Fish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review

Ghaly AE*, Ramakrishnan VV, Brooks MS, Budge SM and Dave D

Department of Process Engineering and Applied Science, Faculty of Engineering Dalhousie University, Halifax, Nova Scotia, Canada

Abstract

The fish processing industry is a major exporter of seafood and marine products in many countries. About 70% of the fish is processed before final sale. Processing of fish involves stunning, grading, slime removal, deheading, washing, scaling, gutting, cutting of fins, meat bone separation and steaks and fillets. During these steps significant amount of waste (20-80% depending upon the level of processing and type of fish) is generated which can be utilized as fish silage, fishmeal and fish sauce. Fish waste can also be used for production of various value added products such as proteins, oil, amino acids, minerals, enzymes, bioactive peptides, collagen and gelatin. The fish proteins are found in all parts of the fish. There are three types of proteins in fish: structural proteins, sacroplasmic proteins and connective tissue proteins. The fish proteins can be extracted by chemical and enzymatic process. In the chemical method, salts (NaCl and LiCl) and solvents (isopropanol and aezotropic isopropanol) are used, whereas during the enzymatic extraction, enzymes (alcalase, neutrase, protex, protemax and flavorzyme) are used to extract proteins from fish. These fish proteins can be used as a functional ingredient in many food items because of their properties (water holding capacity, oil absorption, gelling activity, foaming capacity and emulsifying properties). They can also be used as milk replacers, bakery substitutes, soups and infant formulas. The amino acids are the building blocks of protein. There are 16-18 amino acids present in fish proteins. The amino acids can be produced from fish protein by enzymatic or chemical processes. The enzymatic hydrolysis involves the use of direct protein substrates and enzymes such as alcalase, neutrase, carboxypeptidase, chymotrypsin, pepsin and trypsin. In the chemical hydrolysis process, acid or alkali is used for the breakdown of protein to extract amino acids. The main disadvantage of this method is the complete destruction of tryptophan and cysteine and partial destruction of tyrosine, serine and threonine. The amino acids present in the fish can be utilized in animal feed in the form of fishmeal and sauce or can be used in the production of various pharmaceuticals. The fish oil contains two important polyunsaturated fatty acids called EPA and DHA or otherwise called as omega-3 fatty acids. These omega-3 fatty acids have beneficial bioactivities including prevention of atherosclerosis, protection against maniac–depressive illness and various other medicinal properties. Fish oil can also be converted to non-toxic, biodegradable, environment friendly biodiesel using chemical or enzymatic transesterification.

Introduction

The fish processing industry in Canada is a major exporter of seafood and marine products. Canada exports 75% of its fish products to more than 80 countries. In the year 2012, exports from Canada amounted to 595,615.738 metric tonnes of fish worth $4.15 billion [1]. Canada has the world's longest coastline (244,000 km) which makes 25% of the entire world's coastline. Atlantic Canada represents 40,000 km of coastline which comprises four major provinces. It exports high quality harvested ground fish, shellfish and pelagic fish accounting for 85% of the total Canadian fish landings [2]. The Pacific fishery accounts for 14% of the total fish landings and includes cod, redfish sp., flatfish, hake, herring, tuna, salmon and calms. The freshwater fishery accounted for 1% of the total landings and includes perch, pike, whitefish, yellow pickerel and smelt. The aquaculture production in Canada for the year 2011 reached 161,036 tonnes worth $845,598 [3].

The world marine capture fisheries contribute more than 50% of the total world fish production. About 70% of fish is processed before final sale, resulting in 20-80% of fish waste depending on the level of processing and type of fish [4]. In addition, a significant amount of the total catch from fish farming is discarded each year. Also, fish processing operation require large volumes of potable water which results in significant amounts of waste water [5]. The majority of fish wastes are disposed of in the ocean. The aerobic bacteria present in the water breakdown the organic matter in the presence of oxygen leading to a considerable reduction of oxygen in water. There are also overloads of nitrogen, phosphorous and ammonia, which lead to pH variation, increased turbidity of the water and as a result of the decomposition of algae. The reduction in water oxygen content creates an anaerobic condition that leads to the release of foul gases such as hydrogen sulfide and ammonia, organic acids and greenhouse gases such as carbon dioxide and methane [6].

The discards from the processing plants amount to 20 million tonnes which is equivalent to 25% of the world's total production from marine capture fisheries [4]. These wastes can be used to produce fish protein concentrate, fish oils and enzymes (such as pepsin and chymotrypsin) as well as other value added products. The fish oil is used for products such as margarine, omega-3 fatty acids and biodiesel.

The fish protein concentrate is used as human food and animal feed. Fish protein is also rich in amino acids which are highly suitable for human consumption [7].

Fish Production

World fish production

All around the world, fish is produced from capture fisheries and aquaculture [3,8]. About 154 million tonnes of fish were produced in the year 2011 (Table 1) with a value of $217.5 billion. Approximately 131 (85%) million tonnes were directly utilized as food and the rest (15%) was underutilized as live bait for fishing, ornamental products (pearls and shells), feed for carnivorous farmed species and marine worm. There has been a sustained growth in the fish supply during the last 50 years with an average growth rate of 3.2% each year which is higher than the growth rate of world's population (1.7%). Therefore, the per capita fish supply increased by 17.5% over 10 year period as shown in Table 2. Table 3 shows the top ten fish harvesting countries in the world. The production of fish in China, Indonesia, India and Russia has increased while fish production decreased in other countries over the ten year period. Fish and fishery products are important sources of protein and essential micronutrients. In 2009, fish accounted for 16.6% of the world's intake of animal protein and 6.5% of all the protein available in the world [9].

The world's capture fish production during a ten year period (2000-2011) is shown in Table 1. The total fish capture in 2011 was 90.4 million tonnes or about 58.7% of the total fish production. The inland capture fish production increased by 30.68% whereas the marine capture fish landings decreased by 9.1% which resulted in a net reduction of 5.43% in the total fish capture [9].

Currently, aquaculture accounts for 40.33% of the world's fish production. It is defined as the farming of fish, shellfish and aquatic plants in fresh or saltwater [3]. Aquaculture has grown to 63.6 million tonnes in 2011. Around 600 aquatic species are raised in captivity worldwide. The Americas accounted for 4.30%, Europe accounted for 4.2%, Africa accounted for 2.2% and Oceania accounted for 0.30% of the world aquaculture production in the year 2010. However, Asia accounted for the majority (89%) of world aquaculture production in 2010. The Asian aquaculture fish production comprises of fin fishes (64.6%), molluscus (24.2%), crustaceans (9.7%) and other species (1.5%). The aquaculture production by region for the year 2010 is shown in Table 4. The top ten aquaculture producers of the world in the year 2010 are shown in Table 5 [9].

Canada fish production

In Canada, fish are produced by both commercial fisheries (fresh and sea water) which include capture fisheries and aquaculture.

The total landing from sea fisheries in the year 2011 was 850,533 metric tonnes with a value of $2,107,402. The Atlantic Region accounted for 703,905 metric tonnes (82.76%) with a value of $1,828,714 and the Pacific region accounted for 146,628 metric tonnes (17.24%) with a value of $278,688. The Sea fish production has decreased by 15.24% in the year 2011 when compared to the production in the year 2000. The fresh water fisheries contributed another 25,744 tonnes with a value of $58,206. The amounts of fish produced from sea water and fresh water during the period of 2000-2011 are shown in Figure 1. The amount of fish produced from fresh water fisheries gradually decreased by 36.69% over the 10 year period. On the other hand, the production from sea fisheries increased reaching its highest production (1,176,229 tonnes) in 2004 and gradually decreased reaching (850,553 tons) in 2011, a net loss of 152,967 tonnes (15.24%) compared to that of 2000 [8].

There are different types of aquaculture operations depending upon the species, environment and culture technologies used as shown in Figure 2 [10]. Aquaculture in Canada is carried out in all Canadian Provinces and in the Yukon Territory. Most of the east and west coasts comprise of finfish and shellfish. Trout are found in fresh water aquaculture in all provinces. Finfish aquaculture in Canada also includes active tilapia, sturgeon, Atlantic halibut and other operations. The fish production from aquaculture during the period of 2000-2011

Production	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
CAPTURE												
Inland	8.8	8.9	8.7	9.0	8.6	9.4	9.8	10.0	10.2	10.1	11.2	11.5
Marine	86.8	84.2	84.5	81.5	83.8	82.7	80.2	80.4	79.5	79.2	77.4	78.9
TOTAL	95.6	93.1	93.2	90.5	92.4	92.1	90.0	90.3	89.7	89.6	88.6	90.4
AQUACULTURE												
Inland	21.2	22.5	24.0	25.5	25.2	26.8	31.3	33.4	36.0	38.1	41.7	44.3
Marine	14.3	15.4	16.4	17.2	16.7	17.5	16.0	16.6	16.9	17.6	18.1	19.3
Total	35.5	37.9	40.4	42.7	41.9	44.3	47.3	49.9	52.9	55.7	59.9	63.6
TOTAL WORLD FISHERIES	131.1	131.0	133.6	133.2	134.3	136.4	137.3	140.2	142.6	145.3	148.5	154.0

Table 1: World fish production in million tonnes from 2000 to 2011 [9].

Production	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Human consumption	96.9	99.7	100.7	103.4	104.4	107.3	114.3	117.3	119.7	123.6	128.3	130.8
Non-food uses*	34.2	31.3	32.9	29.8	29.8	29.1	23.0	23.0	22.9	21.8	20.2	23.2
Total fish supply	131.1	131.0	133.6	133.2	134.2	136.4	137.3	140.3	142.6	145.4	150.1	154.0
Population (billions)	6.1	6.1	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.8	6.9	7.0
Per capita food fish supply (kg)	16.0	16.2	16.0	16.3	16.2	16.5	17.4	17.6	17.8	18.1	18.6	18.8

*Live bait for fishing
Live ornamental species
Ornamental products (pearls and shells)
Feed for carnivorous farmed species
Culture of biomass for palanton, *Artemia* and marine worm

Table 2: World fish utilization in million tonnes from 2000 to 2011 [9].

Country	2001	2010	Difference (%)
China	14,403,875.0	15,665,587.0	8.75
Indonesia	4,294,458.0	5,384,418.0	25.38
India	3,817,092.0	4,694,970.0	22.99
USA	4,981,800.9	4,378,683.7	-12.10
Peru	7,991,712.0	4,265,459.0	-46.62
Japan	4,837,830.2	4,141,312.4	-14.39
Russia	3,638,827.0	4,075,541.0	12.00
Myanmar	1,187,880.0	3,063,210.0	157.87
Chile	4,031,799.0	3,048,316.0	-24.39
Norway	2,862,152.0	2,675,292.0	-6.52
Other Countries	39,935,214.0	38,110,903.7	-4.56
World Total	91,982,640.1	89,503,692.8	-2.69

Table 3: Top ten capture fish producers of the world in metric tonnes [8].

Regions	2000		2010	
	Tonnes	(%)	Tonnes	(%)
Africa	399,676	1.20	1,288,320	2.20
Americas	1,423,433	4.40	2,576,428	4.30
Asia	28,422,189	87.70	53,301,157	89.00
Europe	2,050,958	6.30	2,523,179	4.20
Oceania	121,482	0.40	183,516	0.30
World	32,417,738	100.00	59,872,600	100.00

Table 4: World Aquaculture production by region from 1970 to 2010 [9].

World	Tonnes	(%)
China	36,734,215	61.35
India	4,648,851	7.76
Vietnam	2,671,800	4.46
Indonesia	2,304,828	3.85
Bangladesh	1,308,515	2.19
Thailand	1,286,122	2.15
Norway	1,008,010	1.68
Egypt	919,585	1.54
Myanmar	850,697	1.42
Philippines	744,695	1.24
Other	7,395,281	12.35
Total	59,872,600	100.00

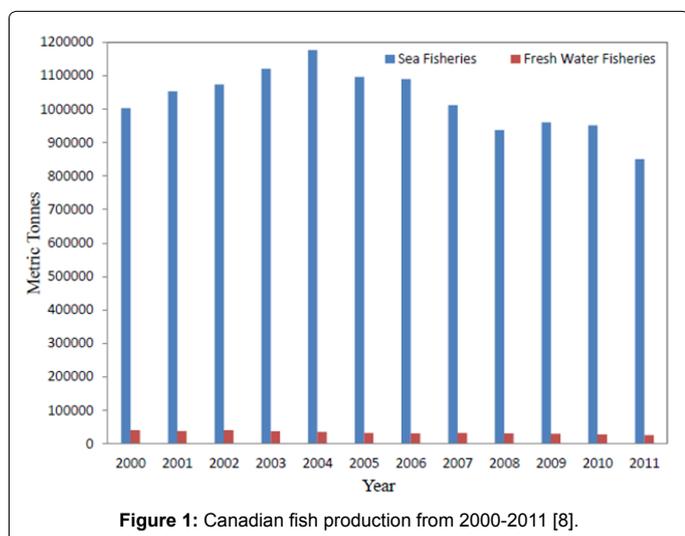**Table 5:** Top ten aquaculture producers in the world in 2010 [9].

Figure 1: Canadian fish production from 2000-2011 [8].
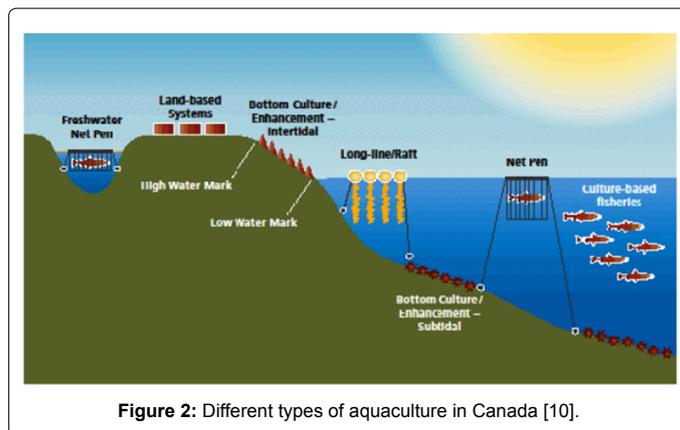

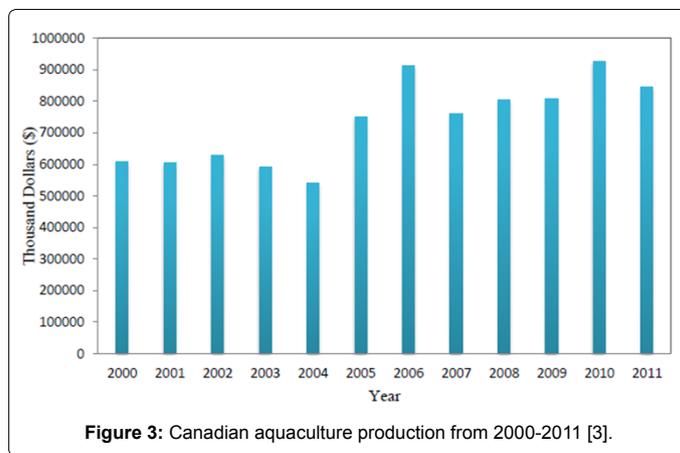**Figure 2:** Different types of aquaculture in Canada [10].

Figure 3: Canadian aquaculture production from 2000-2011 [3].

is shown in Figure 3. In 2011, the total production of fish from aqua culture was 163,036 tonnes with a value of $845,598. In 2010, British Columbia, New Brunswick produced and Newfoundland and Labrador produced 58%, 18% and 13% of the total aquaculture production, respectively [3].

Fish processing

Most fish processing plants process fish using the following steps: stunning of fish, grading, removal of slime, scaling, washing, deheading, gutting, cutting of fins, slicing into steaks, filleting, meat-bone separation, packaging, labelling and distribution (Figure 4). The amount of waste collected from each Canadian province is shown in Table 6.

Stunning

The stunning of fish is the first and most critical step in the processing of fresh water and farmed fish because prolonged agony experienced by the fish causes the production of undesired substances in fish tissues. The oxygen deficiency in the blood and muscle causes accumulation of lactic acid and leads to paralysis of the neural system. Stunning of the fish produces enough movements to break the vertebrae and rupture blood vessels. Red spots appear on the surface of the skin and in the muscle tissues near the backbone [11].

Erikson et al. [12] stated that some of the wild fish are subjected to asphyxiation on board after capture until they die using CO_2. Robb et al. [13] reported that immersion in CO_2 saturated water resulted in narcoss, with a loss of brain function. During the initiation of
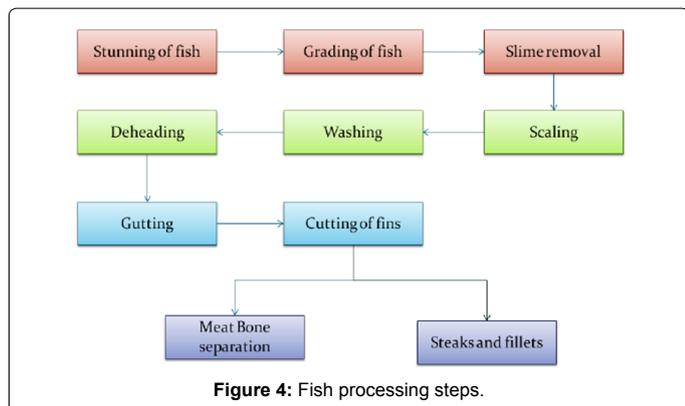
Figure 4: Fish processing steps.

Province	Landing		Product		Waste	
	(Tonnes)	(%)	(Tonnes)	(%)	(Tonnes)	(%)
New Brunswick	113588	13.95	89012	78.36	24576	21.63
New Foundland and Labrador	267959	32.92	120999	45.15	146960	54.84
Nova Scotia	366381	45.01	146708	40.04	219673	59.95
Prince Edward Island	66046	8.11	39000	59.04	27046	40.95
Total	813974	100.00	395719	48.61	418255	51.38

Table 6: Fish waste amount by province in 2001 [4].

narcosis, the fish shows strong aversion lasting from 30sec to 3min after immersion in CO_2. The immersion causes pH imbalances in the blood and thereby disrupting the function of brain. Poli et al. [14] reported that the CO_2 narcosis initiated greater lactic acid production. Roth et al. [15] reported that CO_2 stunning of fish is the most stressful method causing panic and flight reactions due to acidic and hypoxic environment, which leads to quick depletion of energy phosphogens and increases the earlier onset of rigor mortis.

In some cases of farmed fish, the fish is directly plunged into iced water in which the temperature is kept close to 0°C. It is critical that the temperature is kept low because the fish would not die due to temperature shock but by asphyxia which affects the quality and texture of the fish [16].

Borderias and Sanchez-Alonso [16] stated that electrical stunning is recommended for killing salmon and grass carp over CO_2 because it causes earlier onset of rigor mortis and fast eradenosine triphosphate (ATP) stunning carried out on turbot (Scopthalmus maximus) resulted in a rapid drop in the pH and potential increase in fillet gaping. Erikson et al. [12] stated that the efficiency of electrical stunning depended on whether the fish is stunned in the head or through the whole body. Morzel et al. [17] reported that during stunning, the initial pH was low and rapid onset of rigor mortis occurred resulting in flesh that was softer, redder and darker when compared to fish cut by percussion or bleeding. Roth et al. [15] reported that when Atlantic salmon were electrically stunned in water for 1.5 s, there was no accelerated rigor development and no injuries were observed in the fish.

Grading

The second step in fish processing is fish grading by species and size. Grading of fish can be done manually or by mechanical equipment. The mechanical grading is more precise for fish before or after rigor mortis than for fish in a state of rigor mortis. The automated grading instruments are 6-10 times more efficient than manual grading [18,19]. The basic benefits of the automated system are: low production costs

and increased quality of fish products at the end of the processing chain [16,20].

Slime removal

Fish secretes slime on its surface as a protection mechanism against harmful conditions. The slime secretion stops before rigor mortis. Pseudomonas species are one of the potent spoilers, always present in the sea water, and fish slime provides them with a perfect environment to grow [21,22]. Anaerobic bacteria present during fish processing can produce hydrogen sulfide by taking up sulfur compounds from the slime, skin and flesh [23,24]. Therefore, the slime should be removed by continuous washing. Slime present in some of the species such as eel, trout and other fresh water species should be soaked in a solution of 2% baking soda and then washed in a cylindrical rotating washer [16,22].

Scaling

The process of scaling is one of the toughest steps in fish processing and is extremely labour intensive. The scales may harbour bacterial pathogens and removing them will keep the fish fresh while refrigerated or frozen [25,26]. The scaling can be done manually with a hard brush or scaling blades. The scales of some fish such as perch, pike-perch, carp and bream are difficult to remove. These fish are first blanched in boiling water for 3-6 seconds and then scaled using mechanized hand-held scalers in a motion perpendicular to the long body axis. The electrical scalers are more efficient in completely eliminating scales than the manual tools and save lot of time [16].

Washing

The primary goal of washing is to clean and remove the accumulated bacteria on the fish. The effective washing of fish depends upon the fish:water ratio, the quality of water and kinetic energy of the water stream. The recommended washing fish:water ratio is 1:1. However, the amount of water used increases by two fold during processing. Use of potable water is recommended during freshwater fish processing [16]. Washing is carried out using vertical drum, horizontal drum and conveyor belt washers. The washing time is about 1-2 min and these mechanized washers can be used to process whole fish, deheaded and gutted fish as well as fish fillets. Washing action does not cause any physical damage to the product [27]. The washing process is always continuous and is accomplished by spraying pressurized water. The dirty water is collected in the waste basins. The amount of wastewater produced during each step in fish processing is shown in Tables 7-9 [28].

Deheading

The fish head constitutes up to 20% of its weight (Table 10) and it is usually considered as an inedible part [29]. The fish can be deheaded manually or mechanically. Manual cutting is easier for small fresh water fish. Larger fish ranging from 20-40 cm can be deheaded using mechanical devices. Fish can be cut in three different ways: round cut, straight cut and contoured cut. In most fish plants, manual deheading is performed because it causes minimal flesh loss. A cut around the operculum is a called a round cut and it results in the lowest meat loss. The contour cut runs perpendicular to the fish backbone and then at an angle of 45°. This cut is mainly used when the final product is a boneless and skinless fillet [16]. Machines with a guillotine cutter are suitable for larger fish under-going round or contour cuts. Machines with a manually-operated circular saw are suitable for larger fish undergoing straight cuts. The amount of deheaded waste produced from fish processing is 27-32% as shown in Table 7 [28].

Process	Inputs			Outputs					
	Fish (kg)	Energy (kW h)		Wastewater (m³)	BOD (kg)	COD$_5$ (kg)	Nitrogen (kg N)	Phosphorous (kg P)	Solid waste (kg)
White fish filleting	1000	Ice: 10-12 Freezing: 50-70 Filleting: 5		5-11	35	50	-	-	Skin: 40-50 Heads: 210-250 Bones: 240-340
Oily fish filleting	1000	Ice: 10-12 Freezing: 50-70 Filleting: 2-5		5-8	50	85	2.5	0.1-0.3	400-450
Frozen fish thawing	1000	-		5	-	1-7	-	-	-
De-icing and washing	1000	0.8-1.2		1	-	0.7-4.9	-	-	0-20
Grinding	1000	0.1-0.3		0.3-0.4	-	0.4-1.7	-	-	0-20
Scaling of white fish	1000	0.1-0.3		10-15	-	-	-	-	Scales: 20-40
Deheading of white fish	1000	0.3-0.8		1	-	2-4	-	-	Head and debris: 270-320
Filleting of deheaded white fish	1000	1.8		1-3	-	4-12	-	-	Frames and off cuts: 200-300
Filleting of ungutted oily fish	1000	0.7-2.2		1-2	-	7-15	-	-	Entrails, tails, heads and frames: 400
Skinning white fish	1000	0.4-0.9		0.2-0.6	-	1.7-5	-	-	Skin: 40
Skinning oily fish	1000	0.2-0.4		0.2-0.9	-	3-5	-	-	Skin: 40
Trimming and cutting of white fish	1000	0.3-3		0.1	-	-	-	-	-
Packaging of fillets	1000	5-7.5		-	-	-	-	-	-
Freezing and storage	1000	10-14		-	-	-	-	-	-
Handling and storage of fish	1000	10-12		-	-	130-140	-	-	-
Unloading of fish	1000	3		2-5	-	27-34	-	-	-

Table 7: Inputs and outputs of fish production processing [28].

Process	Inputs			Outputs					
	Fish (kg)	Energy (kW h)		Wastewater (m³)	BOD (kg)	COD$_5$ (kg)	Nitrogen (kg N)	Phosphorous (kg P)	Solid waste (kg)
Canning	1000	150-190		15	52	116	3	0.1-0.4	Head: 250 Bones: 100-150
Unloading fish for canning	1000	3		2-5	-	27-34	-	-	-
Precooking of fish to be canned	1000	0.3-11		0.07-0.27	-	-	-	-	Inedible parts: 150
Nobbing and packing in cans	1000	0.4-1.5		0.2-0.9	-	7-15	-	-	Head and entrails: 150 Bones and meat: 100-150
Draining of cans containing precooked fish	1000	0.3		0.1-0.2	-	3-10	-	-	-
Sauce filling	1000	-		-	-	-	-	-	Spillage of sauce and oil: varies
Can sealing	1000	5-6		-	-	-	-	-	-
Washing of cans	1000	7		0.04	-	-	-	-	-
Sterilization of cans	1000	230		3-7	-	-	-	-	-

Table 8: Inputs and outputs of fish canning process [28].

Process	Inputs			Outputs					
	Fish (kg)	Energy (kW h)		Wastewater (m³)	BOD (kg)	COD$_5$ (kg)	Nitrogen (kg N)	Phosphorous (kg P)	Solid waste (kg)
Fish meal and fish oil	1000	Electricity: 32		-	-	-	-	-	-
Cooking of fish	1000	90		-	-	-	-	-	-
Pressing the cooked fish	1000	-		750kg water 150kg oil	-	-	-	-	Press cake: 100 dry matter
Drying of press cake	1000	340.0		-	-	-	-	-	-
Fish oil polishing	1000	Hot water		0.05-0.1	-	5	-	-	-
Stick water evaporation	1000	475		-	-	-	-	-	Concentrated stick water: 250 Dry matter: 50

Table 9: Inputs and outputs of fish meal production process [28].

Gutting

Gutting of the fish is the removal of internal organs and optionally cleaning the body cavity of the peritoneum, kidney tissue and blood. In the gutting process, the fish is cut longitudinally to remove the internal organs on a table made of special material which is easy to wash and does not absorb fluids. The table is rinsed and periodically disinfected. There are some mechanical gutting machines used for trout, eel and other fish, but their use increases the fish processing cost [30]. The internal organs constitutes around 5-8% of the fish weight [29]. The amount of waste in the gutting processing is shown in Table 7 [28].

Cutting of fins

Fins constitute around 1-2% of the fish weight. The amount of fin waste after fish processing is shown in Table 7 [28]. Fins are cut

Component	Average Weight (%)
Head	21
Gut	7
Liver	5
Roe	4
Backbone	14
Fins and lungs	10
Skin	3
Fillet, skinned	36

Table 10: Average composition of fish [29].

manually either by a knife or by mechanized rotating disc knives [31]. This process is mostly carried out after deheading and gutting. This process is difficult for cutting larger fish. The mechanical knives are provided with a slit opening in which the fins are cut when the fish are passed through it manually [16,32].

Steaks and fillets

Fillets are pieces of meat containing only the dorsal and abdominal muscles. The fillets are processed manually or mechanically. Manual filleting is carried out in small fresh water fish industries and mechanical filleting is used for processing marine fish. Deheaded whole fish are sliced into steaks by cutting perpendicular to the backbone. Small and medium-sized fish are cut manually in a concave basin with evenly-spaced slots to facilitate slicing. The average thickness of the fish pieces is 2.5-4.5 cm. Large fish such as cyprinids are sliced mechanically because of their solid and massive backbone. These pieces are more popular in the retail market and the canning industry [16,33]. Once the fillet leaves the filleting stations, three products remain: napes, block and trimmed fillet. Napes are the thinnest part of a fillet that covered the guts before the fish was gutted. Blocks are the parts removed from fillets for aesthetic purposes. Trimmed fillet is the final product in which the napes and pin bone attached to some fillets will be removed [30].

Meat bone separation

Around 30-50% of the meat is usually left along the ribs and backbone during filleting. In smaller fish, the loss of meat is high and so minced fish meat is gaining more attention [34,35]. Minced fish meat can also be produced from less valuable species after deheading and carefully removing their internal organs. In this process, the meat is removed from skin, scales and bones through automated separators. The fish travels along a conveyor belt which runs closely to a perforated cylinder. The meat is squeezed through the holes due to the pressure applied from the conveyer belt and the bones are scraped away [16]. The minced fish meat stability is much less than that of intact fish muscle and so it is frozen immediately. It is used to produce fish burgers, fish sticks, canned fish, vegetable mixes and fish dumplings [36,37].

Composition of Fish Waste

The composition of the fish varies according to the type of species, sex, age, nutritional status, time of year and health. Most of the fish contains 15-30% protein, 0-25% fat and 50-80% moisture [7,38]. Suvanich et al. [39] reported that the composition of catfish, cod, flounder, mackerel and salmon variedaccording to the species (Table 11). Mackerel had the highest fat content (11.7%) and cod had the lowest (0.1%). Salmon had the highest protein content (23.5%) and flounder had the lowest (14%). The moisture content of the five fishes varied between 69 and 84.6% but the ash content of all species was similar.

Solid fish waste consists of head, tails, skin, gut, fins and frames. These by products of the fish processing industry can be a great source of value added products such as proteins and amino acids, collagen and gelatin, oil and enzymes as shown in Table 12 [40,41]. These wastes contain proteins (58%), ether extract or fat (19%) and minerals. Also, monosaturated acids, palmitic acid and oleic acid are abundant in fish waste (22%).

Proteins

Fish frames contain significant amounts of muscle proteins. These muscle proteins are highly nutritious and easily digestible. Therefore, proteins from this part of the fish waste can be extracted by enzymatic hydrolysis rather than being discarded as waste [42]. Proteins derived from fish are nutritionally superior when compared to those of plant sources. They have a better balance of the dietary essential amino acids compared to all other animal protein sources [43,44]. However, fish muscle proteins are more heat sensitive than mammalian muscle proteins [45]. Also, fish muscle proteins from the cold water species are more susceptible to denaturation by heat when compared to those of tropical water fish. The T-50 values (the temperature required for 50% denaturation of the fish muscles) are influenced by the pH and were reported to be in the range of 29-35°C at a pH of 7.0 and in the range of 11-27°C at a pH of 5.5 [46].

Fish muscles consist of two types: light and dark. The proportion of dark muscle is low in white fish such as cod and haddock where there is a small strip of dark or red muscle just under the skin on both sides of the body. In fatty fish, such as herring and mackerel, the percentage of dark muscles is high and the muscles contain more vitamins and fats as shown in Figure 5 [7]. Light muscle is more abundant and contains about 18-23% proteins.

About 70-80% of the fish muscles are made up of structural proteins and the remaining 20-30% are composed of sarcoplasmic proteins with about 2-3% insoluble connective tissue proteins. Myofibrillar proteins

Fish Type	Fat (%)	Ash (%)	Protein (%)	Moisture (%)
Catfish	7.7	0.9	15.4	76.3
Cod	0.1	1.1	18.2	80.8
Flounder	0.7	1.3	14.0	84.6
Mackerel	11.7	1.1	18.8	69.0
Salmon	1.6	1.1	23.5	74.3

Table 11: Composition of the fish fillets determined by standard methods [39].

Nutrient	Fish waste
Crude protein (%)	57.92 ± 5.26
Fat (%)	19.10 ± 6.06
Crude fiber (%)	1.19 ± 1.21
Ash (%)	21.79 ± 3.52
Calcium (%)	5.80 ± 1.35
Phosphorous (%)	2.04 ± 0.64
Potassium (%)	0.68 ± 0.11
Sodium (%)	0.61 ± 0.08
Magnesium (%)	0.17 ± 0.04
Iron (ppm)	100.00 ± 42.00
Zinc (ppm)	62.00 ± 12.00
Manganese (ppm)	6.00 ± 7.00
Copper (ppm)	1.00 ± 1.00

Values in % or mg/kg (ppm) on a dry matter basis.
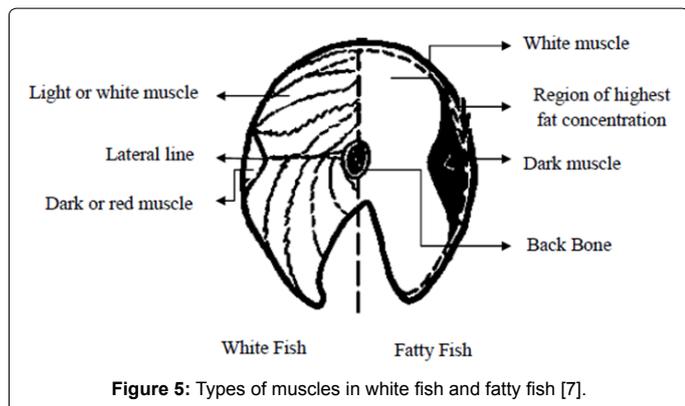
Table 12: Composition of fish waste [40].

Figure 5: Types of muscles in white fish and fatty fish [7].

are the primary food proteins and they make up about 66-77% of the total protein content in the fish meat. These myofibrillar proteins comprise of 50-60% myosin and 15-30% actin [47]. The myosin fibers can be cleaved by proteases trypsin and chymotrypsin on one end and on the other end with papain. During this cleavage, the myosin fibers are divided into heavy meromyosin and light meromyosin with different functional properties. Actin occurs in two forms, G-actin, a spherical monomer and F-actin, a large polymer which connects to myosin [46].

Amino acids

Fish protein contains a well balanced amino acid composition. Fish is composed of 16-18 amino acids based upon the species type and seasonal variations. The amino acid profile of Atlantic mackerel is shown in Table 13 [48,49]. Fish contains well balanced amino acid compositions consisting of eight essential amino acids and eight non-essential amino acids. Due to the rich amino acid content of fish, it is being utilized as fish meal, fish sauce, fertilizer, animal feed and fish silage [50].

Oil

Fish processing by products contain fish oil. The amount generally depends upon the fat content of the specific fish species. Generally, fish contains 2-30% fat. Almost 50% of the body weight generated as waste during the fish processing would be a great potential source for good quality fish oil which can be used for human consumption or production of biodiesel. The fish oil consists of two main fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two fatty acids are polyunsaturated fatty acids and are classified as omega-3 fatty acids. They are mainly found in the marine animals which have high polyunsaturated fatty acid content [51].

Bioactive peptides

Proteins extracted from the fish muscle contain a number of peptides which have manybioactivities such as antihypertensive, antithrombotic, immune modulatory and antioxidative properties [52]. The bioactive peptides obtained from the fish muscle have anticoagulant and antiplatelet properties, which are the main reason behind the capability of peptides obtained from the fish to inhibit coagulation factors in the intrinsic pathway of coagulation [53]. The protein obtained by the enzymatic hydrolysis of the fish muscle has several nutritional and functional properties from which many biologically active peptides can be obtained [54].

Collagen and gelatin

The fish skin waste is a good source for collagen and gelatin which are currently used in food, cosmetic and biomedical industries. Collagen and gelatin are two different forms of same macromolecule in which gelatin is a partially hydrolysed form of collagen. The collagen and gelatin are two unique and more significant forms of proteins in comparison to that of fish muscle proteins.

The significance lies upon the amino acid content; more than 80% are non-polar amino acids such as glycine, alanine, valine and proline [55]. Heat denaturation of collagen easily converts it into gelatin. The collagen and gelatin extracted from bovine sources pose the risk of mad cow disease or bovine spongiform encephalopathy (BSE), whereas the collagen and gelatin extracted from fish skin eliminates these risks of BSE. The gelatin extracted enzymatically from fish skin has better biological activities as antioxidants and antihypertensive agents. The gelatin has a unique repeating sequence of glycine-proline-alanine in their structure compared to the peptides derived from fish muscle protein and it is the main reason behind the antioxidative propertyof gelatin [56,57].

Enzymes

The internal organs of the fish are a rich source of enzymes, many of which exhibit high catalytic activities at relatively low concentrations. The enzymes which are available in fish include: pepsin, trysin, chymotrypsin and collagenase. These enzymes are commercially extracted from the fish viscera in a large scale. They possess better catalytic properties, good efficiency at lower temperatures, lower sensitivity to substrate concentrations and greater stability in a wide range of pH [56-58].

Pepsin is a proteolytic enzyme which is found in the stomach of fish and constitutes 5% of the fish weight. It is used in various extraction processes such as extraction of collagen gelatin and can be used as rennet substitute and can be used to digest proteins. The optimum conditions for pepsin were pH 2-4 and temperature of 30°C [59-63].

Chymotrypsin is an endoprotease which hydrolyze proteins by breaking the central peptide bonds, and yielding a mixture of peptides and amino acids. Fish chymotrypsin consists of two different forms:

Amino acid	Mackerel Fish		
	Whole Tissue	**White Muscle**	**Dark Muscle**
Aspartic acid	11.8	12.2	11.4
Threonine	5.7	5.5	5.5
Serine	4.5	5.2	4.1
Glutamic acid	15.8	18.0	15.6
Proline	1.5	4.6	1.5
Glycine	6.0	6.2	4.6
Alanine	7.7	7.3	7.3
Valine	7.8	6.8	8.5
Methionine	2.7	4.6	2.8
Isoleucine	5.5	6.0	5.6
Leucine	9.4	10.0	8.8
Tyrosine	3.5	3.9	3.4
Phenylalanine	4.2	4.0	3.1
Histidine	4.5	3.8	5.2
Lysine	7.9	8.0	7.6
Arginine	7.6	5.9	7.1

Table 13: Amino acid profile of Atlantic Mackerel (g amino acid/100 g protein) [48].

chymotrypsin A and chymotrypsin B. Fish chymotrypsin has higher catalytic activity, lower thermo stability and differs in polypeptide amino acid composition to bovine chymotrypsin. The optimum conditions for chymotrypsin was pH 7-8 and temperature of 50°C [58,64,65].

Trypsin is an important pancreatic serine protease which is synthesized as proenzyme by pancreatic acinar cells and is secreted to the intestine. Fish trypsin is similar to mammalian trypsin in molecular weight and amino acid composition [66]. Trypsin cleaves the peptide bond on the carboxyl side of arginine and lysine. They hydrolyse synthetic substrates such as Nα-benzoyl-L-arginine-p-nitroanilide and tosyl-arginine methyl ester. Trypsin is susceptible to serine-protease inhibitors such as phenyl-methyl-sulfonyl fluoride (PMSF), soybean trypsin inhibitor (SBTI) and aprotonin. The optimum conditions for trypsin isa pH of 9 and a temperature of 40°C [67,68].

Fish collagenases, also called collagenolytic serine proteases which differ from muscle collagenases, belong to the zinc metalloproteases and are known to hydrolyse triple type I, II and III tropocollagen molecule. They show both trypsin and chymotrypsin like activities. They are most active in the pH range of 6.5-8.0 and at a temperature of 30°C [67].

Minerals

Fish bones are normally separated after removal of muscle proteins from the frames. The fish bones account for 30% of the collagen and are considered an additional source of collagen along with fish skin. Fish bones contain 60-70% minerals including calcium, phosphorous and hydroxyapatite [56]. Generally, calcium is deficient in most of the regular diets and to improve calcium intake, consumption of small whole fish can be nutritionally valuable. The fish bones obtained from the fish processing waste can be used to provide calcium. In order for bones to be a fortified food, they should be converted into edible form by softening their structure with hot water treatment, hot acetic acid solutions or by super heated steam cooking [69]. Fish bones are

a very good source of hydroxyapatite ($Ca_{10}(PO_4)6(OH)_2$) which can be used as a bone graft material in medical and dental applications. Previously autografts, allografts and xenografts were used to solve bone fractures and damages but they were found to be ineffective due to their mechanical instability and incompatibility. The important properties of hydroxyapatite are: it does not break under physiological conditions, it is thermodynamically stable at physiological pH and it plays an active role in bone binding [70].

Current Utilization of Spoiled Fish and Fish Waste

Fish silage

Fish silage is an excellent protein source having high biological properties for animal feeding. Fish silage is a liquid product made from whole fish or parts of fish that are liquefied by the action of enzymes in the fish in the presence of added acid. The enzymes present in the acidic medium breakdown fish proteins into smaller soluble units while the acid helps to speed up their activity and prevent bacterial spoilage. Fish silage can be made from spoiled fish, sub-utilized species, by-products from marine fish, commercial fish waste and industrial residues from the filleting industry [41,71]. The proteins present in the fish silage can also be hydrolysed to free amino acids, making the silage the most available source of amino acids for protein biosynthesis. The composition of amino acids in the various states of fish silage is shown in the Table 14 [71].

During fish silage preparation, the raw material is chopped into small pieces and a 3% by weight solution of 98% formic acid is added and mixed well and then stored for 48 days. The pH of the mixture should be less than 4 to prevent bacterial action [72,73]. Fish silage can also be prepared by a fermentation method in which fish is chopped, minced and mixed with 5% (w/w) sugar beet molasses. A culture of Lactobacillus plantarum is inoculated into molasses and incubated until a population of 10^7 bacteria per g of molasses is obtained. This culture is then added in the ratio of 2 ml/kg to the minced fish. The inoculum is incubated at 30°C for 7 days inside sealed plastic buckets.

Amino acids	SW	FSW	ASW	FW	FFW	AFW	TR	FTR	ATR
Tryptophan	0.79	0.65	0.66	0.97	0.87	1.34	0.52	0.61	0.43
Lysine	10.12	9.16	7.90	7.48	9.92	9.09	9.75	5.94	6.77
Histidine	5.24	5.85	5.70	2.65	3.08	2.75	2.02	2.52	2.20
Arginine	3.03	2.19	6.11	3.62	1.80	7.72	2.46	2.49	7.27
Aspartic acid	9.05	10.79	7.83	10.17	9.62	6.20	10.16	11.79	8.98
Threonine	2.85	4.97	4.58	3.18	5.12	5.28	2.76	4.68	4.72
Serine	2.71	3.23	4.49	3.39	3.52	5.53	2.04	3.72	5.11
Glutamic acid	13.57	14.45	14.04	16.18	13.83	9.26	13.88	14.76	13.10
Proline	3.19	3.66	5.74	4.37	5.57	7.78	7.75	7.22	5.94
Glycine	6.49	5.87	8.17	6.20	6.32	11.55	7.50	9.22	12.32
Alanine	8.60	7.41	7.39	9.27	8.12	6.00	8.81	8.92	7.63
½ Cystine	0.81	0.69	1.54	0.97	1.03	0.63	1.40	0.86	1.34
Valine	6.42	5.77	4.16	5.95	5.83	3.92	6.62	5.06	4.31
Methionine	6.88	6.03	3.75	3.19	4.97	5.31	2.80	5.54	5.37
Isoleucine	5.31	5.05	3.10	5.38	5.00	3.10	6.24	4.63	2.51
Leucine	9.16	8.00	7.33	9.61	9.31	7.57	10.32	6.72	6.23
Tyrosine	1.78	1.90	3.45	2.40	2.02	2.73	1.22	1.70	2.43
Phenylalanine	3.99	4.32	4.08	5.02	4.07	4.26	3.76	3.63	3.35
CP (g/kg)	776.7	596.1	699.1	496.2	420.9	443.8	429.9	358.4	395.9

SW: Commercial Saltwater Fish Waste; FSW: Fermented Saltwater Fish Silage; ASW: Acid Salt Water Fish Silage; FW: Commercial Freshwater Fish Waste; FFW: Fermented Freshwater Fish Silage; AFW: Acid Freshwater Fish Silage; TR: Tilapia Filleting Residue; FTR: Fermented Tilapia Residue Silage; ATR: Acid Tilapia Residue Silage; CP: Crude Protein (Dry Matter)

Table 14: Amino acid composition (g/100 g CP) and protein content of fish silage [71].

The autolysis is later stopped by heating the silage at 90°C for 30 min [74,75]. Fish silage can be mixed with wheat bran and oven dried at 105°C. Co-drying fish silage with cereals reduces the drying times of the silage and improves the nutritional content of the silage. To prevent spoilage of the dried silage it should contain low moisture content. Water levels greater than 120 g/kg can support bacterial, mould and yeast growth [76].

According to Gildberg [77], many bioactive products including peptone, oil and pepsin can be obtained from fish silage. Fish like Atlantic cod and salmon have high amounts of pepsin in the stomach. The optimal storage conditions for the recovery of pepsin are pH of 3 and 25°C for 3 days. By ultra-filtration and spray drying, the stomach silage can provide crude pepsin corresponding to 0.5-1 g of pure pepsin per kg. The purity of the crude pepsin extracted ranges from 2-10%. The cod stomach and viscera silage can also provide 100 g low molecular weight peptone per kg of the raw material.

Fish meal

Fishmeal is a dry powder prepared from whole fish or from fish filleting wastes which are unacceptable for human consumption. The raw materials are transported to the processing factories either fresh or preserved in formaldehyde or sodium nitrate [78]. The production of fish meal is carried out in six steps: heating, pressing, separation, evaporation, drying and grinding. When the fish is heated the protein present in it coagulates and ruptures the fat deposits. This liberates oil and water. The fish is then pressed which removes large amounts of liquid from the raw material. The liquid is collected to separate oil from water. The water which is also known as stick water is evaporated to a thick syrup containing 30 to 40% solids. Then it is subjected to drying using press cake method to obtain a stable meal. This meal is grinded to the desired particle size [79].

Fishmeal obtained from wild-harvested whole fish and shellfish currently makes up the major aquatic protein source available for animal feed. The global fishmeal production was 5 million tonnes in 1976 which increased to 7.48 million tonnes in 1994 and then decreased to 5.74 million tonnes in 2009. In recent years, the fishmeal production from the fisheries by-products has increased drastically and around 6 million tonnes of fish waste have been used for fishmeal production. It is estimated that 25% (1.23 million tonnes in 2008) of the total fish-meal produced is from fish by-products [9].

In 1988, around 80% of the total fishmeal produced was used as feed for pigs and poultry and 10% of the total was used for aquaculture. Currently, 63% of the fishmeal is being consumed by the aquaculture industry, 25% by the pigs, 8% by the poultry and 4% by other animals [80].

Fish sauce

Fish sauce is made from small pelagic fish or by-products using salt fermentation. Fish are mixed with salt in the ratio of 3:1 at 30°C for six months and an amber protein solution is drained from the bottom of the tank. It can be used as a condiment on vegetable dishes and is very nutritious due to the presence of essential amino acids [81]. Fermented fish sauce has various biological activities including angiotensin I-converting enzyme (ACE) inhibitory activity and insulin secretion-stimulating activity. Various studies reported ACE inhibitory activity in the fermented fish sauce from salmon, sardine and anchovy. Three ACE peptides (gly-trp, ile-trp and val-trp) were found in fermented fish sauce [82-84].

Production and utilization of Fish Protein

Fish proteins are extracted from fish using chemically and enzymatic methods. Protein hydrolysates obtained from these processes have various industrial uses such as milk replacers, protein supplements, stabilizers in beverages and flavour enhancers.

Chemical extraction of fish protein

The most common extraction method used for the fish proteins is the solvent extraction method. The standard protocol for the solvent extraction of proteins reported by Sikorski and Naczk [85] is shown in Figure 6. The whole fish is first ground and the protein is extracted using isopropanol. After grinding, the supernatant is collected and extracted three times. The first extraction is carried out at 20-30°C for 50 min in isopropanol. The second extraction is carried out at 75°C for 90 min with isopropanol. The third extraction is carried out at 75°C for 70 min with azeotropic isopropanol. The final supernatant fraction is collected, dried, milled and screened to separate out bone particles. Hermansson et al. [86] reported that the fish protein concentrate can also be produced at a temperature of 50°C but it will have lower emulsifying properties and poor solubility. The disadvantages of this method are poor functionality, off-flavours, high cost of production and traces of the solvent in the final product, making it commercially unsuccessful.

Another chemical method for the production of fish protein concentrate and gelatin (Figure 7) was reported by Arnesen and Gildberg [87]. 2000 g of the Atlantic cod are added to 2000 ml of water and the pH is adjusted to 11 with 62 ml of 3 M NaOH. The first extraction is carried out for 15 min and the sample is centrifuge is then suspended in 2000 ml and the pH is adjusted to 11 with 3 M NaOH (15 ml). The second extraction is carried out for 60 min and the sample is then centrifuged. The pellet is again suspended in 2000 ml of water and the pH is adjusted to 2 with 3M HCl (145 ml). The third extraction is carried out for 15 min and centrifuged. The supernatants from the three extracts are pooled together and the pH was adjusted to 7 with 3 M NaOH. The samples are allowed precipitating for 15 min at room
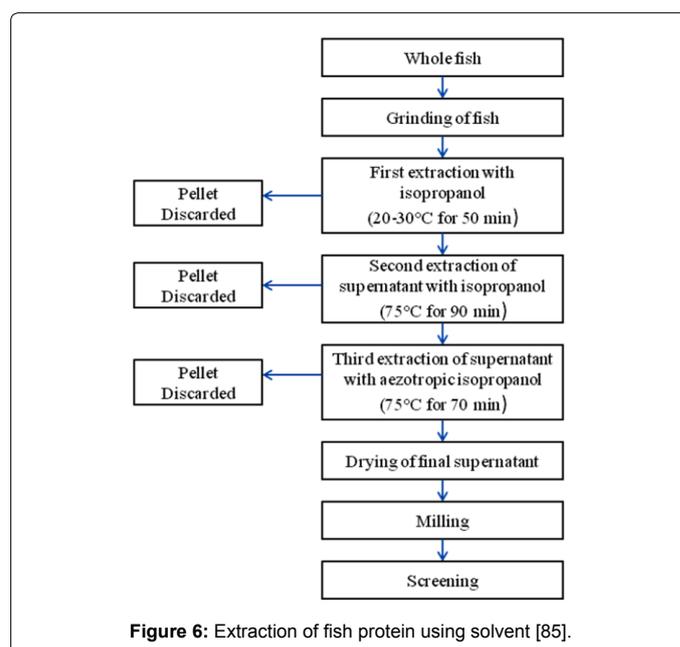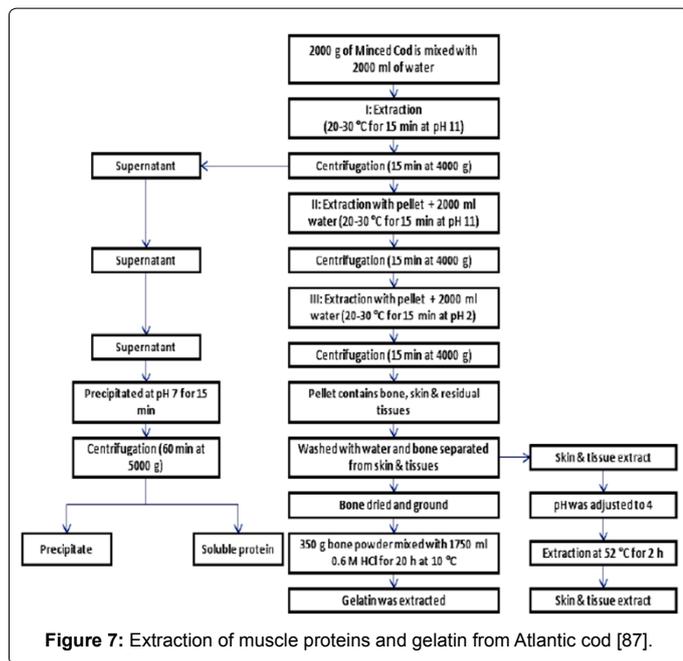

Figure 6: Extraction of fish protein using solvent [85].

Figure 7: Extraction of muscle proteins and gelatin from Atlantic cod [87].

temperature and the soluble protein is separated by centrifugation at 4°C for 60 min at 5000 g. Altogether 47.5% of the total protein is recovered from the pooled extract from muscle and soft tissues. The solids remaining after the third extraction included bone, skin and residual muscle tissues.

Batista [88] reported on the extraction of proteins from hake and monkfish wastes using a chemical method. The minced fish waste was mixed with water in ratios of 20:1 20:5 for a time ranging from 5-120 min. The pH was in the range of 1-12 and the temperature range was in the range of 22-55°C. The extraction was carried out with HCl in the acid phase and with NaOH and $Ca(OH)_2$ in the alkaline phase. After the extraction is completed, the protein extracts were centrifuged for 15 min at 5000 rpm and the supernatant obtained was filtered through glass wool. The results indicated that the minimum solubility for hake waste proteins was seen at a pH in the range of 5-6 for hake fish waste and at pH of 5 for monkfish waste. The amount of proteins solubilised at optimum pH was 17% for hake fish waste and 9% for monkfish waste. The extraction time influenced the amount of protein solubilised in both hake and monk fish wastes. Higher yield was obtained at 45°C when NaOH was used for extraction and at 50°C when $Ca(OH)_2$ was used for extraction. The ratio of 10:1 (fish:water) was found to be more convenient giving better yield.

Undeland et al. [89] extracted proteins with acid and alkali from ground whole herring fish (120-300 g) in a blender using 9 volumes of ice cold distilled water. The proteins present in the homogenate were solubilized by adding 2 N HCl or 2 N NaOH in drops until a pH of 2.8 or 10.8 was reached. The protein suspension was centrifuged for 15 min at 18000 g which gave four layers: a floating emulsion layer, a clear supernatant, soft gel like sediment and harder bottom sediment. The supernatant was separated from the emulsion layer by filtration through a double layer cheese cloth. The solubilised proteins were precipitated by adjusting the pH in the range of 4.8-7. The precipitated proteins were centrifuged at 100000 g for 20 min. The results indicated that the extract solubilised by acid had 92.1 ± 3.4% proteins compared to 88.6 ± 3.8% solubilised by alkali.

Kelleher and Hultin [90] used lithium chloride for the extraction of protein from fish muscle. 40 g of ground muscle were mixed with 760 ml (1:20) of 4.2% LiCl and 0.02 M Li_2CO_3 at a pH of 7.2 and a temperature of 25°C. The contents were homogenized in a blender for 2 min and centrifuged at 2600 g for 20 min. The supernatant was collected and analyzed for protein using the Biuret method. The same procedure was used but LiCl was replaced by 5% NaCl with 0.02 M $NaHCO_3$ and 6% KCl with 0.02 M $KHCO_3$. The results indicated that lithium chloride was much better than sodium or potassium chlorides with respect to blending time. Also, lithium chloride had a stable and consistent protein yield over a wide range of concentrations compared to the other two salts.

Nurdiyana et al. [91] optimized the extraction process of proteins from freeze dried fish waste using response surface methodology. The fish waste obtained was minced in a blender and pre-treated with petroleum ether to remove the fat. The defatted fish waste was freeze dried for 24 h. The freeze dried fish waste was mixed with distilled water in the ratio of 1:10 before adding NaOH. The results from the response surface optimization methodology indicated that the optimum ratio of NaOH:sample was 1.54:1, the optimum speed of rotation was 105 and the optimum extraction time was 49 min at a pH of 10.5. The predicted protein yield under these conditions was 85.02 mg/ml compared to an experimental protein yield of 83.51 mg/ml.

Enzymatic extraction of fish protein

The enzymatic processing of various biopolymers in foodstuffs (such as polysaccharides, proteins and pectins) is an important process which is used to improve the physical, chemical and organoleptic properties of the original food in relation to the nutritive value and the intestinal absorption characteristics. The enzymatic extraction of protein is carried out under controlled pH conditions without degrading their nutritional qualities for the acceptance in the food industry and broad spectrum of products can be produced for a wide range of applications [92]. Many protein hydrolysates are subjected to enzymatic processing to produce special diets for babies and sick adults. This is possible only when the hydrolysates are low in bitterness, osmotically balanced, hypoallergic and have good flavour. Most of these diets are composed of peptides and are rich in amino acids [93].

Although enzymatic processing has been widely applied to various livestock and poultry meat and milk, there are few studies on the production of fish protein hydrolysate using enzymes. Fish processing waste has also been underutilized for use as a feed or a fertilizer [46]. Most of the research studies conducted on the enzymatic processing of fish protein seems to be laboratory or small scale oriented and have their limitations when scaled up to industrial scale [94]. However, the large scale production of fish protein hydrolysates are carried out in various countries including France, Japan and other countries in Southeast Asia. The process has several disadvantages including low yields, initial high cost of enzymes, inactivation of enzymes after hydrolysis either by heat or by pH and the inability to reuse enzymes [95].

The enzymatic processing of fish waste could be helpful in producing a broad spectrum of food ingredients and industrial products for a wide range of applications [96]. The enzymes used in the food industry for the preparation of fish protein hydrolysate are mostly carbohydrases, proteases and lipases.

Proteases are one of the most highly used enzymes in the food industry [97]. Proteases are derived from animal, plant and microbial

sources. Enzymes extracted from plant sources include papain, bromelain and keratinases. Enzymes extracted from animal's sources include trypsin, chymotrypsin, pepsin and renin. Because of the inability of plant and animal proteases to meet current demand in the market, there is an increase in the demand for microbial proteases. Bacterial proteases are often used in the production of protein hydrolysate [46,98]. They are mainly neutral or alkaline and are produced by the genus Bacillus. The neutral proteases are active in the pH range of 5-8 and have low temperature tolerance whereas the alkaline proteases are active in the pH range of 7-10 and have broad specificity [98].

Alcalase is an alkaline enzyme produced from Bacillus licheniformis which is developed by Novo Nordisk (Bagsvaerd, Denmark) for the detergent industry. This enzyme has been proven to be one of the best enzymes used to prepare fish protein hydrolysate [54,99]. Shahidi et al. [92] stated that fish protein hydrolysate produced by alcalase had better functional properties, a high protein content with an excellent nitrogen yield, an amino acid composition comparable to that of muscle and a higher nutritional value than those produced by other enzymes such as Neutrase.

Liaset et al. [100] reported on the extraction of protein hydrolysate from fish frames from Atlantic cod and Atlantic salmon using four different enzymes neutrase, alcalase, pepsin and kojizyme. The study revealed that after 120 min of hydrolysis, salmon treated with alcalase and cod treated with pepsin yielded higher protein recoveries of 67.6% and 64%, respectively.

Shahidi et al. [92] reported on the extraction of protein hydrolysate from capelin (Mallotus villosus) using alcalase, neutrase and papain. The samples were also subjected to autolytic hydrolysis. The results revealed that protein recoveries with commercial enzymes reached 51.6-70% in comparison with the autolytic hydrolysis yield of 22.9%. Alcalase hydrolysis had the highest protein recovery compared to those other enzymes.

The effects of initial inactivation of endogenous enzymes, water and different enzymes on the yield of proteins and oil from cod (Gadusmorhua) were studied by Slizyte et al. [101]. The enzymes used in the hydrolysis were alcalase and lecitase ultra. The results revealed that initial heating of raw material changed its composition

and inactivated the endogenous enzymes. The yield of fish protein hydrolysate had higher amount of lipids such as phospholipids and other polar lipids. Alcalase with the addition of water produced good quality fish protein and oil.

Guerard et al. [102] extracted protein from a yellowfin tuna (Thunnus albacores) waste using alcalase. The freeze dried protein hydrolysate was used as nitrogen substrate for microbial cultures such as E. coli, L. casei, S. cerevisiae, S. odorus, P. roqueforti and A. niger.

Enzymatic hydrolysis was performed on the Atlantic spiny dogfish (Squalus acanthias), which is of low commercial value but potential source for high quality protein by Diniz and Martin [103]. The optimized variables for protein extraction from dogfish waste were a temperature of 35°C, a reaction time of 23.8 h, a rotation speed of 171 rpm and an enzyme: substrate ratio of 1.5. The protein yield was 80.75 g/L [91].

Beak and Cadwallader [104] reported on the extraction of proteins from crayfish processing byproducts using alkaline protease optimase. The optimal conditions for the enzymatic hydrolysis were a temperature of 65°C, a pH of 8-9, a reaction time of 2.5 h and an enzyme concentration of 0.3%. The maximum protein yield was 75%.

Utilization of Fish Protein

Fish protein contains many bioactive peptides that are easily absorbed and can be used for various metabolic activities. They can be used as a functional ingredient in many food items as they have properties such as water holding capacity, oil absorption, gelling activity, foaming capacity and emulsification property. Various nutraceuticals are commercially produced and their applications are shown in Table 15 [105,106]. Kristinsson and Rasco [46] reported that fish protein hydrolysate can be used as a milk replacer with high protein efficiency ratio (PER) value and in a cost effective manner than dried skimmed milk. Due to its rich amino acid composition, fish protein can be used as supplementing cereal proteins and can be used in bakery products, soups and infant formulas. It is also reported that fish proteins can serve as nitrogen source for the growth of micro organisms for the production of extracellular lipases [106].

During the storage of fish under frozen conditions, impart

Product name	Preparation	Applications	Country
Seacure®	Prepared by hydrolyzing deep ocean white fish proteins	Dietary supplement helps to support the cells in the gastrointestinal tract and regulate bowel function.	US & Canada
Amizate®	Produced from Atlantic salmon fish proteins by autolysis	Sports nutrition for muscle anabolism and metabolic recovery.	North America
Stabilium®200	Prepared from Molva dypterygia by autolysis	Supports the body's response to stress and provides nutritional support for memory and cognitive function.	UK
Protizen®	Produced by enzymatic hydrolysis of white fish proteins	It is also called as "mood food" to fight against stress and symptoms such as weight disorders, work pressure, sleep troubles and concentration difficulties	UK
Vasotensin®	Produced from Bonito (Sarda orientalis) by thermolysin hydrolysis	It supports healthy vascular function for optimal blood flow and blood pressure vessel.	US and Japan
Peptace®	Produced from Bonito (Sarda orientalis) by thermolysin hydrolysis	It lowers the blood pressure by inhibiting ACE enzyme.	US and Japan
Nutripeptin®	Manufactured by enzymatic hydrolysis of cod fish fillet	It helps in blood glucose stabilization and weight management.	UK and USA
Liquamen®	Prepared from Molva molva by autolysis	Dietary supplement that helps in reducing oxidative stress, lowering glycemic index and anti stress	UK
Molval®	Produced from North Atlantic fish Molva molva by enzymatic hydrolysis	Dietary supplement recommended for cholesterol equilibrium, stress control and promotes good cardiovascular health	UK
Seagest®	Prepared by hydrolysing deep ocean white fish proteins	It supports the structure of the intestinal lining and health	US

Table 15: Commercial nutraceuticals from fish protein hydrolysates [106].

changes on the texture and functional properties of fish proteins and may eventually denature them. To prevent protein denaturation, cryoprotectants are used to increase the surface tension and amount of bound water to prevent ice formation and migration of water molecules in the protein. Sugar-sorbitol in the ratio of 1:1 is commonly used cryoprotectants. These fish products cannot be served for diabetic patients due to the presence of carbohydrates in the cryoprotectants. The protein hydrolysate from Pacific Hake, shrimp head can be used as cryoprotectants without any loss in the cryoprotective property when compared to other cryoprotectants [107-109]. The fish protein hydrolysates also possess various bioactivities such as antioxidative, antithrombic and antihypertensive properties.

Production and Utilization of Fish Amino Acids

Amino acids can be produced by hydrolyzing proteins. Chemical (acid or alkali) and biological (enzymatic) methods are most commonly used for the hydrolysis of proteins [46]. Microwave induced hydrolysis of protein has also been reported. The aim of the hydrolysis process is to liberate amino acids and recover them without degrading their properties. The factors affecting the hydrolysis of proteins are temperature, time, hydrolysis agent and additives. These factors affect the quality and yield [110].

Acid hydrolysis of fish proteins

Acid hydrolysis is the most commonly used process for the hydrolysis of proteins. The process itself is very harsh and hard to control, but is still the preferred method for hydrolyzing proteins. Acid hydrolysis is normally carried out using hydrochloric acid and in some cases with sulfuric acid [111]. The agents used in the acidic hydrolysis of proteins are shown in the Table 16. The conventional acidic hydrolysis of fish proteins is carried out using 6 M HCl for 20-24

h at 110°C under vacuum [92]. Under these conditions of hydrolysis, asparagine and glutamine are completely hydrolyzed to aspartic acid and glutamic acid, respectively. Tryptophan is completely destroyed and cysteine cannot be directly determined from the acid hydrolysed samples. Tyrosine, serine and threonine are partially hydrolysed. There is usually 5-10% loss in the recovery using acid hydrolysis [110].

The conventional method of acid hydrolysis was modified by adding 50% acetic acid in order to reduce the hydrolysis time [112]. During conventional hydrolysis, the recoveries of amino acids are very low and, therefore, in the presence of organic acid it is possible to reach the hydrophobic regions of proteins. Tsugitaand Scheffler [113] used formic acid, acetic acid, trifluoroacetic acid and propionic acid to hydrolyze proteins. Trifluoroacetic acid was found to be a strong acid with a pKa of 0.23, high vapour pressure and low boiling point (72.5°C). The dipeptide consisting of valine and isoleucine (Val-Val, Val-Ile, Ile-Val and Ile-Ile) in the proteins were hydrolyzed at 160°C for 25 min with various combinations of mixtures of hydrochloric acid and organic acid. The results indicated that combination of trifluoroacetic acid and HCl in the ratio of 1:1-1:2 showed a recovery of 100% when compared to other organic acids as shown in Table 17.

Fountoulakis and Lahm [110] reported that the time and temperature are always important variables to consider in conventional acid hydrolysis processes. Hydrolysis of protein with 6 M HCl at 145°C for 4 h gives comparable recoveries and quantification in comparison to conventional hydrolysis with 6M HCl at 110°C for 24 h. The recoveries of threonine and serine were reduced by 50% after 4 h, whereas the valine and isoleucine recoveries increased by 100%. In comparison, shortened hydrolysis at elevated temperatures gave similar or superior results to those of conventional hydrolysis at 110°C for 24 h.

Csapo et al. [114] stated that in addition to the recovery yield, the

Hydrolysis agent	Hydrolysis Conditions	Additives	Specific Determination
6 M HCl	110°C, 24 h	0.02% Phenol	All residues except of Cys, Trp
6 M HCl or 4 M MSA	110°C, 24 h	0.2% Sodium azide	Cys
6 M HCl	110°C, 18 h	5% Thioglycolic acid, 0.1% phenol, 3,3'-dithiodipropionic acid	Cys
6 M HCl		3-Bromopropylamine	Cys
6 M HCl	145°C, 4 h	Samples previously oxidized with performic acid	Cys, Met, Lys
4 M MSA		3-(2-aminoethyl)indole	Trp, Methionine sulfoxide
4 M MSA	115°C, 22 h	Samples previously alkylated, tryptamine	All residues
4 M MSA	160°C, 45 min		All residues
4 M MSA or 5.7 M HCl	150°C, 90 min	Oxidation with performic acid / 50°C, 10 min	All residues
3 M *p-Toluenesulfonic* acid			Methionine sulfoxide
12 M HCl–propionic acid (1:1)	150°C, 90 min		Resin-bound peptides
12 M HCl–propionic acid (1:1)	840 W, 1–7 min microwave		Resin-bound peptides
p-Toluenesulfonic acid	15 min, microwave		
DCl	medium power, 30 min microwave		Sensitive residues
2.5 M Mercaptoethanesulfonic acid	176°C, 12.5 min	S-Pyridylethylated samples	Cys, Trp
6 M HCl–TFA (6:3)	120°C, 16 h	Dithiodiglycolic acid, 1% phenol	Cys
HCl		Thioglycolic acid	Trp
HCl	110°C, 24 h	0.4% β- Mercaptoethanol	Trp
HCl	166°C, 25 min or 145°C, 4 h	3% Phenol	Trp
HCl	145°C, 4 h	Tryptamine	Trp
6 M HCl	145°C, 4 h gas phase	Tryptamine[3-(2-aminoethyl)]indole	Trp
TFA–HCl (1:2)	166°C, 25-50 min	5% Thioglycolic acid	Trp, Met
7 M HCl, 10% TFA		10% Thioglycolic acid, indole	Trp

Table 16: Hydrolysis agents of protein hydrolysis [110].

Acid Mixture	Composition (v/v)	Recovery (%)
Formic acid: HCl	1:1	85
	1:2	95
Acetic acid: HCl	1:1	97
	1:2	100
Trifluoroacetic acid: HCl	2:1	85
	1:1	100
	1:2	100
Propionic acid: HCl	1:1	90
	1:2	97

Table 17: Recovery of amino acids from valyl- glutamic acid [113].

duration also affects the degree of racemization of the hydrolyzate. When using conventional protein hydrolysis, racemization is 1.2-1.6 times higher compared to the hydrolysis carried out at elevated temperatures of 160-180°C. At higher temperatures, proteins are hydrolyzed rapidly into free amino acids and racemization of free amino acids is always slower than that of amino acid bound to polypeptides. During conventional acid hydrolysis the proteins are hydrolyzed at a much slower rate, and the amino acids bound to polypeptide bonds were exposed to heat for a longer time causing racemization. According to Ozols [115], there are certain peptide bonds that are very tough to cleave, including Ile-Val, Val-Val, Ile-Val, resulting in only 50-70% yield at 110°C in 24 h and therefore hydrolysis must be carried out for 92-120 h in order to obtain higher yields.

Blackburn [116] used Methanesulfonic acid (MSA) in the presence of 3-(2-aminoethyl) indole for the hydrolysis of proteins. The advantage of using methane sulfonic acid in comparison to HCl is that tryptophan is not destroyed and methionine is determined as methionine sulfoxide. To reduce the losses incurred by the acid hydrolysis certain protective agents such as phenol, thioglycolic acid, mercapto ethanol, indole or tryptamine are added to the sample Adebiyi et al. [117] used a single step protein hydrolysis process with 4 M methanesulfonic acid at 115°C for 22 h in the presence of 0.02% tryptamine, in which even tryptophan and cysteine were determined.

Chiou and Wang [118] hydrolyzed proteins using methane sulfonic acid, in which 0.5 mg of protein samples were added with 0.5 ml 4 M methane sulfonic acid containing 0.2% 3-(2-aminoethyl) indole. The hydrolysis tubes were flushed with nitrogen gas for 1 min, closed tightly and heated at 160°C for 45 min. At the end of hydrolysis, the samples were partially neutralized with 8 M sodium hydroxide to a pH of 2. The samples were analyzed using amino acid analyzer and the results were compared with conventional HCl hydrolysis. Hydrolyzing the proteins at a higher temperature and a shorter time, accurate results were produced for all amino acids including tryptophan and half-cystine which are normally degraded in the conventional HCl acid hydrolysis process. An additional advantage of this process is that there is no degradation of serine, threonine and tyrosine as in the case of prolonged hydrolysis. The amino acids recovery were between 97-102% at a temperature of 160°C for 45 min. Methane sulfonic is also non-volatile in nature and cannot be evaporated after hydrolysis and it is, therefore, often neutralized to pH 2 before analysis.

Liu and Chang [119] hydrolyzed proteins using 3 N p-toluenesulfonic acid containing 0.2% 3-(2-aminoethyl)indole at 110°C for 22, 48 and 72 h. At the end of hydrolysis, 2 ml of NaOH were added and the mixture was transferred to 5 ml volumetric flask, in which the total volume was made up to 5ml before analysis. The results indicated that p-toluenesulfonic acid hydrolysis can be carried out to identify and quantify tryptophan but cannot be used for the analysis of proteins which are contaminated with carbohydrates such as those in animal feed.

Ault [120] stated that the extraction method is dependent on the starting material which can include: hair, keratin, feather, blood meal and soybeans. The standard procedure comprises of the hydrolysis with aqueous acid, in which the amino acids are captured when passing the hydrolysate over a strongly acidic ion exchange resin. The resin is later washed with water and eluted with aqueous ammonia which frees the amino acids. This is one of the most economical processes for the production of tyrosine and cysteine. The main drawback of this process is that the raw materials cannot keep up with the increasing demand for amino acids [121].

Sano [122] reported on the extraction of amino acids from wheat gluten which was identified as the major source of glutamate. The process consists of three steps: extraction, isolation and purification. In the extraction process, gluten was first separated from the wheat flour by washing the starch from the dough. The crude gluten was transferred to pottery vessels and mixed with hydrochloric acid and heated for 20 h. The hydrolysates were then filtered to remove the black residue resulting from the reaction of amino acids with carbohydrates. The filtrate was transferred to the same vessel and concentrated for 24 h and then transferred to another vessel to crystallize for 1 month. The crystals of L-glutamic acid hydrochloride were isolated from the liquid through filtration and redissolved in water. The pH was adjusted to 3.2 and stored for 1 week for L-glutamic acid crystallization. The crystals had two polymorphs granular α-form and stable, thin β-form. The α-form contained only glutamate crystals with improved purity. The separated L-glutamic acid α-form crystals were dissolved in water and the pH was adjusted to 7 and filtered and decolorized using activated charcoal. The filtered solution was concentrated by heating and cooled to form monosodium L-glutamate.

Alkaline hydrolysis of fish protein

Hydrolysis of proteins can be carried out using sodium hydroxide, potassium hydroxide or barium hydroxide. The alkaline treatment is specifically used for the determination of tryptophan. It is also applied to the samples which have a higher percentage of carbohydrates as in the case of foods and formulation of pharmaceutical solutions which have higher percentage of monosaccharides. The major disadvantage of this method is that serine, threonine, arginine and cysteine are destroyed and all other amino acids are racemized [123].

Linder et al. [124] stated that many deleterious reactions occur in alkaline solutions during hydrolysis. These reactions are initiated by hydrogen abstraction from the alpha carbon of an amino acid which includes racemization of L- amino acids to produce D- amino acids.

Lahl and Braun [125] reported that D- amino acids are not absorbed by humans and that alkaline hydrolysis also splits disulfide bonds with loss of cysteine, serine and threonine via β-elimination reactions and formations of lysinoalanine, orinithinoalanine, lanthionine and β-amino alanine. These eliminations can also lead to production of toxic substances such as lysinoalanine and are undesirable in foods.

The chemicals that are produced during the alkaline hydrolysis of protein are shown in the Figure 8 [46]. Protein phosphorylation plays a major role in cell biology and biomedical sciences. Addition of phosphate to the amino acid side chain by esterification causes conformational changes to the protein in terms of activity and stability. The typical acceptors of phosphate in their ring structure are the hydrophobic amino acids such as tyrosine, serine, tryptophan, histidine, aspartic acid, glutamic acid, lysine, arginine and cysteine.

Yan et al. [126] reported the partial hydrolysis of amide bonds using alkali can release phosphoamino acids. Fountoulakis and Lahm [110] reported that potassium hydroxide hydrolysis was applied for the quantification of phosphorylated and sulphated tyrosine and for analysis of phosphohistidine. Proteolytic hydrolysis of protein samples, followed by alkali hydrolysis results in superior yields of phosphoamino acids [126].

Enzymatic hydrolysis of fish protein

Kim and Wijesekara [127] reported that enzymatic hydrolysis of fish proteins with appropriate enzymes such as alcalase, pronase, collagenase, pepsin, papain, protamex, bromelain, chymotrypsin and trypsin allows for the preparation of bioactive peptides made up of specific length of amino acids. The main advantage of enzymatic hydrolysis of proteins is that it allows quantification of asparagine and glutamine and other sensitive residues, which are normally destroyed by acid and alkali hydrolysis, and does not cause any racemization during digestion.

Wachirattanapongmetee et al. [128] hydrolysed fish proteins obtained from hybrid Catfish frame. The proteins were mixed with distilled water in the ratio of 1:1.5 (w/v) and the hydrolysis was started by adding 0.5, 1.5 or 3% v/w protex enzyme. The fish proteins were hydrolysed for 60, 120 and 180 min. The experiment was carried to improve the functional properties of fish proteins such as protein solubility, emulsification properties and fat absorption capacity rather than completely breaking down the fish proteins into amino acids.

Cheftel et al. [129] hydrolysed fish protein using enzymes (pronase, pepsin, bromein, ficin, rhozyme P11 and rhozyme 41) to obtain complete solubilisation of proteins. The results indicated the amount of protein hydrolyzed depended upon the type of enzyme used and hydrolysis time. The rate constant of the reaction decreased with increased with time and the hydrolysis did not follow first order reaction. In addition, the formation of products within the system was inhibitory to the rate of reaction. The results indicated that pronase enzyme was able to hydrolyse 94% of the proteins in 23 h.

Yamashita et al. [130] hydrolysed fish protein concentrate (FPC) in a two step hydrolysis process using two enzymes (papain and pronase). The fish proteins were first hydrolysed using papain for 48 h at a temperature of 37°C and a pH of 1.5 with vigorous shaking. The mixture was then hydrolysed using pronase at a temperature of 37°C for another 5 h at various pH conditions ranging from 6 to 9. The protein was completely broken down and was comparable to that of soy bean hydrolysates.

Bhumiratana et al. [131] studied the enzymatic solubilization of fish protein in membrane reactors in batch, semi-batch and continuous mode of operations using trypsin temperature of 50°C and pH of 9.0. The results indicated that the fish proteins were completely solubilized in the reactor and the functional properties of the product improved better than the original substrate.

For complete hydrolysis of the protein, a combination of different proteases is necessary with a longer incubation time. Therefore, this method is not applied for serial analysis [110]. Linder et al. [124] stated that due to the presence of several peptide bonds and their specific accessibility to enzymatic reaction, the enzymatic hydrolysis is a complex process. The specificity of the enzymes is not the only factor affecting the hydrolysis but some environmental factors such as temperature and pH also play an important role in the hydrolysis of the proteins. However, the widespread application of this type of hydrolysis is hampered by the relative specificity of the proteases for certain residues.

To overcome the drawbacks of enzymatic hydrolysis of proteins, enzyme membrane reactors (EMR) can be used to produce amino acids. The set up for membrane reactors used for the production of amino acids includes dead-end membrane; recycle membrane, diffusion membrane and multiphase membrane reactors. In a dead-end reactor, the solution containing enzymes is pushed towards the membrane, the product is obtained on the other side and the enzymes are retained (Figure 9a). In a recycle membrane reactor, the substrate containing enzymes is continuously recycled from and to the reactor through the filtration membrane (Figure 9b). If the soluble enzymes are used, the reaction takes place in both vessel and membrane module but occurs only in the module if immobilized enzyme are used [132,133]. The diffusion membrane reactor allows passive diffusion of substrate molecules through the membrane to the adjacent compartment containing enzymes (Figure 9c). These reactors are used only for low molecular weight substrates (as the substrate is diffused back after catalysis). The multi-phase reactors are capable of creating interfacial contact between enzyme and substrate at the membrane matrix through diffusion (Figure 9d). In this type of reactor, the membrane acts as a support between two liquid phases and in some cases positive pressure can be applied in order to prevent the phases from mixing [133].

The main advantages of the enzyme membrane reactors (EMR) include: (a) development of continuous processes for the production of amino acids, (b) higher productivity, (c)controllable environment, shift in the chemical equilibrium, (d) improved rate of reaction, (e) concentration of process streams (f) possibility to conduct multiphase process reactions, (g) no enzyme fixation costs, (h) interchange ability of substrate/enzyme systems, (i) use of multienzyme system, (j)
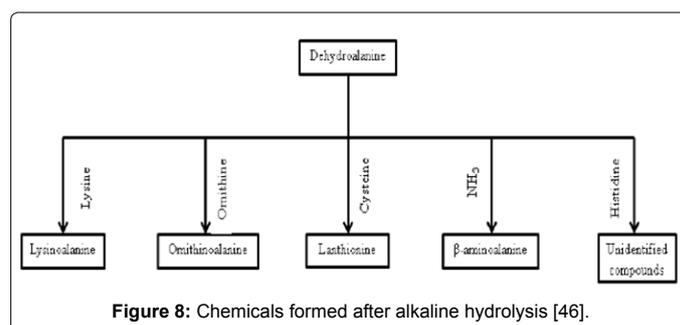
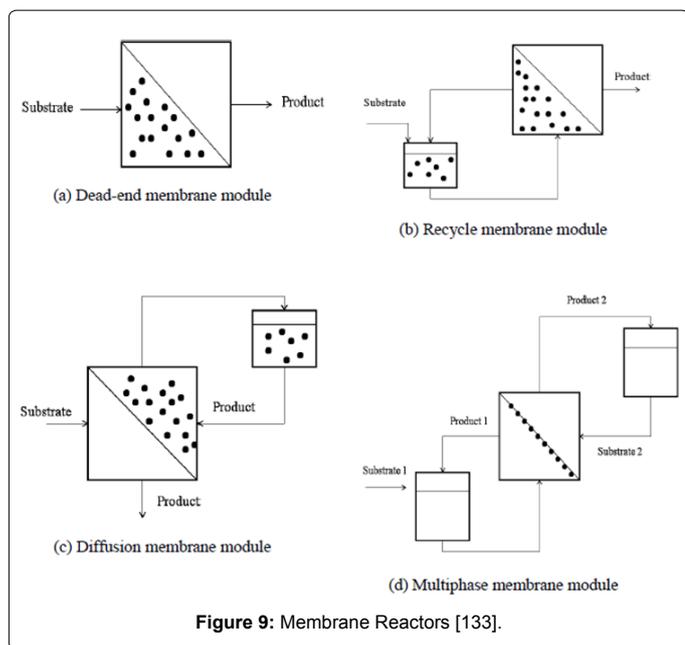**Figure 8:** Chemicals formed after alkaline hydrolysis [46].

Figure 9: Membrane Reactors [133].

sterilizability of the plant and (k) no diffusion limitation. The main drawbacks of EMR are: (a) a specific substrate is used to produce corresponding amino acids, (b) lacks operational stability, the enzymes are resistant towards product inactivation, (c) high substrate and salt concentrations, (d) it cannot withstand high temperature and organic solvents, (e) it is not stable at low or high pH values, (f) poisoning of enzyme, (g) deactivation of enzymes, (h) concentration polarization, (i) fouling and (j) enzyme leakage [133,134].

Microwave induced hydrolysis of fish proteins

In the microwave induced hydrolysis of proteins, substrate can be hydrolysed in either liquid or gas phase mode with HCl or other reagents. In this method, the instant uptake of the radiation energy results in the reduction of the overall hydrolysis time from many hours to few minutes, 1-30 min for the liquid phase hydrolysis and 20-45 min for gas phase hydrolysis. There are no reports in the literature on the use of this method with fish proteins. However, this method has been used with bovine serum albumin, casein, gelatin and recombinant human interferon α_2 and has the potential for application in the fish protein hydrolysis.

Englehart [135] reported that microwave hydrolysis of proteins have been under investigation for determining the amount of amino acids in connective tissues of meat products. The protein collagen in the meat products contains a high concentration of amino acids which can be determined by microwave hydrolysis.

Kaiser and Benner [136] studied microwave induced hydrolysis of proteins (bovine serum albumin). Amino acids were hydrolysed with a CEM Mars 5000 microwave equipped with a protein hydrolysis accessory kit which included four Teflon vessels for the samples. The major advantages of the process were: (a) it allows processing 40 samples in 3 h, (b) only 100 μl was needed for analysis, (c) the samples contain only hydrochloric acid and moisture with less impurities and (d) more feasible for smaller sized samples.

Margolis et al. [137] studied the hydrolysis of proteins (bovine serum albumin) by microwave energy using bovine serum albumin that was hydrolyzed with 10 mol/L HCl in a clean acid leached Teflon

(PFA) pressure vessel which was free of the metal impurities and kept in the microwave system at 125°C for 2-4 h. The results showed that most of amino acids were completely hydrolyzed within 2 h except valine, isoleucine and leucine which were resistant to hydrolysis. Tryptophan was not stable and could not be measured. When the time was increased to 4 h, threonine, serine and tyrosine were stable compared to the conventional hydrolysis process.

Wu et al. [138] used microwave energy to study the cleavage of synthetic peptide. In this study, synthetic peptides (1 mg/ml) were dissolved in dilute hydrochloric acid (0.006, 0.015, 0.03, 0.06 M) and water in 0.3 ml Teflon vials. The vials were flushed with nitrogen gas for about min and placed in microwave oven for time intervals ranging from 1 to 7 min. The results indicate that the peptides in the 0.06 M HCl were completely hydrolyzed in 3 min and the peptides in the 0.03 M HCl were hydrolyzed in 4 min. The peptide content in the neutral solution was less than 15% after 4 min. The microwave energy that is emitted is absorbed into the liquid media by two mechanisms: ionic conduction and dipole rotation. Therefore, the input power during microwave hydrolysis plays an important role and 572-650 W power is very suitable for microwave hydrolysis. This process was developed to control the cleavage sites especially for the peptide bonds connected to aspartic acid residues and it is very useful in obtaining defined acid-cleaved peptide fragments.

Joergensen and Thestrup [139] performed microwave assisted hydrolysis on protein samples (casein and gelatin). The results suggested that a hydrolysis time of 10-30 min is enough to cleave all the peptide linkages with no loss of serine or threonine. Methionine was found to be stable with the addition of thioglycolic acid during the hydrolysis process. As in the case of conventional hydrolysis process, cysteine and tryptophan could not be quantified. To quantify cysteine, the sample was subjected to pre-hydrolysis oxidation in which 20-50 mg of the protein were added to 5 ml ice cold performic acid (0.5 ml 30% H_2O_2 and 4.5 ml formic acid) and 250 μl of 200 m Mn or leucine were also added along with 250 μl of 10% phenol at 0°C for 18 h. After 18 h, the reagents were dried under vacuum using a freeze dryer and 5 ml water and 10 ml 30% HCl were added to the dried samples to perform microwave hydrolysis.

Weiss et al. [140] performed liquid phase and gas phase hydrolysis on protein solutions (recombinant human interferon α_2 from *E. coli*) using a microwave technique. In liquid phase microwave hydrolysis, 500 μl of protein solution were added to 6 M HCl containing 0.02% phenol or to 4 M methane sulfonic acid containing 0.2% 3-(2-aminoethyl)indole. The hydrolysis vial was placed in the microwave oven inside a beaker containing 200 ml water for equal absorption of energy and hydrolysis was carried out at 155°C (900 W) for 4 min. In the gas phase microwave hydrolysis, 300 μl of protein solution were evaporated to dryness, 6 M HCl were added and the hydrolysis was performed at 1000 W for 5 min and 500 W for 15 min after flushing the vials with argon gas. The results indicated that using this microwave technique, the hydrolysis was completed within 4 min and gave results comparable to that of conventional hydrolysis with higher losses of serine and threonine and a higher percentage of racemization of amino acids.

Utilization of amino acids

Amino acids are the building blocks of proteins. They have wide nutritional value, taste, medicinal action and chemical properties. All amino acids are sold in different quantities each year as shown in Table 18. They are used as food additives, in pharmaceutical applications, feed and food supplements. The amino acids such as arginine, glycine,

glutamate and histidine are used in protein pharmaceuticals as an excipient for drug development is shown in Table 19. The largest consumer of amino acids is the food flavoring industry which uses monosodium glutamate, alanine, aspartate and arginine to improve the flavour of food. The second largest consumer of amino acids is the animal feed industry which uses lysine, methionine, threonine, tryptophan and others to improve the nutritional quality of animal feed. The amino acids can also used in various pharmaceutical applications such as protein purification and formulations and production of antibiotics such as jadomycin [141]. The total amino acid market in 1996 was estimated to be $4.5 billion. The market value of amino acids has drastically increased since 1996 [121]. Fermentation products in 2004 were estimated to be $14.1 billion and $17.8 billion and amino acids were the second most important category after antibiotics in 2009 [142].

Production and Utilization of Fish Oil

Chemical extraction of fish oil

Fish oil can be extracted from fish and fish-waste chemically using Soxhlet or Goldfisch, Folch, Bligh and Dryer method and acid digestion method with various solvents such as diethyl ether, petroleum ether, chloroform/methanol and hexane [143].

Goldfisch method: In the Goldfisch method, 10 g of the predried samples is placed in a ceramic extraction thimble. To the sample, 40 ml of hexane or petroleum ether is added and kept for 4-7 h. The sample is cooled and the solvent is evaporated at 95°C for 30 min. The sample is cooled and the weight of the lipid is calculated as shown in Equations 1 and 2 [143-145].

Weight of lipid=(weight of container+extracted lipid)-(weight of container)   (1)

$$Lipid\ content\,(\%) = \frac{Mass\ of\ lipid\ extracted\ (g)}{weight\ of\ the\ original\ sample(g)} \times 100 \quad (2)$$

Hwang and Regenstein [146] reported the use of gold fisch extraction procedure to determine the fat content of the frozen menhaden fish at different temperatures and time intervals. Erickson [147] carried out lipid extraction from catfish minced muscle using the goldfisch method and compared it with other extraction methods of

Amino acid	Amount (ton/year)	Process	Uses
L- Glutamate	1000000	Fermentation	Flavour enhancer
D,L- Methionine	350000	Chemical	Food, Feed supplement and pharmaceutical
L- Lysine HCL	250000	Fermentation	Feed supplement
Glycine	22000	Chemical	Pharmaceutical, soy sauce
L- Phenylalanine	8000	Fermentation, synthesis	Aspartame
L- Aspartic acid	7000	Enzymatic	Aspartame, Pharmaceutical
L- Threonine	4000	Fermentation	Feed supplement
L- Cysteine	1500	Extraction, Enzymatic	Pharmaceutical
D, L- Alanine	1500	Chemical	Flavor, sweetener
L- Glutamine	1300	Fermentation	Pharmaceuticals
L- Arginine	1200	Fermentation	Flavor, Pharmaceuticals
L- Tryptophan	500	Fermentation, Enzymatic	Feed supplement, Pharmaceuticals
L- Valine	500	Fermentation	Pharmaceuticals
L- Leucine	500	Fermentation, extraction	Pharmaceuticals
L- Alanine	500	Enzymatic	Pharmaceuticals
L- Isoleucine	400	Fermentation	Pharmaceuticals
L- Histidine	400	Fermentation	Pharmaceuticals
L- Proline	350	Fermentation	Pharmaceuticals
L- Serine	200	Fermentation	Pharmaceuticals
L- Tyrosine	120	Extraction	Pharmaceuticals

Table 18: Global production of amino acids in 1996 [121].

Amino acids	Concentration	Product	Drug Substance	Company
L-Arginine	55 mg/mL	TNKase®	Human tissue plasminogen activator (tPA)	Genentech
L-Arginine	35 mg/mL	Activase®	Human tissue plasminogen activator (tPA)	Genentech
L-Glycine	21-25 mM	Kogenate®FS	Recombinant antihemophilic factor (rAHF)	Bayer
L-Glycine	3 mM	Synagis®	Human monoclonal antibody	MedImmune
L-Glycine	260 mM	BeneFIX®	Coagulation factor IX	Wyeth
L-Glycine	0.17-0.34 mg/mL	Nutropin®	Human growth hormone (hGH)	Genentech
L-Glycine	23 mg/mL	Neumega®	Interleukin-11	Wyeth
L- Glutamate	1 mg/mL	VariVax®	Varicella virus vaccine live	Merck
L-Glutamate	5 mg/mL	Streptase®	Streptokinase	Aventis
L-Histidine	55 mM	Recombinate	Recombinate antihemophilic factor (rAHF)	Baxter
L-Histidine	18-23 mM	Kogenate®FS	Recombinate antihemophilic factor (rAHF)	Bayer
L-Histidine	47 mM	Synagis®	Human monoclonal antibody	MedImmune
L-Histidine	5 mM	Herceptin®	Human monoclonal antibody	Genentech
L-Histidine	10 mM	BeneFIX®	Coagulation factor IX	Wyeth

Table 19: Approved protein pharmaceuticals containing amino acids as excipient **[141].**

and found the chloroform/methanol method to be the better solvent system for the lipid extraction from catfish.

Paredes and Baker [148] studied the physical, chemical and sensory changes during thermal processing of canned catfish, ocean perch and Atlantic Pollock. In this study, the fat from all the three species was extracted using goldfisch extraction method.

Chloroform/Methanol method: Folch et al. [149] described a chloroform/methanol lipid extraction procedure in which 1.5 g of fish tissue was mixed with 30 mL of 2:1 chloroform/methanol. The mixture was then filtered and washed several times using 2:1 chloroform/methanol and a weak salt solution of 0.88% NaCl was then added to get a final ratio of 8:4:3 chloroform/methanol/water. The final biphasic layer was centrifuged ant the lower phase was collected in a pre-weighed glass tube and evaporated to dryness under nitrogen in a thermostatically controlled water bath maintained at 25–30°C. To completely remove all final traces of solvent and water, the sample tube was then flushed with nitrogen and vacuum suction was applied for 5 min. The lipid content was then determined gravimetrically by weighing the remaining lipids in the tube.

Iverson et al. [150] extracted lipids from fish samples using the original procedure described by Folch et al. [149] and the results were compared to Bligh and Dyer method of lipid extraction. The results suggested that both methods of extractions were highly correlated and the only advantage of using Bligh and Dyer is the reduction in the solvent/sample ratio; 1 part of tissue to 3 parts in the chloroform/methanol method instead of 1 part of tissue to 20 parts of chloroform/methanol in the Folch method.

Bell et al. [151] extracted lipids from the Atlantic salmon (Salmosalar) using the procedure described by Folch et al. [149]. Gigliotti et al. [152] reported on the extraction of lipids from Atlantic krill (Euphausiasuperba) using Folch method. Ramanathan and Das [153] extracted lipids from Scomberomorus commersoni using Folch method to study the lipid oxidation of the ground fish in the presence of antioxidants. Bernardez et al. [154] reported on the extraction of lipids from Atlantic mackerel (Scomberscombrus) using Folch method and analyzed the free fatty acid in the fish. Saify et al. [155] extracted lipids from two species of shark using chloroform/methanol (2:1) extraction method (Folch method) and evaluate lipid composition of both the species.

Bligh and dyer: In the Bligh and Dyer [156] method, 100 g of sample is homogenized with 100 ml of chloroform and 200 ml methanol. To this mixture, 100 ml of chloroform is added and homogenised for 30 seconds. Then 100 ml of distilled water or weak salt solution (0.88% NaCl) is added to the mixture. The homogenate is filtered using Whattman No. 1 filter paper. The filtrate is then transferred to 500 ml measuring cylinder and allowed for separation and clarification. The chloroform later contains the lipid which is then separated through distillation. Lipid content was then determined gravimetrically by weighing the remaining lipids in the tube.

Iverson et al. [150] extracted lipids from fish tissues using the Bligh and Dyer extraction method. Smedes and Askland [157] extracted lipids from cod fillet using Bligh and Dryer method and determined the total lipid content in the fish. Norziah et al. [158] extracted fish oil from wastes of seafood processing industry using Bligh and Dyer method and determined the peroxide and anisidine values.

Ozogul et al. [159] compared the petroleum ether solvent extraction

and Bligh and Dyer extraction for changes in the EPA and DHA. The results indicated that that Bligh and Dyer method was more efficient in extracting polar and non-polar lipids than the petroleum ether method and also prevented losses of polyunsaturated fatty acids by reduction in oxidation.

Aryee and Simpson [160] extracted fish oil from salmon skin using Bligh and Dyer method and determined the total yield. Gruger et al. [161] extracted lipids from 21 species of marine fish, freshwater fish and shellfish using Bligh and Dyer method and determined the fatty acid composition of fish species. Indrati et al. [162] extracted fatty acids from fish oil and cod liver oil using Bligh and Dyer method and determined the fatty acid methyl esters (FAME) present in the oil.

Zhong et al. [163] extracted lipids from the muscles and viscera of steelhead trout using Bligh and Dyer method with a chloroform/methanol ratio of 2:1 to characterize the fatty acid composition of muscles and viscera. The results indicated that muscles had more polyunsaturated fatty acids than viscera.

Acid digestion method: In the acid digestion method, 5 g of ground sample is hydrolysed using 6 N HCl at 80°C for 1 h or at 110°C for 4-24 min until complete dissolution. The lipids are extracted using 1:1 (v/v) chloroform/methanol, retaining the organic layers each time. The organic solvent is removed at 40°C under reduced pressure using arotary evaporator. Then, the weight and content of lipid are calculated [143].

Xiao [144] extracted lipids using acid digestion method, in which 4 g of fish sample was weighed in a flask and dried at 103°C in an oven for one hour. After drying, 100 ml of petroleum ether were to the added to the flask and extraction was carried out for 2 h. The petroleum ether was then distilled and the sample was again heated for 1 h at 103°C. Then, 3 ml of hydrochloric acid were added and the sample was boiled for 1 h in a water bath. The sample was cooled and filtered. The filtrate was washed with distilled until the filtrate is neutral. The sample was again dried for 1 h at 103°C. 100 ml of petroleum ether were again added to the sample and extracted overnight with a condensation rate of approximately 2-3 drops/second. The petroleum ether was distilled and the sample was dried in oven for 2 h at 103°C. The fat content was calculated as follows:

$$\text{Fat} = \frac{a-b}{w} \times 100 \quad\quad\quad (3)$$

Where,

a=Weight of the tube with fat extract (g)

b=Weight of the tube (g)

w=Initial weight of the sample (g)

Radar et al. [164] determined the total fat and saturated fat in various food stuffs including fish using gas chromatography after acid hydrolysis using 6N hydrochloric acid and petroleum ether.

Christie [165] stated that during extraction of lipids, problems may occur due to the presence of highly hydrophilic functional groups such as acyl-coenzyme A esters, lysophospholipids and polyphosphoinositides and gangliosides. Especially with polyphosphoinositides, it always necessary to store the fish tissues in a proper condition, so that enzymatic degradation is minimized. In such cases, the tissues are extracted with chloroform/methanol in the presence of calcium chloride initially and with 1 M hydrochloric acid for better recovery of lipids.

Enzymatic extraction of fish oil

The enzymatic hydrolysis of fish is carried out using various commercially available enzymes such as alcalase, neutrase, lecitase ultra, protex and protamex. In the enzymatic extraction method, the fish samples are minced and 50 g of fish mince is initially heated at 90°C for 5 min to deactivate endogenous enzymes and to the heated samples 50 ml of water or buffer is added. The temperature and pH of the fish mince is adjusted to 55°C and 8.0 for alcalase, 45°C and 6.5 for neutrase, 40°C and 6.8 for lecitase ultra, 60°C and 9.0 for protex and 50°C and 7.5 for protamex. The hydrolysis is started by adding 0.5% (w/w) enzyme to the mixture and proceeded for 1, 2, 3 or 4 h. After each hydrolysis time intervals the samples are heated at 90°C for 5 min to deactivate the added enzymes. The mixture is then centrifuged at 10000 rpm for 20 min to separate oil fraction from the mixture [166-169].

Mbatia et al. [170] used 0.5% bromelain and 0.5% protex to extract oil from nile perch and salmon heads at 55°C. The results indicated that a maximum oil yield of 11.6 g/100 g and 15.7 g/100 g was achieved for bromelain and protex, respectively. The oil recovered from the available total lipids in salmon heads and nile perch using bromelain and protex were 65% and 88% and 81% and 81%, respectively. The study also suggested that increasing the enzyme concentration increased the hydrolysis rate but did not increase the oil yield from fish.

Linder et al. [171] used three different enzymes Neutrase, Alcalase and Flavourzyme at a concentration of 0.05% and three temperatures (45, 55 and 50°C) for 2 h to extract oil from salmon heads. The oil yield using Neutrase, Flavourzyme and Alcalase were 17.2, 17.0 and 17.4%, respectively.

Dauskas et al. [172] extracted oil from cod (Gadusmorhua) by products using alcalase enzyme at 50°C and pH of 6.5 from various parts of cod fish. The study reported maximum oil recovery of 82.8% from cod viscera without digestive tract using flavourzyme enzyme. The lowest oil recovery of 36.4% was achieved by using Neutrase enzyme on viscera with backbone. The authors suggested that at the end of hydrolysis lipids were formed in three forms: free oil, emulsion and sludge. The formation of emulsion is not desirable and increase in the amount of emulsion decreases the amount of free oil produced. The study suggested that addition of water during hydrolysis increased the formation of emulsion and decreased the production of free oil. In this study the highest oil yield (76.26%) was achieved from head and it was less than the oil yield reported by the author which is due to the addition of buffer during the hydrolysis process.

Slizyte et al. [167] extracted oil from cod (Gadusmorhua) by products using alcalase enzyme at 55°C and pH of 7.5 and lecitase ultra at 70°C and pH of 8.5 for 60 min. In this study, the extraction was carried out by varying parameters such as heat inactivation and addition of water both individually and in combination of enzymes. The oil is obtained by centrifugation at 10000 g for 10 min. The results indicated that the alcalase treated samples without addition of water gave best results followed by the combination of alcalase and lecitase without addition of water. The addition of water increased the emulsion formation and increased the protein yield and decreased the oil yield. The heat inactivation prior to start of the hydrolysis increased the amount of oil released into the system.

Slizyte et al. [101] used flavourzyme and Neutrase to extract oil from cod and reported that the decrease in the amount of free oil fraction can be attributed to the presence of large amounts of proteins in the raw material (digestive tracts, flesh and backbones) which together with the oil present in the liver forms various complexes when heated during thermal deactivation of endogenous enzymes. During heat inactivation, the proteins in the raw material were denatured and precipitated. Only a small portion of denatured proteins can be solubilised and the remaining forms a lipid-protein complex which eventually reduces the release of lipids into the oil fraction. The report also suggested that the minimum amount of lipids in the raw material should be more than 8.5 g/100 g to form an emulsion and to decrease the formation of emulsion the amount of protein must be higher than 16.5 g/100 g.

Utilization of Fish Oil

For human consumption

Fish oils are readily available sources for long chain polyunsaturated fatty acids whichconsist of omega-3 fatty acids mainly composed of cis-5,8,11,14,17- eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) [173]. The omega-3 fatty acids have many beneficial bioactivities including prevention of atherosclerosis, protection against arrhythmias, reduced blood pressure, benefit to diabetic patients, protection against manic-depressive illness, reduced symptoms in asthma patients, protection against chronic obstructive pulmonary diseases, alleviating the symptoms of cystic fibrosis, improving survival of cancer patients, reduction in cardiovascular disease and improved learning ability [96,156,174]. The American Heart Association has recommended at least two servings of fish every weekto reduce the effect of cardiovascular diseases [175].

For biodiesel production

Biodiesel is comprised of monoalkyl esters of vegetable oils, animal fats or fish oils which can be synthesized from edible, non-edible and waste oils. It is a non-toxic, biodegradable and renewable energy source. Biodiesel can be produced chemically or enzymatically. Currently, the biodiesel production on an industrial scale is being carried out chemically, using alkali (NaOH) as catalyst due to high conversion ratio of triglycerols (TAG) to methyl esters (biodiesel) and low reaction times (4-10 h). [176]. There are several disadvantages using chemical catalysts including high reaction temperature, soap formation, waste generation and contamination of glycerol with alkali catalysts.

The biodiesel production using enzymatic transesterification is mainly carried out using lipases such as *Canadida Antarctica, Carica papaya, Rhizopusoryzae, Pseudomonas cepacia, Pseusdomonas fluoresces, Rhizomucormiehei* and *Mucormiehei.* The advantages of using enzymatic method are: there is no soap formation, low temperature requirement, no waste generation and high quality of glycerol. The disadvantages of using enzymatic transesterification are: high reaction times (12-24 h) and high cost of enzymes [176-178].

Conclusion

Fish processing is one of the major industries in Canada. Processing of fish involves stunning, grading, slime removal, deheading, washing, scaling, gutting, cutting of fins, meat bone separation and steaks and fillets. During these steps significant amount of waste is generated which can be utilized as fish silage, fishmeal and fish sauce. Fish waste can also be used for production of various value added products such as proteins, oil, amino acids, minerals, enzymes, bioactive peptides, collagen and gelatin.

The fish proteins are found in all parts of the fish. There are three types of proteins in fish: structural proteins, sacroplasmic proteins and connective tissue proteins. The fish proteins can be extracted by chemical and enzymatic process. In the chemical method, salts (NaCl and LiCl) and solvents (isopropanol and aezotropic isopropanol) are used, whereas during the enzymatic extraction, enzymes (alcalase, neutrase, protex, protemax and flavorzyme) are used to extract proteins from fish. These fish proteins can be used as a functional ingredient in many food items because of their properties (water holding capacity, oil absorption, gelling activity, foaming capacity and emulsifying properties). They can also be used as milk replacers, bakery substitutes, soups and infant formulas.

The amino acids are the building blocks of protein. There are 16-18 amino acids present in fish proteins. The amino acids can be produced from fish protein by enzymatic or chemical processes. The enzymatic hydrolysis involves the use of direct protein substrates and enzymes such as alcalase, neutrase, carboxypeptidase, chymotrypsin, pepsin and trypsin. In the chemical hydrolysis process, acid or alkali is used for the breakdown of protein to extract amino acids. The main disadvantage of this method is the complete destruction of tryptophan and cysteine and partial destruction of tyrosine, serine and threonine. The amino acids present in the fish can be utilized in animal feed in the form of fishmeal and sauce or can be used in the production of various pharmaceuticals.

The fish oil contains two important polyunsaturated fatty acids called EPA and DHA or otherwise called as omega-3 fatty acids. These omega-3 fatty acids have beneficial bioactivities including prevention of atherosclerosis, protection against maniac–depressive illness and various other medicinal properties. Fish oil can also be converted to non-toxic, biodegradable, environment friendly biodiesel using chemical or enzymatic transesterification.

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