hydrolysis) to the original pH of the mince (Figure 8). Table 8 shows both the pH of the mince (prior to hydrolysis) and the neutralised hydrolysates for all the samples.

As the table shows the pH of the mince varied between 6.75 and 7.5, a variation which is not unusual. The pH of live or newly-caught fish is slightly alkaline and ranges between 7.05 and 7.35 (Cutting, 1969). After death, however, the pH falls due to the breakdown of muscle glycogen, anaerobially, to lactic acid. The final pH depends on factors such as the nutritional status of the fish. In starving fish there is very little glycogen in the muscle and the pH remains close to neutrality after death. In well fed fish, however, the larger amount of glycogen in the muscle gives rise to considerable amounts of lactic acid and hence low pH. A pH as low as 6.0 in sea-caught fish has been recorded (Love, 1980).

Post mortem pH is associated with various rather complicated phenomena such as *rigor mortis* and gaping in fish (Amlacher, 1961; Love, 1980). For the purposes of this study, however, the two phenomena would not be particularly important because the fish is comminuted and ultimately turned into powder. Measurement of pH of mince, therefore, only serves to establish the "end-point" of the neutralisation process.

The neutralisation step may offer an opportunity to alter the flavour of the final product. Orlova *et al* (1979) working on acid hydrolysis of fish, for example, found that a hydrolysate neutralised to pH 5.8-6.0 gave "acid taste and smell but neutral". A pH range of 6.6-7.0 gave rise to "sweet taste and ammonium smell" and when neutralised to pH 6.0-6.4 the hydrolysate gave a "pleasant meaty/mushroomy flavour and smell".

TABLE 8: pH of mince and neutralised hydrolysates of cooked and uncooked *Haplochromis*

Sample[1]	pH of Mince	pH of Neutralised Hydrolysate
C25	7.4	7.4
C37	7.4	7.4
C46	6.9	6.8
C56	7.0	7.0
C64	7.1	7.0
C74	7.2	7.2
C84	7.2	7.2
U25	7.1	6.8
U38	7.0	7.0
U48	6.9	6.9
U56	6.9	6.9
U65	6.8	6.8
U74	7.0	7.0
U84	6.9	6.9
C2.5M	6.9	6.9
C5M	6.75	6.75
C7.5M	6.85	6.85
U2.5M	6.95	6.95
U5M	6.85	6.85
U7.5M	7.0	7.0

(1) C = cooked fish; U = uncooked. See Appendix 23 for full explanation of codes.

It is also possible at the neutralisation stage to reduce the potential content of sodium (cf NaCl) in the final product (see 4.7.4). By using a mixture of KOH and NaOH solutions to neutralise the hydrolysate it is possible to reduce the amount of NaCl in the final product. Mixtures of NaCl and KCl have been used to produce organoleptically-acceptable low-sodium fish sauces (Chayovan *et al*, 1983).

4.4 HOMOGENIZATION

The homogenization step was incorporated because due to the varied nature of the raw material (flesh, skin, bones and scales) it was necessary to have a homogeneous hydrolysate prior to drying. After homogenization the hydrolysate was sieved using a metal sieve with an aperture of 710 microns. The object of sieving was to ensure that no unhomogenized particles remained in the hydrolysate for these could have blocked the spray drier nozzle.

All the 11.3M and 7.5M acid concentration hydrolysates were easily homogenized and no particles larger than 710 microns remained. (No particles were left behind after sieving). However the hydrolysates of the 5M and 2.5M acid concentrations had small quantities of soft cartilagenous material which had not been homogenized and could not pass through the sieve. It was found, however, that the cartilagenous material could easily be further softened (for subsequent homogenization) when returned to the reaction vessel.

4.5 BLEACHING

In the early stages of the experimental work the hydrolysates were drum dried. On examination, however, it was found that the drum dried products had a grey, mud-like appearance which the consumers might not like. So the idea of bleaching the hydrolysates before drum drying

was introduced. For every bleached sample there was a control which was unbleached. The results of bleaching, from the colour point of view, are discussed appropriately in Section 4.6.5.

As outlined in 3.3.5 the bleached hydrolysates were washed and centrifuged twice to remove residual hydrogen peroxide. It was also envisaged that the washing and centrifuging would result in reduction of total lipid in the final product. The effect of the washing and centrifuging procedures on true protein nitrogen (TCA-insoluble nitrogen TPN) and total nitrogen was also investigated and the results are shown in Figures 30 and 31 and Tables 9 and 10. The immediate observation is that in both cooked and uncooked fish, irrespective of temperature there was a loss (in varying amounts) of total lipid, TPN and total nitrogen during washing and centrifuging. In cooked fish the greatest loss in the amount of total lipid occurred in sample C37 (Table 9 and Figure 30) in which about 50% of the lipid was removed by washing and centrifugation. The least reduction in total lipid occurred in sample C74 where only about 10% of the original lipid was removed by washing and centrifugation. Over 80% of the lipid also remained in samples C56 and C64. In uncooked fish, however, most lipid was lost in sample U74 (Table 10 and Figure 31) where nearly 71% of the lipid was lost through washing and centrifugation. The least amount of lipid was lost in sample U48 where 40% of the original total lipid was washed and centrifuged out.

From Tables 9 and 10 and Figures 30 and 31 it can be seen that washing and centrifugation removed more lipid from uncooked than cooked fish. At first this may seem contrary to what would have been expected. A considerable proportion of lipid would have been expected to be removed from the cooked fish by washing and centrifugation. This is because experience from fish meal production has shown that cooking ruptures the cell walls releasing physiologically bound water and oil (Sikorski *et al*, 1984). However, what was observed in this study was that nearly

TABLE 9: The effect of centrifuging and washing on total lipid (TL), total nitrogen (TN) and true protein nitrogen (TPN) of cooked *Haplochromis* hydrolysate

Sample[1]	A[2]	B[3]	A	B	A	B	Final lipid as % of TL in raw material	Final TPN as % of TN of raw material	Final TN as % of TN of raw material
C Raw material	24.2		9.4		8.5		100	90.4	100
C25	12.0 (0.6)	13.7 (0.3)	8.9 (0.1)	8.0 (0.1)	6.7 (0.1)	6.5 (0.2)	56.6	69.1	85.1
C37	10.8 (0.8	12.2 (0.4)	8.2 (0.2)	7.5 (0.4)	7.1 (0.2)	6.8 (0.2)	50.4	72.3	79.8
C46	14.8 (0.5)	16.2 (0.5)	8.5 (0.3)	7.9 (0.4)	7.3 (0.3)	6.8 (0.2)	66.9	72.3	84.0
C56	17.6 (1.6)	19.4 (1.5)	7.8 (0.4)	7.5 (0.5)	7.0 (0.5)	6.5 (0.6)	80.2	69.1	79.8
C64	17.2 (0.3)	20.2 (0.4)	7.8 (0.6)	7.3 (0.4)	6.8 (0.4)	6.1 (0.2)	83.5	64.9	77.6
C74	11.2 (0.3)	21.6 (1.2)	6.6 (0.8)	5.8 (0.8)	6.3 (0.2)	5.6 (0.3)	89.2	59.6	61.7
C84	11.6 (0.3)	14.1 (0.6)	6.7 (0.4)	6.1 (0.5)	6.0 (0.5)	4.2 (0.4)	58.3	44.7	64.9

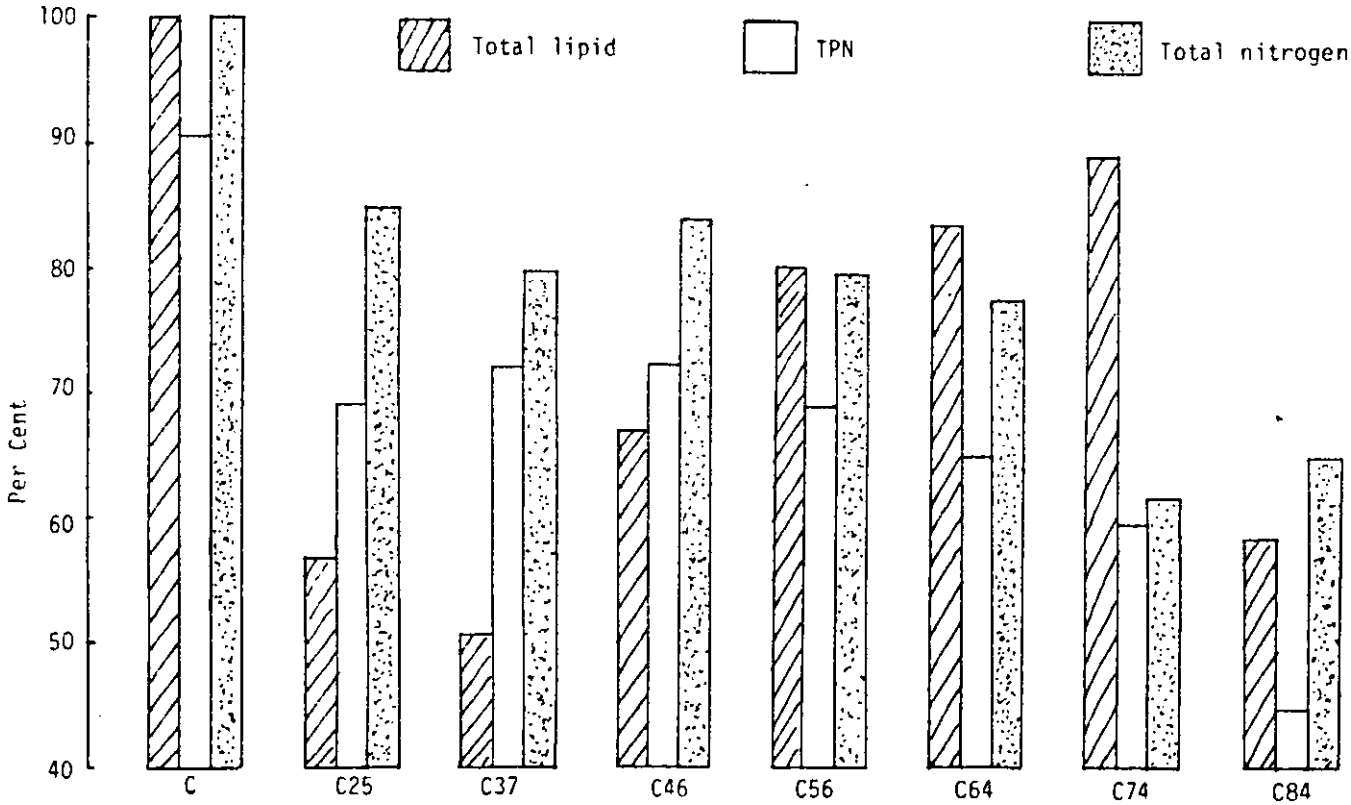
Notes:
(1) See Appendix 23 for explanation of sample codes
(2) Hydrolysate after 1st centrifugation (see 3.3.5)
(3) Hydrolysate after 2nd centrifugation (see 3.3.5)
(4) Mean deviation in parentheses

TABLE 10: The effect of centrifuging and washing on total lipid (TL), total nitrogen (TN) and true protein nitrogen (TPN) of uncooked *Haplochromis* hydrolysate

Sample[1]	TL (%)		TN (%)		TPN (%)		Final lipid as % of TL of raw material	Final TPN as % of TN of raw material	Final TN as % of TN of raw material
	A[2]	B[3]	A	B	A	B			
U Raw material	22.9 (0.4)		8.9 (0.6)		8.0 (0.2)		100	89.9	100
U25	11.5 (0.5)	12.2 (1.1)	7.3 (0.2)	7.2 (0.5)	6.2 (0.2)	5.9 (0.1)	53.3	66.3	80.9
U38	11.9 (0.1)	12.3 (0.9)	7.3 (0.4)	6.9 (0.6)	6.5 (0.4)	6.0 (0.1)	53.7	67.4	77.5
U48	9.8 (1.1)	13.7 (0.6)	7.0 (0.2)	6.8 (0.2)	6.3 (0.1)	6.0 (0.1)	59.8	67.4	76.4
U56	10.3 (1.2)	10.1 (0.7)	7.2 (0.3)	6.7 (0.2)	6.6 (0.3)	6.3 (0.1)	44.1	70.8	75.3
U65	13.7 (0.9)	8.5 (0.7)	5.7 (0.2)	5.5 (0.3)	5.6 (0.1)	5.0 (0.0)	37.1	56.2	61.8
U74	10.1 (0.4)	6.7 (0.6)	6.8 (0.3)	6.2 (0.3)	3.2 (0.2)	2.9 (0.2)	29.2	32.6	69.7
U84	10.4 (2.0)	10.7 (1.6)	5.7 (0.1)	5.2 (0.1)	2.4 (0)	2.3 (0)	46.7	25.8	58.4

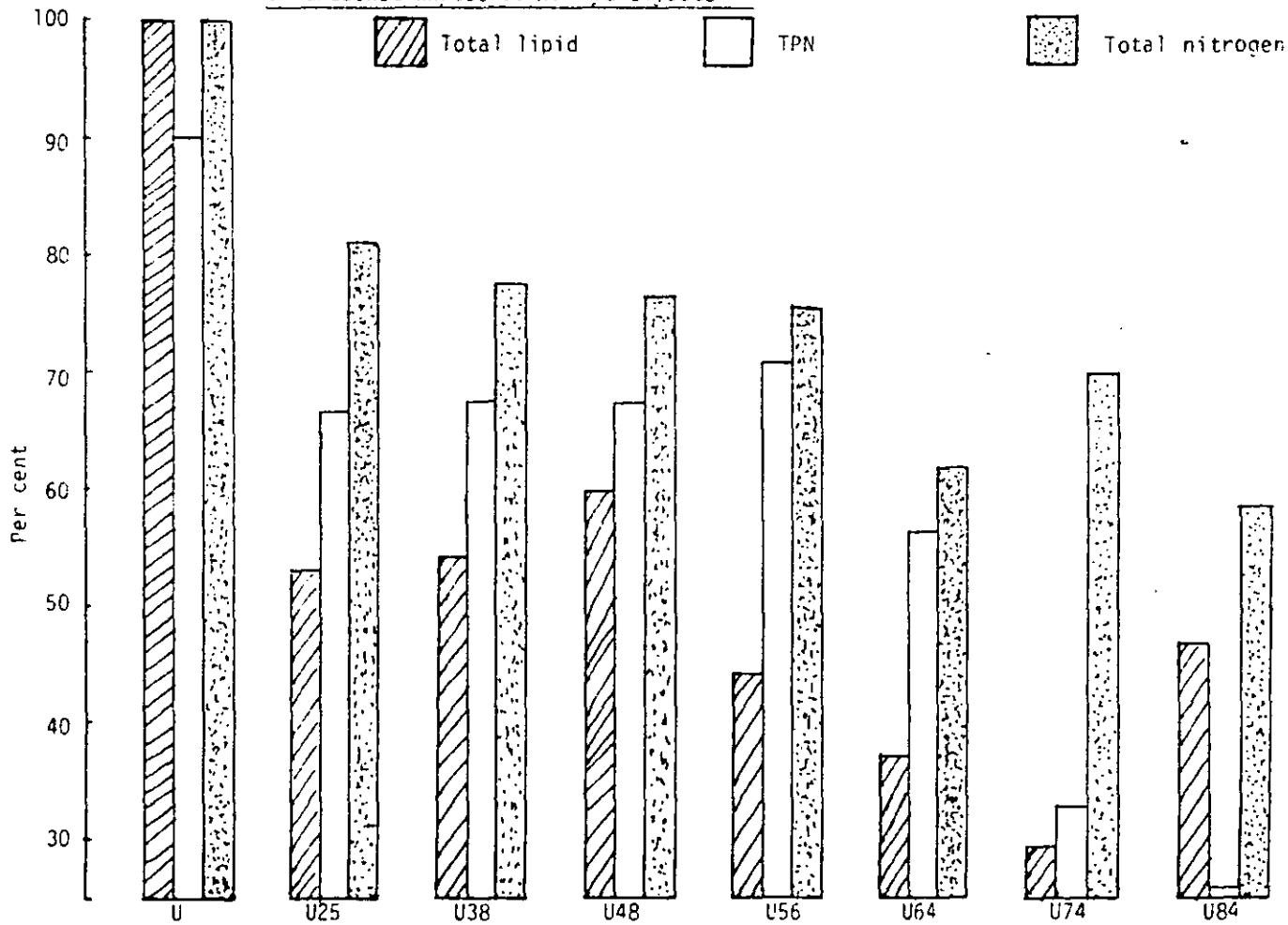
Notes: (1) See Appendix 23 for explanation of sample codes.
(2) Hydrolysate after first centrifugation (see 3.3.5).
(3) Hydrolysate after second centrifugation (see 3.3.5).
(4) Mean deviation in parentheses.

FIGURE 30: The effect of centrifuging and washing on total lipid[1], total nitrogen[2] and TCA-insoluble nitrogen (TPN)[3] of cooked *Aristichromis* hydrolysate[4]



(1) Final lipid content (after 2nd centrifugation - see 3.3.5) as % of total lipid of raw material.
(2) Final total nitrogen (after 2nd centrifugation - see 3.3.5) as % of total nitrogen of raw material.
(3) Final TCA-insoluble N (after 2nd centrifugation - see 3.3.5) as % of total nitrogen of raw material.
(4) See Appendix 23 for explanation of sample codes.

FIGURE 31: The effect of centrifuging and washing on total lipid(1), total nitrogen(2) and TCA-insoluble nitrogen (TPN)(3) of uncooked *Haplochromis* hydrolysate(4)



(1) Final lipid content (after 2nd centrifugation - see 3.3.5) as % of total lipid of raw material.
(2) Final total nitrogen (after 2nd centrifugation - see 3.3.5) as % of total nitrogen of raw material.
(3) Final TCA-insoluble N (after 2nd centrifugation - see 3.3.5) as % of total nitrogen of raw material.
(4) See Appendix 23 for explanation of sample codes.

50% of the lipid was lost in sample C37 through washing and centrifuging but only 10% was lost in sample C74. Yet in uncooked fish, about 46% of the lipid was removed from U38, the sample corresponding to C37 and about 71% of the lipid was removed from U74 the corresponding sample to C74. From the data presented above and also the results in Table 2, it would appear that the fish was not cooked for long enough for the cells to rupture. (In fish meal production the fish is cooked at about 100°C for about 20 minutes (Sikorski *et al*, 1984). The fish in this study was only cooked for 5 minutes (see 3.3.1). It is possible that the cooking weakened the cells enough to be ruptured when mixed with the acid at the lower temperatures, hence the total lipid was reduced to 56.6% and 50.4% of the original amount in samples C25 and C37 respectively. As the temperature increased however some changes in the fish tissues must have taken place which resulted in more lipid being retained even after washing and centrifugation (Figure 30). Theoretically there is a possibility that ionic linkages could be formed between phosphate groups of phospholipids and NH_3^+ of proteins (Asghar and Henrickson, 1982). Ester and amide linkages, also, could have been formed between NH_3^+ and COO^- groups (of protein) and fatty acids. The end result would be the trapping of the lipid in the form of emulsions thus retaining it even after washing and centrifugation. The substantial decrease in the lipid content of C84 could be explained in terms of the increased hydrolytic effect of the acid at 84° (see 4.2.2) resulting in disruption of the lipid-protein bonds.

In uncooked fish, however, the situation was different. Due to the presence of proteolytic enzymes the protein was readily hydrolysed into smaller molecules in such a way that fewer lipid-protein bonds were formed, hence the marked reduction in total lipid as a result of washing and centrifugation.

Although bleached hydrolysates were lighter in colour than unbleached ones (see 4.6.5) the bleaching procedure brought about some major

undesirable effects in the product. In the first place there were considerable losses of TPN ranging between 22% and 68% in cooked fish and 24% and 111% in uncooked fish. Such high protein losses arose mainly from the fact that the hydrates contained salt (NaCl - a by-product of the neutralisation process) which on addition of water formed an ideal extraction medium for the proteins. When the hydrolysates were centrifuged and the supernatant discarded salt soluble proteins were discarded with it, and some of this was TPN. It is significant to note that TPN losses were higher in uncooked fish than cooked fish. TPN losses were related to the amount of hydrolysis that had taken place in the samples. In hydrolysates where appreciable amount of hydrolysis had taken place (as shown by nett increase in TCA-soluble N of sample cf. Table 4) the TPN losses were high. In uncooked fish hydrolysis was about three times as much as that in cooked fish (Table 4 and Section 4.2.1).

Another undesirable effect of the bleaching procedure was the possibility of oxidative rancidity induced by the hydrogen peroxide. The presence of salt, unsaturated fatty acids and hydrogen peroxide (an oxygen donor) in the hydrolysate would favour the initiation and propagation steps of oxidative rancidity (see 2.3.2). Hydrogen peroxide has also been associated with the destruction of certain amino acids particularly methionine, cystine and tyrosine (Koning and Rooijen, 1972). There are reports, however, where it has successfully been used to improve the colour of fishery products such as fish protein concentrate type A (FPC A) (Rasekh *et al*, 1972; Orlova *et al*, 1977) and marinated herring (Sims *et al*, 1975) without affecting the nutritional value of the protein.

It was in the light of the undesirable effects discussed above that the bleaching procedure was abandoned. Since it was introduced as a means of improving the colour of drum dried hydrolysates, in the first place, the drum drying method was also abandoned in favour of spray drying.

4.6 PRODUCT AND PROCESS EVALUATION

Among the objectives of this study was to produce a fish powder of high nutritional value and microbiologically and chemically stable at tropical ambient conditions. Emphasis was placed on chemical analytical work as a means of assessing the nutritional quality of the products. The stability of the products would also be assessed in the light of the chemical composition data.

4.6.1 Protein

4.6.1.1 Total nitrogen (TN), TCA-soluble nitrogen/ non-protein nitrogen (NPN) and TCA-insoluble nitrogen/true protein nitrogen (TPN)

Table 11 and Figure 32 show the TN, NPN and TPN of the raw material and various products. "Crude protein" and "true protein" values were derived by multiplying the TN and TPN values respectively by a factor of 6.25. Normally true protein values are lower than crude protein values because low molecular weight nitrogenous substances are excluded from the latter (see 2.3.1). In Table 11 and Figure 32, however, true protein values are higher than crude protein values except for samples U and C. This is because true protein and crude protein were determined on samples of slightly different composition. In determining true protein the samples were washed with trichloro-acetic acid solution to remove the non-protein nitrogenous compounds and in the process some of the salt contained in the products was eluted. This resulted in the nitrogen fraction increasing at the expense of the ash fraction. Crude protein, however, was determined on samples which contained all the salt effectively reducing the nitrogen fraction.

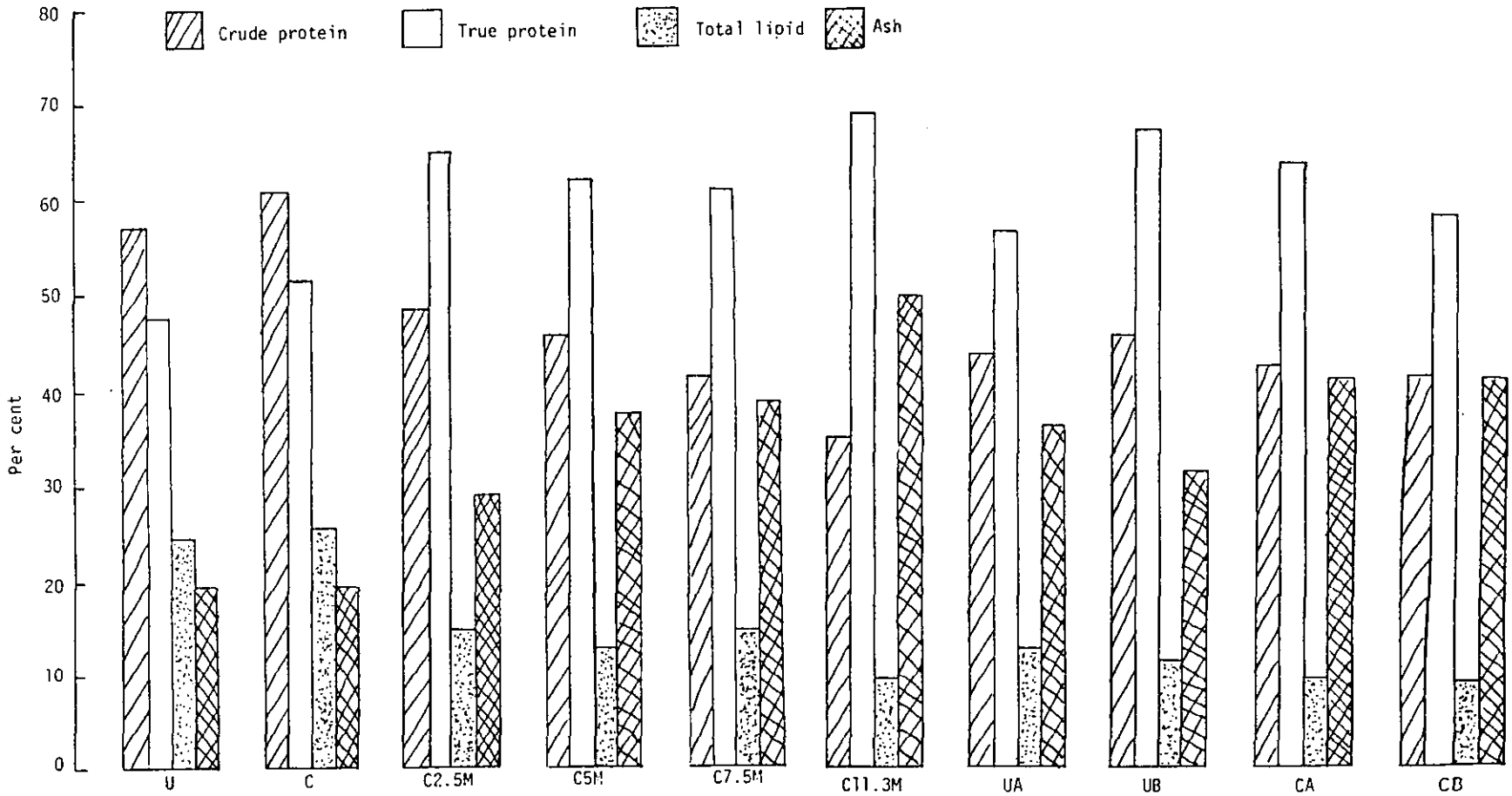
Overall the results indicate that even after processing (true) protein formed the largest single component of the products, just as in the raw material. The crude protein figures, on the other hand, indicate

TABLE 11: Proximate composition of raw material and various products[1] resulting from acid hydrolysis of *Haplochromis*

Sample	Per cent[2]						Water[3] Activity (a_w)
	Total Nitrogen (TN)	Crude Protein (TNx6.25)	"True Protein" (TCA-insoluble Nx6.25)	Total Lipid	Ash	Salt (NaCl)	
Uncooked fish U	9.1 (0.5) n = 11	57.1 (3.4) n = 11	47.5 (0.3) n = 3	24.3 (0.8) n = 4	19.3 (0.1) n = 4	0.2 (0.01) n = 4	-
Cooked fish C	9.7 (0.4) n = 7	60.6 (2.3) n = 7	51.5 (0.2) n = 3	25.3 (1.3) n = 4	19.3 (0.1) n = 4	0.1 (0.05) n = 4	-
C2.5M	7.7 (0.05) n = 3	48.3 (0.2) n = 3	64.9 (0.5) n = 3	14.7 (1.1)	28.6 (0.2) n = 4	4.9 (1.9) n = 4	0.50
C5M	7.3 (0.1) n = 3	45.6 n = 3	61.9 (1.8) n = 3	13.6 (1.8)	37.3 (5.4) n = 4	10.2 (6.0) n = 4	0.59
C7.5M	6.6 (0.1) n = 3	41.4 (0.5) n = 3	60.8 (1.9) n = 3	14.5 (0.6)	38.5 (0.2) n = 4	16.1 (5.0) n = 4	0.47
C11.3M	5.6 (0.2) n = 4	34.9 (1.1) n = 4	69.0 (8.1) n = 4	9.5 (0.4) n = 4	49.9 (1.5) n = 4	38.0 (2.0) n = 4	0.36
UA	7.0 (0) n = 2	43.7 (0) n = 2	56.5 (2.4) n = 2	12.6 (1.2) n = 2	36.1 (0) n = 2	11.4 (0.1) n = 2	0.33
UB	7.3 (0.1) n = 2	45.6 (0.6) n = 2	67.2 (0.6) n = 2	11.2 (1.3) n = 2	31.1 (0.2) n = 2	0.6 (0.05)n = 2	0.34
CA	6.8 (0) n = 2	42.2 (0) n = 2	63.6 (1.3) n = 2	9.5 (0.6) n = 2	40.9 (0.1) n = 2	12.9 (0.25)n = 2	0.32
CB	6.6 (0.2) n = 2	41.2 (1.1) n = 2	58.3 (1.7) n = 2	9.0 (0.4) n = 2	40.9 (0.5) n = 2	1.8 (0.3) n = 2	0.34

Notes: (1) See Appendix 23 for explanation of sample codes. (2) Dry weight basis. (3) Single determination.
(4) Standard/mean deviation in parentheses.

FIGURE 32: Proximate composition of raw material and various products[1] resulting from acid hydrolysis of *Haplochromis*



(1) See Appendix 23 for explanation of sample codes.

that as the acid concentration increased the crude protein effectively decreased. The crude protein content of C2.5M for example was approximately 6%, 15% and 32% higher than that of C5M, C7.5M and C11.3M respectively. The crude protein decreased because of a corresponding increase of the ash and salt after neutralisation.

Samples C11.3M, UA, UB, CA and CB were produced using 11.3M HCl. However, the crude protein content of C11.3M is, on average, 16% lower than that of the others because some of the salt in the latter was removed through washing and centrifuging prior to drying (see 3.3.5 and Table 11).

The crude protein content of all the products is higher than that of salted and dried *Haplochromis* developed by Dhatemwa (1981) (protein content 28%) and roller dried *Haplochromis*/carbohydrate mixtures developed in Tanzania (Jensen *et al*, 1982) (protein content 30%). Both the salted cakes and fish/carbohydrate mixtures are still under development and intended for the East African market.

4.6.1.2 Amino acids

Table 12 shows the amino acid profiles of the raw material and eight final products of acid hydrolysis of whole *Haplochromis*. The amino acid profile of the raw material was included as a reference with which to compare the products. The aspartic acid, serine, glycine and alanine contents of all the products were considerably lower than those of the raw material. Threonine was about 52% and 10% lower in C2.5M and C5M respectively than in the raw material. Methionine was approximately 32% lower in C5M and C7.5M than in the raw material. Lysine was about 11% lower in C2.5M, C5M and CA and 18% lower in C7.5M than in the raw material. The observed lower values in the products may have been due to the destruction of some of the amino acids by the acid during processing. There is also a possibility

TABLE 12: Amino acid profiles of *Haplochromis* and various products resulting from acid hydrolysis of the whole fish(1)

Amino Acid g/100g Protein	Sample								
	C2.5M	C5M	C7.5M	C11.3M	UA	UB	CA	CB	Whole Fish(2)
Aspartic acid	5.1	8.7	8.2	10.6	10.5	9.2	9.7	10.7	11.8
Threonine	3.0	4.6	4.9	5.1	5.8	5.1	5.7	5.5	5.1
Serine	4.6	5.6	7.1	6.9	7.0	6.6	6.4	6.7	9.7
Glutamic acid	17.4	15.6	16.5	18.8	18.6	16.0	17.2	16.6	19.0
Glycine	6.8	6.8	5.9	7.3	4.4	4.7	4.5	3.9	8.5
Alanine	6.9	6.6	5.8	6.6	5.7	5.3	5.7	5.5	7.3
Cystine	0.7	0.8	0.9	0.2	1.2	0.7	1.0	<0.1	0.1
Valine	3.6	3.8	3.5	3.5	4.4	3.7	4.2	4.3	3.5
Methionine	1.8	1.6	1.6	2.2	2.5	1.9	2.2	2.4	2.2
Isoleucine	3.8	3.5	3.1	4.1	4.7	3.8	4.4	5.1	4.2
Leucine	7.6	7.4	6.7	8.2	9.1	7.6	8.6	9.0	8.1
Tyrosine	2.7	2.5	2.4	2.8	3.6	2.9	3.7	3.7	2.9
Phenyl-alanine	3.8	3.6	3.7	4.1	4.8	4.3	4.8	5.7	4.1
Ammonia	0.5	0.5	0.5	0.6	0.4	0.5	0.4	0.4	0.6
Lysine	7.5	7.5	7.0	8.1	8.4	8.7	7.5	7.8	8.4
Histidine	1.8	1.9	1.7	2.3	2.3	2.0	2.1	2.2	2.0
Arginine	5.7	5.8	5.4	6.1	6.6	5.2	5.5	5.6	-
Hydroxy-proline	3.3	3.2	2.5	2.5	-	-	2.5	0.9	4.0
% Protein (N x 6.25)	48.1	45.6	41.2	34.4	43.7	45.6	42.2	41.2	55.6

Notes:

(1) All single determinations.
(2) Whole fish dried in oven and ground.
(3) See Appendix 23 for explanation of sample codes.

that some amino acids are lower than expected due to incomplete hydrolysis of the bonds involving them, during the preparation of the samples for amino acid analysis. The stability of peptide bonds formed by valine, isoleucine and leucine, for example, may lead to low yields of these amino acids in total hydrolysates (Hill, 1965). This may explain why the isoleucine values for C5M and C7.5M were 18% and 30% respectively lower than the raw material values. Leucine values for C5M and C7.5M were also 9% and 19% respectively lower than the corresponding values for the raw material.

Generally more amino acid destruction (or incomplete hydrolysis) occurred in C2.5M, C5M and C7.5M than in the other products. The relative ease with which certain amino acids are destroyed is a direct result of the lability of the bonds involving those amino acids. For example, the peptide bonds formed by the amino groups of serine and threonine are reported to be extremely labile. Hydrolysis of proteins in 10N HCl at 30°C and 12N HCl at 20°C results in rapid liberation of amino groups of the hydroxyamino acids, without much effect on other end groups (Hill, 1965). Studies on total acid hydrolysis of protein for 24 hours with 6N HCl at 110°C, under conditions which rigidly exclude oxygen, non-protein substances and metals have shown that 5-10% of the serine and threonine are destroyed. The results in Table 12, however, show that the serine content of all the products was considerably lower (31-73%) than that of the raw material. The conditions of acid hydrolysis of *Haplochromis* to produce the products in the table favoured the extensive destruction of the amino acids (presence of oxygen and other non-protein substances).

Other amino acids such as cysteine, aspartic acid, glutamic acid, lysine, arginine, tyrosine and proline have also been reported to be destroyed by acid, though to a lesser extent (Hill, 1965).

Hydroxyproline contributed between 2.5% and 3.8% of the amino acids analysed in samples C2.5M, C5M, C7.5M, C11.3M and CA. In the raw

material hydroxyproline accounted for 3.9% of the amino acids analysed. The amino acid is a structural component of collagen (skin, bones, scales and eyeballs).

A certain amount of ammonia was detected in all samples. This was most likely to have arisen from the conversion of glutamine to glutamic acid and asparagine to aspartic acid. Some ammonia is also reported to originate from liberation of α-amino nitrogen of serine and threonine (Smyth *et al*, 1962).

When the amino acid values for whole fish in Table 12 are compared with those obtained from earlier studies (Appendix 24) it is found that there is some appreciable difference. Generally the values in Table 12 are lower than those in Appendix 24. The difference may be due to experimental error or variation between laboratories. It is also important to take into account the fact that the results in Table 12 are based on a single determination.

The purpose of determining the amino acid content of a protein, from a nutritional point of view is to assess the protein as a source of the amino acids essential in man (Appendix 25). Ssali (1981) found that whole *Haplochromis* would provide all the essential amino acids (except tryptophan which was not determined) in good quantities. When the values for the amino acid content of the products of acid hydrolysis in Table 12 are compared with the essential amino acid requirements in man it can be seen that the products still provide the essential amino acids in sufficient quantities. The exceptions are tryptophan which was not determined and cystine (sulphur containing) which was determined; but some cystine may have been destroyed by acid. It would be best to determine cystine as cysteic acid (Moore, 1963; Hirs, 1967) and tryptophan by alkaline hydrolysis or other methods discussed in 2.5. From a nutritional point of view also the lysine content is best evaluated as "available lysine" (Booth, 1971).

The amino acid analysis results in this study should not be regarded as conclusive but should only serve as a guide when assessing the effect of acid hydrolysis (as a processing method) on the nutritional value of *Haplochromis* powder. The results show that the fish powder produced by acid hydrolysis would be a valuable source of essential amino acids in particular. Conclusive data on amino acid content of the fish powder produced by acid hydrolysis would be best obtained using a hydrolytic technique which qualitatively and quantitatively yields all the amino acids in the protein. By using the reagent 4N methanesulphonic acid containing 0.2% 3-(2-aminoethyl) indole instead of 6N HCl to hydrolyse the protein Simpson *et al* (1976) demonstrated that complete amino acid analysis of proteins from a single hydrolysate can be achieved. For complete assessment of the nutritive value of a protein, however, amino acid profiling should be complemented with other studies such as digestibility of the protein.

4.6.1.3 Protein digestibility

Although the amino acid profile of a protein is a useful indicator of its nutritional value, the ultimate test is the digestibility and availability of its amino acids. The classic method for determining protein digestibility is by using rat bioassays but this is an expensive and time consuming procedure. A number of *in vitro* methods, however, have been developed for measuring protein digestibility. Although they have some shortcomings their main advantages are that they are much quicker and cheaper than the bioassay method. In this study an *in vitro* method described by Hsu *et al* (1977) (see 3.4.1.3) was used to assess the digestibility of the proteins of four *Haplochromis* powders (C2.5M, C5M, C7.5M and C11.3M). The results are shown in Figures 33-36 and Appendices 26-29.

The *in vitro* protein digestibility method used in this study is based on measurement of pH drop when enzyme is added to the protein substrate. The pH drop results from the release of carboxyl groups from the protein

FIGURE 33: pH vs time curves obtained by incubation of four *Haplochromis* powders and casein with the multienzyme system

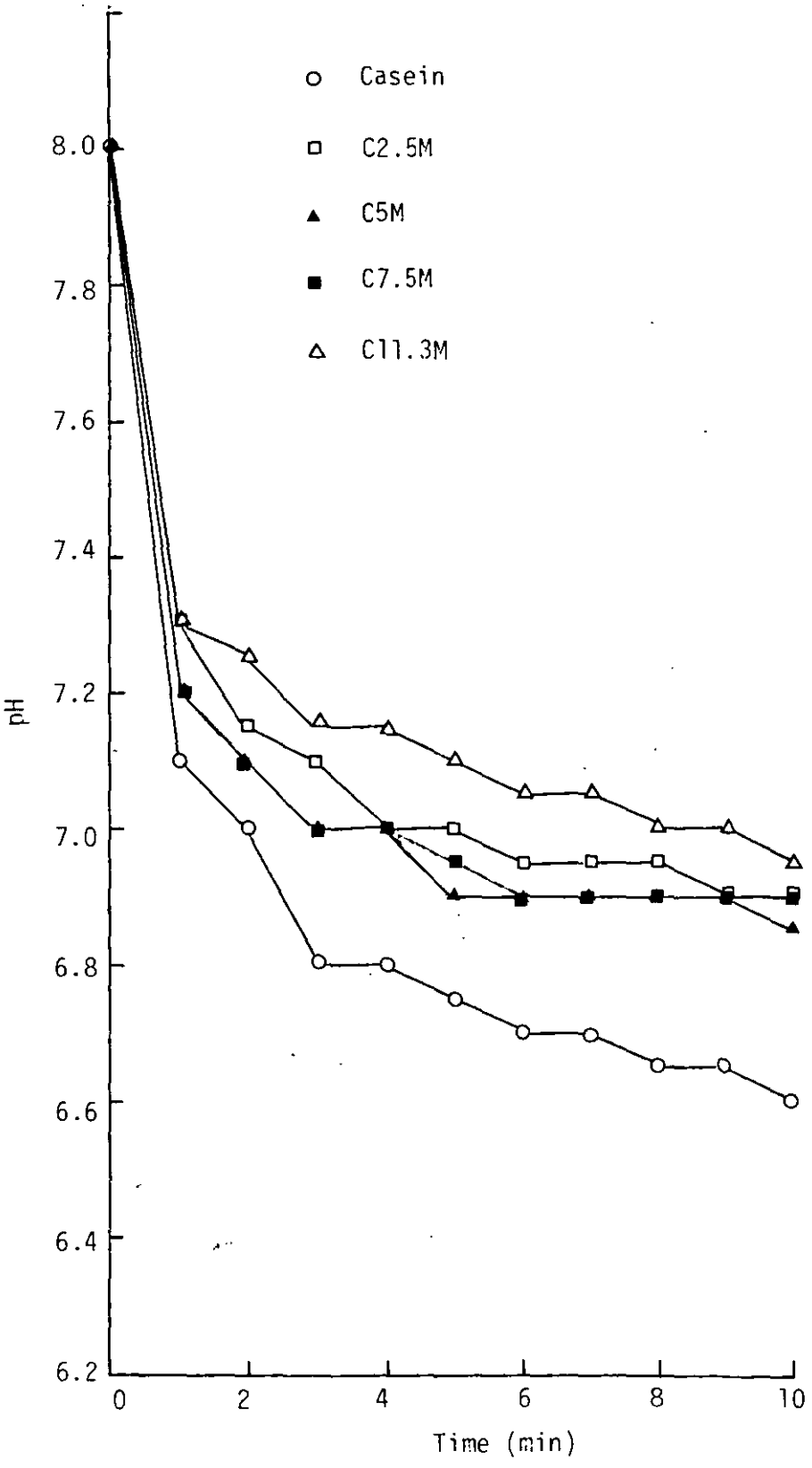


FIGURE 34: pH vs time curves obtained by incubation of four *Haplochromis* powders and casein with chymotrypsin

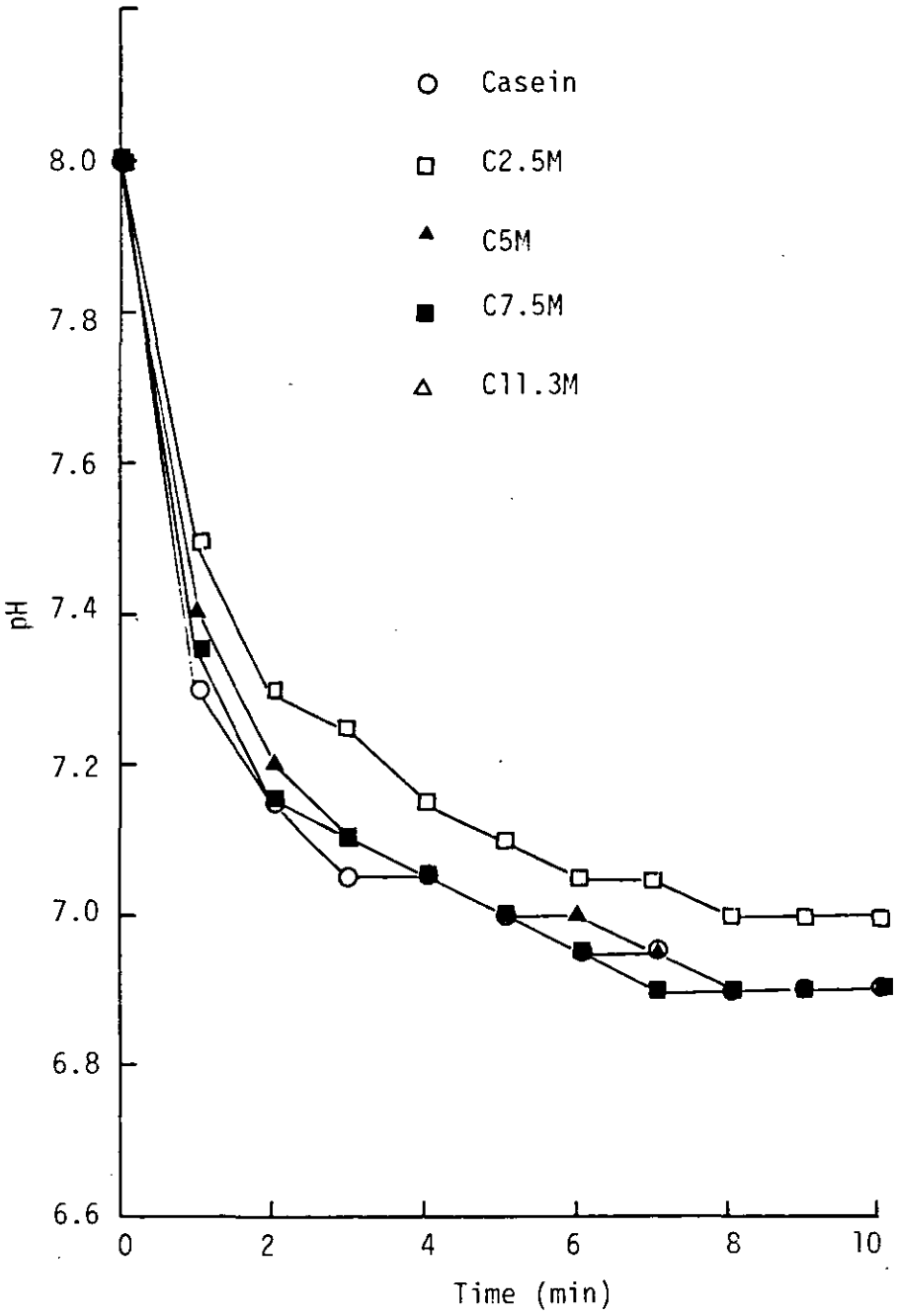


FIGURE 35: pH vs time curves obtained by incubation of four *Haplochromis* powders with peptidase

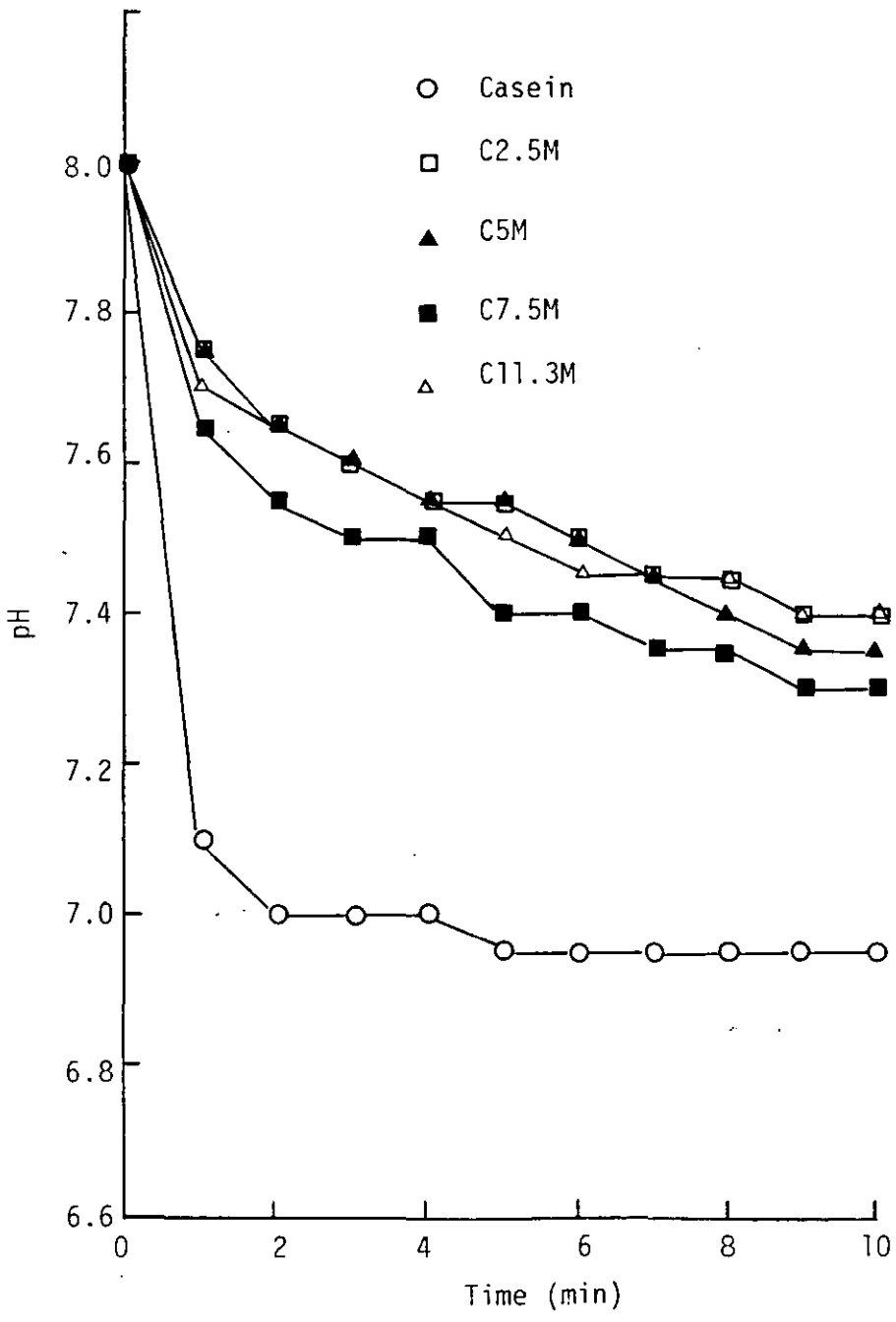
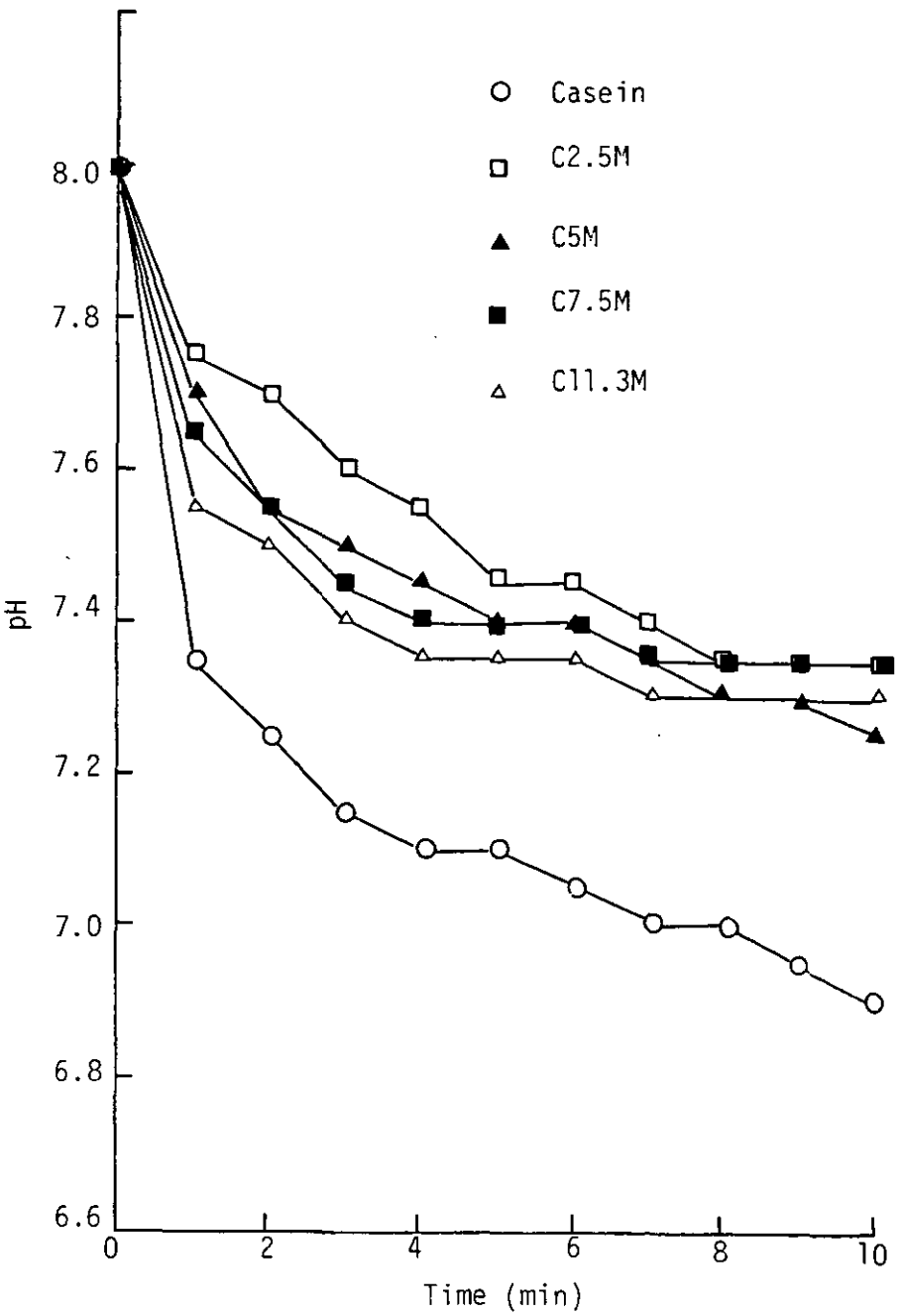


FIGURE 36: pH vs time curves obtained by incubation of four *Haplochromis* powders and casein with trypsin



chain by proteolytic enzyme. The digestibility is characterized by (1) the rate of the pH drop immediately after enzyme addition, (2) the extent of the pH drop 10 minutes after enzyme addition, and (3) the equation describing the pH drop vs time. The *in vitro* results should be correlated with an *in vivo* method result. In this study, however, the procedure was simplified. Only the trends in pH drop for each of the four *Haplochromis* powders and the standard (casein) were considered.

Figure 33 shows the pH drop when the protein samples were incubated with the three enzymes which together formed the multienzyme system. Figures 34, 35 and 36 show the corresponding pH drop during 10 minutes when the protein samples were incubated with chymotrypsin. peptidase, and trypsin respectively.

It can be seen that although the invididual enzymes digested the proteins, as shown by the pH drop curves, the multienzyme system brought about the greatest pH drop. Casein was digested to the greatest extent by all enzymes except chymotrypsin which digested all samples except C2.5M equally (Figure 34).

When the initial rate of pH drop (as shown by the gradient of the curve during the first 0.5 min), is considered, particularly for the multienzyme (Figure 33) it can be seen that the rate is slightly higher for casein than the *Haplochromis* powders. The initial rates of pH drop for the fish powders are almost the same. After the first minute however, the rate of pH drop decreases considerably particularly for the C11.3M sample, indicating a lesser degree of digestibility for the sample protein. Although the order of *in vitro* digestibility by the multienzyme system seems to be casein > C5M > C2.5M = C7.5M > C11.3M when the respective mean deviations for the four samples are taken into account, it becomes apparent that all the four fish powder proteins show the same degree of *in vitro*

digestibility. However, they appear to be less digestible than casein.

The apparent difference in digestibility between casein and the *Haplochromis* powders may not be due to inferior quality of the fish powder but to buffering capacity of the fish powder proteins. (The buffer capacity of a solution is an indication of its effectiveness in minimizing the pH change that results from the addition of a standard quantity of strong acid or strong base). Hsu *et al* (1977) concluded that the buffering capacity of the food proteins they studied did not affect their *in vitro* digestibility. Suyama and Shimizu (1982), however, found that the dipeptides carnosine, anserine and balenine (which are part of the non-protein nitrogen fraction of fish tissue) possess strong buffering capacities above pH 6. They also found that amino acids, organic bases, nucleotides, organic acids, sugars and sodium chloride had some buffering capacity. O'Hare *et al* (1984), also provided evidence suggesting that the buffering capacity of the protein may alter considerably the degree of digestibility obtained using the type of assay described by Hsu *et al* (1977). In food proteins with a high buffering capacity the detection of bond cleavage (pH drop) would be masked. This would be likely in the case of the *Haplochromis* powder samples because of the existence of the compounds investigated by Suyama and Shimizu (1982) and their high content of ash (Table 11). The salts and other compounds in the samples would resist pH change. O'Hare *et al* (1984) suggested a modification of the method described by Hsu *et al* (1977) involving the use of an automatic titrator to overcome the problem of buffering capacity of the proteins.

The results for the protein digestibility of the *Haplochromis* powder protein are not sufficient to enable definite conclusions to be drawn. In addition to solving the equations for the pH vs time curves (Figure 33) and determining the rate of pH drop after the enzyme addition it would be necessary to conduct parallel *in vivo* studies and correlate

the *in vitro* and *in vivo* results. Fidanza (1984) reviewed *in vivo* and *in vitro* methods of evaluating protein quality and suggested that the quality evaluation procedure should start with *in vitro* assays and only move to *in vivo* methods if time, money and sample size are not limiting factors or there are some specific reasons the biological assays represent the final step. Allowing for the possible effect of the buffering capacity of the *Haplochromis* powder proteins the results in this study indicate that the *in vitro* digestibility of the proteins is high.

4.6.2 Total Lipid

Table 11 and Figure 32 show the total lipid content of the raw material and eight products resulting from acid hydrolysis of *Haplochromis*. In all the products there was a reduction in the total lipid content, compared with the raw material. In the case of the spray dried products (C2.5M, C5M, C7.5M and C11.3M) some of the lipid must have been extracted with the moisture during the spray drying process. Of the four spray dried products the greatest reduction in lipid content was in sample C11.3M (62%) and the least in sample C2.5M (43%). The difference between the lipid content of products C2.5M, C5M and C7.5M was within ±5%. In the drum dried products (UA, UB, CA and CB) some of the lipid was removed during the washing and centrifuging (see 3.3.5). Taken as a whole the drum dried products contained about 21% less lipid than the spray dried ones. Some of the apparent decrease in the lipid fraction in all the products may be due to the increased ash content and therefore only relative.

A substantial reduction in lipid content of fishery products such as the ones under discussion is desirable. As pointed out in 2.3.2 fish lipids are highly susceptible to oxidative rancidity due to the presence of polyunsaturated fatty acids. The likelihood of oxidative rancidity taking place in the above products is increased by the fact that salts (cf. ash fraction in Table 11) and haem compounds such as haemoglobin

and cytochrome C which could act as proxidants (Labuza, 1971) are present in appreciable amounts. In this study no oxidative rancidity studies were carried out. However, Dhatemwa (1981) found that in dry salted fish cakes produced from *Haplochromis* mince (see 2.2.2.3) some rancidity developed after a few weeks' storage. He suggested vacuum packing and the use of antioxidants as a solution to the problem. He also pointed out that some communities in Africa can tolerate a certain degree of rancid flavour in foods. In fact the traditional way of processing *Haplochromis* is sun-drying which can take up to six days for the fish to dry adequately (Dhatemwa, 1981). Some degree of rancidity must have taken place by the time the fish dry and yet the consumers accept it.

From a nutritional point of view a certain amount of lipid in food is desirable as a source of energy and essential fatty acids (Aaes-Jørgensen, 1967). Therefore provided rancidity is controlled a certain amount of lipid in the *Haplochromis* powder would be beneficial.

4.6.3 Total Ash and Salt (NaCl)

Originally only one concentration of acid (11.3M) was intended to be used for fish hydrolysis. In fact that is why all the temperature vs extent of hydrolysis experiments were done using 11.3M HCl. When the final prduct C11.3M was submitted to a taste panel, however, the majority of the people indicated that the saltiness in the product was excessive (see 4.6.6). It therefore became apparent that the amount of fish powder that could be added to food would be limited by the salt content. Virtually all the salt in the final product originates from the neutralisation process according to the classic expression:

$$\text{Acid} + \text{Alkali} \xrightarrow{\text{neutralisation}} \text{Salt} + \text{Water}$$

Since the amount of salt formed depends on the concentration of the acid it was decided to use concentrations of acid lower than 11.3M and hence reduce the salt concentration in the final product.

Table 11 and Figure 32 show the ash content of the raw material and eight products of acid hydrolysis of *Haplochromis*. As expected the ash and salt content increase as a function of acid concentration. It must be pointed out that although the term "salt" may cover all the inorganic products of the neutralisation process, for the purposes of this discussion it is used to mean sodium chloride (NaCl).

Although products UA, UB, CA and CB were produced using the 11.3M acid concentration they contain less ash and less salt than C11.3M which was also produced using the same acid concentration. This is because the former products underwent the extra washing and centrifuging process where some of the salt was washed out and discarded in the supernatant (see 3.3.5). The UB and CB samples contained much less salt (0.6% and 1.8% respectively) because they were washed and centrifuged twice as opposed to UA and CA which were centrifuged once.

It is interesting to note that although most of the salt was washed out of UB and CB the ash content still remained high (31.1% and 40.9% respectively), an indication that there were other inorganic products of the neutralisation process. These inorganic products would have originated mainly from bone and scales. The major inorganic constituents of mackerel bone, for example, are calcium, phosphorus, magnesium and carbonate (CO_3) and each constitutes 36.7%, 17.4%, 2.76% and 2.8% of the ash fraction respectively (Long, 1961). In the reaction vessel many possible reactions could take place between the acid, alkali and the bone components increasing the ash fraction.

The results in Table 11 and Figure 32 show that the ash and salt content of the products can be regulated to a certain extent by acid

concentration. The choice of a suitable acid concentration for fish hydrolysis would take into account the preservative role of the salt (see 4.6.4) and the organoleptic test results. The acid concentration of 2.5M would suffice to soften its tissues, and on drying the a_w level (0.50) attained would inhibit microbial growth.

As mentioned in 2.2.4.1.1, however, there has been growing concern about the connection between high dietary sodium and hypertension. It may therefore even be desirable to have a lower sodium chloride content in the final product than that formed even with the lowest acid concentration (4.9 ± 1.9%). In theory this can easily be achieved by substituting KOH for NaOH or by using KOH/NaOH mixtures. Such mixtures have been reported to successfully reduce the Na content of fish sauce without affecting its sensory attributes (Chayovan *et al*, 1983).

4.6.4 Water Activity (a_w)

The aim of producing a microbiologically and chemically stable fish powder under tropical ambient conditions as outlined in the objectives of this study was based on the fact that refrigeration facilities are scarce in Uganda. The poor distribution network in the country also necessitates to aim at producing dry food products whenever possible. The water activities of the products produced in this study were therefore determined in order to assess the success of the process in attaining the objectives. Table 11 shows the water activities of eight products of acid hydrolysis. Generally the products which were produced using the 11.3M acid concentration (C11.3M, UA, UB, CA and CB) had lower water activities than those produced using less concentrated acid. This was because the former had higher solute concentration than the latter. However, it should be taken into account that the results were based on a single determination. The

results also show that drum dried products had lower water activities than spray dried ones, an indication that drum drying was more effective as a dehydrating method than spray drying.

It is significant to note that the water activities of all the products were below 0.65, the level at which very few organisms are known to grow. In fact it is suggested that at a_w = 0.65 food spoilage would be most unlikely to occur in less than 1½ to 2 years (Scott, 1957). It can therefore be assumed that all the eight fish powders in Table 11 would be safe from microbial attack for several months.

However, as discussed in 2.6.1, at low water activity levels in the absence of microbial spoilage foods become more susceptible to oxidative rancidity. The *Haplochromis* powder products in Table 11 would be particularly prone to this type of spoilage, because of the presence of unsaturated lipids (Ssali, 1981) and potential proxidants discussed in 4.6.2. From the work that has been done on mechanisms of lipid oxidation in foods, using model systems, it has been suggested that there is an optimum moisture level for dehydrated foods which reduced the rate at which rancidity developed (Labuza, 1971). This concept is illustrated in Figure 5. According to the figure lipid oxidation in the *Haplochromis* powder (particularly the drum dried samples) would be around the minimum level. But the relative reaction rate increases rapidly between a_w = 0.5 and 0.6. However when discussing chemical stability it is also important to take into account the complex system presented by the fish powder. Although extensive research has been conducted on the chemical stability of dehydrated foods a considerable proportion of that work has been done on simplified model systems. Each food system, on the other hand, has its unique properties which may differ significantly from the model system. The results from model system studies should therefore only serve as a guide when predicting the chemical stability

of real food systems. The best way to understand the microbiological and chemical stability of the *Haplochromis* powder would be to conduct stability studies under Ugandan conditions.

4.6.5 Colour

As mentioned in 4.5 the earlier products of acid hydrolysis had a mud-like appearance to which Ugandan consumers were unaccustomed. (Uganda Ministry of Animal Industry and Fisheries, 1984). A bleaching step prior to drum drying was therefore introduced. However, for reasons discussed in 4.5 both bleaching and drum drying were abandoned in favour of spray drying. The various drum dried and spray dried products were visually different in colour. These visual differences were determined objectively using a colorimeter (see 3.4.6).

The human eye is sensitive to electromagnetic waves in the range 380-780 nm. The eye incorporates two types of light sensitive "receiver"; receivers for brightness i.e. black and white, and receivers for colours. The latter are divided into three categories which are respectively sensitive to blue, green and red. The brain combines the colour signals and the sum of the colours is the resultant colour impression (Billmeyer and Saltzman, 1981). Therefore to quantify a colour impression three measurements are necessary and sufficient. The Hunter (L,a,b) system uses three axes; the L-value is given on a lightness/darkness axis, the a-value represents the red/green measurement and the b-value the blue/yellow measurement. Recognisable colour differences (ΔE) may be calculated using the following formula:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Table 13 shows the ΔE values for 8 samples of *Haplochromis* powder. The table also includes the salt content values for the products because they have a bearing on some of the ΔE values as will be discussed later.

Bleaching improved the colour of drum dried products. Products UB (ΔE = 67.1) and CB (ΔE = 64.1) were lighter in colour than the corresponding unbleached UA (ΔE = 54.2) and CA (ΔE = 60.5). Bleaching was more effective in the uncooked fish than in the cooked fish. (Compare UA with UB and CA with CB). The spray dried products (which were all unbleached, see Figure 10) had a lighter colour than the unbleached drum dried products. In fact the spray dried sample C11.3M (ΔE = 70.1) was the highest of all samples including the bleached ones. Among the spray dried products colour brightness was a function of salt (NaCl) concentration. The lightest product (C11.3M) had the highest salt content and vice versa.

It is difficult to decide which product had the "best" colour because the choice would depend on the consumers. The author is of the opinion, however, that all spray dried products would be readily acceptable to the consumers. Tests would have to be conducted to ascertain this view.

4.6.6 Organoleptic Testing

The C11.3M sample was submitted to an "expert panel" for sensory evaluation as detailed in 3.4.7. The panel consisted of 2 qualified members of staff and students of the Fisheries Training Institute, Entebbe. According to the Institute's curriculum the students would have been taught the principles of sensory evaluation of fish but their

TABLE 13: Results of measurement of colour of *Haplochromis* powders

Sample[1]	ΔE Value[2]	Salt (NaCl) (%)
White Tile (reference)	100	-
C2.5M	60.2 (0.6)	4.9
C5M	63.6 (0.5)	10.2
C7.5M	65.9 (0.5)	16.1
C11.3M	70.1 (0.7)	38.0
UA	54.2 (1.2)	11.4
UB	67.1 (0.9)	0.6
CA	60.5 (1.8)	12.9
CB	64.1 (1.2)	1.8

(1) See Appendix 23 for explanation of sample codes

(2) Mean deviation in parentheses.

experience in the subject was not known to the author. Only one tasting session was conducted.

Table 14 summarises the mean taste panel scores. It can be seen that for sample 1 (13g fish powder approx.) the majority of the people (10 out of 12) thought that the salt content, fish taste and odour were almost satisfactory and only the first traces of rancidity could be detected. When the fish powder was increased to 2 spoonfuls (Sample 2, 25g approx.) however, less than half of the panel (6 out of 15) found the product generally satisfactory. The odour and fish taste were regarded as rather strong, the sample was regarded rancid (mean score 3.1) and the salt was considered to be between "satisfactory" and "slightly salty" (mean score 3.6). Six people stated they definitely did not like the sample but did not state why and 3 people stated it was rancid but did not state whether they liked it or not. However, assuming that both groups did not like the sample it follows that 9 people out of 15 rejected it. When the amount of fish was further increased to 3 spoonfuls (Sample 3, 39g approx.) 8 people out of 14 commented the sample "too salty". The odour and fish taste were also considered "strong". It is interesting that the mean score for rancidity still remained at 3.1 in spite of the fact that the amount of fish powder had been increased by about 50%. When discussing the taste panel results with one of the panelists (Dhatemwa, 1984) it emerged that rancidity may have been interpreted differently among some panel members.

The sensory evaluation results revealed that the salt concentration of Cl1.3M (38%) might be excessive for most people. In fact the results prompted the hydrolysis experiments using lower concentrations of acid (2.5M, 5M and 7.5M) in order to lower the concentration of salt in the final product (see 4.6.3). The sensory test results also indicated that the fish powder may be highly susceptible to oxidative rancidity as discussed in 4.6.2 and 4.6.4. It should be realised, however, that

TABLE 14: Summary of mean taste panel scores for *Haplochromis* powder (C11.3M)

Sample[1]	Parameter Tested[2]				
	Odour	Salt	Rancidity	Fish Taste	Other comments[3]
0	1.5 n = 10 (0.7)	1.3 n = 10 (0.5)	1.2 n = 10 (0.6)	1.5 n = 10 (0.5)	No detectable taste of sample [10]
1(13g)	3.2 n = 12 (0.7)	2.9 n = 12 (0.5)	1.9 n = 12 (0.7)	3.4 n = 13 (1.1)	Good/satisfactory/nice [10] Strong fish flavour [1] Unsatisfactory preparation [1]
2(25g)	3.9 n = 15 (0.8)	3.6 n = 15 (0.6)	3.1 n = 15 (0.8)	4.1 n = 15 (0.8	Good taste but too salty [1] Satisfactory [5] Rancid [3] Not liked [6]
3(39g)	4.2 n = 14 (1.2)	4.1 n = 14 (0.8)	3.1 n = 14 (0.9)	4.3 n = 14 (0.9)	Good taste but too salty [5] Colour good but too salty [3] Not liked [5] NH_3-like smell [1]

(1) See 3.4.7 for explanation of sample codes.
(2) Mean deviation in parentheses.
(3) Figures in square brackets [ ] show number of times comment made.

the fish powder used in the sensory evaluation tests may have been produced from fish which was rancid already. The fish had been in cold store for several months before being processed. As discussed in 2.3.2 oxidative rancidity does take place during prolonged cold storage of fish. The problem can be solved by ensuring that only newly caught fish is used and/or incorporating antioxidant in the product. The antioxidant could easily be added during homogenization (Figure 8).

4.6.7 Particle Size Analysis

The particle size distribution in 8 samples of *Haplochromis* powder was determined and the results are shown in Table 15. The general comment about the results is that the spray dried samples (C2.5M, C5M, C7.5M and C11.3M) were comprised of finer particles than the drum dried ones (UA, UB, CA and CB). For instance more than 75% by weight of the spray dried powders were under 106 µm whereas in the drum dried ones, with the exception of UA, the percentage weight of particles under 106 µm was less than 10%. This major difference was due to the fact that during drum drying products tended to flake, particularly when the temperature rose too high, increasing the particle size. The problem did not occur in spray drying. Although the results were not subjected to statistical analysis it appears as if acid concentration did affect the particle size of spray dried samples; the smallest particles being attributed to the highest concentration and vice versa.

From a practical point of view very small particles would present a problem during preparation of fish powder for consumption. Since the powder would be used in soup formulation or incorporated into dishes such as groundnut sauce as a condiment (see 3.4.7) it should be easily reconstituted in water. The reconstitution process depends on the ability of the powder particle to adsorb water on its surface, a

TABLE 15: Results of particle size analysis of *Haplochromis* powder samples

	Size (μm)	Cumulative Percent Under Size by Weight							
		Sample[1] UA	UB	CA	CB	C2.5M	C5M	C7.5M	C11.3M
Sieve Analysis	*1000		92.3	90.4	88.7				
	* 850	96.8	90.4	87.2	84.6		100		
	* 710	94.6	86.9	79.4	77.5	100	99.8	100	100
	* 500	87.6	73.8	59.8	62.5	99.5	99.1	99.9	99.8
	* 420	83.7	67.3	52.2	56.7	98.9	98.4	99.8	99.6
	* 300	69.7	41.3	29.3	38.8	95.5	95.4	99.3	99.5
	* 212	49.8	16.7	14.7	21.2	89.4	91.6	98.2	99.1
	* 150	36.1	7.2	8.2	16.1	84.7	87.4	94.5	97.6
	* 106	19.1	1.4	4.0	7.3	75.2	75.3	86.0	92.7
Malvern Laser Diffractometer	87.2	13.8	1.0	2.6	5.1	70.4	69.4	81.5	89.6
	53.5	8.0	0.6	1.4	3.0	63.9	60.2	70.3	82.1
	37.6	5.2	0.4	0.8	2.0	55.6	49.5	61.7	75.0
	28.1	3.6	0.3	0.5	1.4	43.4	37.1	48.9	62.3
	21.5	2.4	0.2	0.4	1.1	32.0	26.7	38.1	47.7
	16.7	1.9		0.3	0.8	22.8	18.9	27.8	34.6
	13.0	1.9		0.3	0.8	15.9	14.5	19.4	25.6
	10.1	1.6		0.2	0.5	12.0	10.5	14.8	19.2
	7.9	1.3			0.4	8.1	8.5	10.2	14.4
	6.2	1.2				6.5	7.2	8.5	11.4
	4.8	1.2				6.2	6.5	7.1	9.5
	3.8	1.1				6.2	6.5	6.7	8.0
	3.0	1.0				4.8	5.0	5.6	6.2
	2.4	0.7				2.9	3.1	3.4	4.0
	1.9	0.4				1.8	1.9	2.1	2.4

(1) See Appendix 23 for explanation of sample codes. *Nominal Sieve Diameter

property which largely depends on particle size (Brennan *et al*, 1976). Small particles, due to their large surface area:mass ratio, do not easily wet individually. Instead they clump together, sharing a wetted surface layer which reduces the rate at which water penetrates into the clump of the particles. Increasing the particle size and/or agglomerating the particles can reduce clumping. Spray driers can be designed to the effect if desired.

5. CONCLUSIONS

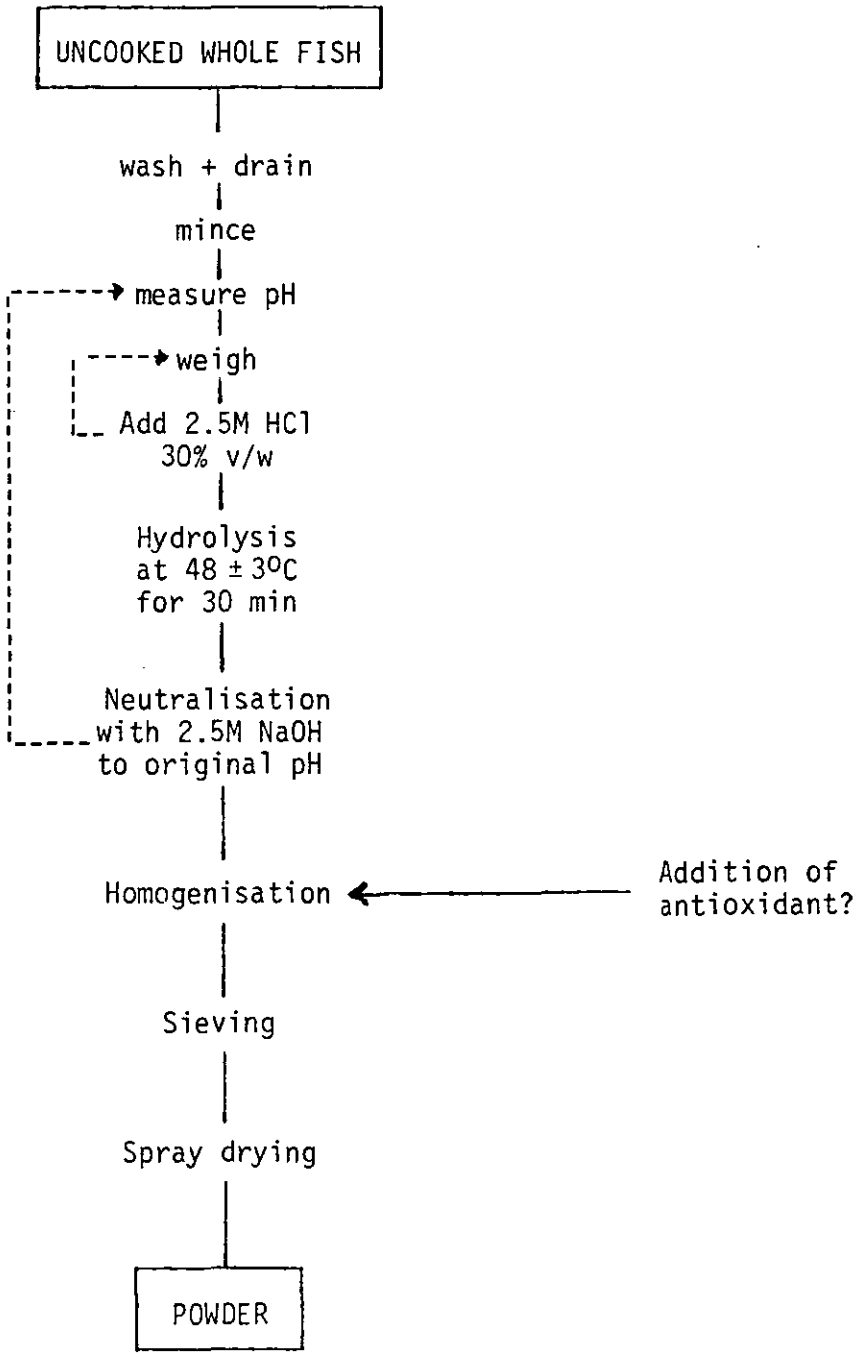
A process for producing fish powder for human consumption from whole *Haplochromis* by partial acid hydrolysis has been developed and is outlined in Figure 37. It offers an effective means of utilizing the underutilized *Haplochromis* resource because by using whole fish wastage is minimized. The process could also be used to process *Engraulicypris argenteus*, another underutilized fish species occurring in L. Victoria.

The proposed process essentially involves simple technology requiring few trained personnel. It could also be easily adapted for producing other stable products such as fish/carbohydrate (cassava, plantain, sweet potato) fish/vegetable and fish/cereal mixtures, thus improving the nutritional quality of some of Uganda's staple foods.

There is a case for fish powder in Uganda. At the cottage level sundried *Haplochromis* is ground in a pestle and mortar and mixed with groundnut sauce. There have also been previous attempts to produce a fish powder from gutted, headed and smoked *Haplochromis*. The product was readily acceptable but too expensive due to labour costs. The concept of fish powder, therefore, is not new in Uganda.

This study has shown that any of the four acid concentrations tested (2.5M, 5M, 7.5M and 11.3M) could soften the fish tissues adequately in 30 minutes for subsequent production of powder (Section 4.2.3). The 2.5M concentration, however, would be more appropriate because it would be cheaper. Secondly the least amount of salt would be formed in the product after neutralisation, this would be desirable both for organoleptic and perhaps health reasons (see Section 4.6.6). Thirdly a less concentrated acid would be safer to handle. Fourthly as the particle sizing results showed (Section 4.6.7) the product

FIGURE 37: Outline of proposed process for production of *Haplochromis* powder by partial acid hydrolysis



from the 2.5M acid concentration seemed to have larger particles than the others and this would be a desirable property during reconstitution.

From the chemical composition data and *in vitro* digestibility studies the fish powder produced by acid hydrolysis is of a high nutritional value. It would be a suitable food supplement particularly in hospital nutrition units and refugee camps.

Haplochromis powder should be microbiologically stable because processing is carried out at very low pH (Section 4.2.4) where no organisms can survive. Secondly water activity is reduced to such low levels that even the xerophilic moulds and yeasts and halophilic bacteria, which would have been a problem in dry salted foods cannot grow at a_w levels below 0.60.

Although the powder is likely to be susceptible to oxidative rancidity, the problem can easily be controlled both by using fresh fish and antioxidants. Therefore provided the powder were packaged properly it should remain microbiologically and chemically stable for several months.

6. SUGGESTIONS FOR FURTHER WORK

1. There is need to obtain corresponding data for *Haplochromis* powder produced from fresh fish.

2. More detailed protein quality studies need to be conducted according to the UN Protein Advisory Group guidelines.

3. Post-processing studies involving storage and packaging need to be carried out; microbiology and lipid oxidation need particular attention.

4. The proposed process needs to be examined at pilot or semi-pilot scale particularly with regard to costing and marketing studies.

7. APPENDICES

APPENDIX I

Date .....

Name .....

EXPERT TASTE PANEL SHEET

Haplochromis fish powder

Sample .....

1.	Odour	1 (no odour)	2	3	4	5 (Strong odour)
2.	Salt	1 (Under-salted)	2 (Slightly under-salted)	3 (Salt satis-factory	4 (Slightly too salty)	5 (Much too salty)
3.	Rancidity	1 (Not rancid)	2 (1st traces of rancidity)	3 (Rancid)	4 (Very rancid)	5 (Much too rancid)
4.	Fish Taste	1 (Bland, no fish taste)	2	3	4	5 (Very strong fish taste)

5. Any other comments:

.....

.....

.....

APPENDIX 2: Acid hydrolysis of cooked *Haplochromis* at 25 ± 1°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	24.8 (1.5)	1.0 (0.1)	7.8 (3.0)	13.3 (1.4)	21.1 (5.4)	33.8 (5.4)	6.9 (2.8)
3	24.7 (1.7)	0.85 (0.25)	7.7 (3.0)	13.3 (1.5)	21.0 (4.5)	35.3 (6.6)	6.9 (2.7)
5	24.6 (1.8)	0.8 (0.2)	8.5 (2.6)	13.1 (1.8)	21.6 (4.4)	38.5 (4.3)	7.5 (2.4)
8	24.7 (1.8)	0.8 (0.2)	7.9 (2.7)	13.3 (1.5)	21.2 (4.2)	36.3 (5.5)	7.1 (2.5)
12	25.0 (1.9)	0.75 (0.15)	8.8 (1.2)	13.0 (1.9)	21.8 (3.1)	40.3 (0.4)	7.8 (1.1)
15	24.8 (1.6)	0.75 (0.05)	8.7 (1.7)	12.2 (0.9)	20.9 (2.8)	41.2 (3.1)	7.7 (1.6)
18	24.1 (0.8)	0.65 (0.05)	8.8 (1.7)	12.9 (1.0)	21.7 (2.8)	40.3 (2.8)	7.8 (1.6)
20	24.5 (1.1)	0.65 (0.05)	8.7 (1.7)	12.8 (1.2)	21.5 (2.9)	40.0 (2.4)	7.7 (1.6)
23	24.6 (1.1)	0.6 (0.05)	8.0 (2.2)	12.5 (2.1)	20.5 (4.4)	38.5 (2.7)	7.1 (2.0)
25	24.3 (0.8)	0.6 (0.0)	9.0 (1.7)	12.0 (2.0)	21.0 (3.7)	42.7 (0.4)	8.0 (1.6)
28	24.6 (1.1)	0.5 (0.0)	9.3 (2.9)	10.0 (0.4)	19.3 (2.5)	46.9 (9.1)	8.3 (2.7)
30	24.5 (0.9)	0.5 (0.0)	8.7 (3.8)	10.6 (0.6)	19.3 (4.4)	42.7 (9.8)	7.7 (3.4)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 7.4.

APPENDIX 3: Acid hydrolysis of uncooked *Haplochromis* at 25 ± 3°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	23.2 (2.7)	0.3 (0.3)	6.8 (1.4)	9.2 (1.6)	16.0 (1.5)	42.1 (5.5)	8.1 (1.8)
5	24.4 (3.1)	0.8 (0.3)	9.3 (2.4)	8.4 (2.3)	17.7 (2.4)	44.5 (7.2)	9.3 (0.7)
7	24.4 (3.1)	0.65 (0.05)	8.6 (0.9)	9.6 (0.9)	18.2 (0.9)	43.4 (3.0)	9.4 (0.5)
9	24.8 (2.7)	0.65 (0.2)	9.9 (3.3)	8.9 (2.8)	18.8 (3.3)	42.1 (9.5)	9.3 (1.3)
12	24.8 (2.7)	0.7 (0.2)	10.2 (1.5)	8.2 (3.5)	18.4 (1.5)	50.5 (5.4)	11.0 (0.7)
14	24.5 (2.8)	0.6 (0.1)	10.6 (0.3)	5.0 (4.8)	15.6 (0.7)	67.9 (1.5)	12.8 (0.9)
17	24.7 (3.4)	0.5 (0.2)	10.6 (0.3)	7.3 (4.1)	17.9 (0.4)	59.1 (0.7)	12.7 (0.4)
19	24.9 (3.3)	0.4 (0.1)	11.6 (0.8)	8.0 (3.8)	19.6 (0.8)	59.0 (1.7)	13.8 (1.0)
22	25.9 (2.9)	0.4 (0.0)	11.1 (2.5)	7.7 (4.6)	18.8 (1.7)	58.7 (3.8)	13.2 (2.0)
25	25.9 (2.9)	0.4 (0.0)	12.0 (0.8)	6.6 (5.5)	18.6 (0.6)	64.5 (1.1)	14.3 (0.7)
27	26.0 (4.3)	0.45 (0.15)	12.0	7.6 (4.9)	19.6	64.4	14.4
30	25.9 (2.9)	0.4 (0.1)	12.4 (1.6)	7.9 (5.2)	20.3 (1.1)	61.1 (2.1)	14.8 (1.3

(1) Mean of triplicate determinations (standard deviation in parentheses)

(2) Initial pH of mince = 7.1.

APPENDIX 4: Acid hydrolysis of cooked *Haplochromis* at 37 ± 3°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	39.8 (3.4)	0.7 (0.1)	8.5 (4.6)	10.9 (1.4)	19.4 (3.2)	41.2 (16.8)	7.5 (4.0)
3	40.8 (0.0)	0.6 (0.1)	13.8 (3.5)	10.8 (1.3)	24.6 (2.2)	55.2 (9.2)	12.1 (3.1)
5	40.7 (0.1)	0.55 (0.05)	14.3 (1.9)	10.7 (0.7)	25.0 (1.2)	57.1 (4.7)	12.6 (1.6)
8	39.6 (0.6)	0.5 (0.0)	13.8 (3.3)	10.5 (1.1)	24.3 (2.2)	55.9 (8.3)	12.1 (2.8)
12	37.1 (0.8)	0.4 (0.1)	12.1 (1.8)	10.4 (1.0)	22.5 (0.7)	53.5 (6.2)	10.6 (1.5)
15	36.0 (0.0)	0.4 (0.1)	14.3 (3.1)	10.6 (1.7)	24.9 (1.4)	57.0 (9.1)	12.6 (2.7)
18	35.7 (0.1)	0.4 (0.1)	13.2 (4.5)	9.9 (1.3)	23.1 (3.3)	55.4 (11.6)	11.6 (4.0)
20	35.7 (0.1)	0.4 (0.1)	13.7 (3.4)	10.4 (1.2)	24.1 (2.3)	56.0 (9.0)	12.1 (3.0
23	35.2 (0.1)	0.4 (0.1)	14.2 (2.9)	11.1 (0.3)	25.2 (2.6)	55.3 (5.6)	12.3 (2.7)
25	35.1 (0.2)	0.4 (0.1)	13.9 (3.1)	11.1 (0.3)	25.0 (2.8)	54.7 (6.4)	12.2 (2.8)
28	36.3 (1.6)	0.4 (0.1)	14.8 (4.9)	11.3 (0.4)	26.1 (4.5)	55.1 (9.3)	13.0 (4.3)
30	36.5 (2.0)	0.4 (0.1)	14.1 (3.7)	13.0 (1.8)	27.1 (5.5)	51.5 (3.2)	12.4 (3.3)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 7.4

APPENDIX 5: Acid hydrolysis of uncooked *Haplochromis* at 38 ± 2°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	27.3 (9.4)	0.6 (0.3)	5.8 (2.9)	7.2 (0.0)	13.0 (2.9)	44.6 (12.3)	6.9 (3.4)
6	38.7 (1.6)	0.6 (0.2)	10.8 (2.5)	9.4 (0.3)	20.2 (2.3)	53.5 (6.5)	12.9 (2.9)
8	38.3 (1.1)	0.4 (0.2)	10.9 (2.3)	10.4 (0.2)	21.2 (2.2)	51.4 (5.5)	12.9 (2.7)
10	38.3 (2.2)	0.3 (0.2)	12.7 (1.8)	9.2 (1.2)	22.0 (1.7)	57.7 (5.5)	15.2 (2.1)
12	38.0 (2.8)	0.3 (0.1)	11.8 (1.2)	10.3 (0.4)	22.1 (1.2)	53.4 (2.6)	14.1 (1.3)
14.5	38.5 (1.4)	0.3 (0.05)	12.7 (1.3)	10.1 (1.2)	22.8 (0.7)	55.7 (5.2)	14.9 (1.9)
16	37.5 (1.4)	0.3 (0.0)	14.3 (3.4)	7.2 (3.5)	21.5 (3.4)	66.5 (4.9)	17.0 (4.1)
18	38.2 (1.0)	0.4 (0.1)	14.1 (0.5)	9.2 (0.4)	23.2 (0.4)	60.8 (4.4)	16.8 (0.6
20	36.5 (1.3)	0.4 (0.1)	15.6 (2.3)	9.5 (0.8)	25.1 (1.8)	62.1 (5.2)	18.7 (2.7)
24	36.6 (1.4)	0.3 (0.0)	14.7 (2.4)	8.1 (0.3)	22.8 (2.6)	64.5 (3.4)	17.5 (2.8)
26	35.2 (0.2)	0.3 (0.0)	16.6 (0.9)	10.3 (1.2)	26.8 (0.9)	61.9 (1.3)	19.7 (1.0)
28	37.3 (0.6)	0.35 (0.05)	15.8 (1.3)	8.5 (2.8)	24.4 (2.5)	64.7 (1.4)	18.9 (1.5)
30	36.2 (0.3)	0.4 (0.05)	14.4 (0.3)	8.8 (3.2)	23.2 (0.3)	62.1 (0.5)	17.2 (0.4)

(1) Mean of triplicate determinations (standard deviation in parentheses)
(2) Initial pH of mince = 7.0

APPENDIX 6: Acid hydrolysis of cooked *Haplochromis* at 46 ± 2°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	44.1 (1.2)	0.55 (0.55)	8.6 (1.3)	10.7 (1.2)	19.3 (2.5)	44.3 (0.9)	7.6 (1.2)
3	44.6 (0.2)	0.375 (0.375)	9.6 (1.4)	11.8 (0.0)	21.4 (1.5)	44.5 (3.7)	8.5 (1.3)
5	45.5 (0.6)	0.3 (0.3)	10.2 (0.9)	11.2 (0.3)	21.4 (0.6)	47.5 (2.9)	9.0 (0.9)
8	45.7 (1.1)	0.275 (0.275)	8.1 (3.8)	10.2 (0.3)	18.3 (3.8)	43.3 (9.9)	7.5 (3.1)
12	45.5 (0.9)	0.3 (0.3)	10.9 (1.3)	10.5 (0.3)	21.4 (1.6)	50.9 (2.3)	9.7 (1.2)
15	46.3 (0.4)	0.25 (0.25)	12.8 (0.6)	11.0 (0.0)	23.8 (0.6)	53.8 (1.1)	11.4 (0.6)
18	47.4 (2.8)	0.275 (0.175)	11.7 (1.1)	11.2 (0.0)	22.9 (1.1)	51.0 (2.2)	10.3 (1.0)
20	47.7 (3.1)	0.25 (0.15)	11.6 (2.3)	11.7 (0.1)	23.3 (2.2)	49.2 (5.1)	10.3 (2.1)
23	46.7 (2.4)	0.25 (0.15)	12.5 (1.3)	10.9 (0.1)	23.4 (1.2)	53.2 (3.0)	11.1 (1.3)
25	46.1 (2.2)	0.25 (0.15)	13.7 (1.3)	11.2 (0.7)	24.9 (2.0)	55.0 (0.9)	12.1 (1.2)
28	45.6 (0.1)	0.275 (0.125)	13.1 (1.0)	11.1 (0.1)	24.2 (1.1)	54.0 (1.8)	11.9 (0.6)
30	44.9 (1.6)	0.325 (0.175)	12.7 (1.7)	10.4 (0.3)	23.1 (2.0)	54.8 (2.7)	11.7 (1.1)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 6.9.

APPENDIX 7: Acid hydrolysis of uncooked *Haplochromis* at 48 ± 3°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	44.0 (5.4)	0.4 (0.3)	4.4 (0.6)	8.1 (1.6)	12.5 (1.1)	35.2 (.7.8)	6.0 (1.3)
4	51.0 (3.6)	0.4 (0.2)	9.7 (1.1)	10.1 (0.3)	19.8 (0.9)	48.9 (3.4)	13.2 (2.4)
6	47.5 (1.5)	0.3 (0.05)	12.8 (0.2)	11.9 (0)	24.7 (0.2)	51.8 (0.3)	17.1 (1.8)
7	51.5 (2.5)	0.35 (0.3)	12.7 (0.5)	9.9 (0.2)	22.6 (0.4)	56.2 (1.3)	17.1 (1.4)
9	48.0 (3.9)	0.35 (0.35)	16.1 (1.6)	9.4 (1.0)	25.5 (1.0)	63.1 (4.7)	21.8 (4.0)
11.5	50.0 (3.2)	0.4 (0.4)	16.1 (1.2)	16.1 (1.2)	28.2 (3.0)	57.1 (10.5)	21.8 (3.5)
13	46.2 (0.7)	0.35 (0.3)	16.2 (1.8)	16.9	33.1 (1.8)	48.9 (2.8)	21.4 (4.2)
15	48.6 (4.1)	0.35 (0.3)	16.4 (1.5)	8.2 (0)	24.6 (1.6)	66.7 (2.0)	22.2 (3.3)
17	47.9 (2.9)	0.4 (0.3)	17.4 (1.9)	11.3 (2.1)	28.7 (2.8)	60.7 (5.3)	23.5 (4.3)
19	45.5 (4.0)	0.45 (0.35)	17.4 (2.7)	9.5	26.9 (2.7)	64.7 (3.6)	23.5 (4.4)
21	47.8 (1.7)	0.45 (0.3)	19.7 (0.2)	8.1	27.8 (0.2)	70.8 (0.2)	26.5 (2.3)
23	47.7 (1.5)	0.4 (0.3)	19.0 (2.8)	8.1	27.1 (2.8)	70.7 (3.1)	25.3 (1.7)
25	47.5 (2.5)	0.35	19.9 (1.4)	-	-	-	-
28	47.4 (1.5)	0.4 (0.3)	19.7 (1.9)	7.6	27.3 (1.9)	72.2 (2.0)	26.5 (3.2)
30	45.0 (1.0)	0.35 (0.3)	19.4 (2.3)	9.0	28.4 (2.3)	68.3 (2.5)	25.9 (1.6)

(1) Mean of triplicate determinations (standard deviation in parentheses)
(2) Initial pH of mince = 6.9.

APPENDIX 8: Acid hydrolysis of cooked *Haplochromis* at 56 ± 2°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	54.4 (4.3)	0.95 (0.95)	14.9 (4.6)	9.4 (0.1)	24.3 (5.2)	60.3 (8.2)	15.3 (0.6)
3	58.1 (1.3)	0.35 (0.35)	16.1 (5.9)	9.8 (0.5)	25.9 (5.7)	60.7 (10.8)	17.1 (0.8)
5	57.8 (0.8)	0.35 (0.15)	18.4 (6.0)	12.3 (3.9)	30.7 (2.4)	59.1 (16.0)	19.1 (0.3)
8	56.7 (0.4)	0.475 (0.1)	19.0 (5.2)	10.0 (0.3)	29.0 (5.1)	64.7 (7.4)	19.3 (0.1)
12	54.1 (1.7)	0.4 (0.3)	18.5 (5.9)	10.2 (0.6)	28.7 (5.1)	63.3 (9.2)	19.2 (0.3)
15	54.7 (0.5)	0.5 (0.1)	19.7 (5.9)	10.2 (0.7)	29.9 (5.6)	64.7 (8.8)	20.2 (0.0)
18	55.4 (1.5)	0.4 (0.2)	18.9 (5.7)	10.1 (0.6)	29.0 (5.5)	64.1 (8.8)	19.4 (0.0)
20	55.5 (1.0)	0.4 (0.25)	19.8 (6.5)	9.5 (0.1)	29.3 (6.5)	66.3 (8.4)	20.6 (0.7)
23	56.0 (1.5)	0.4 (0.3)	19.7 (6.9)	10.5 (0.8)	30.2 (6.6)	63.7 (10.6)	20.8 (0.0)
25	55.3 (1.6)	0.45 (0.3)	20.8 (7.9)	9.0 (0.1)	29.8 (8.3)	69.3 (9.4)	22.6 (1.7)
28	54.3 (1.3)	0.45 (0.3)	21.2 (8.0)	9.8 (0.4)	31.0 (7.9)	66.7 (10.2)	22.5 (1.8)
30	53.8 (1.1)	0.4 (0.3)	20.1 (6.8)	11.0 (1.0)	31.1 (6.5)	63.4 (10.4)	21.1 (0.5)

(1) Mean of triplicate determinations (standard deviation in parentheses)

(2) Initial pH of mince = 7.4.

APPENDIX 9: Acid hydrolysis of uncooked *Haplochromis* at 56 ± 4°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	45.0 (5.3)	0.5	9.4 (2.6)	6.3	15.7	59.9	8.0
5	61.0 (9.0)	0.55 (0.1)	14.1 (0.1)	8.0	22.1	63.8	17.1
7.5	60.2 (5.2)	0.45 (0.1)	16.7 (0.8)	7.8	24.5	68.2	19.0
9	57.8 (2.1)	0.35 (0.05)	18.6 (1.6)	6.6	25.2	73.8	20.2
11	56.5 (0.5)	0.3 (0.05)	19.4 (1.9	6.7 (0.6)	26.1 (2.5)	74.3 (0)	23.1 (2.2)
13	55.5 (0.5)	0.2 (0.15)	20.0 (1.5)	7.4 (0.3)	27.4 (1.2)	72.8 (2.3)	23.9 (1.8)
15	53.0 (1.0)	0.2 (0.05)	19.9 (2.1)	7.3 (0)	27.2 (2.1)	73.1 (2.0)	23.8 (2.5)
17	56.2 (1.7)	0.25 (0)	21.1 (0.9)	9.5 (1.5)	30.6 (2.5)	69.1 (2.7)	25.2 (1.1)
20	54.7 (2.2)	0.3 (0.05)	21.3 (1.0)	8.5 (3.4)	29.8 (4.5)	72.6 (7.5)	25.5 (1.2)
22	54.7 (2.2)	0.2 (0.05)	23.7 (0.9)	8.4	33.6 (2.8)	70.7 (3.2)	28.3 (1.1)
24	55.0 (2.0)	0.2 (0.1)	23.0 (0)	9.4 (1.1)	32.4	71.0	27.6
26	55.7 (1.7)	0.2 (0.1)	24.1 (1.9)	9.6 (1.3)	33.7 (3.2)	71.7 (1.1)	28.8 (2.3)
28	55.7 (0.7)	0.1 (0.05)	25.0 (0.5)	10.3	35.3	71.0	30.5
30	53.2 (0.7)	0.1 (0.05)	22.6 (1.8)	8.3 (1.7)	30.8 (0.1)	73.1 (5.5)	27.0 (2.2)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 6.9.

APPENDIX 10: Acid hydrolysis of cooked *Haplochromis* at 64 ± 4°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	53.8 (1.5)	0.5 (0.5)	13.1 (1.6)	10.3 (0.7)	23.4 (1.0)	55.9 (4.8)	11.7 (1.6)
3	59.8 (2.3)	0.25 (0.25)	14.2 (2.9)	10.6 (0.4)	24.8 (2.5)	56.6 (6.0)	12.6 (2.7)
5	63.2 (4.7)	0.3 (0.05)	14.9 (1.0)	11.2 (0.2)	26.1 (1.3)	56.9 (1.2)	13.2 (1.1)
8	63.3 (2.2)	0.45 (0.05)	15.9 (0.7)	10.8 (0.3)	26.7 (0.4)	59.5 (1.8)	14.1 (0.8)
12	65.4 (2.0)	0.4 (0.0)	16.0 (0.2)	11.0 (0.3)	27.0 (0.5)	59.3 (0.2)	14.1 (0.3)
15	64.2 (0.9)	0.4 (0.05)	17.4	10.7 (0.3)	27.8	62.5	15.6
18	65.4 (1.2)	0.45 (0.05)	17.3 (0.9)	10.8 (0.4)	28.1 (0.5)	61.5 (2.0)	15.3 (0.9)
20	66.2 (0.7)	0.4 (0.05)	18.2 (1.1)	13.3 (2.5)	31.5 (3.7)	58.1 (3.2)	16.1 (1.1)
23	67.4 (0.3)	0.25 (0.15)	17.8 (1.6)	13.5 (2.6)	31.3 (4.2)	57.1 (2.4)	15.8 (1.6)
25	65.9 (0.6)	0.35 (0.05)	17.2 (2.1)	13.0 (2.1)	30.2 (4.3)	57.1 (1.0)	15.2 (2.0)
28	65.1 (0.6)	0.4 (0.0)	21.6 (1.0)	13.0 (2.3)	34.5 (1.3)	62.6 (5.2)	19.1 (0.7)
30	64.5 (0.2)	0.45 (0.05)	18.5 (2.4)	13.8 (2.9)	32.3 (5.3)	57.6 (1.9)	16.4 (2.3)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 7.1.

APPENDIX 11: Acid hydrolysis of uncooked *Haplochromis* at 65 ± 4°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	53.8 (14.9)	0.7 (0.2)	8.1 (0.0)	10.0 (2.2)	18.1 (2.1)	45.2 (5.4)	9.7 (0.1)
4	69.0 (8.5)	0.65 (0.35)	9.9 (3.0)	10.5 (4.1)	19.4 (0.4)	46.2 (13.8)	10.7 (3.0)
7	60.9 (5.1)	0.6 (0.3)	15.9 (1.8)	9.8 (3.7)	25.5 (0.9)	61.6 (8.8)	18.8 (2.1)
9	66.7 (1.7)	0.6 (0.2)	17.5 (2.7)	10.9 (3.4)	28.1 (0.2)	61.0 (9.0)	20.6 (3.3)
12	67.1 (0.2)	0.6 (0.2)	19.1 (3.8)	9.6 (1.2)	26.8 (0.9)	64.0 (4.5)	20.6 (2.2)
15	65.6 (5.7)	0.55 (0.2)	20.9 (5.6)	10.9 (1.2)	28.6 (0.2)	61.9 (4.5)	21.3 (1.8)
17	68.7 (1.6)	0.5 (0.2)	23.5 (3.8)	10.5 (1.9)	32.2 (0.3)	67.3 (6.3)	26.1 (2.8)
20	66.4 (2.1)	0.45 (0.3)	21.7 (3.0)	10.4 (2.0)	30.6 (0.6)	66.1 (5.9)	24.3 (1.8)
23	65.8 (2.0)	0.4 (0.2)	22.6 (4.4)	9.3 (0.1)	29.6 (1.8)	68.4 (2.3)	24.3 (2.4)
26	66.6 (1.8)	0.4 (0.3)	23.2 (4.1)	9.6 (1.3)	30.5 (0.6)	68.5 (3.5)	25.1 (0.8)
28	64.4 (5.1)	0.5 (0.3)	23.8 (7.1)	8.9 (0.9)	28.8 (1.2)	69.0 (4.3)	24.0 (2.6)
32	65.2 (1.9)	0.45 (0.3)	24.0 (5.0)	9.1 (1.4)	30.3 (0.4)	74.3	26.8

(1) Mean of triplicate determinations (standard deviation in parentheses).

(2) Initial pH of mince = 6.8.

APPENDIX 12: Acid hydrolysis of cooked *Haplochromis* at 74 ± 4°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	67.0 (3.2)	0.5 (0.1)	10.8 (0.1)	10.4 (1.1)	21.2 (1.0)	51.1 (2.9)	9.5 (0.1)
3	68.3 (3.9)	0.3 (0.1)	13.5 (0.3)	10.4 (0.3)	23.9 (0.0)	56.3 (1.2)	11.8 (0.2)
5	72.2 (0.3)	0.35 (0.15)	13.6 (0.5)	10.9 (0.2)	24.5 (0.4)	55.6 (1.3)	11.9 (0.4)
8	73.9 (0.9)	0.25 (0.05)	14.2 (0.0)	10.3 (0.1)	24.5 (0.1)	58.0 (0.4)	12.4 (0.0)
12	74.7 (0.4)	0.2 (0.0)	15.5 (0.0)	10.7 (0.1)	26.2 (0.2)	59.1 (0.2)	13.6 (0.0)
15	79.1 (2.6)	0.2 (0.1)	16.1 (1.3)	10.5 (0.2)	26.5 (1.0)	60.2 (2.5)	14.0 (1.1)
18	78.7 (0.8)	0.3 (0.1)	15.0 (2.4)	10.0 (0.3)	25.1 (2.1)	59.5 (4.7)	13.2 (2.2)
20	76.6 (0.3)	0.3 (0.05)	16.2 (0.7)	9.9 (0.2)	26.1 (0.5)	62.0 (1.6)	14.2 (0.6)
23	75.1 (0.0)	0.3 (0.1)	17.2 (0.4)	9.8 (0.0)	27.0 (0.4)	63.6 (0.6)	15.0 (0.4)
25	76.3 (0.4)	0.3 (0.1)	17.7 (0.5)	9.7 (0.1)	27.4 (0.6)	64.6 (0.4)	15.6 (0.5)
28	75.3 (2.5)	0.35 (0.05)	18.2 (0.1)	9.7 (0.1)	27.9 (0.0)	65.2 (0.3)	15.8 (0.4)
30	73.4 (2.8)	0.35 (0.05)	18.1 (0.4)	10.0 (0.1)	28.1 (0.5)	64.4 (0.3)	15.9 (0.4)

(1) Mean of duplicate determinations (mean deviation in parentheses).

(2) Initial pH of mince = 7.2.

APPENDIX 13: Acid hydrolysis of uncooked *Haplochromis* at 74 ± 6°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	65.4 (8.6)	0.75 (0.35)	7.2 (0.7)	9.7 (1.1)	16.9 (1.4)	42.5 (2.8)	8.6 (0.8)
3	68.7 (6.3)	0.65 (0.05)	13.1 (1.9)	8.5	23.6	63.7	18.0
5	74.7 (6.6)	0.60 (0.2)	17.9 (2.2)	10.5 (2.1)	28.4 (3.0)	63.1 (5.2)	21.4 (2.6)
8	70.1 (6.3)	0.70 (0.3)	22.6 (1.4)	10.7 (1.8)	30.1	70.5	25.4
10	76.3 (4.6)	0.5 (0.2)	21.7 (3.9)	9.0 (0.3)	30.7 (3.8)	70.3 (3.9)	26.0 (4.7)
13	74.6 (4.5)	0.4 (0.1)	23.4 (5.2)	9.4 (1.1)	35.7	75.8	32.4
15	75.5 (1.7)	0.4 (0.1)	27.3 (2.3)	11.0 (2.2)	38.2 (2.6)	71.4 (5.3)	32.7 (2.7)
17	76.5 (2.2)	0.35 (0.1)	24.8 (4.7)	10.6 (1.7)	35.4 (5.1)	69.8 (5.8)	29.8 (5.8)
23	70.1 (8.8)	0.4 (0.2)	28.8 (2.0)	11.1 (1.7)	35.3 (3.2)	73.3 (5.4)	34.4 (3.4)
25	76.5 (0.6)	0.4 (0.2)	27.2 (4.4)	10.4 (2.3)	37.6 (4.4)	72.1 (6.6)	32.5 (5.2)
27	76.4 (0.5)	0.4 (0.3)	28.2 (2.9)	8.7 (3.0)	35.3 (3.2)	75.8 (6.3)	31.8 (0.2)
31	77.8 (5.8)	0.4 (0.3)	29.5 (4.9)	10.7 (1.2)	40.2 (5.8)	70.4 (5.0)	34.1 (7.3)

(1) Mean of triplicate determinations (standard deviation in parentheses).

(2) Initial pH of mince = 7.0.

APPENDIX 14: Acid hydrolysis of cooked *Haplochromis* at 84 ± 4°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2],[3]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	73.7 (0.8)	1.2	15.0 (0.4)	10.5 (1.1)	25.5 (1.6)	59.0 (2.0)	13.1 (0.3)
3	81.0 (1.5)	0.75	17.1 (0.3)	10.8 (0.1)	27.9 (0.2)	61.2 (0.5)	15.0 (0.2)
5	84.9 (2.7)	0.65	19.1 (0.5)	10.4 (0.1)	29.4 (0.5)	64.7 (0.4)	16.7 (0.4)
8	84.8 (1.7)	0.6	17.4 (0.5)	9.3 (0.5)	26.7 (1.0)	65.1 (0.5)	15.2 (0.4)
12	84.2 (2.2)	0.7	18.8 (1.2)	9.0 (0.7)	27.8 (1.8)	67.6 (0.2)	16.4 (1.0)
15	84.1 (2.7)	0.7	20.9 (0.0)	8.8 (0.0)	29.7 (0.0)	70.3 (0.0)	18.2 (0.0)
18	85.6 (0.8)	0.6	21.6 (0.8)	10.6 (0.5)	32.3 (1.2)	67.0 (0.2)	18.9 (0.6)
20	85.1 (1.4)	0.7	22.2 (1.1)	7.6 (1.4)	29.8 (0.3)	74.5 (4.4)	19.4 (0.8)
23	86.1 (1.1)	0.65	22.4 (2.1)	9.3 (0.2)	31.7 (1.9)	70.4 (2.3)	19.6 (1.8)
25	85.0 (0.5)	0.65	24.1 (2.7)	9.3 (0.3)	33.4 (2.8)	72.0 (1.5)	21.1 (2.3)
28	82.6 (0.3)	0.6	26.5 (1.5)	9.7 (0.1)	36.2 (1.6)	73.1 (1.0)	23.2 (1.3)
30	83.3 (3.8)	0.6	26.8 (2.7)	9.6 (0.3)	36.4 (3.0)	73.6 (1.3)	23.5 (2.3)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 7.2.

(3) Single determination

APPENDIX 15: Acid hydrolysis of uncooked *Haplochromis* at 84 ± 4°C (11.3M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	70.9 (3.2)	0.70 (0.7)	7.6 (3.3)	9.5 (1.6)	17.0 (4.4)	43.1 (11.2)	9.1 (4.1)
3	77.4 (2.5)	0.8 (0.8)	16.0 (2.4)	9.0 (1.4)	25.0 (1.3)	63.9 (6.8)	19.1 (3.2)
6	80.7 (1.7)	0.7 (0.7)	19.6 (1.6)	9.9 (3.0)	29.6 (1 8)	66.8 (8.2)	23.4 (2.3)
8	82.8 (1.2)	0.7 (0.7)	23.2 (1.5)	8.0 (2.6)	31.9 (1.1)	75.2 (5.0)	28.6 (0.9)
12	85.4 (2.2)	0.7 (0.7)	26.9 (3.4)	9.0 (1.4)	35.9 (4.4)	73.7 (0.9)	31.4 (3.5)
15	86.6 (0.9)	0.6 (0.6)	28.1 (0.9)	9.5 (2.3)	37.6 (2.9)	75.0 (4.1)	33.4 (0.8)
17	86.7 (2.7)	0.6 (0.6)	29.3 (1.7)	9.0 (1.7)	38.3 (3.0)	76.6 (3.0)	34.8 (1.5)
18	83.7 (3.3)	0.6 (0.6)	31.3 (4.0)	9.1 (1.5)	40.5 (4.4)	77.3 (3.3)	37.2 (4.8)
21	85.0 (1.6)	0.6 (0.6)	32.0 (1.4)	8.6 (1.6)	41.2 (2.7)	77.8 (1.7)	37.7 (1.5)
25	82.6 (1.3)	0.6 (0.6)	31.9 (3.5)	8.3 (2.1)	40.2 (5.3)	79.5 (2.8)	37.9 (3.5)
27	82.6 (3.8)	0.7 (0.6)	35.3 (3.3)	8.0 (1.9)	43.3 (4.1)	81.5 (3.4)	41.9 (4.2)
30	85.2 (7.7)	0.75 (0.6)	36.4 (2.1)	8.2 (1.7)	44.6 (3.4)	81.7 (2.6)	43.2 (2.5)

(1) Mean of triplicate determinations (standard deviation in parentheses).

(2) Initial pH of mince = 6.9.

APPENDIX 16: Acid hydrolysis of cooked *Haplochromis* at 58 ± 2°C (7.5M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	55.9 (0.8)	0.1 (0.0)	19.4 (4.0)	9.3 (0.4)	28.7 (4.4)	66.8 (3.7)	17.1 (3.3)
3	59.3 (0.3)	0.05 (0.05)	22.6 (0.5)	9.2 (0.1)	31.8 (0.6)	71.0 (0.3)	20.1 (0.2)
5	60.2 (0.3)	0.075(0.05)	24.9 (2.1)	9.3 (0.2)	34.2 (2.3)	72.6 (1.2)	22.1 (1.6)
8	60.3 (0.5)	0.1 (0.0)	24.2 (1.0)	8.8 (0.2)	33.0 (0.8)	73.4 (1.2)	21.5 (0.5)
12	59.6 (0.3)	0.15 (0.05)	25.5 (0.8)	9.1 (0.0)	34.6 (0.9)	73.7 (0.5)	22.7 (0.4)
15	60.0 (1.3)	0.4 (0.3)	26.9 (1.8)	9.1 (0.1)	36.0 (1.7)	74.6 (1.5)	23.9 (1.2)
18	58.9 (0.7)	0.5 (0.3)	26.8 (2.3)	9.0 (0.0)	35.8 (2.3)	74.7 (1.7)	23.8 (1.7)
20	58.3 (0.1)	0.5 (0.3)	29.2 (1.4)	8.9 (0.3)	38.1 (1.7)	76.6 (0.2)	25.9 (1.5)
23	57.4 (0.8)	0.5 (0.4)	26.4 (1.8)	9.1 (0.5)	35.5 (1.3)	74.2 (2.3)	23.5 (1.3)
25	56.7 (1.1)	0.55 (0.35)	26.6 (2.4)	8.7 (0.0)	35.4 (2.4)	75.1 (1.8)	23.6 (1.8)
28	55.2 (1.4)	0.55 (0.35)	25.4 (3.8)	9.0 (0.0)	34.4 (3.8)	73.5 (2.8)	22.5 (3.0)
30	54.7 (1.7)	0.55 (0.35)	27.4 (3.9)	9.5 (0.4)	36.9 (3.4)	74.0 (3.6)	24.3 (3.1)

(1) Mean of duplicate determinations (mean deviation in parentheses).

(2) Initial pH of mince = 6.85.

APPENDIX 17: Acid hydrolysis of uncooked *Haplochromis* at 60 ± 4°C (7.5M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	60.0 (0.0)	0.7 (0.1)	24.0 (0.0)	8.9 (0.0)	32.9 (0.0)	73.0 (0.1)	27.3 (0.0)
3	60.9 (0.7)	0.65 (0.05)	27.4 (0.3)	8.8 (0.1)	36.2 (0.4)	75.5 (0.0)	31.2 (0.3)
5	60.6 (0.9)	0.65 (0.05)	25.0 (1.3)	8.7 (0.2)	33.7 (1.1)	74.2 (1.5)	28.5 (1.5)
8	59.5 (1.6)	0.65 (0.05)	29.9 (2.6)	8.9 (0.0)	38.8 (2.6)	76.9 (1.4)	34.1 (2.9)
12	62.0 (2.8)	0.65 (0.05)	27.6 (0.3)	8.7 (0.0)	36.3 (0.3)	76.1 (0.2)	31.5 (0.4)
15	64.3 (6.1)	0.65 (0.15)	29.5 (0.1)	8.6 (0.1)	38.1 (0.0)	77.5 (0.2)	33.7 (0.0)
18	62.7 (5.4)	0.75 (0.05)	33.4 (2.5)	8.4 (0.1)	41.8 (2.6)	79.8 (1.1)	38.1 (2.8)
20	61.1 (4.6)	0.7 (0.1)	29.4 (0.1)	8.3 (0.1)	37.8 (0.2)	77.9 (0.1)	33.6 (0.1)
23	59.3 (4.5)	0.8 (0.1)	31.0 (1.9)	8.3 (0.1)	39.3 (1.9)	78.7 (0.8)	35.3 (2.1)
25	59.3 (2.5)	0.8 (0.1)	31.3 (2.3)	8.4 (0.1)	39.7 (2.4)	78.7 (1.0)	35.7 (2.6)
28	59.2 (0.1)	0.8 (0.1)	30.7 (1.2)	8.5 (0.0)	39.1 (1.1)	78.3 (0.7)	35.0 (1.3)
30	58.2 (0.8)	0.75 (0.05)	31.5 (1.8)	8.4 (0.0)	39.9 (1.8)	78.9 (0.8)	36.0 (2.0)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 7.0

APPENDIX 18: Acid hydrolysis of cooked *Haplochromis* at 59 ± 4°C (5M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	55.5 (8.9)	0.4 (0.3)	15.6 (2.1)	8.7 (0.2)	24.3 (1.9)	64.0 (3.6)	13.9 (2.1)
3	59.7 (6.2)	0.6 (0.5)	19.1 (4.3)	8.8 (0.1)	27.9 (4.4)	67.8 (4.8)	17.1 (4.1)
5	60.9 (4.4)	0.75 (0.65)	19.9 (3.9)	8.9 (0.0)	28.8 (3.9)	68.4 (4.2)	17.7 (3.7)
8	61.7 (1.8)	0.9 (0.7)	18.7 (0.3)	9.1 (0.3)	27.9 (0.6)	67.1 (0.4)	16.7 (0.5)
12	60.5 (0.0)	1.0 (0.6)	22.1 (3.3)	8.8 (0.0)	30.9 (3.4)	71.0 (3.0)	19.7 (3.3)
15	59.8 (0.4)	1.0 (0.6)	21.8 (3.4)	8.9 (0.1)	30.7 (3.4)	70.6 (3.0	19.5 (3.3)
18	58.6 (1.0)	1.0 (0.6)	21.6	9.1 (0.2)	30.9	70.0	19.6
20	57.9 (1.2)	1.0 (0.6)	21.7 (1.1)	8.0 (1.0)	29.7 (2.1)	73.0 (1.4)	19.3 (1.2)
23	57.9 (0.2)	1.0 (0.5)	20.6 (0.0)	9.1 (0.0)	29.7 (0.0)	69.4 (0.1)	18.3 (0.2)
25	57.0 (0.5)	1.0 (0.5)	22.0 (0.2)	9.3 (0.0)	31.3 (0.2)	69.9 (0.3)	19.5 (0.1)
28	60.1 (4.0)	1.3 (0.3)	18.3 (0.2)	9.5 (0.1)	27.8 (0.4)	65.8 (0.0)	16.3 (0.5)
30	62.3 (5.9)	1.5 (0.1)	19.1 (2.7)	9.6 (0.1)	28.7 (2.5)	66.5	17.0 (2.1)

(1) Mean of duplicate determinations (mean deviation in parentheses).

(2) Initial pH of mince = 6.75.

APPENDIX 19: Acid hydrolysis of uncooked *Haplochromis* at 59 ± 3°C (5M HCl)(1)

Time (min)	Temperature (°C)	pH(2)	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	59.6 (3.3)	1.45 (0.75)	19.4 (1.8)	8.2 (0.4)	27.6 (2.3)	70.2 (0.8)	22.1 (2.1)
3	63.2 (0.4)	1.65 (0.15)	25.2 (2.0)	8.3 (0.6)	33.5 (2.6)	75.1 (0.0)	28.7 (2.3)
5	62.2 (0.2)	1.75 (0.25)	26.6 (0.7)	8.2 (0.4)	34.8 (1.1)	76.5 (0.5)	30.6 (0.9)
8	61.2 (0.5)	1.8 (0.2)	24.1 (3.9)	8.3 (0.7)	32.5 (4.6)	74.1 (1.5)	27.5 (4.4)
12	59.1 (1.2)	1.85 (0.15)	27.1 (1.3)	7.9 (0.7)	35.0 (2.0)	77.5 (0.5)	30.9 (1.5)
15	57.3 (1.5)	1.95 (0.15)	28.1 (0.5)	8.2 (0.5)	36.3 (1.1)	77.4 (0.9)	32.0 (0.5)
18	59.4 (1.6)	1.9 (0.2)	25.3 (1.3)	7.9 (0.4)	33.3 (1.8)	76.2 (0.1)	28.9 (1.5)
20	59.1 (2.2)	1.9 (0.2)	28.7 (1.2)	8.3 (1.0)	36.9 (0.2)	77.6 (2.9)	32.7 (1.4)
23	57.4 (2.2)	1.95 (0.25)	27.0 (2.1)	8.0 (0.9)	35.1 (3.0)	77.1 (0.5)	30.8 (2.3)
25	56.6 (1.9)	2.0 (0.2)	28.0 (0.1)	7.8 (0.8)	35.9 (0.7)	78.2 (1.8)	31.9 (0.1)
28	56.8 (0.0)	2.0 (0.2)	28.5 (0.5)	7.7 (0.6)	36.2 (0.2)	78.7 (1.7)	32.5 (0.5)
30	56.6 (0.5)	2.0 (0.2)	27.7 (0.6)	7.9 (0.7)	35.6 (0.0)	77.7 (1.9)	31.6 (0.8)

(1) Mean of duplicate determinations (mean deviation in parentheses).

(2) Initial pH of mince = 6.85.

APPENDIX 20: Acid hydrolysis of cooked *Haplochromis* at 58 ± 4°C (2.5M HCl)(1)

Time (min)	Temperature (°C)	pH(2)	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	50.1 (7.1)	0.7 (0.2)	14.2 (2.3)	8.8 (0.1)	23.0 (2.4)	61.5 (3.7)	12.8 (1.7)
3	60.1 (2.0)	2.0 (1.0)	14.1 (2.1)	9.2 (0.1)	23.3 (2.0)	60.0 (3.8)	12.7 (1.5)
6	61.7 (2.1)	2.2 (1.0)	17.7 (1.3)	9.6 (0.2)	27.3 (1.5)	64.8 (1.2)	16.0 (0.7)
9	62.0 (2.4)	2.4 (0.9)	17.6 (0.3)	9.6 (0.3)	27.2 (0.0)	64.6 (1.1)	15.9 (0.2)
13	60.6 (3.1)	2.6 (0.8)	17.8 (1.7)	9.7 (0.4)	27.5 (2.1)	64.7 (1.3)	16.1 (1.0)
15	59.5 (2.3)	2.6 (0.8)	19.5 (1.8)	9.2 (0.4)	28.7 (1.4)	67.8 (2.9)	17.6 (1.0)
18	59.4 (1.4)	2.7 (0.7)	19.5 (1.1)	9.3 (0.2)	28.7 (0.9)	67.7 (1.7)	17.6 (0.4)
20	59.1 (0.9)	2.7 (0.7)	18.9 (2.3)	9.2 (0.1)	28.2 (2.4)	66.9 (2.5)	17.0 (1.5)
23	57.9 (1.0)	2.75 (0.65)	20.5 (0.6)	9.1 (0.3)	30.0 (1.3)	69.7 (0.4)	19.0 (0.3)
25	56.9 (0.9)	2.75 (0.65)	18.6 (1.4)	9.1 (0.5)	27.7 (0.9)	66.9 (3.0)	16.8 (0.7)
28	55.9 (0.5)	2.75 (0.65)	21.0 (3.2)	9.2 (0.1)	30.2 (3.1)	69.3 (3.5)	18.9 (2.2)
30	56.2 (0.8)	2.75 (0.65)	22.1 (4.8)	9.4 (0.4)	31.5 (4.4)	69.5 (5.6)	19.9 (3.7)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 6.9.

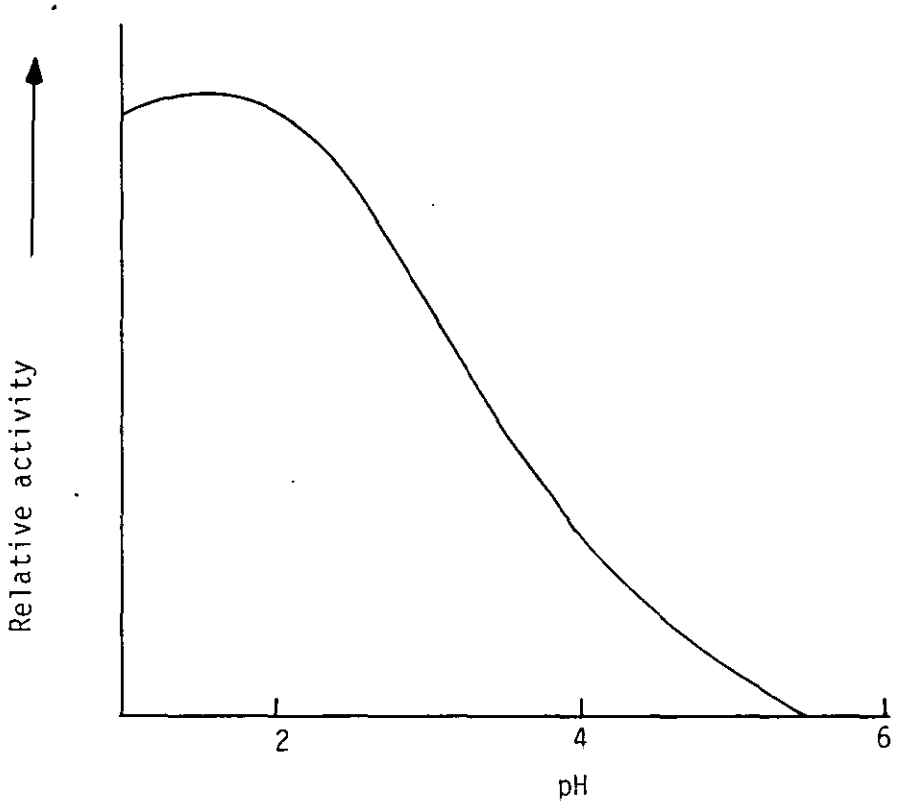
APPENDIX 21: Acid hydrolysis of uncooked *Haplochromis* at 58 ± 4°C (2.5M HCl)[1]

Time (min)	Temperature (°C)	pH[2]	4 ml SAMPLE ALIQUOT				REACTION VESSEL CONTENTS
			TCA-soluble Nitrogen (mg)	TCA-insoluble Nitrogen (mg)	Total Nitrogen (mg)	TCA-soluble N at given time (%)	TCA-soluble Nitrogen as % of Total Nitrogen
0	50.8 (1.7)	2.6 (0.1)	18.5 (3.5)	7.1 (0.5)	25.6 (3.0)	71.4 (5.2)	22.1 (4.2)
3	55.0 (3.2)	3.0 (0.0)	24.7 (2.7)	8.3 (0.4)	33.0 (2.3)	74.5 (3.3)	29.5 (3.2)
6	56.3 (3.1)	3.1 (0.0)	24.2 (1.4)	8.5 (1.2)	32.7 (0.2)	73.9 (3.8)	28.9 (1.7)
9	57.7 (1.9)	3.15 (0.05)	26.7 (0.8)	7.9 (0.6)	34.6 (1.5)	77.3 (0.9)	32.0 (1.0)
13	61.1 (3.0)	3.2 (0.0)	27.5 (0.2)	8.1 (1.0)	35.6 (1.3)	77.4 (2.1)	32.9 (0.3)
15	60.7 (2.5)	3.25 (0.0)	29.2 (2.2)	8.1 (1.1)	37.3 (3.2)	78.2 (0.9)	34.9 (2.6)
18	59.8 (2.0)	3.25 (0.0)	28.0 (0.5)	7.7 (1.1)	35.7 (0.7)	78.4 (2.7)	33.4 (0.5)
20	60.5 (0.2)	3.35 (0.05)	24.5 (3.9)	8.0 (0.6)	32.5 (3.3)	74.8 (4.4)	29.3 (4.7)
23	59.9 (0.8)	3.35 (0.05)	27.3 (1.8)	8.1 (1.1)	35.5 (0.6)	77.0 (3.5)	32.7 (2.1)
25	58.6 (0.7)	3.4 (0.0)	31.4 (1.5)	7.9 (0.6)	39.3 (2.1)	79.9 (0.5)	37.5 (1.7)
28	57.3 (0.4)	3.45 (0.0)	23.2 (6.1)	8.3 (0.7)	31.5 (5.4)	72.4 (6.9)	27.7 (7.3)
30	56.2 (0.7)	3.5 (0.1)	26.0 (1.5)	8.6 (0.5)	34.6 (1.0)	75.2 (2.2)	31.1 (1.7)

(1) Mean of duplicate determinations (mean deviation in parentheses)

(2) Initial pH of mince = 6.95.

APPENDIX 22: The pH-activity profile of Pepsin[1]



(1) Lehninger (1975)

APPENDIX 23: Coding of samples as referred to in text

C = cooked fish
U = uncooked fish
B = bleached hydrolysate
A = unbleached hydrolysate

C25 = cooked fish processed at 25°C with 11.3M HCl
C37 = cooked fish processed at 37°C with 11.3M HCl
C46 = cooked fish processed at 46°C with 11.3M HCl
C56 = cooked fish processed at 56°C with 11.3M HCl
C64 = cooked fish processed at 64°C with 11.3M HCl
C74 = cooked fish processed at 74°C with 11.3M HCl
C84 = cooked fish processed at 84°C with 11.3M HCl

U25 = uncooked fish processed at 25°C with 11.3M HCl
U38 = uncooked fish processed at 38°C with 11.3M HCl
U48 = uncooked fish processed at 48°C with 11.3M HCl
U56 = uncooked fish processed at 56°C with 11.3M HCl
U65 = uncooked fish processed at 65°C with 11.3M HCl
U74 = uncooked fish processed at 74°C with 11.3M HCl
U84 = uncooked fish processed at 84°C with 11.3M HCl
*C2.5M = cooked fish processed at 58°C with 2.5M HCl
*C5M = cooked fish processed at 59°C with 5M HCl
*C7.5M = cooked fish processed at 58°C with 7.5M HCl
*C11.3M = C56 cooked fish processed at 56°C with 11.3M HCl

U2.5M = uncooked fish processed at 58°C with 2.5M HCl
U5M = uncooked fish processed at 59°C with 5M HCl
U7.5M = uncooked fish processed at 60°C with 7.5M HCl
U11.3M = uncooked fish processed at 56°C with 11.3M HCl

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* Final products produced and evaluated in 4.6.

Appendix 23 ... continued

*CB = cooked and bleached fish processed at 56°C with 11.3M HCl
*CA = cooked and unbleached fish processed at 56°C with 11.3M HCl
*UB = uncooked and bleached fish processed at 56°C with 11.3M HCl
*UA = uncooked and unbleached fish processed at 56°C with 11.3M HCl

* Final products produced and evaluated in 4.6.

APPENDIX 24: Amino acid profiles of *Haplochromis*

Amino Acid g/100g Protein	Sample		
	Whole Fish[1] 6.0-8.9g	Whole Fish[1] 14g and over	Deboned, Pressed dried & ground (2)
Aspartic Acid	11.4	10.9	9.9
Threonine	5.2	5.2	4.6
Serine	5.3	5.1	4.8
Glutamic Acid	17.8	18.4	14.3
Glycine	10.3	10.4	8.8
Alanine	8.8	9.7	7.2
Cystine[3]	-	-	1.1
Valine	5.4	5.2	5.6
Methionine	3.3	3.3	3.2
Isoleucine	5.0	4.9	5.0
Leucine	8.7	8.6	7.5
Tyrosine	3.9	3.6	3.7
Phenylalanine	4.7	4.8	5.1
Lysine	9.0	9.4	7.9
Histidine	2.5	2.5	2.3
Arginine	8.2	7.5	6.2

(1) Ssali (1981)

(2) Freshwater Fisheries Institute, Nyegezi, Tanzania (1975), for deboned pressed, dried and ground *Haplochromis* from Lake Victoria

(3) Cystine + tryptophan = 2% of total amino acids

APPENDIX 25: FAO/WHO suggested pattern of amino acid requirements in man[1]

Amino Acid g/100g protein	Infant (0-2 yrs)	Child (10-12 yrs)	Adult
Histidine	1.4	0	0
Isoleucine	3.5	3.7	1.8
Leucine	8.0	5.6	2.5
Lysine	5.2	7.5	2.2
Methionine[2]	2.9	3.4	2.4
Phenylalanine[3]	6.3	3.4	2.5
Threonine	4.4	4.4	1.3
Tryptophan	0.9	0.5	0.7
Valine	4.7	4.1	1.8

(1) FAO/WHO (1973)

(2) Figures are for total sulphur amino acids (methionine and cystine).

(3) Figures are for total phenylalanine and tyrosine.

APPENDIX 26: pH drop during 10 minutes incubation of four *Haplochromis* powders and casein with a multienzyme system

Time (min)	Sample and pH[1]				
	Casein	YC2.5M	YC5M	YC7.5M	YC11.3M
0	8.0 (0)	8.0 (0)	8.0 (0)	8.0 (0)	8.0 (0)
1	7.1 (0.4)	7.3 (0.1)	7.2 (0.15)	7.2 (0.15)	7.3 (0)
2	7.0 (0.4)	7.15 (0.1)	7.1 (0.15)	7.1 (0.15)	7.25 (0)
3	6.8 (0.4)	7.1 (0.1)	7.0 (0.1)	7.0 (0.15)	7.15 (0)
4	6.8 (0.3)	7.0 (0.1)	7.0 (0.1)	7.0 (0.15)	7.15 (0.05)
5	6.75 (0.3)	7 0 (0.1)	6.9 (0.15)	6.95 (0.15)	7.1 (0.05)
6	6.7 (0.3)	6.95 (0.1)	6.9 (0.15)	6.9 (0.15)	7.05 (0)
7	6.7 (0.3)	6.95 (0.05)	6.9 (0.1)	6.9 (0.15)	7.05 (0)
8	6.65 (0.3)	6.95 (0.05)	6.9 (0.1)	6.9 (0.1)	7.0 (0)
9	6.65 (0.3)	6.9 (0.05)	6.9 (0.1)	6.9 (0.1)	7.0 (0.05)
10	6.6 (0.3)	6.9 (0.05)	6.85 (0.1)	6.9 (0.1)	6.95 (0)

(1) Mean of triplicate determinations, standard deviation in parentheses.

APPENDIX 27: pH drop during 10 minutes incubation of four *Haplochromis* powders and casein with chymotrypsin

Time (min)	Sample and pH[1]				
	Casein[2]	YC2.5M	YC5M	YC7.5M	YC11.3M
0	8.0	8.0 (0)	8.0 (0)	8.0 (0)	8.0 (0)
1	7.3	7.5 (0)	7.4 (0.1)	7.35 (0.05)	7.3 (0.1)
2	7.15	7.3 (0.05)	7.2 (0.05)	7.15 (0.05)	7.2 (0.1)
3	7.05	7.25 (0.05)	7.1 (0.05)	7.1 (0.05)	7.1 (0.1)
4	7.05	7.15 (0.05)	7.05 (0.1)	7.05 (0.05)	7.0 (0.05)
5	7.0	7.1 (0)	7.0 (0.1)	7.0 (0.1)	7.0 (0.05)
6	6.95	7.05 (0.05)	7.0 (0.1)	6.95 (0.05)	6.95 (0.05)
7	6.95	7.05 (0.05)	6.95 (0.1)	6.9 (0.05)	6.95 (0.05)
8	6.9	7.0 (0)	6.9 (0.05)	6.9 (0.05)	6.95 (0.1)
9	6.9	7.0 (0)	6.9 (0)	6.9 (0)	6.9 (0.1)
10	6.9	7.0 (0)	6.9 (0)	6.9 (0)	6.9 (0.1)

(1) Mean of duplicate determinations, mean deviation in parentheses.

(2) Single determination.

APPENDIX 28: pH drop during 10 minutes incubation of four *Haplochromis* powders and casein with peptidase

Time (min)	Sample and pH[1]				
	Casein	YC2.5M	YC5M	YC7.5M	YC11.3M
0	8.0 (0)	8.0 (0)	8.0 (0)	8.0 (0)	8.0 (0)
1	7.1 (0.4)	7.75 (0)	7.75 (0.05)	7.65 (0.05)	7.7 (0.05)
2	7.0 (0.5)	7.65 (0.05)	7.65 (0.05)	7.55 (0.05)	7.65 (0.05)
3	7.0 (0.5)	7.6 (0)	7.6 (0.05)	7.5 (0.05)	7.6 (0)
4	7.0 (0.5)	7.55 (0)	7.55 (0.05)	7.5 (0.05)	7.55 (0)
5	6.95 (0.5)	7.55 (0)	7.55 (0)	7.4 (0.05)	7.5 (0)
6	6.95 (0.5)	7.5 (0)	7.5 (0)	7.4 (0)	7.45 (0.05)
7	6.95 (0.45)	7.45 (0.05)	7.45 (0)	7.35 (0.05)	7.45 (0.05)
8	6.95 (0.45)	7.45 (0.05)	7.4 (0)	7.35 (0)	7.4 (0.05)
9	6.95 (0.45)	7.4 (0)	7.35 (0.05)	7.35 (0)	7.4 (0.05)
10	6.95 (0.45)	7.4 (0)	7.35 (0.05)	7.3 (0)	7.4 (0.05)

(1) Mean of duplicate determinations, mean deviation in parentheses.

APPENDIX 29: pH drop during 10 minutes incubation of four *Haplochromis* powders and casein with trypsin

Time (min)	Sample and pH[1]				
	Casein	YC2.5M	YC5M	YC7.5M	YC11.3M
0	8.0 (0)	8.0 (0)	8.0 (0)	8.0 (0)	8.0 (0)
1	7.35 (0.1)	7.75 (0.05)	7.7 (0.05)	7.65 (0.05)	7.55 (0.05)
2	7.25 (0.15)	7.7 (0)	7.55 (0.1)	7.55 (0.05)	7.5 (0.05)
3	7.15 (0.15)	7.6 (0)	7.5 (0.05)	7.45 (0.05)	7.4 (0.1)
4	7.1 (0.15)	7.55 (0.05)	7.45 (0.05)	7.4 (0.05)	7.35 (0.1)
5	7.1 (0.2)	7.45 (0.05)	7.4 (0.05)	7.4 (0)	7.35 (0.1)
6	7.05 (0.2)	7.45 (0.05)	7.4 (0)	7.4 (0)	7.35 (0.1)
7	7.0 (0.2)	7.4 (0.05)	7.35 (0.05)	7.35 (0.05)	7.3 (0.1)
8	7.0 (0.2)	7.35 (0.05)	7.3 (0.05)	7.35 (0)	7.3 (0.1)
9	6.95 (0.25)	7.35 (0.05)	7.3 (0.05)	7.35 (0.05)	7.3 (0.1)
10	6.9 (0.2)	7.35 (0.05)	7.25 (0.05)	7.35 (0.05)	7.3 (0.1)

(1) Mean of duplicate determinations, mean deviation in parentheses.

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