CC7;Unit 6; FERMENTED FISH PRODUCTS

Fermentation is one of the methods of preservation of foods. The fermentation process is based on traditional knowledge and later on the scientific basis of the action of ingredients was understood. The preservation of fish by fermentation depends on the interaction of a number of environmental and microbiological factors including pH, water activity, presence of preservatives and competitive microflora. Preservation is usually achieved by a combination of fermentation, with the use of water activity-lowering compound usually salt. The selection of favourable conditions to encourage the growth and development of a desirable safe microflora is particularly important, as fish may not be cooked before or after fermentation.

The topic covers the following aspects.

1. Fermentation process
2. Fermentation of fish
3. Fermented fish products.
4. Fish silage
5. Fish protein hydrolysate

1. FERMENTATON PROCESS

Some types of microorganisms thrive in foods and produce metabolites. These metabolites may be injurious to competing spoilage species and thus help preserve food. Such preservation depends on the anaerobic breakdown of carbohydrates to yield various alcohols and organic acids (formic, acetic, propionic or lactic), ie., fermentation by non-pathogenic microorganisms. Indigenous microorganisms were responsible for the fermentation traditionally. Desired sensory attributes can now be assured by adding cultures of predetermined composition, under strictly controlled operating conditions. Meat is a post-mortem aspect of muscle. Lactic acid is naturally produced in muscle during its postmortem conversion to meat. Lactic acid is also the organic acid which is most frequently produced by the microbial fermentation of fish.The traditional fermentation took considerable amount of time to obtain desired changes in meat. Process of fermentation can be accelerated by adding known type of microbial starter cultures, providing appropriate environmental conditions and precisely controlling the process of fermentation.The advantagesof fermentation process are production process is simple, cost effective, products have long storage life and suitable for both large and small scale operation. The disadvantages are skilled and very strict operating conditions in order to produce safe food.

2. FERMENTATION OF FISH

The principal attributes of eating quality of meat are color, texture (including tenderness and juiciness) and flavor. Muscle in live animal is generally microbiologically sterile. Meat may be infected by microorganisms by subsequent postmortem contamination. Postmortem contamination of meat is the major cause of both sensory quality spoilage and of food poisoning. The survival and growth of microorganisms in meat is determined by the availability of nutrients, temperature, moisture content, pH and nature of gaseous environment to which the organisms are exposed.

The different types of microorganism associated with meat are psychrotrophs (temperature optima, -2 to 7ºC), mesophils (temperature optima, 10 – 40ºC) and thermophils (temperature optima, 43 – 66ºC). In effectively chilled meat, the spoilage microflora will not normally be pathogenic. With ineffective refrigeration in the immediate postmortem period, mesophils may grow causing offensive odor. The availability of water (water activity, aw) is another major determinant of microbial growth. When the concentration of soluble compounds such as salts and sugars increase, microorganisms have increasing difficulty in obtaining sufficient water for their metabolism. The aw is the ratio of the water vapour of the medium to that of pure water at the same temperature. Bacteria grow from aw of under 1.0 to 0.75; yeasts and moulds will grow slowly at an aw of 0.62. Fresh meat has aw of about 0.99 and hence can spoil through the growth of a wide range of microorganisms. The rate of growth of microbes falls rapidly as the aw is lowered. The pH of meat markedly affects microbial growth. Most microbes grow optimally at pH 7 and little below pH 4 or above pH 9.

3. FERMENTED FISH PRODUCTS

There are several fish products based on traditional technologies involving enzymes, acids and bacteria. In most of these products the protein present in fish flesh is broken down into simpler substances and the products will be in the form of liquid or semisolid. These products are collectively referred to as proteolysed or fermented fish products.

In Eastern Border States of India there a few traditional fermented fish products based on small sized fresh water fish species, which are not suitable or relished as table fish. Hentak and Ngari are two such products well relished by the people of these states. Table 1 lists the chemical composition of Hentak and Ngari fish paste products. Hentak and Ngari contain mainly about 33% (5.3 X 6.25) and 38 % (6.1 X 6.25) proteins and 13.6 % and 13.3 % oils, respectively. Several fermented fish products are produced in South East countries like Malaysia, Indonesia, Philippines, Thailand, Vietnam, Cambodia and Myanmar. Ngapi of Myanmar,Budu of Indonesia, Patis of Philippines, Nampla of Thailand and Nuocman of Vietnam are the examples of fish sauces. Trassi of Indonesia, Bagoong of Philippines, Kapi of Thailand, Mamea ofVietnam and Prohoc of Cambodia are the examples of fish pastes.

4. FISH SILAGES

Ensilaging or ensiling or silaging is a process of preserving food by using acid. The vessel where ensiling is done is silo and the product is silage. If inorganic acids (hydrochloric,

sulphuric, phosphoric acids) are used for ensiling, the product is called acid silage. Fermented silage is the product preserved by using organic acids such as formic, propionic and acetic acids. Since organic acids are expensive, judicious combination of both inorganic and organic acids are used for the production of fish silage. Fish silage is a product obtained by acid preservation of fish of low nutritional or commercial value. Only soft tissues such as flesh, viscera (intestines, livers, roes or fish eggs etc) and blood are amenable for ensiling. Hard tissues such as bones and scales are not suitable for ensiling.

Generally fermented fish silages are produced using pure culture of lactic acid bacteria (LAB) and sugar source such as molasses. Lactic acid produced by LAB lowers the pH and aid liquefaction process.The acidity of the product inhibits microbial activity and favours enzymatic reaction. Silages are of semisolid consistency. The type of fish, fat content, temperature and freshness of fish influence the rate of liquefaction.Compared to fish meal, silages are advantageous as silage production does notneed complicated machineries. The process is simple. The disadvantages of silage are (i) The silage product is bulky with about 70-75% moisture content and (ii)Silages need acid resistant containers for storage and transportation.Table 2 lists some chemical quality characteristics of fermented products from different fish species. Fermented fish products from different fish contain 13.8 – 19.4 % protein (Total nitrogen X 6.25) and 5.8 – 19.3 % salt.

If silage is made from oily fish containing considerable amount of oil, it is better to separate oil. Very oily silage is not suitable as an ingredient in animal feed as it causes fishy taint in the flesh of animals. However, the fishy taint can be metabolically turned over by feeding the animals during the last weeks before slaughtering with the rations without silage. As oil is economically valuable, its separation will give additional revenue. The antioxidants such as butylated hydroxytoluene (BHT) or ethoxyquin may be incorporated in the silaging mixture to prevent / minimise oxidation of fats. Silaging also promotes oil separation and heating and pressing the material may not be necessary. Fish silage is stable if the pH is below 4.5. The silages at pH 4.5 and above can be used as feed ingredient directly without neutralisation. Acid silage has pH much below 4.5 and hence need neutralisation prior to use in feed. Fermented fish silage product is of medium acidity of pH 4.5 and does not need neutralisation.It can be directly incorporated in feed as a substitute for fish meal.The diet containing silage products has shown better growth and performance in fish and poultry compared to conventionally used fish meal as a source of valuable animal proteins.

In the fish silage production, the raw material is minced and mixed with acids or bacterial cultures and carbohydrate source which produce acids. Adequate mixing ensures that the enzymes naturally present in the raw material digest the material under favourable conditions provided by the acids. The composition of silage is mainly influenced by the composition of raw material itself and to some extent the amount of additives primarily carbohydrate source added to effect fermentation. The proximate composition is normally sufficient for the assessment of the silage product value as an ingredient in animal feed. The treatment conditions and composition of acid and fermented silages of fish processing byproductsare presented in Tables3 and 4.Acid silages from different fish byprocducts contain 12.2 – 15.5%

proteins. Fermented silages are more rich in nutrients with 12.0 – 13.4 % proteins and 5.2 – 23.9% fats.

5. FISH PROTEIN HYDROLYTES (FPH)

When proteins are hydrolysed, the end products are the amino acids; the intermediate products are metaprotein, peptones and polypeptides. The FPH are produced using powerful proteolytic enzymes. The enzymes are derived from plants, animals and microorganisums.The commonly used plant based enzymes are papain from papayalatex, ficin from figs and bromelin from pineapple peels. The enzymes from animal source are trypsin and chymotrypsin. Certain microbial enzymes such as pronase and alcalase also produce good hydrolysates. In the conventional enzymatic method, commercial enzymes are directly applied under predefined conditions such as pH, temperature, incubation time and enzyme / substrate ratio. The essential processing steps for the production of FPH are provided in Fig. 1. Proteases producing microbial strains are used as starter culture and incubated in preferred conditions to grow microbes on fish. The yield of FPH depends on various factors such as enzyme used, its activity, duration of hydrolysis, species of fish, its size and physiological condition, cooking and pressing. The yields of dry hydrolysateon the round weight of a few species of fish varies from 7.5 – 13.5 % (Table 5).The yield of hydrolysates is in the range of 7.5 – 13.3%.Table 6 provides proximate composition of fish protein hydrolysates. The hydrolysates are very rich in proteins (83.1 – 91.2%) and poor in fat contents (0.1 – 4.2 %).

Physicochemical as well as functional properties of enzymatic hydrolysates vary with the degree of hydrolysis which determines size of protein fractions. Over hydrolysis may impair some functional properties of fish proteins or develop off – flavours in hydrolysates. The protein hydrolysatespossess bitter taste. Certain flavouring agents are used to mask the bitterness for human consumption.The FPHs possess potent antioxidant activity. The FPHs also reduce risk of cardiovascular diseases by modulating blood clot and platelet formation, cholesterol metabolismand regulation of blood pressure.

Fish sauce or paste: Fish sauce or paste is salted and fermented fish product. Marine fish (anchovies, clupeids, mackerel, lizard fish) and fresh water fish are the raw materials commonly used for the preparation of fish sauces. Quality of the product depends on the type of fish, the amount of salt used and fermentation time. The product may be (a) high salt filtered fermented product, (b) unfiltered fish sauce, (c) *shiokara* paste involving hydrolysis with or without frinding / drying and (d)*narezushi*, fermented products based on fish, salt and carbohydrates.

Fish sauce or paste production involves mixing of whole fish in tubs with salt in the various ratios of 1:1 to 1: 5 (salt:fish, w/w). The mix is left sealed from air at ambient temperature (~30ºC) and allowed to autolyse for 2 to 18 months. Mainly the proteolytic enzymes present in the muscle cause autolysis, since bacterial action is minimal due to high salt content. Proteases isolated from different fish are listed in Table 7.The first liquid is drawn off and

filtered. This filtrate is the first grade product. The residue after the first liquid is drawn off, is left with 25% brine for a few days or weeks. The accrued filtrate is a second grade product. The residue at this stage may go for use as a fertilizer or is further extracted with boiling brine. This brine is then passed through new residue. These are mixed with different amounts of the first grade liquid to obtain products of different grades.

The sauce contains a high level of soluble nitrogenous compounds. The autolysis can be accelerated by the addition of exogenous proteases. Addition of proteolytic enzymes such as fungal protease, pronase, trypsin, chymotrypsin, or squid protease to mince and salted fish increase the rate of protein solubilisation. This accelerated autolysis reduces the incubation time. A first grade fish sauce contains 2 - 3 % nitrogen, 20 - 25% salt with a pH below 6.0.

The liquids which will have malty odour and golden yellow color are termed fish sauce. The solids which remain on the cloth are soft with pasty consistency and are called fish pastes. The sauces and pastes have different names. They are used as condiments and important as a source of animal protein in the predominant rice diet of South East Asian countries.The products are packed in glass containers and stored for use in daily diet. The typical fishy flavour, malty taste and high salt content are the desirable sensory characteristics of the fermented fish products. They are the favourite dish in every menu as a source of high quality protein in easily digestible form.

The essential components of the process are:

i. Restriction of the natural microbial spoilage flora by the addition of high amount of salt. The low water activity and anaerobic conditions result in a decreasing microbial count throughout the period of fermentation.
ii. The use of uneviscerated fish and the autolytic breakdown of the fish proteins result in the separation of a nitrogen – rich liquid phase. The high concentration of salt slows down the action of endogenous proteases and less important role of bacteria.
iii. Ammonia, trimethylamine, volatile fatty acids amino acids help development of correct flavor, taste and aroma of sauce. Although bacteria are not responsible for proteolysis, a special bacteria (Clostridia) are responsible for the peculiar and special flavor and taste of these products.

Conclusion: Fish and fish processing byproducts are rich in valuable proteins and oils as nutrients. They also contain high value biomolecules such as enzymes. Small fish is not relished as human food and such material is generally rendered for the production of fish meal for use in animal diets. Rendering is expensive due to severe heat input. Enzymatic hydrolysis of these products seems to be better alternative. The fish and its soft byproducts such as flesh, viscera (intestines, livers,) and blood can be subjected to such mild treatments. Such materials can be treated with appropriate bacterial cultures especially of lactic acid bacteria and a suitable carbohydrate source (for example, sugar industry by-roduct molasses).

The treated mixture is allowed to ferment under suitable conditions to obtain protein hydrolysates and / or silage products. This mild treatment will help preserve the nutrients almost in their native state. Fish silage from low value fish and byproducts has been used as an ingredient in animal feed formulations replacing conventionally used fish meal. Silage products have resulted in to better growth and performance in fish and poultry when included in diets compared to fish meal diet.

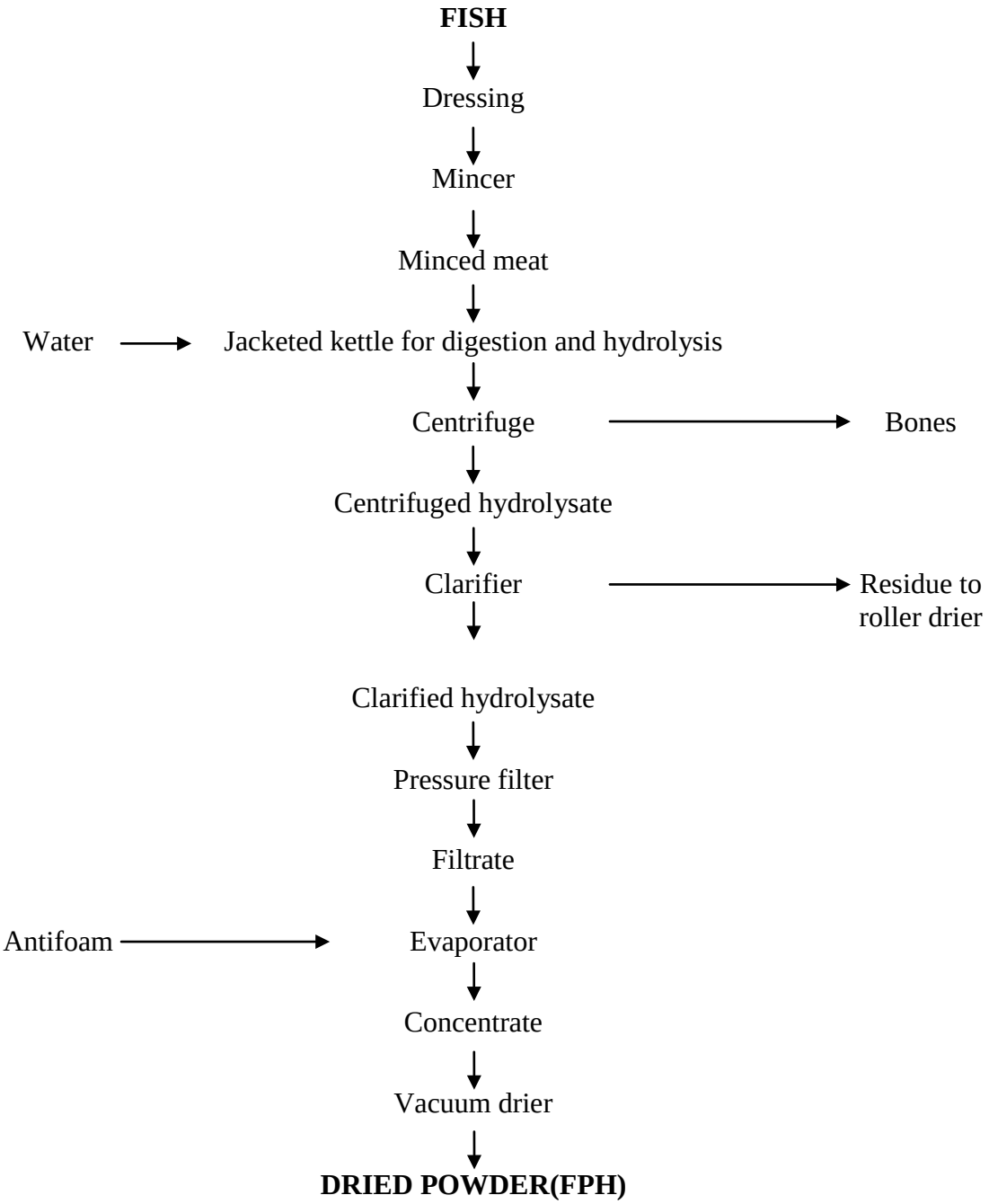

Fig. 1. Essential processing steps for the production of fish protein hydrolysate (FPH)
Source: Sen 2005

T**able 1.** Chemical composition of Hentak and Ngari fermented fish pastes

	Hentak	**Ngari**
Water, %	36.3	36.0
Total nitrogen, %	5.3	6.1
Non – protein nitrogen, %	1.3	3.6
Total lipids, %	13.6	13.3
Ash, %	11.4	5.5
Total soluble sugar, %	1.5	1.1
Total amino acids expressed as glycine, mg / g	6.0	8.1
pH	6.7	7.5

Note: Fermented fish pastes are rich source of valuable proteins and oils.

Source: Sen 2005

Table 2. Chemical quality characteristics of fermented fish products

Name of fish	**pH**	**Total titrable acidity, mg lactic acid / g**	**Salt, %**	**Moisture, %**	**Total nitrogen, %,**	**Non-protein nitrogen, %**
Common carp	4.4	22.6	8.6	59.3	3.1	0.27
Cat fish	4.2	28.0	5.8	69.4	2.4	0.28
Snake head fish	5.2	16.3	12.3	62.4	2.21	0.73
Gourami	5.4	16.3	19.3	53.6	2.66	0.68

Note: Fermented fish products from different fish contain 13.8 – 19.4 % protein (Total nitrogen X 6.25) and 5.8 – 19.3 % salt.
Source: Sen 2005

Table 3. Proximate composition (%) of acid silages of fish byproducts from different varieties of fish.

Type of byproduct	**Treatment conditions**	**Moisture**	**Protein**	**Ether extract (Fat)**	**Ash**
Cod viscera - deoiled	1.5%FA+1.5%PA, 30ºC, 8 days	83.6	12.2	0.2	1.7
Saithe viscera - deoiled		83.6	12.2	0.2	1.7
Herring offal	3.5%FA, 25ºC, 2 days	75.4	13.5	8.7	2.6
Herring offal – deoiled		80.0	14.5	2.0	2.8
White fish offal		78.9	15.0	0.5	4.2
Otholitus fish waste	1.5-2.0%FA+1.5-2.0%PA+250ppmBHT, 25-18ºC, 15 days	67.2	15.5	6.0	8.5
Fresh water fish viscera (mixed)	0.75%SA+0.75%FA+1.5%PA, 26ºC, 7 days	67.8	12.3	14.0	2.5

BHT: Butylated hydroxytoluene; FA: Formic acid; PA: Propionic acid; SA: Sulfuric acid

Note: Acid silages are rich in proteins.

Source: Mahendrakar and Rathina Raj 2015

Table 4. Proximate composition (%) of fermented silages of fish byproducts from different varieties of fish.

Type of byproduct	Treatment conditions	Moisture	Protein	Ether extract (Fat)	Ash
Otholitus fish waste	15%M+250 ppmBHT, 25-28ºC 15 days	71.7	12.5	5.2	7.7
Fresh water fish viscera (mixed)	10%M+ 0.5%PA +0.02%EQ, 27ºC, 3 days	67.5	13.4	10.4	3.0
Fresh water fish viscera (mixed)	10%M+ 0.5%PA +0.02%EQ, 26ºC, 8 days	67.1	13.4	10.6	2.9
Fresh water fish viscera (mixed)	7.5%M, 37ºC, 14 days	60.3	12.0	23.9	3.3

BHT: Butylated hydroxytoluene; EQ: Ethoxyquin; M: Molasses; PA: Propionic acid.

Note: Fermented silages are rich in proteins and oils.

Source: Mahendrakar and Rathina Raj 2015

Table 5. Yield of protein hydrolysates from miscellaneous fish by enzymatic digestionmethod with papain

Common name of fish	Scientific name of fish	Yield of hydrolysate (Dry soluble as % of wet whole fish or fish waste)
Anchovy	*Thrissocles spp.*	9.7
Cat fish	*Tachysurus spp.*	10.9
Jew fish	*Johnius spp.*	9.9
Lizard fish	*Saurida tumbil*	13.3
Perch	*Nemipterus bleekeri*	7.5
Sole	*Nemipterus japonicus*	12.0
Ribbon fish	*Trichiurus spp.*	9.9

Source: Sen 2005

Table 6. Proximate composition of a few samples of fish protein hydrolysates

Common name of fish	Scientific name	Portion of fish	Moisture, %	Protein, %	Fat, %	Ash, %
Cod fish	*Galus spp.*	Filleting byproduct	8.5	81.8	4.2	6.9
Jew fish	*Johnius spp.*	Filleting byproduct from whole fish	1.4	91.2	Trace	8.6
Lizard fish	*Saurida tumbil*	Whole fish	5.0	83.1	0.1	5.4
Thread fin bream	*Nemipterus japonicus*	Whole fish	3.5	86.4	0.1	7.6
Tilapia	*Oreohcromis mossambicus*	Mechanically deboned washed flesh	3.2	90.1	0.5	6.2

Note: The hydrolysates are rich in valuable proteins.
Source: Sen 2005.

Table 7. Proteases isolated from different fish

Enzyme	Type of enzyme	Fish source
Pepsin	Aspartic protease	Sardine, Capelin, Salmon, Cod, Mackerel, Tuna, Rainbow trout, Shark
Pepsinogen	Aspartic protease	Rainbow trout, Tuna, Shark
Trypsin	Serine protease	Sardine, capelin, Salmon, Cod, Anchovy, Carp, Mackerel
Chymotrypsin	Serine protease	Carp, Capelin, Herring, Rainbow trout, Grass carp, Catfish, Atlantic cod
Alkaline protease	Serine protease	White croakers, Chum salmon, Tilapia
Neutral protease	Metallo – proteases, cystein proteases	Tilapia

Source: Venugopal 2015